Original Article
Obstet Gynecol Sci 2014;57(6):457-463
http://dx.doi.org/10.5468/ogs.2014.57.6.457
pISSN 2287-8572 · eISSN 2287-8580

Obstetrics & Gynecology Science

Thrombocytosis as a prognostic marker in stage III and IV serous ovarian cancer

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Objective
Thrombocytosis is an adverse prognostic factor in many types of cancer. We investigated if pre-treatment increased platelet counts provide prognostic information specifically in patients with stage III and IV serous ovarian cancer which is the most common clinical presentation of ovarian cancer.

Methods
Platelet number on diagnosis of stage III and IV serous ovarian adenocarcinoma was evaluated in 91 patients for whom there were complete follow-up data on progression and survival. Survival and progression free survival of patients with normal platelet counts (150–350 $\times 10^9$/L) was compared with that of patients with thrombocytosis (>350 $\times 10^9$/L) by X^2 and logrank tests.

Results
The median age of the patients was 66 years-old. From the 91 patients, 52 (57.1%) had normal platelet counts (median, 273 $\times 10^9$/L; range, 153–350) at diagnosis of their disease and 39 patients (42.9%) had thrombocytosis (median, 463 $\times 10^9$/L; range, 354–631). In the group of patients with normal platelet counts, 24 of the 52 patients had died with a median survival of 43 months (range, 3–100). In the group of patients with thrombocytosis, 24 of the 39 patients had died with a median survival of 23 months (range, 4–79). In the entire group of 91 patients there was a statistically significant difference of the overall survival and progression-free survival between the two groups (logrank test P=0.02 and P=0.007, respectively).

Conclusion
In this retrospective analysis of stage III and IV ovarian cancer patients, thrombocytosis at the time of diagnosis had prognostic value regarding overall survival and progression-free survival.

Keywords: Blood platelets; Ovarian neoplasms; Prognosis; Serous histology; Thrombocytosis

Introduction

Platelets play an important role in hemostasis and vascular integrity. They have a unique mechanism of derivation as fragments from the cytoplasm of bone marrow megakaryocytes. Abnormalities in platelet number, either increase (thrombocytosis) or decrease (thrombocytopenia), accompany diverse pathologic conditions and may aid in their diagnosis [1]. Cancer is a pathology that is often associated with thrombocytosis. This relates to the cytokine context of several malignancies that stimulates thrombopoiesis. Possibly due to this fact of association with a particular cytokine setting, thrombocytosis has been found to be an adverse prognostic factor in many common malignancies.

Received: 2014.1.15. Revised: 2014.3.5. Accepted: 2014.3.6.
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This study was partially presented as a poster at the 17th European Society for Medical Oncology and European Cancer Organization meeting, Amsterdam, The Netherlands, September 27 to October 1, 2013.

Ovarian cancer is one of the most common female malignancies. It is asymptomatic in initial stages and as a result it is diagnosed in many patients after intra-abdominal dissemination or even after becoming metastatic in distant sites. Ovarian carcinoma presents as localized or regional disease in about 30% of cases and as extensive disease in the rest [2]. Histologically ovarian adenocarcinomas are divided to several sub-types such as serous, endometrioid, mucinous and clear cell, in decreasing order of frequency [3]. Ovarian cancer is treated with debulking surgery and often with adjuvant chemotherapy to prolong the time to progression or decrease the risk of recurrence [4]. Stage is the main prognostic factor and determinant for the administration of chemotherapy after ovarian cancer surgery [5]. Histologic subtypes possess also prognostic information with some being more aggressive than others [6,7] but additional markers are needed to further promote prognostication of ovarian cancer in the individual patient inside each histologic group. In patients with epithelial ovarian cancer of all stages and histologic sub-types, pre-treatment thrombocytosis was an independent prognostic factor for overall survival (OS) and progression-free survival [8]. We investigated if pre-treatment thrombocytosis provide prognostic information specifically in patients with advanced stage serous ovarian cancer who represent the most frequent setting of presentation.

Materials and methods

Platelet number at diagnosis of stage III and IV serous ovarian adenocarcinoma was evaluated in 91 patients treated in our hospital and for whom there were complete follow-up data on progression free (PFS) and OS. Case records of women with ovarian cancer treated over the last 12 years were retrospectively reviewed. PFS and OS of patients with normal platelet counts (150–350 $\times 10^9$/L) were compared with that of patients with thrombocytosis (>350 $\times 10^9$/L) by X^2 and logrank tests. Patients with other histologic types of ovarian cancer or earlier stages of disease were excluded.

Follow-up was considered complete if a patient was followed till her death or was seen within the last 6 months from data collection. Data on patients' age, menopause status, histologic characteristics of tumor, stage, site(s) of metastases, tumor marker CA-125 at diagnosis and completeness of surgery (defined as maximal residual disease of less than 1 cm) were recorded. Platelet number on diagnosis of ovarian cancer (before the start of any therapy) was also recorded. All patients had received standard peri-operative chemotherapy with carboplatin and paclitaxel. OS was defined as the interval from the date of diagnosis to death. PFS was defined as the interval from the date of diagnosis to disease progression or death, whichever happened first. Survival plots of patients with normal platelet counts and thrombocytosis were constructed using the Kaplan-Meier method and were compared using the logrank test [9]. The X^2 test was used to evaluate differences in clinical and biologic characteristics in the two groups [10]. A Cox regression proportional hazard multivariate analysis was performed to identify statistically significant factors associated with overall and PFS survival. All *P*-values were considered to be significant at the level of $P<0.05$. Statistical calculations were performed with on-line tools available from the Technical University of Denmark (https://statcom.dk/K-M_plot.php) and a non-commercial site (http://www.statpages.org).

Results

The median age of the patients was 66 years old. From the 91 patients, 52 (57.1 %) had normal platelet counts (median, 273 $\times 10^9$/L; range, 153–350) at diagnosis of their disease and 39 patients (42.9 %) had thrombocytosis (median, 463 $\times 10^9$/L; range, 354–631) (Table 1). The median age of the patients with normal counts was 65 years old (range, 27–87) and of those with thrombocytosis was 70 years old (range, 41–90). In the normal platelet group 50% of patients were older than 65 years-old while in the thrombocytosis group 59% were older than 65 years old (X^2 test $P=0.39$). Stage was III in 61.5% and IV in 38.5% of patients with normal platelets while 71.8% of patients with thrombocytosis had stage III disease and 28.2% had stage IV disease (X^2 test $P=0.3$). Surgery was complete in 49% and 69.2% of normal platelet and thrombocytosis patients respectively (X^2 test $P=0.052$). In the group of patients with normal platelet counts, 24 of the 52 patients (46.2%) had died with a median survival of 43 months (range, 3–100) and 28 patients were alive at last follow-up with a median follow-up of 32.5 months (range, 1–140). In the group of patients with thrombocytosis, 24 of the 39 patients (61.5%) had died with a median survival of 23 months (range, 4–79) and 15 patients were alive at last follow-up with a median follow-up of 20 months (range, 4–69). Among the 48 patients (24 patients in each group) who had died, the 3 year survival of patients with normal platelet counts was 54.2% and of pa-

tients with thrombocytosis was 33.3%.

In the entire group of 91 patients there was a statistically significant difference of the OS between the two groups (logrank test *P*=0.02) (Fig. 1). In the multivariate Cox regression analysis, stage (IV vs. III) and thrombocytosis were statistically significantly associated with reduced OS while increasing age (as a continuous variable), menopausal status, tumor marker CA-125 at diagnosis and completeness of surgery were not (Table 2). A CA-125 cut-off of 1,000 IU/L that, in some centers, influences the decision of proceeding with neo-adjuvant chemotherapy was used in the model.

PFS was similarly in favor of the normal platelets group (logrank test *P*=0.007) (Fig. 2). In the multivariate Cox regression analysis, stage IV, incomplete surgery and thrombocytosis

Table 1. Characteristics and outcome of patients in the series

	All[a]	Normal platelets	Thrombocytosis	*P*-value
Patients	91 (100)	52 (57.1)	39 (42.9)	
Age (yr)	66 (27–90)	65 (27–87)	70 (41–90)	0.39
Stage				
III	60 (65.9)	32 (61.5)	28 (71.8)	0.3
IV	31 (34.1)	20 (38.5)	11 (28.2)	
Completeness of surgery number (%)	52 (51.7)	25 (49)	27 (69.2)	0.05
CA-125 >1,000	36 (42.4)	20 (40.8)	16 (44.4)	0.11
Overall survival in months	28.5 (3–100)	43 (3–100)	23 (4–79)	

Values are presented as n (%) or median (range).
[a]All includes the totality of patients.

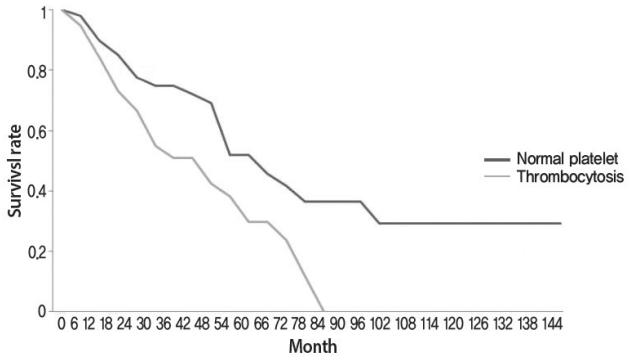

Fig. 1. Kaplan-Meier overall survival (OS) curves in months from diagnosis of ovarian cancer in patients with normal platelet counts (150–350 $\times 10^9$/L) versus patients with thrombocytosis. Logrank test *P*=0.02.

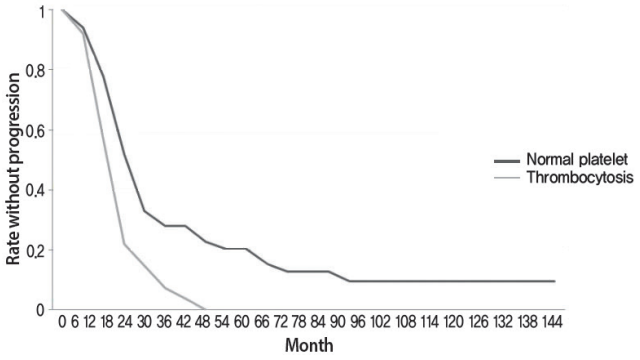

Fig. 2. Kaplan-Meier progression free survival curves in months from diagnosis of ovarian cancer in patients with normal platelet counts (15–350 $\times 10^9$/L) versus patients with thrombocytosis. Logrank test *P*=0.0007.

Table 2. Multivariate Cox regression analysis of parameters possibly related to overall survival of ovarian cancer patients CA-125 cut-off point for this analysis was 1000 IU/L

Variable	Hazard ratio	95% confidence interval		*P*-value
		Lower limit	Upper limit	
Age	1.01	0.98	1.04	0.31
Menopause status	2.61	0.65	10.4	0.17
Stage	2.83	1.41	5.70	0.0034
CA-125[a]	0.87	0.45	1.68	0.69
Completeness of surgery	1.54	0.73	3.24	0.24
Thrombocytosis	2.07	1.08	3.97	0.02

[a]Cut-off point for this analysis was 1,000 IU/L.

Table 3. Multivariate Cox regression analysis of parameters possibly related to progression free survival of ovarian cancer patients

Variable	Hazard ratio	95% confidence interval		*P*-value
		Lower limit	Upper limit	
Age	0.99	0.96	1.02	0.69
Menopause status	1.05	0.40	2.77	0.91
Stage	2.53	1.37	4.66	0.002
CA-125	1.12	0.67	1.85	0.65
Completeness of surgery	2.01	1.07	3.76	0.02
Thrombocytosis	2.21	1.28	3.81	0.004

were statistically significantly associated with reduced PFS while increasing age, menopausal status and tumor marker CA-125 at diagnosis were not (Table 3).

Discussion

Cancer is together with infection, trauma or surgery and iron deficiency a cause of secondary thrombocytosis. In one study, occult cancer was present in 40% of patients with thrombocytosis of more than 400 $\times 10^9$/L when other common conditions were excluded [11]. In addition, emerging data reveal that thrombocytosis is a reverse prognostic factor in a wide range of carcinomas such as localized [12] and metastatic breast cancer [13], gastrointestinal cancers [14-16] and genitourinary cancers [17,18].

Several studies have dealt with the significance of thrombocytosis in epithelial ovarian cancer with conflicting results. In a study of 619 patients with ovarian carcinoma of all stages and various histologic types, thrombocytosis (defined as a platelet count of more than 450 $\times 10^9$/L) was associated with more advanced stage and higher preoperative CA-125 [8]. In addition the median OS in patients with thrombocytosis was 2.62 years while in the group with normal platelets it was 4.65 years (P<0.001). In a multivariate analysis thrombocytosis, more advanced stage (III or IV), high grade and suboptimal cytoreduction emerged as statistically significant. In another study in ovarian cancer across stages and histologic types, thrombocytosis (defined as platelets more than 400 $\times 10^9$/L) was also associated with advanced stage and grade and reduced OS [19]. This was in contrast to a study of 136 patients also of all stages and histologies that showed that, although thrombocytosis was associated with reduced overall and disease-free survival in univariate analysis, this association was no longer present in multivariate analysis [20]. Still another study of 183 advanced epithelial ovarian cancer patients of all histologies associated thrombocytosis (defined as platelets more than 400 $\times 10^9$/L) with both decreased overall and disease-free survival [21]. In a more recent analysis of 587 patients (various histologies) focusing on peri-operative factors, thrombocytosis (platelets more than 450 $\times 10^9$/L) was associated with decreased OS only in stage I/ II but not in stage III/ IV disease [22].

In order to clarify some of these conflicting results we undertook the current investigation which represents the first series including only advanced stage patients with serous histology. In this retrospective study we show that thrombocytosis is associated with decreased OS and PFS in 91 patients with this specific stage and histology representing the most common clinical presentation of ovarian cancer. The two groups with normal platelets and thrombocytosis were similar regarding the age of patients and the distribution of stage III and IV. The thrombocytosis group had a border line statistically significant higher percentage of patients with complete surgery. In the multivariate analysis, stage IV disease and thrombocytosis were associated with decreased OS while these two factors together with incomplete surgery were associated with decreased progression-free survival. Completeness of surgery was not associated with OS possibly because of effectiveness of multiple lines of chemotherapy in controlling the disease.

Concerning the pathogenesis of thrombocytosis, the above mentioned investigation of 619 patients has shed some light. Thrombocytosis was significantly correlated with plasma levels of interleukin (IL)-6 [8]. In mouse models bearing human ovarian cancer, human IL-6 stimulates hepatocytes through the IL-6 receptor to trigger thrombopoietin production. Thus a proposed model stipulates that ovarian cancer tumor cells produce IL-6 which stimulates hepatic thrombopoietin production. Ovarian cancer patients have higher serum thrombopoietin

levels than patients with benign ovarian masses and these levels fall after surgical rejection [23]. Thrombopoietin increases thrombopoiesis through stimulation of megakaryocyte progenitors in the bone marrow [8]. In other cancers IL-6 may also play a similar role in favoring thrombocytosis and increased serum levels or tumor positivity by immuno-histochemistry have been detected in a variety of cancer types [24-26].

The mechanistic basis of platelets contribution to carcinogenesis is a subject of investigation [27]. Circulating tumor cells may use platelets as a protective shield from the attack of the immune system and as facilitators for attachment to endothelial cells at metastatic sites. Platelets have also roles in carcinogenesis directly related to their normal function in promotion of vascular integrity [28]. Newly formed tumor vasculature lacks the normal architecture and robustness of resident vasculature and platelets have been shown to be indispensable for preventing hemorrhage in tumor beds [29]. Both alpha and dense granules of platelets carry bioactive molecules and growth factors [30,31]. These include vascular endothelial growth factor (VEGF), epidermal growth factor, platelet derived growth factor, hepatocyte growth factor, insulin-like growth factor, transforming growth factor β (TGFβ), IL-1β, IL-8, CXC motif containing ligand 12, sphingosine 1-phosphate and lysophosphatidic acid [30,32]. Each of these molecules may actively facilitate metastatic progression. An example is platelet-derived TGFβ which promotes an epithelial to mesenchymal transition (EMT) program in cancer cells through transcription factors Smad and nuclear factor-κB signaling [33]. EMT constitutes a program endowing epithelial cells with a mesenchymal phenotype that promotes mobility and metastasis while protecting them from anoikis (Apoptosis due to lack of adhesion) [34]. There exist quantitative differences in platelet cargo of bioactive factors in malignancy and platelets from patients with cancer have a higher VEGF level than platelets from individuals without cancer [35]. As a result, both increased platelet counts and bioactive factor content may contribute to increased VEGF concentrations in the tumor and metastases sites environment where they are activated.

In conclusion, this retrospective analysis of a series of advanced serous ovarian cancer patients shows that thrombocytosis (defined in this paper as platelets more than 350 $\times 10^9$/L) at the time of diagnosis has prognostic value regarding OS and PFS. The presence of thrombocytosis is independent in multivariate analysis of other known prognostic characteristics and thus has value beyond these characteristics. An important question for future investigation is whether thrombocytosis can serve as a predictive marker of specific treatments, for example of anti-VEGF therapies. Indeed a recent study in metastatic renal cell carcinoma has shown that patients with thrombocytosis had a higher risk to present a primary refractoriness to anti-VEGF treatments (odds ratio, 1.7; P=0.0068) than patients with normal platelets [36]. It remains to be seen if thrombocytosis could be a predictive factor for anti-VEGF therapies in other cancers and in ovarian cancer in particular where the anti-VEGF monoclonal antibody bevacizumab has been recently approved for clinical use [37].

Conflict of interest

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

References

1. Bleeker JS, Hogan WJ. Thrombocytosis: diagnostic evaluation, thrombotic risk stratification, and risk-based management strategies. Thrombosis 2011;2011:536062.
2. Jelovac D, Armstrong DK. Recent progress in the diagnosis and treatment of ovarian cancer. CA Cancer J Clin 2011;61:183-203.
3. Chan JK, Teoh D, Hu JM, Shin JY, Osann K, Kapp DS. Do clear cell ovarian carcinomas have poorer prognosis compared to other epithelial cell types? A study of 1411 clear cell ovarian cancers. Gynecol Oncol 2008;109:370-6.
4. Fader AN, Rose PG. Role of surgery in ovarian carcinoma. J Clin Oncol 2007;25:2873-83.
5. Baldwin LA, Huang B, Miller RW, Tucker T, Goodrich ST, Podzielinski I, et al. Ten-year relative survival for epithelial ovarian cancer. Obstet Gynecol 2012;120:612-8.
6. Mackay HJ, Brady MF, Oza AM, Reuss A, Pujade-Lauraine E, Swart AM, et al. Prognostic relevance of uncommon ovarian histology in women with stage III/IV epithelial ovarian cancer. Int J Gynecol Cancer 2010;20:945-52.
7. Karabuk E, Kose MF, Hizli D, Taskin S, Karadag B, Turan T, et al. Comparison of advanced stage mucinous epithelial ovarian cancer and serous epithelial ovarian cancer with regard to chemosensitivity and survival outcome: a matched case-control study. J Gynecol Oncol 2013;24:160-6.

8. Stone RL, Nick AM, McNeish IA, Balkwill F, Han HD, Bottsford-Miller J, et al. Paraneoplastic thrombocytosis in ovarian cancer. N Engl J Med 2012;366:610-8.
9. Dawson B, Trapp RG, Dawson-Saunders B. Basic & clinical biostatistics. Norwalk: Appleton & Lange; 1994.
10. Lawless JF. Statistical models and methods for lifetime data. New York: Wiley; 1982.
11. Levin J, Conley CL. Thrombocytosis associated with malignant disease. Arch Intern Med 1964;114:497-500.
12. Taucher S, Salat A, Gnant M, Kwasny W, Mlineritsch B, Menzel RC, et al. Impact of pretreatment thrombocytosis on survival in primary breast cancer. Thromb Haemost 2003;89:1098-106.
13. Stravodimou A, Voutsadakis IA. Pretreatment thrombocytosis as a prognostic factor in metastatic breast cancer. Int J Breast Cancer 2013;2013:289563.
14. Shimada H, Oohira G, Okazumi S, Matsubara H, Nabeya Y, Hayashi H, et al. Thrombocytosis associated with poor prognosis in patients with esophageal carcinoma. J Am Coll Surg 2004;198:737-41.
15. Hwang SG, Kim KM, Cheong JH, Kim HI, An JY, Hyung WJ, et al. Impact of pretreatment thrombocytosis on blood-borne metastasis and prognosis of gastric cancer. Eur J Surg Oncol 2012;38:562-7.
16. Brown KM, Domin C, Aranha GV, Yong S, Shoup M. Increased preoperative platelet count is associated with decreased survival after resection for adenocarcinoma of the pancreas. Am J Surg 2005;189:278-82.
17. Symbas NP, Townsend MF, El-Galley R, Keane TE, Graham SD, Petros JA. Poor prognosis associated with thrombocytosis in patients with renal cell carcinoma. BJU Int 2000;86:203-7.
18. Hernandez E, Lavine M, Dunton CJ, Gracely E, Parker J. Poor prognosis associated with thrombocytosis in patients with cervical cancer. Cancer 1992;69:2975-7.
19. Gungor T, Kanat-Pektas M, Sucak A, Mollamahmutoglu L. The role of thrombocytosis in prognostic evaluation of epithelial ovarian tumors. Arch Gynecol Obstet 2009;279:53-6.
20. Qiu J, Yu Y, Fu Y, Ye F, Xie X, Lu W. Preoperative plasma fibrinogen, platelet count and prognosis in epithelial ovarian cancer. J Obstet Gynaecol Res 2012;38:651-7.
21. Li AJ, Madden AC, Cass I, Leuchter RS, Lagasse LD, Karlan BY. The prognostic significance of thrombocytosis in epithelial ovarian carcinoma. Gynecol Oncol 2004;92:211-4.
22. Allensworth SK, Langstraat CL, Martin JR, Lemens MA, McGree ME, Weaver AL, et al. Evaluating the prognostic significance of preoperative thrombocytosis in epithelial ovarian cancer. Gynecol Oncol 2013;130:499-504.
23. Mermer T, Terek MC, Zeybek B, Ergenoglu AM, Yeniel AO, Ozsaran A, et al. Thrombopoietin: a novel candidate tumor marker for the diagnosis of ovarian cancer. J Gynecol Oncol 2012;23:86-90.
24. Paule B, Belot J, Rudant C, Coulombel C, Abbou CC. The importance of IL-6 protein expression in primary human renal cell carcinoma: an immunohistochemical study. J Clin Pathol 2000;53:388-90.
25. Nakashima J, Tachibana M, Horiguchi Y, Oya M, Ohigashi T, Asakura H, et al. Serum interleukin 6 as a prognostic factor in patients with prostate cancer. Clin Cancer Res 2000;6:2702-6.
26. Benoy I, Salgado R, Colpaert C, Weytjens R, Vermeulen PB, Dirix LY. Serum interleukin 6, plasma VEGF, serum VEGF, and VEGF platelet load in breast cancer patients. Clin Breast Cancer 2002;2:311-5.
27. Buergy D, Wenz F, Groden C, Brockmann MA. Tumor-platelet interaction in solid tumors. Int J Cancer 2012;130:2747-60.
28. Ho-Tin-Noe B, Demers M, Wagner DD. How platelets safeguard vascular integrity. J Thromb Haemost 2011;9 Suppl 1:56-65.
29. Ho-Tin-Noe B, Carbo C, Demers M, Cifuni SM, Goerge T, Wagner DD. Innate immune cells induce hemorrhage in tumors during thrombocytopenia. Am J Pathol 2009;175:1699-708.
30. Gay LJ, Felding-Habermann B. Contribution of platelets to tumour metastasis. Nat Rev Cancer 2011;11:123-34.
31. Battinelli EM, Markens BA, Italiano JE Jr. Release of angiogenesis regulatory proteins from platelet alpha granules: modulation of physiologic and pathologic angiogenesis. Blood 2011;118:1359-69.
32. Gunsilius E, Petzer A, Stockhammer G, Nussbaumer W, Schumacher P, Clausen J, et al. Thrombocytes are the major source for soluble vascular endothelial growth factor in peripheral blood. Oncology 2000;58:169-74.
33. Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. Cancer Cell 2011;20:576-90.

34. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009;119:1420-8.
35. Niers TM, Richel DJ, Meijers JC, Schlingemann RO. Vascular endothelial growth factor in the circulation in cancer patients may not be a relevant biomarker. PLoS One 2011;6:e19873.
36. Heng DY, Mackenzie MJ, Vaishampayan UN, Bjarnason GA, Knox JJ, Tan MH, et al. Primary anti-vascular endothelial growth factor (VEGF)-refractory metastatic renal cell carcinoma: clinical characteristics, risk factors, and subsequent therapy. Ann Oncol 2012;23:1549-55.
37. Sato S, Itamochi H. Bevacizumab and ovarian cancer. Curr Opin Obstet Gynecol 2012;24:8-13.