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ORIGINAL ARTICLE

The clinical significance of vascular endothelial growth factor in malignant ascites

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Abstract Ascites can be caused by many kinds of diseases. Patients with undetermined ascites represent a diagnostic challenge. The aims of this study were to determine the diagnostic value of vascular endothelial growth factor (VEGF) in differentiation of malignant ascites from benign ascites and to investigate the clinical value of ascitic VEGF as an independent prognostic parameter. The study included 462 consecutive patients with malignant ascites and 550 patients with benign ascites, VEGF level in ascites were determined by a sandwich enzyme-linked immunosorbent assay. The survival rate was calculated by the Kaplan-Meier method and the log-rank test. Multivariate survival analysis was performed using the Cox hazards model. In our study, we found VEGF levels in malignant ascites (676.59±303.86 pg/ml) were significantly higher than those in benign ascites (218.37± 98.15 pg/ml) ($P<0.001$). Meanwhile, we also found that VEGF levels in malignant ascites from patients with ovarian cancer were higher than those with other cancers. Areas under the receiver operating characteristic (ROC) curves of ascitic VEGF was 0.940. At a cutoff value of 319.5 pg/ml, VEGF yielded a sensitivity of 89.2 % and a specificity of 88.4 %. Patients associated with the high-level VEGF value (≥613.38 pg/ml) in malignant ascites exhibited poor mean survival rates (8.3±0.52 vs 15.11±0.66 months, $P<0.001$). In a multivariate Cox regression model, higher ascitic VEGF was an independent prognostic factor for overall survival. Planned subgroup analysis was performed for patients with tumor node metastasis (TNM) stage I. In the univariate analysis, only ascitic VEGF was associated with overall survival. VEGF was found to have a highly accurate sensitivity and specificity, suggesting that it could be considered as a new biomarker to differentiate malignant ascites from the benign one. The high level of VEGF value in malignant ascites may be used as an independent prognostic factor in patients with all stages of cancer.

Keywords Ascites · VEGF · Differential diagnosis · Prognosis

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The differentiation between malignant and benign ascites is a common clinical problem of considerable importance for further diagnostic and therapeutic procedures. Cytological examination, despite its high specificity, has been found to have a low sensitivity in the diagnosis of malignant ascites and the high percentage of false negative results. So it is indispensable to find appropriate cancerous markers in ascites. Among them, angiogenic cytokines, such as vascular endothelial growth factor (VEGF), are considered to have a great potential. The aim of this study was to evaluate the usefulness of vascular endothelial growth factor as a malignancy marker in ascites with different etiologies and to assess the association between VEGF value and poor prognosis.

Introduction

Ascites, which can be caused by several kinds of disease, is a major factor affecting the patients' quality of life [1, 2]. In order to make appropriate diagnostic and therapeutic procedures, it is essential to differentiate malignant ascites from a benign one. Currently, the differential diagnosis of ascites is based on the symptoms of the patient, biochemical tests, fluid cytology, culture of ascites, and laparoscopy. Cytopathology

analysis is the major diagnostic method for neoplasias in effusions. However, only 50–60 % malignant ascites can be detected by cytopathology according to a reported series [3, 4]. Clinical symptoms may be atypical or unreliable. Current biochemical tests lack sufficient specificity. Indeed, differentiating malignant ascites from a benign one might be somewhat challenging without invasive testing.

Angiogenesis is essential for tumor invasion and metastasis, tumor cells have been shown to secrete a variety of angiogenic factors. Among these factors, vascular endothelial growth factor (VEGF, also called vascular permeability factor, VPF), a bifunctional cytokine, is recognized as one of the most important molecules in the growth, invasion, metastasis, and recurrence of human tumors. It was considered to play a major role in the formation of ascites and peritoneal metastases [5] and be a useful marker in differentiating malignant from benign ones [6]. A number of studies have analyzed the diagnostic value of VEGF in differentiation of malignant ascites from benign ascites. However, the literatures were conflicting in this respect, and the number of patients included in these studies was relatively low. Recently, serum VEGF was shown to be associated with a dismal prognosis of cancers, such as ovarian cancer, non-small cell lung cancer, non-Hodgkin's lymphoma, esophageal cancer, and colorectal carcinoma [7–11]. The relationship between the value of VEGF, and the prognosis is yet unclear.

The aim of this study is to explore the diagnostic value of vascular endothelial growth factor (VEGF) in differentiating malignant ascites from benign ones and to investigate the relationship between the level of VEGF value in malignant ascites and the survival rates. Specially, we also compared VEGF level in different stages of cancer progression.

Materials and methods

Patients and human approval

This study was performed with the approval of the Ethics Committee of Renmin Hospital, Wuhan University. All patients had been diagnosed by cytology, histopathology, and medical imaging examination. A number of 1012 consecutive patients with ascites were recruited from the Renmin Hospital of Wuhan University from January 2007 to December 2012. For all cases, we reviewed age, gender, primary disease. Only specimens diagnosed as primary malignancies were included; otherwise, they were excluded. All patients were divided into two groups (Table 1): group I (malignant group) and group II (benign group). The clinical data are summarized in Table 1. The patients with malignant ascites were staged according to tumor node metastasis (TNM) classification and were regularly followed up for 2 years with intervals of 4–5 months by means of clinic records and patient or family contact. A number of 354 Chinese patients with malignant ascites were finally selected in survival analysis study except 108 patients lost to follow-up. The informed consent was obtained from all patients.

Table 1 Patient characteristics

Diagnosis	No. of patients	Mean years	Female/male
Group I (malignant ascites)	462	52.17±11.96	253/209
Ovarian cancer	148	52.85±11.51	148/0
Gastric cancer	103	52.66±10.09	36/67
Colorectal cancer	91	57.81±12.95	30/61
Hepatocarcinoma	71	44.87±9.55	23/48
Others	49	49.16±12.42	16/33
Group II (benign ascites)	550	46.75±13.35	161/389
Liver cirrhosis	189	48.38±9.89	51/138
Tuberculosis	167	40.89±10.67	53/114
Heart failure	97	61.90±10.44	27/70
Pancreatitis	56	38.48±9.75	16/40
Others	41	38.56±15.66	14/27

Methods

Ascitic fluid samples were collected under sterile conditions and immediately centrifuged at 1500*g* for 15 min and stored at −80 °C until analysis and were obtained preoperatively from the patients or those without preoperative radiation or chemotherapy. The VEGF levels in ascites were determined with a sandwich enzyme-linked immunosorbent assay (Quantikine, R&D systems). All assays were performed in duplicate.

Statistical analysis

The mean±standard deviation (SD) expressed all values. *T* test or one-way ANOVA were used to determine the statistical difference. The areas under receiver operating characteristic (ROC) curves were used to assess the feasibility of using VEGF levels as a diagnostic tool for detecting malignant versus benign ascites. For univariate survival analysis, survival rates were calculated using the Kaplan-Meier method and the curves were compared by the log-rank test. The Cox proportional hazards regression model was performed using multivariate survival analysis. A value of $P<0.05$ was considered significant. All statistical analyses were performed using SPSS 11.0.

Results

Comparison of VEGF level in ascites between the malignant and the benign groups

As shown in Fig. 1a, the VEGF levels of ascites in the malignant group (676.59±303.86 pg/ml) were significantly higher than those in the benign group (218.37±98.15 pg/ml) ($P<0.001$). Furthermore, VEGF levels in malignant ascites from patients with ovarian cancer (827.18±291.12 pg/ml) were higher than those with gastric cancer (600.60±274.36 pg/ml), colorectal cancer (609.44±287.78 pg/ml), hepatocarcinoma (631.77±300.28 pg/ml), and other cancers (566.43±280.20 pg/ml) ($P<0.001$, respectively), while there was no significant difference between gastric cancer, colorectal cancer, hepatocarcinoma, and other cancers ($P>0.05$, Fig. 1b).

ROC curves for VEGF levels in ascites

ROC curves can be used to assess the performance of VEGF levels in detecting malignant and benign ascites. Our research showed that the values of VEGF levels in ascites for the AUC were 0.940. Standard error was 0.08. Asymptotic 95 % confidence interval included 0.924 and 0.956 (Fig. 2). These results suggested VEGF might be helpful in differentiating malignant ascites from benign ones. At a cutoff value of 319.5 pg/ml, a sensitivity and a specificity of VEGF were 89.2 and 88.4 %. The cutoff values of VEGF in ascites in terms of sensitivity and specificity were shown in Table 2.

Univariate Kaplan-Meier analysis of VEGF

In a univariate analysis, sex, different tumor types, ascitic VEGF, and tumor TNM stage were associated with overall survival (Table 3). For 1-year survival, analysis was performed on 354 patients whose mean of VEGF level is 613.38±314.67 pg/ml and survival rate is 12.09±0.47 months. They were divided into survival group ($n=110$) and death group ($n=244$). VEGF levels in the death group (711.72±301.03 pg/ml) were higher than that in the survival group (395.25±221.30 pg/ml) ($P<0.001$, Fig. 3). According to a cutoff value of VEGF (613.38 pg/ml), patients associated with the high-level VEGF value (≥613.38 pg/ml) in malignant

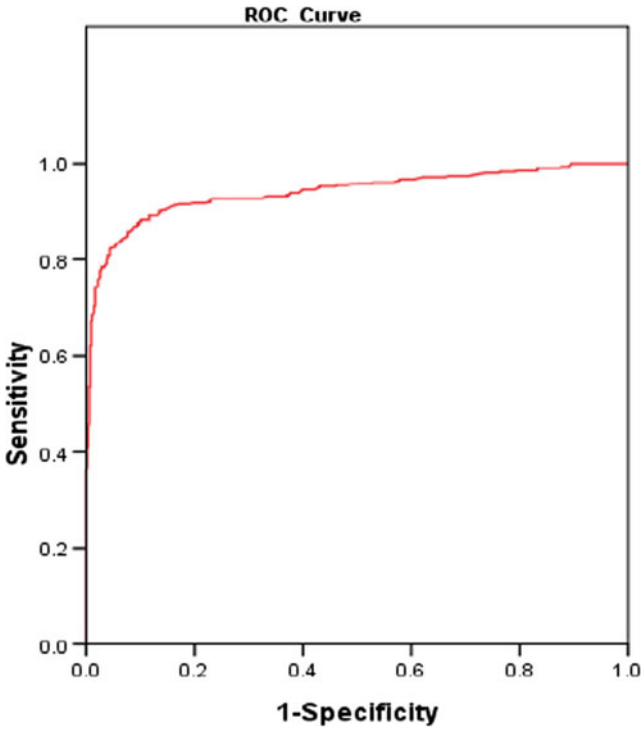

Fig. 2 ROC curves for ascitic VEGF in the malignant and the benign groups

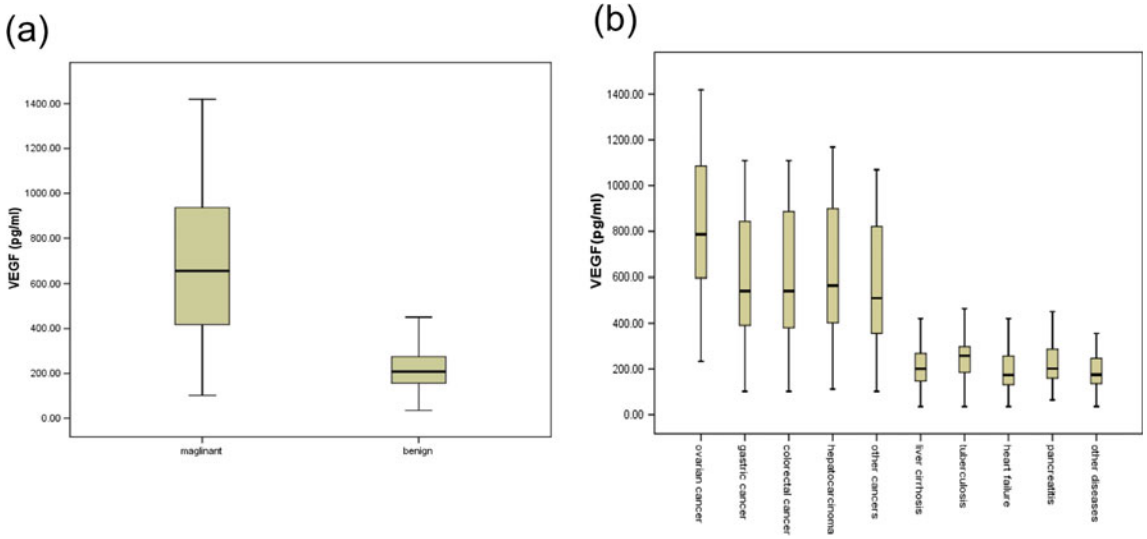

Fig. 1 Comparisons of ascitic VEGF levels in different etiologies. **a** VEGF levels in the malignant and the benign groups. **b** VEGF levels in different malignant ascites

Table 2 Performance of ascitic VEGF levels for predicting malignant ascites at optimal cutoff values

	Cutoff value	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
VEGF (ascites)	319.5 pg/ml	89.0	89.2	88.4 %	87.0	91.3

ascites exhibited poor mean survival rates (8.3±0.52 vs 15.11 ±0.66 months, $P<0.001$). The survival curves are shown in Fig. 4. These results suggest the level of VEGF value may be used as an independent prognostic factor for cancer patients with malignant ascites in short-term survival.

Multivariated analysis

In a multivariate Cox regression model, sex, different carcinoma types, TNM stage, and higher ascitic VEGF value were associated with shortened overall survival (Table 3). VEGF level in malignant ascites was independently correlated to overall survival (HR 1.002, 95 % CI 1.002–1.003, $P<0.001$), showing that patients with higher levels in general had poorer prognosis than patients with lower levels.

Ascitic VEGF and tumor TNM stage

A number of 462 patients with malignant ascites were staged according to TNM classification. Among these patients, the distribution of ascitic VEGF stratified by TNM stage was as follows: stage I, 73 (419.49±210.72 pg/ml); stage II, 131 (673.47±387.15 pg/ml); stage III, 148 (671.14±411.65 pg/ml); and stage IV, 110 (968.35±611.18 pg/ml). As shown in Fig. 5, patients with stage I had a significantly lower ascitic VEGF level compared to patients with stages II, III, IV ($P=$ 0.001), while patients with stage IV had a significantly higher ascitic VEGF level than other stages ($P<0.001$). In univariate analysis, only ascitic VEGF was associated with overall survival (Table 4). In a multivariate Cox regression model, tumor type and higher serum VEGF were associated with shortened overall survival (Table 4).

Table 3 Univariate Kaplan-Meier analysis and multivariate Cox regression model of prognostic covariates in patients with malignant ascites

	Overall survival			
	Univariate	Multivariate		
	P	HR	HR (95 % CI)[a]	*P*[a]
Sex	0.003[b]	0.649	0.472–0.892	0.008
Age	0.457[c]	1.009	0.998–1.019	0.102
Different cancer types	0.004[b]	1.362	1.216–1.526	<0.001
VEGF	<0.001[c]	1.002	1.002–1.003	<0.001
TNM stage	<0.001[b]	1.299	1.127–1.497	<0.001

[a] Multivariate Cox regression model

[b] Log-rank test

[c] Univariate Cox regression model

Discussion

Frequently, ascites is the first physical sign of a malignant intra-abdominal process [12]. Studies reported that 52 % of patients have obvious ascites with initial cancer diagnosis. Ascitic fluid analysis is essential for the diagnosis of malignant ascites. The presence of malignant cells in the ascitic fluid is the gold standard for the diagnosis. The yield of cytology is greater with primary peritoneal tumors. The sensitivity of cytology is only 60 % because not all tumors shed cells into the peritoneum [13, 14]. In most instances, ascites can be diagnosed by a careful history and physical examination. However, malignant ascites is associated with a wide variety of neoplasms such as colorectal, stomach, pancreatic, ovarian, breast, and lung cancers [15]. It is indistinguishable by physical examination from ascites caused by benign conditions. Radiographic techniques also have limitations on their ability to distinguish the two. Cirrhosis, congestive heart failure, nephrosis, tuberculosis, pancreatitis, and peritonitis from pyogenic organisms, to mention a few, can produce intra-abdominal fluid accumulation. Indeed, differentiating between malignant and nonmalignant ascites might be somewhat challenging without invasive testing. Although, some serum or ascitic tumor markers may be used for additional diagnostics in patients with malignant ascites in which the cytologic examination was negative such as CA125, CA19-9, CA15-3, α-fetoprotein, tissue polypeptide-specific antigen, soluble interleukin-2 receptor α, soluble aminopeptidase N/CD13, carcinoembryonic antigen, and several cytokines [16–19], most of these markers are not specific enough to differentiate the malignant ascites from the benign ones.

VEGF is recognized as one of the most important molecules in the growth, invasion, metastasis, and recurrence of human tumors [20]. Review of the literature supports the fact that angiogenesis promoted by VEGF is associated with fluid accumulation in human tumor effusions and malignant ascites is accompanied by high levels of VEGF [21]. Zebrowski and associates reported markedly increased VEGF levels in the ascitic fluid obtained from gastric, colon, and ovarian cancer patients compared with levels in nonmalignant cirrhotic ascites serving as controls [22]. Some researchers have highlighted that the detection of ascitic VEGF levels may provide a novel molecular approach to supplement cytological examination in the evaluation of ascites, especially in the

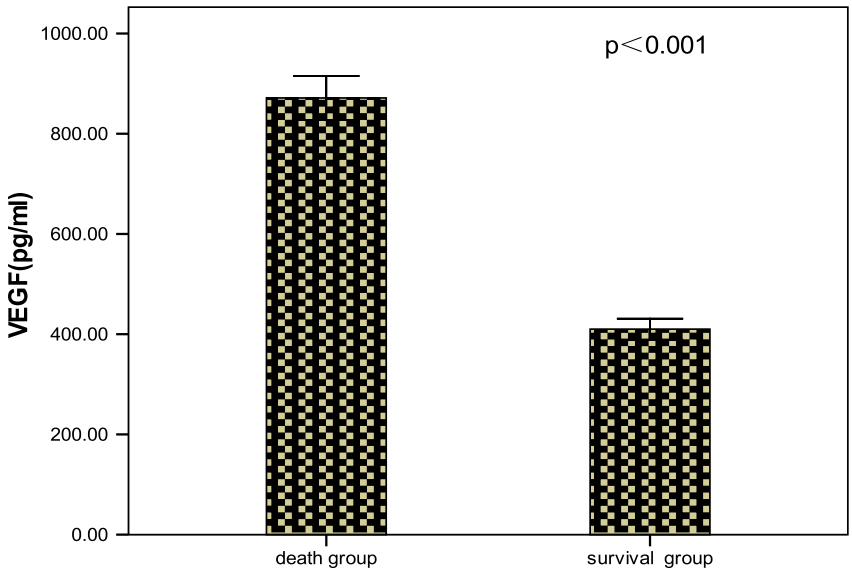

Fig. 3 The level of VEGF value in malignant ascites between survival group and death group

differentiation of benign and malignant ascites [23–30]. Unfortunately, the number of patients included in these studies was relatively low, and the clinical value of preoperative ascitic VEGF was not adequately assessed. Therefore, in our study, a large number of samples (1012 ascites) was analyzed, which is different from the former researches. We found that ascitic VEGF levels were markedly higher in malignant ascites than those in benign ascites (676.59±303.86 pg/ml vs 218.37±98.15 pg/ml, $P<0.001$). These experimental data suggest that the detection of VEGF levels probably provides a new approach to diagnose malignant ascites. Our result of a retrospective study with a large sample is a powerful improvement and complement for previous results. A review of 209 patients [31] showed that malignant ascites was more common in females than in males (67 vs 33 %), probably due to the high prevalence of ascites in ovarian cancer. In our studies, the ratios of malignant ascites in females were not higher markedly than in males (54.8 vs 45.2 %). However, the VEGF levels in malignant ascites from patients with ovarian cancer were higher than those with gastric cancer, colorectal cancer, hepatocarcinoma, and other cancers ($P<0.05$, respectively). But in terms of those with gastric cancer, colorectal cancer, hepatocarcinoma, and other cancers, no significant difference could be seen ($P>0.05$). We think ovarian cancer is known to be highly dependent on VEGF-mediated angiogenesis. The detection of VEGF values is particularly important for those ovarian cancer patients with ascites.

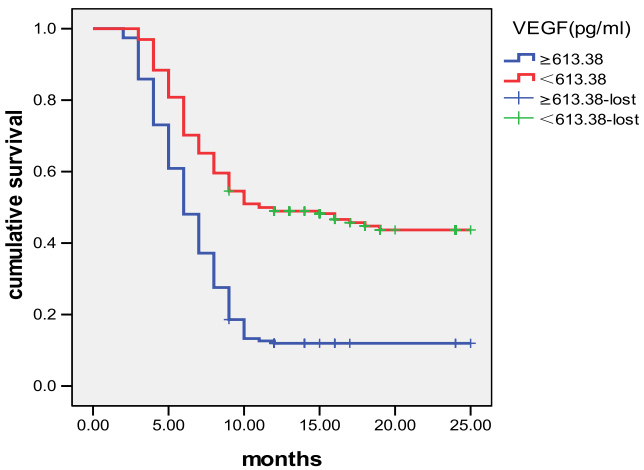

Fig. 4 Kaplan-Meier analysis for short-time survival in 354 patients with malignant ascites according to ascitic VEGF level

For being as a diagnostic marker, we also evaluated the diagnostic values of VEGF with ROC curves. The areas under the ROC curves of VEGF were 0.940. Therefore, we think VEGF may be better than other makers in detecting malignant ascites. The detection of VEGF in ascites may provide a new and useful molecular approach to supplement cytological examination in the estimation of ascites, especially in the differentiation of benign ascites and malignant ones. Our study also showed that ascitic VEGF levels achieved sensitivities of 89.2 % and specificities of 88.4 %, respectively. This result suggested that VEGF is an ideal marker with higher sensitivity and specificity for diagnosing malignant ascites at optimal cutoff values.

As we known, VEGF expression is associated with tumor growth and aggression, as well as poor survival [32–34]. However, few studies have shown an association between VEGF level in malignant ascites and poor prognosis. In this study, we also analyzed the relationship between short-term survival and VEGF level. VEGF levels in 1-year survival group (395.25±221.30 pg/ml) were lower significantly than those in death group (711.72±301.03 pg/ml) ($P<0.001$). With respect to overall survival, patients associated with the high-level VEGF value (≥613.38 pg/ml) in malignant ascites

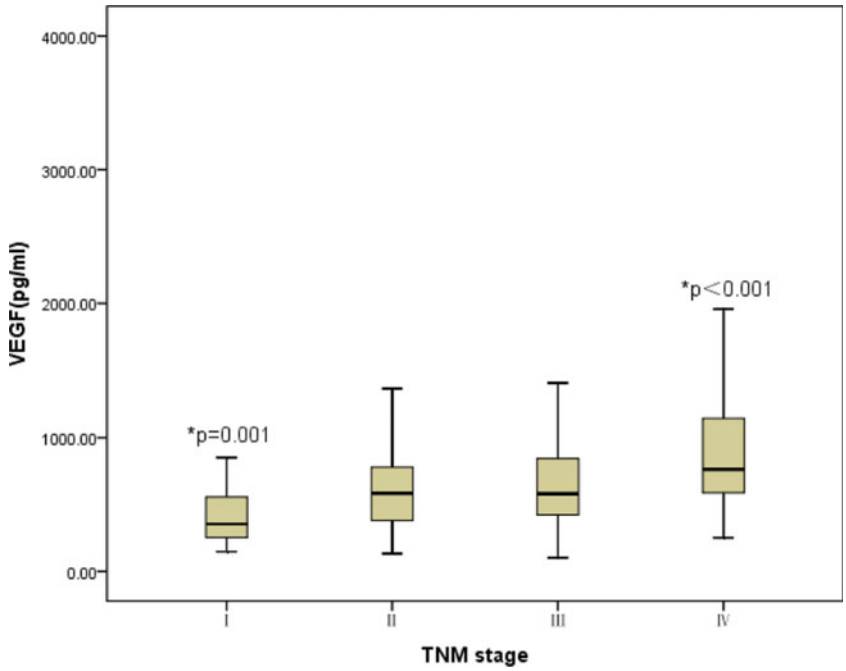

Fig 5 Comparisons of ascitic VEGF levels in TNM stage

exhibited poor mean survival rates (8.30±0.52 vs 15.11± 0.66 months, $P<0.001$). Both univariate Kaplan-Meier analysis and multivariate Cox regression model showed the level of VEGF value may be used as an independent prognostic factor for cancer patients with malignant ascites. Unfortunately, our follow-up time is not long. The cutoff value of VEGF also must be considered carefully and depends on more researches. In addition, we compared ascitic VEGF level in different tumor stages sought to establish the prognostic impact of serum VEGF in early-stage disease. Our results showed that advanced cancer patients have higher VEGF in ascites than the patients in earlier stage of cancer, and ascitic VEGF might provide independent prognostic information in the "low-risk" group of patients with TNM stage I disease.

Table 4 Univariate Kaplan-Meier analysis and multivariate Cox regression model of prognostic covariates in patients with TNM stage I

	Overall survival			
	Univariate	Multivariate		
	P	HR	HR (95%CI)[a]	*P*[a]
Sex	0.143[b]	0.732	0.218–2.415	0.612
Age	0.581[c]	1.009	0.975–1.044	0.618
Different cancer types	0.167[c]	1.786	1.103–2.891	0.018
VEGF	<0.001[c]	1.008	1.005–1.011	<0.001

[a] Multivariate Cox regression model

[b] Log-rank test

[c] Univariate Cox regression model

In summary, these experimental data suggest that the detection of VEGF levels probably provides a new approach to diagnose malignant ascites, which remains a knotty problem all the time. VEGF may be a new biomarker to differentiating malignant ascites from the benign, which has a highly accurate sensitivity and specificity with a strong ROC curve. The high level of VEGF value may be used as an independent prognostic factor in those tumors with malignant ascites.

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Conflicts of interest None

Authors' contributions NZ designed the study, evaluated the ELISA results, and wrote the manuscript. XGL performed the statistical analyses. WGD designed the study. JW evaluated the clinical records.

All authors read and approved the manuscript.

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