Arch. Immunol. Ther. Exp. (2013) 61:165–174
DOI 10.1007/s00005-012-0215-9

ORIGINAL ARTICLE

Pretreatment Levels of Vascular Endothelial Growth Factor in Plasma Predict a Complete Remission Rate and Time to Relapse or Progression in Patients with Diffuse Large B-Cell Lymphoma

Ewa Lech-Maranda · Jacques Bienvenu · Florence Broussais-Guillaumot · Anne-Sophie Michallet · Krzysztof Warzocha · Przemysław Biliński · Peter Boyle · Bertrand Coiffier · Gilles Salles

Received: 15 July 2012 / Accepted: 20 December 2012 / Published online: 3 January 2013

Abstract The aim of this study was to verify whether pretreatment plasma levels of vascular endothelial growth factor (VEGF) correlate with prognosis and survival of patients with diffuse large B-cell lymphoma (DLBCL). Plasma VEGF levels were assessed at the time of diagnosis in 157 DLBCL patients treated with anthracycline-based chemotherapy. Plasma VEGF levels greater than or equal to the highest quartile (high VEGF levels) were associated with lower probability of a complete remission achievement (odds ratio 0.3; 95 % confidence interval [CI]: 0.1–0.6; $p = 0.002$) in univariate as well as in multivariate analysis ($p = 0.04$). The estimated 3-year progression-free survival (PFS) rate of patients with high VEGF levels was 31.7 % (95 % CI 17–51) compared to the 62.5 % 3-year PFS rate (95 % CI 53–71; $p = 0.0004$) in the patients with lower values. The former group of patients demonstrated an estimated 3-year overall survival (OS) rate of 47.1 % (95 % CI 30–65) in contrast to the 3-year OS rate of 64.3 % (95 % CI 54–73; $p = 0.02$) in the latter. In multivariate analysis, the high VEGF level retained its independent impact on shorter PFS ($p = 0.02$). Our results suggest that VEGF plays an important role in the clinical course of DLBCL. VEGF may be a useful marker for selecting the patients for whom new treatment approaches, especially those based on VEGF inhibitors, could be recommended.

Keywords Vascular endothelial growth factor · Diffuse large B-cell lymphoma · Chemotherapy · Prognosis

E. Lech-Maranda · J. Bienvenu · B. Coiffier · G. Salles
Equipe d'Accueil "Pathologie des Cellules Lymphoïdes", Université Claude Bernard, Lyon, France

E. Lech-Maranda (✉) · K. Warzocha · P. Biliński
Department of Hematology, Institute of Hematology and Transfusion Medicine, Indiry Gandhi 14, 02-776 Warsaw, Poland
e-mail: ewamaranda@wp.pl

E. Lech-Maranda
Department of Hematology, Medical University of Lodz, Lodz, Poland

J. Bienvenu
Laboratoire d'Immunologie, Centre Hospitalier Lyon-Sud, Pierre-Bénite, France

F. Broussais-Guillaumot · A.-S. Michallet · B. Coiffier · G. Salles
Hospices Civils de Lyon, Service d'Hématologie, Centre Hospitalier Lyon-Sud, Pierre-Bénite, France

P. Biliński
Chief Sanitary Inspectorate, Warsaw, Poland

P. Boyle
International Prevention Research Institute, Lyon, France

Introduction

The importance of angiogenesis for malignant tumor growth and the development of metastatic lesions have been reported in a variety of solid tumors as well as in hematologic malignancies (Hanahan and Folkman 1996; Koster and Raemaekers 2005; Podar and Anderson 2005). Numerous pro- and anti-angiogenic factors regulate physiological angiogenesis, however, during tumorigenesis, the balance among these factors shifts to neovascularization in response to increasing of proangiogenic stimuli (Hanahan and Folkman 1996). One such stimulus is vascular endothelial growth factor (VEGF) which is a central mediator of tumor-induced angiogenesis. VEGF is a multifunctional

cytokine that is active as an endothelial cell-specific mitogen and inducer of vascular permeability (Dvorak et al. 1995).

In non-Hodgkin lymphoma (NHL), VEGF plays a crucial role in mediating angiogenesis and stimulating growth and survival of malignant cells cells (Koster and Raemaekers 2005; Podar and Anderson 2005). Microvessel density (MVD), a histological marker of angiogenesis has been found to increase in NHL tissues, particularly in high-grade NHL subtypes (Etto et al. 2008; Gratzinger et al. 2007; Hazar et al. 2003; Vacca et al. 1999;). The level of MVD also correlated with lymphoma stage and outcome (Etto et al. 2008; Gratzinger et al. 2007; Negaard et al. 2009). Moreover, lymphoma cells have been shown to express both VEGF ligand and VEGF receptors (Bellamy et al. 1999; Gratzinger et al. 2007; Paydas et al. 2009). Circulating VEGF levels are also increased in the serum or plasma of NHL patients in comparison to healthy controls (Bertolini et al. 1999; Niitsu et al. 2002; Salven et al. 1997, 2000; Wróbel et al. 2004). Interestingly, elevated VEGF levels have been reported to predict poor prognosis in patients with different histological subtypes of NHL, however, conflicting findings regarding the correlation between circulating VEGF levels and response to treatment or survival have been published (Etto et al. 2008; Labidi et al. 2010; Niitsu et al. 2002; Pedersen et al. 2005; Salven et al. 2000; Wróbel et al. 2004). The aim of this study was therefore to verify if pretreatment plasma VEGF levels are associated with the clinical course and prognosis in patients with diffuse large B-cell lymphoma (DLBCL).

Materials and Methods

Subjects

The study group consisted of 157 consecutive patients with de novo DLBCL accrued prospectively between 1995 and 1999 and treated at the Department of Hematology of the Centre Hospitalier Lyon-Sud in France. Plasma samples for VEGF evaluation were taken at the time of diagnosis and kept at −80 °C. All patients gave their written informed consent prior to peripheral blood collection. This study was performed in accordance with the guidelines for studies on human subjects at our institution and with French law.

The initial medical evaluation consisted of a complete history and physical examination, bone marrow biopsy, computed tomography of the chest, abdomen and pelvis, blood morphology and chemistry. Patients were graded according to the Ann Arbor classification, and performance status was assessed using criteria from the Eastern Cooperative Oncology Group (ECOG). The International Prognostic Index (IPI) system was used to divide patients into a low/intermediate low-risk (IPI score 0–2) and intermediate high/high-risk (IPI score 3–5) groups. Clinical characteristics of the patients enrolled in the study are presented in Table 1.

After completion of the treatment, patients were followed-up every 3 months during the first year, then every 6 months for two additional years and then yearly afterward. Follow-up information included the response to initial therapy based on Cheson criteria (Cheson et al. 1999), progression-free survival (PFS) and overall survival (OS). PFS was determined from the onset of treatment until relapse, progression, or at the last follow-up evaluation. OS was determined from the onset of treatment until the last follow-up evaluation or death from any cause.

Treatment

All patients included in this study received anthracycline-containing regimens, consisting of CHOP (cyclophosphamide, adriamycine, vincristine, prednisone) or high-dose CHOP (Coiffier et al. 1989) according to the age and number of IPI factors, as outlined in the Groupe d'Etude des Lymphomes de l'Adulte prospective trials. All patients were treated with curative intent. No patients received rituximab as a part of the first-line treatment.

Evaluation of Plasma VEGF Levels

All blood samples from 157 patients were taken at the time of DLBCL diagnosis before administration of treatment. Citrated plasma was processed within 1 h of venepuncture. Samples were frozen at −80 °C soon after collection, thawed immediately prior to analysis and tested by a quantitative sandwich enzyme immunoassay technique for determination of VEGF (Quantikine Human VEGF Immunoassay; R&D Systems, Mineapolis, MN, USA). The detection limit of the test was 9 pg/mL.

Statistical Analysis

Associations between VEGF levels and clinical or biological variables were assessed with the χ^2 test with Yates's correction when a cell frequency was less than 20 unless any expected frequency was less than 5 when Fisher's Exact Test was used. Receiver operating characteristic (ROC) curves were used to provide the specific cutoff points of VEGF levels. Logistic regression was used to estimate the influence of various clinical or biological parameters on a complete remission (CR) rate. Survival (PFS and OS) were estimated by the Kaplan–Meier method and compared using the log-rank test. 95 % confidence intervals (95 % CI) were calculated. A multivariate regression analysis with the Cox proportional hazard model was used to adjust the effect of

Table 1 Characteristics of 157 patients with DLBCL and their association with plasma VEGF levels at the time of diagnosis

Characteristic	Patients with VEGF < the highest quartile *n* (%)	Patients with VEGF ≥ the highest quartile *n* (%)	*p*
Sex			
Male	56 (75)	19 (25)	0.02
Female	73 (89)	9 (11)	
Age			
60 years or younger	67 (90.5)	7 (9.5)	0.01
Older than 60 years	62 (75)	21 (25)	
Performance status (ECOG score)			
<2	98 (90)	11 (10)	<0.0001
2 or more	25 (61)	16 (39)	
Unknown	7		
Ann Arbor stage			
I, II	55 (93)	4 (7)	0.004
III, IV	66 (74)	23 (26)	
Unknown	9		
No. of extranodal sites			
<2	81 (90)	9 (10)	0.003
2 or more	37 (70)	16 (30)	
Unknown	14		
Serum LDH level			
1× or less than normal	56 (86)	9 (14)	0.3
>1× normal	62 (78.5)	17 (21.5)	
Unknown	13		
Serum β2-microglobulin level			
3.0 mg/mL or less	84 (88)	11 (12)	0.005
>3.0 mg/mL	31 (67)	15 (33)	
Unknown	13		
Bulky tumor (10 cm)			
Absent	73 (89)	9 (11)	0.03
Present	48 (74)	17 (26)	
Unknown	10		
B symptoms			
Absent	101 (89)	12 (11)	<0.0001
Present	28 (64)	16 (36)	
Serum albumin level			
>35.0 g/L	85 (88.5)	11 (11.5)	0.008
35.0 g/L or less	30 (68)	14 (32)	
Unknown	17		
Hemoglobin level			
>12 g/dL	71 (91)	7 (9)	0.004
12 g/dL or less	49 (72)	19 (28)	
Unknown	11		
Platelet count			
Normal	103 (87)	15 (13)	0.002
Greater than normal	14 (58)	10 (42)	
Unknown	15		
IPI risk groups			
Low/intermediate low	71 (96)	3 (4)	<0.0001
Intermediate high/high	44 (68)	21 (32)	
Unknown	18		

n number of patients, χ^2 test, *p* values are two-sided

VEGF levels along with the prognostic variables for potential independent factors. Statistical tests with $p < 0.05$ were considered significant. All reported p values were two sided. Statistical analysis was performed using the SPSS 14.0 package (SPSS Inc., Chicago, IL, USA).

Results

Plasma VEGF Levels in DLBCL Patients and Healthy Controls

The median value of VEGF levels in 157 patients with DLBCL was 85 pg/mL (range 9–1454 pg/mL) as compared to 12 pg/mL (range 9–26 pg/mL) in 20 healthy subjects ($p < 0.001$, Mann–Whitney test). One-third of the patients had VEGF levels greater than or equal to 132 pg/mL (the highest tertile), and one-fourth of the patients had VEGF levels greater than or equal to 173 pg/mL (the highest quartile).

To determine the pretreatment VEGF cutoff values with the highest discriminating power for achieving of a CR, risk of relapse or progression and death, we performed a ROC analysis. The VEGF concentrations less than or equal to 194 pg/mL as well as greater than 217 pg/mL, and 291 pg/mL were associated with the highest prediction accuracy for CR, relapse or progression, and death, respectively ($p = 0.01$, $p < 0.0001$, and $p < 0.001$, respectively). Determined sensitivity and specificity for these values were 93.75 and 28.89 pg/mL for CR, 23.88 and 98.89 pg/mL for relapse or progression, 17.54 and 97 pg/mL for death. The ROC analysis confirmed the significant influence of pretreatment VEGF levels on clinical prognosis of this group of patients. However, since the ROC-determined cutoff values with the highest sensitivity and specificity varied for different end-points of the study, for consistency reasons, we chose to utilize the median, the highest tertile and the highest quartile of VEGF as cutoff values for the further analysis of patients' outcome.

The association between VEGF levels and clinical prognostic factors incorporated in the IPI, as well as other disease features known to correlate with the clinical prognosis of lymphoma was analyzed in this study. Numerous prognostic factors correlated with VEGF levels when the median, the highest tertile and the highest quartile of VEGF were used as a cutoff value, however, the strongest statistical differences were reached when the highest quartile of VEGF level was used (Table 1).

Since VEGF was reported to be abundant in platelets (Banks et al. 1998; Salven et al. 1999), a possible association of plasma VEGF levels with peripheral blood platelet counts was assessed in the patients with DLBCL. High VEGF levels were strongly correlated with high platelet counts ($p = 0.0001$; Spearman rank correlation).

Plasma VEGF Levels and Response to First-Line Treatment

One hundred and twelve patients (71 %) achieved a CR, whereas 45 (29 %) did not. Sixty-seven patients (43 %) experienced disease progression, and 57 (36 %) patients died. The median follow-up for the patients remaining alive was 36 months (range 7–80 months).

In univariate logistic regression analysis, VEGF levels greater than the median value as well as being greater than or equal to the highest tertile, or the highest quartile were significantly associated with a lower probability of achieving a CR ($p = 0.008$, $p = 0.006$, $p = 0.002$, respectively). Other potential prognostic factors that showed an adverse impact on a CR were age >60 years ($p < 0.0001$), ECOG performance status ≥2 ($p < 0.001$), Ann Arbor advanced disease stage ($p = 0.001$), number of extranodal sites ≥2 ($p = 0.05$), elevated LDH levels ($p = 0.001$), elevated β2-microglobulin levels ($p < 0.0001$), the presence of B symptoms ($p < 0.0001$), bulky tumor mass ($p = 0.03$), low serum albumin levels ($p = 0.002$), anemia ($p = 0.02$) and the intermediate high/high IPI risk group ($p < 0.0001$) (Table 2). Multivariate logistic regression analysis showed that age >60 years ($p < 0.0001$), elevated LDH levels ($p = 0.008$), the presence of B symptoms ($p = 0.03$), and VEGF levels greater than or equal to the highest quartile ($p = 0.04$) retained their independent impact on achieving a CR (Table 3).

Plasma VEGF Levels and DLBCL Outcome

The prognostic parameters associated with shorter PFS and OS from univariate analysis are listed in Table 4. Plasma VEGF levels were associated with a shorter PFS when the median ($p = 0.01$) or the highest tertile ($p = 0.002$) of VEGF levels were used as cutoff values as compared to the patients with VEGF levels lower than these values. Of note, the estimated 3-year PFS rate of patients with VEGF levels greater than or equal to the highest quartile was 31.7 % (95 % CI 17–51) in comparison to the 62.5 % 3 year PFS rate (95 % CI 53–71; $p = 0.0004$; log-rank test) found among patients with lower VEGF levels (Fig. 1a). When the 3-year OS rate was analyzed, the trend toward a shorter survival was observed in the patients with VEGF levels greater than the median value ($p = 0.07$) as well as in those with VEGF levels greater than or equal to the highest tertile ($p = 0.07$) compared to the patients with lower VEGF levels. However, this tendency was more pronounced when the highest quartile of VEGF was used as a cutoff value (47.1 % 95 % CI 30–65 vs. 64.3 % 95 % CI 54–73; $p = 0.02$; log-rank lest; Fig. 1b).

In a multivariate Cox regression analysis, after incorporating all variables that were significant in the univariate

Table 2 Probability of achieving complete remission using univariate logistic regression analysis

Characteristic	*n*	OR (95 %CI)	*p*
Sex			
Male	75	1.0	
Female	82	0.6 (0.3–1.3)	0.2
Age			
60 years or younger	74	1.0	
Older than 60 years	83	0.1 (0.05–0.3)	<0.0001
Performance status			
>2	109	1.0	
2 or more	41	0.2 (0.1–0.4)	<0.0001
Ann Arbor stage			
I, II	59	1.0	
III, IV	89	0.2 (0.1–0.5)	0.001
Number of extranodal sites			
<2	90	1.0	
2 or more	53	0.5 (0.2–1.0)	0.05
Serum LDH level			
1× or less than normal	65	1.0	
>1× normal	79	0.2 (0.1–0.5)	0.001
Serum β2-microglobulin level			
3.0 mg/mL or less	95	1.0	
>3.0 mg/mL	46	0.1 (0.07–0.3)	<0.0001
Bulky tumor (≥10)			
Absent	82	1.0	
Present	65	0.4 (0.2-0.9)	0.03
B symptoms			
Absent	113	1.0	
Present	44	0.2 (0.1–0.5)	<0.0001
Serum albumin level			
>35.0 g/L	96	1.0	
35.0 g/L or less	44	0.3 (0.1–0.6)	0.002
Hemoglobin level			
>12 g/dL	78	1.0	
12 g/dL or less	68	0.4 (0.2–0.9)	0.02
Platelet count			
Normal	118	1.0	
Greater than normal	24	1.06 (0.4–2.8)	0.9
VEGF level (the median)			
≤85 pg/mL	99	1.0	
>85 pg/mL	58	0.4 (0.2–0.8)	0.008
VEGF level (the highest tertile)			
<132 pg/mL	118	1.0	
≥132 pg/mL	39	0.3 (0.2–0.7)	0.006
VEGF level (the highest quartile)			
<173 pg/mL	129	1.0	
≥173 pg/mL	28	0.3 (0.1–0.6)	0.002
IPI risk groups			
Low/intermediate low	74	1.0	
Intermediate high/high	65	0.08 (0.03–0.2)	<0.0001

n number of patients, *OR* odds ratio, *95 % CI* 95 % confidence interval

Table 3 Probability of achieving complete remission using multivariate logistic regression analysis

Factors	*n*	OR (95 %CI)	*p*
Age			
60 years or younger	61	1.0	<0.0001
Older than 60 years	69	0.1 (0.03–0.3)	
Serum LDH level			
1× or less than normal	60	1.0	0.008
>1× normal	70	0.2 (0.1–0.7)	
B symptoms			
Absent	96	1.0	0.03
Present	34	0.3 (0.1–0.9)	
VEGF level (the highest quartile)			
<173 pg/mL	107	1.0	0.04
≥173 pg/mL	23	0.4 (0.1–0.9)	

n number of patients, *OR* odds ratio, *95 % CI* 95 % confidence interval

analysis, elevated β2-microglobulin levels ($p < 0.0001$) along with VEGF levels greater than or equal to the highest quartile ($p = 0.02$) retained their independent impact on shorter PFS. Using the significant variables for the analysis of overall survival, only elevated β2-microglobulin levels ($p < 0.0001$) followed by disease stage III/IV ($p = 0.02$) and age >60 years ($p = 0.04$) were found to be independent factors predicting shorter OS (Table 5).

Discussion

In this study, we have validated the predictive value of pretreatment VEGF levels in a group of 157 DLBCL patients uniformly treated with anthracycline-based chemotherapy. Two major points can be concluded from this study. Firstly, high VEGF levels were significantly associated with clinical and laboratory parameters reflecting lymphoma dissemination or tumor burden and the host–tumor relationship. This is in line with the observations indicating that angiogenesis occurring in B-NHL increases with tumor progression (Gratzinger et al. 2008; Hazar et al. 2003). It has been reported that many lymphoid tumors secrete VEGF and express two types of receptors (VEGFR-1 and VEGFR-2) (Bellamy et al. 1999). Gratzinger et al. (2007) showed that DLBCL cells co-ordinately express high levels of VEGF, VEGFR-1 and VEGFR-2, particularly the more aggressive DLBCL subtypes (non-germinal center B cells). Importantly, MVD was significantly higher in DLBCL specimens showing higher VEGF expression (Gratzinger et al. 2007). In addition, the recent immunohistochemical study revealed that VEGF was highly expressed in neoplastic lymphocytes and strongly associated with the expression of phosphorylated form of VEGFR/KDR (pVEGFR-2/KDR) in DLBCL patients (Giatromanolaki et al. 2008b).

Based on these observations, a dual model of VEGF action in lymphoma might be suggested. Firstly, VEGF via an autocrine VEGF/VEGFR-1 and VEGF/VEGFR-2/KDR loop directly stimulates lymphoid tumor cell growth by release of growth hormones and cell survival signals (Gerber et al. 2002; Giatromanolaki et al. 2008b; Masood et al. 2001). Indeed, DLBCL cell lines in culture proliferate in response to VEGF stimulation (Wang et al. 2004). Secondly, VEGF/VEGFR-2 activates, via a paracrine loop, angiogenesis and mitogenesis of endothelial cells as well as enhancing vessel permeability (Gerber et al. 2002; Masood et al. 2001; Wang et al. 2004). Moreover, in the developing vasculature, VEGFR-2-mediated signaling is the predominant growth signal, and VEGFR-1 appears to sequester VEGF ligand, decreasing its availability to trigger VEGFR-2-mediated signaling (Gratzinger et al. 2010; Roberts et al. 2004). The association of VEGF levels with factors reflecting the host–tumor relationship observed in our study can be explained by the immune response to the presence of lymphoma and also the possible production of VEGF from bystander cells. Of note, Vacca et al. (1999) observed that peritumoral inflammatory infiltrate consisting of macrophages, fibroblasts, and other leukocytes may contribute to the induction of angiogenesis by secreting several angiogenic factors, especially VEGF. Furthermore, VEGF was found to inhibit maturation of dendritic cells, therefore exposure of host immune system to high levels of VEGF, both local and circulating, may prevent tumor from being guarded by the immune system (Gabrilovich et al. 1996; Salven et al. 1997).

The second finding of our study is the independent impact of high pretreatment VEGF levels on a lower probability of achieving a CR and shorter PFS. Of note, the VEGF levels along with serum LDH levels retained their independent impact on achieving a CR in multivariate analysis. This may support the previous observation that the expression of lactate dehydrogenase 5 was highly upregulated and significantly related to VEGF and VEGFR-2/KDR expression in DLBCL patients (Giatromanolaki et al. 2008a). Although in our study, the VEGF levels greater than the median value did not reach statistical significance in terms of OS, the patients with VEGF levels greater than or equal to the highest quartile demonstrated shorter OS. Our findings confirm the prognostic value of circulating VEGF in DLBCL patients and suggest that lymphangiogenesis is important in clinical outcome. In the literature, inconsistent reports describing the relationship between circulating VEGF levels and the clinical course of lymphoma, especially with regard to the treatment response and PFS have been published (Bertolini et al. 1999; Etto et al. 2008; Negaard et al. 2009; Niitsu et al. 2002;

Table 4 Probabilities of 3-year progression-free survival (PFS) and overall survival (OS) of 157 patients with DLBCL according to prognostic factors

Characteristics	*n*	PFS 95 % CI (%)	*p*	*n*	OS (%), 95 % CI	*p*
Sex						
Male	75	54.3 (42–66)	0.8	75	61.6 (48–73)	0.6
Female	81	59.1 (48–70)		82	60.5 (49–71)	
Age						
60 years or younger	74	74.0 (62–83)	<0.0001	74	77.4 (65–86)	<0.0001
Older than 60 years	82	41.5 (31–53)		83	46.5 (35–58)	
Performance status						
<2	108	66.6 (56–74)	<0.0001	109	68.1 (57–77)	<0.0001
2 or more	41	33.8 (21–50)		41	42.7 (28–59)	
Ann Arbor stage						
I, II	58	69.4 (55–81)	0.002	59	76.4 (61–87)	0.0003
III, IV	89	48.3 (38–59)		89	51.1 (40–62)	
Number of extranodal sites						
<2	89	57.9 (47–68)	0.4	90	64.3 (52–75)	0.1
2 or more	53	56.1 (43–69)		53	56.3 (42–69)	
Serum LDH level						
1× or less than normal	64	65.7 (52–77)	0.003	65	74.2 (60–85)	0.0002
>1× normal	79	47.9 (37–59)		79	49.0 (38–60)	
Serum β2-microglobulin level						
3.0 mg/mL or less	94	70.9 (60–80)	<0.0001	95	74.0 (63–83)	<0.0001
>3.0 mg/mL	46	26.1 (15–41)		46	31.5 (19–48)	
Bulky tumor (≥10)						
Absent	81	64.5 (53–75)	0.02	82	69.0 (56–79)	0.004
Present	65	47.9 (36–60)		65	51.2 (39–63)	
B symptoms						
Absent	112	63.2 (53–72)	0.0003	113	67.3 (57–76)	0.0001
Present	44	41.9 (28–57)		44	46.7 (32–62)	
Serum albumin level						
>35.0 g/L	95	64.0 (53–73)	0.006	96	67.9 (57–77)	0.004
35.0 g/L or less	44	41.7 (27–58)		44	46.8 (31–63)	
Hemoglobin level						
>12 g/L	77	66.2 (54–76)	0.02	78	71.1 (58–81)	0.002
12 g/dL or less	68	45.6 (34–58)		68	48.9 (37–62)	
Platelet count						
Normal	118	56.3 (47–65)	0.8	118	61.9 (52–71)	0.3
Greater than normal	24	52.4 (33–71)		24	52.1 (33–71)	
VEGF level (the median)						
85 pg/mL	99	62.3 (51–72)	0.01	99	65.1 (53–75)	0.07
>85 pg/mL	57	46.7 (34–60)		58	53.8 (40–66)	
VEGF level (the highest tertile)						
<132 pg/mL	117	62.5 (53–71)	0.002	118	63.7 (53–73)	0.07
≥132 pg/mL	39	39.4 (25–56)		39	52.9 (37–68)	
VEGF (the highest quartile)						
<173 pg/mL	128	62.5 (53–71)	0.0004	129	64.3 (54–73)	0.07
≥173 pg/mL	28	31.7 (17–51)		28	47.1 (30–65)	
IPI risk groups						
Low/intermediate low	73	71.6 (59–81)	<0.0001	74	77.1 (64–86)	<0.0001
Intermediate high/high	65	39.3 (28–52)		65	42.3 (31–55)	

n number of patients, *p* groups were compared using the log-rank test

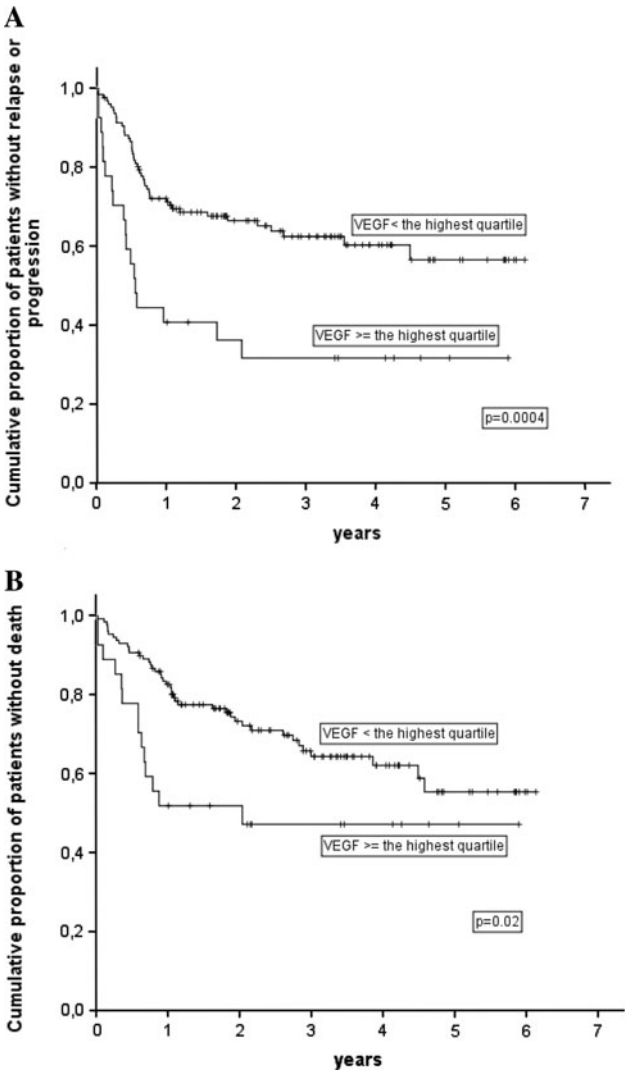

Fig. 1 Progression-free survival (**a**) and overall survival (**b**) of 157 patients with DLBCL according to initial plasma levels of VEGF

Pedersen et al. 2005; Salven et al. 1997, 2000). Of note in these studies, the influence of VEGF levels on outcome of patients with different histological types of NHL was also investigated. Our results in fact are similar to those obtained by Bertolini et al. (1999) who observed that pretreatment plasma VEGF levels among NHL patients in a CR were significantly lower than those of patients with progressive disease. Another study of 64 patients with aggressive NHL has revealed that the significant decrease of VEGF levels as well as interleukin 6 within the first 3 weeks after initiation of CHOP therapy influences the achievement of a CR (Pedersen et al. 2005). Furthermore, Niitsu et al. (2002) observed that serum VEGF levels were lower in CR than in non-CR patients and that elevated VEGF levels at the time of diagnosis independently predicted shorter overall and disease-free survival in patients with aggressive NHL. On the other hand, Salven et al.

Table 5 Cox model describing progression-free and overall survival

Factor	*n*	Progression-free survival	
		RR (95 % CI for RR)	*p*
Serum β2-microglobulin			
3.0 mg/mL or less	90	1.00	<0.0001
>3.0 mg/mL	43	4.0 (2.2–7.2)	
VEGF level (the highest quartile)			
<173 pg/mL	109	1.00	0.02
≥173 pg/mL	24	2.0 (1.1–3.8)	
Factor	*n*	Overall survival	
		RR (95 % CI for RR)	*p*
Serum β2-microglobulin level			
3.0 mg/mL or less	90		
>3.0 mg/mL	40		<0.0001
Ann Arbor stage			
I, II	53		
III, IV	77		0.02
Age			
60 years or younger	61		
Older than 60 years	69		0.04

n number of patients, *RR* relative risk

(1997, 2000) did not find any correlation of pretreatment serum VEGF levels with the response to treatment, however, they did show that elevated serum VEGF levels were associated with shorter OS in patients with DLBCL and other histological types of NHL.

Of note, VEGF was reported to be abundant in platelets (Banks et al. 1998; Salven et al. 1999) and the strong correlation between VEGF levels and peripheral blood platelet count was observed in the present study. However, when we assessed whether platelet count had any prognostic value in the current series, no associations were found between platelet count and a CR rate, PFS nor OS.

In our study, all patients were treated with anthracycline-based regimens with a curative intent and none of them received rituximab as a part of front-line treatment. Recent randomized trials demonstrated that rituximab in combination with the CHOP regimen is the best treatment option both in patients younger than 60 years and in elderly patients with DLBCL (Coiffier et al. 2002; Pfreundschuh et al. 2006). It has also been reported that rituximab in combination with chemotherapy may overcome the prognostic value of some biological markers. Indeed, Gratzinger et al. (2010) have recently found that the negative prognostic import of high MVD observed in CHOP-treated patients has been abrogated in R-CHOP-treated patients. In another recent study, no statistical differences between VEGF-C, VEGF-D, VEGF-R3 levels after R-CHOP treatment as compared to initials cytokines values

were found in 55 patients with DLBCL (Wosztyl et al. 2012). Therefore, it would be interesting to investigate whether the prognostic impact of plasma VEGF still remains significant in DLBCL patients treated with rituximab in addition to anthracycline-based chemotherapy.

In conclusion, our results suggest that VEGF plays an important role in the clinical course of DLBCL. High pretreatment levels of VEGF can be used not only for identifying patients with a lower probability of achieving a CR and higher risk of disease progression but also for selecting those who may benefit best from anti-VEGF therapy (Ganjoo et al. 2006; Stopeck et al. 2009).

References

Banks RE, Forbes MA, Kinsey SE et al (1998) Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. Br J Cancer 77:956–964

Bellamy WT, Richter L, Frutiger Y et al (1999) Expression of vascular endothelial growth factor and its receptors in hematopoietic malignancies. Cancer Res 59:728–733

Bertolini F, Paolucci M, Peccatori F et al (1999) Angiogenic growth factors and endostatin in non-Hodgkin's lymphoma. Br J Haematol 106:504–509

Cheson BD, Horning SJ, Coiffier B et al (1999) Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol 17:1244–1253

Coiffier B, Gisselbrecht C, Herbrecht R et al (1989) LNH-84 regimen: a multicenter study of intensive chemotherapy in 737 patients with aggressive malignant lymphoma. J Clin Oncol 7:1018–1026

Coiffier B, Lepage E, Briere J et al (2002) CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 346:235–242

Dvorak HF, Brown LF, Detmar M et al (1995) Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 146:1029–1039

Etto L, Lacerda E, Baiocchi O et al (2008) Clinical correlations and prognostic relevance of HGF, VEGF AND FGF expression in Brazilian patients with non-Hodgkin lymphoma. Leuk Lymphoma 49:257–264

Gabrilovich D, Chen HL, Girgis KR et al (1996) Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat Med 2:1096–1103

Ganjoo KN, An CS, Robertson MJ et al (2006) Rituximab, bevacizumab and CHOP (RA-CHOP) in untreated diffuse large B-cell lymphoma: safety, biomarker and pharmacokinetic analysis. Leuk Lymphoma 47:998–1005

Gerber HP, Malik AK, Solar GP et al (2002) VEGF regulates haematopoietic stem cell survival by an internal autocrine loop mechanism. Nature 417:954–958

Giatromanolaki A, Koukourakis MI, Pezzella F et al (2008a) Lactate dehydrogenase 5 expression in non-Hodgkin B-cell lymphomas is associated with hypoxia regulated proteins. Leuk Lymphoma 49:2181–2186

Giatromanolaki A, Koukourakis MI, Pezzella F et al (2008b) Phosphorylated VEGFR2/KDR receptors are widely expressed in B-cell non-Hodgkin's lymphomas and correlate with hypoxia inducible factor activation. Hematol Oncol 26:219–224

Gratzinger D, Zhao S, Marinelli RJ et al (2007) Microvessel density and expression of vascular endothelial growth factor and its receptors in diffuse large B-cell lymphoma subtypes. Am J Pathol 170:1362–1369

Gratzinger D, Zhao S, Tibshirani RJ et al (2008) Prognostic significance of VEGF, VEGF receptors, and microvessel density in diffuse large B cell lymphoma treated with anthracycline-based chemotherapy. Lab Invest 88:38–47

Gratzinger D, Advani R, Zhao S et al (2010) Lymphoma cell VEGFR2 expression detected by immunohistochemistry predicts poor overall survivalin diffuse large B cell lymphoma treated with immunochemotherapy (R-CHOP). Br J Haematol 148:235–244

Hanahan D, Folkman J (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 86:353–364

Hazar B, Paydas S, Zorludemir S et al (2003) Prognostic significance of microvessel density and vascular endothelial growth factor (VEGF) expression in non-Hodgkin's lymphoma. Leuk Lymphoma 44:2089–2093

Koster A, Raemaekers JM (2005) Angiogenesis in malignant lymphoma. Curr Opin Oncol 17:611–616

Labidi SI, Ménétrier-Caux C, Chabaud S et al (2010) Serum cytokines in follicular lymphoma. Correlation of TGF-beta and VEGF with survival. Ann Hematol 89:25–33

Masood R, Cai J, Zheng T et al (2001) Vascular endothelial growth factor (VEGF) is an autocrine growth factor for VEGF receptor-positive human tumors. Blood 98:1904–1913

Negaard HF, Iversen N, Bowitz-Lothe IM et al (2009) Increased bone marrow microvascular density in haematological malignancies is associated with differential regulation of angiogenic factors. Leukemia 23:162–169

Niitsu N, Okamato M, Nakamine H et al (2002) Simultaneous elevation of the serum concentrations of vascular endothelial growth factor and interleukin-6 as independent predictors of prognosis in aggressive non-Hodgkin's lymphoma. Eur J Haematol 68:91–100

Paydas S, Seydaoglu G, Ergin M et al (2009) The prognostic significance of VEGF-C and VEGF-A in non-Hodgkin lymphomas. Leuk Lymphoma 50:366–373

Pedersen LM, Klausen TW, Davidsen UH et al (2005) Early changes in serum IL-6 and VEGF levels predict clinical outcome following first-line therapy in aggressive non-Hodgkin's lymphoma. Ann Hematol 84:510–516

Pfreundschuh M, Trümper L, Osterborg A et al (2006) CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomized controlled trial by the MabThera International Trial (MInT) Group. Lancet Oncol 7:379–391

Podar K, Anderson KC (2005) The pathophysiologic role of VEGF in hematologic malignancies: therapeutic implications. Blood 105:1383–1395

Roberts DM, Kearney JB, Johnson JH et al (2004) The vascular endothelial growth factor (VEGF) receptor Flt-1 (VEGFR-1) modulates Flk-1 (VEGFR-2) signaling during blood vessel formation. Am J Pathol 164:1531–1535

Salven P, Teerenhovi L, Joensuu H (1997) A high pretreatment serum vascular endothelial growth factor concentration is associated with poor outcome in non-Hodgkin's lymphoma. Blood 90:3167–3172

Salven P, Orpana A, Joensuu H (1999) Leukocytes and platelets of patients with cancer contain high levels of vascular endothelial growth factor. Clin Cancer Res 5:487–491

Salven P, Orpana A, Teerenhovi L et al (2000) Simultaneous elevation in the serum concentrations of the angiogenic growth factors VEGF and bFGF is an independent predictor of poor prognosis in non-Hodgkin lymphoma: a single-institution study of 200 patients. Blood 96:3712–3718

Stopeck AT, Unger JM, Rimsza LM et al (2009) A phase II trial of single agent bevacizumab in patientswith relapsed, aggressive non-Hodgkin lymphoma: Southwest oncology group study S0108. Leuk Lymphoma 50:728–735

Vacca A, Ribatti D, Ruco L et al (1999) Angiogenesis extent and macrophage density increase simultaneously with pathological progression in B-cell non-Hodgkin's lymphomas. Br J Cancer 79:965–970

Wang ES, Teruya-Feldstein J, Wu Y et al (2004) Targeting autocrine and paracrine VEGF receptor pathways inhibits human lymphoma xenografts in vivo. Blood 104:2893–2902

Wosztyl A, Wolowiec D, Ziolkowska E et al (2012) Plasma concentration of cytokines involved in lympho- and angiogenesis in diffuse large B-cell lymphoma. Acta Haematol Pol 43:277–284

Wróbel T, Mazur G, Usnarska-Zubkiewicz L et al (2004) Vascular endothelial growth factor (VEGF) serum concentration in non Hodgkin's lymphoma patients. Pol Arch Med Wewn 112:919–923