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Concurrent Elevations of VEGF, Osteopontin and MCP-1 Serum Levels Are Independent Predictors of Survival in Patients with Diffuse Large B-Cell Lymphoma

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Key Words
Chemokines · CCL2 · Diffuse large B-cell lymphoma · MCP-1 · Osteopontin · VEGF

Abstract
Background: Diffuse large B-cell lymphomas (DLBCL) are heterogeneous diseases, and the identification of additional DLBCL risk factors is especially important. ***Methods:*** In this pilot study, we determined pretreatment serum levels of vascular endothelial growth factor (VEGF), osteopontin (OPN) and macrophage chemotactic protein-1 (MCP-1) in 67 newly diagnosed DLBCL patients before treatment with standard chemoimmunotherapy and in 30 healthy persons. ***Results:*** Serum levels of all three cytokines were significantly elevated in untreated patients compared to controls. VEGF and OPN concentrations were higher in patients with advanced Ann Arbor stage, B symptoms, Eastern Cooperative Oncology Group score ≥2, International Prognostic Index (IPI) ≥3 and partial/no remission. A high MCP-1 level was associated with advanced stage, increased IPI and bone marrow infiltration. In univariate analysis, elevated OPN and VEGF, and concurrent elevation of all three biomarkers, were identified as significant predictors of poor survival. Multivariate Cox analysis revealed that elevated OPN combined with elevated VEGF levels was one of the best parameter subsets predicting poorest survival. ***Conclusion:*** According to our preliminary results, serum levels of VEGF and OPN before treatment predict response to therapy and survival after chemoimmunotherapy, and may help to further stratify DLBCL patients into risk groups.

Introduction

Diffuse large B-cell lymphomas (DLBCL) are heterogeneous diseases that vary in their biological expression and clinical course. The introduction of chemoimmunotherapy for the treatment of DLBCL has dramatically improved the outcome of these patients compared to chemotherapy alone [1, 2]. However, a significant proportion of these patients (20–30%) become refractory to treatment or eventually relapse [3]. Because of the cura-

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tive intent of treatment and the dismal prognosis of patients who do not achieve complete remission (CR) or who have relapse, the identification of risk factors is particularly important [4]. However, currently applied prognostic factors are, for the most part, clinical variables that reflect disease development but do not have a role in pathogenesis. Therefore, the identification of factors, either biological or clinical, that can identify patients at a higher risk of progressive disease is a priority. Different prognostic factors for response and survival have been described for DLBCL, but in the rituximab era, the role of these biological factors in prognosis has yet to be determined [5, 6].

The tumor microenvironment has been increasingly recognized as a factor affecting neoplastic progression in both solid and hematological malignancies [7, 8]. Recently, two gene expression signatures that reflect the character of nonmalignant cells in DLBCL, stromal-1 and stromal-2, have been shown to predict survival in DLBCL patients treated with the R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone) regimen [9]. In contrast to the stromal-1 signature, which is associated with a favorable prognosis and reflects a monocyte-rich host reaction, the stromal-2 signature is associated with increased tumor blood vessel density and an adverse outcome, and comprises genes that encode molecules related to angiogenesis [9]. Angiogenesis (the formation of new blood vessels) is essential for the growth and dissemination of cancer. It is regulated by various pro- and antiangiogenic factors [10, 11].

Vascular endothelial growth factor (VEGF) is the most specific and critical regulator of angiogenesis; it controls normal endothelial proliferation, permeability and survival, but it is also an angiogenic mediator in tumors, and has been implicated in the pathogenesis and progression of cancer. Increased serum VEGF levels are associated with an unfavorable outcome in various solid tumors and hematological malignancies, including DLBCL [12–16]. Another potentially angiogenic cytokine is osteopontin (OPN), a chemokine-like protein that is present in multiple tissue types and involved in a variety of physiological and pathological processes, including tumor progression [17], enhanced mobility and adhesion of tumor cells, acceleration of tumor growth, division and survival, and promotion of neoangiogenesis [17]. In vitro studies suggest that OPN and VEGF cooperatively enhance angiogenesis in various cancer tissues, including hematological malignancies [18, 19]. OPN has shown promise as a diagnostic and prognostic indicator that reflects tumor progression and is associated with reduced survival [20].

Table 1. Clinicopathological characteristics of the patients with newly diagnosed, untreated DLBCL

Variable	n	%
Age (>60/≤60 years)	45/22	67/33
Gender (female/male)	38/29	57/43
Ann Arbor stage (I–II/III–IV)	26/41	39/61
B symptoms (yes/no)	35/32	52/48
ECOG score (≥2/0–1)	35/32	52/48
IPI (3–5/0–2)	34/33	51/49
LDH (>250/≤250 IU/l)	37/30	55/45
Bone marrow infiltration (yes/no)	17/50	25/75
Response to therapy (CR/PR + no resp.)	47/20	70/30
Outcome (dead/survived)	23/44	34/66

CR/PR + no resp. = Complete remission/partial response + no response to therapy.

Macrophage chemotactic protein-1 (MCP-1) is a CC chemokine that induces chemotaxis of macrophages and a variety of lymphoid cells through its receptor CCR2 [21]. Furthermore, it is the first CC chemokine reported to play a direct role in tumor angiogenesis [22, 23], and some studies have described mechanisms by which MCP-1 may induce angiogenesis [23]. Although the expression and roles of OPN and MCP-1 in non-Hodgkin's lymphoma (NHL) have been investigated in some studies, their biological roles and prognostic potential with respect to this malignancy have not been elucidated yet [24, 25].

The use of novel biomarkers to predict the biological behavior of DLBCL and patient prognosis, and to assist in choosing optimal treatment options has lately attracted increased attention. However, despite some promising results, the majority of these cytokines or combinations thereof does not have adequate sensitivity or specificity to justify their routine usage.

The aim of this pilot study was to analyze the prognostic value of serum VEGF, OPN and MCP-1 levels in DLBCL patients. Serum levels of these three angiogenic proteins were measured before treatment and correlated with clinicopathological characteristics and patient outcome.

Patients and Methods

Patient Characteristics

The study included 67 newly diagnosed, previously untreated patients with DLBCL (38 women, 29 men; median age 69 years, range 18–87 years) treated at the Department of Hematology of the Clinical Hospital Center in Rijeka. Their median follow-up lasted

Table 2. Serum levels of VEGF, OPN and MCP-1 (pg/ml) in the controls and DLBCL patients

	Patients (n = 67)			Controls (n = 30)			p value
	mean ± SD	median	range	mean ± SD	median	range	
VEGF	472.0±342.8	423.4	153.6–636.4	184.4±81.7	192.6	107.8–277.6	0.002
OPN	98.4±59.3	81.5	51.0–140.5	30.0±9.7	29.5	22.0–37.0	<0.001
MCP-1	1,319±980	1,005	780–1,550	793±291	790	585–915	0.044

The Mann-Whitney U test was employed. Range = 25th–75th percentiles.

35 months (range 1–53). The control group consisted of 30 age-matched healthy individuals (17 women, 13 men; median age 67 years, range 19–83 years). Histological classification of NHL was performed in accordance with World Health Organization guidance [26]. The control group comprised healthy volunteers who were treated as outpatients because of altered blood findings, but none of them had any hematological disease. Patients with liver or renal impairment, other current or previous malignancies or infectious diseases, or patients who were incapable of providing informed consent were excluded from the control group. Written informed consent was obtained from each patient and healthy volunteer prior to their inclusion in the study. The study was approved by the local ethics committee. The initial staging was performed by medical interview and examination of complete blood and platelet counts, serum chemistry, measurement of β_2-microglobulin, C-reactive protein and lactate dehydrogenase (LDH) levels, chest X-ray, computerized tomography (CT) of the chest, abdomen and pelvis, bone marrow aspiration and biopsy. Follow-up evaluations included a medical interview, a complete physical examination and positron emission tomography combined with CT. After therapy, control bone marrow aspiration and biopsy were performed only in patients presenting initially with bone marrow infiltration. For each patient, data obtained from the initial evaluation of disease extent were used to determine Ann Arbor stage [27], International Prognostic Index (IPI) [28], presence of B symptoms (presence of at least one of the following symptoms: unexplained fever >38°C, drenching night sweats or unintentional weight loss of >10% of body weight in the preceding 6 months), and Eastern Cooperative Oncology Group (ECOG) score [29]. Patients were treated with 6–8 cycles of the R-CHOP regimen.

The relationships between VEGF, OPN and MCP-1 levels and outcome (response criteria) – complete remission, partial remission, progressive disease (no responders) and overall survival (OS) – were examined according to the International Workshop of Standardized Response Criteria for NHL [30]. Clinicopathological characteristics of the patients with new, untreated DLBCL are summarized in table 1.

Enzyme-Linked Immunosorbent Assay

Peripheral venous blood samples were collected from all participants before treatment initiation. All samples were centrifuged at 3,000 *g* for 10 min and then stored at −70°C.

A VEGF, OPN and MCP-1 eBioscience ELISA kit (Bender MedSystems GmbH, Vienna, Austria) was used to determine serum VEGF, OPN and MCP-1 levels according to the manufacturer's protocol. Color intensity was measured with a microplate reader (MRX II; Dynex Technologies, Germany) at 450 nm. The blank was subtracted from the duplicate readings for each standard and sample. A standard curve was generated for each set of samples assayed using standard and control readings. VEGF, OPN and MCP-1 concentrations were determined at the point of the intersection of the standard curve and the absorbance value. Concentrations are reported as picograms per milliliter. Serum VEGF, OPN and MCP-1 levels were considered to be elevated when they were above the upper limit of the median value of the DLBCL patients (>423.4, >81.5 and >1,005 pg/ml, respectively).

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Declaration of Helsinki of 1975 (as revised in 2008).

Statistical Analysis

Statistical evaluation of data was performed with the data analysis software package Statistica v.12 (StatSoft, Inc.). In cases of normally distributed numerical variables, comparisons were made using the t test or ANOVA. Otherwise, the Mann-Whitney U test or the Kruskal-Wallis ANOVA was used. Frequencies of categorical variables were analyzed by Fisher's exact test. The distribution of survival times was calculated by the Kaplan-Meier method. For survival analysis, all patients still alive were censored at the date of the last follow-up. The Cox-Mantel test was used for comparisons of survival times. A univariate regression model for censored data and Cox proportional hazard regression were used to obtain parameters for predictor variables.

Results

Serum VEGF, OPN and MCP-1 Levels in Controls and Patients with DLBCL

The 30 volunteers in the control group had a median serum VEGF level of 192.6 pg/ml, while patients with DLBCL had a median serum VEGF level of 423.4 pg/ml. Serum VEGF levels of patients with DLBCL were significantly higher than those of the controls (p = 0.002; table 2). Serum OPN levels in lymphoma patients (median

Table 3. Serum levels of VEGF, OPN and MCP-1 (pg/ml) before treatment in different sample subgroups with respect to demographic and disease characteristics

	n	OPN		MCP-1		VEGF	
		median	range	median	range	median	range
Age							
≤60 years	22	52.5	19.5–235.5	1,333	330–5,240	320.0	47.4–1,204.6
>60 years	45	51.0	17.0–230.0	935	120–6,030	467.0	70.8–1,512.6
p value		0.848		0.246		0.186	
Gender							
Female	38	86.3	17.0–235.5	1,090	375–6,030	441.9	47.4–1,189.0
Male	29	78.5	25.0–199.0	960	120–2,535	398.6	50.0–1,512.6
p value		0.780		0.056		0.712	
Ann Arbor stage							
I–II	26	61.0	17.0–186.5	935	120–1,710	316.0	47.4–1,157.8
III–IV	41	104.5	21.0–235.5	1,315	330–6,030	444.8	70.8–1,512.6
p value		0.006		0.009		0.038	
B symptoms							
Yes	32	119.8	21.0–235.5	1,155	330–6,030	482.5	100.6–1,512.6
No	35	54.0	17.0–186.5	990	120–2,865	385.0	47.4–1,157.8
p value		<0.001		0.075		0.011	
ECOG score							
0–1	32	63.8	19.5–188.5	980	120–2,865	290.5	47.4–1,157.8
≥2	35	99.5	17.0–235.5	1,230	330–6,030	472.8	70.8–1,512.6
p value		0.027		0.092		0.027	
IPI							
0–2	33	53.5	19.5–186.5	935	120–1,895	241.2	47.4–1,157.8
3–5	34	127.5	17.0–235.5	1,433	330–6,030	465.6	74.2–1,512.6
p value		<0.001		0.007		0.018	
LDH							
≤250 IU/l	30	53.8	19.5–186.5	935	385–2,695	425.5	70.8–1,189.0
>250 IU/l	37	121.0	17.0–235.5	1,315	120–6,030	423.4	47.4–1,512.6
p value		<0.001		0.051		0.364	
Bone marrow infiltration							
No	50	70.8	17.0–235.5	958	120–5,240	425.5	47.4–1,512.6
Yes	17	133.0	34.0–230.0	1,435	810–6,030	423.4	100.6–1,174.6
p value		0.008		0.016		0.649	
Response to therapy							
PR + no resp.	20	129.5	17.0–230.0	1,390	330–6,030	618.2	142.2–1,512.6
CR	47	69.0	19.5–235.5	990	120–5,240	390.8	47.4–1,157.8
p value		0.007		0.240		0.004	

CR = complete remission; PR + no resp. = partial response + no response to therapy.

81.5 pg/ml) were also significantly higher than those of the controls (29.5 pg/ml; $p < 0.001$; table 2). Serum MCP-1 levels were significantly higher in patients with DLBCL than in the controls; the median MCP-1 level in patients was 1,005 pg/ml, and in controls it was 790 pg/ml ($p = 0.044$; table 2). Serum VEGF, OPN and MCP-1 levels were considered to be elevated when they were above the upper limit of the median value of the DLBCL patients (>423.4, >81.5 and >1,005 pg/ml, respectively).

Relationship between VEGF, OPN and MCP-1 Serum Levels before Treatment and Disease Characteristics

Serum VEGF levels were significantly higher in patients with more advanced Ann Arbor stage ($p = 0.038$), B symptoms ($p = 0.011$), ECOG score ≥2 ($p = 0.027$) and IPI ≥3 ($p = 0.018$; table 3). Higher serum OPN was associated with advanced Ann Arbor stage ($p = 0.006$), B symptoms ($p < 0.001$), ECOG score ≥2 ($p = 0.027$), IPI ≥3 ($p < 0.001$), elevated serum LDH ($p < 0.001$) and bone

Table 4. Association of serum levels of VEGF, OPN and MCP-1 with patient survival

	n	Survival[a], months	p	3-year survival[a], %	RR (at 36 months)	95% CI for RR
OPN						
≤81.5 pg/ml	34	NR	<0.001	85 (73–97)	1.79	1.46–2.21
>81.5 pg/ml	33	26 (11–32)		48 (30–65)		
MCP-1						
≤1,005 pg/ml	34	NR	0.196	73 (58–88)	1.22	0.97–1.52
>1,005 pg/ml	33	30 (15–36)		60 (43–77)		
VEGF						
≤423.4 pg/ml	33	NR	0.022	79 (66–93)	1.46	1.17–1.82
>423.4 pg/ml	34	29 (12–39)		54 (37–71)		
OPN + MCP-1 +VEGF						
0 elevated	12	NR	0.005	100		
1 elevated	19	NR		79 (61–97)	1.27	1.05–1.52
2 elevated	28	NR		53 (34–71)	1.90	1.36–2.66
3 elevated	8	15.5 (11–30)		38 (4–71)	2.67	1.91–3.73

RR = Relative risk; NR = not reached. [a] Median (95% CI).

marrow infiltration (p = 0.008; table 3). High MCP-1 levels were associated with Ann Arbor stage III–IV (p = 0.009), IPI ≥3 (p = 0.007) and bone marrow infiltration (p = 0.016; table 3).

CR was attained by 47 (70%) patients, and partial remission/no response to therapy was noted in 20 (30%) patients. Patients with CR had significantly lower VEGF (median 390.8 pg/ml) and OPN (median 69.0 pg/ml) values compared to patients with partial remission/no response to therapy (median values 618.2 and 129.5 pg/ml for VEGF and OPN, respectively; p = 0.004 and p = 0.007, respectively; table 3). Serum MCP-1 levels were not associated with response to therapy (p = 0.240; table 3).

Association of Serum VEGF, OPN and MCP-1 Levels with Patient Survival

The median follow-up was 35 months (range 1–53 months). OS of patients with a serum VEGF level lower than the median value of the DLBCL patients was superior to that of patients with elevated serum VEGF levels (above the upper-limit median value of DLBCL patients; p = 0.022; table 4; fig. 1a). The 3-year survival rate of patients with an elevated pretreatment serum VEGF level was 54% compared to a survival rate of 79% in patients with lower levels of VEGF, which indicates a significant relative risk of dying in the former group.

OS of DLBCL patients with a serum OPN level lower than the median value (≤81.5 pg/ml) was significantly longer (p < 0.001) than in patients with elevated serum OPN levels (>81.5 pg/ml). The 3-year survival rate of patients with elevated pretreatment serum OPN levels was 48%, which is in contrast to the 85% in patients with a pretreatment OPN level of ≤81.5 pg/ml, again indicating an increased and significant relative risk of dying (table 4; fig. 1b).

Neither elevated nor lower serum MCP-1 levels (>1,005 or ≤1,005 pg/ml MCP-1, respectively) were significantly associated with the survival time of the patients (p = 0.196; table 4; fig. 1c). Survival times were significantly different among patients who had concurrently elevated values of all three biomarkers (VEGF, OPN and MCP-1), or of two, one or none of the biomarkers at the time of diagnosis (p = 0.005; table 4). Moreover, the combination of elevated biomarker levels was associated with a significantly lower 3-year survival and an increased relative risk of dying (p = 0.005; table 4; fig. 1d).

Univariate analysis of OS identified the following independent prognostic factors as significant: age (p = 0.036), tumor grade (p = 0.004), B symptoms (p < 0.001), performance status (p = 0.001), IPI (p = 0.001), LDH levels (p = 0.003), bone marrow infiltration (p = 0.021), response to therapy (p < 0.001), elevated OPN levels (p = 0.002), elevated VEGF levels (p = 0.029) and concurrent elevation of all three biomarkers (p = 0.013; table 5). Moreover, in patients with no response to therapy and B symptoms, concurrent elevation of all three biomarkers was found to have the highest hazard ratio (5.02, 95% confidence interval 1.41–17.86) for patient survival. Nevertheless, multivariate Cox proportional hazard regression showed that

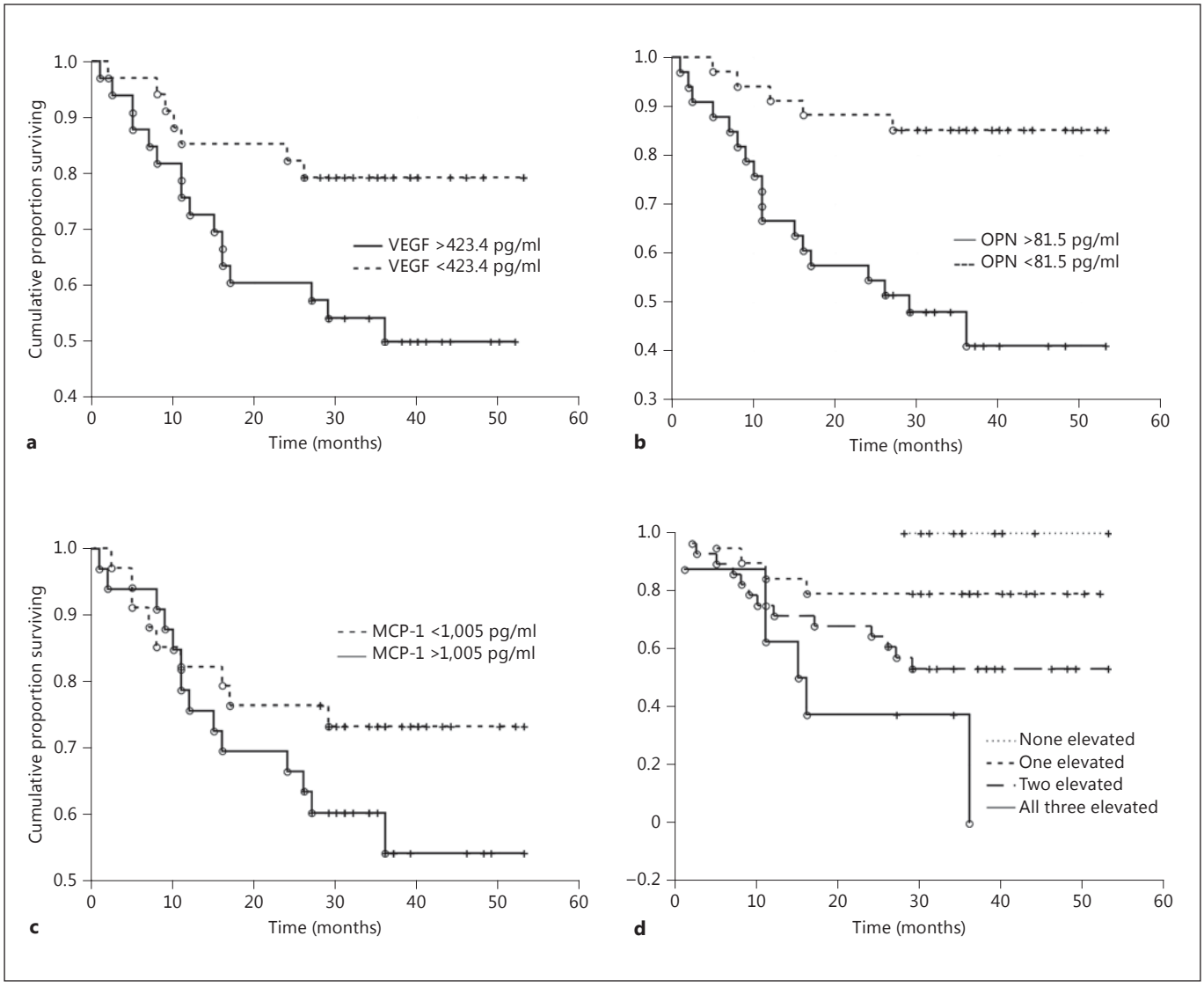

Fig. 1. OS curve according to VEGF (**a**), OPN (**b**) and MCP-1 levels (**c**), and concurrently elevated values of all three, two, one or none of the biomarkers at the time of diagnosis (**d**).

patient status identified as no response to therapy and elevated LDH (p = 0.006), elevated OPN (p = 0.009) and elevated VEGF (p = 0.017) was the best subset of parameters for predicting the poorest OS (table 5).

Discussion

In the present pilot study, we found significantly higher serum levels of the angiogenic cytokines VEGF and OPN, as well as the chemokine MCP-1, in patients with DLBCL compared to healthy controls. These results confirmed our hypothesis that the serum levels of the investigated cytokines play an active role in lymphoma biology, which is in agreement with those of previous investigations [31–34]. However, the prognostic value of the investigated biomarkers, either alone or in combination, for patients with DLBCL treated with chemoimmunotherapy has not been completely elucidated. Our preliminary results indicate the possibility to identify subgroups of patients with a poor prognosis according to the serum levels of VEGF, OPN and MCP-1.

In the present study, patients with high VEGF levels at the time of diagnosis showed a significantly worse re-

Table 5. Value of demographic and disease-related parameters as predictors of OS (univariate Cox regression) and the subset of disease-related parameters as significant predictors of OS (multivariate Cox regression)

Predictor	*b*	SE *(b)*	p value	e^b, HR	95% CI for e^b
Univariate analysis					
Age	0.041	0.019	0.036	1.04	1.00–1.08
Ann Arbor stage	2.161	0.741	0.004	0.12	0.03–0.49
B symptoms	–1.962	0.552	<0.001	7.11	2.42–20.87
ECOG score	2.145	0.621	0.001	0.12	0.03–0.39
IPI	3.516	1.024	0.001	0.03	0.004–0.22
LDH elevated	1.657	0.551	0.003	5.24	1.79–15.36
Bone marrow infiltration	0.977	0.422	0.021	2.66	1.17–6.04
Response to therapy	–3.285	0.536	<0.001	26.71	9.40–75.91
OPN elevated	1.604	0.508	0.002	4.98	1.85–13.40
MCP-1 elevated	0.544	0.428	0.204	1.72	0.75–3.97
VEGF elevated	0.992	0.454	0.029	2.69	1.11–6.53
OPN + MCP-1 + VEGF					
2 elevated	0.964	0.573	0.092	2.62	0.86–8.02
3 elevated	1.613	0.651	0.013	5.02	1.41–17.86
Multivariate analysis					
Response to therapy (no response)	3.873	0.714	<0.001	48.09	11.96–193.43
LDH elevated	1.724	0.629	0.006	5.61	1.65–19.13
OPN elevated	1.467	0.564	0.009	4.34	1.44–13.03
VEGF elevated	1.213	0.507	0.017	3.36	1.25–9.04

HR = Hazard ratio. Multivariate Cox regression, overall model fit $\chi^2 = 70.1$ ($p < 0.001$).

sponse to therapy and a shorter OS and 3-year survival. Several study groups have found that elevated serum VEGF is associated with an unfavorable outcome and a poor prognosis in DLBCL, but all of these studies were performed before the rituximab era [13, 15, 16]. Recently, Riihijärvi et al. [35] demonstrated that a high serum VEGF level is an adverse prognostic factor for patients with high-risk DLBCL treated with dose-dense chemoimmunotherapy. They confirmed the impact of serum VEGF levels on progression-free survival in different IPI subgroups and especially in patients with the nongerminal center subtype of DLBCL. However, the authors did not observe a corresponding prognostic effect of serum VEGF levels on OS [35].

Serum levels of VEGF, which reflect the production of this cytokine in the body, can influence the biological behavior of DLBCL in several ways, as it plays a role in tumor growth. Our results, as well as those of others [31, 35], showed that high serum VEGF levels were associated with advanced Ann Arbor stage, high IPI and the presence of B symptoms, which all somehow represent a measure of tumor burden. These results are in concordance with data showing that VEGF signaling is coupled to growth of lymphoma tissue [36]. Furthermore, VEGF in the tumor microecosystem can stimulate the proliferation of the vasculature and enhance vascular permeability [37], facilitating the hematogenic dissemination of tumor cells. An abnormal vessel structure in the tumor locus, which may be a result of VEGF activity, can lead to lower drug delivery [38]. Finally, there is some evidence that high VEGF concentrations play a role in immune evasion of lymphoma cells [35, 39]. Taken together, these findings indicate a significant tumor-promoting role for VEGF in DLBCL, and serum levels of this proangiogenic cytokine may thus be an important biomarker of tumor aggressiveness and act as a predictor of survival. These findings also emphasize that the addition of rituximab to chemotherapy does not reduce the capacity of serum VEGF to predict outcome.

OPN is a marker of cancer progression with high sensitivity but limited specificity, as it is elevated in about 30 different cancer types [20, 40]. Although serum levels and tumor expression do not always correlate, OPN from both sources can act as a prognostic indicator, a measure of tumor burden, reflecting tumor progression and reduced survival [20, 40, 41]. Previous studies have investi-

gated the pathogenic role of OPN in central nervous system lymphomas and DLBCL with nodal and intravascular involvement, as well as in hepatitis C virus-associated B-cell NHL [34, 42–44]. Furthermore, elevated OPN and CD44 levels at diagnosis may predict an unfavorable outcome in childhood lymphomas and leukemia [33]. However, to the best of our knowledge, our study is the first to investigate the association between serum levels of OPN, other prognostic parameters and survival in a significant number of adult patients with DLBCL. High OPN levels at the time of diagnosis were confirmed in 55% of our patients and were associated with advanced Ann Arbor stage, presence of B symptoms, unfavorable ECOG and IPI scores, elevated serum LDH and bone marrow infiltration. In addition, our patients with higher serum OPN levels showed a worse response to therapy and decreased 3-year survival and OS. This observation is consistent with studies that concluded that low serum OPN levels before treatment are associated with response to treatment and better survival in other cancer types [20, 40, 45]. Like VEGF levels, OPN levels in the present study showed a positive association with parameters that reflect tumor burden, suggesting a possible role for this molecule in lymphoma growth. The well-known proangiogenic role of OPN in human neoplasms [20], as well as its role in enhancing the invasive, adhesive and migratory capabilities of tumor cells [45, 46], may also contribute to the progression of DLBCL and compromise patient survival.

The pathogenetic roles of VEGF and OPN in DLBCL may be mutually dependent, as suggested by some previous papers. Chakraborty et al. [45] showed that OPN promotes VEGF-dependent breast tumor growth and angiogenesis via autocrine and paracrine mechanisms. Their results suggested that OPN might be necessary for maximal induction of neovascularization by inducing VEGF expression through activation of Brk/NF-κB/ATG-4 pathways. The simultaneous targeting of VEGF and OPN and their regulatory signaling networks, in combination with chemoimmunotherapy, may be an important challenge in the treatment of patients with DLBCL in the future.

Chemokines and their receptors have been detected in many tumor types [22]. MCP-1, a chemokine involved in macrophage infiltration of different tissues, is secreted not only by tumor cells, but also by stromal and inflammatory cells, such as fibroblasts, endothelial cells and monocytes [47]. Moreover, MCP-1 is the first CC chemokine reported to play a direct role in tumor angiogenesis [21]. To date, the pathophysiological role of MCP-1 in hematological tumors, including lymphomas, has not been well explored. Luciani et al. [24] described the presence of RNA transcripts for MCP-1 in the majority of biopsy samples from patients with NHL and Hodgkin's disease. Our group also showed that NHL tissue produced MCP-1 mRNA, while lymphoma cells and stromal/inflammatory cells expressed this chemokine [25]. We found higher levels of MCP-1 cDNA in patients with aggressive NHL than in indolent tumors. In addition, the amount of MCP-1 cDNA detected in this preliminary study was higher in patients with early-stage NHL, good IPI and normal levels of LDH; however, these results were based on a small number of cases, which limits any definitive conclusions [25]. Mazur et al. [48] demonstrated that MCP-1 gene expression in lymphomas was higher than in reactive lymph nodes, and patients with high gene expression of this chemokine had a shorter survival. Thus, previous studies suggest that NHL has the potential to produce MCP-1, but the mechanisms by which this chemokine may influence the pathogenesis of NHL remain unclear. To date, there have been no reports regarding the role of serum levels of this chemokine before treatment of DLBCL. In the present study, elevated serum levels of MCP-1 were found in 42% of the patients and had a positive association with advanced Ann Arbor stage, IPI ≥3 and bone marrow infiltration. Thus, we found associations between MCP-1 levels and parameters connected with tumor burden, but serum concentrations of this chemokine were not associated with response to therapy or OS. Previously it was demonstrated that the majority of DLBCL tumors are immunohistochemically positive for MCP-1, VEGF, OPN or all three, and that tumor cells and benign stromal and inflammatory cells also have the capacity to produce these molecules [25, 43, 44, 48–50]. Thus, we can speculate that elevated serum levels of MCP-1 may reflect the host response to the presence of a growing lymphoma and some type of an immunological reaction to the tumor.

An important result of the present study is our finding that elevated values of the biomarkers that we investigated have prognostic power. Namely, survival was significantly different among patients with concurrent elevations of all three, two, one or none of these biomarkers, and combinations of elevated markers were associated with worse OS and 3-year survival. In addition, elevated OPN and VEGF, and concurrent elevation of all three biomarkers were confirmed as independent prognostic factors for OS by univariate analysis, while multivariate Cox proportional hazard regression revealed that elevated OPN and VEGF levels, as well as response to therapy and elevated LDH, predicted the poorest OS.

Important limitations of the present study are the rather small sample size with missing MYC and BCL2 status, the lack of serial monitoring of protein levels, as well as its retrospective design, which limits any definite conclusions.

Our preliminary data provide evidence that elevated serum levels of VEGF and OPN, as well as simultaneous elevation of all three biomarkers before treatment are powerful independent prognostic factors in patients with DLBCL, and predict response to therapy and OS. It is possible that patients with significantly elevated concentrations of these cytokines warrant more aggressive therapeutic regimens and very careful follow-up. The question of whether the combination of VEGF, OPN and MCP-1 serum levels can be used alone or whether they should be added to standard prognostic factors such as IPI or gene expression profiling with the purpose of improving their prognostic significance should be resolved in a larger prospective study.

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