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ORIGINAL ARTICLE

Significance of tumor burden, vascular endothelial growth factor, lactate dehydrogenase and beta-2 microglobulin serum levels in advanced diffuse large B cell lymphoma

N. Milanovic[1], S. Matkovic[1], D. Ristic[1], S. Jelic[1], +M. Petrovic[2]

[1]Institute for Oncology and Radiology of Serbia, Medical Oncology Service, Department of Hematological Oncology, Belgrade; [2]Institute for Hematology, Clinical Center of Serbia, Belgrade, Serbia
+ Dr. M. Petrovic passed away

Summary

Purpose: *Lactate dehydrogenase (LDH) and beta-2 microglobulin (B2M) are incorporated in the so-called "serologic staging system", as independent parameters for predicting time to treatment failure (TTF) and overall survival (OS) for aggressive non-Hodgkin's lymphoma (NHL) patients. Elevated values of serum vascular endothelial growth factor (sVEGF) was associated with poor survival in the largest histological subgroup, the diffuse large B cell (DLBCL) and immunoblastic lymphomas. sVEGF has independent influence on survival in multivariate models when tested together with the components of the International Prognostic Index (IPI). The purpose of this study was to define possible correlations between LDH, B2M levels and the novel prognostic parameter sVEGF, with assessed tumor burden, as another parameter of aggressiveness for advanced-stage DLBCLs.*

Methods: *Serum samples were collected from 29 patients with DLBCL, Ann Arbor clinical stages III and IV, to measure pretreatment serum levels of LDH, B2M and sVEGF. Tumor burden was defined as low and high according to criteria's defined by Jagannath and colleagues.*

Results: *A trend toward significant correlation between high initial levels of sVEGF and high tumor burden was observed (p=0.077). High serum LDH level was strongly associated with high tumor burden (p=0.0091), but B2M correlation with either low or high tumor burden was not confirmed (p=0.249). Complete response (CR) rates (CR vs. non CR) and OS according to tumor burden (low vs. high) showed no statistically significant differences (p=0.245 and p=0.202).*

Conclusion: *Our preliminary data confirmed association between serum LDH level and DLBCL burden with a satisfactory sensitivity-specificity relationship. The other two parameters, sVEGF and B2M, failed to demonstrate significant relationship with tumor burden.*

Key words: B cell lymphoma, beta-2 microglobulin, lactate dehydrogenase, serum vascular endothelial growth factor, tumor burden

Introduction

DLBCL is the most common type of NHL, representing about 30-40% of these lymphomas worldwide, with increasing incidence in the last two decades. Approximately 75% of patients with DLBCL have advanced disease on presentation (Ann Arbor clinical stages bulky II, III and IV). However, 50-65% of such patients achieve long-lasting remissions with anthracycline-based chemotherapy [1].

In the last decade, chemotherapy is combined with the anti CD20 monoclonal antibody rituximab, which has resulted in increased median 5-year OS up to 58% [2].

In order to anticipate progression-free survival (PFS) and OS in DLBCL patients, a 5-factor prognostic score (age, stage, performance status, LDH, number of extranodal sites) was established as IPI [3].

Four risk categories were formed (from low to high) with 4-year OS from 82 to 59%. The revised IPI version is defined for patients treated with rituximab+cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP) with 3 risk categories –very good, good, and poor– with 4-year OS 94, 79, and 55%, respectively [4].

Correspondence to: Nenad Milanovic, MD, MSc. Institute for Oncology and Radiology of Serbia, Medical Oncology Service, Department of Hematological Oncology, Pasterova 14, 11000 Belgrade, Serbia. Tel: +381 11 2067 223; Fax: +381 11 2067 294; E-mail: nmilanovic@ncrc.ac.rs

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Elevated LDH was established as adverse prognostic factor in the mid-eighties for high grade NHLs with B symptoms, bulky disease, hepatosplenomegaly, bone marrow involvement and leukemic forms at initial presentation, as well as in relapse [5].

Elevated levels of B2M also correlated with aggressive initial presentation of the disease such as lung and mediastinal involvement, with relatively poor outcome (8-year OS 59% and 8-year mortality rate 27%) [6]. Initial elevation of B2M (>3.5 mg/l) strongly correlated with advanced clinical stages of disease (III and IV) and later resistant disease [7,8].

"Serologic staging system", based on pretreatment serum levels of B2M and LDH, defines 3 risk groups: low (both parameters normal), intermediate (one parameter elevated) and high risk (both parameters elevated), with 2-year OS 100, 54 and 19%, respectively [9].

In the pre-IPI era, Jagannath and colleagues developed a scoring system for tumor burden in advanced stages of aggressive NHLs using 2 crucial parameters – extensive nodal disease and extranodal disease– which discriminate patients in 2 categories: low and heavy tumor burden.

A novel prognostic parameter, sVEGF, has shown clear positive impact on longer OS in patients with lower pretreatment levels [10,11].

Not enough data exist concerning the correlation between "serologic staging system" and sVEGF levels in patients with advanced stages of DLBCL and different tumor burden.

The aim of this study was to see for possible correlations between sVEGF, LDH and B2M and tumor burden in patients with advanced DLBCLs.

Methods

Twenty-nine previously untreated adult patients with advanced DLBCLs (clinical stages III and IV) were prospectively enrolled in this study from February 2006 to July 2007. Clinical staging was assessed according to the Ann Arbor staging system.

Patients were treated with R-CHOP (rituximab 375 mg/m^2 day 1, cyclophosphamide 750 mg/m^2 day 1, doxorubicin 50 mg/m^2 day 1, vincristine 2 mg (flat dose) day 1, prednisolone 60 mg/m^2, days 1-5, or CHOP.

One patient was treated with R-ESHAP due to anthracycline contraindications (rituximab 375 mg/m^2 day 1, etoposide 40 mg/m^2 days 1-4, cisplatin continuous infusion 25 mg/m^2 days 1-4, methylprednisolone 40 mg days 1-4, cytarabine 2 g/m^2 day 5).

Therapy response was assessed by the criteria defined by Cheson and colleagues [12].

Tumor burden was defined as low and high, with 2 main variables:

1. Extensive nodal disease defined as T3 (nodes >4 cm) and T4 (disease invading adjacent structures defined under specific site)
   a) TNM AJC tumor of Waldeyer's ring (this is assessed according to the 1997 UICC/AJC TNM staging system);
   b) cervical and/or axillary nodal mass > 7 cm;
   c) any mediastinal mass;
   d) any palpable abdominal mass;
   e) abdominal nodal mass > 7 cm;
   f) paraaortic and pelvic nodal involvement;
   g) and abdominal organ displacement.
2. Any extranodal site of disease (according to Jagannath et al) [13].

For Ann Arbor clinical stage III, low tumor burden was considered as the presence of only one site of extensive nodal involvement, and high as two or more.

For Ann Arbor clinical stage IV, low tumor burden was defined as one extranodal site of disease and only one area of extensive nodal disease, and high as two or more areas of extensive nodal disease, three or more extranodal sites or one area of extensive nodal disease and two extranodal sites.

"Serologic staging system" was defined by two parameters: pretreatment levels of LDH (normal up to 460 U/l) and B2M (normal up to 1.9 mg/l) [9].

Blood samples for sVEGF testing were collected on day 1, one hour before starting the first chemotherapy cycle. Serum samples were processed by centrifugation at 3000 rpm (1500 g) and then stored at –70°C until ELISA testing (Quantikine Human VEGF Immunoassay: R&D Systems, Minneapolis, MN, USA).

Statistical analyses

Statistical analyses included normality testing of sample distribution (Kolmogorov-Smirnov and Shapiro-Wilk tests), parameters description (frequencies, percentages, mean, median, standard deviation [SD], range) and testing the differences between the parameters (Wilcoxon rank sum test for sVEGF, LDH, B2M and Fisher exact test for CR rate estimation). Curves of cumulative probabilities for OS were constructed using the Kaplan-Meier product-limit method, and log-rank test was applied for group differences estimations. Receiver-operating-characteristic (ROC) analysis was performed to assess the capacity of each of the sVEGF, LDH and B2M values for predicting high tumor burden. Data analysis was performed using the statistical program R, version 2.13.1 (2011-07-08); Copyright (C) 2011, The R Foundation for Statistical Computing, ISBN 3-900051-07-0.

Results

Patients were predominantly male, aged over 62 years. The majority of patients were in advanced clinical stage IV, with high IPI (Table 1).

Most patients were treated with standard R-CHOP. The CR rate was 51.7 % (Table 2).

Most patients had high tumor burden. Tumor burden, sVEGF, LDH and B2M characteristics are shown in Table 3.

A trend toward correlation between high initial levels of sVEGF and high tumor burden was observed, yet without statistical significance. High values of LDH were strongly associated with high tumor burden, but no B2M correlation with either low or high tumor burden was confirmed (Table 4).

Table 1. Patient and disease characteristics

Characteristics	*N (%)*
Gender	
Male	17 (70.6)
Female	12 (29.4)
Age (years)	
Mean (±SD)	62.65 (±11.3)
Median (range)	62 (33-79)
Clinical stage	
III	7 (24.1)
IV	22 (75.9)
IPI	
≤ 2	9 (31.0)
> 2	18 (62.1)
No data	2 (6.9)

IPI: international prognostic index, SD: standard deviation

Table 2. Treatment characteristics

Characteristics	*N (%)*
Chemotherapy regimens	
R-CHOP	23 (79.3)
CHOP	5 (17.2)
R-ESHAP	1 (3.5)
Response to therapy	
CR	15 (51.7)
PR	5 (17.2)
SD	2 (6.9)
PD	5 (17.2)
No data	2 (6.9)

CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease. For chemotherapy regimens see text

ROC analysis showed that only LDH was a significant factor for predicting high tumor burden; the same analysis also showed a trend towards statistical significance of sVEGF (Table 5 and Figure 1).

CR was not significantly different in relation to the tumor burden categories (Table 6). Patients with low tumor burden lived longer, but differences in OS were not statistically significant (Table 6 and Figure 2).

Table 3. Tumor burden, sVEGF, LDH and B2M

Parameters	
Tumor burden, N (%)	
Low	10 (34.5)
High	19 (65.5)
sVEGF (ng/ml)	
Mean (±SD)	259.6 (±219.1)
Median (range)	234.2 (25.2-1010.0)
LDH (U/l)	
Mean (±SD)	773.8 (±688.3)
Median (range)	500 (168.0-3626.0)
B2M (mg/l)	
Mean (±SD)	3.69 (±2.7)
Median (range)	3.1 (0.7-11.6)

SD: standard deviation, sVEGF: serum vascular endothelial growth factor, LDH: lactate dehydrogenase, B2M: β-2 microglobulin

Table 5. ROC analysis for sVEGF, LDH and B2M according to high tumor burden

Parameters	*ROC analysis for high tumor burden*		
	AUC	*95% CI for AUC*	*p-value*
sVEGF	0.706	0.513-0.898	p=0.076
LDH	0.783	0.587-0.979	p=0.014
B2M	0.636	0.414-0.858	p=0.240

AUC: area under the ROC curve. For other abbreviations see footnote of Table 3

Table 4. sVEGF, LDH and B2M according to tumor burden categories

sVEGF, LDH	*Tumor burden Low*	*Tumor burden High*	*Wilcoxon rank sum test*
sVEGF			
N	10	19	
Mean (±SD)	157.9 (±106.6)	313.2 (±245.4)	W=56
Median (range)	134.4 (32.9-310.1)	241.5 (25.2-1010.0)	p=0.077
LDH			
N	10	19	
Mean (±SD)	485.0 (±333.7)	925.7 (±781.4)	W=39
Median (±range)	377.0 (168.0-1235.0)	663.0 (297.0-3626.0)	p=0.0091
B2M			
N	10	18	
Mean (±SD)	3.3 (±3.1)	3.7 (±2.5)	W=65.5
Median (range)	2.8 (0.7-11.6)	3.3 (1.3-11.0)	p=0.250

For abbreviations see footnote of Table 3

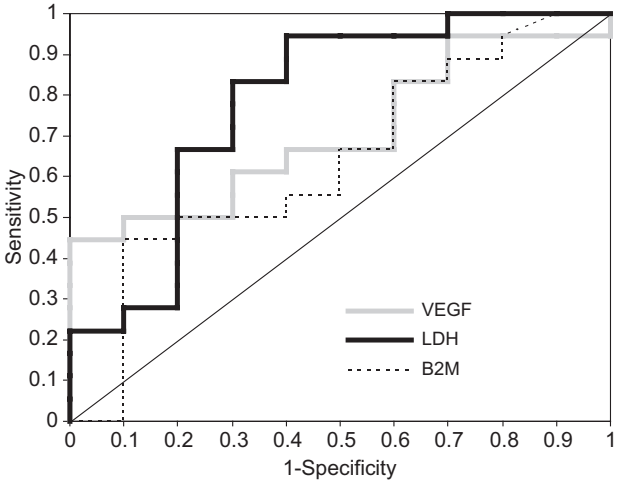

Figure 1. ROC curves for sVEGF, LDH and B2M for predicting high tumor burden.

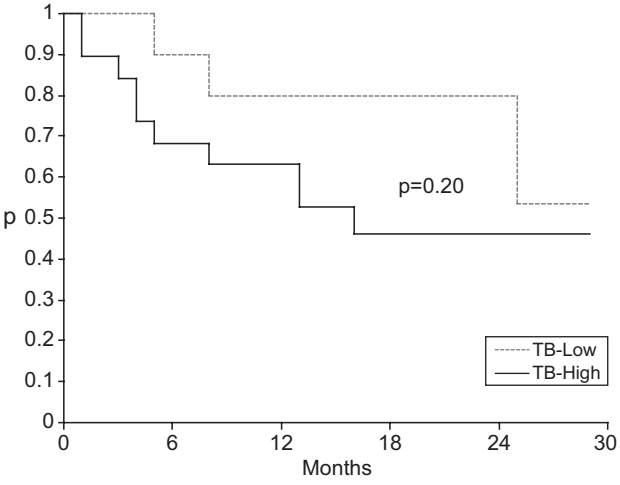

Figure 2. Overall survival according to tumor burden TB categories.

Table 6. Complete response rate and overall survival according to tumor burden categories

VEGF, LDH	*Tumor burden Low N (%)*	*Tumor burden High N (%)*	*p-value*
Complete response			
CR	7 (70.0)	8 (42.1)	Fisher Exact Test
Non CR	3 (30.0)	9 (47.4)	p=0.424
No data	–	2 (10.5)	
OS (months)			Log-rank test
Median (95% CI)	Inf*	16 (>8)	p=0.202

*Not reached. OS: overall survival, CR: complete response

Discussion

Although response to therapy and clinical outcome has been improved, only about half of the patients with newly diagnosed aggressive NHL can be cured with standard induction therapy. It is well known that CR obtained after first-line treatment is associated with a longer OS [3].

Assessment of tumor burden is an old idea considered by many authors and in many entities in hematological oncology, in order to make a prognostic system for response to therapy and survival.

The Ann Arbor staging system for aggressive NHL is not good prognostic indicator because it lacks criteria for evaluating the true extent of disease. The initial presentation of DLBCL commonly includes massive tumor or extranodal involvement.

Jagannath et al. reported 5- and 8-year OS in 50 and 43% of patients with low and high tumor burden, respectively. CR was achieved by 74/105 patients and 37/105 were alive and disease-free at 72 months of follow up. There was no difference in clinical outcome between clinical stage III and IV. These authors also reported two independent risk factors for OS, achievement of remission and relapse-free survival: LDH level and tumor burden. The subgroup of patients with low tumor burden and low LDH level (under cutoff value) had the best outcome, with 5-year survival of 87% [13]. Our results are in concordance with this finding, since we also found a significant correlation between these two parameters. The sensitivity and specificity analysis of the LDH level confirmed this parameter as appropriate for the tumor burden assessment.

VEGF is an established prognostic parameter in lymphomas. Elevated pretreatment levels above the median anticipate poor patient outcome with significantly shorter OS [10,11]. Our results of sVEGF related to high tumor burden showed that there was a trend to statistical significance.

The prognostic power of sVEGF was improved when serum basic fibroblast growth factor (b FGF) was incorporated into the prognostic model. Patients with increased level of sVEGF had a significantly shorter 5-year OS compared with patients with decreased value of this growth factor [14].

In Hodgkin's lymphoma patients with elevated

pretreatment sVEGF values, a statistically significant greater tumor burden (p=0.009) and extended disease (more than 4 involved areas) (p=0.036) was found [15]. sVEGF was also elevated in advanced-stage disease, in patients with B symptoms, and poor-risk groups, but none of them showed statistical significant. A very important observation was that there were no statistically significant differences between bulky and non-bulky disease at presentation (p=0.904), and between advanced disease (CS III/IV) and localized disease (CS I/II) (p = 0.680).

It was concluded that pathologic angiogenesis is an activated pathway in Hodgkin's lymphoma and participates in Hodgkin's lymphoma progression. This has been shown by an elevated VEGF level in patients before the first treatment, with greater tumor burden, and with a higher number of involved areas [15].

In patients with DLBCLs, the presence of extranodal disease and bulky tumor were obviously considered as high tumor burden cases [11]. Statistically significant correlation between absence or presence of bulky tumors (p=0.02) and sVEGF levels 132 vs. 224 pg/ml (p=0.02) was shown [11]. sVEGF was significantly higher in patients with high IPI score and the presence of extranodal disease and bulky tumor [11].

To our knowledge there are no studies dealing with DLBCLs patients correlating sVEGF and tumor burden, precisely defined by the Jagannath criteria [13], and evaluation of their impact on response rate and OS.

Conclusion

The present study revealed a trend to statistical significance between high tumor burden and high pretreatment sVEGF level. Strong statistical correlation between high serum LDH levels and high tumor burden was noted. According to the sensitivity and specificity analyses, only the serum LDH was appropriate for tumor burden assessment.

Our data indicate the need for further relevant studies with larger groups of patients.

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