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ORIGINAL ARTICLE

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VEGFR2 and VEGFA polymorphisms are not associated with an inferior prognosis in Caucasian patients with aggressive B-cell lymphoma

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Abstract

Purpose: Previous published data showed an impact of single-nucleotide polymorphisms in the VEGF A and VEGFR2 genes on the survival of patients with various malignancies, among others diffuse large B-cell lymphoma (DLBCL).

Patients and Methods: We investigated the role of four VEGF-A and two VEGFR-2 gene polymorphisms on the outcome of 273 patients with diffuse large B-cell lymphoma who were treated with R-CHOP within a prospective, randomized trial of the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL). The genomic DNA samples were analyzed using commercial DNA Probes (Applied Biosystems, USA) to detect single-nucleotide polymorphisms in the VEGF A rs699947, rs1570360, rs2010963, rs3025039 and rs1870377, and rs2305948 in the VEGFR2 receptor. Hundred healthy blood donors served as a control.

Results: There was no difference between the SNP allele frequencies in lymphoma patients compared to the control group for all investigated SNPs. None of the investigated SNPs was significantly associated with EFS or OS. After adjusting for the International Prognostic Index risk factors in a multivariate analysis, these results could be confirmed.

Conclusion: Single-nucleotide polymorphisms of the VEGF and VEGFR2 were not associated with a worse outcome in Caucasian patients with DLBCL.

KEYWORDS

diffuse large B-cell lymphoma, polymorphism, single nucleotide polymorphisms, vascular endothelial growth factor, vascular endothelial growth factor receptor type 2, VEGF gene

Kaddu-Mulindwa and Rosolowski equally contributed to this work.

1 | INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma. The addition of the CD20 antibody rituximab to conventional chemotherapy has substantially improved the outcome of these patients in the last 15 years and is now the standard of care.[1-5] Despite this improvement, more than 30% of patients with DLBCL will ultimately relapse and are in need for a salvage treatment.[6] The International Prognostic Index (IPI), which was introduced in 1993,[7] is still the standard clinical tool to predict outcomes in patients with aggressive lymphomas.[8] Nevertheless, there is a constant pursuit of new prognostic markers, for example, ABC and GCB,[9] vitamin D,[10] or Fc-gamma receptor.[11] Angiogenesis is of utmost importance in the progression of many malignancies. The vascular endothelial growth factor (VEGF) is known as a regulator of endothelial cell proliferation and plays a major role in angiogenesis.[12] Furthermore, single-nucleotide polymorphisms (SNP) of the VEGF pathway have been associated with incidence and prognosis of many solid and hematologic malignancies,[13,14] and the inhibition of angiogenesis pathways for example by VEGF antibodies or tyrosine kinase inhibitors has shown clinical benefit in colon [15] and kidney cancer.[16,17] In addition, VEGF and its cellular receptor, and the vascular endothelial growth factor receptor type 2 (VEGFR2) play a key role in leukemia-associated angiogenesis,[18] and the VEGF gene (VEGFA) polymorphism was reported to predict the prognosis in patients with acute myeloid leukemia patients.[19] Despite the success of VEGF antibodies for the treatment of solid tumors, it failed to prove its efficacy in hematologic neoplasias like DLBCL. Previously published studies showed no significant treatment effect of bevacizumab whether as a single agent in patients with relapsed, aggressive non-Hodgkin lymphoma [20] or in combination with R-CHOP as first-line treatment for patients with aggressive B-cell lymphoma.[21] There have been several reports in the literature[22-24] that in aggressive lymphoma, in particular in DLBCL, high VEGF serum levels, and elevated expression of VEGF in tissue biopsies are associated with a higher tumor burden and inferior overall survival (OS). Kim et al observed in a Korean population of lymphoma patients that the VEGF-receptor 2 polymorphism rs1870377 major allele TT had an inferior prognosis. It was hypothesized that the inferior binding of minor allele AA to VEGF impaired lymphoma angiogenesis and conferred the benefit.[25] Therefore, the aim of this study was to investigate the effects of VEGF and VEGF-receptor polymorphisms on the prognosis of Caucasian patients with aggressive B-cell lymphoma treated with R-CHOP protocol within the prospective RICOVER-60 trial of the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL).

Novelty Statements

1. What is the NEW aspect of your work?
VEGF polymorphisms in Caucasian patients with aggressive B-cell lymphoma.
2. What is the CENTRAL finding of your work?
We investigated the prognostic impact of six potentially functional polymorphisms in the VEGF and VEGFR2 genes in Caucasian patients with aggressive B-cell lymphoma.
3. What is (or could be) the SPECIFIC clinical relevance of your work?
VEGF polymorphisms do not have a prognostic value in Caucasian patients with diffuse aggressive B-cell lymphoma.

2 | MATERIALS, METHODS AND PATIENTS

In the RICOVER-60 trial of the DSHNHL, 1222 patients aged 61-80 years with aggressive B-cell lymphoma were recruited between July 2000 and June 2005 by 203 institutions. The patients were randomized to 6 or 8 cycles of CHOP-14 (cyclophosphamide, vincristine, doxorubicin, and prednisone) with and without eight cycles of rituximab in a 2 × 2 factorial design. The study was performed in accordance with the Declaration of Helsinki. All protocols had been approved by the ethics committee of each participating institution, and all patients had given written informed consent.[5] From these 1222 patients, 533 genomic DNA samples were available for further analysis. From these 533 patients, 273 received rituximab and were included in the final analysis. Patients with primary central nervous system lymphoma (PCNSL) and post-transplantation lymphoproliferative disorders (PTLD) were excluded in the RIVOCER-60 trial and therefore not included in our analysis. The DNA samples were analyzed using commercial DNA Probes (Applied Biosystems, USA) to detect single-nucleotide polymorphisms in the VEGF A- (rs699947, rs1570360, rs2010963, and rs3025039) and in the VEGFR2 receptor (rs1870377 and rs2305948). Minor allele frequencies were compared with a control cohort of 100 healthy blood donors.

2.1 | Statistics

Event-free survival (EFS) was defined as time from randomization to disease progression, start of salvage treatment, additional (unplanned) treatments, relapse, or death from any cause. Overall survival (OS) was defined as time from randomization to death from any cause. EFS and OS according to SNPs were estimated according to the Kaplan-Meier method, and global log-rank tests were performed. Cox regression models to analyze the SNPs adjusted for the factors of the IPI were used for EFS and OS. Hazard ratios (HRs) with 95% confidence intervals (CI) and *P*-values are presented. For differences regarding patient characteristics, we used chi-square tests and for age the Mann-Whitney *U* test. The two-sided significance level was $P < .05$. Haplotype analysis was performed for SNPs with high linkage disequilibrium. Individual haplotypes were inferred using the PHASE program (available at http://stephenslab.uchicago.

edu/phase/download.html). Statistical analysis was performed using SPSS software (version 11.5) and R (version 3.6.3).

3 | RESULTS

The characteristics of the 273 patients included in this study are shown in Table 1. This cohort did not significantly differ from the entire RICOVER-60 population (n = 1222) with respect to demographics and IPI risk factors.

SNP allele frequencies were not different in lymphoma patients compared to healthy controls (data not shown). In univariate analysis, no significant impact of the SNPs on EFS and OS was observed (Figure S1). Similarly, a multivariate analysis for EFS and OS adjusted for the factors of the IPI showed no impact of VEGF and VEGF-receptor polymorphisms on prognosis of this series of patients with aggressive B-cell lymphoma (Table 2).

The three neighboring VEGFA SNPs rs699947, rs1570360, and rs2010963 were closely linked with each other but not with the VEGFA SNP rs3025039 (Table S1). VEGFR rs1870377 and rs2305948 polymorphisms were moderately linked (r^2 = .078, Lewontin's D' = 0.524). Four haplotypes of the gene VEGFA for alleles rs699947, rs1570360 and rs2010963 were estimated and considered for further analysis: AAG (34.2%), CGC (31.1%), AGG (16.7%), and CGG (17.8%) (AAC was not analyzed, because only one its copy was inferred in one patient). None of the haplotypes was significantly associated with EFS, which was the primary endpoint of the RICOVER-60 trial. Only the haplotype CGG was significantly associated with OS in a univariate analysis (P = .034) as well as adjusting for IPI factors (haplotype copy number 1 (n = 79) vs. 0 (n = 181), HR, 0.5; 95% CI, 0.2-0.9; P = .019; Figure S2, Table S2). However, this association would no longer be significant, if multiple testing was taken into account.

TABLE 1 Patient characteristics

	Analyzed cohort n = 273	RICOVER-60 trial n = 1222	*P*-value[a]
Age median (range)	68 (61-80)	68 (61-80)	.550
Male	147 (54%)	650 (53%)	
Female	126 (46%)	572 (47%)	.859
LDH ≤ N	146 (53%)	618 (51%)	
LDH > N	127 (47%)	604 (49%)	.307
Stages I, II	140 (51%)	603 (49%)	
Stages III, IV	133 (49%)	619 (51%)	.511
ECOG 0,1	239 (88%)	1046 (86%)	
ECOG > 1	34 (12%)	176 (14%)	.346
Extralymphatic sites > 1			
No	223 (82%)	1006 (82%)	
Yes	50 (18%)	216 (18%)	.823
IPI score			
1	96 (35%)	372 (30%)	
2	67 (25%)	339 (28%)	
3	64 (23%)	313 (26%)	
4, 5	46 (17%)	198 (16%)	.203
No bulk	168 (62%)	759 (62%)	
Bulk	105 (38%)	463 (38%)	.880
B-symptoms no	187 (68%)	823 (67%)	
B-symptoms yes	86 (32%)	399 (33%)	.699
6× CHOP-14	128 (47%)	613 (50%)	
8× CHOP-14	145 (53%)	609 (50%)	.246

[a]*P*-value for comparison of patients analyzed (n = 273) and not analyzed (n = 949) from RICOVER-60 trial.

4 | DISCUSSION

We have investigated the prognostic impact of six potentially functional polymorphisms in the VEGF and VEGFR2 genes in Caucasian patients with aggressive B-cell lymphoma who were treated with R-CHOP. We could not find any impact of these polymorphisms on prognosis within these uniformly treated patients with aggressive B-cell lymphoma. Similarly, to certain solid malignancies for example ovarian [26] or head and neck cancers,[27] VEGF polymorphisms do not have any prognostic value in Caucasian patients with DLBCL.

This is in contrast to previously published data by Kim et al which showed a significant impact on OS and PFS for the polymorphism VEGFR2 rs1870377T > A. In this Korean cohort, which had a comparable IPI risk and had been uniformly treated with R-CHOP as well, the three-year PFS for AA was 76,7%, for TA 68,0% and TT 59,7%, the OS for AA was 81,6%, for TA 73,4% and for TT 63,7%. This was independent of the IPI score in a multivariate analysis.[25] In our cohort the three-year EFS for AA was 53.8%, for TA 73.5% and TT 71,6%, the overall survival for AA was 64.1%, for TA 82% and for TT 77%. Contrary to the expectation the AA genotype in our cohort had a trend to a worse OS compared to AT + TT. However, this polymorphism had no impact on EFS and OS in our multivariate analysis.

The other assessed polymorphism (VEGFA rs699947, rs2010963 and rs3025039, and VEGFR2 rs2305948) by Kim et al did not show any significant impact on OS or PFS supporting our own results. The EFS and OS were in a similar range in both cohorts indicating similar IPI risk and treatment conformity.

A validation cohort was missing in the publication by Kim et al, which underlines the necessity of validation cohorts for confirmation of prognostic factors in hematologic malignancies. However, we cannot exclude that ethnicity (Korean vs Caucasian) may play a role in these findings even though there is no evidence in the literature described yet. We therefore would encourage a validation of the results of Kim et al to clarify this point because according to our results VEGF polymorphism do not have a prognostic value for patients with DLBCL.

TABLE 2 Cox regression models for SNPs adjusted for IPI factors

	EFS		OS	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
VEGFR2 (RS1870377)				
AA (n = 13) vs. TT (n = 155)	2.0 (0.9; 4.7)	.103	1.4 (0.5; 3.8)	.481
AT (n = 105) vs. TT (n = 155)	1.4 (0.9; 2.2)	.197	1.2 (0.7; 2.0)	.594
VEGFR2 (rs2305948)				
CC (n = 228) vs. CT (n = 45)	0.7 (0.4; 1.3)	.250	0.8 (0.4; 1.4)	.392
VEGFA (rs699947)				
AA (n = 72) vs. CC (n = 80)	1.2 (0.7; 2.1)	.538	1.8 (0.9; 3.4)	.09
AC (n = 121) vs. CC (n = 80)	0.8 (0.5; 1.4)	.397	1.0 (0.5; 1.9)	.99
VEGFA (rs15707360)				
AA (n = 33) vs. GG (n = 126)	0.9 (0.5; 1.8)	.774	1.2 (0.6; 2.5)	.598
AG (n = 114) vs. GG (n = 126)	0.7 (0.5; 1.2)	.210	0.9 (0.5; 1.5)	.651
VEGFA (rs2010963)				
CC (n = 27) vs. GG (n = 124)	1.4 (0.7; 2.9)	.390	1.3 (0.6; 3.1)	.489
CG (n = 122) vs. GG (n = 124)	1.1 (0.7; 1.8)	.668	0.9 (0.5; 1.5)	.660
VEGFA (rs3025039)				
CC (n = 196) vs. CT (n = 74)[a]	0.9 (0.5; 1.4)	.516	0.7 (0.4; 1.3)	.278

[a]Three samples with TT were excluded due to the small sample size.

Nevertheless, our study has some limitations. First, it is small sample size in comparison to the Korean cohort (n = 494 vs. n = 273) and the fact that only six SNPs were studied even though nowadays genome-wide association study offers the opportunity to analyze a large number of SNPs at the same time. Second, due to the design of the RICOVER-60 trial gene-expression profiling information about the cell of origin (COO) was not available in all patients, which did not allow us to correlate our findings with the COO. Earlier studies showed that this phenotypic distinction is associated with overall survival after R-CHOP chemotherapy.[28] Nevertheless, an already published analysis including patients from the RICOVER-60 and other German trials showed no clinical impact of COO on patients with DLBCL.[29] Furthermore, we were not able to correlate our findings with soluble isoform of VEGF, which has been shown to be a predictor for outcome in patients with de novo DLBCL,[30] or relapsed/refractory DBLCL[31]—this should be addressed in future studies.

The strengths of our study are its collection of patient samples within a randomized controlled trial with uniform and well-documented treatment and clinical outcome data, which minimize a probably existing selection bias in previous published data addressing this issue.

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[Correction added on 17 November 2020, after first online publication: Projekt Deal funding statement has been added.]

CONFLICT OF INTEREST

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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