

Does Fascin Expression in Diffuse Large B-Cell Lymphomas have a Clinical Impact in Patients Treated with Anthracyclin-Based Chemotherapy Plus Rituximab?

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ABSTRACT

Fascin is an actin-bundling protein that is expressed by dendritic cells of the lymphoid tissue. Fascin expression is also seen in the neoplastic cells of classical Hodgkin lymphomas, nearly half of the anaplastic lymphomas and in some diffuse large B-cell lymphomas. We aimed to investigate the clinical significance of fascin expression in CD20 positive diffuse large B-cell lymphomas (DLBCL) in patients treated with cyclophosphamide, doxorubicin, vincristine, and prednisolone plus rituximab (R-CHOP). Thirtyfour of the 55 patients included in the study showed fascin expression, 16 of them being diffuse and strong. There was no statistically significant correlation between fascin expression and overall survival and disease-free survival, sex, disease stage, chemotherapy response, whether or not having bulky disease, extranodal involvement, and international prognostic index (IPI) score. The study has failed to show prognostic significance of fascin expression in DLBCL patients treated with R-CHOP. However, because of the alterations in fascin expression in a variety of benign and malignant lymphoid entities, regardless of its prognostic impact, studies on fascin expression may help us to understand tumor biology of lymphomas better.

Keywords: Fascin, Non-Hodgkin lymphoma, Prognosis

ÖZET

Antrasiklin Temelli Kemoterapi ile Birlikte Rituksimab Tedavisi Alan Diffüz Büyük B-Hücreli Lenfoma Olgularında Fascin Ekspresyonunun Klinik Önemi Var mı?

Fascin, lenfoid dokunun dendritik hücreleri tarafından eksprese edilen, aktin paketleyici bir proteindir. Klasik Hodgkin lenfomalarının neoplastik hücrelerinin, anaplastik lenfomaların yaklaşık yarısının ve diffüz büyük B-hücreli lenfomaların bir kısmının da fascin eksprese ettiği gösterilmiştir. Bu çalışmanın amacı, siklofosfamid, doksorubisin, vinkristin, prednizolon ile birlikte rituksimab (R-CHOP) tedavisi alan CD20 pozitif diffüz büyük B-hücreli lenfomalı (DLBCL) hastalarda fascin ekspresyonunun klinik önemi olup olmadığını ortaya koymaktır. Çalışmaya dahil edilen 55 hastaya ait tümör örneklerinin 34'ünde immünohistokimyasal yöntemle fascin pozitifliği saptandı. Otuzdört vakanın 16'sındaki pozitiflik yaygın ve kuvvetli idi. İstatistiksel değerlendirmede fascin ekspresyonu ile toplam yaşam süresi, hastalıksız yaşam süresi, cinsiyet, hastalığın evresi, kemoterapiye yanıt, bulky hastalık olup olmaması, ektranodal tutulum ve enternasyonal prognostik indeks (IPI) skoru arasında anlamlı bir ilişki saptanmadı. Çalışmada R-CHOP tedavisi alan DLBCL'li hastalarda fascin ekspresyonunun prognostik önemi olduğuna dair bulgu saptanmamıştır. Farklı benign ve malign lenfoid antitelere fascin ekspresyonunda bildirilen değişiklikler ve aynı hastalığa sahip hastalarda saptanan ekspresyon farklılıkları, prognostik önemi olmasa da, fascin üzerine yapılacak çalışmaların lenfomaların tümör biyolojisini daha iyi anlamamıza yardımcı olacağını düşündürmektedir.

Anahtar Kelimeler: Fascin, Non-Hodgkin lenfoma, Prognoz

INTRODUCTION

Fascin is an actin-bundling protein that is expressed by dendritic cells of the lymphoid tissue.¹ Although it has been once proposed as a specific marker for Reed-Sternberg cells and variants in classical Hodgkin lymphoma, it is now known that some of the diffuse large B-cell lymphomas (DLBCL) and nearly half of the anaplastic large cell lymphomas show fascin expression.^{2,3,4,5} Patients with DLBCL, the largest subtype of non-Hodgkin lymphomas, show widely variable clinical outcomes.⁶ So it is important to investigate markers that may have a potential prognostic impact on the clinical course of the disease for a better management of the patients. There are only few studies regarding the fascin expression in DLBCL and there is no data in the literature whether fascin expression in DLBCLs have a clinical impact or not.

The clinical applicability of a prognostic factor may depend on the specific therapy that the patient receiving⁷, so we aimed to investigate the clinical significance of fascin expression in CD20 positive diffuse large B-cell lymphomas in patients treated with cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) plus rituximab (R), the current standard regimen for DLBCL.

PATIENTS AND METHODS

Slides taken from the representative blocks belonging to the fiftyfive patients who were diagnosed as CD20 positive DLBCL between 2005 and 2010 at the Baskent University, were immunohistochemically stained with fascin monoclonal antigen (clone 55K-2, DAKO, Carpinteria, Ca, USA) in Dako Autostainer Link48. Slides were scored semi quantitatively regarding the strength of the cytoplasmic staining (0: no staining, +:weak staining, ++: moderate staining, +++: strong staining) and the percentage of tumor showing fascin expression (no staining, staining in < 25% of tumor, staining in 25-50% of tumor, staining in > 50% of tumor).

All results are presented as rate for categorical values or mean and median for continuous variables. Overall survival (OS) was determined as time between histological diagnosis and death. Disease free survival (DFS) was determined as time between histological diagnosis and relapse. Survival curves

were estimated according to the Kaplan-Meier method and log-rank tests were used for univariate statistical comparisons. Fisher's exact chi-square test was also used for the statistical analysis of categorical values. Adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs) were used for estimation. All data were analyzed using SPSS 11.1 statistical package program and a p value of <0.05 was considered statistically significant.

RESULTS

Patients

Median age of patients was 56 years (range 24-85). Thirty six (65.5%) were male and 19 (31.7%) patients were female. Six patients (10.9%), 11 patients (20%), 20 patients (36.4%), and 18 patients (32.7%) were staged as stage I, II, III, and IV at the time of diagnosis, respectively. Twenty two patients (40%) had bulky disease and 44 patients (80%) had extranodal involvement. ECOG performance status of patients were; 0 in 25 patients (45.5%), 1 in 6 patients (10.9%), 2 in 15 patients (27.3%), and 3 in 9 patients (16.4%). All of the patients were treated with rituximab containing regimen initially. The median overall survival was 48 months (Table 1).

Immunohistochemical Studies

Total 35 of 55 cases (61.8%) showed positive staining with in fascin different strength and percentages of the tumors (Figure 1 and 2), while 20 of the cases were negative (Figure 3). Cases according to the stained percentage of the tumor and strength of staining were; staining in < 25% of tumor cells: 1 case (+), 1 case (++), 3 cases (+++), staining in 25-50% of tumor cells: 1 case (+), 2 cases (++), 6 cases (+++), staining in >50% of tumor cells: 1 case (+), 3 cases (++), 16 cases (+++).

Statistical Analysis

Sex, ECOG performance status, stage, whether or not having bulky disease, response to initial chemotherapy and IPI score showed statistically significant effect on overall survival in univariate analyses (p= 0.015, p= 0.043, p= 0.010, p= 0.035, p< 0.0001, and p= 0.006, respectively) (Table 1). The median disease-free survival was 23 months. None

Table 1. Demographic and clinical characteristics of 55 patients with DLBCL

	n (%)	p value*
Gender (male)	36 (65.5)	p= 0.015
Stage		p= 0.010
I	6 (10.9)	
II	11 (20)	
III	20 (36.4)	
IV	18 (32.7)	
Bulky disease	22 (40)	p= 0.035
Extranodal involvement	44 (80)	p= 0.201
ECOG		p= 0.043
0	25 (45.5)	
I	6 (10.9)	
II	15 (27.3)	
III	9 (16.3)	
Response to initial chemotherapy (n= 50)		p< 0.0001
PD	4 (7.3)	
SD	1 (1.8)	
PR	8 (14.5)	
CR	37 (67.3)	
Fascin expression		p= 0.509
0	24 (43.6)	
1	5 (9.1)	
2	7 (12.7)	
3	19 (34.5)	
Median overall survival, months		48 (95%CI)
*: p-values from Kaplan-meier method and log-rank test, OS(overall survival)		
ECOG= eastern cooperative oncology group performance scale		
PD= Progressive disease, SD= Stable disease, PR= Partial remission, CR= Complete remission		

of the parameters showed significant effect on disease-free survival. Statistical analysis failed to show any significant correlation neither between the presence and strength nor the percentage of fascin expression and overall survival and disease-free survival ($p > 0.05$). Also none of the following parameters (i.e. sex, disease stage, chemotherapy response,

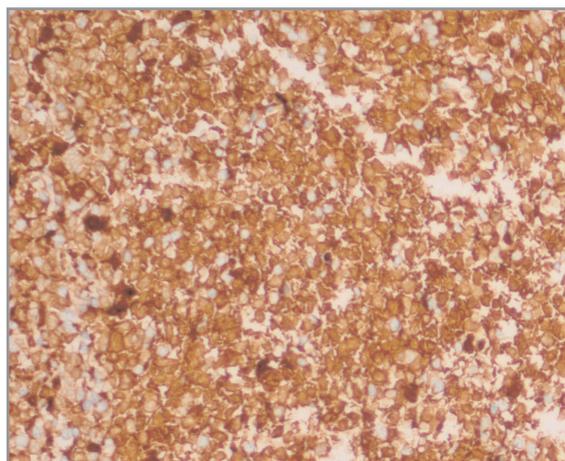


Figure 1. Diffuse and mostly strong cytoplasmic fascin expression in a diffuse large B-cell lymphoma (Fascin x100)

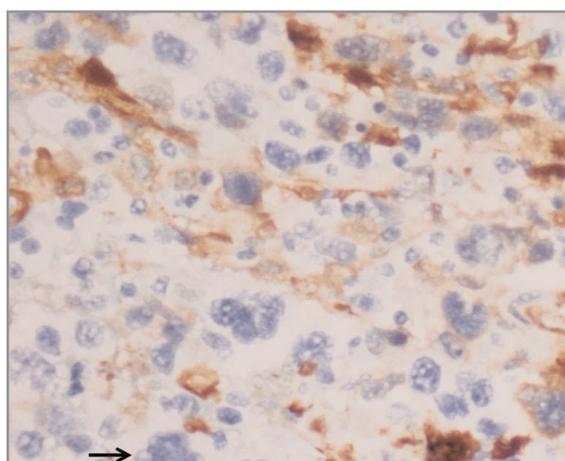


Figure 2. Neoplastic cells exhibiting (+) staining, (++) staining and no staining with fascin (Fascin x400, arrow: (+) staining, asterix: (++) staining, double arrows: no staining)

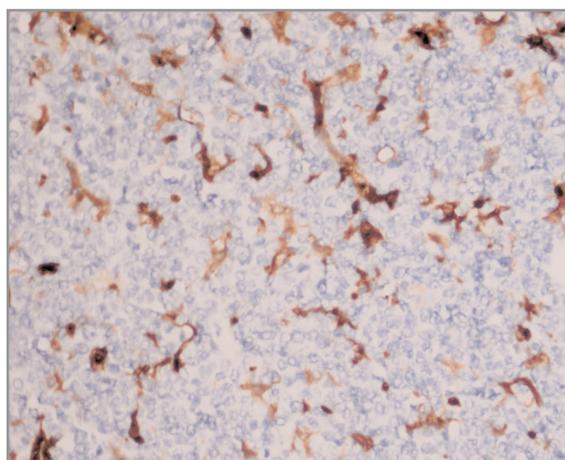


Figure 3. Photomicrograph reveals staining with fascin in capillary walls and dendritic cells but not in the neoplastic lymphocytes (Fascin x100)

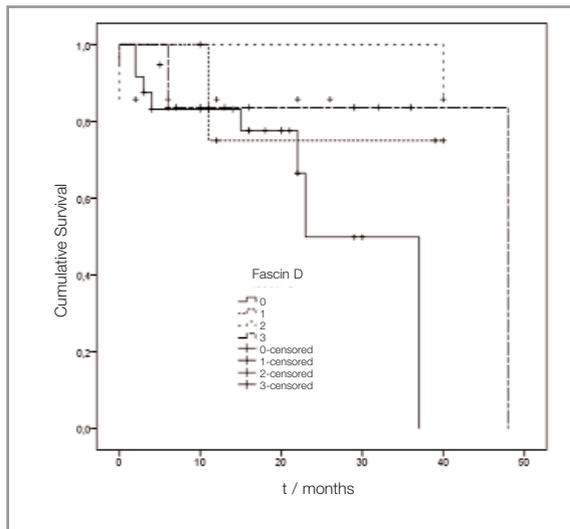


Figure 4. Graph showing relationship between fascin expression and survival ($p= 0.058$)

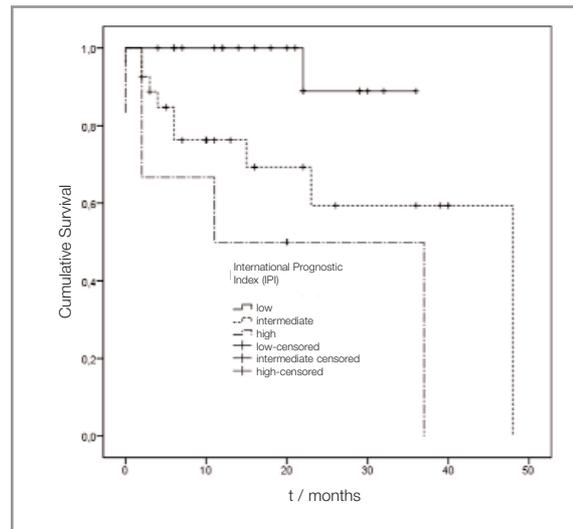


Figure 5. Graph showing relationship between IPI and survival ($p= 0.006$)

whether or not having bulky disease, extranodal involvement, and IPI score) showed statistically significant correlation either with the strength of fascin expression or the percentage of tumor showing fascin expression ($p > 0.05$) (Figure 4, 5).

DISCUSSION

Fascin is expressed in the germinal center dendritic cells of the normal lymphoid tissue. Alterations in fascin expression were reported in neoplastic follicles of follicular B-cell lymphomas as well as follicular hyperplasia.⁸ Fascin is expressed consistently in Reed-Sternberg cells of classical Hodgkin lymphoma but rarely and weak in “L&H” or “popcorn” cells of lymphocyte predominant Hodgkin lymphoma.^{2,9} There are few studies revealing fascin expression in a subset of diffuse large B-cell lymphomas and anaplastic large cell lymphomas.^{3,4,5} Taking into the consideration that some classical Hodgkin lymphomas may even express cytotoxic molecules, differential diagnosis between classical Hodgkin lymphoma and anaplastic large cell lymphoma may be challenging.¹⁰ Fascin appears to have a limited usefulness in the differential diagnosis between these two entities as its negativity may help the exclusion of classical Hodgkin lymphoma.^{2,5,9}

Fascin staining was observed in 34 of 55 (61.8%) DLBCLs in our study and 20 (36.3%) of them were diffuse strong. Bakshi et al. observed staining in 6 of 41 cases (14.6%), 1 case was weak positive, 1 case showed focal to diffuse staining and the remaining 3 were strong positive.² Idrees et al. studied fascin in 26 DLBCLs and observed staining in 8 cases, all of which were strong (30.8%).⁹ With smaller number of cases studied, Idrees et al. found nearly two folds staining more in terms of percentages than Bakshi et al. found.^{2,9} Our serial is the largest among the three studies on fascin expression in DLBCLs. Larger series, questioning fascin expression particularly in various histopathologic subtypes of DLBCL and comparing the rate and pattern of the staining, will add more to the literature.

International prognostic index (IPI) is considered as the dominant prognostic factor in high grade lymphomas.¹¹ Patients with DLBCL may show highly variable clinical outcomes even if they are in the same IPI group and taking the same therapy regimen.⁶ Efforts to explain this heterogeneity in biological behavior, revealed some prognostic factors such as bcl-2 expression, CD5 expression, myc rearrangements, and the germinal center (GC) or non-germinal center (non-GC) origin of the tumor.¹¹⁻¹⁵ Results of the studies after the addition of rituximab to the anthracyclin-based chemotherapy showed that R-CHOP regimen overcame the negative im-

pact of some of these prognostic factors^{7,16-19}, while prognostic value of some (i.e. GC, non-GC phenotype) remains controversial.^{7,15} Double-hit and triple-hit lymphomas have had a poor response to R-CHOP therapy.¹⁸ Some markers such as beclin-1 were shown to be the predictors of favorable clinical outcome in patients treated with R-CHOP.²⁰

Fascin was shown to be a poor prognostic factor in certain epithelial tumors due to its role cell adhesion, cell motility and invasiveness.²¹⁻²³ However, in lymphoid malignancies, fascin expression was observed in the entities representing the two distinct ends of biological behavior spectrum.^{2,5,9}

In this study, there was no statistically significant correlation between IPI score and state of fascin expression, between any component (age, stage, ECOG performance status, extranodal sites, serum LDH level) of the IPI score alone, in addition to gender, state of fascin expression, chemotherapy response, and state of fascin expression. In our study, fascin expression did not appear as a stand alone prognostic factor independent from IPI, while IPI was the dominant predictive factor of clinical outcome.

This study has failed to show any prognostic impact of fascin expression in DLBCL patients, treated with R-CHOP regimen. Studies, may be conducted to evaluate the results of fascin expression in pathological archival materials and survival data obtained from the files of DLBCL patients treated in the pre-rituximab era. Whether fascin expression in DLBCLs have a prognostic impact or not, considering the alterations seen in fascin expression from follicular hyperplasia to neoplastic follicles of follicular B-cell lymphomas, as well as its expression in neoplastic cells of distinct hematolymphoid malignancies, the role of fascin remains to be elucidated. Further studies on fascin expression may help us to understand the tumor biology of lymphomas better.

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