Expression IRF/MUM1>25% Predictor to Three-year Survival of Diffuse Large B Cell Lymphoma in the Immunchemotherapy Era

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ABSTRACT

Introduction: Non Hodgkin lymphoma-Diffuse large B cell lymphoma (DLBCL) is composed of more varieties of one disease. Analysis and understanding of a wide range of characteristics of the disease, which include: clinical, immunohistochemical, cytogenetic and molecular characteristics may improve treatment results. Aim: achieving the estimated three-year survival and influence of IRF/MUM1 expression to three-year survival. Material and methods: A study was retrospective–prospective, patients were followed for seven years a period of time. The study included 60 patients de novo DLBCL. Age was 18–72 years old, the average age 45 years, male 31 (51.7%) and female 29 (48.3%).Median follow-up was 47 months (3–91 months). To determine differentiation immunophenotype antibodies those were used: anti-CD20, anti-CD10, anti-Bcl-6, IRF-4/MUM1, CD 138. Results: Included the GCB type was 65%. Impact prognostic index IPI>2 GBC vs non GBC p=0,038 X². Statistically significant difference was confirmed compared to the IPI> 2 to 3 year OS p<0,0005 X². Significantly longer three-year survival was provided in the group GCB 36 (92,3%) vs. non GCB 8 (38,1%) p=0,003 X². Clinical and immunohistochemical factors showed a significant impact to three-year survival by univariate: LDH p=0,005, MUM1 p=0,003, while CD10 p=0,069 was confirmed on the level of borderline impact. Using multivariate analysis, expression MUM1 has the greatest impact p<0.0005 OR=0.083 (95% CI 0.23–0.303) on the disease outcome – three-year survival. Conclusion: expression MUM1 >25% has the greatest impact on the disease outcome – three-year survival.

Key words: DLBCL, MUM1 expression, three-year survival.

1. INTRODUCTION

According to the latest classification of the World Health Organization Classification of Tumors published 2008th in Lyon, molecular, biological and clinical studies have recognized within Non Hodgkin lymphoma - Diffuse large B cell lymphoma (DLBCL) morphological, molecular and immunohistochemical subgroups and entities (1). Despite great progress in treatment of DLBCL in last 20 years of immunochemothera, failure free survival (FFS) remains around 50% with a particularly poor prognosis for those who have not been treated with immunotherapy and autologous transplantation.

In current practice immunochemothery (R-CHOP) is considered the standard first-line treatment of NHL built of CD20 + B cells, and thus DLBCL. Although the R-CHOP is the best existing first-line therapy for DLBCL, it is curative in about
half of patients, while in others it does not achieve a permanent and/or complete remission. Approximately 30-40% of patients respond very well to treatment and live long, while 60-70% die from the disease. Patients with high and intermediate IPI (>2) have a high rate of relapsing. In these patients, refractory disease requires more aggressive therapeutic approach. Advances in treatment can potentially be achieved using autologous peripheral blood stem cell transplantation (APBSCT), but it is accompanied by an increased rate of mortality and delayed improvement of survival (2).

Therefore, first-line therapy requires further training, which includes understanding the pathophysiological mechanisms of the disease, distinguishing patients with favorable between those with unfavorable characteristics, in which the standard therapeutic approach is not sufficient. It should provide proper patient selection and choice of adequate treatment, which is important in achieving a better and longer therapeutic response, reducing morbidity and treatment costs, avoiding disability and other late effects of treatment, including secondary malignancies.

If we go back to the fact that the NHL is composed of more varieties of one disease, since progress can only be achieved from a critical analysis and understanding of a wide range of characteristics of the disease, which include: clinical, immunohistochemical, cytogenetic and molecular characteristics. It is appropriate to assume that a good choice of therapy, with the definition of prognostic indicators, may improve treatment results.

Prognosis and predictions of NHL is represented by clinical factors expressed by International Prognostic Index (IPI). However, it is evident that clinically and pathologically standard parameters themselves are not sufficient. Biological properties of the tumor largely determine its clinical behavior, indicate prognosis and treatment outcome. It can identify new “targets” that will target the so-called biological therapy.

List of biomarkers with potential prognostic and predictive significance of Diffuse large B cell lymphoma (DLBCL) is huge (3). Based on differentiation cell type determined by immunohistochemical technique and fluorescence in situ hybridization (FISH) by two groups of DLBCL are determined: germinal center B cell type like (GCB ) DLBCL with identification of two cell type: bcl-6+/CD10+/MUM1- and bcl-6+/CD10+/MUM1+/CD138-, which has a favorable prognosis, and activated B cell type (ABC) DLBCL immunophenotype bcl-6+/CD10-/MUM1+ and CD138-, which has a poor prognosis with a small third untested group with a poor prognosis, which is considered unique with ABC (4). However, the prognostic value of some immunohistochemical characteristics of DLBCL is fully defined.

The 2008 WHO classification of tumors of hematopoietic and lymphoid tissues along with data from a study by Rosenwald, Coloma, Hans, Hummel, and al (5-8) point out that the cases with the expression of CD10 cells are considered GCB type, as well as cases that were CD10-, bcl6+, MUM1-. All other cases are considered non-GCB type. Hans's algorithm uses three antibodies: CD10, MUM1 and BCL6 on which form non GBC and GBC subgroups.

Today, the determination of cell type differentiation is an important part of the diagnostic work-up of DLBCL with the recent studies testing and the importance of molecular markers involving FOXP1 and GCET1, whose significance is tested in many studies (9, 10).

In addition, noteworthy is the importance of the other biological characteristics of the B cell NHL as well as understanding of programmed cell death-apoptosis.

Understanding the complexity of carcinogenesis has a positive impact on finding the right option in choosing the right therapy for the treatment and improving the lives of patients.

Recognition of apoptotic deregulation as a fundamental element in the development of cancer today is the most important guide in the study of cancer and finding targeted therapeutic options.

2. MATERIALS AND METHODS

The study was clinical retrospective-prospective. Patients were followed in relation to the clinical characteristics and the data of histopathologic diagnosis until the completion of the study. In this study we analyzed 60 patients who had been diagnosed de-novo diffuse large B cell lymph (DLBCL) and who were treated and followed up at the Hematology Clinic, University Clinical Center of Sarajevo. Median follow-up was 47 months (3-91 months). At the end of the study 44 (73.35%) patients were alive. Patients were divided into two groups: the origin of germinal center - GCB and non germinal center - non GCB. According to the latest WHO classification in relation to subtypes and entities, the study included patients who belonged: DLBCL NOS with subtype T-rich and entities: Mediastinal large B cell lymphoma 3 patients and ALK positive DLBCL 1 patient. The study included patients aged 18-72 years.

It was a homogeneous group of patients in comparison to the first line of treatment. In the first-line treatment patients received immunochemotherapy per protocol R-CHOP (rituximab 375mg/m2 iv day 1 + CHOP / day 1 Cyclophosphamide 750 mg/m2 iv, 50mg/m2 iv Doxorubicin, Oncovin max. 2 mg / iv, 1–5th day Prednisone 100 mg per os).

Radiotherapy was administered at: bulky, extra nodal sites and the residual mass.

Post-treatment restaging consisted of a repetition of earlier pathological tests and/or biopsy.

Response was assessed according to conventional criteria (normalization of metabolic tests and the absence of previously existing tumor mass).

Biopsy material was first analyzed in several different centers to diagnose DLBCL using the following markers: CD20, bcl-2, bcl-6, cyclin D1, very rare CD10, according to the indications: CD5, bcl-1, CD3, CD30, S-100, CK-HMW, CK/AE1/AE3, CK, CD79a/CD15, ALK, EMA, LCA/CD45, CD43, TTF-1, vimentin, TDT, CD99, CD23, cap, lambda, synaptophysin, CK7, NSE, HMB45, desmin, ASMA.
Additional immunohistochemical staining were performed at the Institute of Pathology and Cytology of Clinical Center of University of Sarajevo on the same histopathological material. Biopsy samples were further analyzed by immunohistochemistry for the markers: BCL6, CD10, IRF/MUM1, CD138 at the Institute of Pathology, Clinical Center University of Sarajevo.

The sections were incubated with primary antibody including:
- Anti-CD20 (1:150, clone L26, DakoCytomation, Glostrup, Denmark),
- Anti-CD10 (1:150, clone 56C6, Novocastra Laboratories, Newcastle, Tyne, UK),
- Anti-Bcl-6 (1:40, clone PG.B6p, DakoCytomation, Glostrup, Denmark),
- CD138 (1:10 dilution, Clone AM 411-10 M, BIOGENEX, CA USA),
- IRF/MUM1 (1:40 dilution, clone sc 6059, Santa Cruz Biotechnology, INC, CA, USA),

The project provides visualization which was performed with EnVision method (DakoCytomation, Glostrup, Denmark) with the manufacturer’s instructions. Appropriate positive and negative controls were used.

Bcl-6 and CD10 was quantified using the H score (histo-score) system, according to the method described by McCarty et al. Positive expression of the MUM1 and CD138 was considered when more than 25% neoplastic cells. Microscopy was performed on a microscope ZEISS Scope A1. Microscopy preparation had next appearance:

Expression bcl6

Expression CD138

Expression IRF-4/MUM1

Expression CD10

Statistical analysis:
When it comes to statistical analysis we used univariate methods for evaluation of significant difference (X² test, binary logistic regression analysis). We assessed the overall survival with Kaplan-Meier methods and unstratified long-rank test. We used a multivariate backward Wald model to assess the significance for the efficacy variables and to establish th Odds ratio (OR) and 95% CI for each subgroup. P<0.05 was considered as significant

3. RESULTS
This study included 60 patients diagnosed with de novo diffuse large B cell lymphoma (DLBCL). The age of the respondents was 18-72 years and the average age prevalence was 45 years old. We analyzed 31 (51.7%) males, 29 (48.3%) were women.

Responses of total period of monitoring
During the period of examination, with 60 patients who were treated by immunochemotherapy and who had DLBCA, complete remission 47 (78.3%), PR-partial remission 8 (13.3%), PB-progressive disease 5 (8.3) was achieved. Statistically significant difference was confirmed compared to the IPI> 2 (low: high) 39 (65%) vs 21 (35%) χ²p= 0.014, clinical stage I/II vs III/IV x² 26 (43.3%) vs 36 (56.7%) p<0.0005, ECOG >2 (11.7%) vs 53 (88.3%) p=0.008 and level LDH normal vs. increased 38(63,3%) vs 22 (36,7%) p=0.003 compared to achieve first complete remission.

Difference in survival length of the examinees with MUM1>25% is statistically significant χ² (Mantel-Cox)=19.2 p<0.0005. Examinees with MUM1>25% vs 22 (36,7%) p=0.003 compared to achieve first complete remission.

Table 1. Clinical features DLBCL according to immunohistochemical profile

| Clinic characteristics | Immunohistochemical groups | sign. p=x2 |
|------------------------|---------------------------|-----------|
| Age, years, median     | GCB (n=39) % | non-GCB % (n=21) % |           |
| Gender                 | Male 23 (59.0%) | 8 (38.1%) | N.S.     |
|                        | Female 16 (41.0%) | 13 (61.9%) | N.S.     |
| Primary extranodal site| 21 (53.8%) | 11 (52.4%) | N.S.     |
| High clinical stage Ann Arbor III/IV % | 18(46.2%) | 16(76.2%) | 0.023 |
| General condition according to ECOG scale (>2) | 2(5,1%) | 5(23,8%) | 0.045 |
| Bulky (>5 cm) | 11(28,2%) | 7(33,3%) | N.S.     |
| Infiltration of bone marrow Positive | 5(12,8%) | 3(14,3%) | N.S.     |
| B symptoms Positive   | 29(74,4%) | 17(81,0%) | N.S.     |
| International prognostic index IPI (> 2) | 2(5,1%) | 5(23,8%) | 0.038 |

Figure 1A. Kaplan-Meier curves for three year overall survival
Expression IRF/MUM1 >25% Predictor to Three-year Survival

Using Multivariate Cox’s Regression Method on three-year survival, MUM1 has the strongest influence \( p<0.0005 \) OR = 0.083 (0.23-0.303), then LDH \( p=0.002 \) OR = 5.8 (19.3-17.5) (Table 2).

In comparison to three-year survival, immunohistochemical features in relation to expression bcl6, CD10, CD138 i MUM1, only expression MUM1 had a significant independent influence. Expressions bcl6 and CD138 did not have significant influence to three-year survival DLBCL in the immunochemotherapy era. The influence of CD10 expression is confirmed on the bordering significant level \( p=0.069 \).

4. DISCUSSION

In the presented study the relationship between clinical status and immunohistochemical profiles has been analyzed in the expression: Bcl-6/CD10 / MUM1/CD138 of diffuse large B-cell lymphoma.

Group GCB which has similar subtypes with B cell origin of germinative center was correlated with group non-GCB, which has subtypes of non germinative center. Immunohistochemical groups were comparable with data from the last sub-classification nomenclature of lymphoma (1).

At the molecular level there are two large groups of patients with DLBCL: germinal center B cell type like (GCB) DLBCL, which has a favorable prognosis, and activated B cell type (ABC) DLBCL, which has a poor prognosis, with a third small untested group with poor prognosis, which is considered unique with ABC. In previous studies these two groups were analyzed (4-7).

Because of its expensive technology molecular analysis is more rarely used than immunohistochemical analysis related to CD10, bcl-6, MUM1 CD138.11 in some studies (12-14). Whether is this a good division is still not clear.

Rate of GCB defined immunohistochemical phenotype is variable from study to study (18% in the study Borovečki to 49% in the study Paeppe et al) (15).

In our study, the group A (GCB) was 39 (65%) patients in group B (non-GCB) 21 (35%) patients. Study was preceded by a pilot study, “Prognostic and predictive significance of CD10 expression in diffuse large B cell lymphoma” (2007), which confirmed the significant impact of CD10 expression in relation to the achievement of CR1. Abstract of the study is published in the journal Leukemia Research - Clinical and Laboratory studies (16).

Analysis of clinical response

Median follow-up was 47 months (3-91 month), which is satisfactory considering the fact that relapse usually occurs within the first two years after our initial treatment. We analyzed 60 patients with DLBCL (median age was 45 with most patients in the age group 46-65 years, significantly impacts of age on the results of this study was not confirmed. We analyzed 31 (51.7%) males, 29 (48.7%) were women. Good response to first-line therapy is confirmed with the achievement of CR1 in 78.3% of investigated, which is comparable with the results of reference centers. A study by the 2007th published Coiffier (17). The difference in achieving CR1 can be explained by
the fact that the study included a small group of subjects in which more patients belonging to group A (GCB) 39 (65%). Statistically significant difference was confirmed compared to the IPI> 2 (low: high) 39(65%) vs 21 (35%) χ²p= 0.014, clinical stage I/II vs III/IV X² 26 (43.3%) vs 36 (56.7%) p<0.012 and ECOG>2 (11.7%) vs. 53 (88.3%) p=0.008 compared to achieve first complete remission.

Statistically significant difference confirmed IPI >2 (low: high) χ²p= 0.014, clinical stage I/II vs III/IV X² 26 (43.3%) vs 36 (56.7%) p<0.012 and ECOG>2 (11.7%) vs. 53 (88.3%) p=0.008 compared to achieve first complete remission. Using Univariate Binary Logistic Regression Analysis it is confirmed statistically significant influence of IPI>2 p<0.0005 OR: 4.5 95% CI (1.98-10.3) to three year survival (Table 2).

Using Univariate Binary Logistic Regression Analysis it is confirmed that the age and sex of the examinees do not have impact to three-year survival: ages p=0.903 OR 0.956 (0.465-1.966), gender p=0.322 OR 0.593 (0.211-1.667), with patients with DLBCL, while ECOG>2 has statistically significant influence to three-year survival p=0.002 OR=6.390 (2.02-20.194) as well as the level LDH p=0.005 OR=4.6) in the expression of MUM1 p=0.003 OR 0.082 95%CI (0.16-0.430 1.5-13.6) (Table 2). Using backward Wald multiple regression analysis on clinical and immunophenotypic features in 60 D LBCL patients in relation to three-year overall survival, the significant impact of MUM1 expression is confirmed p<0.0005 OR=0.083(0.23-0.303), then LDH p=0.002 OR=5.8 (1.93-17.5) and IPI p=0.002 OR=4.47 95%CI (1.8-12.7) (Figure 1A), Kaplan-Meier curve in the group GBC/MUM–the survival average 37.5 months 95%CI (34-40) months, compared with examinees with <25% MUM1 live shorter (23 months; 95% (16-29 months), compared with examinees with <25% MUM1 who live in average 37; 95% (34-40 months) (Figure 1B).

High expression of CD10 is confirmed in group GCB X² =21.538 p <0.0005 where it reached a better response to therapy compared to CR. In relation to three-year survival the expression of CD10 was confirmed with impact on borderline impact X² p=0.069 OR 2.331 (0.936-5.808). Expression of MUM1 was> 25% in group non-GCB, and <25% in group GCB, as well as in the study of Alizadah (4). Difference in the survival length examinees with expression MUM1 >25% vs. <25% is statistically significant χ²(Mantel-Cox)=19,2 p=0,0005. The examinees with >25% MUM1 live shorter (23 months; 95% (16-29 months), with patients with >25% MUM1 who live in average 37; 95% (34-40 months) (Figure 1B).

High expression of MUM1 is confirmed in group non-GCB, which has reduced sensitivity, poorer response to treatment, in relation to the achievement of CR1 and OS in the application of immunotherapy in treating DLBCL. Our results in relation to expression MUM1 on three-year survival are comparable with the study of author Berglund M (18). He states that the expression of bcl-6 and CD10 are better predictors of response and that expression of MUM1 is not associated with a good prognosis. Results of this study are comparable with the results of the above authors in relation to expression of MUM1 and partially CD10, while not comparable to the results related to the expression of bcl-6. The data obtained in our study confirmed that immunohistochemical profile affects the achievement of three-year survival with significant influence of expression MUM1 and borderline impact expression of CD10 and in applying immunohemotherapy in treating DLBCL. Good predictors of response are confirmed in the application of immunohemotherapy: expression of MUM1-, which is comparable with the results obtained by Muris JJ (24) which in his study concludes that the strongest predictors are CD10 expression, MUM1, bcl-2 and IPI.

The results of multiple regression analysis

Using Multivariate Cox’s Regression backward Wald, impact of independent clinical and immunophenotype factors on three-year survival was examined. Independent factors showed statistically significant influence univariately ECOG>2 p=0.002, LDH p=0.005, MUM1 p=0.003. Using multiple regression analysis of clinical and immunophenotypic features DLBCL in relation to three-year overall survival, the significant impact of MUM1 expression is confirmed p<0.0005 OR=0.083 (0.23-0.303), then LDH p=0.002 OR=5.8 (1.93-17.5) (Table 2). Pre-treatment prognostic and predictive value
of immunohistochemically defined GCB and non GCB group within DLBCL was confirmed significant independent by univariate analysis and by multivariate analysis effect of the expression of MUM1.

The results of multiple regression analysis, the independent influence of MUM1 expression confirm significant impact of the planned three-year survival rate, where the impact associated with immunohistochemical markers of GCB and non GCB was analyzed and in which the analysis confirmed significant difference in the achievement of CR1 and OS.

5. CONCLUSIONS

Immunohistochemical profile in the expression: Bcl-6/CD10/ MUM1/ has a significant impact on therapeutic response DLBCL in relation to three year overall survival when applied immunochemothery. The significant impact of MUM1>25% expression is confirmed and particular influence the expression of CD10 to three-year survival. In this study significant impact to three-year survival of expression bcl6 in the era of immunochemothery in DLBCL was not confirmed.

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