

Original article

Prognostic impact of MYD88 mutation, proliferative index and cell origin in diffuse large B cell lymphoma



Laura Fogliatto^{a,b,c,*}, Kamila Castro Grokoski^{c,d}, Yuri Machado Strey^b, Tito Vanelli^a, Christina Garcia da Silva Fraga^a, Marínes Bizarro Barra^a, Fernanda Correa Pinto^a, Israel Bendit^e, Cláudia Giuliano Bica^b

^a Irmandade Santa Casa de Misericórdia, Porto Alegre, RS, Brazil

^b Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil

^c Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, RS, Brazil

^d Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

^e Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brazil

ARTICLE INFO

Article history:

Received 20 February 2018

Accepted 14 May 2018

Available online 24 October 2018

Keywords:

Diffuse large B-cell lymphomas

MYD88

DLBCL

Tumor markers

ABSTRACT

Background: Diffuse large B-cell lymphoma, among non-Hodgkin lymphomas, is one of the most frequent subtypes. Clinical laboratory data and post-treatment outcomes are scarce in the Brazilian population.

Objective: The main objective of this retrospective study was to assess the impact of tumor markers, including the Myeloid differentiation primary response 88 (MYD88) mutation.

Method: Eighty-three patients were included and treated with R-CHOP or R-CHOP-like regimens.

Results: Median age was 64-years old and 58% were female patients. The median follow-up was 42 months. The progression free survival (PFS) at this time was 63% and overall survival (OS), 66%. In the patients with tumors expressing Myc proto-oncogene protein (MYC) and B-cell lymphoma 2 (BCL2), assessed by immunohistochemistry (IHC), known as dual protein expressers, median post-progression survival was 31 (15–45) months. An increased proliferative index were associated with a high rate of progression (hazard ratio 2.31 [95% confidence interval 1.05–5.12]; $p = 0.04$). The cell of origin (COO), identified by IHC, was not able to predict PFS ($p = 0.76$). The MYD88 L265P mutation was present in 10.8% (9/83) of patients and did not show a prognostic correlation.

Conclusion: In conclusion, the MYD88 mutation, although an important tool for diagnosis and a possible target drug, presented at a low frequency and was not a prognostic marker in this population.

© 2018 Published by Elsevier Editora Ltda. on behalf of Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. This is an open access article under CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author at: Serviço de Hematologia, Hospital Santa Rita, Irmandade Santa Casa de Misericórdia, Rua Sarmento Leite, 187 - Centro Histórico, Porto Alegre CEP 90050-170, RS, Brazil.

E-mail address: fogliattolaura@gmail.com (L. Fogliatto).

<https://doi.org/10.1016/j.htct.2018.05.014>

2531-1379/© 2018 Published by Elsevier Editora Ltda. on behalf of Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. This is an open access article under CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma in adults, accounting for 40% of all cases.¹ Multiple genetic lesions of pathogenic significance segregate with different DLBCL subtypes, suggesting that these tumors rely on distinct oncogenic pathways.² The DLBCL cell of origin (COO) can be distinguished according to the gene expression profiling into two major subtypes, the germinal center B-cell-like (GCB) type and the activated B-cell-like (ABC) type.³ Constitutive activation of the nuclear transcription factor κ B (NF- κ B) pathway, which is normally transiently activated by antigen-dependent stimulation in B cells, is characteristic of ABC DLBCL.⁴ The survival of ABC DLBCL is known to be inferior to that of GCB DLBCL, and the poor response to chemotherapy has been attributed to the anti-apoptotic effect of NF- κ B.⁵

The myeloid differentiation primary response gene 88 (MYD88) is an adaptor protein that activates the NF- κ B signaling through most of the Toll-like receptors (TLRs).⁶ Mutations in this gene have been implicated in the pathogenesis and development of therapy resistance in B-cell neoplasms, mainly Waldenstrom's Macroglobulinemia and ABC-DLBCL.⁷ The L265P variant is the most prevalent MYD88 mutation in patients with non-Hodgkin lymphomas.⁸

In DLBCL, the MYD88 L265P mutation occurs at various frequencies, ranging from 0 to 94% and a general consensus on clinical pathology implications has not been reached because it varies according to the population included.⁹ Some recent studies demonstrated the presence of mutation in association with an immune-privileged anatomical compartment, such as the central nervous system (CNS) or the testis, a non-GCB subtype and older age. No clear relation to prognosis was demonstrated.^{8–11} A study with relapsed refractory DLBCL submitted to autologous stem cell transplantation showed that the MYD88 L265P mutation alone had no survival impact in patients before or after transplantation.¹²

This study aims to evaluate the MYD88 L265P gene mutation prevalence in patients with DLBCL with focus on possible associations with this gene mutation and clinicopathologic parameters.

Material and methods

Study design and sample

This was a retrospective study, with consecutive cases of DLBCL. Patients were diagnosed and treated at Santa Casa de Misericórdia de Porto Alegre, Hospital Santa Rita, a regional cancer reference center, from 2011 to 2016 and had follow-up data and tumor DNA available. We excluded all cases that were not treated at this hospital, had no tumor sample available, had transformed disease or composite lymphomas, were patients with other active cancers or those with CNS involvement and those for whom the MYD88 mutation had not been tested.

Clinical parameters and biochemical analysis

The medical records were reviewed to collect clinical data, pathology and immunohistochemical information. Since this is a hospital-based study, two qualified and experienced pathologists reviewed the cases.

Immunohistochemistry was performed in all cases, selecting the representative areas with the highest percentage of tumor cells. Sections were subjected to staining protocols with the following antibodies: CD10 (clone 56C6 – Diagnostic Biosystem, USA), MUM1 (mum1P – Dako, Denmark), BCL2 (clone 124 – Ventana, USA), BCL6 (clone PG-B6 – Dako, Denmark) and Ki-67 (clone 30-9 – Ventana, USA) and MYC (clone Y-69 – Biocare, USA). The positivity of the immunostaining was detected by the percentage of positive cells and intensity of staining. For the Bcl-2, the positivity cutoff was considered to be when the reactivity of lymphoma cells with antibodies was $\geq 40\%$, $\geq 40\%$ for MYC^{13,14} and for Bcl-6 $> 30\%$.¹⁵ According to the expression of IHC markers, the cases were stratified as GCB and non-GCB using the algorithm described by Hans et al. (2004).¹⁵ The Ki-67 antibody was used to determine the proliferation cell index, and Ki-67 $\geq 95\%$ was defined as a “high-proliferation” tumor.¹⁶ Ki-67-positive cells were evaluated among 1000 tumor cells by nuclear staining and counted under high magnification (400 $\times$) in the highest labeling area. The Ki-67 labeling index was calculated as positive nuclei $\times$ 100/total number of counted nuclei (%).^{17,18} Cases with positive expression for MYC and BCL2 were considered dual protein expressers.

Mutation analysis

The mutation analysis technique was described in a previous study.¹⁹ Genomic DNA from 5 micrometers of formalin-fixed

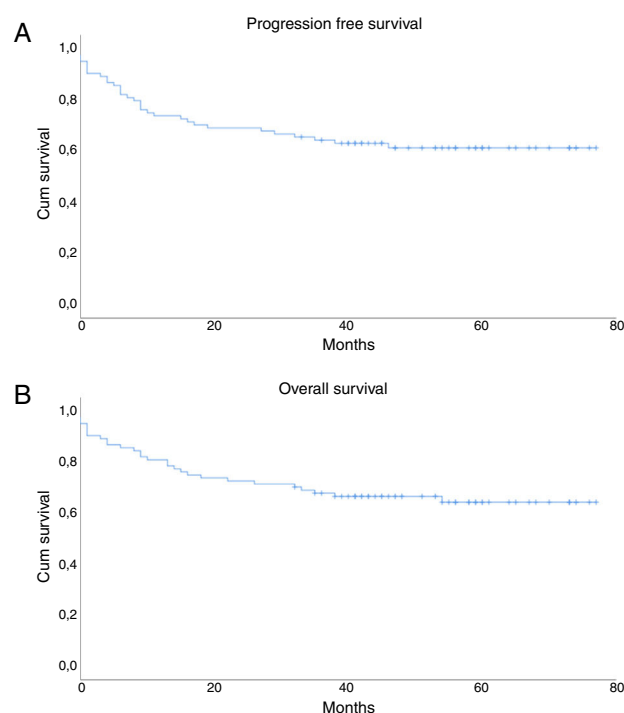


Figure 1 – Progression Free Survival (A) and Overall Survival (B) in the total population.

Table 1 – MYD88 mutation analyses status and clinicopathologic parameters.

Clinicopathologic parameters	All casesn (%)	MYD88 wild typen (%)	MYD88 mutatedn (%)	p
Gender				
Female	48 (57.8)	41 (55.4)	7 (77.8)	0.29
Male	35 (42.2)	33 (44.6)	2 (22.2)	
Age				
<60 years	33 (39.8)	30 (40.5)	3 (33.3)	1.00
≥60 years	50 (60.2)	44 (59.5)	6 (66.7)	
LDH				
Normal	38 (50.0)	34 (50.0)	4 (50.0)	1.00
Elevated	38 (50.0)	34 (50.0)	4 (50.0)	
R-IPI				
Very Good	7 (10.0)	6 (9.7)	1 (12.5)	1.00
Good	43 (61.4)	38 (61.3)	5 (62.5)	
Poor	20 (28.6)	18 (29.0)	2 (25.0)	
Double expressers (MYC+BCL2+)				
Positive	15 (18.1)	14 (18.9)	1 (11.1)	1.00
Negative	68 (81.9)	60 (81.1)	8 (88.9)	
Ki67 labeling index				
≥95%	19 (23.5)	18 (25.0)	1 (11.1)	0.67
<95%	62 (76.5)	54 (75.0)	8 (88.9)	
Biopsy site				
Nodal	45 (54.9)	43 (58.9)	2 (22.2)	0.07
Extranodal	37 (45.1)	30 (41.1)	7 (77.8)	
Subtype				
Non-GCB	48 (59.3)	42 (58.3)	6 (66.7)	0.73
GCB	33 (40.7)	30 (41.7)	3 (33.3)	
Non-GCB: non-germinal center B-cell; GCB: germinal center B-cell; LDH: lactate dehydrogenase enzyme; R-IPIP: revised international prognostic index.				

paraffin-embedded (FFPE) DLBCL sample was extracted with the QIAamp DNA FFPE Kit (QIAGEN GmbH, Germany) following the manufacturer specifications. The minimum necessary DNA concentration needed for the polymerase chain reaction (PCR) assay was 10 ng/mL.²⁰ Isolated DNA was subjected to PCR amplification in a final volume of 25 µL, containing 1 U of Platinum Taq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 10 pMol of primers flanking the codon 265 mutation region, 200 mM dNTPs, MgCl₂ 1.5 mM, 1× of the Taq DNA polymerase buffer, and 10–50 ng of genomic DNA. The primers were as follows: Forward 5'-AGACTGGGCTTGTCCAC-3' and Reverse 5'-AGATTTGGTGCAGGGTTG-3', generating a 175 bp amplicon. The reaction consisted of 45 °C of an initial denaturation step of 15 min at 95 °C, followed by 45 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s and a final extension step at 72 °C for 10 min. The PCR amplicons were confirmed after electrophoresis on agarose gel. Ten microliters of the PCR product were then sequenced using primer 5'-AGACTGGGCTTGTCCAC-3' and pyrosequencing was performed using the PyroMark Vacuum Prep Workstation and the PyroMark Q24 (Qiagen) according to the manufacturer's instructions.

Statistical analysis

Data are displayed as mean ± standard deviation or median (interquartile range). Comparisons between groups (MYD88 positive and negative) were made using the Student t-test

for continuous variables and the chi-square test for categorical variables. Survival analysis was performed using the Kaplan–Meier method and log-rank test to assess significance. Multivariate analysis was performed using a Cox proportional hazards model. Factors significant at the $p=0.20$ level in univariate analysis were included in a stepwise regression multivariate analysis with entry and removal limits set at $p=0.20$. A p -value of 5% was set for statistical significance. All the analysis was conducted using the Statistical Package for Social Sciences 21.0 (SPSS® Inc, Chicago, IL).

Ethical considerations

This research project was conducted in accordance with resolution number 466/12 of the Brazilian National Health Council and preserves the ethical principle of confidentiality. It was approved by research ethics committee at Santa Casa de Misericórdia de Porto Alegre (CAE: 24896013.0.0000.5335). The study was conducted in accordance with the provisions of the Declaration of Helsinki and all experiments described herein comply with the current laws of Brazil.

Results

From 2011 to 2016, according to the WHO 2016 criteria,¹ 151 patients were diagnosed with DLBCL at the Pathology Unit, Hospital Santa Rita. Sixty-five patients were excluded from

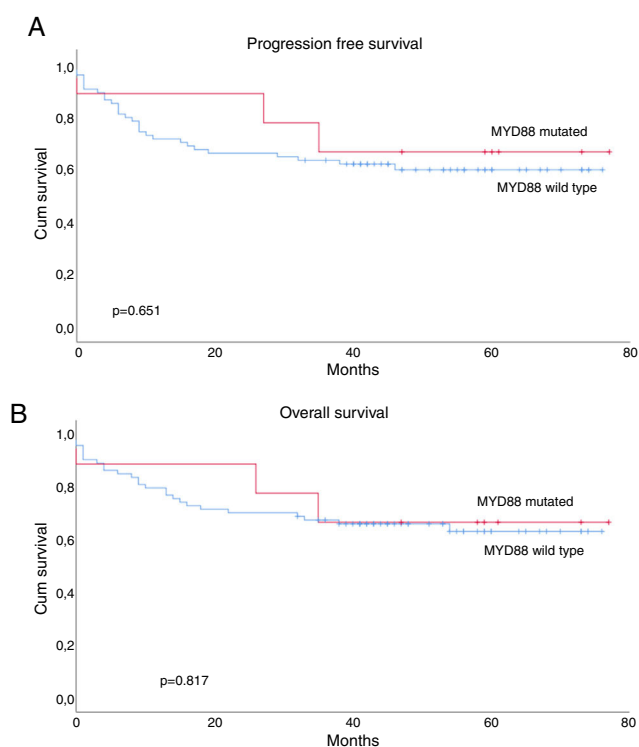


Figure 2 – Progression Free Survival (A) and Overall Survival (B) stratification according to MYD88 mutated vs MYD88 wild type.

the analysis, including 6 CNS lymphomas, 4 transformed and composite lymphomas, 6 cases where tumor tissue samples were unavailable, 26 patients not treated in the hospital and 23 cases lacking the MYD88 mutation analysis. A final group of 83 DLBCL patients was analyzed for this study. The median follow-up was 42 (0–77) months.

The median age was 64 (15–92) years old. Fifty patients (60%) were 60-year-olds or older and the majority of the patients were female (58%). The median time from first symptoms to treatment initiation was 3 (0–32) months. Very good, good and poor R-IPI scores were found in 7%, 61% and 32% of the population, respectively. The BCL-2 and MYC were expressed in 18% of patients, assessed by IHC, and 23% of the sample showed high Ki67%. Non-GCB COO were demonstrated in 59.3%. R-CHOP was the first line treatment in 75 (85.2%) patients. Seven patients (9.2%) did not receive rituximab upfront due to B hepatitis positivity serology. In this study, the PFS was 63% and the OS was 66% at the median follow-up of 42 months (Figure 1).

The prevalence of the MYD88 L265P mutation was 10.8%. The mutated cases were more frequent in older patients, the non-GCB subtype and extranodal lymphoma, however no significant difference in clinicopathologic parameters between patients with mutated and wild type MYD88 was observed (Table 1). The MYD88 status mutation did not impact on treatment response or survival, although MYD88-mutated patients did slightly better (Figure 2).

The COO, identified by IHC, was not associated with prognosis. The PFS, at median follow-up, was 63 months for the GCB subtype and 62 months for the non-GCB ($p = 0.76$).

Poor R-IPI category and high Ki67 index were associated with a decreased PFS in univariate and multivariate analysis (Table 2 and Figure 3). An expression of BCL2 and MYC in IHC was a significant prognostic marker in the univariate analysis (Figure 4).

Discussion

Although the use of rituximab was approved later in Brazil in the public health system, RCHOP has been the first-line treatment in our DLBCL patients; according to international guidelines. DLBCL is the predominant subtype of non-Hodgkin's lymphomas in Brazil, but clinical and laboratory data, as well as outcomes, are scarce.^{21,22} This study contributes to demonstrated results of the DLBCL patient characteristics, prognostic implications and outcomes. Considering a regional reference cancer center, with the providing of public and private health care, the PFS of 63% and OS of 66% in a 42-month median follow-up were consistent with international results.

The MYD88 mutation can be a powerful driver of high NF- κ B activity, a characteristic of DLBCL's ABC or non-GCB subtypes. The incidence of the MYD88 L265P mutation in DLBCL patients varies from tumor site to tumor site. Kraan et al. (2013)²³ suggested that the MYD88 L265P mutation in DLBCL is associated with an immune-privileged anatomical compartment, such as the CNS or the testis. In this study, we excluded primary CNS DLBC and those patients with a CNS DLBCL secondary involvement because the treatment and outcome of this group of patients are significantly different. There was a paucity of testis cases. The MYD88 mutation was more frequent in extranodal lymphoma and in the non-GCB subtype as described previously,^{8,24,25} nonetheless this association was not significant in this sample. In the authors opinion, this can be explained by the low frequency of the mutation in the studied population (10.8%). The prognostic value of the MYD88 mutation has been a matter of controversy. Some studies have reported that the MYD88 L265P mutation is significantly associated with lower survival rates, but this was not proved in a meta-analysis conducted by Lee et al. (2017).⁹ Their conclusion was that more studies are needed to demonstrate the negative prognostic effect of the MYD88 L265P mutation in DLBCL patients. In our study, no prognostic relevance of MYD88 was demonstrated.

The DLBCL classification, according to the COO, into GCB and ABC, has been studied by gene expression profiling (GEP). The ABC subtype, in Lenz et al. (2008),²⁵ was associated with an inferior PFS with traditional RCHOP. Following this landmark study, numerous other groups have reported similar findings showing inferior outcomes.^{3,26–28} Although this suggests a worse prognosis for the ABC subset, in recent prospective randomized trials in DLBCL, the COO determination failed to identify prognostic subgroups.^{29,30} Because GEP is expensive and not readily available in routine practice, several algorithms have been proposed in recent years; these algorithms have been based on IHC staining or tissue microarray analysis, which is a surrogate for GEP analysis.³¹ Although these algorithms have been validated in many studies, the reproducibility of scoring such parameters across laboratory

Table 2 – Multivariate analysis by Cox model for progression and death in the population with poor R-IPI, double-expressers and high Ki67.

Variable	Progression		Death	
	HR (95% CI)	p	HR (95% CI)	p
Poor R-IPI ^a	2.45 (1.17–5.15)	0.02	3.34 (1.54–7.25)	0.02
Double expressers	2.05 (0.91–4.63)	0.08	1.78 (0.76–4.16)	0.18
Ki67 > 95%	2.31 (1.05–5.12)	0.04	2.20 (0.95–5.03)	0.06

HR: hazard ratio; CI: confidence interval; R-IPI: revised international prognostic index.

^a Good and very good R-IPI were considered a single category.

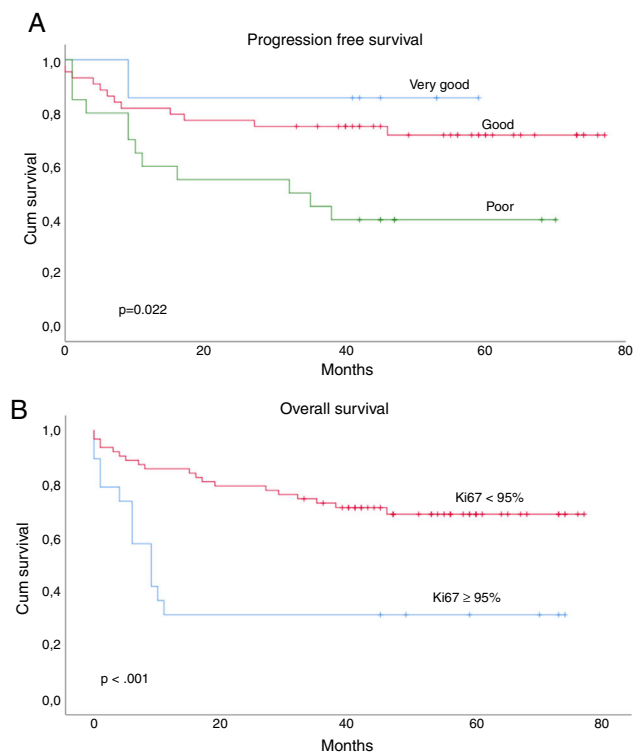


Figure 3 – Progression Free Survival and R-IPI score in the total population (A); Progression Free Survival stratification according to Ki67 proliferative index $\geq$ or < than 95% (B).

techniques is not completely uniform.¹⁷ This study, using the Hans algorithm, could not associate non-GCB DLBCL with a worse outcome and in part this could be explained by the methodology used. The COO prognostic impact is not yet a consensus in literature and this biomarker needs to be evaluated in future prospective clinical trials.

“Double-expressers” refers to immunohistochemical detection of MYC and BCL2 overexpression phenotype in DLBCL of lymphoid neoplasms. The WHO classification defines overexpression as greater than 40% of MYC-expressing cells and greater than 50% of BCL2-expressing cells.¹ However, the cutoff points to define overexpression vary from study to study, resulting in heterogeneous groups of patients.^{32–34} Probably due to this limitation, data on double-expresser lymphoma outcome with traditional treatment are still controversial. In our study, double-expressers of DLBCL presented inferior outcome in a univariate analysis. The IHC analysis

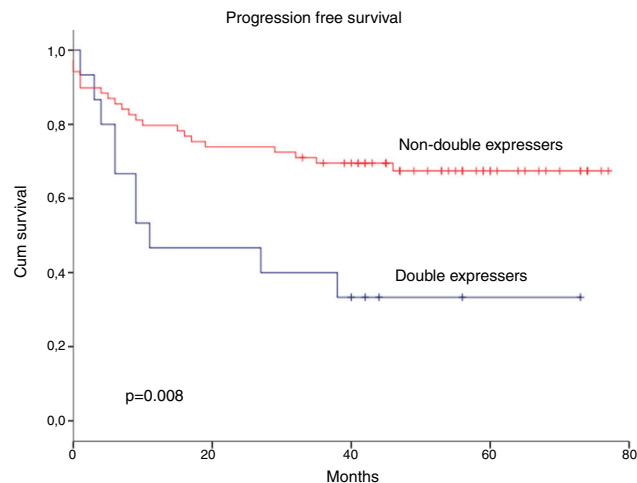


Figure 4 – Progression Free Survival in MYC+/BCL2+ expression by IHC (dual protein expressers) vs no coexpression of these markers.

methodology, the lower BCL2 cutoff positivity ($\geq 40\%$),³⁵ and the small number of double expressers cases may have interfered in this result.

In the present report, we analyzed the relationship between Ki-67 expression and clinical outcomes of DLBCL patients treated with rituximab plus chemotherapy. Ki-67 is a nuclear protein antigen present in all proliferating cells during the active part of the cell cycle. It has been associated with proliferative cellular activity.³⁶ In DLBCL, the wide variation of prognostic significance of Ki-67 among study groups may be explained by diverse study populations, sample sizes, method of Ki-67 staining/assessment, chosen Ki-67 cutoff, and heterogeneous therapeutic regimens.^{17,37,38} A recent meta-analysis was conducted to explore the correlation between Ki-67 expression and the survival outcome in lymphoma. The results revealed that high Ki-67 expression in patients with lymphoma was associated with worse prognosis, both for the OS (HR = 2.19, 95%CI: 0.95–5.03; $p = 0.000$) and the PFS (HR = 1.727, 95%CI: 1.159–2.571; $p = 0.007$).¹⁶ A large prospective study from the Lunenburg Lymphoma Biomarker Consortium, including 2451 patients, also identified high Ki-67 expression as a predictive factor of response to Rituximab in DLBCL.¹⁸ However, an optimal cutoff point needs to be further defined and validated for lymphoma.³⁹ In this study, we are able to find a correlation between high Ki67 and a higher rate of progression (HR 2.31, CI95% 1.03–5.59, $p = 0.04$). As Ki67 is done

for all patients in our center, although not valuable in making treatment decisions, can assist in the closer monitoring of this group of patient treatment responses.

Conclusion

This unique center and retrospective study contributes to the demonstration of results in a Brazilian cohort of DLCLBCL patients, where all the limitations related to the public health system do not negatively impact on the PFS and OS. The MYD88 mutation in this group of patients presented a low frequency and was not associated to clinical laboratory features, and neither was it a prognostic marker. The DLBCL COO, determined by IHC was not able to discriminate poor response to RCHOP treatment patients. Patients with expression of BCL2/MYC demonstrated an inferior PFS in a univariate analysis. High R-IPI and high Ki67 proliferative index were associated with increased risk in progression and death rates. Evaluation of prognostic or predictive biomarkers in the management of DLBCL within prospective clinical trials will be important in the future.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

We are also immensely grateful to David Scott for his comments on an earlier version of the manuscript, although any errors are our own and should not tarnish the reputation of this esteemed person.

REFERENCES

- Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood*. 2002;100(7):2292–302.
- Pasqualucci L, Zhang B. Genetic drivers of NF- κ B deregulation in diffuse large B-cell lymphoma. *Semin Cancer Biol*. 2016;39:26–31.
- Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000;403(6769):503–11.
- Lim KH, Yang Y, Staudt LM. Pathogenetic importance and therapeutic implications of NF- κ B in lymphoid malignancies. *Immunol Rev*. 2012;246(1):359–78.
- Baldwin AS. Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. *J Clin Invest*. 2001;107:241–6.
- Jeelall YS, Horikawa K. Oncogenic MYD88 mutation drives Toll pathway to lymphoma. *Immunol Cell Biol*. 2011;89(6):659–60.
- Nagao T, Oshikawa G, Ishida S, Akiyama H, Umezawa Y, Nogami A, et al. A novel MYD88 mutation L265RPP, in Waldenström macroglobulinemia activates the NF- κ B pathway to upregulate Bcl-xL expression and enhances cell survival. *Blood Cancer J*. 2015;5:e314.
- Kim Y, Ju H, Kim DH, Yoo HY, Kim SJ, Kim WS, et al. CD79B and MYD88 mutations in diffuse large B-cell lymphoma. *Hum Pathol*. 2014;45(3):556–64.
- Lee JH, Jeong H, Choi JW, Oh H, Kim YS. Clinicopathologic significance of MYD88 L265P mutation in diffuse large B-cell lymphoma: a meta-analysis. *Sci Rep*. 2017;7(1):1785.
- Fernández-Rodríguez C, Bellosillo B, García-García M, Sánchez-González B, Gimeno E, Vela MC, et al. MYD88 (L265P) mutation is an independent prognostic factor for outcome in patients with diffuse large B-cell lymphoma. *Leukemia*. 2014;28(10):2104–6.
- Yu S, Luo H, Pan M, Palomino LA, Song X, Wu P, et al. High frequency and prognostic value of MYD88 L265P mutation in diffuse large B-cell lymphoma with R-CHOP treatment. *Oncol Lett*. 2018;15(2):1707–15.
- Lee YS, Liu J, Fricano KA, Webb EM, Toolsie DR, Jones S, et al. Lack of a prognostic impact of the MyD88 L265P mutation for diffuse large B cell lymphoma patients undergoing autologous stem cell transplantation. *Biol Blood Marrow Transplant*. 2017;23(12):2199–204.
- Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127(20):2375–90.
- Zhou K, Xu D, Cao Y, Wang J, Yang Y, Huang M. C-MYC aberrations as prognostic factors in diffuse large B-cell lymphoma: a meta-analysis of epidemiological studies. *PLoS One*. 2014;9(4):e95020.
- Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004;103(1):275–82.
- He X, Chen Z, Fu T, Jin X, Yu T, Liang Y, et al. Ki-67 is a valuable prognostic predictor of lymphoma but its utility varies in lymphoma subtypes: evidence from a systematic meta-analysis. *BMC Cancer*. 2014;14:153.
- Yoon DH, Choi DR, Ahn HJ, Kim S, Lee DH, Kim SW, et al. Ki-67 expression as a prognostic factor in diffuse large B-cell lymphoma patients treated with rituximab plus CHOP. *Eur J Haematol*. 2010;85(2):149–57.
- Salles G, de Jong D, Xie W, Rosenwald A, Chhanabhai M, Gaulard P, et al. Prognostic significance of immunohistochemical biomarkers in diffuse large B-cell lymphoma: a study from the Lunenburg Lymphoma Biomarker Consortium. *Blood*. 2011;117(26):7070–8.
- Kim Y, Jub H, Kim D, Yoo H, Kim S, Kim W, et al. CD79B and MYD88 mutations in diffuse large B-cell lymphoma. *Hum Pathol*. 2014;45:556–64.
- Pulido JS, Salomao DR, Frederick LA, Viswanatha DS. MyD-88 L265P mutations are present in some cases of vitreoretinal lymphoma. *Retina*. 2015;35(4):624–7.
- Gouveia GR, Siqueira SA, Pereira J. Pathophysiology and molecular aspects of diffuse large B-cell lymphoma. *Rev Bras Hematol Hemoter*. 2012;34(6):447–51.
- Instituto Nacional de Câncer (INCA) Estimativa 2018. Incidência de Câncer no Brasil. Available from: <http://www.inca.gov.br/estimativa/2018> [cited March 2018].
- Kraan W, Horlings HM, van Keimpema M, Schilder-Tol EJ, Oud ME, Scheepstra C, et al. High prevalence of oncogenic MYD88 and CD79B mutations in diffuse large B-cell lymphomas presenting at immune-privileged sites. *Blood Cancer J*. 2013;3:e139.
- Bohers E, Mareschal S, Bouzeflen A, Marchand V, Ruminy P, Maingonnat C, et al. Targetable activating mutations are very frequent in GCB and ABC diffuse large B-cell lymphoma. *Genes Chromosomes Cancer*. 2014;53(2):144–53.
- Lenz G, Wright GW, Emre NC, Kohlhammer H, Dave SS, Davis RE, et al. Molecular subtypes of diffuse large B-cell lymphoma

- arise by distinct genetic pathways. *Proc Natl Acad Sci U S A*. 2008;105(36):13520–5.
26. Gutiérrez-García G, Cardesa-Salzmänn T, Climent F, González-Barca E, Mercadal S, Mate JL, et al. Gene-expression profiling and not immunophenotypic algorithms predicts prognosis in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. *Blood*. 2011;117(18):4836–43.
 27. Scott DW, Mottok A, Ennishi D, Wright GW, Farinha P, Ben-Neriah S, et al. Prognostic significance of diffuse large B-cell lymphoma cell of origin determined by digital gene expression in formalin-fixed paraffin-embedded tissue biopsies. *J Clin Oncol*. 2015;33(26):2848–56.
 28. Fu K, Weisenburger DD, Choi WW, Perry KD, Smith LM, Shi X, et al. Addition of rituximab to standard chemotherapy improves the survival of both the germinal center B-cell-like and non-germinal center B-cell-like subtypes of diffuse large B-cell lymphoma. *J Clin Oncol*. 2008;26(28):4587–94.
 29. Staiger AM, Ziepert M, Horn H, Scott DW, Barth TFE, Bernd HW, et al. Clinical Impact of the Cell-of-Origin Classification and the MYC/BCL2 Dual Expresser Status in Diffuse Large B-Cell Lymphoma Treated Within Prospective Clinical Trials of the German High-Grade Non-Hodgkin's Lymphoma Study Group. *J Clin Oncol*. 2017;35(22):2515–26.
 30. Cunningham D, Hawkes EA, Jack A, Qian W, Smith P, Mouncey P, et al. Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone in patients with newly diagnosed diffuse large B-cell non-Hodgkin lymphoma: a phase 3 comparison of dose intensification with 14-day versus 21-day cycles. *Lancet*. 2013;381(9880):1817–26.
 31. Visco C, Li Y, Xu-Monette ZY, Miranda RN, Green TM, Li Y, et al. Comprehensive gene expression profiling and immunohistochemical studies support application of immunophenotypic algorithm for molecular subtype classification in diffuse large B-cell lymphoma: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. *Leukemia*. 2012;26(9):2103–13.
 32. Herrera AF, Mei M, Low L, Kim HT, Griffin GK, Song JY, et al. Relapsed or refractory double-expressor and double-hit lymphomas have inferior progression-free survival after autologous stem-cell transplantation. *J Clin Oncol*. 2017;35(1):24–31.
 33. Sarkozy C, Traverse-Glehen A, Coiffier B. Double-hit and double-protein-expression lymphomas: aggressive and refractory lymphomas. *Lancet Oncol*. 2015;16(15):e555–67.
 34. Johnson NA, Slack GW, Savage KJ, Connors JM, Ben-Neriah S, Rogic S, et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. *J Clin Oncol*. 2012;30(28):3452–9.
 35. Tsuyama N, Sakata S, Baba S, Mishima Y, Nishimura N, Ueda K, et al. BCL2 expression in DLBCL: reappraisal of immunohistochemistry with new criteria for therapeutic biomarker evaluation. *Blood*. 2017;130(4):489–500.
 36. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol*. 2000;182(3):311–22.
 37. Jerkeman M, Anderson H, Dictor M, Kvaløy S, Akerman M, Cavallin-Ståhl E, Nordic Lymphoma Group study. Assessment of biological prognostic factors provides clinically relevant information in patients with diffuse large B-cell lymphoma—a Nordic Lymphoma Group study. *Ann Hematol*. 2004;83(7):414–9.
 38. Broyde A, Boycov O, Strenov Y, Okon E, Shpilberg O, Bairey O. Role and prognostic significance of the Ki-67 index in non-Hodgkin's lymphoma. *Am J Hematol*. 2009;84(6):338–43.
 39. Spyrtas F, Ferrero-Poüs M, Trassard M, Hacène K, Phillips E, Tubiana-Hulin M, et al. Correlation between MIB-1 and other proliferation markers: clinical implications of the MIB-1 cutoff value. *Cancer*. 2002;94(8):2151–9.