Virchows Archiv
https://doi.org/10.1007/s00428-024-03945-y

ORIGINAL ARTICLE

Combined immunohistochemical profile CD10/LMO2/MYC is a useful tool to screen *MYC* rearrangements in aggressive large B-cell lymphomas

Natalia Papaleo[1,2,6,7] · Andrea Molina-Alvarez[1] · Gustavo Tapia[3] · Ricard Onieva[2] · Marta Salido[1] · Carmen Lome-Maldonado[1] · Xavier Ara-Mancebo[1] · Anna Puiggros[1] · Blanca Espinet[1] · Carmen Blazquez[2] · Diana Fuertes[4] · Blanca Sanchez-Gonzalez[5] · Jose Yelamos[1] · Xavier Calvo[1] · Luis Colomo[1,7]

Received: 16 August 2024 / Revised: 1 October 2024 / Accepted: 10 October 2024
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2024

Abstract

Aggressive large B-cell lymphomas (LBCL) are a heterogeneous group of lymphomas with variable biological characteristics, for which the identification of *MYC* rearrangements (*MYCr*) is a defining and prognostic feature. Both the International Consensus Classification and the 5th edition of the World Health Organization Classification of Hematolymphoid Tumors recommend performing cytogenetic studies in all aggressive LBCL to detect *MYCr*. Since *MYC*r incidence is low, cost-effective screening tools are necessary. We asked whether the immunohistochemical combined profile of CD10, LMO2, and MYC could be a useful tool to screen for *MYCr*. For this purpose, we used two strategies: first, a scoring system assigning 0 points each for CD10 −, LMO2 +, and MYC − and 1 point for CD10 +, LMO2 −, and MYC +, adding the results, and second, an algorithm that selected tumors with CD10 + /LMO2 − profile and/or MYC overexpression. All analyses were performed in a training series including 482 cases from a single center and a validation series of 124 patients from two centers. The resulting system classified cases in scores from 0 to 3. Scores 0 and 1 had low *MYCr* (0/92 and 7/224, 3%, respectively), being higher for scores 2 (40/98, 41%) and 3 (61/68, 90%) ($P < 0.001$) in the training cohort. The incidence of *MYCr* in the validation series was as follows: score 0, 0/29 cases; score 1, 3/64 (5%); score 2, 10/23 (43.5%); score 3, 8/8 ($P < 0.001$). Sensitivity and negative predictive values were respectively 93.5% and 97.8% for the training and 85.7% and 96.8% for the validation cohorts. The algorithm rescued 2 and 1 *MYCr* cases included in score 1 from both series. In conclusion, we suggest that both approaches combining the interpretation of CD10/LMO2/MYC by immunohistochemistry are useful to screen for *MYCr*.

Keywords Large B-cell lymphoma · Algorithm · CD10 · MYC · LMO2

Introduction

Aggressive large B-cell lymphomas (LBCL) is a heterogeneous group of tumors comprising different types of diffuse large B-cell lymphoma (DLBCL), transformed DLBCL (tDLBCL), high-grade B-cell lymphomas (HGBCL) carrying *BCL2* and/or *BCL6* rearrangements, HGBCL not otherwise specified (HGBCL-NOS), and Burkitt lymphoma (BL) [1, 2]. *MYC* is a relevant gene defining some of these entities and the presence of *MYC* rearrangements (*MYCr*) confers an

✉ Luis Colomo
lcolomo@psmar.cat

1 Department of Pathology, Hospital del Mar, Hospital del Mar Research Institute-IMIM, Barcelona, Spain

2 Department of Pathology, Consorci Hospitalari Parc Tauli, Institut d'Investigacio I Innovacio Parc Tauli (I3PT), Sabadell, Spain

3 Department of Pathology, Hospital Universitari Germans Trias I Pujol, Badalona, Barcelona, Spain

4 Research Unit Support, Institut d'Investigacio I Innovacio Parc Tauli (I3PT), Sabadell, Spain

5 Department of Hematology, Hospital del Mar, Hospital del Mar Research Institute-IMIM, Barcelona, Spain

6 Department of Morphological Sciences, Universitat Autonoma de Barcelona, Barcelona, Spain

7 Department of Medicine and Life Sciences, Universitat Pompeu Fabra, Barcelona, Spain

Published online: 22 October 2024

unfavorable prognosis for these diseases. However, *MYCr* incidence is low among the whole group of aggressive LBCL, ranging from 5 to 15% in most papers [3–5].

Intermediate, blastoid, and large cell morphology are the common features of aggressive LBCL. This morphological approach, in addition to distinct clinical features, is the starting point in the classification of this group of tumors with the subsequent use of ancillary techniques, first using immunohistochemistry (IHC) and later adding cytogenetic and molecular techniques, if possible.

The current classification systems, the International Consensus Classification (ICC) and the 5th edition of the World Health Organization Classification of Hematolymphoid Tumors (WHO-HAEM5), recommend performing cytogenetic studies in all aggressive LBCL to detect *MYCr*, mainly by fluorescence in situ hybridization (FISH). However, because of the low prevalence of *MYCr*, since MYC IHC detection alone is not a good surrogate marker, and due to FISH testing not being affordable everywhere, cost-effective screening tools are necessary. Several studies have used different approaches to identify *MYCr*, namely the evaluation of double expression of MYC and BCL2, the utilization of varied cutoffs for MYC IHC, the usage of other methods such as qRT-PCR, or the contribution of gene expression profiling to the IHC approach [3, 6–9]. Some of these methods have shown very good results but are even more difficult than FISH to be translated into clinical practice and are not readily applicable as screening tools.

Previous studies undertaken by our own group and others have observed the utility of the detection of LMO2 by IHC to identify *MYCr* [10–14]. Whereas LMO2 was highly efficient to detect *MYCr* among CD10 positive aggressive LBCL and GCB-like cases according to the Hans algorithm, MYC IHC remained useful among CD10 negative as well as non-GCB-like cases. LMO2 IHC detection was particularly useful when evaluated in whole tissue sections, and offered very high intra and interlaboratory reproducibility, as well as high interobserver reproducibility [11, 15]. All these results were similar or even better when compared with MYC IHC protein expression.

In the present study, we have added LMO2 to MYC IHC, benefiting from the utility of both markers. With this, we have assessed whether the combination of CD10, LMO2, and MYC expression detected by IHC offers superior results as a screening tool to identify *MYCr* in comparison to the individual detection of LMO2 or MYC based on CD10 expression. We have used two strategies: first, by using a scoring system and analyzing its association with *MYCr*, and second, using an algorithm that indicated when cases should be evaluated by *MYC* FISH. We have first studied a training series including 482 cases from a single center, evaluated by the same group of pathologists and cytogenetists. To validate the results, we have studied an additional series of 124 cases evaluated by two different groups of interpreters from two centers. Both approaches suggested that the combined interpretation of CD10/LMO2/MYC by IHC is useful to screen *MYCr*.

Materials and methods

Case selection

All cases were selected based on clinical information and availability of formalin-fixed paraffin-embedded (FFPE) tissue. A total number of 606 cases were evaluated by IHC and FISH methods. A training cohort of 482 cases was selected from the files of the Hospital del Mar, Barcelona, Spain. Cases were studied between September 2014 and January 2024. The validation series included 124 cases selected from the Consorci Sanitari Parc Tauli, Sabadell, Spain (54 cases) and Hospital Germans Trias I Pujol, Badalona, Spain (70 cases). Cases were consecutively diagnosed in whole tissue sections. The diagnoses were updated following the criteria of the ICC and WHO-HAEM5 classifications, and patients with primary mediastinal large B-cell lymphoma, T-cell/histiocyte-rich LBCL, plasmablastic lymphomas, HHV8-positive DLBCL, primary effusion lymphomas, intravascular LBCL, DLBCL associated with chronic inflammation, fibrin-associated DLBCL, and lymphomatoid granulomatosis were not included. The incidence of *MYCr* was slightly higher in the training series (22%) compared with the validation series (17%) because the training series was enriched in external cases sent for consultation and cytogenetic studies.

The Ethics Committee of the Hospital del Mar (2017/7481/I) approved the study. Informed consent to use both clinical data and histological material was obtained in accordance with the Declaration of Helsinki.

As the clinical behavior of the cases was not evaluated in this study, we decided to abbreviate HGBCL with *MYC* and *BCL2* rearrangements, HGBCL with *MYC* and *BCL6* rearrangements, and cases carrying simultaneous *MYCr*, *BCL2r*, and *BCL6r* as HGBL-DH/TH.

Immunohistochemical panel

Each case comprised a set of slides including hematoxylin and eosin and a panel of IHC markers including CD10 (clone SP67), BCL6 (clone GI191E-A8), MUM-1/IRF4 (clone MRQ-43), LMO2 (clone SP51), and MYC (clone Y69), all from Ventana, Roche, Tucson, AZ, USA. The cutoff used for CD10, BCL6, MUM1/IRF4, and LMO2 was 30% and cases with staining over the cutoff were rated as positive. For MYC evaluation, we used a cutoff of 40%.

Cell of origin (COO) was determined by IHC according to the Hans algorithm [16], performed consecutively during routine diagnostics, and included in pathology reports.

IHC scoring system and algorithm construction with CD10, LMO2, and MYC

We generated a score for each case based on the sum of CD10, LMO2, and MYC IHC expression. The score assigned 0 or 1 point to each marker based on the results obtained in our previous studies. Thus, CD10 and MYC negative IHC expression and LMO2 positive expression received 0 points each. As an example, as shown in Fig. 1A, a CD10 − /LMO2 + /MYC − profile added 0 points. A score of 1 point was assigned for CD10 and MYC expression, and LMO2 negative expression.

We designed an algorithm that is shown in Fig. 1B. The decision tree was based on two or three steps, aiming to select or exclude cases for *MYC* FISH testing. The first step depended on CD10 expression and the second depended on LMO2 expression. All cases with the profile CD10 + / LMO2 − were elected for FISH testing. In the third step, all LMO2 + cases were selected for FISH if MYC was simultaneously positive. Among CD10 − /LMO2 − cases, MYC expression indicated FISH and a MYC negative result was not tested. Cases with CD10 + /LMO2 + /MYC − profile were not selected for testing. To summarize, the algorithm selected cases for *MYC* FISH testing when MYC protein expression was over 40% and/or cases with CD10 + / LMO2 − profile.

Fluorescence in situ hybridization (FISH)

FISH analyses using break-apart probes specific for MYC (8q24) and *BCL6* (3q27) and dual fusion probes *IGH/BCL2* (all from Vysis, Abbott Molecular, Des Plainescity, IL, USA) were performed during routine diagnostics as previously described [12]; the cutoff values for interphase FISH

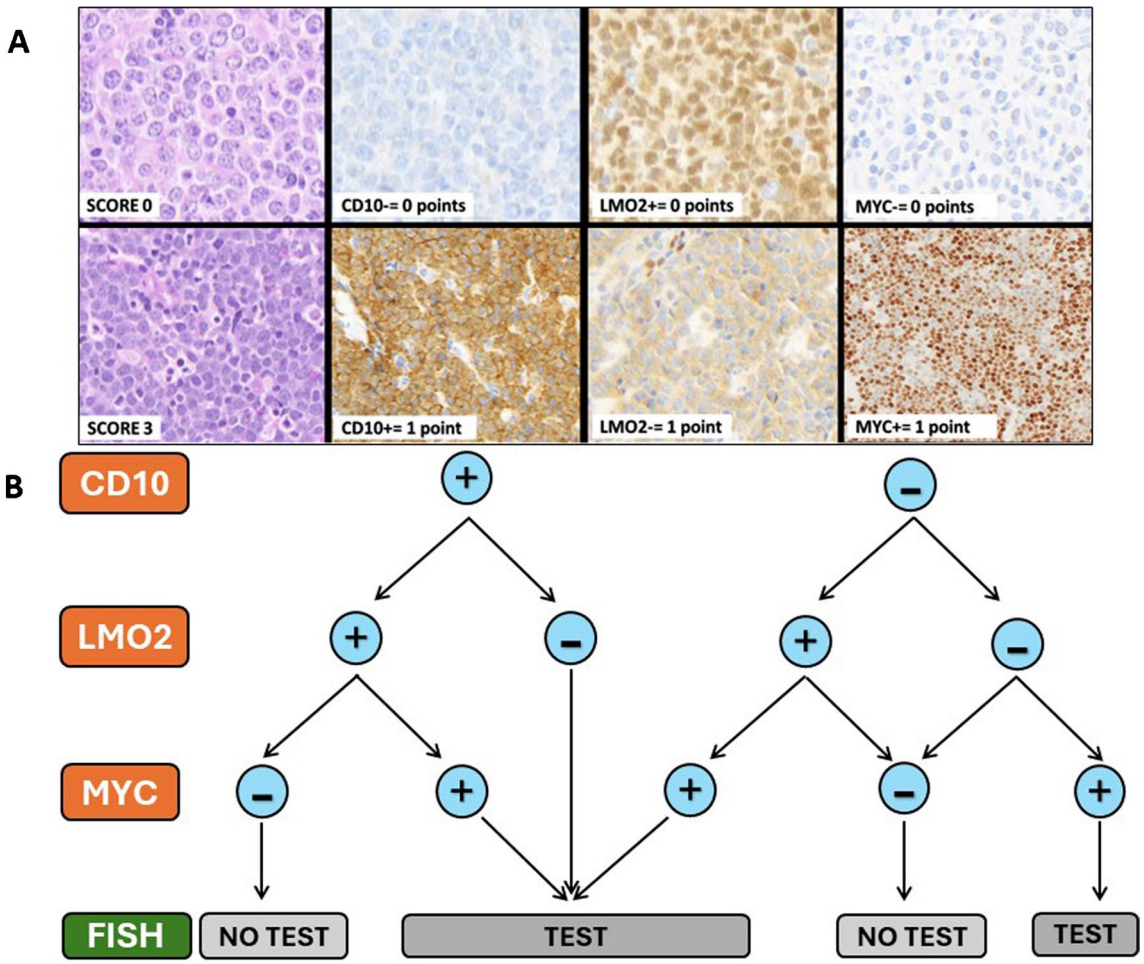


Fig. 1 Examples of the scoring system (**A**). In the upper line, the CD10 − /LMO2 + /MYC − profile received a score of 0. In the lower line, the CD10 + /LMO2 − /MYC + profile was scored 3. Algorithm designed (**B**). This can be summarized as follows: test cases with CD10 + /LMO2 − profile and/or MYC expression

analyses were established following the criteria of Ventura et al. [17].

In this study, we have emphasized the detection of *MYCr*, which can be the starting point for the detection of the additional rearrangements of *BCL2* and *BCL6* in aggressive LBCL.

Statistical analysis

Multivariate analysis was carried out using R studio 2023.03.0 software, R version 4.3.0 (2023–04-21 ucrt), and SPSS statistics v28 (IBM Corp., Armonk, NY, USA). Data was also analyzed using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). The decision trees were generated using the rpart.plot package and visualized using the rpart function. To estimate the area under the curve and to determine the optimal threshold in relation to the established score, the pROC package was used. Youden's method was used to calculate the optimal threshold. The ROC curves allowed us to analyze if the scoring method distinguished properly between cases carrying *MYCr* and no-*MYCr* cases [18].

Accuracy, sensitivity, specificity, and positive/negative predictive ratios were calculated for the scoring system and the algorithm designed. Likelihood positive and negative ratios were calculated to evaluate the diagnostic accuracy of the results obtained. *P* values < 0.05 were considered statistically significant for all tests [19].

Results

Clinicopathological features

The training cohort included the following: 82 tDLBCL (81 transformed follicular lymphomas-tFL- and 1 transformed marginal zone lymphoma), 330 DLBCL, 39 HGBCL-DH/TH, 7 HGBCL-NOS, and 24 BL. The median age of the patients was 65 years (range 6–96 years), and there were 277 (57%) men and 205 (43%) women. The site of diagnosis included lymph nodes in 245 (51%) cases and extranodal sites in 237 (49%). The validation series included the following: 10 tDLBCL (all tFL), 96 DLBCL, 10 HGBCL-DH/TH, 3 HGBCL-NOS, and 5 BL. The median age of the patients was 65 years (range 24–91 years), and there were 80 (64.5%) men and 44 (35.5%) women. Table 1 shows the immunophenotypical and FISH results of both series. The distribution of the entities and the prevalence of *MYCr* were similar among series. The Pearson correlation coefficient was 0.62 between the series and is shown in supplementary Figure S1.

In both series, there was a significant statistical association between CD10, LMO2, and MYC expression with the presence of *MYCr* among the lymphoma types ($P < 0.001$). In addition, the Hans algorithm, calculated in the training series, is also significantly associated with *MYCr* ($P < 0.001$).

As opposed to what was expected, in the training series, MYC was not expressed over the cutoff in 12 (11%) cases carrying *MYCr* (2 tDLBCL, 2 DLBCL, 1 HGBCL-NOS, 2 HGCBL with *MYCr/BCL2r* and 5 BL), and LMO2 was positive in 22 (20%) cases with *MYCr* (7 tDLBCL, 1 DLBCL, 1 HGBCL-NOS, 13 HGCBL with *MYCr* and *BCL2r/BCL6r*). According to the Hans algorithm, *MYCr* were more frequently identified in GCB-like cases 95/108 (88%) than in non-GCB-like cases (13/108, 12%; $P < 0.001$). MYC was also expressed in 87/324 (23%) cases non-*MYCr* (37/87, 42% GCB-like), and LMO2 was negative in 103/374 (27.5%) non-*MYCr*, mostly non-GCB-like cases (76/103, 74%).

Scoring system

Using the scoring system described above, we obtained 4 different scores, from 0 to 3, which arise from the 8 possible combinations of the markers, that we included in 8 categories, from 1 to 8, as shown in Table 2. Tables 1 and 2, as well as supplementary Figure S1 and Fig. 2, show the distribution of the results.

In the training series, score 0 included 92 (19%) cases; score 1, 224 (47%); score 2, 98 (20%); and score 3, 68 (14%) cases. The presence of *MYCr* had a strong statistical association with the scoring system ($P < 0.001$), as well as the categorization in the 8 combinations of the markers ($P < 0.001$). Additional significant results were obtained comparing the scoring system with the Hans algorithm ($P < 0.001$), *BCL2r* ($P < 0.001$), and *BCL6r* ($P = 0.008$).

Figure 2 and Table 2 show the results of the scoring system approach related to *MYCr*. Cases with score 0 did not present *MYCr*, whereas score 1 included 7/224 (3%) *MYCr* cases, representing 7/108 (6.5%) of all *MYC* rearranged cases and 7/482 (1%) cases when considering the whole training series. Such cases were as follows: 2 tDLBCL (1 *MYCr/BCL2r*, 1 *MYCr/BCL2r/BCL6r*), 2 DLBCL, and 3 HGBCL with *BCL2r*, two of them with no expression of MYC protein. Scores 2 and 3 were better at *MYCr* detection, with an incidence of 41% (40/98 cases) and 90% (61/68), respectively ($P < 0.001$). This approach offered a higher ability to detect *MYCr* in comparison to the individual detection of the CD10 + /LMO2 − profile, that identified 68 out of 88 cases (77%), or MYC overexpression, that captured 96/108 (89%) *MYCr*. All CD10 + /LMO2 − cases and most MYC IHC positive cases (146/183, 80%) had scores 2 or 3.

The validation series obtained very similar results. Again, contrary to what was expected, MYC was not expressed in 9/21 (43%) cases carrying *MYCr* (1 tDLBCL, 2 DLBCL, 2 HGCBL with *MYCr/BCL2r* and 1 HGBCL *MYCr/BCL6r*,

Table 1 Clinicopathological features and scoring system results of the cohorts and lymphoma types studied

	Total	tDLBCL	DLBCL	HGBCL-DH/TH	HGBCL-NOS	BL
Training series, *N* (%)	482	82 (17%)	330 (68%)	39 (8%)	7 (2%)	24 (5%)
IHC						
CD10	252/482 (52%)	62/82 (76%)	129/330 (39%)	31/39 (79.5%)	7/7 (100%)	23/24 (96%)
LMO2	293/482 (61%)	62/82 (76%)	216/330 (65.5%)	13/39 (33%)	2/7 (29%)	0/24
MYC	183/482 (38%)	23/82 (28%)	98/330 (30%)	37/39 (95%)	6/7 (86%)	19/24 (79%)
Cell-of-origin*						
GCB-like	283/481 (59%)	65/82 (80%)	152/330 (46%)	35/39 (90%)	7/7 (100%)	24/24 (100%)
NON-GCB-like	198/481 (41%)	17/82 (20%)	177/330 (54%)	4/39 (10%)	0/7	0/24
FISH						
MYCr	108/482 (22%)	18/82 (22%)	21/330 (6%)	39/39 (100%)	6/7 (86%)	24/24 (100%)
BCL2r	142/426 (33%)	50/78 (64%)	60/292 (20.5%)	32/38 (84%)	0/5	0/12
BCL6r	109/406 (27%)	19/77 (27.5%)	76/276 (27.5%)	14/36 (39%)	0/5	0/12
Scoring system						
Score 0	92/482 (19%)	6/82 (7%)	86/330 (26%)	0/39	0/7	0/24
Score 1	224/482 (47%)	53/82 (66%)	167/330 (51%)	3/39 (8%)	0/7	0/24
Score 2	98/482 (20%)	15/82 (18%)	57/330 (17%)	17/39 (43%)	3/7 (43%)	6/24 (25%)
Score 3	68/482 (14%)	7/82 (9%)	20/330 (6%)	19/39 (49%)	4/7 (57%)	18/24 (75%)
Validation series, *N* (%)	124	10 (8%)	96 (77%)	10 (8%)	3 (2%)	5 (4%)
IHC						
CD10	45/124 (36%)	5/10 (50%)	25/96 (26%)	9/10 (90%)	1/3 (33%)	5/5 (100%)
LMO2	59/124 (48%)	4/10 (40%)	52/96 (54%)	2/10 (20%)	1/3 (33%)	0/5
MYC	24/124 (19%)	1/10 (10%)	12/96 (12.5%)	7/10 (70%)	2/3 (67%)	2/5 (40%)
FISH						
MYCr	21/124 (17%)	1/10 (10%)	4/96 (4%)	10/10 (100%)	1/3 (33%)	5/5 (100%)
Scoring system						
Score 0	29/124 (23%)	0/10	29/96 (30%)	0/10	0/3	0/5
Score 1	64/124 (53%)	8/10 (80%)	54/96 (56%)	1/10 (10%)	1/3 (33%)	0/5
Score 2	23/124 (18.5%)	2/10 (20%)	12/96 (13%)	4/10 (40%)	2/3 (67%)	3/5 (60%)
Score 3	8/124 (6.5%)	0/10	1/96 (1%)	5/10 (50%)	0/3	2/5 (40%)

*Available in 481 cases; HGBCL DH/TH, HGBCL with *BCL2* and/or *BCL6* rearrangements

Table 2 Distribution of *MYCr* cases and total number of cases according to the scores and categories defined by the scoring system

Category	CD10/LMO2/MYC IHC profile*	Score	Training series *MYCr*/score	Validation series *MYCr*/score
1	0/1/0	0	0/92	0/29
2	0/0/0	1	3/67	2/36
3#	0/1/1	1	2/37	1/4
4	1/1/0	1	2/120	0/24
5	1/0/0	2	7/20	7/11
6	1/1/1	2	18/44	1/2
7	0/0/1	2	15/34	2/10
8	1/0/1	3	61/68	8/8

*0 means no IHC expression of the marker and 1 means IHC expression

#Cases included in category 3 are then rescued by the algorithm

and 3 BL), and LMO2 was positive only in 2 (9.5%) cases (1 HGCBL with *MYCr/BCL2r* and 1 HGBCL *MYCr/BCL6r*).

Out of 124 cases, score 0 included 29 (23%); score 1, 64 (52%); score 2, 23 (18.5%); and score 3, 8 (6.5%) cases (Tables 1 and 2). Also, the presence of *MYCr* had a strong statistical association with the scoring system and the categories defined ($P < 0.001$, each). *MYCr* occurred in 21 cases and was associated mostly with scores 2 and 3 (10/23, 43.5% for score 2 and 8/8 cases for score 3), compared with scores 0 and 1 (0/29 and 3/64, 5%, respectively) ($P < 0.001$).

Table 3 shows the statistical measures of the performance of the scoring system compared with the presence of *MYCr* as the gold standard in the training and validation series. With this approach, the sensitivity was 93.5% and 85.7%, the specificity was 82.7% and 87.4%, and the negative predictive values (NPV) were 97.8% and 96.8%, respectively. To determine whether the model was useful, we calculated the ROC curves for both cohorts, with the area under the curve

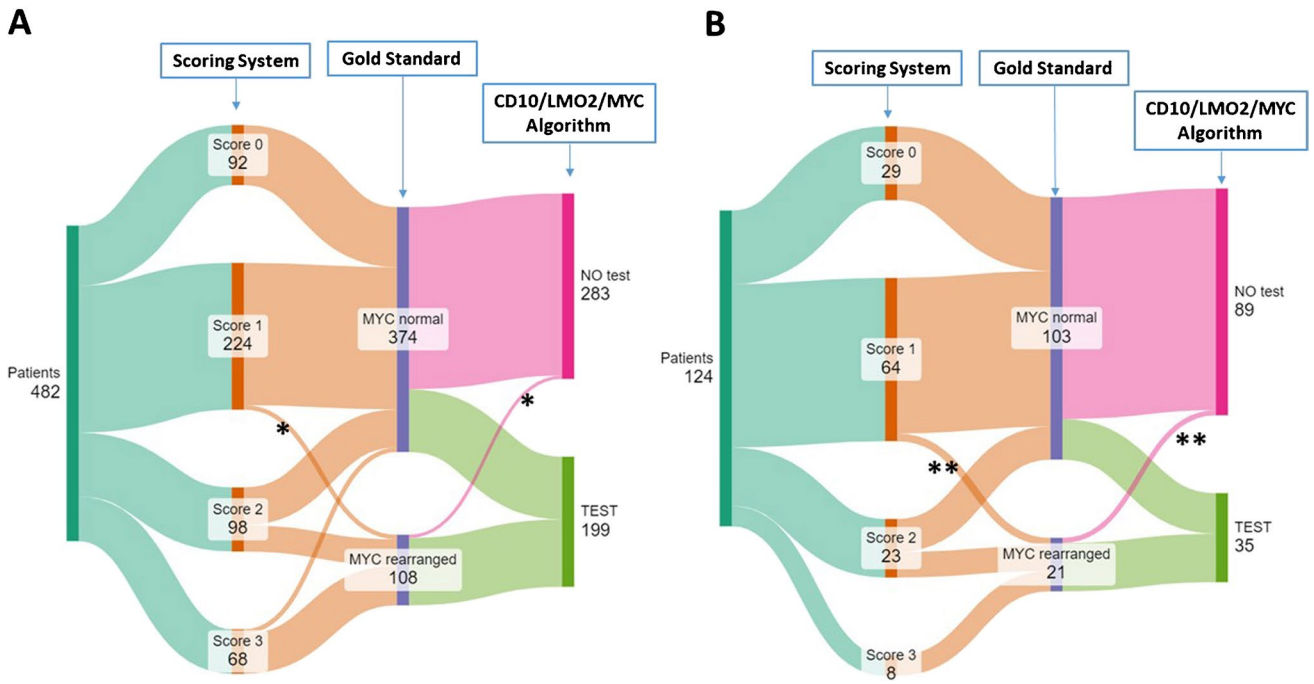


Fig. 2 Distribution of the results of the scoring system and algorithm according to *MYC* rearrangements as a gold standard (blue line) in the training (**A**) and the validation cohorts (**B**). The asterisk (*) indicates 7 and 5 patients from the scoring and algorithm in the training series, respectively; the asterisks (**) indicate 3 and 2 patients from scoring/algorithm in the validation series

Table 3 Statistic measures of the performance of the scoring system and algorithm compared with the presence of *MYC* rearrangements as a gold standard in training and validation series

Category	Training series Score (%)	Validation series Score (%)	Training series Algorithm (%)	Validation series Algorithm (%)
Sensitivity	93.5	85.7	95.4	90.5
Specificity	82.7	87.4	73.3	84.5
PPV	60.8	58.1	50.7	54.3
NPV	97.8	96.8	98.2	97.7
Accuracy	85	87.1	78.2	85.5
PLR	5.38	6.79	3.57	5.82
NLR	0.08	0.16	0.06	0.11

PPV positive predictive value, *NPV* negative predictive value, *PLR* positive likelihood ratio *NLR* negative likelihood ratio

being 0.93 (confidence interval (CI) 0.9–0.96, $P < 0.001$) in the training series and 0.91 in the validation series (CI 0.84–0.98, $P < 0.001$). The Youden index was 0.76 and 0.73 in the training and validation series, respectively, and the cutoff was 1.5 for each series (supplementary Figure S2).

Algorithm approach

After obtaining the scoring system approach, we wanted to decrease the number of false negative cases. Thus, as 80% of MYC overexpressing cases fell into scores 2–3 and the CD10 + /LMO2 − profile was already included in score 2, we designed the algorithm including MYC positive cases of category 3, that corresponded to score 1. This approach allowed rescuing 2 cases in the training series and 1 from the validation series. The total number of cases salvaged by the algorithm from the training test was 1 tFL carrying *MYC*, *BCL2*, and *BCL6* rearrangements and 1 HGBCL with *MYC* and *BCL2* rearranged. One HGBCL with *MYC* and *BCL6* rearrangements was rescued from the validation series. Two HGBCL with *MYC* and *BCL2* rearrangements were not detected by any approach in the training series and no other HGBCL with *MYCr/BCL2r* and/or *BCL6r* remained undetectable in the validation cohort.

Therefore, as summarized in Figs. 1B and 2, and supplementary Figures S3 and S4, all MYC + and/or CD10 + /

LMO2 − cases were eligible for FISH testing. The statistical measures of the performance of the algorithm compared with the presence of *MYCr* as a gold standard in the training and validation series, shown in Table 3, were as follows: sensitivity was 95.4% and 90.5%, the specificity was 73.3% and 84.5%, and the NPV 98.2% and 97.8%, respectively. The NPV improved, although the specificity decreased because the number of false positive cases increased.

We also evaluated the possibility of excluding LMO2 from the algorithm, selecting only all CD10 + cases and cases with the profile CD10 − /MYC + for FISH testing. The analysis of this approach is shown in supplementary Table S3 and can be followed in supplementary Figures S3 and S4. This could also be a useful approach that would maintain the values of sensitivity, NPV, and NLR, but the other statistical measures of performance decreased. Moreover, the number of FISH tests increased respectively in 120 and 24 cases in the training and validation series when compared with the CD10/LMO2/MYC algorithm. Therefore, we conclude that the inclusion of LMO2 in the algorithm adds value to our approach.

Discussion

In this study, we aimed to evaluate the utility of the immunohistochemical combined profile of CD10/LMO2/MYC as a screening tool to detect *MYCr* in aggressive LBCL. The current recommendation of the ICC and WHO-HAEM5 about the need to determine *MYCr* by cytogenetic methods in all cases with aggressive LBCL morphology may be difficult to sustain because of the low incidence of this genetic alteration, the costs of the technique, and the need of expertise and facilities, which are not easily affordable elsewhere [20]. However, screening approaches have yet to be defined, and additional guidelines are necessary.

For our purpose, we first analyzed a scoring system based on ours and other authors' results with respect to the utility of LMO2 to detect *MYCr*. All these papers emphasized the utility of the lack of expression of LMO2 to detect *MYCr*. The downregulation of LMO2 in aggressive LBCL may be consistent with the physiological reentry into the dark zone (DZ) of the germinal center cells expressing MYC located in the light zone. As most aggressive LBCL-carrying *MYCr* may arise in the DZ and show a DZ molecular signature [21], the unexpected LMO2 downregulation identified by IHC may be normal and associated with specific functions of *MYC* and *LMO2* related to mechanisms of DNA repair [22, 23]. In a recent study of our group using chromogenic *LMO2* mRNA in situ hybridization, we observed that the cells located in the DZ had very low levels of *LMO2* mRNA, which was even lower in lymphomas carrying *MYCr*, offering the produced results a reasonable explanation to the lack of expression of LMO2 detected by IHC in those tumors [24].

The scoring system proposed in this study identified two major groups, scores 0–1 vs 2–3, that were highly statistically associated with the presence of *MYCr* in both training and validation cohorts. If scores 0–1 were excluded from FISH testing, only 7 *MYCr* cases would have been missed from the training series and 3 from the validation set, representing 1% of all cases included in each cohort. Therefore, the hypothetic strategy of selecting cases for FISH testing based on this scoring system would point to the need of testing only cases with scores 2 and 3, and this approach would decrease to 66.5% and 75% of the number of cases needing FISH. Thus, only 166 cases and 31 cases would be eligible for testing following this strategy. An alternative approach would be testing only cases over score 0, which would mean saving FISH tests in 92/482 cases (19%) and 29/124 cases (23%).

To diminish the number of false negative cases, we designed an algorithm based on MYC expression and the CD10 + /LMO2 − profile. This approach rescued a few cases from each cohort allowing a mild increase in the sensitivity and the NPV of the test but decreasing the positive predictive value and accuracy because of the increase in the number of false positive cases. Although these changes occurred, high sensitivity and very low NPV are desirable for a screening test, implying that false negatives are minimized, which is particularly important in the present scenario of the detection of *MYCr* in aggressive LBCL [25].

In this study, we have widened the available information of MYC with the produced on LMO2. MYC is the most used IHC marker trying to identify *MYCr*. However, as MYC protein expression does not properly capture *MYCr*, it is necessary to be cautious when using MYC IHC for this purpose [26–28]. One of the reasons for the downregulation of MYC protein in *MYCr* cases is genetic complexity. The development of clonal evolution during the course of the disease has been associated with low MYC expression, particularly in cases carrying double and triple rearrangements involving *MYC*, *BCL2*, and *BCL6* [29]. This is relevant since the screening tools should not miss such cases. Nevertheless, the unique non-detected cases by any method in our series were 2 HGBCL with *MYCr/BCL2r* that showed a profile CD10 + / LMO2 + /MYC − (score 1, category 4). Both patients were treated with R-CHOP schemes only and had a particularly favorable behavior of the disease, being alive with no evidence of disease 25 and 66 months after treatment. This specific profile associated with long survival after treatment was noticed in one of our previous studies [11], and further studies may clarify this observation and the potential protective role of LMO2 in hematological neoplasms, which overexpression associates with a favorable behavior in some aggressive tumors [23, 30, 31].

Different studies have untangled the importance of MYC IHC as well as the limitations of FISH [3, 8, 32, 33]. The higher levels of MYC expression have been associated with higher specificity for the detection of *MYCr* [8, 28]. However, this is not a consistent finding, and some studies identify *MYCr* appropriately by maintaining a cutoff of 30 to 40% for MYC expression [5]. In this study, we also performed all the analyses using a cutoff of 50% for MYC without obtaining better results than the ones described (data not shown). Another limitation of MYC IHC is the differences in the reproducibility among interpreters, as reported [15, 34] and evidenced here when comparing the results of MYC expression and *MYCr* cases between the two series. However, the discrepancies should not undervalue the role of the IHC approach since it is also known that FISH does not detect *MYC* cryptic rearrangements [33]. Finally, the usage of additional markers such as LMO2, CD38, or TCL1 should help to identify *MYCr* even in apparently non-altered cases [35].

In summary, we are proposing an immunohistochemical algorithm along with a scoring system that attempts to identify cases which are likely to have a *MYC* break. Our study needs to be correlated with the clinical data and further results of other series should confirm the utility of the combined CD10/LMO2/MYC profile. We have performed all the analyses in academic centers and based on the background of experienced hematopathologists and cytogenetists. We think that both the scoring system and the algorithm may be useful as a starting point for the workup of aggressive LBCL and an affordable approach for many laboratories.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00428-024-03945-y.

Author contribution Conceptualization: Natalia Papaleo, Luis Colomo; methodology: Natalia Papaleo, Ricard Onieva, Andrea Molina-Alvarez, Gustavo Tapia, Diana Fuertes, Marta Salido, Blanca Espinet, Blanca Sanchez-Gonzalez, Anna Puiggros, Carmen Blazquez, Xavier Calvo; formal analysis and investigation: Natalia Papaleo, Luis Colomo; writing—original draft preparation: Natalia Papaleo, Andrea Molina-Alvarez, Carmen Lome-Maldonado, Xavier Ara-Mancebo, Carmen Blazquez, Marta Salido, Xavier Calvo, Luis Colomo; writing—review and editing: Natalia Papaleo, Luis Colomo; funding acquisition: Luis Colomo, Xavier Calvo; supervision: Luis Colomo.

Funding This research was funded by grant Fondo de Investigacion Sanitaria (FIS), Instituto de Salud Carlos III PI17/313 (L.C.) and PI23/199 (X.C.)

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Campo E, Jaffe ES, Cook JR et al (2022) The International Consensus Classification of Mature Lymphoid Neoplasms: a report from the Clinical Advisory Committee. Blood 140:1229–1253. https://doi.org/10.1182/BLOOD.2022015851
2. Alaggio R, Amador C, Anagnostopoulos I et al (2022) The 5th edition of the World Health Organization Classification of haematolymphoid tumours: lymphoid neoplasms. Leukemia 36:1720–1748
3. Scott DW, King RL, Staiger AM et al (2018) High-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements with diffuse large B-cell lymphoma morphology. Blood 131:2060–2064. https://doi.org/10.1182/blood-2017-12-820605
4. Copie-Bergman C, Cuillière-Dartigues P, Baia M et al (2015) MYC-IG rearrangements are negative predictors of survival in DLBCL patients treated with immunochemotherapy: a GELA/LYSA study. Blood 126:2466–2474
5. Agarwal R, Lade S, Liew D et al (2016) Role of Immunohistochemistry in the era of genetic testing in MYC-positive aggressive B-cell lymphomas: a study of 209 cases. J Clin Pathol 69:266–270. https://doi.org/10.1136/jclinpath-2015-203002
6. Di Napoli A, Remotti D, Agostinelli C et al (2019) A practical algorithmic approach to mature aggressive B cell lymphoma diagnosis in the double/triple hit era: selecting cases, matching clinical benefit. Virchows Arch 475:513–518. https://doi.org/10.1007/s00428-019-02637-2
7. Ambrosio MR, Lazzi S, Lo BG et al (2019) MYC protein expression scoring and its impact on the prognosis of aggressive B-cell lymphoma patients. Haematologica 104:e25–e28. https://doi.org/10.3324/haematol.2018.195958
8. Kluk MJ, Chapuy B, Sinha P et al (2012) Immunohistochemical detection of MYC-driven diffuse large B-cell lymphomas. PLoS ONE 7:e33813. https://doi.org/10.1371/journal.pone.0033813
9. Green TM, Nielsen O, De Stricker K et al (2012) High levels of nuclear MYC protein predict the presence of myc rearrangement in diffuse large B-cell lymphoma. Am J Surg Pathol 36:612–619. https://doi.org/10.1097/PAS.0b013e318244e2ba
10. Colomo L, Vazquez I, Papaleo N et al (2017) LMO2-negative expression predicts the presence of MYC TRANSLOCATIONS IN AGGRESSIVE B-Cell lymphomas. Am J Surg Pathol 41:877–886. https://doi.org/10.1097/PAS.0000000000000839
11. Vazquez I, Papaleo N, Garcia E et al (2020) Clinical interest of LMO2 testing for the diagnosis of aggressive large B-cell lymphomas. Cancers (Basel) 12(4):884. https://doi.org/10.3390/cancers12040884
12. Vazquez I, Papaleo N, Lop J et al (2021) Lack of expression of LMO2 clone SP51 identifies MYC rearrangements in aggressive large B-cell lymphomas. Virchows Arch 479:1073–1078. https://doi.org/10.1007/s00428-021-03091-9
13. Chapman J, Verdun RE, Lossos IS (2021) Low LIM-domain only 2 (LMO2) expression in aggressive B cell lymphoma correlates with MYC and MYC/BCL2 rearrangements, especially in germinal center cell-type tumors. Leuk Lymphoma 62:2547–2550
14. Liu Y, Bian T, Zhang Y et al (2019) A combination of LMO2 negative and CD38 positive is useful for the diagnosis of Burkitt lymphoma. Diagn Pathol 14:100. https://doi.org/10.1186/s13000-019-0876-3
15. Papaleo N, Climent F, Tapia G et al (2023) Round-robin testing for LMO2 and MYC as immunohistochemical markers to screen MYC rearrangements in aggressive large B-cell lymphoma. Virchows Arch 485:307–314. https://doi.org/10.1007/s00428-023-03584-9
16. Hans CP, Weisenburger DD, Greiner TC et al (2004) Confirmation of the molecular classification of diffuse large B-cell lymphoma

by immunohistochemistry using a tissue microarray. Blood 103:275–282. https://doi.org/10.1182/blood-2003-05-1545
17. Ventura RA, Martin-Subero JI, Jones M et al (2006) FISH analysis for the detection of lymphoma-associated chromosomal abnormalities in routine paraffin-embedded tissue. J Mol Diagn 8:141–151. https://doi.org/10.2353/jmoldx.2006.050083
18. Zweig MH, Campbell G (1993) Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 39:561–577. https://doi.org/10.1093/clinchem/39.4.561
19. McGee S (2002) Simplifying likelihood ratios. J Gen Intern Med 17:646–649. https://doi.org/10.1046/j.1525-1497.2002.10750.x
20. Falini B, Martino G, Lazzi S (2023) A comparison of the International Consensus and 5th World Health Organization classifications of mature B-cell lymphomas. Leukemia 37:18–34. https://doi.org/10.1038/s41375-022-01764-1
21. Alduaij W, Collinge B, Ben-Neriah S et al (2023) Molecular determinants of clinical outcomes in a real-world diffuse large B-cell lymphoma population. Blood 141:2493–2507. https://doi.org/10.1182/blood.2022018248
22. Jin Z, May WS, Gao F et al (2006) Bcl2 suppresses DNA repair by enhancing c-Myc transcriptional activity. J Biol Chem 281:14446–14456. https://doi.org/10.1074/jbc.M511914200
23. Parvin S, Ramirez-Labrada A, Aumann S et al (2019) LMO2 Confers synthetic lethality to PARP inhibition in DLBCL. Cancer Cell 36:237-249.e6. https://doi.org/10.1016/j.ccell.2019.07.007
24. Papaleo N, Molina-Alvarez A, Onieva R et al (2024) Chromogenic LMO2 mRNA ISH expression correlates with LMO2 protein and gene expression and captures their survival impact in diffuse large B-Cell lymphoma. NOS Cancers (Basel) 16:2378. https://doi.org/10.3390/cancers16132378
25. Trevethan R (2017) Sensitivity, specificity, and predictive values: foundations, pliabilities, and pitfalls in research and practice. Front Public Health 5:307. https://doi.org/10.3389/fpubh.2017.00307
26. Sakr H, Cook JR (2019) Identification of "double Hit" lymphomas using updated WHO Criteria: insights from routine MYC immunohistochemistry in 272 consecutive cases of aggressive B-cell lymphomas. Appl Immunohistochem Mol Morphol 27:410–415. https://doi.org/10.1097/PAI.0000000000000657
27. Chisholm KM, Bangs CD, Bacchi CE et al (2015) Expression profiles of MYC protein and MYC gene rearrangement in lymphomas. Am J Surg Pathol 39:294–303. https://doi.org/10.1097/PAS.0000000000000365
28. Wang XJ, Medeiros LJ, Lin P et al (2015) MYC cytogenetic status correlates with expression and has prognostic significance in patients with MYC/BCL2 protein double-positive diffuse large B-cell lymphoma. Am J Surg Pathol 39:1250–1258. https://doi.org/10.1097/PAS.0000000000000433
29. Valera A, Epistolio S, Colomo L et al (2016) Definition of MYC genetic heteroclonality in diffuse large B-cell lymphoma with 8q24 rearrangement and its impact on protein expression. Mod Pathol 29:844–853. https://doi.org/10.1038/modpathol.2016.71
30. Latchmansingh KA, Wang X, Verdun RE et al (2022) LMO2 expression is frequent in T-lymphoblastic leukemia and correlates with survival, regardless of T-cell stage. Mod Pathol 35:1220–1226. https://doi.org/10.1038/s41379-022-01063-1
31. Natkunam Y, Farinha P, Hsi ED et al (2008) LMO2 protein expression predicts survival in patients with diffuse large B-cell lymphoma treated with anthracycline-based chemotherapy with and without rituximab. J Clin Oncol 26:447–454. https://doi.org/10.1200/JCO.2007.13.0690
32. Muñoz-Mármol AM, Sanz C, Tapia G et al (2013) MYC status determination in aggressive B-cell lymphoma: the impact of FISH probe selection. Histopathology 63:418–424. https://doi.org/10.1111/his.12178
33. Hilton LK, Tang J, Ben-Neriah S et al (2019) The double-hit signature identifies double-hit diffuse large B-cell lymphoma with genetic events cryptic to FISH. Blood 134:1528–1532. https://doi.org/10.1182/blood.2019002600
34. Mahmoud AZ, George TI, Czuchlewski DR et al (2015) Scoring of MYC protein expression in diffuse large B-cell lymphomas: concordance rate among hematopathologists. Mod Pathol 28:545–551. https://doi.org/10.1038/modpathol.2014.140
35. Rodig SJ, Vergilio JA, Shahsafaei A, Dorfman DM (2008) Characteristic expression patterns of TCL1, CD38, and CD44 identify aggressive lymphomas harboring a MYC translocation. Am J Surg Pathol 32:113–122. https://doi.org/10.1097/PAS.0b013e3180959e09

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.