Diffuse Large B Cell Lymphoma: Immunohistochemical Classification According to Hans Algorithm and Association With Outcome in A Moroccan Institution

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ABSTRACT

BACKGROUND: The most prevalent subtype of non-Hodgkin lymphoma is diffuse large B-cell lymphoma (DLBCL). Germinal center B-cell (GCB) and non-germinal center B-cell (non GCB) are the two main biologically different molecular subtypes identified utilizing an immunohistochemistry-based approach.

AIM: Our objective in this study is to analyze the impact of immunohistochemical subtypes of DLBCL (GCB or non GCB) on demographic and clinicopathological parameters, response to chemotherapy and survival outcomes.

SUBJECTS AND METHODS: This is a retrospective study including 106 cases of DLBCL collected in the department of pathology, Hassan II university hospital, Fez (Morocco), over a period of 12 years (January 2010-September 2022). The subtypes of DLBCLs were defined according to Hans algorithm, using immunohistochemistry by three biomarkers (CD10, BCL6, MUM1).

STATISTICAL ANALYSIS USED: Independent t tests and analyses of variance were used for the comparison of mean values. We employed the SPSS 26.0 program to achieve this. A statistically significant value was set at $P < .05$.

RESULTS: Seventy-five patients (71%) were non-GCB subtype, while thirty-one patients (29%) had the GCB immunosubtype. We have found a significant ($P < .05$) correlations between DLBCL immunosubtypes and treatment responses on one hand and survival in the other hand. In the GCB subtype, the response rate and survival were significantly improved. A significant association was found between Ki 67 expression and survival on univariate analysis. On multivariate analysis, we note a correlation between Ki 67 expression, DLBCL immunohistochemical subtypes and survival outcome.

CONCLUSION: Non GCB subtype is associated with poor response to treatment and inferior survival outcome compared to GCB subtype in Moroccan context, especially when combined with high expression of Ki 67 marker.

KEYWORDS: Diffuse large B cell lymphoma, germinal center B cell, non germinal center B cell, non Hodgkin lymphoma, Hans algorithm, immunohistochemistry

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Introduction

Diffuse Large B cell lymphoma (DLBCL), which accounts for 30% to 40% of all cases of Non-Hodgkin lymphomas (NHL), is the most prevalent NHL in the world.[1-3] It is a malignancy of medium or large B lymphoid cells with a diffuse growth pattern, nuclear size nearly equal to or more than the typical macrophage nucleus, and even twice the size of a normal lymphocyte.[4]

DLBCL has remarkable biological heterogeneity at the pathological, morphological, immunophenotypic, and molecular levels.[5] By employing gene expression profiling, it may be divided into the prognostically important subtypes of germinal center B cell-like (GCB) and activated B cell-like (ABC) lymphoma.[6] In reason of their high price and restricted availability, molecular methods are still difficult to apply in everyday practice. Because it is a practicable procedure to apply at any pathology laboratory, the immunohistochemistry (IHC) based algorithm is used.

Hans and al. classified DLBCL into GCB and non GCB subtypes in 2004 using cDNA microarray data and three

antibodies, including the cluster of differentiation 10 (CD10), polyclonal B cell lymphoma 6 (BCL 6), and multiple myeloma oncogene 1 (MUM1). Numerous studies examined the ratio of GCB and non-GCB in different populations using the Hans et al and al algorithm; however, the results were unclear regarding its predictive prognostic significance.[7] While some studies have shown that the GCB subgroup has a significantly higher survival rate, other studies have found no difference in survival rates between the two groups.[8,9]

The GCB and non-GCB subtypes have also been correlated to various treatment outcomes, demonstrating that patients with the GCB subtype responded better to rituximab in combination with the CHOP chemotherapy (Cyclophosphamide, Doxorubicin, Vincristine, and Prednisolone) than patients with the non-GCB subtype.[10,11] These two diffuse large cell B-cell lymphoma immunohistochemistry subtypes have predictive significance that is influenced by both biological and molecular factors as well as clinical considerations.[8,11,12]

Most of studies evaluating the prognostic value of the two-immunohistochemical subtypes of diffuse large cell B lymphoma have been performed in Western countries and only a few studies have been reported in the African countries. Moroccan data on the immunophenotypes of DLBCL are particularly rare; a single study has recently been carried out at the Mohamed V Military Hospital in Rabat, exploring the impact of the cell of origin on the prognosis of DLBCL patients in Morocco.[13] In this regard, the current study will attempt to determine the correlation between immunohistochemical DLBCL subtypes, GCB or non GCB, and many important factors such as clinical and biological characteristics, evolution and survival aspects.

Materials and Methods

Patients

This is a retrospective study including 106 patients diagnosed with DLBCL according to the Word Health Organization (WHO) classification between 2010 and 2022 in Hassan II University Hospital.

The choice of patients was based on the availability of a good quality specimen, clinical, and follow-up data. Among 240 DLBCL patients diagnosed at Hassan II University Hospital, we identified 106 patients who had accessible data.

Diagnostic of DLBCL

The diagnostic of DLBCL was based on:

1) Analysis of general signs: weight loss of more than 10% of body weight in less than six months, fever over 38°C for more than a week with no obvious infectious source, nocturnal sweating, etc.
2) Clinical examination listing all invaded lymph nodes and looking for hepatosplenomegaly.
3) Full biological panel (hemogram, coagulation test, renal function, uric acid and lactate dehydrogenase levels) and tests for HIV and B and C hepatitis.
4) Thoraco-abdominal and cervical scans with injection of medium contrast if there is no contraindication. The same applies to 18fluorodeoxyglucose (18FDG)-labeled positron emission tomography (PET), a technique that can reveal unrecognized disease and thus help the treatment strategy.
5) Bone biopsy, gastrointestinal endoscopy in the event of suggestive symptoms, an exploratory lumbar puncture in the presence of mammary, testicular or ORL lesions, when the patient has more than two extraganglionic lesions or a medullary lesion, and when the lymphoma occurs during the course of HIV infection.
6) Pathological examination of a tissue biopsy to examine morphological appearance of a diffuse proliferation of large B cells, ie, a nucleus at least twice the size of a small lymphocyte.
7) Immunohistochemical study: The immunohistochemical markers needed to diagnose DLBCL are CD20, which marks B lymphocyte differentiation, CD79a and Pax5, Ki67 for estimation of cell proliferation, CD5 to make a differential diagnosis of mantle cell lymphoma if cyclin D1 is also positive.

Staging

Staging was based on Ann Arbor criteria:

- **Stage I:** Involvement of a single lymph node region or a single extralymphatic organ or site.
- **Stage II:** Involvement of two or more lymph node regions on the same side of the diaphragm or localized involvement of an extralymphatic organ or site.
- **Stage III:** Involvement of lymph node regions or structures on both sides of the diaphragm.
- **Stage IV:** Diffuse or disseminated involvement of one or more extralymphatic organs, or either:
  - Isolated extralymphatic organ involvement without adjacent regional lymph node involvement, but with disease in distant sites.
  - Involvement of the liver, bone marrow, pleura or cerebrospinal fluid.

Treatment

Treatment for DLBCL was determined as the following approach: For advanced Ann Arbor stage (III-IV), treatment was based on chemotherapy comprising 6 to 8 cycles of R-CHOP14 or 21 (Rituximab + CHOP [Cyclophosphamide, doxorubicin), vincristine (Oncovin) and Prednisone] every 14 or 21 days. For less advanced stages, under 6 cycles of chemotherapy was recommended.

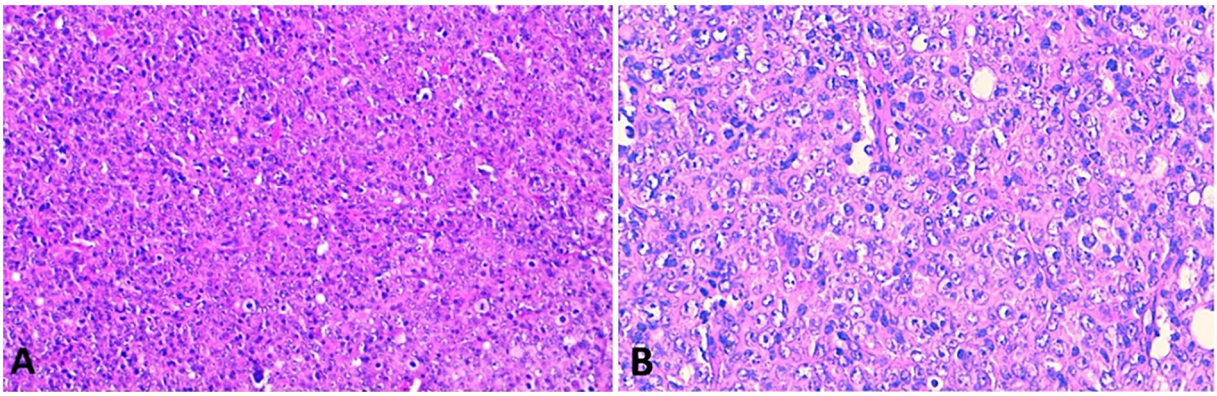

Figure 1. DLBCL cells: (A) proliferation of diffuse large B cells (HESx200) and (B) large tumor cells (HESx400).

After receiving the recommended cycles of chemotherapy, a PET scan was performed to measure the response to treatment:

- If there is a complete response, treatment is finished and there is no need for further cycles of treatment.
- If a partial response is observed, additional cycles of chemotherapy are administered.
- If there is no response to the R-CHOP protocol, that is, if the DLBCL progresses or remained constant, the chemotherapy used is that for treating relapsed or refractory disease.

If DLBCL responds to R-CHOP-based chemotherapy but recurs following treatment, this is a recurrent disease. If the DLBCL does not respond to R-CHOP, the disease is said to be refractory.

The chemotherapy combinations used for relapsed or refractory DLBCL are:

- RDHAOX: Rituximab Dexamethasone Cytarabine Oxaliplatin.
- RACVBP: Doxorubicine, Cyclophosphamide, Vindesine, Bleomycine, Prednisone.
- R-ICE: Rituximab, Ifosfamide (Ifex), Carboplatin and Etoposide.
- RDAEPOCH: Rituximab Dose Adjusted, Etoposide, Prednisone, Oncovin, Cyclophosphamide, Hydroxyadriamycine.

For elderly subjects who cannot tolerate full-dose R-CHOP or those suffering from cardiac problems or in poor health, less intense regimens are used, in particular R-miniCHOP (comprising reduced doses of cyclophosphamide and doxorubicin).

In our population, 60 patients received RCHOP chemotherapy. The number of cycles was less than 6 for 18 patients and more than 6 for 42 patients. Seven patients received RCHOP chemotherapy followed by the RDHAOX protocol. Three patients received RCHOP chemotherapy followed by RDHAOX followed by other protocols (RICE, RACVBPP and/or RDAEPOCH). Four patients received RminiCHOP and 11 patients received chemotherapy without RCHOP with the following protocols: RACVBP, RDAEPOCH.

In our population, there was no difference in treatment protocols between the two-immunohistichemical subtypes GCB and non GCB.

Morphological features

The tissue samples were formalin-fixed, normally treated, paraffin-embedded, and cut into 4 µm slices before being stained with hematoxylin and eosin (HE). After reviewing the HE-stained slides from each tumor block, representative regions with the highest level of tumor cells were chosen for an immunohistochemical analysis (Figure 1).

Immunohistochemistry study

Formalin-fixed, paraffin-embedded tissue sections were used for the immunohistochemistry process. CD10 (clone 56C6), BCL6 (clone LN22), and MUM1 (clone MUM1p) were used as markers. In an automated Ventana Benchmark, immunoreaction was performed for these antibodies.

Classification as GCB versus non GCB subtype was based on the algorithm of Hans using CD10, BCL6 and MUM1 expression with a cutoff of 30% of positive cells. According to this algorithm, cases were allocated to the GCB subgroup if CD10 was positive, or if CD10 was negative, BCL6 positive and MUM1 negative. Then, cases were allocated to non GCB subgroup if CD10 was negative and BCL6 negative, or if CD10 was negative, BCL6 was positive and MUM1 was positive (Figure 2).

In addition to immunohistochemical classification, immunohistochemistry was carried out with anti-Ki67 antibody (clone K2). The percentage of tumor cells with nuclear Ki-67 staining was evaluated. We examined various cutoff points of Ki-67 expression from the lower to the upper quartile with a

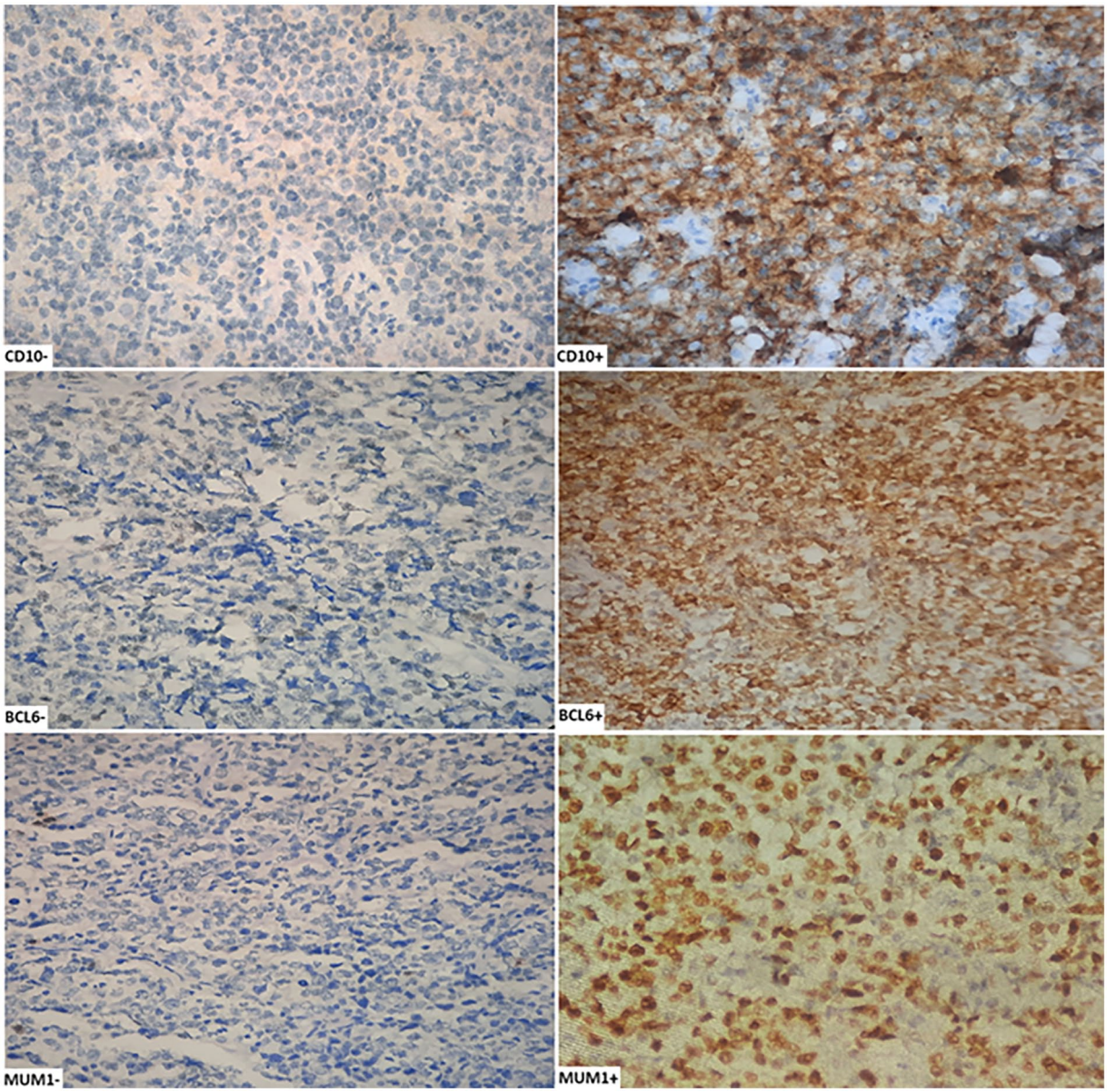

Figure 2. Immunostaining with cluster of differentiation CD10, B-cell lymphoma 6 (BCL6), and multiple myeloma oncogene 1 (MUM1) in diffuse large B-cell lymphoma cell blocks. In positive cases, >30% of the diagnostic cells show nuclear (BCL6 and MUM1) and cytoplasmic (CD10) positivity. In negative cases, occasional diagnostic cells or small cells in the background represent positive internal controls (CD10-BCL6-MUM1 immunostain, ×430). - indicates negative; 1, positive.

rising gradient constructed using 5% steps (60%, 65%, 70%, 75%, 80%, 85%, 90% and 95%; Figure 3). The most significant statistical difference in survival was observed at a cutoff value of 85%, employing the log-rank test.

Survival analysis

From the date of diagnosis to the first occurrence of progression, relapse, or death, the event-free survival (EFS) period was determined. From the date of diagnosis until the date of death, the overall survival (OS) period was calculated. For some patients who had an antecedent history of diffuse large cell B lymphoma prior to the starting year of our study (2010), survival was calculated from the date of initial diagnosis.

Statistical analysis

Descriptive statistics were used to describe the demographic, clinical, and biological parameters. In univariate analysis, the classical parametric tests (Chi2 test and Student test) were used to study the association between the different variables, and GCB and non GCB subtypes. Our software of choice for this was SPSS 26. Statistics were judged significant if $P < .05$.

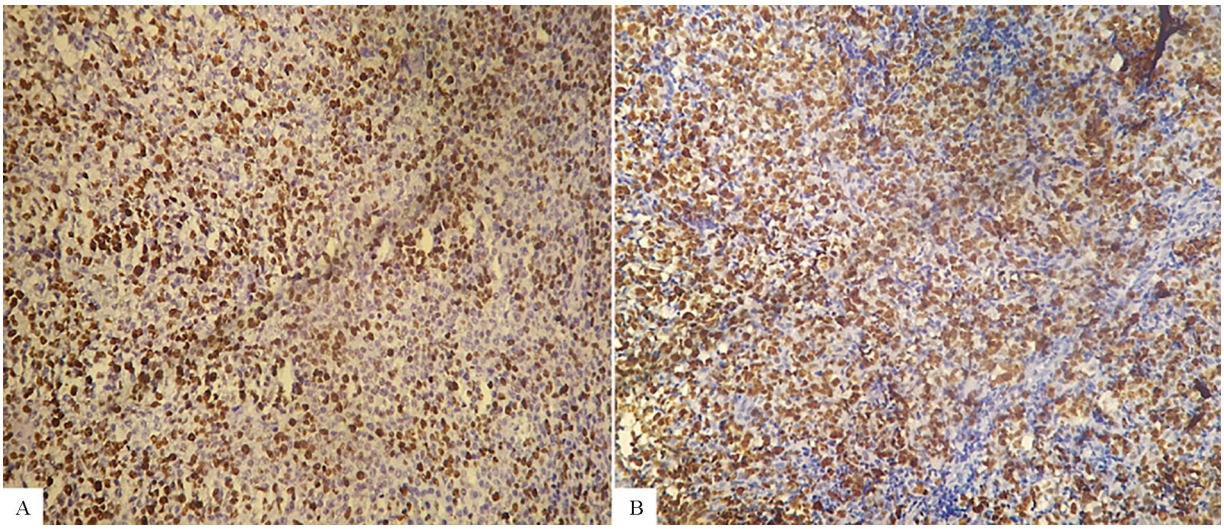

Figure 3. Immunostaining with Ki67 expression: (A) Ki 67 expression at 70% and (B) Ki67 expression at 90% (×400).

The Kaplan-Meier technique of survival analysis was used, and the relevant log-rank test p-values were obtained. The defined p-values are only intended for descriptive purposes. The statistical software program SPSS version 26 was used for all statistical operations.

Results

Clinical and biologic al characteristics

Age and gender. The median age at diagnosis was 54 years (range, 2-85 years), and there were 59 men (55.7%) and 47 women (44.3%), the male to female ratio was 1.25. The age group most affected by diffuse large-cell B lymphoma is the third (between 41 and 60 years old) with a frequency of 40.5% (43 cases; Table 2).

Clinical presentation. B symptoms are present in 63% of cases (67 cases), weight loss represent the most frequent sign present in 58.5% of cases (62 cases). The OMS index performance status was 0 to 1 in 78% of the cases (62 cases). Lymphadenopathy (LAP) was the most common sign, seen in 82% (87 cases), followed by splenomegaly and hepatomegaly (18%, 19 cases).

Location and stage. Regarding the primary lymph node location, it represent 62.26% (66 patients) of all cases. Among these cases, the cervical location was the most frequent with a percentage of 63.64% (42 patients).

Primary extranodal involvement was present in almost 37.73% of cases (40 patients). Among the primary extranodal involvement, digestive location was the most frequent (45%, 18 cases).

Patients in our population have advanced stages of the disease; 80% of patients had stage III/IV disease (58.5% had stage IV and 21.5% had stage III). Moreover, 20% had stage I/ II disease.

Biological characteristics. The LDH level was high in 80% (85) of all cases. Anemia was present in 35% (37) of cases, and high level of CRP was observed in 38.7% (41) of all cases.

International prognostic index. International prognostic index (IPI) score was low (IPI 0,1) in 22.5% of patients (24 cases), while 52% were intermediate risk (IPI 2,3; 55 cases), and 25.5% were high risk (IPI 4,5; 27 cases).

Immunohistochemical classification

Results indicate that 75 patients (71%) had non-GCB subtypes and 31 patients (29%) had GCB subtypes in our population. CD10 positivity was used to categorize the majority of GCB cases (n = 25), however CD10 negative cases (n = 6) were also categorized as the GCB subtype based on the combination of BCL 6 positivity and MUM1 negativity. The majority of non-GCB cases (n = 47) were categorized similarly based on CD10 and BCL6 negativity, while BCL6 positive cases (n = 28) were categorized as non-GCB subtype based on MUM1 positivity (Table 1).

Evolution

In treated patients, evolution was marked by a complete remission in 32.9% of cases (28 patients) and a partial remission in 25.9% of cases (22 patients). The tumor remained constant in 23.5% of cases (20 patients). Progression and relapse were noted in 22.35% and 20% of cases respectively (19 and 17 patients respectively). Untreated patients were lost to follow-up or died before starting treatment.

Of all cases, the median of the overall survival (OS) was 11.5 months (range, 0.25-216 months), and the median of the

Table 1. Classification of cases of DLBCL based on the presence or absence of three antibodies.

IMMUNOSTAINING	GCB (N=31)	NON GCB (N=75)
CD10+	25	-
BCL6+/MUM1-	6	-
CD10-/BCL6-	-	47
CD10-/BCL6+/MUM1+	-	28

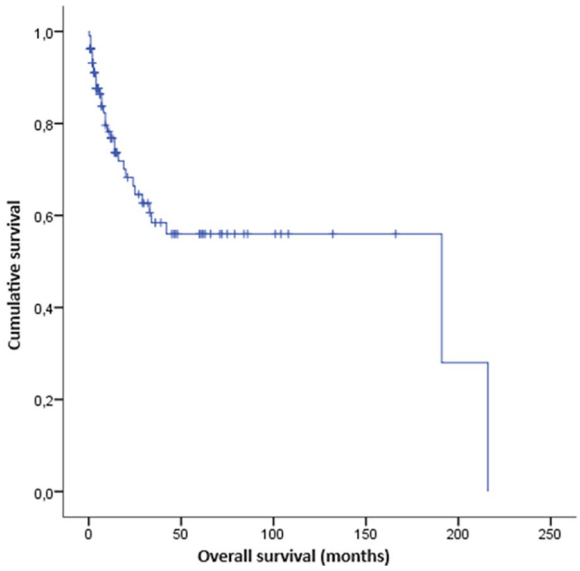

Figure 4. Overall survival of patients with DLBCL.

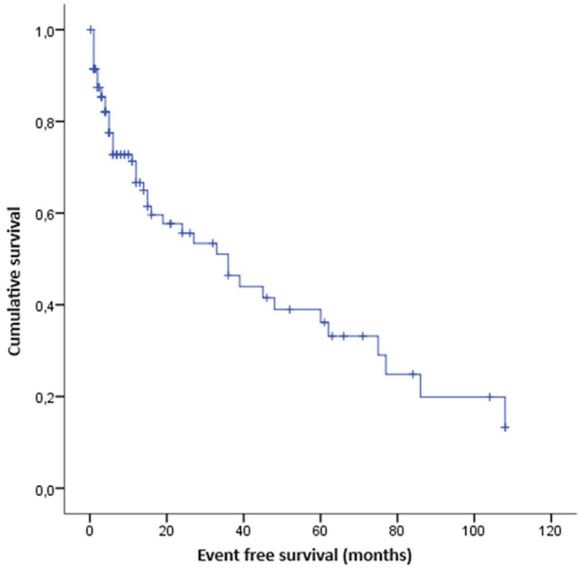

Figure 5. Event free survival of patients with DLBCL.

event free survival (EFS) was 9 months (range, 0.25-108 months; Figures 4 and 5).

In treated patients, the median of OS was 12 months and the median of EFS was 9 months. However, in untreated patients, the median of OS was 2 months and the median of EFS was 1 month. At the end of our study, there were 33 cases of death. Among these cases, five did not receive any treatment. We note that among the cases who died, 22 were classified as non GCB subtype and 11 as GCB subtype.

Association between GCB and non GCB subtypes and clinicobiological characteristics

Association of DLBCL (subtypes) is found to be statistically insignificant with gender, age groups, presence of B symptoms, lymphadenopathy, hepato-splenomegaly, stage, OMS index, LDH, CRP and hemoglobin levels ($P > .05$). These results are shown in Table 2.

In our population, around 60% of CGB patients had an IPI score between 3 and 4, while 57% of non GCB patients have an IPI score between 2 and 3. The correlation between the IPI score and the immunohistochemical subtypes of DLBCL was non-significant ($P > .05$).

Association between GCB and non GCB subtypes and response to treatment

Highly significant ($P = .006$) association between remission and DLBCL immunophenotype was found. In the GCB subtype compared to non-GCB subtype, the response rate was much higher. Regarding the complete remission, a significant difference between GCB and non GCB subtypes was found ($P = .02$). The non-GCB subtype had a much worse response to treatment; progression and relapsed disease were both significantly related to this subtype ($P = .05$ and $P = .025$, respectively; Table 3).

Association between GCB and non GCB suptypes and survival

Association between survival and DLBCL immunophenotypes showed that event free survival was significantly associated with GCB subtypes ($P = .028$). Of all DLBCL cases studied in our population, 27% had an event-free survival of 2 years or more. This was strongly correlated with the GCB subtype with a *P*-value of .002.

On Kaplan-Meier survival analysis based on cell of origin, there was no effect on overall survival ($P = .056$; Figures 6 and 7).

Association between GCB and non GCB subtypes/ Ki67 expression and survival

Regarding Ki67 expression, it was noted that high Ki67 expression was insignificantly associated with GCB or non GCB subtypes ($P = .133$). However, in univariate analysis, high Ki67 expression was associated with poor overall survival and event free survival ($P = .038$ and $P = .041$ respectively; Figures 8 and 9). Furthermore, in multivariate analysis, we have found an association between immunohistochemical subtypes and Ki67 expression with overall survival and event

Table 2. Clinical and biological characteristics of GCB and non GCB DLBCLs.

CHARACTERISTICS	GCB		NON GCB		*P* VALUE
	NO. OF PATIENTS	%	NO. OF PATIENTS	%	
Sex:					.238
-Male	20	64.52	39	52	
-**Femele**	11	35.48	36	48	
Age:					
-Median:	53	54			
-Ranges:					.063
-0-18 y	3	9.68	3	4	
-19-40 y	2	6.45	21	28	
-41-60 y	13	41.93	21	28	
-61-90 y	13	41.93	30	40	
B symptoms	17	16	50	47	.324
-**Weight loss**	17	27.4	45	72.6	.624
OMS index:					.431
-0-2	25	80.65	65	86.67	
-3-4	6	19.35	10	13.33	
Localizations					
-Intra nodal	28	51.85	64	48.85	.490
-**Extra nodal**	26	48.15	67	51.15	.435
Ann Arbor stage:					.585
-I	3	9.68	4	5.34	
-II	3	9.68	11	14.66	
-III	5	16.13	18	24	
-IV	20	64.51	42	56	
IPI score:					.056
-0	5	16.13	3	4	
-1	3	9.68	13	17.33	
-2	3	9.68	19	25.33	
-3	9	29.03	24	32	
-4	10	32.26	12	16	
-5	1	3.22	4	5.3	
High LDH level	22	26.2	62	73.8	.177
Anemia	20	29	49	71	.936
High CRP	16	24.6	49	75.4	.187
High Ki67 (⩾85%)	10	32.26	55	73.33	.133

Table 3. Response to treatment of GCB and non GCB DLBCL.

RESPONSE TO TREATMENT	GCB		NON GCB		*P* VALUE
	NO. OF PATIENTS	%	NO. OF PATIENTS	%	
Remission:	20	64.52	17	22.66	.006
-Complete	13	65	12	70.59	.020
-Partial	7	35	5	29.41	.410
Tumor remained constant	6	19.35	26	34.66	.118
Progression	4	12.9	23	30.66	.050
Relapse	3	9.68	14	18.66	.025

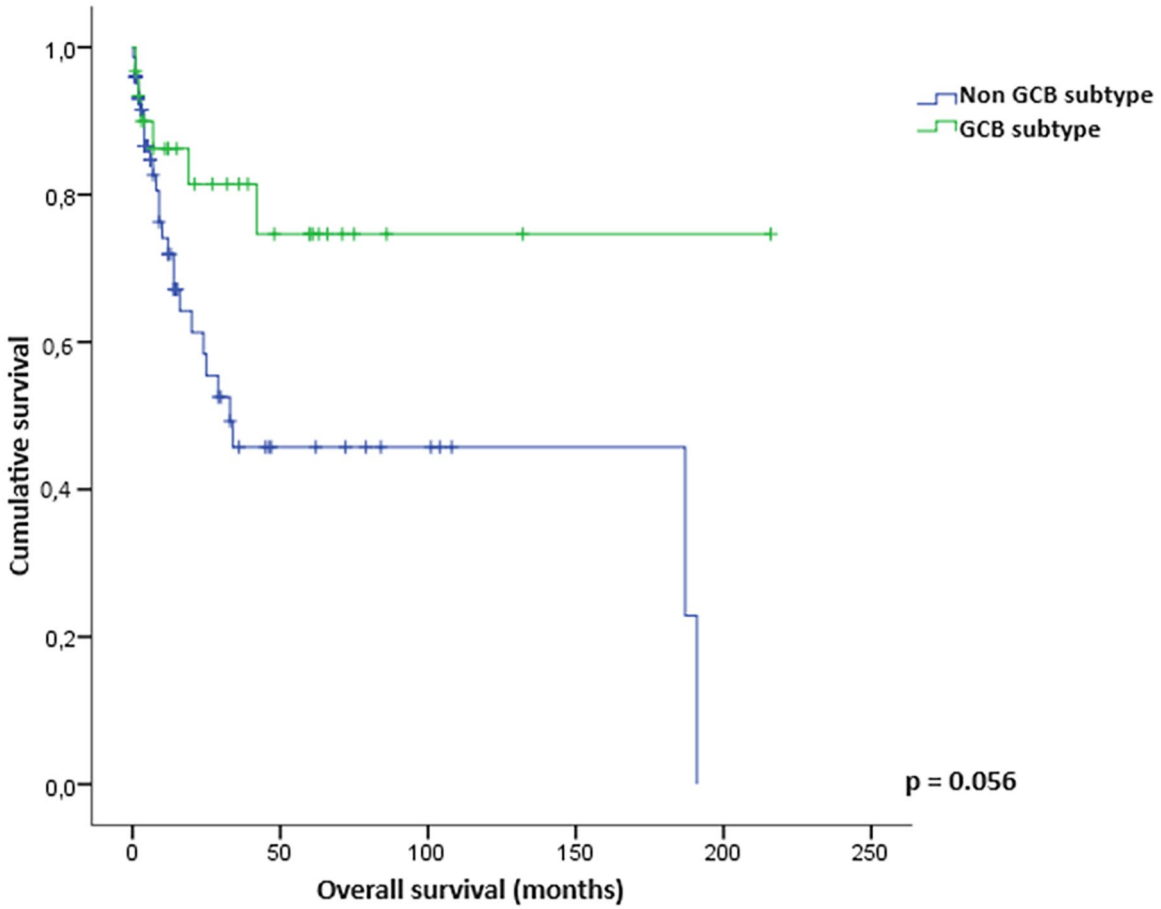

Figure 6. Overall survival of germinal center B-cell (GCB) versus non-GCB immunophenotype according to Hans algorithm.

free survival (*P* = .046 and 0.027 respectively). In this regard, the group with GCB subtype and low Ki67 expression shows better overall and event free survival in comparison to the others 3 groups. Moreover, the group with non GCB subtype and high Ki67 expression had the poorer survival outcome in our population (Figures 10 and 11).

Association between response to treatment and treatment protocols

A significant association (*P* = .001) was observed between the type of treatment administered to patients in our population and response to treatment. The best response as a remission was observed in patients who received more than 6 cycles of

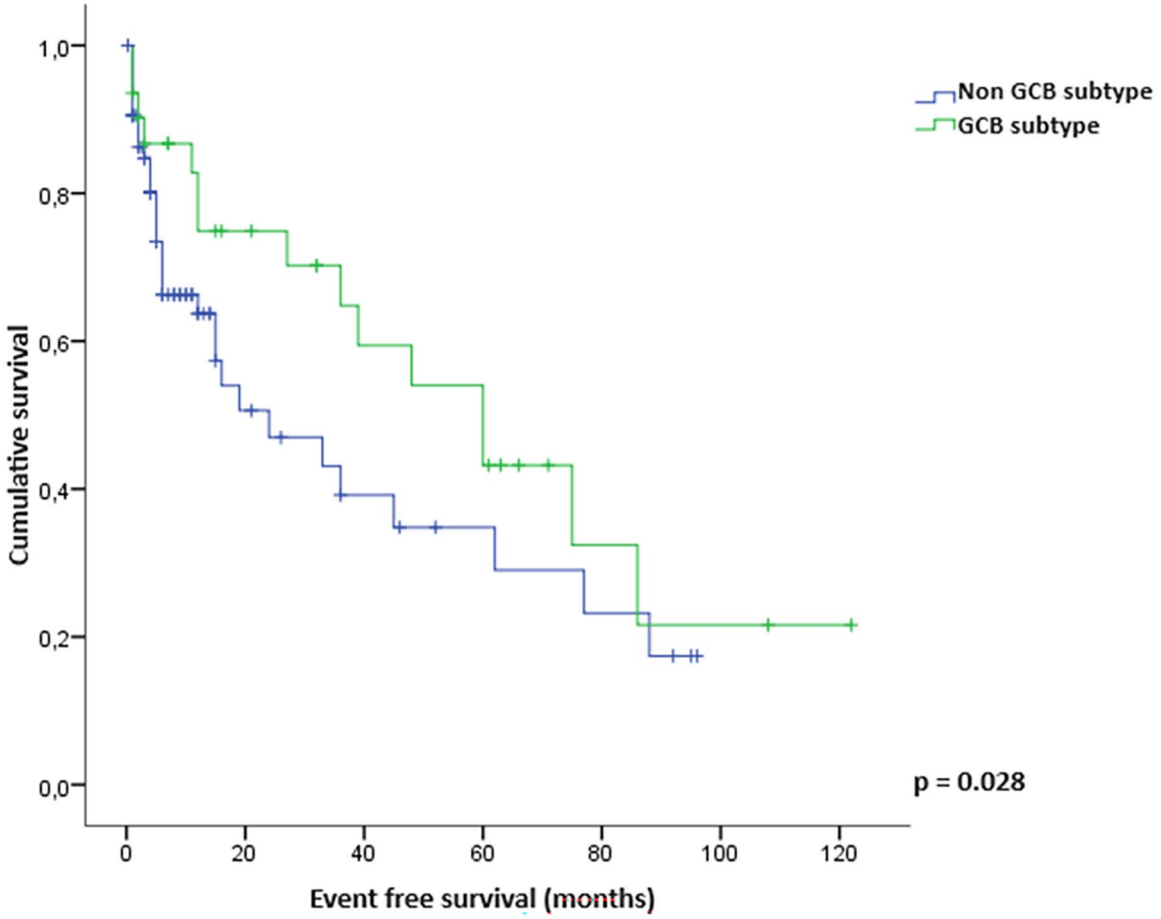

Figure 7. Event free survival of germinal center B-cell (GCB) versus non-GCB immunophenotype according to Hans algorithm.

chemotherapy using RCHOP protocol or using chemotherapy combining RCHOP protocol with other chemotherapies such as RDHAOX, compared to those using other protocols without RCHOP. Beyond that, response to treatment as remission in our population was estimated at 60%. This percentage is in concordance with the literature, where the percentage of remission varies between 50% and 60%.

Discussion

The aim of this study was to divide cases with DLBCL into GCB and non GCB subgroups using immunohistochemistry. Additionally, the study intended to assess the impact of these subtypes on prognosis and treatment response. This study will have a contribution in the field of the immunohistochemical classification of DLBCLs and its subsequent correlation with prognostic variables, treatment response, and survival outcomes in the Moroccan context.

Within our population, it was observed that 71% of patients are classified in non-GCB group, whereas 29% were categorized as GCB group. Nevertheless, the research conducted in Western countries has indicated that the proportion of GCB subtype of DLBCL is notably higher than that of the non-GCB subtype.[1-4] In contrast, previous research in Asian populations have consistently found a higher prevalence of non GCB subtype and a lower prevalence of GCB subtype, which aligns with the findings of our study.[5-7] A recent investigation conducted at the Mohammed V Military Hospital in Rabat, Morocco examined 140 cases of DLBCL. The findings revealed that 31% of patients with DLBCLs were classified as GCB subtype, while the remaining 68% were categorized as the non-GCB subtype.[13] Consequently, it can be concluded that the non-GCB subtype is more prevalent within the Moroccan population. The observed disparity in the distribution of DLBCL subtypes across different countries can potentially be attributed to genetic, environmental, ethnic, and demographic factors that contribute to the development of lymphoma. However, it is possible that this discrepancy has arisen as a result of the varying approaches employed in the classification of DLBCL. Several studies have indicated that certain cases, which are categorized as non-CGB using immunohistochemistry, are instead classified as activated B-cell-like (ABC) using gene expression profiling (GEP).[9-11]

The median age at the time of diagnosis in our population was found to be 54 years, while the Mahtat et al.'s moroccan study reported a median age of 58 years. The median age of Moroccan patients diagnosed with DLBCL is observed to be

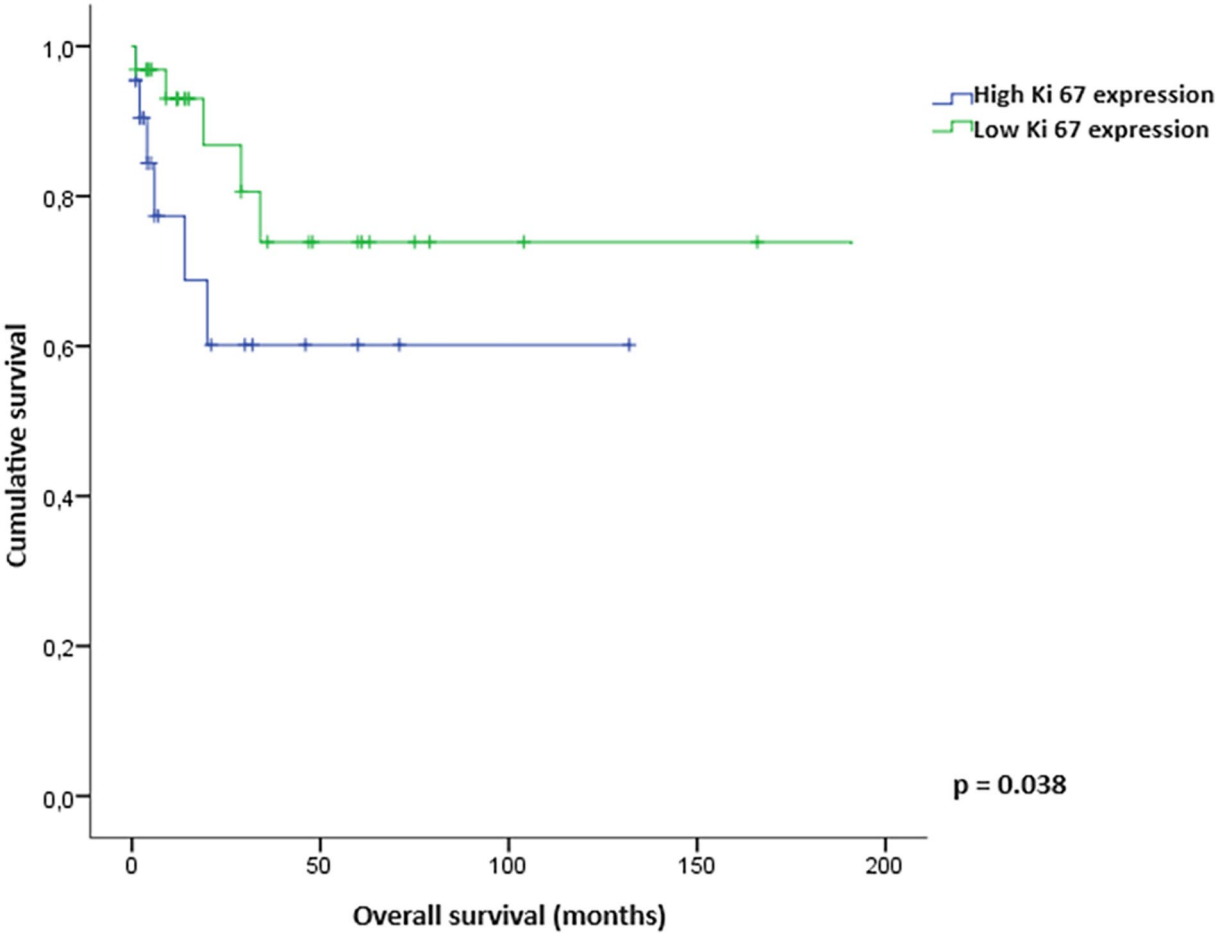

Figure 8. Overall survival of high Ki67 expression versus low Ki 67 expression.

significantly lower than that reported in Western studies,[12-14] but is found to be equivalent to the findings of Asian studies.[7,8,15] The underlying cause of this phenomenon can also be attributed to genetic and environmental influences. In addition, while examining research conducted on a global scale, it was determined that the median age of patients diagnosed with DLBCL was between 50 and 60 years. We note a male predominance in our population, which is comparable to the literature in worldwide.[2,13,14,16,17]

B signs was present in 63% of patients, which is higher than other series that show a percentage of B signs around 14% to 42%.[4,6,10] Primary extranodal involvement was observed in 37.73% of all patients in our series. Which is similar to other studies in literature.[11,12,14,15] Eighty percent of patients had a high Ann Arbor stage (III/IV) which is higher than previous series.[8,11,12] These results can be explained by the long delay between the time of appearance of symptoms in patients in our population and the time of consultation. The average time to consultation in our population was 6 months, which could affect the stage of the disease.

In our study population, there was a poor response to treatment compared with other series. Remission after treatment was observed in 58.8% of patients treated, including complete and partial remission. Gogia et al. reported an estimated remission of 85% and He Sang Hwang and al reported a remission of 95.4%. These results allow us to conclude that the patients studied in our sample are less responsive to treatment. The genetic and environmental characteristics of our population may be implicated in this poor response to treatment, as may the advanced stage of our patients at the time of diagnosis and the high frequency of the non GCB subtype over the GCB subtype.

The present study observed a statistically significant correlation between DLBCL subtypes and response to chemotherapy, specifically in terms of remission. Among the cases that achieved remission, around 66% were of GCB subtype, while the remaining 34% were of non-GCB subtype. In addition, it was shown that 62.5% of patients who achieved complete remission were classified as having the GCB subtype, whereas 85.2% of cases showing disease progression and 82.4% of cases of relapsed disease were attributed to the non-GCB subtype. Previous research has indicated that patients with the CGB subtype exhibit a higher frequency of complete remission following treatment.[12,16] The study findings presented here align

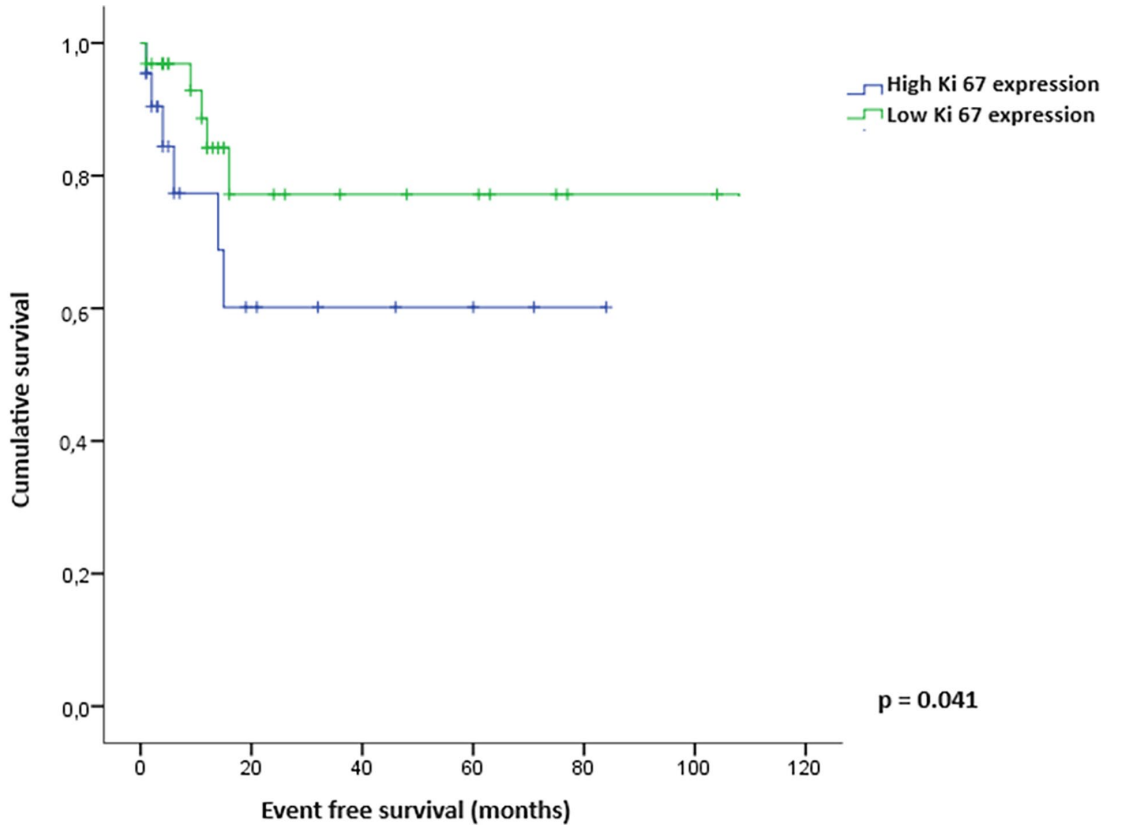

Figure 9. Event free survival of high Ki67 expression versus low Ki 67 expression.

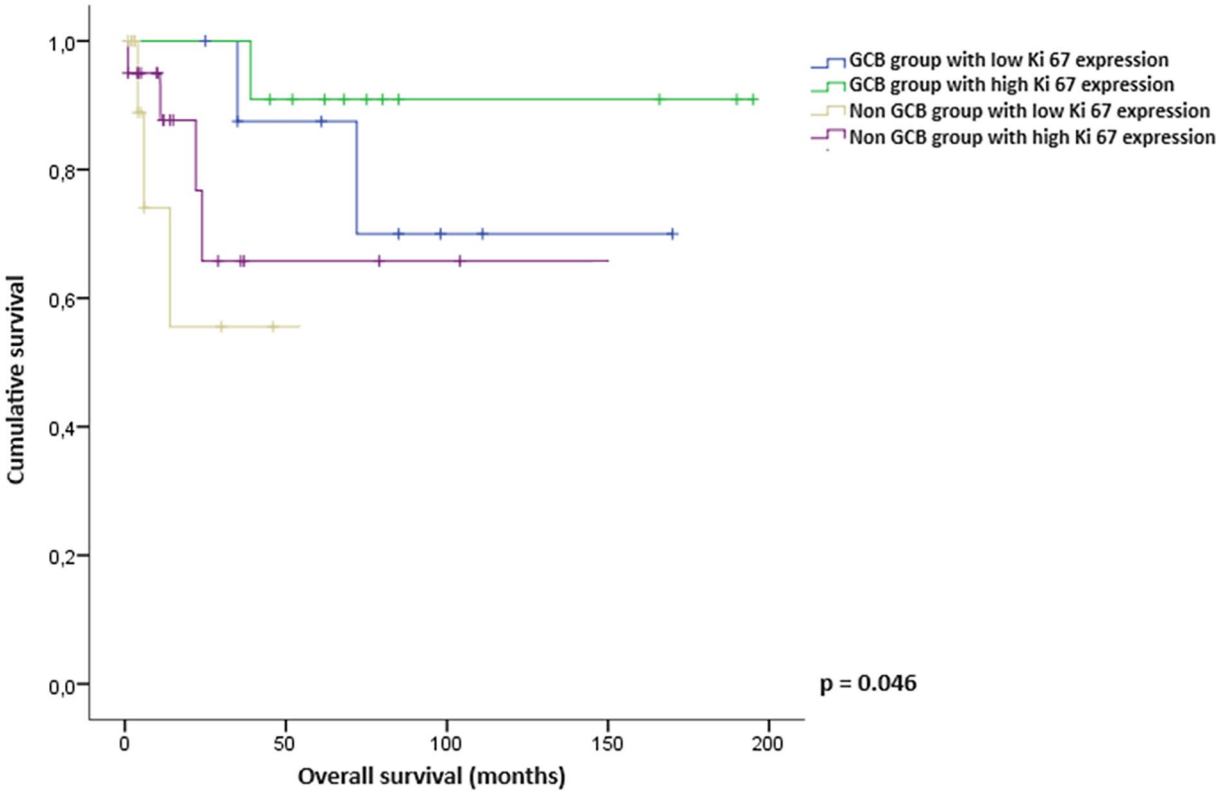

Figure 10. Overall survival of immunohistochemical subtypes in comparison to Ki 67 expression.

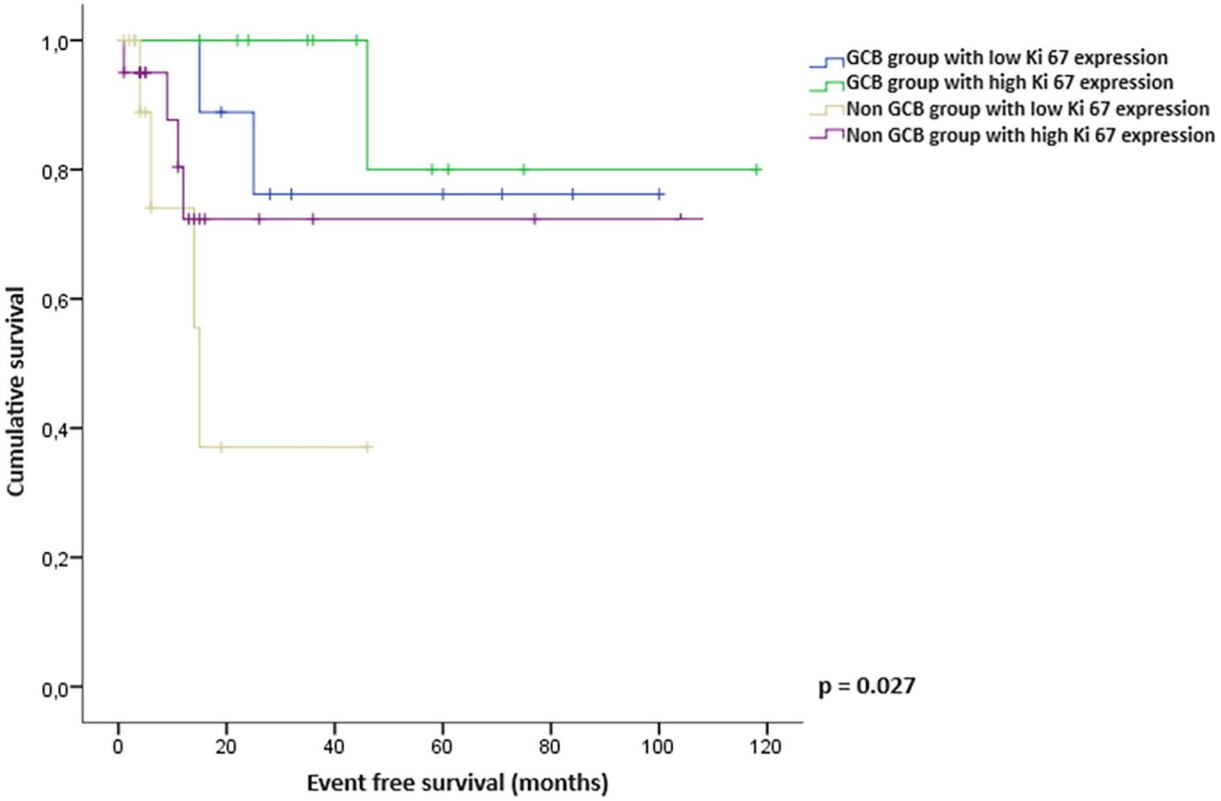

Figure 11. Event free survival of immunohistochemical subtypes in comparison to Ki 67 expression.

with earlier research, which has indicated that the GCB subtype is associated with a more favorable treatment response as compared to the non GCB subtype (Table 4).

A significant difference was observed regarding survival outcomes. GCB subtype was correlated with better event free survival in comparison to non GCB subtype. Moreover, better overall survival tends to be correlated with GCB subtype (P= .061; Figures 3 and 4). These results are in line with the majority of studies that have assessed the prognostic impact of cell of origin on overall survival and event-free survival.[6,8,11,17] However, some studies have shown no significant association between these parameters.[12] Others studies showed better but non-significant EFS and OS than those in the non GCB subgroup.[18]

In addition to the impact of DLBCL immunohistochemical subtypes on outcome, the expression of Ki 67 was considered as an independent prognostic factor in our series showing that low Ki 67 expression is associated with better survival outcome. When combining DLBCL subtypes and Ki 67 expression, results in our series showed that high Ki 67 expression in the non GCB subtype is associated with the worst survival outcome in comparison to others groups. This results are in concordance with numerous previous studies.[19-22]

No significant association was found between the immunohistochemical subtypes of DLBCL and age, sex, presence or absence of B symptoms, extranodal involvement, IPI score, high LDH and Ann Arbor stage. In the literature, the results are inconclusive about the prognostic value of these variables; some studies have shown that the non-CGB subtype is associated with the presence of more than one extra-nodal location, higher IPI score or LDH level and advanced Ann Arbor stage.[16,17] Other studies found no significant association between these variables and immunohiostochemical subtypes, as found in our study.[6,8,18]

Finally, based on the results of previous studies and our own, we can confirm the importance of immunohistochemical classification in the prognosis of patients with DLBCL. However, immunohistochemical classification alone is not sufficient. Molecular classification is essential for a better therapeutic approach and providing personalized treatment for patients. This classification aims to classify DLBCL cases into molecular subtypes: DHL, THL and HGBL NOS. To achieve this, FISH testing for rearrangements of C-MYC, BCL2 and BCL6 genes is required. In our context, this classification was not carried out due to the unavailability of FISH kit and C-MYC, BCL2 and BCL6 probes.

Conclusion

Our analysis revealed a higher prevalence of non GCB subtype in comparison to GCB subtype. The later exhibited significantly higher response rate and better event free survival compared to the former. Conversely, non GCB subtype had much

Table 4. Comparative analysis of patient characteristics, response and survival.

	VAN IMHOFF ET AL.[11]	YAN ET AL.[17]	BERGLUND ET AL.[6]	TYAGI ET AL.[16]	FU ET AL.[8]	GOGIA ET AL.[12]	HWANG ET AL.[18]	OUR STUDY
Total of patients	66	160	161	236	243	417	150	106
-GCB	-58%	-37.5%	-51%	-41.5%	-52%	-43%	-34%	-29%
-Non GCB	42%	62.5%	49%	58.5%	-48%	-47%	-55.3%	71%
-UC						10%	10.7% (unclassifiable)	
Sex ratio	1.35	1.19	1.04	1.3	1.06	2	1.7	1.25
Median Age	50 y	-	62 y	56 y	68 y	48 y	59 y	54 y
B symptoms	-	-	36%	-	-	42.9%	14%	63%
Extranodal involvement	36%	-	64%	55.9%	35%	50.8%	38%	33%
Ann Arbor stage III/IV	-	-	48%	-	45%	51%	51.3%	80%
Response to treatment:								
-CR	-	-	-	-	-	– 74.82%	– 86.7%	– 32.9%
-PR	-	-	-	-	-	– 10.18%	– 8.7%	– 25.9%
-RC	-	-	-	-	-	-	-	– 23.5%
-P		-	-	-	-	– 1.43%	– 3.3%	– 22.35%
-R		-	-	-	-	– 7.2%	-	– 20%
Survival (Median):								
-OS	-	-	-	-	-	-	- 72.1 mo	-11.5 mo
-EFS	-	-	-	-	-	-	-	- 9 mo
Sex	-	$P = .838$	$P = .57$	$P = .24$	$P = .31$	-	$P = .398$	$P = .238$
Age (Median)	-	$P = .045$	-	-	$P = .41$	-	-	$P = .653$
B symptoms	-	-	$P = .55$	-	-	-	$P = .510$	$P = .324$
Extranodal involvement	-	$P = .014$	-	$P = .04$	$P = .51$	-	$P = .42$	$P = .435$
IPI score	-	$P = .004$	$P = .6$	-	-	-	$P = .49$	$P = .056$
High LDH level	-	$P = .035$	-	$P = .00$	$P = .93$	-	$P = .262$	$P = .177$
Ann Arbor stage	-	$P = .002$	$P = .79$	-	$P = .2$	-	$P = .177$	$P = .602$
Response to treatment:								
-CR	- $P = .15$	-	-	$P = .00$	-	$P < .05$	$P > .05$	$P = .02$
-PR	-	-	-	$P = .00$	-	-	-	$P = .410$
-RC	-	-	-	$P = .00$	-	-	-	$P = .118$
-P	-	-	-	$P = .00$	-	-	-	$P = .05$
-R	-	-	-	-	-	-	-	$P = .025$
Survival (Median):								
-OS	- $P = .04$	- $P = .091$	$P = .002$	-	$P = .032$	$P = .15$	$P = .307$	$P = .061$
-EFS	-	-	$P = 00001$	-	$P = .11$	$P = .07$	$P = .281$	$P = .028$

worse prognosis with a lower survival rates and responsiveness to treatment. In addition, non GCB subtype with high Ki 67 expression is an indicator of poor survival outcome.

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None.

Author contributions

Mahat Taybi—Investigation, Methodology, Validation, Writing—Original Draft, Review & Editing; **Hind Bourkhime**—Statistical Analysis, Validation, Review & Editing; **Zineb Khammar**—Visualization, Review & Editing; **Noufissa Alami Drideb**—Visualization, Review & Editing; **Rhizlane Berrady**—Visualization, Review & Editing; **Sanae Benmiloud**—Visualization, Review & Editing; **Sanae El Fakir**—Visualization, Review & Editing; **Laila Bouguenouch**—Visualization, Review & Editing; **Laila Tahiri**—Visualization, Review & Editing; **Laila Chbani**—Visualization, Review & Editing; **Nawal Hammas**—Supervision, Validation, Visualization, Review & Editing.

Ethics

The University Hospital Ethics Committee of Fez has approved the project under No. 07/2024. The investigation was conducted in accordance with the Declaration of Helsinki of 2008.

Consent

The authors certify that they have obtained written informed consent forms from the patient's family for the publication of this article.

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