A clinicopathological study of B-cell differentiation markers and transcription factors in classical Hodgkin's lymphoma: a potential prognostic role of MUM1/IRF4

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ABSTRACT

Background and Objectives

Although most patients with classical Hodgkin's lymphoma (CHL) are cured, a significant minority are refractory to treatment. The investigation of biological markers could improve the predictive capacity of clinical staging systems. The aim of our study was to detect B-cell differentiation markers and transcription factors in CHL in order to define subgroups with different histogeneses and prognoses.

Design and Methods

We evaluated 107 cases of CHL for BCL6, CD79a, MUM1/IRF4 and B-cell transcription factors BOB.1, OCT.2 expression by immunohistochemistry. Statistical analysis was performed using Fisher's exact test, the Mann-Whitney test, the Kaplan-Meier method and the log rank test. Univariate and multivariate regression analyses were performed to identify variables with a significant effect on survival.

Results

CD79a was expressed in 5.8%, BCL6 in 14.7%, MUM1/IRF4 in 92.3%, BOB.1 in 53.4% and OCT.2 in 12.6% of cases. There was no significant association between CD79a or BCL6 expression and clinical characteristics. Univariate analysis showed that age of 45 or more, stage III and IV disease and MUM/IRF4 negative status were associated with significantly shorter time to progression (TTP) and overall survival (OS). On multivariate analysis the lack of MUM/IRF4 expression was associated with significantly shorter TTP while age of 45 or more and the presence of extralymphatic sites of disease were associated with significantly shorter OS.

Interpretation and Conclusions

Our study has confirmed that MUM1/IRF4 is expressed in most cases of CHL and shows that lack of this expression in a minority of cases may be a potential adverse prognostic factor.

Key words: classical Hodgkin's lymphoma, immunohistochemistry, MUM1/IRF4, prognostic factors, survival.

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Classical Hodgkin's lymphoma (CHL) is a malignant lymphoma characterized by the presence of mononuclear Hodgkin and multinucleated Reed-Sternberg cells (H/RS) residing in a complex admixture of inflammatory cells.[1] The majority of patients with CHL are cured. Nevertheless, treatment fails in a significant minority and most of these patients eventually die as a result of progressive disease or therapy-related complications.[2] The ability to identify more accurately patients at risk of primary treatment failure might allow the application of more aggressive treatment or minimize treatment toxicity in those with low-risk disease. Several studies have attempted to identify biological parameters that could be helpful in the prognostic evaluation of patients at initial diagnosis and in planning treatment.[3,4] The aims of the present study were to clarify the histogenetic origin of H/RS cells in CHL and identify subgroups with different clinical characteristics and prognoses.

The origin of H/RS cells has been enigmatic for a long time, as they often express markers of different hematopoietic lineages.[5] Only recently have analyses of immunoglobulin and T-cell receptor loci of single H/RS cells revealed that they represent monoclonal populations of tumor cells of B-cell (98%) or T-cell (2%) origin.[6,7] In most instances CHL is a B-cell lymphoma, H/RS cells are derived from germinal center (GC) or post-GC B-cells, they harbor clonally rearranged immunoglobulin genes and carry a high load of somatic mutations.[6,8] However H/RS cells frequently lack the expression of B-cell specific markers (such as CD20, CD79a, and J-chain),[4,9,10] they variably express B-cell histogenetic markers related to different stages of B-cell differentiation such as BCL6, CD10, MUM1/IRF4 and CD138[4,5,8,11-13] and they also lack immunoglobulin light and heavy chain mRNA.[6]

CD79a is a signal transduction portion of the B-cell receptor and is expressed almost exclusively on B cells and B-cell neoplasms.[14] In CHL, despite the B-cell origin of H/RS cells, only a small minority of cases express the CD79a B-cell marker.[9,10,15] BCL6 protein is a POZ/zinc finger transcription repressor and is required for GC formation and T-helper-2-mediated responses.[16-18] Strong positivity for BCL6 is detected in tumor [lymphocytic and histiocytic (L&N)] cells of nodular lymphocyte-predominant Hodgkin's lymphoma, but only in a small fraction of H/RS cells in CHL.[4,5,8,11] MUM1/IRF4 (multiple myeloma-1/interferon regulatory factor-4) protein is encoded by the *MUM1/IRF4* gene, which has been identified as a myeloma-associated oncogene activated at the transcriptional level as a result of t(6;14)(p25;q32).[19] MUM1/IRF4 is expressed in the final step of intra-GC B-cell differentiation, in subsequent steps of B-cell maturation towards plasma cells, in lymphoid neoplasms thought to be derived from these cells and in activated T cells.[12,13] MUM1/IRF4 protein is expressed in almost all cases of CHL.[11-13] Transcription factor OCT.2 (octamer-binding transcription factor-2) and its co-activator BOB.1 (B-cell Oct binding protein), are necessary for the octamer-dependent transcription of immunoglobulin and other important lymphoid-specific genes of B cells, involved in proliferation and differentiation.[20,21] Expression of both factors in H/RS cells in CHL is seen in only a subset of cases.[9,10,22,23]

In the present study we analyzed the CD79a/BCL6/MUM1 B-cell differentiation immunophenotypes and the B-cell transcription factors BOB.1/OCT.2 in H/RS cells in 107 cases of CHL aiming to elucidate the histogenesis of CHL and correlate the pattern of expression of these markers with clinical and laboratory characteristics and the patients' outcome. Most previous studies[8-13,22] have shown a variety of results regarding the expression of all these markers and furthermore their clinical significance is not clear.

Design and Methods

Patients

Clinical data and biopsy samples from 107 patients with CHL diagnosed between 1992-2003 were collected from the participating centers. All cases were reclassified according to the updated WHO classification.[1] Data recorded included sex, age, WHO/ECOG performance status[24] histological subtype, presence of B-symptoms, involvement of extralymphatic sites and bone marrow status. Patients with advanced stage disease were risk stratified according to the International Prognostic Score (IPS),[25] and those with early stage disease according to EORTC/GELA.[26] Laboratory values considered in the analysis included hemoglobin levels, leukocyte, lymphocyte and platelet counts, erythrocyte sedimentation rate (ESR), lactate dehydrogenase (LDH), serum albumin, IgA, IgG and IgM levels. The patients were treated according to the current established protocols for CHL therapy. A total of 106 out of 107 patients included in the study received combination chemotherapy: 66 patients (61.7%) received the ABVD regimen,[27] 20 patients (18.7%) received baseline BEACOPP,[28] 15 patients (14%) received hybrid doxorubicin-containing regimens, and 4 patients (3.7%) received MOPP or variants. Seventy-two patients with early stage disease were treated as follows: 1 patient received only radiotherapy, 25 only chemotherapy and 46 combined modality therapy. Thirty of 34 patients with advanced stage disease were treated with chemotherapy alone; 4 radiotherapy was added in the other four patients due to residual tumor mass.

Immunohistochemical analysis

Immunohistochemical staining was performed using five different antibodies: CD79a (JCB 117, DAKO, Glostrup, Denmark; dilution 1:40), BCL6 (P6-B6p, DAKO; dilution 1:20), MUM1/IRF4 (clone MUM1p,

DAKO; dilution 1:20), BOB.1 (C-20 sc-955, Santa Cruz, CA, USA; dilution 1:400) and OCT.2 (C-20 sc-233, Santa Cruz; dilution 1:600). Immunostaining was performed on paraffin-embedded tissue as previously described.[22] At least 100 neoplastic H/RS cells per section, as defined by histologic and immunohistologic criteria (CD30 positivity), were independently counted by two of the authors. Positivity was defined as staining of at least 10% of the cells of interest. Intensity of staining (weak, moderate, strong) was also recorded with reference to that observed in normal cells.[22]

Statistical analysis

Overall survival (OS) was estimated from the date of initial diagnosis until the date of last follow-up or until the patient's death. Surviving patients were censored at the date of last contact. Time to disease progression (TTP) was defined as the time between the date of the initial diagnosis and the date of documented recurrence or death from a disease-related cause without there documentation of disease progression. Complete response (CR) was defined as the resolution of clinical and visual evidence of disease for a minimum of 4 weeks. Duration of response was calculated for patients with CR. It was defined as the time between the date of obtaining CR and the date of documented recurrence or death from a disease-related cause without there being previous documentation of disease progression.

Fisher's-exact test was used to test differences in proportions between two groups. In case of continuous variables, differences in median values were assessed by the non-parametric Mann-Whitney test.

The Kaplan-Meier method was used to calculate TTP, duration of response and survival curves, while the log-rank test was used to compare time to event distributions. p values of 0.05 or less were considered statistically significant.

In order to identify variables with significant effect on TTP and OS, univariate and multivariate regression analyses were performed, using the Cox proportional hazards model. Variables included in the models were: age (equal or above 45 vs below 45 years old), sex (male vs female), stage (III and IV vs I and II), B-symptoms (yes vs no), extra-lymphatic sites of disease (yes vs no), and CD79a, BCL6, MUM1/IRF4, BOB.1 and OCT.2; all markers were included as dichotomous variables (positive vs negative). A backwards selection procedure identified the subclass of significant variables. The significant factors were kept in the model if the maximum likelihood ratio criterion had a p-value below 0.10. All analyses were performed using SPSS 11.0.1 software.

Results

The main clinical features of the 107 patients are listed in Table 1. The median period of follow-up was 43 months. The results of the immunohistochemical analyses are shown in Table 2. CD79a was expressed in six out of 104 cases (5.8%)(Table 2, Figure 1B). There were no significant correlations between the expression of CD79a and clinical and laboratory disease characteristics. BCL6 was found to be positive in 15 (14.7%) of 102 cases examined as shown in Table 2 (Figure 1D). In most

Table 1. Clinical data of the 107 patients with classical Hodgkin's lymphoma.

Characteristic	*No. of patients*	*%*
Sex		
Male	55	51.4
Female	52	48.6
Age, median (range)	37 (13- 79)	
WHO/ECOG perfomance status		
0	49	45.8
1	46	43.0
2	6	5.6
3	2	1.9
Unknown	4	3.7
Ann Arbor stage		
Iπ	6	5.6
II	66	61.7
III	12	11.2
IV	22	20.6
Unknown	1	0.9
Histological subtype		
NS	75	70.1
MC	18	16.8
Other*	14	13.1
B-symptoms		
Yes	49	45.8
No	55	51.4
Extra-lymphatic sites of disease		
Yes	24	22.4
No	81	75.7
Unknown	2	1.9
Enlarged/bulky mediastinum		
Yes	14	13.1
No	89	83.2
Unknown	4	3.8
Bone marrow involvement		
Yes	10	9.3
No	93	86.9
Unknown	4	3.7
EORTC/GELA classification		
Advanced	34	31.8
Early unfavorable	40	37.4
Early favorable	23	21.5
Not applicable	6	5.6
Unknown	4	3.7
International Prognostic Score°		
0	1	2.9
1	2	5.9
2	14	41.2
3	8	23.5
4	6	14.7
5	4	11.8

**Other histological subtypes were lymphocyte-depleted (n=3), lymphocyte-rich (n=8), composite lymphomas (n=2) of which one was chronic lymphocytic leukemia (CLL)/lymphocyte-depleted CHL and the other CLL/nodular sclerosis CHL, and CLL transformed to CHL (n=1). °Percentages are calculated over the number of patients with advanced stage disease (n=34). WHO: World Health Organization; ECOG: Eastern Cooperative Oncology Group; NS: nodular sclerosis; MC: mixed cellularity; EORTC: European Organization for Research and Treatment of Cancer; GELA: Groupe d'Etude des Lymphome de l'Adulte.*

Table 2. Immunohistochemical results.

Molecular Markers	*Number of positive patients*	*Total number of patients*	*Percentange*
CD79a	6	104	5.8%
BCL6	15	102	14.7%
MUM1/IRF4	96	104	92.3%
BOB.1	55	103	53.4%
OCT.2	13	103	12.6%

MUM1/IFR4: multiple myeloma–1/interferon regulatory factor-4; BOB.1: B-cell Oct binding protein; OCT.2: octamer-binding transcription factor 2.

BCL6-positive cases (86.6%), weak or moderate nuclear expression was observed (*data not shown*). There were no significant associations between BCL6 expression and different disease features, patients' characteristics, OS, TTP or duration of response.

Positive MUM1/IRF4 nuclear staining was observed in 96 (92.3%) of 104 CHL cases examined (Table 2, Figure 1C). H/RS cells showed moderate or strong nuclear positivity in the majority of positive cases (92.7%) (data not shown). There was a significant negative correlation between MUM1/IRF4 and CD79a expression (p=0.005). MUM1/IRF4-expressing patients developed progressive disease more rarely (p<0.001) (data not shown), and had better TTP (p<0.001) (Figure 2a), and OS (p=0.03) (Figure 2B). Clinical characteristics of patients according to MUM1/IRF4 expression are shown in Table 3.

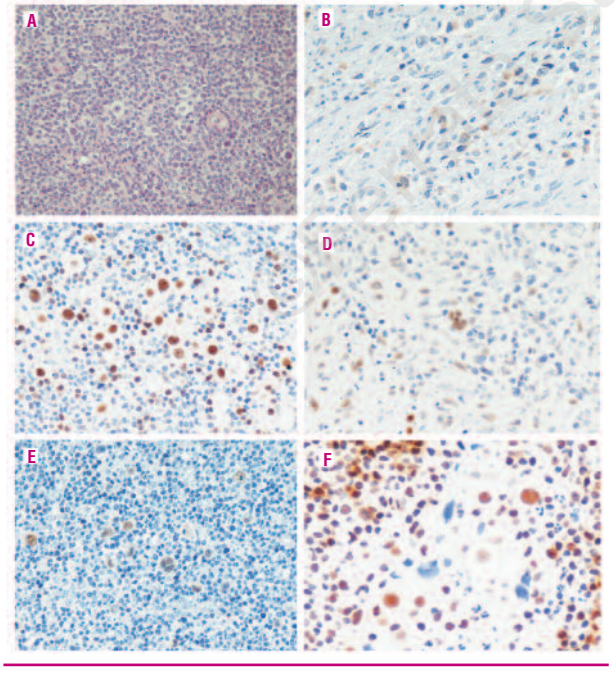

Figure 1. Classical Hodgkin's lymphoma (CHL). A. Lacunar variants of Reed-Sternberg cells (RS) (H+E×400). B. Membrane expression of CD79a (CD79a×400). C. RS cells with nuclear positivity for MUM1/IRF4(MUM1×400). D. Nuclear expression of BCL6 (BCL6×400). E. OCT.2 expression was weak and seen in only a subset of the RS cells (OCT.2×400). F. Expression of BOB.1 was seen in a subset of the RS cells (BOB.1×400).

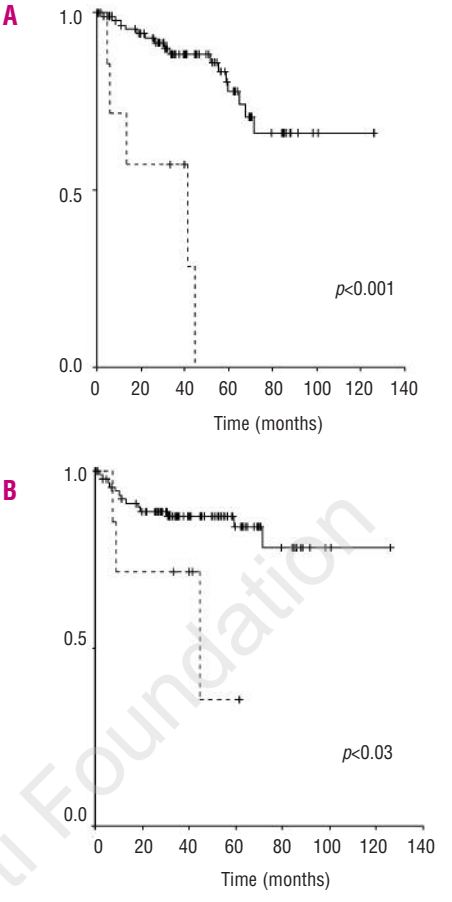

Figure 2. Kaplan- Meier curves for (A) the time to disease progression ($p<0.001$), and (B) overall survival ($p=0.03$) according to MUM1/IRF4 expression. Dashed lines correspond to MUM1/IRF4-negative cases, while solid lines correspond to MUM1/IRF4-positive cases.

The transcription factor BOB.1 was expressed in 55 (53.4%) of 103 CHL cases studied (Table 2, Figure 1F). Intensity of staining was evaluated in all positive cases as weak, moderate, strong in 16.4%, 61.8% and 21.8%, respectively (*data not shown*). There were no significant associations between BOB.1 expression and different patients' characteristics, disease features, TTP or OS. However the percentage of relapsing patients following achievement of CR was greater in BOB.1-positive patients ($p=0.027$) (Figure 3A). This association was more significant in cases with strong BOB.1 expression ($p=0.001$) (Figure 3B).

The transcription factor OCT.2 was found positive in 13 (12.6%) of 103 CHL cases studied (Table 2, Figure 1E). The OCT.2-positive patients were older than the OCT.2-negative ones ($p=0.001$). There was a borderline significant association between OCT.2 negativity and nodular sclerosis histology ($p=0.048$). There was a significant positive correlation between OCT.2 and CD79a expression ($p=0.026$). OCT2 expression was not significantly related to duration of response, TTP or OS. Co-expression of BOB.1 and OCT.2 was observed in seven

Table 3. Clinical characteristics of the patients according to MUM1/IRF4 expression.

	MUM1/IRF4 expression		
	Negative N=8	*Positive N=96*	*p*
Median age (range)	44 (16-79)	36 (13-77)	0.449
Sex (%)			0.719
Male	5 (62.5)	50 (52.1)	
Female	3 (37.5)	46 (47.9	
Stage (%)			0.434
I or II	4 (50)	65 (68.4)	
III or IV	4 (50)	30 (31.6)	
Histology (%)			0.247
NS	4 (50)	69 (71.9)	
MC	2 (25)	16 (16.7)	
Other*	2 (25)	11 (11.5)	
WHO/ECOG perfomance status (%)			0.719
0 or 1	3 (37.5)	44 (47.8)	
2 or 3	5 (62.5)	48 (52.2)	
Extra- lymphatic sites (%)			0.086
No	4 (50)	74 (78.7)	
Yes	4 (50)	20 (21.3)	
EORTC/GELA (%)			0.717
Early favorable	1 (12.5)	20 (20.8)	
Early unfavorable	3 (37.5)	36 (37.5)	
Advanced	4 (59)	30 (31.3)	

**Other histological subtypes were lymphocyte-depleted (n=3), lymphocyte-rich (n=8) and composite lymphomas (n=2) of which one CLL/lymphocyte-depleted CHL and the other CLL/nodular sclerosis CHL. WHO: World Health Organization; ECOG: Eastern Cooperative Oncology Group; NS: nodular clerosis; MC: mixed cellularity; CLL: chronic lymphocytic leukemia; CHL: classical Hodgkin's lymphoma; EORTC: European Organization for Research and Treatment of Cancer; GELA, Groupe d'Etude des Lymphome de l'Adulte.*

of the 103 cases (6.7%) (*data not shown*).

In order to identify variables that have a significant effect on TTP and OS, univariate and multivariate regression analyses, using the Cox proportional hazards model, were performed. Table 4 lists the results of the univariate survival analysis. As expected, age 45 years or more and stage III and IV disease were associated with significantly shorter TTP and worse OS (p=0.009 and p=0.017, respectively). Patients with B-symptoms, extra-lymphatic sites of involvement and MUM1/IRF4 negative status had significantly worse OS (p=0.049, p=0.01 and p=0.043, respectively). MUM1/IRF4-negative status was also associated with shorter TTP (p<0.001). Stepwise multivariate analysis was performed to identify independent predictors for TTP and OS. Age and extra-lymphatic sites of involvement were independent predictive factors for OS (p=0.015 and p=0.018 respectively), while MUM1/IRF4 status was an independent predictor factor for TTP (p<0.001), as shown in Table 5.

Discussion

In the present study, the CD79a/BCL6/ MUM1 B-cell differentiation immunophenotypes and the B-cell transcription factors BOB.1/OCT.2 in H/RS cells were ana-

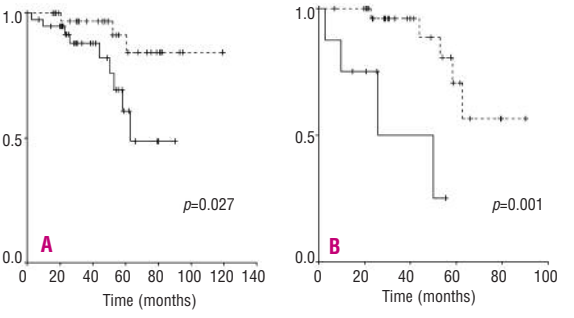

Figure 3. Kaplan-Meier curves for duration of response according to (A) BOB.1 expression, where the dashed line corresponds to BOB.1-negative cases and the solid line corresponds to BOB.1-positive cases (p=0.027) and (B) staining intensity of BOB.1 where the dashed line corresponds to weak or moderate staining and the solid line corresponds to strong staining (p=0.001).

Table 4. Univariate Cox regression analysis for time to disease progression and overall survival (significance level 0.05).

	TTP			OS		
	HR	*95% CI*	*p value*	*HR*	*95% CI*	*p*
Age, years						
≥45	1	–	–	1	–	–
< 45	0.27	0.10- 0.72	0.009	0.27	0.10- 0.72	0.009
Sex						
Female	1	–	–	1	–	–
Male	0.76	0.32- 1.80	0.759	0.95	0.36- 2.53	0.918
Performance Status						
0-1	1	–	–	1	–	–
≥2	2.13	0.85- 5.40	0.107	3.02	0.96- 9.49	0.059
Ann Arbor stage						
I- II	1	–	–	1	–	–
III- IV	2.08	0.87- 4.94	0.017	3.33	1.24- 8.98	0.017
B-symptoms						
No	1	–	–	1	–	–
Yes	2.14	0.88- 5.17	0.092	2.89	1.00- 8.34	0.049
Extra-lymphatic sites						
No	1	–	–	1	–	–
Yes	2.36	0.90- 6.20	0.081	3.72	1.37- 10.11	0.010
BCL6*						
-	–	–	–	1	–	–
+	–	–	–	0.75	0.10- 5.72	0.783
MUM1/IFR4						
-	1	–	–	1	–	–
+	0.11	0.04-0.32	<0.001	0.27	0.08- 0.96	0.043
BOB.1						
-	1	–	–	1	–	–
+	2.24	0.90-5.6	0.084	1.60	0.58- 4.43	0.366
OCT.2						
-	1	-	-	1	-	-
+	0.41	0.05-3.04	0.380	0.57	0.08- 4.37	0.592
CD79a						
-	1	-	-	1	-	-
+	0.56	0.08-4.23	0.578	0.89	0.12- 6.74	0.906

*TTP: time to disease progression; OS: overall survival; HR: hazard ratio; CI: confidence interval; MUM1/IFR4: multiple myeloma-1/interferon regulatory factor-4; BOB.1: B-cell Oct binding protein; OCT.2: octamer-binding transcription factor 2. *In case of TTP, for BCL6 coefficients did not converge.*

Table 5. Multivariate Cox regression analysis for time to disease progression, and overall survival.

	HR	TTP 95% C.I.	p value
Sex			
Female	1	–	–
Male	0.40	0.14- 1.14	0.087
MUM1/IFR4			
–	1	–	–
+	0.04	0.01- 0.17	<0.001
CD79a			
–	1	–	–
+	0.15	0.02- 1.30	0.085
	HR	**OS 95% CI**	**p value**
Age, years			
≥45	1	–	–
< 45	0.29	0.11- 0.79	0.015
Extra-lymphatic sites			
No	1	–	–
Yes	3.31	1.23- 8.95	0.018

TTP: time to disease progression; OS: overall survival; HR: hazard ratio; CI: confidence interval; MUM1/IFR4: multiple myeloma–1/interferon regulatory factor-4.

lyzed in 107 cases of CHL aiming to elucidate the histogenesis of CHL and correlate the pattern of expression of these markers with clinical and laboratory characteristics and patients' outcome. Although H/RS in CHL are of B-cell origin in most cases,[9-11] it is well documented that expression of B-cell specific markers such as CD79a occurs only in a minority of cases. In our study CD79a was expressed in 5.8% of the CHL cases, which is in accordance with published data.[9,10,15]

The transcription factors BOB.1 and OCT.2 were expressed in 53.4% and 12.6%, respectively, of the 103 CHL cases studied. There is variation in the expression of these transcription factors reported in the literature due to small number of cases studied and/or to differences in immunohistochemical methods used.[9,10,22,23] We found a significant positive correlation between OCT.2 and CD79a expression (p=0.026), suggesting the existence of a common B-cell lineage down-regulating factor in H/RS cells. The latter observation was also made by Garcia-Cosio M *et al.*[10] However, we did not find an association between BOB.1 and CD79a in our series. This discrepancy could be explained by the fact that BOB.1 is a co-activator of immunoglobulin gene transcription and is mainly involved, as indicated by gene knock-out data, in the late steps of B-cell differentiation.[29]

BCL6 protein and MUM1/IRF4 have been used as phenotypic markers for the characterization of B-cell lymphoma histogenesis.[12,30,31] BCL6 is a valuable marker of B-cells of GC-origin as its expression is restricted to the GC B cells.[16,17] The presence of MUM1/IRF4 marks the final step of intra-GC differentiation and subsequent steps of B-cell maturation towards plasma cells.[12,13] In our study BCL6 and MUM1/IRF4 positivity was found in 14.7% and 92.3%, respectively, of CHL cases studied. This is in accordance with published data.[4,5,8,10,13] A comparison of BCL6 and MUM1/IRF4 expression in each individual revealed that the majority of CHL cases studied (80/102, 78%) were BCL6$^-$/MUM1IRF4$^+$, consistent with a late GC or post-GC B-cell like immunophenotype. Only one case of 102 studied (1%) displayed the BCL6$^+$/MUM1IRF4$^-$ GC-like immunophenotype (*data not shown*). Even though H/RS cells have been shown in many cases to be derived from GC B cells, they show low or no expression of typical GC B-cell differentiation proteins (such as BCL6) and almost constant expression of the late GC/post-GC B-cell differentiation protein, MUM1/IRF4.[5,6,11] These data are strongly supported by the results of recent gene expression profiling in CHL cell lines which revealed a gene expression profile similar to that of cell lines derived from diffuse large B-cell lymphoma (DLBCL) showing features of activated B cells.[32] However, although in DLBCL, groups of distinct biological behavior can be distinguished based upon the histogenetic origin,[31] in our study such a correlation could not be assessed, since only one case displayed the GC B-cell phenotype, making a statistical evaluation impossible.

In our study 14 cases (13.7%) were BCL6$^+$/MUM1IRF4$^+$ and seven cases (6.9%) were BCL6$^-$/MUM1IRF4$^-$ (*data not shown*). The latter two groups display heterogeneous, indeterminate BCL6/MUM1IRF4 immunophenotypic profiles that do not correspond to the differentiation immunophenotypes of normal B cells, suggesting that the differentiation process of H/RS cells is not complete in a fraction of these cells and/or is still ongoing at the time of observation.[11,12,30]

Our analysis confirms the findings of previous studies demonstrating the importance of age more than 45 years and extralymphatic sites of disease involvement as independent prognostic factors for OS in multivariate analysis and advanced clinical stage (III and IV) and the presence of B-symptoms in univariate Cox regression analysis.[3,25,33]

Moreover, as shown in Figure 2A-B, our study demonstrated that lack of MUM1/IRF4 expression was associated with significantly shorter TTP and significantly worse OS (p<0.001 and p=0.03, respectively). These results were further confirmed by using a Cox proportional hazard model which on univariate analysis demonstrated that lack of MUM1/IRF4 expression was associated with both shorter TTP (p<0.001) and OS (p=0.043) (Table 4). In addition, in a multivariate Cox regression analysis, lack of MUM1/IRF4 expres-

sion was associated with significantly shorter TTP (p<0.001) (Table 5). To our knowledge this is the first study demonstrating that lack of MUM1/IRF4 expression in CHL has a negative prognostic impact on TTP and OS. However, the result is only indicative given the small number of MUM1/IRF4-negative cases.

The prognostic impact of MUM1/IRF4 expression has been studied in other lymphoproliferative disorders. In B-cell chronic lymphocytic leukemia MUM1/IRF4 negativity was mostly associated with the unmutated phenotype and significantly worse OS.[34] A significant association between MUM1/IRF4 expression and reduced OS was observed in a group of patients with cutaneous large B-cell lymphoma using tissue microarray methodology.[35] In DLBCL MUM1/IRF4 expression indicates an activated B-cell origin and more aggressive clinical behavior. This observation has been confirmed by several studies using either immunohistochemistry[31] or gene expression profiling.[36,37] We could speculate that in our analysis of CHL, the lack of MUM1/IRF4 expression may be immunophenotypically indicative of a GC-cell origin. However this is not true since almost all (7/8) MUM1/IRF4-negative cases were also BCL6-negative, indicating an indeterminate immunophenotypic profile not corresponding to the normal B-cell differentiation program.

As revealed by their gene expression profiles, a striking and unexpected relationship was found between CHL and primary mediastinal B-cell lymphoma (PMBL). This subtype of lymphoma differs from the other DLBCL subgroups. Immunophenotypically a large number of PMBL cases show a variable expression of MUM1/IRF4.[38] In addition, over one third of the genes that distinguish PMBL from the other DLBCL are also expressed in CHL cell lines.[37] Moreover PMBL and CHL also share several oncogenic mechanisms, such as the NFκB signaling pathway.[39] Taking into consideration the clinical and molecular similarities between CHL and PMBL, it is not surprising that MUM1/IRF4 is expressed in the majority of cases in both these entities.

In conclusion, our study has confirmed that MUM1/IRF4 is expressed in the majority of CHL. In our series lack of expression of MUM1/IRF4 in a minority of cases was associated with shorter TTP and OS, suggesting that this B-cell differentiation marker has a potential negative prognostic role. However our findings need to be confirmed in larger series of cases in order to shed light on the prognostic role of MUM1/IRF4 in the context of CHL.

Authors' contributions

SV, VP, DR and TE: substantial contributions to the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, revising the study critically for important intellectual content, and final approval of the version to be published; FK: analysis and interpretation of data, drafting the article, final approval of the version to be published; EP: substantial contributions to the conception and design of the study, revising the study critically for important intellectual content; and final approval of the version to be published; JD, NH, NX, PT, CG and EN: acquisition of data, revising the study critically for important intellectual content, and final approval of the version to be published; TM: acquisition of data, analysis and interpretation of data, revising the study critically for important intellectual content, and final approval of the version to be published; SP: analysis and interpretation of data, revising it critically for important intellectual content; and final approval of the version to be published.

Conflicts of Interest

The authors reported no potential conflicts of interest.

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