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## Expression of heat shock proteins in classical Hodgkin lymphoma: correlation with apoptotic pathways and prognostic significance

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**Title Page****Expression of heat shock proteins in classical Hodgkin lymphoma: correlation with apoptotic pathways and prognostic significance**

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**Running title**

Heat shock proteins in Hodgkin lymphoma

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## Abstract

*Aims:* Heat shock proteins (HSPs), which are known to inhibit apoptosis and promote cellular survival, are over-expressed in many tumours. We analyzed the expression of relevant HSPs and heat shock factor 1 (HSF1) in classical Hodgkin lymphoma (cHL) and their relationship with caspase signalling pathways and patient outcome.

*Methods and results:* Using tissue-microarrays, most cases showed strong immunohistochemical expression of HSPs (10, 27, 40, 60, 70, 90, 110, HO1, CDC37) and HSF1, which points to cHL as a potential candidate to stress-response inhibitors. Active caspases 3, 8, and 9 were detected in 55.1%, 55.4%, and 96.2% of cases; however, cleaved PARP was observed only in 16.1%, suggesting improper functioning of apoptosis. Statistical analysis showed associations of HSP70 with active caspase 3 ( $p= 0.000$ ); HSP40 with active caspase 9 ( $p= 0.031$ ) and p53 ( $p= 0.003$ ); HO1 with p53 ( $p= 0.006$ ) and p21 ( $p= 0.005$ ); and p53 with p21 ( $p= 0.015$ ).

*Conclusions:* Correlations between the expression of apoptotic markers and HSPs may suggest a role for the latter in modulating apoptosis in cHL, mainly through the HSP70-HSP40 system, and in the stabilization of p53. Survival analyses showed that absence of active caspase 8 and HO1 had a negative impact in patient outcome.

## Introduction

Classical Hodgkin lymphoma (cHL) is characterized by the presence of scarce neoplastic Hodgkin/Reed-Sternberg (HRS) cells in a background of reactive cells. HRS cells are clonal germinal center-derived B cells with rearranged and somatically mutated but nonproductive immunoglobulin genes that escape from CD95-mediated apoptosis.<sup>1</sup> The antiapoptotic phenotype of HRS cells has been mainly attributed to constitutive NF- $\kappa$ B activation, and is mediated by several molecules including c-FLIP and XIAP.<sup>2-4</sup> The dysregulation of apoptosis is a key feature of lymphoma pathogenesis and many therapeutic approaches are focused on neutralizing mediators of the antiapoptotic and pro-proliferative phenotype of tumour cells. Promising targets for anti-cancer drug design are heat shock proteins (HSPs) due to their cytoprotective function.

HSPs are highly conserved proteins which besides functioning as molecular chaperones play a pivotal role in apoptosis inhibition and cell survival. HSPs are classified into 5 groups according to their molecular size: HSP100, HSP90, HSP70, HSP60, and small HSPs.<sup>5</sup> Under the control of the heat-shock factor 1 (HSF1), which is the major regulator of cellular response to stress, HSPs are rapidly induced in response to different damaging stimuli. The cytoprotective function of HSPs reflects their ability to suppress the apoptotic signalling pathway at multiple points. For instance, at the pre-mitochondrial stage HSPs regulate pro-survival signalling cascades mediated by AKT, JNK, and NF- $\kappa$ B; at the mitochondrial level inhibit the mitochondrial outer membrane permeabilization and the release of pro-apoptotic proteins and, finally, at the post-mitochondrial level, prevent caspase activation.<sup>6,7</sup>

In normal, non-stressed cells inducible HSPs are poorly expressed; in contrast, over-expression of HSPs has been reported in many tumours and in some types this is linked with worse prognosis and chemo-resistance. Although pharmacological blockade of HSPs is an

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attractive strategy for cancer treatment, up to now only inhibitors of HSP90 have been clinically tested.<sup>8-10</sup>

Little is known about the expression of HSPs in cHL and the role that HSPs may play in the establishment and maintenance of the malignant HRS phenotype. Abundant expression of HSP90 and HSP60, and lower levels of HSP27 have been described in a small group of HL cases using immunohistochemical (IHC) methods;<sup>11</sup> however, the IHC expression profile of HSF1 and most HSPs, as well as the possible prognostic significance of such expression are not established in cHL.

Using tissue microarrays, we analyzed the IHC expression of HSP families and HSF1 in HRS cells of cHL and their relationship with different markers for the status of the extrinsic and intrinsic apoptotic pathways and with patient outcome.

## Materials and Methods

This work was approved by the Ethics Committee of our institution.

### PATIENTS AND TISSUE SAMPLES

Diagnostic biopsy samples from a total of 89 patients with cHL were retrospectively collected from the files of the Pathology Department of Ramón y Cajal Hospital during the period between 1989 and 2002.

Complete clinical, analytical, therapeutic, and follow-up data were available from 76 of them. Data recorded in the database included: sex, age, Ann Arbor stage, presence of B symptoms, histological type, number of lymph nodes and extranodal sites, analytic variables included in the international prognostic score (IPS), response to therapy (complete response versus treatment failure), disease free survival (DFS) from the achievement of complete remission until relapse, death or last follow-up, and overall survival (OS) from the time of

diagnosis to last follow-up or death. Complete remission (CR) was defined as the absence of clinical and radiological evidence of disease for a minimum period of 4 weeks.

All patients were treated with standard polychemotherapeutic regimens (adriamycin-containing regimens or MOPP regimens) with or without adjuvant radiotherapy. Some patients with refractory disease also received autologous peripheral stem-cell transplantation.

The histological confirmation of cHL was made in pre-treatment standard tissue sections from lymph node biopsies in 86 cases and from an extranodal site in 3 cases, using H&E, CD20, CD3, CD30, CD15 and PAX5 staining, and according to the World Health Organization (WHO) classification. There were 51 nodular sclerosis, 24 mixed cellularity, 10 lymphocyte-rich, 2 lymphocyte depletion and 2 unclassifiable HL.

Reactive lymphoid tissue and different B- and T-cell lymphoma samples were also collected from the tissue archive.

#### TISSUE MICROARRAY DESIGN

A Tissue Arrayer device (Beecher Instrument, Silver Spring, MD, USA), kindly provided by Centro Nacional de Investigaciones Oncológicas, was used to construct the tissue microarrays (TMA). The suitability of TMA for the study of HL has been proved elsewhere.<sup>12</sup> Paraffin blocks were selected only on the basis of the availability of formalin-fixed, paraffin-embedded tissue (at least 1mm thick). Two different TMA blocks were constructed, each containing duplicate cores from the areas richest in neoplastic cells of the selected 89 cHL cases. Abundant tumour cells had to be identified in the two cylinders for a case to be recorded in the study.

Cylinders of external controls were also included in the TMA blocks and consisted on a representation of reactive lymphoid tissue (2 tonsils and 3 reactive lymphadenitis) and different samples of non-Hodgkin lymphomas (2 diffuse large B-cell lymphoma and 2 peripheral T-cell lymphoma, NOS), all of them collected from the archive.

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IHC staining was performed on the TMA sections following previously described methods.<sup>12, 13</sup> Twenty different antibodies were used whose source, dilution and pattern of positivity are listed in Table 1.

Antibodies were incubated overnight at 4°C or 30 minutes at room temperature (HSPs 10, 27, 60, 90, and 110, CDC37, BCL2, p53 and p21); immunodetection was subsequently performed with the ENVISION DUAL LINK Visualization System (DAKO, Glostrup, Denmark).

Immunodetection of HSP70 and HSP40 was accomplished incubating 30 minutes at room temperature with a peroxidase-conjugated rabbit anti-rat (HSP70) or anti-goat (HSP40) secondary antibody at 1/100 dilution (Jackson ImmunoResearch, West Grove, PA, USA).

Internal controls were provided in each case by the reactivity of the accompanying lymphocytes, plasma cells, macrophages or endothelial cells.

The staining of the TMA sections for the different antibodies was independently evaluated by two pathologists (M.G-C. and A.P.) according to previously published criteria.<sup>3, 4, 12-15</sup> Concurrent results in the two cores were essential to be considered suitable for the study.

For HSF1 and HSPs, samples were considered positive if 10% of more of the malignant cells expressed the corresponding protein as least as intense as reactive accompanying cells. Although an arbitrary 10% cut-off was established, in positive samples these proteins were strongly expressed virtually by all tumour cells with relatively lower levels by the reactive cells.

Epstein-Barr virus (EBV) was detected by *in situ* hybridization with FITC-conjugated *EBER* probes (DAKO) on tissue microarray sections as previously described.<sup>13</sup> The pattern of nuclear staining was recorded in HRS cells as: +, positivity in most cells; -, completely absent. The positivity in bystander lymphocytes was not recorded.

#### STATISTICAL ANALYSIS

The relationships between the expression of the different markers included in the study were analyzed by Pearson chi-square test and Fisher exact test.

A univariate analysis was performed for each clinical variable and for the tumour expression of the analyzed markers. Actuarial survival curves in terms of OS and DFS were constructed according to the Kaplan-Meier method. Comparison of the survival curves for each variable was performed using the log-rank test and Cox univariate analysis.

A backward multivariate analysis was performed using the Cox proportional hazards model to identify factors that might be of independent significance in influencing OS and DFS. The maximal model was formed by the variables found significant in the univariate analysis. Proportional hazard assumption was assessed by Schoenfeld residual.

All values were based on 2-tailed statistical analysis, with values of  $p < 0.05$  considered statistically significant. The statistical analyses were performed using the SPSS statistical software package (SPSS Inc, Chicago, IL).

## Results

### CLINICAL FINDINGS

Briefly, 57.5% of patients were males and 42.5% were females. The range of age at diagnosis varied between 7 and 83 years, with a median of 47 years. At diagnosis, most patients presented with stage II (44.4%) and had low IPS (0 to 2, 77.5%). B symptoms were present in 49.4% of cases. Most patients were treated with conventional chemotherapeutic regimens containing adriamycin (85.5%) with or without radiotherapy. Two patients (2.6%) presenting with low-stage disease were treated only with excisional surgery and 3 (3.9%) received only radiotherapy. Seven patients (9.1%) in advanced-stage received autologous peripheral stem-cell transplantation either as consolidation therapy or as escalating treatment after partial response. CR was achieved by 67 patients (88.2%) and 25 (32.9%) experienced relapse. The median follow-up was 89 months. The clinical data of the patients are summarized in Table 2.

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The IHC evaluation of the different markers tested in the TMAs showed that almost all cHL cases displayed in HRS cells strong expression of HSF1 (98.7%), HSP60 (100%), HSP90 (89.3), HSP110 (98.7%), HO1 (96.3%), HSP10 (100%), and CDC37 (92.4%) (Figure 1). The frequency of cases showing positive staining for the remaining HSPs was lower: 55.4% for HSP27, 78.6% for HSP70, and 78.2% for HSP40. As expected, correlation was found between the expression of HSP60, HSP70 and HSP90, and their corresponding co-chaperones HSP10, HSP40 and CDC37, respectively ( $p=0.000$ ).

EBV was detected by *EBER in situ* hybridization in the neoplastic HRS cells in 50.6% of cHL cases (42 of 83); however, its presence was not associated with any histological subtype. Although *EBERs* have been shown to up-regulate BCL2 and to increase resistance to apoptosis, we did not find any relationship between *EBER* expression and BCL2, c-FLIP, XIAP, p53 or p21.

Active caspases 3, 8, and 9 were detected in 55.1%, 55.4%, and 96.2% of cases, respectively. Despite the detection of active caspase 3 in approximately half of the samples, cleaved PARP (the main product of caspase 3 proteolytic activity) was observed only in 16.1% of cases.

Apoptosis inhibitors BCL2, XIAP, and c-FLIP were expressed in 57.8%, 73.4%, and 66.7% of biopsies, respectively. The phosphorylated form of the pro-survival protein AKT was present in 97.4% of cases.

P53 was expressed in 89.5% of patients and p21 in 96.25%; a significant association between both markers was found ( $p=0.015$ ).

Statistical analysis showed direct significant associations of HO1 with p53 ( $p=0.006$ ) and p21 ( $p=0.005$ ); HSP40 with p53 ( $p=0.003$ ), active caspase 9 ( $p=0.031$ ) and c-FLIP ( $p=0.036$ ); and HSP70 with active caspase 3 ( $p=0.000$ ).

## SURVIVAL ANALYSIS

The study was performed in 76 patients from the initial 89 for which adequate clinical and laboratory data from the time of diagnosis, as well as follow-up information were available.

Consistent with previous studies, advanced stage at presentation ( $\geq 3$ ) and age 45 years or more were strong prognostic markers associated with worse OS ( $p = 0.001$  and  $p = 0.006$ , respectively). Stage 3 or 4 also associated with shorter DFS ( $p = 0.043$ ).

OS was significantly longer in patients expressing HO1 (Hazard Ratio (HR) = 0.172,  $p = 0.005$ ); p53 (HR=0.298,  $p = 0.010$ ); p21 (HR= 0.250,  $p = 0.025$ ); active caspase 8 (HR= 0.413,  $p = 0.039$ ); active caspase 9 (HR= 0.224,  $p = 0.049$ ) and p-AKT (HR= 0.061,  $p = 0.001$ ). Longer DFS was associated with HSP27-negative status ( $p = 0.014$ ).

In order to identify independent predictors for OS and DFS, a multivariate analysis was performed including in the model the variables found significant in the univariate analysis. Advanced stage at presentation (HR= 11.89;  $p = 0.000$ ), lack of active caspase 8 (HR=4.67,  $p = 0.001$ ) and absence of HO1 expression (HR=20.83,  $p = 0.001$ ) remained as independent adverse prognostic factors for OS (Figure 2). To compensate for the heterogeneity in treatment regimens of our series, multivariate analysis was subsequently performed taking into account only the patients treated with adriamycin-containing regimens. This analysis showed that the three former variables: advanced stage at presentation (HR= 16.605,  $p = 0.000$ ), lack of active caspase 8 (HR= 8.197,  $p = 0.000$ ) and absence of HO1 expression (HR=62.5,  $p = 0.000$ ) hold and even improved their significance. The relevance of HO1 as a prognostic marker is only indicative and must be taken with caution since few negative cases were identified. IHC staining showed that in these cases HO1 expression was completely absent from all neoplastic cells.

For DFS, only advanced stage at diagnosis appeared as independent predictor of poor outcome (HR= 2.406,  $p = 0.043$ ).

## Discussion

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Despite great clinical progress in the treatment of cHL, current therapeutic regimens have long-term adverse effects.<sup>16</sup> In this setting, the identification of accurate prognostic factors and the development of non-genotoxic therapeutic agents are major goals in the management of cHL. HSPs are promising targets for the treatment of cancer because the inhibition of HSP function allows the simultaneous modulation of multiple client proteins involved in growth control and cell survival.<sup>9, 10</sup>

Over-expression of HSPs relative to normal tissues is a common feature of human cancers and is associated with poor prognosis and resistance to therapy.<sup>17-19</sup> However, few data are available regarding the expression of HSPs in cHL and their correlation with prognosis. In an early work, abundance of HSP90, HSP60 and HSP27 was described in HRS cells in a group of 25 HL cases.<sup>11</sup> More recently, strong IHC expression of HSP90 was reported in 35 out of 37 (95%) cHL patients.<sup>20</sup> In the present study, we confirm that main HSPs (27, 60, 70, 90, 110 and HO1) and co-chaperones (CDC37, HSP10 and HSP40) are strongly expressed in HRS cells, so too is the major transactivator of HSP induction, HSF1. This finding is of interest since it points to cHL as a possible candidate to stress-response inhibitors. To date, only HSP90 small-molecule inhibitors have been available to be clinically tested.<sup>21</sup> Taking into account that such inhibitors are potent inducers of HSF1 and antiapoptotic HSP27 and HSP70, and that cancer cells show much greater dependence on HSF1 than their non-transformed counterparts, the ablation of the heat shock response through HSF1 inhibition is therefore an attractive strategy for anticancer therapy.<sup>22, 23</sup>

The strong expression of HSF1 and HSPs in HRS cells may be attributed to an increased demand for HSPs to stabilize over-expressed oncoproteins. Over-expression of NF- $\kappa$ B is a hallmark of HRS cells and we have also found abundant expression of p-AKT in most cases; HSP90 and its co-chaperone CDC37 are directly involved in the formation of active NF- $\kappa$ B and help also in the phosphorylation of the pro-survival protein AKT. CDC37 expression in

HRS cells is a surprising finding since this co-chaperone is an oncogene in itself and is over-expressed in a number of cancers.<sup>24</sup> CDC37 mediates carcinogenesis by stabilizing the catalytic domains of oncogenic kinases within HSP90 complexes and provides a target for the design of selective inhibitors of multiple kinase cascades.<sup>25, 26</sup>

Given the essential roles that HSPs play in promoting cell proliferation and inhibiting death pathways, we studied the possible interactions of the major HSPs with components of extrinsic and intrinsic apoptotic pathways in HRS cells. Active caspases 3, 8 and 9 were detected in 55.1%, 55.4% and 96.2% of cHL cases; however, cleaved PARP – the main product of caspase-3 proteolytic activity – was observed only in 16.1% of cases, which seemed to confirm an alteration of apoptotic pathways in HRS cells. Different frequencies of caspase-3 activation in cHL have been reported in the literature; our result resembles that of Bai *et al* (67%) and is lower than those reported by others.<sup>14, 27</sup> However, IHC detection of cleaved PARP was reported only in one work and, contrary to our result, strongly correlated with the expression of active caspase 3.

The major inhibitory molecule that prevents proper caspase 3 activation in HRS cells through the intrinsic pathway is XIAP<sup>4</sup> which was present in our series in 73.4% of cases. However, we did not find any significant association of XIAP or antiapoptotic BCL2 with lack of active caspases 3 or 9. Strikingly, a strong direct association existed between the expression of active caspase 3 and that of HSP70 ( $p= 0.000$ ). Elevated HSP70 does not preclude the activation of caspase 3 but prevents cleavage of PARP.<sup>10, 28</sup> HSP70 can rescue cells from a latter phase of apoptosis than any known survival-promoting protein and protection by HSP70 has been reported to occur in some cases downstream of caspase 3 activation and cleavage of its substrates.<sup>29</sup> Although functional studies are indeed required, it is tempting to speculate that in HRS cells inhibition of the intrinsic apoptotic pathway may be at least partially attributed to the anti-apoptotic effect of HSP70 expression.

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To check proper functioning of the stress-induced apoptotic pathway upstream caspase 3 activation and PARP processing, we studied the expression of active caspase 9 and also of p53 and its effector p21. Although active caspase 9 was detected in most cHL cases (96.2%), this caspase seems not to be truly functional as suggested by activation of caspase 3 only in half of samples. The functional significance of the association between active caspase 9 and HSP40 in our series can only be speculative, but it might point to the HSP70-HSP40 system as a negative regulator of apoptosis since it is well known that HSP40 works in concert with HSP70, helping to lock in the binding of the chaperone to its protein substrate.

Although we have not directly sequenced p53, its expression in HRS cells was associated with that of p21, which seem to suggest that p53 is functionally active and is able to trans-activate the *p21* target gene, what would support the presence of wild-type p53.<sup>14, 30</sup> It could be inferred from our study that high levels of probably wild-type p53 may be stabilized by members of the HSP families as it has been described for the HSP70-HSP40 system.<sup>31, 32</sup> This could be supported by the direct association found between HSP40 and p53. HO1 was also associated with p53 and p21; in some HO1 over-expressing cell lines, induction of p21 has been observed and may result in improved resistance to apoptotic stimuli.<sup>33, 34</sup>

Concerning the role of HSPs as prognostic markers, in our study multivariate analysis showed a final model in which advanced stage at presentation, lack of caspase 8 activation and absence of HO1 expression were the only independent variables with prognostic significance and associated with shorter OS. Despite the fact that in cHL constitutive expression of c-FLIP results in the inhibition of caspase 8 mediated apoptosis,<sup>3</sup> we did not find significant association between presence of c-FLIP and lack of active caspase 8. Other mechanisms of caspase 8 inactivation like mutations, alternative splicing and epigenetic silencing have been reported; impaired function of caspase 8 is detected in a variety of cancers and can promote tumour progression as well as resistance to current treatment approaches.<sup>35</sup> Regarding HO1,

aside from its antioxidative action, it can directly affect cell viability by blocking apoptosis; this tumourigenic potential could seem contradictory to the protective effect of HO1 expression in our series of cHL patients. However, expression of HO1 in squamous cell carcinomas was associated with a higher sensitivity of patients to radiotherapy, whereas lack of HO1 was much more common in non-responders<sup>36</sup> and associated with lymph node metastasis.<sup>37</sup> Similarly, HO1 expression might be a useful indicator of treatment response in cHL, although this result is only indicative given the small number of HO1 negative cases in our series; the clinical relevance of HO1 as a prognostic marker should be explored in a large cohort of patients.

In conclusion, HSF1 and major HSPs are strongly expressed in HRS cells, which highlights the potential use of HSP inhibitors as an attractive strategy to treat cHL. Although functional studies are indeed required, we confirm improper functioning of apoptotic machinery in HRS cells. The correlation between protein expression of different markers of the apoptotic pathway and relevant members of the HSP family might imply a role for HSPs in modulating apoptosis in HRS cells, mainly through the involvement of antiapoptotic HSP70-HSP40 system. HSPs could also be implied in the stabilization of possibly wild-type p53. Finally, advanced stage at presentation, absence of active caspase 8 and lack of HO1 expression have a negative impact in OS in our series of cHL patients.

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## **Disclosure/Duality of Interest**

The authors report no potential conflicts of interest.

**Table 1.** Antibodies used, staining conditions, positive controls and patterns of reactivity.

Protein	Clone	Source	Titer	Pre-treatment	Positive control	Staining pattern
HSF1	PC	Cell Signaling	1:250	TE	GC-B cells	N
HSP27	G31	Cell Signaling	1:50	TE	Endothelial cells	C
HSP60	LK1	Lab Vision	1:200	TE	GC-B cells	C
HSP70 §	6B3	Cell Signaling	1:100	TE	RL	C/N
HSP90	JPB24	Novocastra	1:400	TE	GC-B cells	C
HSP110	58F12	Novocastra	1:200	Citrate	GC-B cells	C, C/N
HSP10	PC	Stressgen	1:500	Citrate	GC-B cells	C
HO1	HO-1-2	Stressgen	1:50	TE	RL	N
HSP40 *	PC	Santacruz	1:250	TE	Squamous epithelia (tonsil)	C/N
CDC37	PC	Lab Vision	1:100	Citrate	GC-B cells	C
Cleaved caspase (Asp175)	3 5A1	Cell Signaling	1:100	TE	RL	N, C/N
Cleaved Caspase (Asp391)	8 18C8	Cell Signaling	1:80	TE	RL	N, C/N
Cleaved caspase (Asp330)	9 PC	Cell Signaling	1:80	TE	GC-B cells	N, C/N
Cleaved-PARP (Asp214)	PC	Cell Signaling	1:50	TE	RL	N, C/N
FLIP	NF6	Alexis	1:80	TE	DLBCL	C
XIAP	2F1	MBL	1:50	TE	RL	C
P21	CP74	Lab Vision	1:50	TE	Scattered GC cells	N
BCL2	124	Dako	Pre-diluted	TE	Small lymphocytes	C
P53	DO-7	Novocastra	1:20	TE	Scattered GC cells	N
p-AKT	Ser473	Cell Signaling	1:50	Citrate	RL	C

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<sup>§,\*</sup> Immunodetection was accomplished with a peroxidase–conjugated rabbit <sup>§</sup>anti-rat or <sup>\*</sup>anti-goat secondary antibody at 1/100 dilution.

Abbreviations:

PC: polyclonal; TE: Tris/EDTA 10mM/1mM, pH 9; Citrate: 10 mM citrate buffer pH 6.5; GC-B cells: germinal center B cells; DLBCL: diffuse large B-cell lymphoma; RL: Reactive lymphadenitis; N: nuclear staining; C: cytoplasmic staining; C/N: both cytoplasmic and nuclear staining.

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**Table 2.** Characteristics of patients.

Clinical characteristics	No./total	%
Median age, years (range)		
47 (7-83)		
Sex		
Males	49/86	57.5
Females	37/86	42.5
Stage		
I	8/81	9.9
II	36/81	44.4
III	17/81	21
IV	20/81	24.7
B symptoms		
Yes	39/79	49.4
No	40/79	50.6
IPS		
0-2	62/80	77.5
≥3	18/80	22.7
Therapy		
Radiotherapy	40/76	52.6
Chemotherapy		
No*	5*/76	6.6
Adriamycin-containing	65/76	85.5
MOPP	5/76	6.6
Other	1/76	1.3
APSCT	7/76	9.2
Complete Remission	67/76	88.2
Relapse	25/76	32.9
Death	28/76	36.4

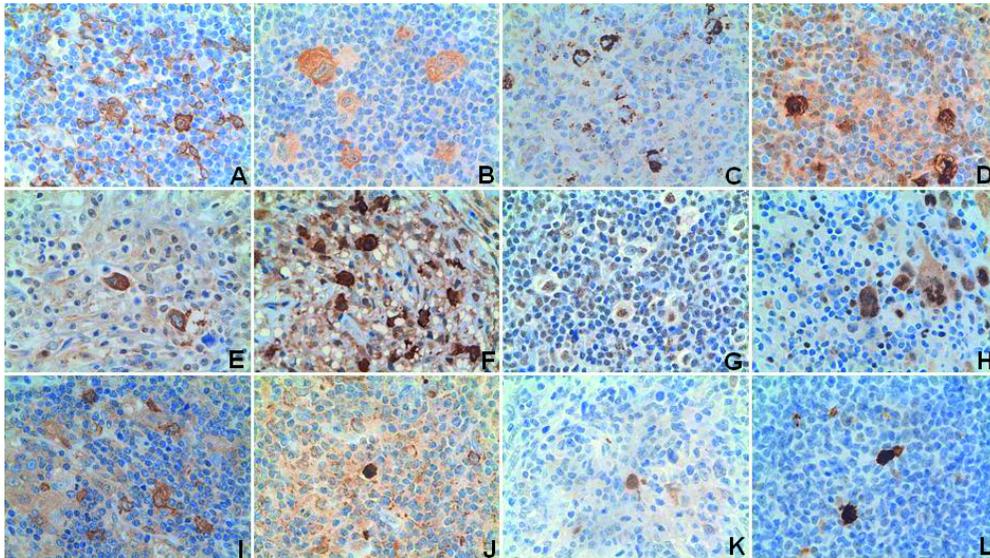
\* Two patients were treated only with excisional surgery and 3 received only radiotherapy

APSCT: autologous peripheral stem-cell transplantation.

### **Titles and legends to figures**

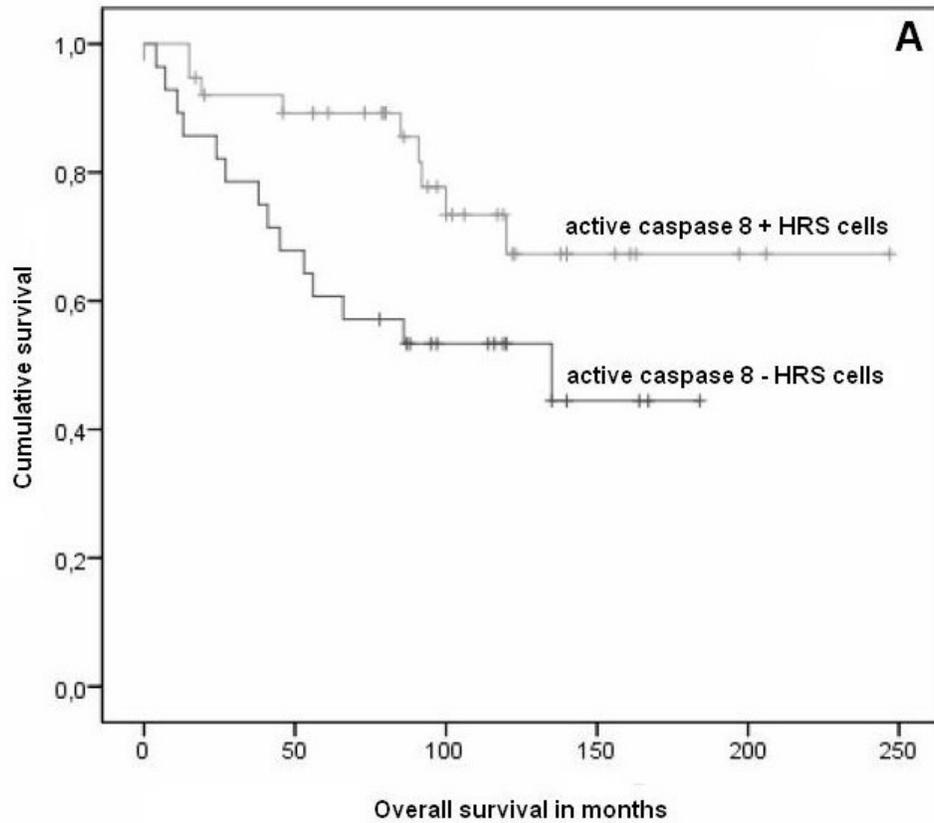
**Figure 1.** Immunohistochemical staining patterns of the main markers studied in our classical Hodgkin lymphoma cases (X600): (A) HSP27, (B) HSP40, (C) HSP60, (D) HSP70, (E) HSP90, (F) HSP110, (G) HO1, (H) HSF1, (I) CDC37, (J) active caspase 3, (K) active caspase 8, (L) cleaved PARP.

**Figure 2.** Comparison of overall survival time according to active caspase 8 expression in Hodgkin/Reed-Sternberg cells.



Immunohistochemical staining patterns of the main markers studied in our classical 250x140mm (96 x 96 DPI)

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Comparison of overall survival time according to active caspase 8 expression in Hodgkin/Reed-Sternberg cells.  
56x51mm (300 x 300 DPI)

