A molecular risk score based on four functional pathways for advanced
classical Hodgkin lymphoma

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Running title: Molecular risk score for classical Hodgkin Lymphoma

Keywords: classical Hodgkin lymphoma, quantitative RT-PCR, outcome prediction, cell cycle, apoptosis, microenvironment.

Footnote: Members of the Spanish Hodgkin Lymphoma Study Group are listed in the Supplemental Appendix.

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Abstract

Despite improvement in the treatment of advanced classical Hodgkin Lymphoma, around 30% relapse or die as result of the disease. Current predictive systems, based on clinical and analytical parameters, fail to identify these high-risk patients accurately. We took a multistep approach to design a quantitative RT-PCR assay to be applied to routine formalin-fixed paraffin-embedded samples, integrating genes expressed either by the tumor cells and their microenvironment. The significance of 30 genes chosen on the basis of previously published data was evaluated in a set of 282 samples (divided into estimation and validation sets) to build a molecular risk score to predict failure. Adequate RT-PCR profiles were obtained from 262 of 282 cases (92.9%). Best predictor genes were integrated into an 11-gene model including four functional pathways (Cell Cycle, Apoptosis, Macrophage Activation and IRF4) able to identify low- and high-risk patients with different rates of 5-year FFS: 74% versus 44.1% in the estimation set (p < 0.0001); and 67.5% vs. 45.0% in the validation set (p = 0.0217). Moreover, this biological model can be combined with stage IV into a final predictive model able to identify a group of patients with very bad outcome (5-year FFS probability 25.2%).
INTRODUCTION

Classical Hodgkin lymphoma (cHL) is assumed to be a curable tumor, but an important fraction of patients with advanced disease do not respond favorably to the current standard chemotherapy regimens based on adriamycin. The most widely used and reproducible prognostic score is based on clinical and analytical parameters integrated in the International Prognostic Score (IPS), but this still fails to identify accurately, at the moment of diagnosis, a significant fraction of patients with very poor prognosis.\(^1-^3\) Thus, the identification of biomarkers that, at diagnosis, may be consistently associated with failure, is essential for the recognition of patients at high risk of treatment failure, in order to establish a more rational risk-adapted treatment strategy.

Classical HL represents a distinctive model of histological complexity, with a minor population of the neoplastic Hodgkin and Reed-Sternberg (HRS) cells diluted in a reactive inflammatory background composed of non-neoplastic B- and T-cells, macrophages, eosinophils, neutrophils and plasma cells. The complex relationship between the HRS cells and their microenvironment is only partially understood, although important, if fragmentary, advances in our understanding are steadily being made.\(^4\) Clinical outcome of cHL has been found to be related with the expression of multiple biological markers alone\(^5-^8\) or in combination,\(^9\) expressed either by the tumor HRS cells, macrophages, regulatory T-cells or other non-neoplastic cell subpopulations.\(^10-^13\)

Some of these previous analyses rely on array-based gene expression analyses, which use frozen tissue and in most cases can only provide retrospective information. Other previous studies have used immunohistochemical staining, with some inherent limitations to the reproducibility of the data thus generated. It is now feasible to apply multigenic predictive molecular tests in advanced cHL patients in a routine setting, using a
quantitative RT-PCR assay as we here describe, incorporating a selected number of genes that capture information from tumor and microenvironment cell components, designed for application to routine formalin-fixed paraffin-embedded (FFPE) samples and that can be used at the moment of the initial diagnosis.

**MATERIAL AND METHODS**

**Patients and samples**

Previous studies allowed us to identify a group of genes whose expression was associated with the response of patients with advanced cHL to standard first-line treatment. Thus, the selection of genes to be analyzed was based on results previously obtained in two independent series of 29 and 52 advanced cHL patients.\textsuperscript{10,11}

The patients included in this study fulfilled stringent, previously described criteria: age >16 yr, advanced cHL, Ann Arbor stage IV, III or IIB with bulky masses,\textsuperscript{1} proven HIV-negative status, who have been treated with a first-line standard chemotherapy regimen that included adriamycin —ABVD (adriamycin, bleomycin, vinblastine and dacarbazine) or ABVD variants— and for whom information was available about the achievement of Complete Remission (CR) and a follow-up of at least 12 months thereafter, which is a well known and accepted surrogate indication of the course of the disease. With respect to the latter, patients were considered to have had a favorable course if they had achieved CR and maintained it for at least 12 months, or with an unfavorable course, if they had either not achieved CR or if they had once had it but had relapsed during the following 12
months. All tissue samples consisted of representative pretreatment lymph node biopsies collected after revision and approved by the Institutional Review Board of the participating institutions of the Spanish Hodgkin Lymphoma Study Group. The study initially included 282 FFPE patients, who were randomly split and assigned to the training (194 cases) or validation sets (88 cases) on the basis of the minimum estimated sample size, to derive the final model (Table 1).

Additional exclusion criteria were insufficient RNA quality (purity ratio A260:A230 < 1.7) or a weak RT-PCR signal (average cycle threshold > 35) for the reference genes or in more than 10 genes of the assay. As a result, 20 patients were excluded and the remaining 262 patients (183 in the training group and 79 in the validation group) meeting these criteria were included in the statistical analysis (Table 1).

**Gene selection**

The genes included in the assay were initially selected from two preliminary expression-profiling studies \(^{10,11}\) that rendered a list of genes expressed by HRS and microenvironment cell subpopulations identified in unfavorable cHL patients. Selected genes were primarily chosen on the basis of their prognostic ability and capacity to represent biological functions identified as relevant in cHL pathogenesis.\(^{11}\) Additionally, the strength and consistency of primer and probe performance were also taken into account.\(^{11}\) The initial selection consisted of 30 genes, including genes expressed by the neoplastic cells involved in the cell cycle (G2/M), apoptosis, histones, chaperones, drug metabolism and MAPK signatures, and from microenvironment genes expressed by different cellular or functional populations of T-cells, monocytes, macrophages and dendritic cells (details of the RT-PCR assays are available in Supplementary Table 1).
Analysis of gene expression

Gene expression was analyzed using a customized TaqMan low-density array platform (Micro Fluidic Cards, Applied Biosystems, CA) on FFPE as previously described. A preamplification step (PreAmp, Applied Biosystems, CA) was used to improve the sensitivity of our assay for low-abundance target genes available from FFPE samples. Reactions were performed using the ABI PRISM 7900HT Sequence Detection system (Applied Biosystems, CA), measuring the expression of each gene in triplicate and then normalizing with a set of two reference genes (HMBS and GUSB) whose uniform expression in cHL tumor samples was tested in previous studies. Missing values were imputed using the K-nearest neighbor (KNN) algorithm.

Statistical analysis

Differences in the distributions of standard clinical parameters (age, gender, stage, IPS, the individual variables contained in IPS and outcome) in the estimation and validation datasets were tested by the Pearson chi-square test (Table 1).

The first endpoint of this study was the response to standard first-line treatment considering favorable response (F) and unfavorable response (U), as mentioned above. Data from second-line and salvage therapies and/or bone-marrow transplantation were not considered.

The selection of the best predictive genes and the logistic regression model was based only on the data from the training group of 183 patients, without any previous survival analysis using information from the validation group. Univariate regression analysis was
performed with treatment response (F versus U) as the dependent variable to identify genes significantly associated (p < 0.05) with outcome. In addition, final gene selection analysis was performed by cross-validation using three prediction algorithms (http://tnasas.bioinfo.cnio.es/): diagonal linear discriminant analysis (DLDA), support vector machines (SVM) and KNN. Cross-validation was used to test the classification ability of the initial set of significant genes in order to choose the strongest predictor genes, which were classified into functional groups on the basis of their known biological relationship and their coregulated expression as estimated by the Pearson correlation coefficient. Individual genes from each functional group were weighted using linear discriminant analysis (LDA). Finally, these functional gene clusters associated with cHL outcome were analyzed in a multivariate logistic regression model with response to therapy (F versus U) as a dependent variable. In this way, an algorithm was derived that combines these measurements into a quantitative “molecular risk score” (MRS), which can be used as a continuous variable to estimate the probability of treatment response. The MRS cut-off points were prespecified by using area under the receiver operating characteristic curve (ROC) analysis to define different risk groups. (See the Supplementary Appendix for details of the statistical analysis and methods). Finally, performance of the logistic regression model was tested in the validation group of patients (N=79).

For graphical representation, survival analyses were done by the Kaplan-Meier method and long-rank test separately in the training and validation series, and in the entire series. Since the primary objective of the study was to identify patients at high risk of treatment failure, we used failure-free survival (FFS) as the fundamental endpoint for survival analysis. FFS was defined as the time interval between treatment initiation and treatment failure or last follow-up. Failure was defined as either the failure to achieve CR, or the occurrence of progressive disease, irrespective of whether there had been an initial CR. Overall survival (OS), an end-point whose significance is imperfect since it is conditioned
by the effect of subsequent eventual treatments and complication of treatment, was included as a secondary end point in the survival analyses, defined as the time interval between diagnosis and death due to the lymphoma.

Finally, in the whole series a multivariate Cox’s proportional hazards model, including the data at diagnosis, the IPS stratified as previously defined (0-2 vs. ≥ 3), and its seven individual variables (hemoglobin < 10.5 g/dl; albumin < 4 g/dl; leucocytosis ≥ 15,000/mm³; lymphopenia < 600/mm³; age ≥ 45 yr; male gender; stage IV), was applied to test the independence of the MRS, including the remaining significant variables in a final integrative model.

All statistical analyses were two-sided, taking values of p < 0.05 to be significant. These were performed with SPSS 15.0 (SPSS Inc., Chicago, IL). Survival curves were assessed by the Kaplan-Meier method and risk groups were compared by the log-rank test. Plots were generated using GraphPadPrism v.5 (GraphPad Software, Inc.).
RESULTS

Gene selection and development of the Predictor Model

In training series, univariate regression analysis of the expression data for the 30 initially selected genes revealed 20 genes to significantly predict failure to first line treatment (Supplementary Table 3). When cross-validation was applied, the genes most frequently found in prognostic models consisted of a panel of 11 genes that were included in the final model: BCL2, BCL2L1, CASP3, HMMR, CENPF, CCNA2, CCNE2, CDC2, LYZ, STAT1 and IRF4.

To derive the model, we took a two-step approach, first combining individual gene-expression patterns into precise functional pathways, and then subsequently correlating these functional groups with the clinical outcome using multivariate logistic regression. Final selected genes were weighted using LDA and clustered into their corresponding functional pathways defined as Macrophage Activation (LYZ, STAT1), Cell Cycle (HMMR, CENPF, CCNA2, CCNE2, CDC2) and Apoptosis (BCL2, BCL2L1, CASP3) (Figure 1A). The Pearson correlation coefficient was significant for the genes included in each of the signatures (p < 0.001) apart from IRF4, which was included as an independent predictive gene since there were neither distinct functional relationships nor statistically significant correlations with other genes or pathways (Supplementary Table 4). These functional groups captured information about the tumoral HRS cells and their non-tumoral microenvironment, in agreement with previous studies. The multivariate logistic regression analysis integrating these pathways showed that Cell Cycle and Apoptosis terms were associated with an unfavorable outcome of patients while Macrophage Activation and IRF4 signatures had protective effects.

Thus, the optimized final model was based on the relative contributions of each of the four functional terms, as described in the following equation: Constant (-0.913) + (0.401 x
Apoptosis) + (0.284 x Cell cycle) + (-0.301 x Macrophage Activation) + (- 0.143 x IRF4).

The continuous probability function generated by the logistic regression was defined as the MRS to treatment failure and ranged from 0.06 to 0.813 (Figure 1B). ROC analysis was used to define a threshold for stratifying patients and the largest area under the curve (AUC) was obtained using 0.3 as the threshold, thus dividing the series into high-risk (MRS ≥ 0.3) and low-risk (MRS < 0.3) cases (Figure 1B and 1C).

Validation of the Molecular Risk Score

The MRS differed significantly between the various outcome groups (Supplementary Figure 2) and predicted treatment response with an accuracy of 68.9 percent in the estimation dataset and 70 percent in the validation dataset.

Since treatment failure was the main endpoint used to derive the logistic regression function, FFS as previously defined was used for validation of the model and Kaplan-Meier analysis of survival. Predicted probabilities also identified two risk groups associated with FFS in both the estimation and validation sets (Figure 1C and Supplementary Figure 3). FFS probabilities at 5 yr were 74.0 percent vs. 44.1 percent (p < 0.0001) in the training set and 67.5 percent vs. 45.0 percent (p = 0.0217) in the validation set.

Additionally, the analyses of OS showed different risk groups identified by the MRS in the estimation group of patients. The differences were not significant in the validation series, probably due to the limited number of events (Supplementary Figure 3).

There were no significant statistical differences in the MRS distributions of the two histological subtypes, nodular sclerosis and mixed cellularity cHL (p = 0.186, t-test), and the differences in survival between risk groups identified by the MRS remained significant.
in both the estimation and validation sets stratified by histological subtype (Supplementary Figure 4).

**Integrative model using Molecular Risk Score and clinical variables**

In the whole series a multivariate Cox proportional hazards model with FFS as the dependent variable, and including the MRS and the IPS, only the MRS was significant (Table 2).

No interaction was observed between the IPS, or the individual IPS variables and the MRS low- and high-risk groups (Supplementary Table 5). Thus, to compare the molecular risk algorithm and the individual IPS components, a backward stepwise selection Cox model was tested, with FFS as the dependent variable and including the MRS and the individual components of the IPS in the global series of samples. Only MRS and Stage IV were statistically significant (Table 2) and so were retained in the final Cox regression model.

Patient stratification into quartiles based on the Cox model identified a subgroup of advanced cHL patients (fourth quartile) with a very poor outcome: 5-year FFS of 24.3 percent (p < 0.0001) (Figure 2).
DISCUSSION

Here we describe, in a series of patients with advanced cHL, a 4-cluster / 11-gene model—derived from an initial selection of 30 potentially predictive markers—that can be detected by RT-PCR and integrated into a molecular risk algorithm that can identify patient subgroups with very different probabilities of treatment failure. This approach is based on reliable quantitative RT-PCR techniques applicable to paraffin-embedded diagnostic samples and follows similar approaches taken in breast cancer and other tumor types. This benefits from previous expression profiling studies performed in cHL, and the improved knowledge about the role of the tumoral cell and the microenvironment in the pathogenesis and outcome of this disease. This MRS, calculated in an initial estimation set, was confirmed in an independent set. Genes included in the score were selected on the basis of previous gene expression profiling data generated in independent sets of patients, and can be classified in four functional pathways.

The final 4-cluster / 11-gene model can additionally incorporate one of the well established clinical variables (stage IV), thus integrating the main molecular characteristics of the tumors related with treatment response and tumor burden estimation in a single scoring system. The multivariate Cox model indicates that most patients with stage IV cHL and with a high MRS ($\geq 0.3$) will have a very poor outcome, with 5-year FFS probability of 24.3 percent and OS probability of 76.3 percent. Therefore this combination of stage IV and high MRS identifies a group of patients with very bad outcome, who could have been initially missed by consideration of the IPS alone.

It is of note that in the present series the IPS did not show any significant prognostic influence on FFS. From the individual components of the IPS, only stage IV disease remained significant in multivariate analyses. This finding is in agreement with recent studies showing that IPS is of limited utility in advanced HL cases treated in the modern era, where more accurate pathologic diagnosis, improved control of therapy, use of growth
factors and enhanced supportive care are yielding better outcomes when compared with historic results.\textsuperscript{3} Thus deviations from the standard therapy cannot be justified based on the survival prediction capacity of the IPS, and identification of high-risk populations needs to be supplemented with molecular markers.

In the model, the expression of BCL2, BCL2L1, CASP3, HMMR, CENPF, CCNA2, CCNE2 and CDC2 included in the Apoptosis and Cell Cycle pathways, respectively, were correlated with short FFS. The expression of various antiapoptotic BCL2 family proteins has been repeatedly reported in HRS cells,\textsuperscript{7,28,29} thereby contributing to the survival of HRS cells. BCL2 and BCL2L1 (BCL-XL) are both frequently expressed by HRS cells in cHL and their levels have been associated with inferior FFS in patients treated with ABVD or equivalent regimens,\textsuperscript{5} thus confirming the importance of this group of apoptotic regulators for cHL outcome prediction. The second signature, Cell Cycle, is mainly comprised of genes coding for regulatory proteins of the S and G2/M phases of the cell cycle, thus directly related with cell proliferation. Again, expression of some of these markers has been previously described at the protein level in cHL,\textsuperscript{7,30,31} and a significant prognostic value for CDC2 (CDK1) and CCNA2 (Cyclin A2) protein expression in non-Hodgkin lymphomas was also found for both disease-free and overall survival. Interestingly, this aberrant association between increased expressions of antiapoptotic proteins and growth fraction-associated proteins in HRS cells provides further evidence that cell cycle and apoptosis regulation are profoundly disturbed and closely related in the disease, further justifying the inclusion of these two pathways in the predictive model. Moreover, pathways involved in cell cycle and apoptosis regulation are rational therapeutic targets. Indeed, inhibitors of Cyclin-Cdk complexes (including Cyclin E – Cdk2, Cyclin A – Cdk2, and Cyclin B – Cdk1 complexes) are currently under preclinical and clinical investigation in different cancer types,\textsuperscript{32-34} and these drugs could be considered for the treatment of advanced and refractory cHL patients. Likewise, the potential for targeting BCL2-related proteins in lymphoma is promising. Small molecule inhibitors of the Bcl-2
family have demonstrated high target affinity and an improved toxicity profile, and clinical trials of these agents are yielding interesting results.\textsuperscript{35}

Confirming previous observations about the importance of the reactive microenvironment for cHL patient outcome,\textsuperscript{9,11,12} LYZ and STAT1 genes, expressed at high levels in a subset of tissue monocytes and activated macrophages, are also included in this model, and correlated with prolonged FFS and better outcome. The relevance of the cell composition of the reactive background in cHL has been reinforced by the data recently reported by Steidl et al.\textsuperscript{36} They used gene-expression profiling to identify a gene signature of tumor-associated macrophages that is associated with treatment failure, in an approach methodologically similar to previous reports from our group and others.\textsuperscript{10,37} The discrepancy in the results concerning the role of macrophages may have arisen from technical differences (RT-PCR vs. microarray gene-expression and immunohistochemistry), or the selection of markers such as LYZ and STAT1 in this study, reflecting a specific functional status of the monocyte-macrophages.\textsuperscript{38}

In addition, IRF4 (MUM1) expression was associated with longer FFS. This gene is an interferon regulatory factor, lymphocyte-specific, induced after NF-kB activation that controls B-cell proliferation and differentiation and has recently been shown to be up-modulated by CD40 engagement in HL cells.\textsuperscript{39} Interestingly, the lack of IRF4 protein has been previously associated with outcome in cHL,\textsuperscript{40} representing a potential adverse prognostic factor. In addition, both IRF4 and BCL2L1 represent well-known NF-kB target genes\textsuperscript{41,42} whose expression is induced after NF-kB pathway activation. Thus, our final model includes important subrogates from the NF-kB activation, which is thought to be an essential pathogenetic mechanism in this disease. It is of note that Bernarski et al. recently described how the inhibition of the canonical NF-kB pathway enhances the proapoptotic effects of adriamycin,\textsuperscript{43} thus also identifying NF-kB inhibition as an interesting therapeutic approach.
In conclusion, we have developed a molecular risk algorithm, based on feasible and reproducible molecular techniques, that is capable of stratifying at the moment of diagnosis advanced cHL patients with different outcome. Moreover, combination of this algorithm with the presence of clinical stage IV disease, identifies a group of cHL patients with a very bad outcome who could benefit from more intensive therapeutic approaches.

These results are promising, but further validation in larger and independent series of patient is needed for the model to become established as part of the clinical routine. Also the predictive value of this model should also be tested in patients treated with modern intensive chemotherapy.
ACKNOWLEDGEMENTS

We thank Nuria Malats, from the Genetic and Molecular Epidemiology Group at the CNIO, for her useful advice and supervision of the statistical analysis. We also thank Marién Castillo and Laura Cereceda, at the CNIO Tumour Bank for collecting the human tumor samples, and their excellent assistance with data management. Members of the Spanish Hodgkin Lymphoma Study Group are cited in the Supplemental Appendix. This work was supported by grants from the Fondo de Investigaciones Sanitarias (PI08/1985, PI05/1623, PI05/2800, PI05/2327, RETIC RD06/0020/0107), and the Ministerio de Ciencia y Tecnología (SAF2008-03871), Spain. BS-E is supported by a grant from the Ministerio de Ciencia e Innovación (FIS), Spain.

AUTHORSHIP CONTRIBUTIONS

BSE, CM, JFG and MAP contributed to the conception and design of the study, the analysis and interpretation of the data, and the drafting of the article. AL and BSE did the statistical analysis. MMM managed tissue banking. JM, PS, CRM, AL, RR, JR, AC, CC, MC, JA, RA, AA, AS, SS, AB, JMM, PSG, FB, CR, MFF, JGL, MGC, CS, JLL, MLI, MM, JGC, AM, JF, RGC and JFT managed patients databases, and contributed with tumor samples and clinical follow-up. All the authors read and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest
REFERENCES


TABLES

Table 1. Clinical characteristics of patients with adequate RT-PCR profiles. Summary of the clinical characteristics of the patients in the estimation and validation sets that yielded suitable analyzable data (262 out of 282, 92.90 percent). Differences in distribution of standard clinical parameters (age, gender, stage, IPS and outcome) between estimation and validation datasets tested by Pearson chi-square with Yates correction were not statistically significant (IPS values of 0-2 classified as low IPS; IPS values > 2 classified as high IPS).

Table 2. The Molecular Risk Score (MRS) and the IPS variables. (A) Multivariate Cox regression analysis considering the MRS and the IPS. (B) Univariate Cox regression analyses considering the individual IPS variables and MRS. (C) Cox regression analysis of the final variables included in the integrative model (Molecular Risk + Stage IV) obtained by backward stepwise selection.
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Table 2. The Molecular Risk Score (MRS) and the IPS variables

| A. International Prognostic Score (IPS) and Molecular Risk Score (N = 262) |
|-----------------------------------|------------------|-------------------|
|                                   | p value | Hazard ratio (95% CI) |
| Molecular Risk Score              | 0.000   | 24.362 (6.268-94.691) |
| IPS                               | 0.205   | 1.111 (0.944-1.309)   |

| B. IPS variables and Molecular Risk Score (N = 262) |
|-------------------|------------------|-------------------|
|                                   | p value | Hazard ratio (95% CI) |
| Molecular Risk Score              | 0.000   | 24.715 (6.804-89.768) |
| Hemoglobin (< 10.5 g/dl)          | 0.352   | 1.243 (0.787-1.963)    |
| Albumin (< 4 g/dl)                | 0.504   | 1.157 (0.755-1.772)    |
| Leucocytosis (≥ 15,000/mm³)      | 0.256   | 1.312 (0.821-2.095)    |
| Lymphopenia (< 600/mm³)          | 0.555   | 0.820 (0.425-1.583)    |
| Age (≥ 45 yr)                    | 0.369   | 1.227 (0.785-1.916)    |
| Stage IV                         | 0.041   | 1.552 (1.018-2.360)    |
| Male gender                      | 0.753   | 0.936 (0.618-1.416)    |

| C. Integrative Cox model (N = 262) |
|-----------------------------------|------------------|-------------------|
|                                   | p value | Hazard ratio |
| Molecular Risk Score              | 0.000   | 23.782 (6.041 - 94.340) |
| Stage IV                          | 0.025   | 1.409 (1.044 - 1.900)  |
FIGURE LEGENDS

Figure 1. Panel of 11 genes and the molecular risk algorithm. A) The molecular risk algorithm is based on the relative contributions of each of the four gene functional groups from the tumoral HRS and their reactive microenvironment as follows: Molecular Risk Score = exp (fx)/ [1+exp (fx)], where fx = (-0.319) + (0.401 x Apoptosis) + (0.284 x Cell Cycle) + (-0.301 x Monocyte) + (-0.143 x IRF4). Coefficients were derived from a multivariate analysis in which positive values indicate that a higher level of expression is correlated with a worse outcome, and negative coefficients indicate that a higher level of expression of the pathways is associated with a better outcome. B) Molecular Risk Score as a continuous function was used to set a threshold for stratifying patients by ROC analysis. Patients were stratified according to the levels of the molecular risk score into low-risk (< 0.3) and high-risk (≥ 0.3) groups. C) and D). Survival estimates of failure-free survival in patients from estimation (N = 183) and validation (N = 79) sets after classification into risk groups. Kaplan-Meier analysis and the log-rank test gave significant results in both estimation and validation sets, indicating the potential prognostic capacity of the algorithm developed here.

Figure 2. Integrative Risk Model of cHL. The final Cox model integrates the molecular risk score (MRS) and clinical variable Stage IV. Patients in quartiles 1, 2 and 3 have comparable failure-free survival (FFS) rates at 5 yr (76.4, 79.3 and 69.7 percent, respectively) while patients in quartile 4 show a 5-year FFS of 24.3 percent (p < 0.001).
Figure 1

A

<table>
<thead>
<tr>
<th>Apoptosis</th>
<th>Macrophage activation</th>
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<tbody>
<tr>
<td>BCL2</td>
<td>LYZ</td>
</tr>
<tr>
<td>BCL2L1</td>
<td>STAT1</td>
</tr>
<tr>
<td>CASP3</td>
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<tr>
<td>Others</td>
<td>IRF4</td>
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Cell cycle

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<tr>
<th>CCNA2</th>
<th>CDC2</th>
<th>HMMR</th>
<th>CCNE2</th>
<th>CENPF</th>
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<tbody>
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Reference

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<th>HMBS</th>
<th>GUSB</th>
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B

Molecular Risk Probability
N=183

Risk Probability

Cut-off=0.3

High Risk

Low Risk

C

Estimation Set
N=183

Probability of FFS

Month

Low Risk

| Number at risk | 131 | 103 | 99  | 99  | 99  |

High Risk

| Number at risk | 52  | 28  | 24  | 22  | 22  |

D

Validation Set
N=79

Probability of FFS

Month

Low Risk

| Number at risk | 56  | 41  | 39  | 39  | 39  |

High Risk

| Number at risk | 23  | 12  | 11  | 11  | 11  |

p value <0.0001

p value = 0.0217
Figure 2

Integrative cHL predictive model (MRS and Stage IV)

Probability of FFS

Number at Risk | Months
---|---
q1 | 65  | 55  | 50  | 48  | 48
q2 | 66  | 58  | 54  | 54  | 54
q3 | 64  | 52  | 50  | 50  | 50
q4 | 67  | 19  | 19  | 19  | 19

p value <0.0001
A molecular risk score based on four functional pathways for advanced classical Hodgkin lymphoma

Beatriz Sánchez-Espiridión, Carlos Montalbán, Ángel López, Javier Menárguez, Pilar Sabin, Carmen Ruiz-Marcellán, Andrés Lopez, Rafael Ramos, Jose Rodríguez, Araceli Cánovas, Carmen Camarero, Miguel Canales, Javier Álvarez, Reyes Arranz, Agustín Acevedo, Antonio Salar, Sergio Serrano, Agueda Bas, Jose M. Moraleda, Pedro Sánchez-Godoy, Fernando Burgos, Concepción Rayón, Manuel F. Fresno, José García Laraña, Mónica García-Cosío, Carlos Santonja, Jose L. López, Marta Llanos, Manuela Mollejo, Joaquín González-Carrero, Ana Marín, Jerónimo Forteza, Ramón García-Sanz, Jose F. Tomás, Manuel M. Morente, Miguel A. Piris and Juan F. García