MAL Is Expressed in a Subset of Hodgkin Lymphoma and Identifies a Population of Patients With Poor Prognosis

Eric D. Hsi, MD,1 Stephen J. Sup, MD,1 Carlos Alemany, MD,2 Elisa Tso, MD,2 Marek Skacel, MD,1 Paul Elson, ScD,2 Miguel A. Alonso, PhD,3 and Brad Pohlman, MD2

Key Words: MAL; Hodgkin lymphoma; Prognosis; Microarray

Abstract

Classic Hodgkin lymphoma (cHL) and mediastinal (thymic) large B-cell lymphoma (MLBL) have clinical, histopathologic, and molecular genetic similarities. MAL, a gene that encodes a protein associated with lipid rafts in T and epithelial cells, is overexpressed in a majority of MLBLs and has been reported in a minority of cHLs. To study the clinical significance of MAL in cHL, we immunostained 86 cases; 16 cHLs (19%) expressed MAL. Expression correlated with nodular sclerosis subtype, and within this subtype, with grade 2 histology. Univariable analysis revealed association of age of 45 years or older, MAL expression, and an International Prognostic Score of more than 2 with worse failure-free survival. Age of 45 years or older, MAL expression, and stage III or IV were associated with worse overall survival (OS). Cox proportional hazards modeling showed age (P = .04 and P = .03, respectively) and MAL expression (P = .03 and P = .01, respectively) as independent predictors of failure-free survival and OS. Stage was of borderline significance in OS (P = .08). MAL expression seems to identify a subset of cHL with an adverse outcome and provides additional evidence for a link between cHL and MLBL.

In the vast majority of cases, Hodgkin lymphoma (HL) is a B-cell lymphoma that is derived from germinal center stage B cells that have defective immunoglobulin transcription.1 Studies suggest a relationship between HL and mediastinal (thymic) large B-cell lymphoma (MLBL), a particular type of diffuse large B-cell lymphoma (DLBL).2-4 HL and MLBL share many clinical, histologic, immunophenotypic, and genetic features. MLBL manifests in younger (often female) patients with a large mediastinal mass—a presentation that also is common in classic HL.4 Furthermore, MLBL characteristically contains fibrosis, like nodular sclerosis HL. As with HL, MLBL often expresses CD30 and lacks detectable immunoglobulin.5,6 Similar genetic abnormalities such as gain of 2p and 9p involving the cREL and JAK2 genes are described in MLBL and HL.2,7-11 In fact, recent studies suggest that the gene expression profile of MLBL differs from typical DLBL and actually has similarities to HL.2,12 Cases of “gray” zone lymphomas, in which the pathologic differential diagnosis between mediastinal classic HL and MLBL is difficult, are well-recognized and support some degree of overlap between these 2 entities.13,14

A 1999 study identified MAL expression in cases of MLBL.15 MAL is a gene that encodes a membrane protein involved in lipid raft organization during T-cell activation and signal transduction.16-18 It also is important in apical transport in epithelial cells.18,19 By immunohistochemical analysis, the MAL gene product is expressed in the majority of MLBLs but not in nodal DLBLs.15 It is interesting that MAL has been reported to be expressed in a subset of HL,17 further supporting a link between HL and MLBL.

To date, the clinical significance of MAL expression in HL is unknown. We evaluated MAL expression by immunohistochemical analysis in a series of DLBL,
Hematopathology / ORIGINAL ARTICLE

MLBL, and HL cases. We then analyzed the clinical significance of MAL expression in HL, and we report that it is a marker of adverse outcome.

Materials and Methods

Cases and Tissue Samples

Paraffin blocks from 33 cases of DLBL, 41 cases of MLBL, and 86 cases of classic HL obtained at the time of initial diagnosis (between January 1988 and December 2002) were retrieved from the archives of the Division of Pathology and Laboratory Medicine, Cleveland Clinic, Cleveland, OH. Tissue microarrays (TMAs) were constructed using two to four 1.5-mm cores per case. For HL, areas rich in Reed-Sternberg (RS) cells were specifically selected. Whole sections of all cases were reviewed by a hematopathologist (E.D.H.) using World Health Organization criteria.20 Nodular sclerosis HL cases were subtyped by grade (grade 1 vs grade 2) according to the British National Lymphoma Investigation criteria.21,22 For MLBL, cases were selected based on DLBL involving primarily the mediastinum.

For HL cases, medical records were reviewed, and the following clinical and laboratory data from the time of diagnosis were obtained: age, sex, stage, bulky tumors (defined as a mediastinal widening of ≥one third of the thoracic diameter or a mass ≥10 cm in maximum dimension), hemoglobin level, WBC count, lymphocyte count, and albumin level. The International Prognostic Score (IPS)23 was calculated based on the 7 defined high-risk features (ie, serum albumin level, <4 g/dL [<40 g/L]; hemoglobin, <10.5 g/dL [<105 g/L]; male sex; stage IV disease; age, ≥45 years; WBC count, ≥15,000/µL [≥15.0 × 10⁹/L]; and lymphocyte count, <600/µL [<0.6 × 10⁹/L]), and cases were divided into a low-risk group (0-2 factors) and a high-risk group (3-7 factors). Initial treatment for each case was reviewed. All patients were treated with curative intent and according to standards of care at the time. Of the patients, 14% were treated with radiation alone, 47% with multiagent chemotherapy alone (mechlorethamine, vincristine [Oncovin], procarbazine, and prednisone [MOPP]; doxorubicin [Adriamycin], bleomycin, vinblastine, and dacarbazine [ABVD], or hybrid therapy), and 40% received radiation therapy and chemotherapy. No statistically significant differences in therapy were present between MAL+ and MAL– cases. The characteristics of the 86 patients with HL are shown in Table I.

Table I

<table>
<thead>
<tr>
<th>Factor</th>
<th>MAL–</th>
<th>MAL+</th>
<th>P†</th>
<th>No. of Failures²</th>
<th>5-Year Failure-Free Survival (%)</th>
<th>P†</th>
<th>No. of Deaths</th>
<th>5-Year Survival (%)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>37 (86)</td>
<td>13</td>
<td>.41</td>
<td></td>
<td>71 ± 7</td>
<td>.49</td>
<td>8</td>
<td>85 ± 6</td>
<td>.83</td>
</tr>
<tr>
<td>Female</td>
<td>33 (77)</td>
<td>9</td>
<td></td>
<td></td>
<td>81 ± 6</td>
<td>.49</td>
<td>7</td>
<td>86 ± 6</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>56 (84)</td>
<td>13</td>
<td>.33</td>
<td></td>
<td>79 ± 5</td>
<td>.04</td>
<td>7</td>
<td>91 ± 4</td>
<td>.002</td>
</tr>
<tr>
<td>≥45</td>
<td>14 (74)</td>
<td>9</td>
<td></td>
<td></td>
<td>63 ± 11</td>
<td></td>
<td>8</td>
<td>67 ± 11</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I or II</td>
<td>42 (81)</td>
<td>9</td>
<td>1.00</td>
<td></td>
<td>82 ± 5</td>
<td>.21</td>
<td>5</td>
<td>92 ± 4</td>
<td>.08</td>
</tr>
<tr>
<td>III or IV</td>
<td>28 (82)</td>
<td>13</td>
<td></td>
<td></td>
<td>66 ± 8</td>
<td>.21</td>
<td>10</td>
<td>77 ± 8</td>
<td></td>
</tr>
<tr>
<td>IPS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.31</td>
<td></td>
<td>.03</td>
<td>.20</td>
</tr>
<tr>
<td>≤2</td>
<td>57 (84)</td>
<td>15</td>
<td></td>
<td></td>
<td>79 ± 5</td>
<td>.47</td>
<td>11</td>
<td>89 ± 4</td>
<td>.55</td>
</tr>
<tr>
<td>&gt;2</td>
<td>13 (72)</td>
<td>7</td>
<td></td>
<td></td>
<td>61 ± 12</td>
<td></td>
<td>4</td>
<td>75 ± 11</td>
<td></td>
</tr>
<tr>
<td>Bulky disease (n = 84)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>42 (84)</td>
<td>12</td>
<td></td>
<td></td>
<td>79 ± 6</td>
<td></td>
<td>10</td>
<td>83 ± 6</td>
<td>.55</td>
</tr>
<tr>
<td>Yes</td>
<td>26 (76)</td>
<td>10</td>
<td></td>
<td></td>
<td>68 ± 9</td>
<td></td>
<td>5</td>
<td>91 ± 5</td>
<td></td>
</tr>
<tr>
<td>Mediastinal biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
<td></td>
<td>.38</td>
<td>.20</td>
</tr>
<tr>
<td>No</td>
<td>62 (82)</td>
<td>21</td>
<td></td>
<td></td>
<td>74 ± 5</td>
<td></td>
<td>15</td>
<td>84 ± 4</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8 (80)</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>0</td>
<td>84 ± 4</td>
<td></td>
</tr>
<tr>
<td>Histologic diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.02</td>
<td></td>
<td>.12</td>
<td>.11</td>
</tr>
<tr>
<td>Nodular sclerosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>52 (76)</td>
<td>19</td>
<td></td>
<td></td>
<td>72 ± 6</td>
<td>.12</td>
<td>13</td>
<td>82 ± 5</td>
<td></td>
</tr>
<tr>
<td>MAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.01</td>
<td></td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IPS, International Prognostic Score.

Data are given as number (percentage) unless otherwise indicated.

Fisher exact test.

Relapse or death.

Generalized Wilcoxon test.

5-year rate cannot be calculated.

Mixed cellularity (n = 13), lymphocyte rich (n = 4), and unclassified (n = 1).
Immunohistochemical Analysis

Immunohistochemical analysis for MAL was performed as previously described. Cases were reviewed by 2 hematopathologists (E.D.H. and M.S.) and scored as positive for MAL if any MAL staining was present in neoplastic cells. Nonneoplastic small lymphocytes, which express MAL, were used as internal positive control cells. Immunohistochemical analysis for OCT2 (rabbit polyclonal, dilution 1:200, pH 8.0, heat-induced epitope retrieval [HIER], Santa Cruz, Santa Cruz, CA), CD20 (L26, dilution 1:400, HIER, DAKO, Carpinteria, CA), CD30 (BerH2, dilution 1:10, HIER, DAKO), CD15 (LeuM1, dilution 1:10, HIER, Becton Dickinson, San Jose, CA), and CD45 (PD7/26, dilution 1:80, DAKO) was performed using automated Ventana Benchmark immunostainers (Ventana Medical Systems, Tucson, AZ). Immunostaining for BOB1 (rabbit polyclonal, dilution 1:500, HIER, Santa Cruz) was performed using the MACH3 peroxidase polymer detection system (Biocare Medical, Walnut Creek, CA) according to the manufacturer’s specifications. Immunoreactivity for OCT2 and BOB1 was considered strong if reactivity in the RS cells was of equal or greater intensity than that for internal positive control cells (B cells).

Statistical Methods

Overall survival (OS) was measured from the date of initial diagnosis to the date of death or the date of last follow-up. Failure-free survival (FFS) was measured from the date of diagnosis to the date of first relapse or the date of death, whichever came first. Patients alive and free of disease at analysis were censored as of their last follow-up. The Kaplan-Meier method was used to summarize FFS and OS data and to estimate 5-year survival rates. The generalized Wilcoxon rank sum test was used to compare OS and FFS for individual factors, and the Cox proportional hazards model with step-wise variable selection was used to simultaneously assess multiple factors. Comparisons of categorical variables, such as sex and IPS, between cases expressing and not expressing MAL were performed using the Fisher exact test. Because we were evaluating prognostic factors for outcome, P values less than .1 were considered statistically significant. All analyses were performed using SAS (Statistical Analysis Software, version 8.0, SAS Institute, Cary, NC).

Results

MAL Is Expressed in MLBL and HL but Only Rarely in DLBL

To confirm previous findings of MAL expression and relative specificity for MLBL, we stained a series of TMAs containing cases of DLBL and MLBL. MAL was expressed in 22 (54%) of 41 MLBL cases and only 1 (3%) of 33 DLBL cases. This DLBL case was from a cervical lymph node of a 45-year-old patient with stage IV disease. Extensive adenopathy was present, including in the mediastinum, but a dominant mass (12 × 14 cm) was present in the pelvis. Only scattered MAL+ lymphoma cells were present. Expression of MAL in HL ranged from only a few positive cells in some cases to intense positivity in the majority of cases.

HL Expressing MAL Has a Poor Outcome Compared With HL Lacking MAL

Overall, in 22 patients with HL, therapy failed (relapse or death), and 15 patients died. The median follow-up of patients alive and free of disease was 6.9 years (range, 1.0-16.9 years).
The 5-year FFS and OS were 75% ± 5% and 86% ± 4%, respectively. In univariable analyses, an age of 45 years or older, stage III or IV, an IPS of more than 2, and MAL expression were associated with a worse prognosis (Table 1). Within the nodular sclerosis subtype, grade 2 histologic features were not associated with 5-year FFS ($P = .94$) or 5-year OS ($P = .59$). Specifically for MAL, the 5-year FFS was 80% ± 5% for MAL− cases and 56% ± 13% for MAL+ cases ($P = .01$), and the 5-year OS was 92% ± 3% for MAL− cases and 57% ± 14% for MAL+ cases ($P = .002$).

Because a number of factors seem to influence outcome, Cox proportional hazards models were used to assess their simultaneous impact and identify independent predictors of outcome. The results of these analyses are summarized in Table 2. An age of 45 years or older and MAL expression were independent predictors of OS ($P < .05$). Advanced stage also was of borderline significance for OS ($P = .08$). Kaplan-Meier FFS and OS curves for MAL are shown in Figure 1 and Figure 2, respectively.

MAL+ and MAL− HL Specimens Have Similar Expression of OCT2 and BOB1

To more fully characterize the MAL+ cases of HL, we performed more extensive phenotyping in these cases to determine whether other differences in diagnostic markers might be present. First, we phenotyped these cases with antibodies typically used in routine diagnosis: CD15, CD30, CD20, and CD45. Staining of the MAL+ cases revealed the expected expression patterns. All 16 MAL+ HL cases strongly expressed CD30, and 15 of 16 lacked CD45. The single case expressing CD45 did so only weakly in 10% of RS cells. CD15 was expressed in more than 10% of RS cells (usually >75%) in 12 (75%) of 16 cases. CD20 was expressed only focally (<50% of RS cells) in 3 cases (19%) and was negative in the remaining cases. Thus, MAL+ HL cases resembled typical HL cases using these standard immunohistochemical markers for HL.

Expression of OCT2 and BOB1 have been suggested to be diagnostically useful in HL. These transcription factors are

### Table 2

**Multivariable Analysis of Failure-Free and Overall Survival**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Failure-Free Survival</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parameter Estimate</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>Age (&gt;45 y)</td>
<td>0.92 ± 0.44</td>
<td>2.51</td>
</tr>
<tr>
<td>MA+</td>
<td>1.00 ± 0.47</td>
<td>2.72</td>
</tr>
<tr>
<td>Stage (III/IV)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
expressed strongly in B cells but show weak or decreased expression in RS cells in the great majority of cases. In the TMA, strong expression of OCT2 was seen in 7 (9%) of 80 evaluable cases, all MAL−. Strong expression of BOB1 was seen in 5 (6%) of 84 evaluable cases, 3 MAL− and 2 MAL+. There was no difference in OCT2 or BOB1 expression between MAL+ and MAL− cases (P = .34 and P = .24, respectively).

Discussion

HL is, in virtually all cases, a malignant B-cell neoplasm. Single-cell polymerase chain reaction analysis has proven that the RS cell is a mature B cell of germinal center origin. MLBL is a recognized subtype of DLBL, which manifests in relatively young patients with a mediastinal mass. The MLBL cells often have a clear cell appearance with background sclerosis, often express CD30, and lack surface immunoglobulin. Many of these features are similar to those of nodular sclerosis HL. At the genetic level, the pathobiology of HL and MLBL involves the activation of the NFκB pathway and amplification of JAK2. In fact, a recent study suggests that HL and MLBL have similar genetic expression profiles. The MAL gene, which is involved in lymphoid development, is expressed in MLBL but rarely in “typical” DLBL. Only 1 study examined MAL expression in HL; MAL was expressed in fewer than 10% of cases. By using immunohistochemical analysis, we showed expression of MAL in 54% of MLBL cases but only 3% of DLBL cases, similar to previous findings. Our data further support the contention that MLBL is distinct from other DLBLs. We also found that MAL is expressed in 19% of classic HL cases, but only in the nodular sclerosis subtype. Immunophenotypically, these MAL+ cases were typical for classic HL using standard markers such as CD15, CD20, CD30, and CD45.

OCT2 and BOB1, transcription factors that are expressed at low levels in RS cells compared with normal B cells, also showed decreased expression in our cases: 9% and 6% of HL cases strongly expressed OCT2 and BOB1, respectively. These findings are in keeping with other reports showing only a minority of cases with strong expression of these markers in classic HL (0%-16% for OCT2 and BOB1). Furthermore, no difference in OCT2 or BOB1 expression was found between MAL+ and MAL− cases. These data suggest that MAL indeed might have a role in the biology of a subset of HL. One possibility is an effect on the extracellular environment given the presence of fibrosis in MLBL and nodular sclerosis HL; however, no direct or indirect role has been ascribed for MAL in development of this fibrosis.

It is interesting that MAL expression also correlated statistically (albeit imperfectly) with histologic grade 2 nodular sclerosis HL. This finding may help explain some reports of a poorer prognosis for patients with this histologic grade, whereas other groups found no prognostic significance. One may speculate that some series included more cases of MAL+ HL in the population of grade 2 cases, accounting for the finding of a poor outcome. Other series may have had fewer MAL+ cases and, therefore, might have found no difference in outcome.

The variable expression of MAL in classic HL suggests a biologic heterogeneity in this disease. We speculate that cases of MAL-expressing HL, in particular, may have more in common with MLBL than other cases of HL. Thus, one might expect that patients with MAL+ HL might have a

![Figure 1](image1.png) Kaplan-Meier failure-free survival analysis according to MAL expression. The 5-year failure-free survival was 80% ± 5% for MAL− cases and 56% ± 13% for MAL+ cases (P = .01).

![Figure 2](image2.png) Kaplan-Meier overall survival analysis according to MAL expression. The 5-year overall survival was 92% ± 3% for MAL− cases and 57% ± 14% for MAL+ cases (P = .002).
worse prognosis than other patients with HL when treated with HL therapy. Indeed, compared with other clinical features, expression of MAL was an independent risk factor for adverse outcome. As found in other studies, advanced age (≥45 years) also was a risk factor for OS in our series. Advanced stage was of borderline significance. Lack of a stronger association between stage and outcome likely is because of the relatively few cases in this series. Overall, our outcome data are in line with the published literature, with patients with low-stage disease having a 92% 5-year OS and patients with high-stage disease having a 77% 5-year OS (Table 1).31

HL is not the first tumor system in which MAL has been associated with adverse outcome. Tracey and colleagues32 created interferon-α-resistant cutaneous T-cell lymphoma cell lines and found that MAL was overexpressed compared with interferon-α-sensitive cells. In addition, expression of MAL in a series of cutaneous T-cell lymphomas was associated with longer time to remission in interferon-treated patients.32 Recently, gene expression profiling in ovarian carcinoma revealed that MAL overexpression was associated with poor survival.33 The biologic mechanisms involved in conferring an adverse outcome in these studies are yet to be elucidated.

We recognize the limitations of this study, given its retrospective nature. If confirmed, however, this information could have therapeutic implications, in addition to risk stratification. A recent retrospective international study suggested that patients with MLBL who are treated with more intensive “third-generation” chemotherapy regimens (eg, methotrexate, doxorubicin, cyclophosphamide, vincristine, and prednisone [MACOP-B]) had a better outcome than patients treated with “first-generation” regimens (eg, cyclophosphamide, doxorubicin, vincristine, and prednisone [CHOP]).34 If, in fact, MAL+ HL is biologically closely related to MLBL, perhaps a treatment strategy that is effective in MLBL might lead to better outcomes in MAL+ HL.

MAL is expressed in just more than half of MLBL cases and absent in typical DLBL cases. MAL is expressed in a minority of classic HL cases, but only the nodular sclerosis type, often in cases with grade 2 histologic features. These data further support a relationship between a subset of HLs and MLBLs. Furthermore, MAL expression in HL predicted a relatively poor prognosis in our study population. The function of MAL in RS cells is unknown. However, its involvement in lymphoid development and signal transduction suggests an important role in the pathobiology of HL and MLBL and also suggests a potential target for specific therapies. Further study of MAL-expressing HL is warranted.

References


