Prognostic Significance of Lung Resistance Protein (LRP) and Multidrug Resistance Protein (MRP1) in Patients with Diffuse Large B-Cell Lymphomas (DLBCL)

WAEL H. ELSAWY, M.D.; FOUAD M. ABOU TALEB, M.D.; MOHAMAD ABDEL KADER, M.D.; ALAA A. OMRAIN, M.D. and AMAL F. GHARIB, M.D.

The Departments of Clinical Oncology, Medical Oncology, Clinical Pathology, Medical Biochemistry, Faculty of Medicine, Zagazig University; Beni-Sweef Branch of Cairo University.

ABSTRACT

Aim of the work: Drug resistance of non-Hodgkin’s lymphomas may involve mechanisms of the multidrug resistance phenotype including the lung resistance protein (LRP) and the multidrug resistance protein (MRP1). To determine the prognostic significance of these multidrug resistance factors, we studied LRP and MRP1 expression and their impact on clinical outcome in previously untreated 48 patients with diffuse large B-cell lymphomas.

Patients and methods: LRP and MRP1 expression were immunohistochemically assessed by means of the monoclonal antibodies LRP-56 and MRPr1, respectively.

Results: LRP was positive in 23% and MRP1 in 44% of the patients. LRP expression was associated with higher tumor stage (p = 0.03), elevated serum lactate dehydrogenase levels (p = 0.01) and the International Prognostic Index (p = 0.0001). LRP-positive patients had a lower complete response rate to chemotherapy than LRP-negative patients (18 versus 65%; p = 0.006) and a shorter overall survival (median of 0.9 years versus undetermined, p = 0.001). MRP1 expression was independent of clinical and laboratory parameters and had no impact on the outcome of chemotherapy or survival of the patients.

Conclusion: Our results suggest that LRP expression may be an important mechanism of drug resistance and is associated with a worse clinical outcome in previously untreated diffuse large B-cell lymphomas. Thus, the reversal of LRP-mediated drug resistance may improve clinical outcome in diffuse large B-cell lymphoma in the future.

Key Words: Non-Hodgkin’s lymphoma - Chemotherapy - LRP - MRP1.

INTRODUCTION

Diffuse large B-cell lymphomas (DLBCL) can be effectively treated with conventional combination chemotherapy regimens with or without radiotherapy. In addition, high-risk patients may benefit from either high-dose consolidation treatment with hematopoietic stem cell support after having achieved complete response from initial chemotherapy [17] or initial high-dose induction chemotherapy with stem cell support [15]. Despite these improvements, 40-50% of the patients are not cured by chemotherapy because of drug-resistant disease [17].

Multidrug resistance (MDR) is an important type of drug resistance that is clinically relevant in leukemias [30,36] and several solid tumors [12]. Different mechanisms can contribute to MDR; some of them have already been studied in non-Hodgkin’s lymphomas. MDR1/P-glycoprotein expression occurs with various frequencies in lymphomas and is associated with clinical drug resistance to various anticancer drugs including anthracyclines and Vinca alkaloids [12,41]. Clinical trials to overcome P-glycoprotein-mediated resistance in drug-refractory lymphoma by combining chemotherapy with resistance modifiers indicated that, at least in a subset of patients with drug-refractory lymphoma, modulation of P-glycoprotein function is feasible. This suggests that P-glycoprotein expression plays a role in the drug resistance of lymphomas [32,49,51]. MRP1, another important factor involved in MDR, is also expressed in lymphomas [52], but its impact on clinical outcome remains to be determined. Alterations in apoptosis and cell cycle regulation are also involved in drug resistance of lymphomas [22,33,39,50]. p53 mutations were associated
The clinical characteristics of the patients are summarized in Table (1). All patients were clinically examined and subjected to routine laboratory and radiological investigations for proper staging. All patients received standard therapy with CHOP protocol. Cycles were repeated every 21 days. Patients with stage I with bulky disease received 3-4 cycles of chemotherapy plus involved field radiotherapy (30 Gy, 2 Gy/fraction over 3 weeks).

Patients with stage II-IV disease received 6 cycles of chemotherapy. Two of them with bulky disease who achieved a complete response were treated with additional involved field radiotherapy after 6 cycles of chemotherapy. All of the patients were evaluable for response. Response to chemotherapy was assessed according to standard criteria [4,47]. Complete response was defined as the absence of clinical and radiological evidence of disease for a minimum of at least 2 months. Age, tumor stage, serum lactate dehydrogenase, performance status and the number of extranodal sites of the disease were used to determine the International Prognostic Index [46]. For statistical analysis, patients were grouped into low-risk (International Prognostic Index, 0-1), intermediate-risk (International Prognostic Index, 2-3) and high-risk (International Prognostic Index, 4-5) patients.

**Immunohistochemical detection of LRP and MRP1:**

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded lymphoma specimens. Paraffin sections were mounted on poly-L-lysine-coated glass microslides. Sections were deparaffinized and rehydrated by consecutive submersions in xylene (two changes, 10 min each), absolute ethanol (two changes, 5 min each), 70% ethanol (two changes, 5 min each) and distilled water (3 min). Endogenous peroxidase activity was blocked by incubation in 0.06% H$_2$O$_2$ for 10 min at room temperature and slides were washed in PBS. The tissues were preincubated for 20 min in normal serum (normal goat serum 1:50; Dako, Glostrup, Denmark) prior to incubation for 2 h with either the LRP-56 monoclonal antibody (Alexis, Läufelfingen, Switzerland) or the MRP1 monoclonal antibody (Alexis). Antibody binding was detected by the avidin-biotin-peroxidase method. Bound peroxidase was developed with 3,3'-diaminobenzidine (Dako). The slides were

**PATIENTS AND METHODS**

Forty-eight previously untreated patients (21 females, 27 males) with DLBCL, diagnosed between 1997 and 1999, were included in this study. All of the biopsy samples were classified according to the criteria provided in the Revised European-American Lymphoma (REAL) classification [1,18,19].
was no significant association between LRP expression and age, sex, or β2-microglobulin (Table 1). However, LRP expression was more frequently observed in patients with stage III and IV disease and in patients with elevated serum lactate dehydrogenase (> 240 units/liter; Table 1). Whereas, 24 of 25 low-risk patients (International Prognostic Index, 0-1) were LRP-negative, 6 of 18 intermediate-risk (International Prognostic Index, 2-3) and 4 of 5 high-risk patients (International Prognostic Index, 4-5) were LRP-positive (p = 0.0001; Table 1).

MRP1 expression was independent of age, sex, β2-microglobulin, serum lactate dehydrogenase and the International Prognostic Index (Table 2). In addition, no correlation between MRP1 and LRP expression was observed.

LRP and MRP1 expression and response to chemotherapy:

All of the patients were evaluable for response to chemotherapy. The complete response rate was 54%. Partial responses and no responses were seen in 21 and 25% of the patients, respectively. The complete response rate was 65% for patients without LRP expression but only 18% for patients with LRP expression (p = 0.006; Table 3). Partial responses and no responses occurred in 8 (22%) and 5 (13%) of LRP-negative patients but in 2 (18%) and 7 (64%) of LRP-positive patients. With regard to MRP1, the complete response rate was 56% for MRP1-negative patients and 52% for MRP1-positive patients (p = 0.8; Table 3). Tumor stage (p = 0.001), serum lactate dehydrogenase (p = 0.01) and the International Prognostic Index (0.0001) were also significantly associated with complete response Table (3).

A logistic regression analysis that included LRP and the International Prognostic Index was performed. In the univariate analysis, the odds ratios for no complete response were 8.3 for LRP (p = 0.006) and 9.5 for the International Prognostic Index (p = 0.0001; Table 4). In the multivariate analysis, the odds ratios for no complete response were 2.3 for LRP (p = 0.4) and 7.6 for the International Prognostic Index (p = 0.004; Table 4).

LRP and MRP1 expression and survival:

Overall survival was estimated according to Kaplan-Meier. Fifteen patients died (7 LRP-negative patients, 8 LRP-positive patients).
Overall survival was significantly shorter in patients with LRP expression (Fig. 3). At a median follow-up of 2.1 years, median overall survival of all of the patients was not reached. Median overall survival was 0.9 years for LRP-positive patients and was not reached for LRP-negative patients ($p = 0.001$). As regard MRP1, 8 MRP1-negative patients and 7 MRP1-positive patients died. Median overall survival was not different between patients with MRP1 expression and those without MRP1 expression ($p = 0.9$) (Fig. 4). In patients with stage II-IV disease ($n = 37$), overall survival remained significantly shorter in LRP-positive patients than in LRP-negative patients (median 0.9 years versus median not reached; $p = 0.03$).

In the univariate Cox regression analysis, the relative risk for death was 4.9 for LRP ($p = 0.001$) and 4.6 for the International Prognostic Index ($p = 0.0001$; Table 5). In the multivariate Cox regression analysis that included LRP and the International Prognostic Index, the relative risk for death was 1.4 for LRP ($p = 0.6$) and 4.0 for the International Prognostic Index ($p = 0.005$; Table 5).

Table (1): Correlation of LRP expression and characteristics of patients with DLBCL.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>LRP-negative patients n (%)</th>
<th>LRP-positive patients n (%)</th>
<th>$p$</th>
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<tbody>
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<td>Number of patients</td>
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<td>37 (100)</td>
<td>11 (100)</td>
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</tr>
<tr>
<td>$\leq 60$ years</td>
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<td>24 (65)</td>
<td>5 (45)</td>
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</tr>
<tr>
<td>$&gt; 60$ years</td>
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<td>6 (55)</td>
<td>19</td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
<td>20 (54)</td>
<td>7 (64)</td>
<td>0.7$^a$</td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
<td>17 (46)</td>
<td>4 (36)</td>
<td></td>
</tr>
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<td></td>
</tr>
<tr>
<td>I + II</td>
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<td>2 (18)</td>
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<tr>
<td>III + IV</td>
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<td>16 (43)</td>
<td>9 (82)</td>
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</tr>
<tr>
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<tr>
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<td>7 (78)</td>
<td>1.0$^a$</td>
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<td>7 (23)</td>
<td>2 (22)</td>
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<td>24 (65)</td>
<td>1 (9)</td>
<td>0.0001$^c$</td>
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<td>2+3</td>
<td>18</td>
<td>12 (32)</td>
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<tr>
<td>4+5</td>
<td>5</td>
<td>1 (3)</td>
<td>4 (36)</td>
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$^a$ $p$ of Fisher's exact test.

$^b$ $p$ of $x^2$ test.

$^c$ Exact Mann-whitney test.
Table (2): Relationship of MRP1 and characteristics of patients with DLBCL.

<table>
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<tr>
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<th>Total</th>
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<th>MRP1-positive patients n (%)</th>
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<td>Number of patients</td>
<td>48</td>
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<td>21 (100)</td>
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<tr>
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</tr>
<tr>
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<td>14 (67)</td>
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<td>&gt; 60 years</td>
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<td>12 (57)</td>
<td>0.9^a</td>
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<tr>
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<td>21</td>
<td>12 (44)</td>
<td>9 (43)</td>
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<tr>
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<td>14 (52)</td>
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<td></td>
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<tr>
<td>Normal ((\leq 240) units/liter)</td>
<td>20</td>
<td>12 (44)</td>
<td>8 (38)</td>
<td>0.7^a</td>
</tr>
<tr>
<td>Elevated (&gt; 240 units/liter)</td>
<td>28</td>
<td>15 (56)</td>
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<td>5 (23)</td>
<td>4 (22)</td>
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<td>\textbf{International prognostic index:}</td>
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<td>0.5^c</td>
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<td>2+3</td>
<td>18</td>
<td>10 (37)</td>
<td>8 (38)</td>
<td></td>
</tr>
<tr>
<td>4+5</td>
<td>5</td>
<td>2 (7)</td>
<td>3 (14)</td>
<td></td>
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</table>

\(p\) of \(\chi^2\) test.  ^a \(p\) of Fisher's exact test.  ^b Exact Mann-whitney test.

Table (3): Relationship of various predictors and outcome to chemotherapy.

<table>
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<tr>
<th></th>
<th>Total</th>
<th>Complete response n (%)</th>
<th>No complete response n (%)</th>
<th>(p)</th>
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<td>\textbf{LRP:}</td>
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<td>24 (65)</td>
<td>13 (35)</td>
<td>0.006^a</td>
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<td>Positive</td>
<td>11</td>
<td>2 (18)</td>
<td>9 (82)</td>
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<tr>
<td>\textbf{MRP1:}</td>
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<td></td>
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<td>11 (52)</td>
<td>10 (48)</td>
<td></td>
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<tr>
<td>\textbf{International prognostic index:}</td>
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<td>12 (67)</td>
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</tr>
<tr>
<td>4+5</td>
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<td>0 (0)</td>
<td>5 (100)</td>
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<tr>
<td>\textbf{Age:}</td>
<td></td>
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</tr>
<tr>
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<td>29</td>
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<tr>
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<td>11 (58)</td>
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<td>27</td>
<td>12 (44)</td>
<td>15 (56)</td>
<td>0.1^a</td>
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<td>7 (33)</td>
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<td>\textbf{Stage:}</td>
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<tr>
<td>I + II</td>
<td>23</td>
<td>18 (78)</td>
<td>5 (22)</td>
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<td>III + IV</td>
<td>25</td>
<td>8 (32)</td>
<td>17 (68)</td>
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<td>\textbf{Lactate dehydrogenase:}</td>
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<tr>
<td>Elevated (&gt; 3 mg/liter)</td>
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<td>2 (22)</td>
<td>7 (78)</td>
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\(p\) of \(\chi^2\) test.  ^a \(p\) of Fisher's exact test.  ^b Exact Mann-whitney test.
### Table (4): Logistic regression analysis of no complete response.

<table>
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<th>Multivariate</th>
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<td></td>
<td>Odds ratio</td>
<td>95% CIa</td>
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<tr>
<td>LRP</td>
<td>8.3</td>
<td>1.6-44.3</td>
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<tr>
<td>International Prognostic index</td>
<td>9.5</td>
<td>2.7-33.7</td>
</tr>
</tbody>
</table>

*a* CI, confidence interval.  
*b* Test for trend.  
For this analysis, the International Prognostic Index was grouped into low risk (0-1), intermediate risk (2-3) and high risk (4-5).

### Table (5): Cox regression analysis of overall survival.

<table>
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<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>95% CIa</td>
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<td>1.7-14.3</td>
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<tr>
<td>International Prognostic index</td>
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<td>2.2-9.9</td>
</tr>
</tbody>
</table>

*a* CI, confidence interval.  
*b* Test for trend.  
For this analysis, the International Prognostic Index was grouped into low risk (0-1), intermediate risk (2-3) and high risk (4-5).

Fig. (1): LRP expression by lymphoma cells LRP-56 monoclonal antibody,  
A) x 100 (low-power) and B) x 400 (high-power).  

Fig. (2): MRP1 expression by lymphoma cells using MRP1 monoclonal antibody (x 400).  

Fig. (3): LRP and overall survival estimated according to Kaplan-Meier in 48 patients. Survival data based on LRP expression are shown. Statistical comparison between survival curves was done by the log-rank test.
phomas has been examined in previous studies. Immunohistochemical studies reported P-glycoprotein expression that ranged from 0 to 49% of samples from untreated patients [3,7,16,6,32,34,35,38,44]. Conflicting results with regard to the clinical importance of MDR1/P-glycoprotein in lymphomas have been reported. MDR1/P-glycoprotein predicted a poor response to induction chemotherapy in two studies [3,35] but not in other studies [34,38]. In pretreated lymphomas, MDR1/P-glycoprotein expression was increased because of induction or selection of P-glycoprotein expressing clones [26,32].

Mutations or overexpression of the p53 gene have been described as predictors of poor response to chemotherapy and shorter survival of lymphoma patients [22,33,50]. In aggressive B-cell lymphomas, patients with p53 mutations had a lower complete response rate and a shorter overall survival as compared with patients with wild-type p53 [22]. A multivariate analysis that included p53 and factors of the International Prognostic Index demonstrated that mutant p53 was an independent predictive and prognostic factor [22].

Overexpression of bcl-2 confers drug resistance in vitro by inhibiting apoptosis [48]. Although an association between bcl-2 and response to chemotherapy could not be demonstrated [14,20,21,40,50], bcl-2 expression correlated with a higher relapse-rate [21], shorter disease-free survival [14,20,40] and shorter overall survival [14].

In conclusion, LRP expression is associated with poor response to chemotherapy and with shorter survival of the patients with DLBCL with statistical significance in univariate analysis and therefore, may prove to be an important mechanism of drug resistance in this disease if large sample was examined. Thus, the development of strategies to clinically overcome LRP-mediated drug resistance may be attempted and might improve clinical outcome in DLBCL in the future.

**REFERENCES**

Prognostic Significance of Lung Resistance Protein (LRP)


