LR11: a novel biomarker identified in follicular lymphoma

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Summary

LR11 (also called SorLA or SORL1) is a type I membrane protein, which was recently identified as a biomarker for atherosclerosis and Alzheimer’s disease. We recently reported that LR11 is highly expressed in leukemic blasts and its soluble form (sLR11) is significantly elevated in acute leukemia patients. Here, we confirmed LR11 expression in follicular lymphoma (FL) cells and retrospectively evaluated the prognostic significance of serum sLR11 levels in FL patients. LR11 was detected in FL cells by immunohistological staining and flow cytometry. Serum sLR11 levels, associated with tumor burden and bone marrow invasion, were significantly elevated in untreated FL patients than in normal controls and reduced to the normal range at disease remission. Serum sLR11 level was a strong predictor of relapse or progression, with a cut-off level of 16.7 ng/mL (AUC 0.9). The 2-year progression-free survival (PFS) was significantly lower in patients with high sLR11 levels (≤16.7 ng/mL vs. >16.7 ng/mL; 100% vs. 31%). Patients with low serum sLR11 levels showed significantly better PFS, regardless of the Follicular Lymphoma International Prognostic Index (FLIPI)-2 score. LR11 is a novel lymphoma-derived molecule reflecting the tumor burden and organ invasion and may be a promising biomarker for FL.

Key Words: LR11, Follicular lymphoma, biomarker, progression free survival, FLIPI-2
Introduction

Follicular lymphoma (FL) constitutes approximately 20% of newly diagnosed lymphoma patients, making it the second most frequent subtype of non-Hodgkin’s lymphoma (Morton, et al 2006). It is characterized by an indolent clinical course, with the presence of a chromosomal translocation, t(14;18)(q32;q21), or its variants in 85% patients (Yunis, et al 1982), which results in the juxtaposition of the immunoglobulin heavy chain gene (IgH) on chromosome 14 with the BCL2 oncogene on chromosome 18, leading to constitutive expression of the BCL2 protein (Weiss, et al 1987).

Although the majority of FL patients are indolent for a long period of time, those with advanced-stage FL are still incurable by combination chemotherapies, even with rituximab-containing regimen (Salles and Ghesquieres 2012). Moreover, clinical behavior is markedly heterogeneous, with some patients undergoing progression or transformation early and 15% of them dying within 2 years of diagnosis (Al-Tourah, et al 2008, van Oers, et al 2010). These variable outcomes in FL patients have prompted us to establish reliable prognostic and predictive indicators.

In 2004, the Follicular Lymphoma International Prognostic Index (FLIPI) was proposed on the basis of data from more than 4000 FL patients diagnosed between 1985 and 1992 (Solal-Celigny, et al 2004). Recently, FLIPI was revised to FLIPI-2 by a recent multicenter cohort receiving rituximab-containing regimen, incorporating 5 clinical parameters of β2-microglobulin, lymph node size over 6 cm, bone marrow involvement, anemia, and age over 60 years (Federico, et al 2009). Although FLIPI-2 was shown to be predictive in progression-free survival (PFS), it still remains a clinical surrogate marker, and the development of prognostic
indicators that better reflect the biological characteristics of FL may enable a more accurate prediction of disease progression. For this purpose, various genetic markers have been proposed by gene expression profiling (Byers, et al 2008, Glas, et al 2007). However, these are only detectable in tumor specimens obtained by biopsy, when the disease is evident. A widely adapted prognostic biomarker that is directly released from FL tumor cells and can be easily quantified repeatedly by a simple method has not been established till date.

LR11 (also called SorLA or SORL1) is a type I membrane protein, and a large extracellular part of it is released from the membrane after shedding. It has been shown that LR11 plays a key role in the migration of undifferentiated vascular smooth muscle cells (SMCs) and that the circulating soluble form of LR11 (sLR11) is a biomarker for atherosclerosis, coronary stenosis, and diabetic retinopathy (Jiang, et al 2008, Ohwaki, et al 2007, Takahashi, et al 2012, Yamazaki, et al 1996, Zhu, et al 2004). The potent actions of sLR11 in enhancing the migration of SMCs and infiltration of macrophages are mediated by the urokinase-type plasminogen activator receptor (uPAR)/integrin-mediated activation of focal adhesion kinase (FAK)/ERK/Rac1 cascades (Jiang, et al 2008, Ohwaki, et al 2007). Circulating sLR11 levels are directly proportional to the fractions of migrating vascular SMCs in atherogenesis or to those elicited by vascular damage in humans and mice (Jiang, et al 2008, Matsuo, et al 2009, Takahashi, et al 2010, Takahashi, et al 2012). Moreover, increased sLR11 levels in the cerebrospinal fluid have been suggested to predict neurodegeneration in the brain of Alzheimer’s disease patients (Guo, et al 2012, Ikeuchi, et al 2010, Matsuo, et al 2009). Thus, serum sLR11 levels appear to reflect the pathological conditions of undifferentiated or degenerated cells in various diseases. Along with the fact that circulating sLR11 levels represent the accumulation of vascular immature cells, human CD34+CD38− immature hematopoietic precursors reportedly express high levels of LR11.
mRNA (Zhang, et al 2000). However, little is known about the expression and role of LR11 in human hematopoietic cells.

We have recently found that LR11 is highly expressed in leukemic cells and that serum sLR11 levels in acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL) patients are significantly increased at diagnosis and decreased to normal levels at remission (Sakai, et al 2012). We have also found that sLR11 levels are associated with the proportion of peripheral leukemic cells (Sakai, et al 2012). This suggests that circulating sLR11 levels are not simply associated with numbers or activities of proliferating cells but rather with pathogenic properties of immature cells, including their migration and attachment activities. Recent studies on humans and animals have shown that sLR11 is produced by myeloid cells after G-CSF treatment and that the released sLR11 induces G-CSF-induced leukocyte mobilization into circulation through the activation of cell mobility (Shimizu et al, unpublished data). Because approximately 40 % of FL patients show bone marrow invasion, we hypothesized that the release of sLR11 may play an important role in the development of FL. Subsequently, we analyzed the expression profile of LR11 in FL cells and retrospectively evaluated the clinical importance of serum sLR11 levels in FL patients.

**Methods**

**Patients**

Tumor specimens were collected from 16 patients, and serum samples were collected from 61 patients with newly diagnosed, untreated FL at Chiba University Hospital and affiliated hospitals from 2002 to 2012. All patients gave written informed consent for general human bio-specimen protocol in accordance with the Declaration of Helsinki. This study was approved by the Human
Investigation Review Committee of the Chiba University Graduate School of Medicine or the affiliated hospitals. The clinical data were collected retrospectively.

Clinical stages of the patients were determined according to the Ann Arbor classification (Carbone, et al 1971) by means of a physical examination; systemic computed tomography (CT) examination; bone marrow aspiration and biopsy; hemogram and differential cell counts; and routine biochemical tests. Fifty-two patients (85.2%) were treated with the R-CHOP regimen (rituximab, cyclophosphamide, Adriamycin, vincristine, and prednisolone), 3 patients (4.9%) were treated with irradiation only, and 6 patients (9.8%) were observed without treatment. The treatment outcome was evaluated by the revised response International Working Group criteria (Cheson, et al 2007). Serum samples of the patients were collected at the time of diagnosis, and of those who received R-CHOP therapy, paired serum samples could be obtained at the time of complete response in 20 patients. As a normal control group, serum samples were collected from 75 healthy adult volunteers who had given informed consent.

**Immunostaining by anti-LR11 antibody**

Formalin-fixed, paraffin-embedded tumor specimens were immunostained with antibody against LR11 (clone, A 2-2-3, 50 ng/mL) (Jiang, et al 2008). The specimens were counterstained with hematoxylin. As a positive control, each staining batch included a cell block of the U937 human leukemic cell line, which shows strong immunostaining against LR11. Samples for LR11 expression were stratified into three categories, depending on the intensity of LR11 staining. The intensity of immunostaining was scored as follows; “weak”, diffuse cytoplasmic staining identified only by magnification greater than ×200; “moderate”, cytoplasmic staining with a granular pattern; and “strong”, cytoplasmic staining with a granular pattern and juxtamembrane
reinforcement.

**Flow cytometry**

Lymph node cells were suspended and stained with the fluorescein isothiocyanate (FITC)-conjugated anti-LR11 monoclonal antibody M3 (Matsuo, et al 2009, Sakai, et al 2012). To identify lymphoma cells, the cells were also stained with antibodies against CD10, CD19, or CD20 (Becton Dickinson, CA, USA). The cells were analyzed on BD FACSCalibur (Becton Dickinson).

**ELISA**

Serum sLR11 levels were determined by sandwich ELISA method, as reported previously (Matsuo, et al 2009, Sakai, et al 2012).

**Statistical analysis**

Comparisons of serum sLR11 levels between subgroups were made using the Mann–Whitney U test. Serum sLR11 levels at diagnosis and remission were compared using Wilcoxon’s signed-rank test. Multivariate analysis for factors associated with serum sLR11 levels was conducted using a linear regression model. For variable selection, the stepwise procedure was set to a threshold of 0.05 for inclusion or exclusion in the model. Overall survival (OS) was defined as the time from diagnosis to death due to any cause. PFS was defined as the time from diagnosis to death due to any cause or disease relapse or progression. OS and PFS were analyzed by the Kaplan–Meier method and the log-rank test, and hazard ratios were calculated using Cox’s proportional hazard model. All comparisons were planned, and the tests were 2-sided. \( P \) values
<.05 were considered statistically significant. Data were analyzed using JMP (version 7.0.2, SAS Institute Inc.) and SAS (version 9.2, SAS Institute Inc.) software programs.

**Results**

**Immunostaining and surface expression of LR11 in FL cells**

We evaluated immunohistological staining of LR11 in lymph nodes obtained from 16 FL patients. Surface expression and immunohistological profiles of the patients’ specimens are described in Table I. LR11 expression was detected in the nodular structures of FL and in the cytosol of CD20-positive tumor cells (Figure 1A-C). LR11 staining was detected in all specimens with various immunological intensities. “Weak”, “moderate”, and “strong” staining pattern was detected in 6 (37.5%), 8 (50%), and 2 (12.5%) samples, respectively. (Figure 1D-F). The intensity of staining did not seem to associate with histological grading (Table I); however we could not statistically analyze because of the small sample number. Next, we investigated the surface expression of LR11 in lymphoma cells obtained from lymph node specimens of 3 FL patients by flowcytometry. LR11 expression was confirmed on the surface of CD19-positive FL cells (representative data is shown in Figure 2). Thus, LR11 is shown to be expressed in FL cells by immunoanalysis using 2 antibodies react against different epitopes of LR11.

**Measurement of serum sLR11 levels in FL patients**

Then, we examined the circulatory sLR11 levels of FL patients. Serum sLR11 levels of FL patients at diagnosis were significantly higher than those of normal controls (19.4 ± 17.1 vs. 8.8 ± 1.8 ng/mL, \( P < 0.0001 \), Figure 3A).
Paired sample analysis of 20 patients revealed that sLR11 levels at diagnosis were significantly decreased at the time of complete remission (20.7 ± 22.2 vs. 8.2 ± 3.1 ng/mL, $P < 0.0001$, Figure 3B). Furthermore, sLR11 levels at complete remission showed no significant differences in normal control samples.

**Correlations between serum sLR11 levels and clinical parameters of FL**

Next, we evaluated the correlations between serum sLR11 level and various clinical parameters to identify the factors associated to serum sLR11 levels. The characteristics of 61 FL patients and their individual serum sLR11 levels are shown in Table II. Serum sLR11 levels were significantly higher among patients with advanced Ann Arbor staging ($P = 0.001$), larger numbers of nodal sites ($P = 0.001$), the presence of bone marrow invasion ($P < 0.001$), the presence of bulky disease ($P = 0.035$), lower hemoglobin levels ($P = 0.018$), higher serum LDH levels ($P < 0.001$), and higher β2-microglobulin levels ($P < 0.001$). No significant relationships with sex, age, the presence of B symptoms, performance status, and histological grading were observed. For determining factors associated with serum sLR11 levels at diagnosis, all significant variables were included in multivariate regression analysis, except for “Ann Arbor staging,” which showed a relatively strong correlation with “number of nodal sites” ($\rho = 0.75$, $P < 0.001$). The multivariate linear regression model showed that the presence of bone marrow invasion, lower hemoglobin levels, and higher serum β2-microglobulin levels were associated with serum sLR11 levels, independently of other risk factors at diagnosis ($R^2 = 0.48$, bone marrow invasion: $P = 0.033$, hemoglobin levels: $P < 0.001$, serum β2-microglobulin levels: $P = 0.029$). When the patients were divided according to FLIPI-2, a previously adopted prognostic index, patients with “high” FLIPI-2 scores showed significantly higher sLR11 levels than those
with “intermediate” and “low” FLIPI-2 scores (29.2 ± 23.9 vs. 13.8 ± 7.7 ng/mL, P < 0.001, Figure 4).

**Correlation of sLR11 levels with disease progression**

With a median follow-up period of 20.1 months, 2-year OS and PFS of all patients was 96% and 69%, respectively. Considering the results obtained from factors associated with serum sLR11 levels, we evaluated whether serum sLR11 could be a powerful predictor of disease relapse or progression. Eleven patients with “watch and wait” policy and Histological grade 3b were excluded from the analysis, since an event for PFS in the “watch and wait” patients and treated patients were not comparable, and grade 3b patients were known to follow different clinical courses from other histological grading (Horn, et al 2011). Overall, 50 patients were included, with a median follow up period of 19.7 months. Their 2-year OS and PFS was 95% and 66 %, respectively. Receiver operating characteristic curve (ROC) analysis established 16.7 ng/mL as the cut-off level for disease relapse or progression, with 76% sensitivity and 100% specificity, and the maximum area under the curve was 0.9.

Subsequently, we analyzed the survival according to 2 subgroups divided by serum sLR11 levels, with the cut-off level of 16.7 ng/mL defined by ROC analysis. Only 2 patients died during the observation; therefore, OS between the 2 sLR11 subgroups were not statistically significant (2-year OS: 100% vs. 89%, P = 0.096, Figure 5A).

However, patients with sLR11 levels higher than 16.7 ng/mL at diagnosis showed significantly inferior PFS than those with sLR11 levels equal to or lower than 16.7 ng/mL (2-year PFS: 100% vs. 31%, Hazard ratio: 2.3 × 10^7, 95% CI: not available, P < 0.001, Figure 5B). Furthermore, when the patient data were analyzed separately by “low-intermediate” and “high” FLIPI-2
scoring, those with higher serum sLR11 levels showed significantly inferior PFS in both the FLIPI-2 categories. The 2-year PFS divided by serum sLR11 levels was 100% vs. 75% among the “low-intermediate” scoring group (Hazard ratio: \(6.85 \times 10^7\), 95% CI: not available, log-rank \(P=0.046\), Figure 6A), and the 2-year PFS divided by serum sLR11 levels was 100% vs. 13% among the “high” FLIPI-2 scoring group (Hazard ratio: \(9.91 \times 10^6\), 95% CI: not available, log-rank \(P=0.005\), Figure 6B). Notably, patients with low sLR11 at diagnosis showed an excellent PFS of 100%, regardless of their FLIPI-2 scoring.

**Discussion**

In this study, we identified LR11 as a novel biomarker, which is expressed in the cytoplasm and on the cell surface of FL cells, and the soluble form of which is released and detectable in the patients’ serum. Serum sLR11 levels in FL patients were associated with bone marrow invasion, lower hemoglobin levels, and elevated serum \(\beta_2\)-microglobulin levels. This finding indicates that sLR11 levels are not only associated with tumor burden but also possibly with their migration and attachment activities, particularly with the affinity for the bone marrow.

Various predictive indicators for lymphoma have been proposed (Sehn, et al 2012). Histological grading and cytogenetics are suggestive for predicting clinical aggressiveness; however, the results have been conflicting because of the recent curative potential of anthracycline-based treatment strategies (Ganti, et al 2006, Shustik, et al 2011). Improved biological insights into the pathogenesis of FL highlighted that interactions between follicular tumor cells and non-malignant cells such as T cells, histiocytes, and macrophages modulate the growth and survival of FL cells, which has been suggested by gene expression profiling and
immunohistological analysis (Camacho, *et al* 2011, Farinha, *et al* 2005, Glas, *et al* 2007, Korenberg, *et al* 2007). However, these strategies still have limitation of technical complexities and the results are also conflicting, subsequently limiting the value of molecular indicators. Soluble biomarkers are useful tools for evaluating tumor burden and its character. The most distinguishable advantage is that they are easy to measure in the patients’ blood sample and can be repeatedly evaluated during the clinical course. Various soluble biomarkers have also been suggested as prognostic indicators (Charbonneau, *et al* 2012, Masuda, *et al* 2008, Shah, *et al* 2012, Yang, *et al* 2011); however, their clinical potential remains to be established.

It has been considered that lymphoma cells metastasized to the bone marrow may not have been randomly distributed but rather localized to the niches, similar to normal hematopoietic precursor cells. Consequently, these micrometastatic lymphoma cells in the bone marrow are shown to achieve chemoresistance through VLA-4/VCAM-1-mediated interaction (Weekes, *et al* 2001).

LR11 can bind to and form complexes with uPAR (Zhu, *et al* 2004). uPAR is known to stimulate tumor cell migration by interacting with integrin-mediated signal transduction (Yebra, *et al* 1999). It is also found to be expressed in disseminated cancer cells in the bone marrow of patients with solid cancers, predicting an early relapse (Heiss, *et al* 2002), and high soluble uPAR levels are reported to be associated with poor prognosis in ovarian cancer patients and AML (Graf, *et al* 2005).

sLR11 interacts with the uPAR/integrin complex and enhances the mobility of monocytes by activating the FAK/ERK/Rac1 cascade in THP-1 macrophages. In addition, sLR11 has been shown to increase the migration and adhesion of THP-1 macrophages in a dose-dependent manner (Ohwaki, *et al* 2007). Furthermore, we have recently found that sLR11 stabilize bone
marrow niche by regulating hypoxia-induced attachment of hematopoietic stem cells to bone marrow stroma cells, via uPAR-mediated pathway (Nishii, et al 2013). These findings suggest that sLR11 may enhance cell adhesion-mediated drug resistance by regulating the uPAR/integrin-mediated interaction of lymphoma and bone marrow stromal cells.

LR11 was immunohistologically detected in various intensities within tumor cells presenting various histological grading. Although the sample number was not enough to evaluate statistically, immunohistological intensities of LR11 seemed not to have an association with histological grading. Moreover, no significant relationship was shown between serum sLR11 levels and histological grading. These findings suggest that LR11 reflexes different character of FL cell from histological grading.

Although few patients were able to compare, immunohistological intensity did not showed significant association with their serum sLR11 levels. We can consider several reasons for this phenomenon. First, LR11 might also be released from non-malignant cells; however, this hypothesis can be denied because we could not find any non-malignant cells specifically reacted against LR11 antibody in the tumor specimens. Second, the release of serum sLR11 might be regulated by not only intracellular LR11 expression, but also cell surface shedding mechanism of LR11. We have found in several leukemic cell lines that the shedding of LR11 from cell surface is regulated by specific tetraspanin co-expressed with LR11 (Tsukamoto et al, unpublished data). Therefore, the same mechanism might be found in FL cells. Third, the sensitivity of immunostaining might not be enough to reflect the range of serum sLR11 levels. To answer this question, we are now evaluating in larger patient cohort to determine the sensitivity and specificity of immunostaining method.

Elevated serum sLR11 levels were a strong predictor of disease relapse, and patients with
high serum sLR11 levels showed significantly inferior PFS. The identification of this novel circulating biomarker, which directly reflects the biological character of tumor cells, may enable a more accurate prediction of tumor burden and activity by a simple method, and it provides new insights into the mechanism of tumor progression in FL.

Histological grade 3B is generally considered to be more clinically and biologically separate from grade 1 to 3A, and similar to diffuse large B cell lymphoma (DLBCL), and a recent study addressed that grade 3B showed a plateau in survival curve (Wahlin, et al 2012). Therefore, we excluded grade 3B patients from the survival analysis.

Variables associated with serum sLR11 levels were included in the 5 variables comprising FLIPI-2, a widely adapted prognostic indicator for PFS established by a large patient cohort (Federico, et al 2009, Numata, et al 2012, Solal-Celigny, et al 2004). The FLIPI-2 index is a combination of variables represented by the patient’s condition (age), tumor burden (longest diameter of the tumor, β2-microgloblin, and hemoglobin levels), and the character of lymphoma cells (bone marrow invasion). Therefore, we consider that serum sLR11 levels more accurately reflect the tumor burden and character of lymphoma cells than FLIPI-2. Notably, all patients with low sLR11 levels survived without disease progression, regardless of their FLIPI-2 score, whereas those with elevated sLR11 levels showed a significantly higher risk of relapse in all FLIPI-2 categories. By combining serum sLR11 levels and previous prognostic indexes, we may be able to distinguish populations with a high risk of disease progression who require more intensive chemotherapy or aggressive strategy such as allogeneic stem cell transplantation. The major limitation of our study is that these findings were based on retrospective analysis with a limited number of patients. Therefore, a prospective validation study is now in progress to reveal the clinical impact of the novel marker LR11 in combination with the established FLIPI-2 score.
for the determination of patient prognosis.

In conclusion, sLR11 is a potential prognostic factor, particularly for identifying patients with higher tumor burden and disease activity. Given the current lack of widely established functional circulating biomarkers for FL, the identification of a novel lymphoma-derived biomarker will elucidate the mechanism of tumor migration and invasion and will ultimately enable us to establish a new therapeutic strategy and a potential molecular target.
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Authorship

• TK was the principal investigator and takes primary responsibility for the paper
• TK, CO, MT, NS, ES, YT, SS, ST, AY, and YS performed the research by recruiting the patients
• TK, MT, MH, and JT performed the research by contributing to laboratory works
• TK and CO analyzed the data and performed statistical analysis
• KY, HB, and CN designed the research and gave approval of the submitted and final versions
• TK, CO, and CN wrote the paper.

Competing interests

The authors have no competing interests.
References


Masuda, A., Nakamura, K., Izutsu, K., Igarashi, K., Ohkawa, R., Jona, M., Higashi, K., Yokota, H., Okudaira, S., Kishimoto, T., Watanabe, T., Koike, Y., Ikeda, H., Kozai, Y., Kurokawa,


Table I. Immunohistological profiles of LR11 and other surface markers in specimens of FL patients

<table>
<thead>
<tr>
<th>Pt. No.</th>
<th>Histological grading</th>
<th>Surface expression by flow cytometry</th>
<th>Immunohistochemical profile</th>
<th>Intensity of LR11 immunostaining</th>
<th>sLR11 level (ng/ml)</th>
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<tr>
<td>1</td>
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<td>CD20,CD79a,bcl-2</td>
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<td>23</td>
</tr>
<tr>
<td>2</td>
<td>3a</td>
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<tr>
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NA; not available
Table II. Patient characteristics and serum soluble LR11 levels

<table>
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<tr>
<th>Variables</th>
<th>No. of patients (n = 61)</th>
<th>sLR11 level (ng/mL) (mean ± SD)</th>
<th>P value</th>
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<td><strong>Age</strong></td>
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<td></td>
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<tr>
<td>≤60</td>
<td>22</td>
<td>19.8 ± 21.4</td>
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<td>&gt;60</td>
<td>39</td>
<td>19.1 ± 14.4</td>
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<td><strong>ECOG Performance status</strong></td>
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<td>19.6 ± 17.6</td>
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<td>17.9 ± 14.3</td>
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<td>23.4 ± 20.2</td>
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<td><strong>B symptoms</strong></td>
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<td><strong>Number of nodal areas</strong></td>
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<td>0–4</td>
<td>28</td>
<td>13.8 ± 8.3</td>
<td>0.001</td>
</tr>
<tr>
<td>&gt;4</td>
<td>33</td>
<td>24.1 ± 21.0</td>
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<tr>
<td><strong>Bone marrow invasion</strong></td>
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<td></td>
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<tr>
<td>absent</td>
<td>42</td>
<td>13.5 ± 5.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>present</td>
<td>19</td>
<td>32.4 ± 25.4</td>
<td></td>
</tr>
<tr>
<td><strong>Bulky disease (&gt; 6cm)</strong></td>
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<tr>
<td>absent</td>
<td>51</td>
<td>18.7 ± 18.0</td>
<td>0.035</td>
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<tr>
<td>present</td>
<td>10</td>
<td>22.9 ± 11.5</td>
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<td><strong>Hemoglobin level</strong></td>
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<td>≥120 g/L</td>
<td>49</td>
<td>15.8 ± 8.2</td>
<td>0.018</td>
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<tr>
<td>&lt;120 g/L</td>
<td>12</td>
<td>34.1 ± 31.7</td>
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<td><strong>Histological grading</strong></td>
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<tr>
<td>1</td>
<td>21</td>
<td>14.9 ± 9.7</td>
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<tr>
<td>2</td>
<td>16</td>
<td>24.6 ± 20.6</td>
<td>NS</td>
</tr>
<tr>
<td>3a</td>
<td>7</td>
<td>16.3 ± 7.8</td>
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</tr>
<tr>
<td>3b</td>
<td>5</td>
<td>15.7 ± 4.2</td>
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<td><strong>Serum LDH level (IU/L)</strong></td>
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<tr>
<td>≤ULN</td>
<td>40</td>
<td>14.2 ± 6.8</td>
<td>&lt;0.001</td>
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<td>&gt;ULN</td>
<td>21</td>
<td>29.2 ± 25.2</td>
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<td><strong>β2-MG level (mg/dL)</strong></td>
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<td>≤ULN</td>
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<td>11.5 ± 3.7</td>
<td>&lt;0.001</td>
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<tr>
<td>&gt;ULN</td>
<td>37</td>
<td>24.5 ± 20.1</td>
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</table>

LLN: lower limit of normal, ULN: upper limit of normal, NS: not significant
Figure legends

Figure 1. (A-C) Lymph node biopsy specimens from FL patients (Pt. No. 8, described in Table I, FL grade 1). (A) Hematoxylin–eosin staining (magnification ×100), (B) CD20 staining (magnification ×400), (C) LR11 staining (magnification ×100).

(D-F) Lymph node biopsy specimens showing various intensities of immunostaining (magnification ×400). (D) Intensity score “weak” (Pt. No. 12, FL grade 2), (E) “moderate” (Pt. No. 13, FL grade 1), and (F) “strong” (Pt. No. 9, FL grade 1).

Figure 2. Flow cytometric analysis of FL cells obtained from FL patient (Pt. No. 13) The solid (black) line represents staining with anti-LR11 antibody, whereas the dotted (gray) line represents the negative control.

Figure 3. (A) Serum sLR11 levels of FL patients compared with those of normal control subjects. (B) Paired sample analysis of serum sLR11 levels at diagnosis and complete remission (CR).

Figure 4. Serum sLR11 levels according to FLIPI-2 scoring. (“int”: intermediate).

Figure 5. (A) OS and (B) PFS according to serum sLR11 at diagnosis.

Figure 6. PFS according to serum sLR11 at diagnosis in patients defined by (A) “low-intermediate” and (B) “high” FLIPI-2 scores.
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(D-F) Lymph node biopsy specimens showing various intensities of immunostaining (magnification ×400). (D) Intensity score “weak” (Pt. No. 12, FL grade 2), (E) “moderate” (Pt. No. 13, FL grade 1), and (F) “strong” (Pt. No. 9, FL grade 1).
**Figure 2.** Flow cytometric analysis of FL cells obtained from FL patient (Pt. No. 13) The solid (black) line represents staining with anti-LR11 antibody, whereas the dotted (gray) line represents the negative control.
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**Figure 4.** Serum sLR11 levels according to FLIPI-2 scoring. (“int”: intermediate).

![Box plot](image)

<table>
<thead>
<tr>
<th>Serum sLR11 (ng/mL)</th>
<th>Median</th>
<th>Mean±SD</th>
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</thead>
<tbody>
<tr>
<td>“low” &amp; “int” (n = 39)</td>
<td>12.6</td>
<td>13.8±7.7</td>
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<tr>
<td>“high” (n = 22)</td>
<td>19.8</td>
<td>29.2±23.9</td>
</tr>
</tbody>
</table>

P<0.001
Figure 5. (A) OS and (B) PFS according to serum sLR11 at diagnosis.
Figure 6. PFS according to serum sLR11 at diagnosis in patients defined by (A) “low-intermediate” and (B) “high” FLIPI-2 scores.