Clinica Chimica Acta 463 (2016) 47–52

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Prognostic impact of serum soluble LR11 in newly diagnosed diffuse large B-cell lymphoma: A multicenter prospective analysis

Yasumasa Sugita [a], Chikako Ohwada [a,*], Takeharu Kawaguchi [b], Tomoya Muto [a], Shokichi Tsukamoto [a], Yusuke Takeda [a], Naoya Mimura [c], Masahiro Takeuchi [a], Emiko Sakaida [a], Naomi Shimizu [d], Hiroaki Tanaka [b], Daijiro Abe [e], Motoharu Fukazawa [f], Takeaki Sugawara [g], Nobuyuki Aotsuka [h], Kaichi Nishiwaki [i], Katsuhiro Shono [j], Hiroyuki Ebinuma [k], Kengo Fujimura [k], Hideaki Bujo [l], Koutaro Yokote [m], Chiaki Nakaseko [a]

[a] Department of Hematology, Chiba University Hospital, 1-8-1 Chuo-ku, Chiba City, Chiba, Japan
[b] Department of Hematology, Oami Municipal Hospital, 884-1 Tomita, Oamishirasato City, Chiba, Japan
[c] Department of Transfusion Medicine and Cell Therapy, Chiba University Hospital, 1-8-1 Chuo-ku, Chiba City, Chiba, Japan
[d] Department of Blood Transfusion, Toho University Medical Center Sakura Hospital, 564-1 Shimoshizu, Sakura City, Chiba, Japan
[e] Department of Hematology, Yokohama Rousai Hospital, 3211 Kozukue-cho, Kohoku-ku, Yokohama City, Kanagawa, Japan
[f] Department of Hematology, Funabashi Central Hospital, 6-13-10 Kaijin, Funabashi City, Chiba, Japan
[g] Department of Hematology Oncology, Chiba Cancer Center, 666-2 Nitona-cho, Chuo-ku, Chiba City, Chiba, Japan
[h] Department of Hematology, Japanese Red Cross Narita Hospital, 90-1 Iida-cho, Narita City, Chiba, Japan
[i] Clinical Oncology and Hematology, Jikei University Kashiwa Hospital, 163-1 Kashiwashita, Kashiwa City, Chiba, Japan
[j] Department of Hematology, Chiba Aoba Municipal Hospital, 1273-2 Aoba-cho, Chuo-ku, Chiba City, Chiba, Japan
[k] Tsukuba Research Institute, Sekisui Medical Co. Ltd, 3-3-1 Koyodai, Ryugasaki City, Ibaraki, Japan
[l] Department of Clinical Laboratory Medicine, Toho University Medical Center Sakura Hospital, 564-1 Shimoshizu, Sakura City, Chiba, Japan
[m] Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, 1-8-1, Chuo-ku, Chiba City, Chiba, Japan

ARTICLE INFO

Article history:
Received 9 July 2016
Received in revised form 5 October 2016
Accepted 6 October 2016
Available online 08 October 2016

Keywords:
Diffuse large B-cell lymphoma
Circulating molecule
Bone marrow invasion
Tumor burden
Progression-free survival
Overall survival

ABSTRACT

Background: LR11 (also called SorLA or SORL1) is a type I membrane protein, originally identified as a biomarker for atherosclerosis and Alzheimer's disease. We recently found that LR11 was specifically expressed in Diffuse Large B-cell lymphoma (DLBCL) cells, and high serum sLR11 concentrations in retrospective cohort indicated inferior survival. In this study, we prospectively validated the clinical impact of serum sLR11 in 97 patients with newly-diagnosed, untreated DLBCL.
Results: Serum sLR11 concentrations were increased in DLBCL patients compared to normal controls (mean ± SD: 21.2 ± 27.6 vs. 8.8 ± 1.8 ng/ml, $P < 0.0001$), and significantly reduced at remission (mean ± SD: 17.4 ± 16.4 vs. 10.9 ± 4.5 ng/ml, $P = 0.02$). Increased serum sLR11 concentrations were affected by tumor burden and bone marrow invasion. The 2-y OS and PFS were significantly lower in patients with high sLR11 concentrations (≤18.1 ng/ml vs. >18.1 ng/ml; 2-y OS: 89.0% vs. 56.4%, $P < 0.0001$; 2-y PFS: 85.8% vs. 56.9%, $P < 0.0001$).
Conclusions: Serum sLR11 is a tumor-derived biomarker for predicting the survival of newly diagnosed patients with DLBCL.

1. Introduction

Diffuse large B cell lymphoma (DLBCL) is the most common histologic subtype of non-Hodgkin lymphoma (NHL), accounting for approximately 30% of all newly diagnosed cases and >80% of aggressive lymphomas [1]. It is widely appreciated that DLBCL is heterogeneous in terms of morphology, genetics, and biologic behavior, therefore patients with DLBCL exhibit a wide range of clinical presentations and outcomes [2]. Although the development of rituximab- CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone, R-CHOP) chemotherapy has improved the prognosis of patients with DLBCL [3], patients whose lymphoma is not cured with the first-line therapy require salvage therapy and high-dose chemotherapy followed by autologous stem cell transplantation [4]. Therefore, risk groups for patients with DLBCL need to be accurately identified to select an appropriate therapeutic strategy.

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

* Corresponding author at: 1-8-1 Inohana, Chuo-ku, Chiba City, Chiba, Japan.
E-mail address: chikako_ohwada@faculty.chiba-u.jp (C. Ohwada).

http://dx.doi.org/10.1016/j.cca.2016.10.008
0009-8981/

circulating soluble form of LR11 (sLR11) is a biomarker for atherosclerosis, coronary stenosis, and diabetic retinopathy [5–8]. 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 [5,6]. Human $CD34^{+}CD38^{-}$ immature hematopoietic precursors also express high concentrations of LR11 mRNA [9]; however, little is known about the expression and role of LR11 in human hematopoietic cells.

We recently found that LR11 is highly expressed in hematological malignant cells, and serum sLR11 concentrations were highly increased in patients' with acute leukemia and NHL [10,11]. Especially in DLBCL and follicular lymphoma (FL), two major subtypes of NHL, increased serum sLR11 concentrations were associated with tumor burden and bone marrow invasion, and patients with high serum sLR11 concentrations significantly showed inferior survival in our previous retrospective cohort [12,13]. Furthermore, serum sLR11 concentrations represent a powerful diagnostic indicator for intravascular large B-cell lymphoma, a rare subtype of aggressive NHL [14].

2. Patients and methods

2.1. Patients

Ninety-seven consecutive patients with newly-diagnosed and untreated DLBCL were enrolled from 2010 to 2013 in a multicenter prospective observational study conducted in Chiba University Hospital and 7 affiliated institutions in Japan. All patients provided written informed consent for a general human bio-specimen protocol in accordance with the Declaration of Helsinki. The study was approved by the Human Investigation Review Committee of the Chiba University Graduate School of Medicine, where this study was organized, and by each participating institution. This trial was registered with the UMIN Clinical Trials Registry.

Clinical stages of the patients were determined according to the Ann Arbor classification by physical examination; systemic computed tomography (CT) and fluorodeoxyglucose positron emission tomography (FDG-PET) examination of the neck, chest, abdomen, and pelvis; bone marrow aspiration; and bone marrow biopsy. Routine hematology and serum chemistry data were obtained at diagnosis. Chemotherapeutic regimens were determined by their attending physicians, and response assessments were performed according to the Revised Response Criteria for Malignant Lymphoma [15].

2.2. Measurement of serum sLR11 concentrations

Serum samples were collected at diagnosis and when response assessments were performed. Serum sLR11 concentrations were determined by the sandwich enzyme-linked immunosorbent assay (ELISA) method, as reported previously [11]. As a normal control group, serum samples were collected from 75 healthy adult volunteers who provided informed consent.

2.3. Statistical analysis

Comparisons of serum sLR11 concentrations between subgroups were performed using the Mann–Whitney *U* test. Serum sLR11 concentrations at diagnosis and remission were compared using Wilcoxon's signed-rank test. Multivariate analysis for factors associated with serum sLR11 concentrations was conducted using a multiple linear regression model. For variable selection, the stepwise procedure was set to a threshold of 0.05 for inclusion or exclusion in the model. Serum sLR11 concentrations according to IPI risk categories were compared by Dunnett's test. Overall survival (OS) was defined as the time from diagnosis to death due to any cause. Progression free survival (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. All comparisons were planned, and the tests were 2-sided. A $P < 0.05$ was considered statistically significant. Data were analyzed using JMP (ver 7.0.2, SAS Institute Inc.) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [16], which is a graphical user interface for R (The R Foundation for Statistical Computing) software programs.

3. Results

3.1. Patient characteristics

Ninety-seven patients enrolled in the study. The median age was 69 years (range: 18–94 years), 58 patients (60%) were male. 52 patients (54%) had advanced disease (clinical stage > 2), and 28 patients (29%) were in the "High" risk group categorized by IPI. In total, 91% of the patients were treated with R-CHOP-based regimens, and complete remission and partial remission were obtained in 75 (77%) and 14 (14%) patients, respectively. Progressive disease was observed in 2 (2%) patients, and 6 (7%) patients were not able to assess the responses.

3.2. Measurement of serum sLR11 concentrations in patients with DLBCL

Serum sLR11 concentrations of patients with DLBCL were significantly increased compared to those of normal controls (mean ± SD: 21.2 ± 27.6 vs. 8.8 ± 1.8 ng/ml, $P < 0.0001$; Fig. 1). Paired serum samples at diagnosis and complete remission were obtained from 51 patients. Increased serum sLR11 concentrations at diagnosis were significantly decreased at complete remission (mean ± SD: 17.4 ± 16.4 vs. 10.9 ± 4.5 ng/ml, $P = 0.02$; Fig. 2).

3.3. Correlations between serum sLR11 concentrations and clinical parameters

The characteristics of various clinical parameters and their individual serum sLR11 concentrations are shown in Table 1. Serum sLR11

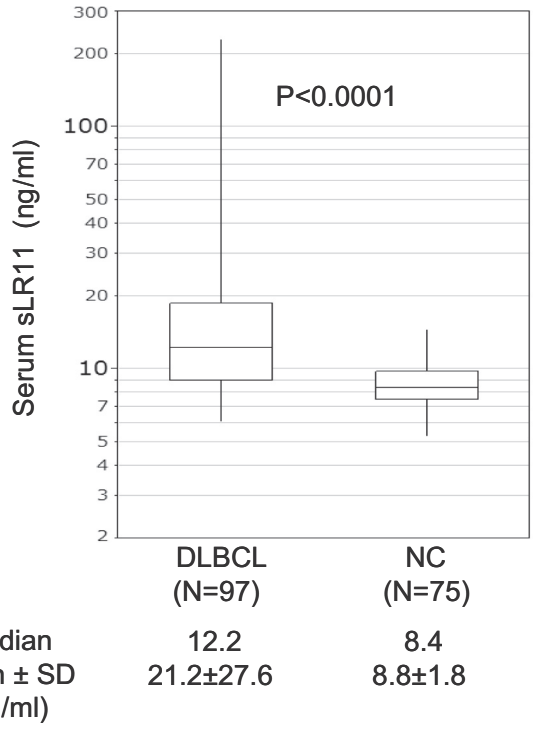

Fig. 1. Serum sLR11 levels in newly diagnosed DLBCL patients compared with normal controls (NC).