

Quantitative Features of serum sIL-2R level in Patients with Mature B-Cell Lymphoma– Involvement of LDH

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Serum Lactate Dehydrogenase (LDH) activity and soluble IL-2 Receptor-alpha (sIL-2R) levels are monitored as a marker of disease activity in patients with lymphoma. Although adult T-cell leukemia (ATL) cells are well known to release large amounts of sIL-2R, it remains unclear to what extent B-cell lymphoma cells shed sIL-2R in sera. Subtypes of mature B-cell lymphoma, including CD25⁺ hairy leukemic cells, were examined for the characteristics of sIL-2R levels in each subtype. In normal controls, the serum sIL-2R mean value was 260 u/mL. The median serum sIL-2R value for 64 B-cell lymphoma cases was 506 u/mL; by subtype the median values were as follows: 1157 u/mL for 7 cases of chronic lymphocytic leukemia/hairy cell leukemia (CLL/HCL), 451 u/mL for 38 cases of diffuse large B-cell lymphoma (DLBCL), and 456 u/mL for 19 cases of follicular Lymphoma (FL). The median values of serum LDH activity by the above subtypes were 175 IU/mL, 204 IU/mL, and 198 IU/mL, respectively. There was distinct inter-subtype and inter-patient variation of serum sIL-2R. In particular, inter-case variation could be grouped into value for three concentration ranges: less than 300 u/mL, 300-1000 u/mL, and greater than 1000 u/mL. Cases with serum sIL-2R values of 1000 u/mL or more tended to have an especially high sIL-2R to LDH ratio, suggesting a close relationship between high sIL-2R and CD25-expressing lymphoma cells. With respect to sIL-2R and LDH levels, CLL/HCL, DLBCL, and FL showed similar distributions. Moreover, for sIL-2R levels exceeding 1000 u/mL, sIL-2R levels were randomly high according to the LDH status. Conclusively, the combination of serum sIL-2R level and LDH activity can provide a better understanding of characteristics of subtypes of mature B-Cell Lymphoma and can be used as a reliable surrogate marker for evaluating numerical and biological data.

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Introduction

Recently, the role of bio-markers has been changing, such as for identifying theranostics or TAT (turn-around-time) diagnosis. Cancer growth and development is associated with stimulation of oncogenic signal pathways, including enhanced interleukin 2-receptor (IL-2R) expression in T-cells and its shedding into the circulation in a soluble form of IL-2Ralpha(1,2,3). In 1994, we identified elevated levels of sIL-2R even in healthy HTLV-1 seropositive carriers, whereby the presence of ATL became overt once levels attained

1000 u/mL(4). Recently, elevated sIL-2R levels have been reported not only in lymphoid tumors but also in cancers, and degenerative and unknown disorders(5,6). Moreover, tumor-infiltrating lymphocytes involved in anti-immunity also are likely to be associated with sIL-2R(7). Also, there is a new attractive theory on the interaction of the sIL-2R/IL-2 complex with the heterogeneous clinical course in follicular lymphoma(8). Of interest, the most common mature lymphomas, diffuse large B-cell lymphoma (DLBCL)- and Follicular lymphoma (FL)-cells, have been reported to express CD25 (9,10). Lactate Dehydrogenase (LDH) is also a

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useful biomarker for lymphoma oncology(11). Most cancer cells use aerobic glycolysis to fuel their growth via pyruvate to lactate by LDH. Although the molecular mechanism has been elusive, the ability of malignant cells to overproduce lactic acid was recognized about seven decades ago. Increases in glucose transport and hexokinase activity in malignant cells appear to account for the increase flux of transport through the malignant cells (12). Accordingly, based on our experience of ATL and CD25 bearing T-cells(13,14), we evaluated the relationship between serum sIL-2R level and serum LDH activity in subtypes of mature B-cell leukemia/lymphoma.

Methods and Material

Study design: Our study's subjects were healthy Nagasaki City employees as normal controls and patients diagnosed by lymph node biopsy. The results were evaluated by numerical analysis only. In general, a high numerical laboratory value implies the presence of lymphoma's characteristic features; therefore, normal controls to produce a reference interval (RI) were collected from the Nagasaki City employees, whose samples were brushed-up by HTLV-1, LDH, and CRP test data. Samples taken from 3 HTLV-1-seropositive persons, 16 high LDH samples, and 29 high CRP samples were discarded. Ages ranged from 20 to 65 with a mean of 45.8 years.

sIL-2R was measured at our hospital by a commercially available kit manufactured by Siemens Healthcare Diagnostics, Tokyo, Japan. Histological diagnosis was conducted at the Center for Diagnostic Imaging in Kurume, Japan.

The B-cell lymphomas of the patient subjects included classical chronic lymphocytic leukemia/hairy cell leukemia (CLL/HCL), diffuse large-B cell lymphoma (DLBCL), and follicular lymphoma (FL). The software used for statistical analysis was StatFlex version 6.0 (Osaka, Japan).

Results

• Age-related study design of sIL-2R

First, as sIL-2R level increase of 18% (239 u/mL – 283 u/mL) was observed across five decades in adulthood from the 20s to the 60s in normal controls brushed-up. No sIL-2R measurements were below 150 u/mL (Figure 1, see red broken line). However, the upper limit measurements increased from 300 u/mL in the 20s to 450 u/mL in the 40s, as denoted by the yellow box. The RI calculation resulted in a median value of 260 ± 41 u/mL, at the age of 46.2 years.

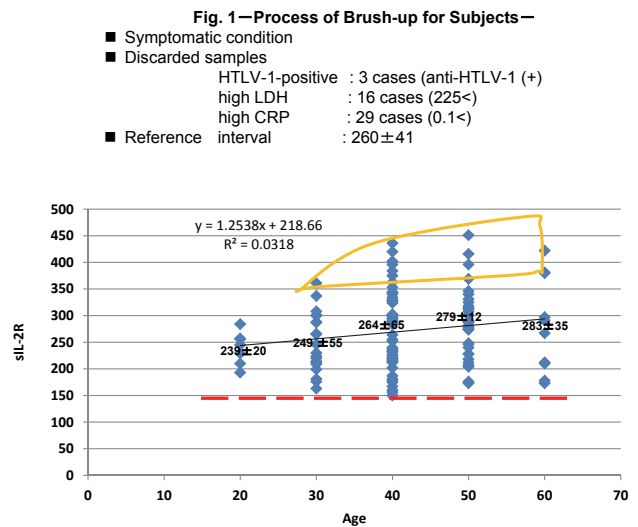


Figure 1. Age-related reference intervals for serum sIL-2R (parametric estimate)

• General status of serum sIL-2R in mature B-ML

We conducted a cross-sectional examination of serum sIL-2R levels and serum LDH activity across all cases. In contrast to the extremely high sIL-2R levels in ATL, for all 64 mature B-ML cases the highest sIL-2R value was at most 4000u/mL and the median serum sIL-2R level was only 506u/mL. Figure 2 is a histogram showing each individual patient's levels of sIL-2R and LDH, in ascending order of sIL-2R level. Nine B-ML samples had a lower sIL-2R value than 260u/mL, the normal RI. The 300 u/mL to 1000 u/mL range showed a continuous increase in level values, but the range above 1000 u/mL showed more random step-like increases. This suggests a substantial transition in sIL-2R kinetics around 1000 u/mL. To confirm significance of this apparent critical line, a single argument dot graph juxtaposing cases by ascending sIL-2R and LDH levels was evaluated (Figure 3). As of the 1000 u/mL critical threshold, such discontinuous events were observed in the sIL-2R levels (red solid circle array), but not immediately in the LDH levels (blue solid circle array) until further along the x-axis. In spite of poor statistical correlation between sIL-2R and LDH ($R^2=0.0917$, slope=0.0041), juxtaposing cases by ascending sIL-2R and LDH levels suggests a positive association with identifiable similarities.

• Features of subtype of B-ML

We examined whether there is a difference between the 2 biomarkers among the 3 subtypes of mature B-ML. The stair-like step-up pattern was also observed in the subtypes (Figure 4), where the median sIL-2R values for CLL/HCL, DLBCL, and FL subtypes were 1157, 451, and 456, respec-

tively (differences between CLL/HCL group vs DLBCL and FL groups significant by Mann-Whitney); Table 1. The critical line was considered to be about 1100u/mL in CLL/HCL, and about 450u/mL in DLBCL and FL. The median values of serum LDH activity by the above subtypes were 175 IU/mL, 204 IU/mL, and 192 IU/mL, respectively. Of interest, cases with sIL-2R values of 1000 or more tended to be separated in two groups: high sIL-2R with or without high LDH activity (Figure 5). Table 2 summarizes the characteristics of the 3 subtypes examined by the present study. In examining the 3 subtypes by the ratio of sIL-2R/LDH, it appeared as if CLL/HCL could be distinguished from the two other subtypes DLBCL and FL.

Table 1: All mature B-ML Subtypes and median values with percentiles

*; The median values of sIL-2R were almost same, but the distribution of individual measurements was different among subtypes

Bio-marker	Mature B-ML	n	2.5%tile	Median	97.5% tile
sIL-2R					
	CLL/HCL	7	582	1157	1974
	DLBCL	38	166	451 *	2799
	FL	19	146	456 *	3810
LDH					
	CLL/HCL	7	169	175	238
	DLBCL	38	136	204	678
	FL	19	192	192	411
Ratio(sIL-2R/LDH)					
	CLL/HCL	7	3.33	5.76	11.34
	DLBCL	38	0.55	2.24	12.49
	FL	19	0.47	2.07	12.53

CLL/HCL 1157; other two; 0.0001 (Mann-whitney)
2.07 significant vs 2.24 & 5.76 (Mann-Whitney)

Individual sIL-2R and LDH data

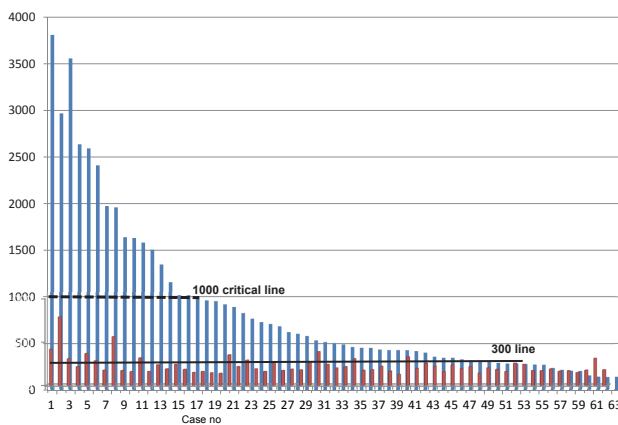


Figure 2. Histogram arranged in descending order of sIL-2R measurements. Blue bar(sIL-2R) becomes high from 142 u/mL and then gradually are being come up. Exceeded 1000u/mL line, their randomly becomes higher. (Red bar; LDH aitiy)

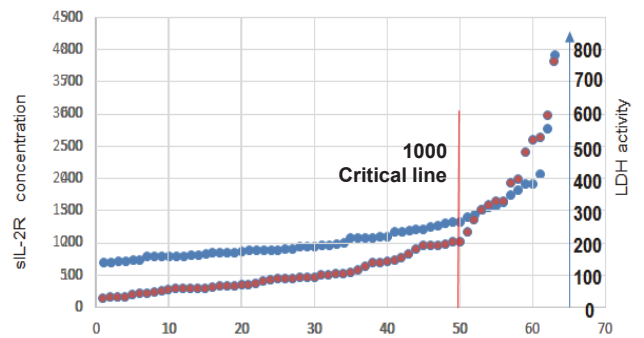


Figure 3. A single argument dot-plot graph (Blue solid ;LDH, Red solid circle; sIL-2R). Two arraries changed after red solid line (1000 critical line).

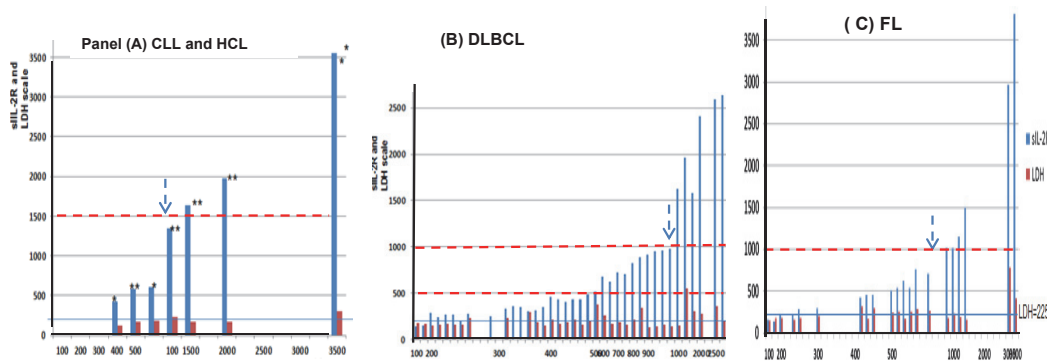


Figure 4. Histograms in each subtype. The association of individual sIL-2R(Blue bar) and LDH (Red bar) among the subtypes. sIL-2R levels were classified into three density groups; less than 300, 300 - 500, 500-1000 , and 1000 or more.

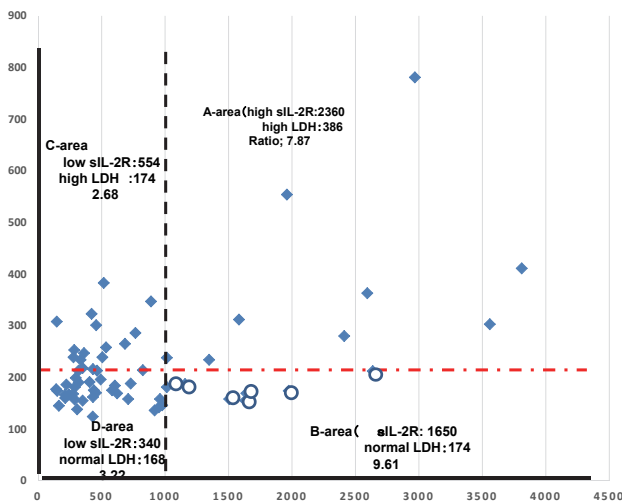


Figure 5. Twin-dot plot graph between sIL-2R and LDH. Exceeded 1000u/mL(broken black line), high sIL-2R group was divided into two groups, high sIL-2R group with high LDH (A-area) and without high LDH (B-area).

Table 2. Characteristics of three subtypes of mature B-ML

features	CLL/HCL	DLBCL	FL
sIL-2R u/ml (median)	1157	451	456
critical line	1500	1000	1000
LDH IU/ml (Median)	188	198	234
individual sIL-2R leve distribution			
-299	0	8(21.6%)	6(31.2%)
300-499	1(14.3)	12(32.1%)	2(10.2%)
500-999	2(28.6%)	12(32.4%)	4(20.4%)
1000-1499	1(14.3%)	0	5(26.3%)
1500-	3(42.8%)	5(13.5%)	2(10.2%)
individul LDH activity distribution site			
-225	5(71%)	26(70%)	8(42%)
226- 382	1(14%)	8(22%)	8(42%)
383-762	0	2(5%)	2
763--	0	1(2%)	1
Ratio of sIL-2R/LDH	5.76	2.24	2.07

Discussion

For a long time,much effort has been devoted to identifying potential biomarkers for lymphoma. However, there do not appear to be indicators readily excelling LDH and sIL-2R. The elevation of serum LDH activity and sIL-2R levels have been used for diagnosing, monitoring therapy, and predicting prognosis.

To precisely interpret clinical data in the present study, we established an RI in healthy controls because high sIL-2R levels are not necessarily associated with any specific symptoms. For example, one healthy sample donor had a serum sIL-2R level of 860u/mL, suggesting some kind of occult condition. Such sampling results were omitted as outliers in establishing the RI.By this approach,we concluded that RI is age-related while establishing 260± 41u/mL as the median adulthood RI.

Our fundamental interest is the applicability of sIL-2R as a laboratory test. Recently, the development of monoclonal antibodies defined the positive-expression of CD25 for many kinds of normal cells and cancer cells. Through flow-cytometric studies, Yoshida et al(9) also reported the presence of CD25-positive expression induced by DLBCL- and FL-leukemic cells. Interestingly, our study’s results have similarities to those of Yoshida et al., with respect to the origins of sIL-2R.

More recently, sIL-2R-related research has been advanced through the study of T-cell growth factors and Tac antigens by Uchiyama et al (16), where Tac was later designated as IL-2R alpha. Although the biological function is not yet completely understood, recently Yang Z-Z et al (8) reported that sIL-2R facilitates IL-2-mediated immune responses, showing the potential impact of sIL-2R on CD4+T-cell-mediated inhibition of CD8+T-cells. The clinical application of sIL-2R, in general, is used as a surrogate marker of tumor burden in oncology. However, the extent by which serum level of sIL-2R reflects the degree of tumor burden differs by disease. For applicability to ATL, we found the potentiality to predict ATL development through longitudinal observation of sIL-2R.Furthermore, since this receptor appears to be influenced by a wide range of diseases, we expedites to use as a marker to attain some measure of university.

Clinical significance of double assays for LDH and sIL-2R

Both of the biomarkers discussed are considered to be equally useful in clinical practice of lymphoma. To our knowledge, there is no report about the possibility that the kinetics of sIL-2R may be modulated by LDH status, yet LDH is one of the major enzymes catalyzing the biochemical process of pyruvate to lactate conversion. Unfortunately, in this study, such research between sIL-2R and LDH was not performed. Our study was noteworthy in that elevated sIL-2R appeared to be affected by LDH status.

Currently, LDH is widely employed in laboratory tests for clinical chemistry as a tumor or death marker. In contrast,the IL-2/IL-2R pathways associated with activation and prolifer-

eration. The pioneering translational research of the IL-2/IL-2R signal mechanism has opened new windows into molecular biology, and clinical applications of IL-2R, as a receptor of various kinds of cells, include tumor marker functionality. Thus, contrasting both biomarkers' progression is expected to promote biomarker applicability. The present study has not established direct correlations with respect to clinical oncological findings or explained origins of sIL-2R in terms of lymphoma cells alone; namely, if the elevation of sIL-2R is over 1000 u/mL accompanied with high LDH, the high sIL-2R is thought to be derived from a tumor. In contrast, high sIL-2R with normal LDH is thought to be derived from the pathology of an intra-tumor microenvironment.

In conclusion, the present study discloses the following: 1) serum sIL-2R level in mature B-cell lymphomas is not very high, equivalent to that of 1/10 to 1/100 in ATL, 2) there is inter-case and inter-subtype variation of sIL-2R level, that is, since an individual's sIL-2R level can fall within a normal healthy limit, above the 1000 u/mL critical threshold, or within the 300 to 1000 u/mL middle range, a serum sIL-2R level may help identify individual conditions. However, the ratio of sIL-2R to LDH may also be of high distinctive value in assessing the state of B-cell lymphomas. Such numerical data may additionally provide qualitative biological information as well.

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