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A Strong Correlation between Serum soluble IL-2 Receptor (sIL-2R) and Atypical Lymphocytosis

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Activated lymphocytes morphologically change into large aberrant cells known as atypical lymphocytes (atyLy). AtyLy are seen in various non-neoplastic conditions such as viral infection of Epstein-Barr virus, cytomegalovirus and hepatitis viruses. These activated cells release various cytokines or soluble receptors such as soluble interleukin-2 receptor (sIL-2R) and Fas-receptor (Fas-R). Accordingly, we measured serum sIL-2R in 25 pediatric patients. The data and other hematological/biochemical parameters were analyzed by the statistical processing method of Principle Component Analysis (PCA). 23 out of 25 patients with atypical lymphocytosis-related conditions (atyLy/lymphocyte ratio >5%) were found to have higher serum sIL-2R levels than the cut-off-value of 400 U/mL. The correlation between sIL-2R and the atyLy/lymphocyte ratio was the best indicator for discriminating the severity of disease. The first component (contribution ratio: 0.384) of PCA showed that lymphocyte activity was mostly represented by sIL-2R, lactate dehydrogenase, white blood cell count, lymphocyte count, lymphocyte percentile and atyLy/lymphocyte ratio.

Conclusively, these findings suggest a strong correlation between serum sIL-2R level and atypical lymphocytosis.

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Key words: sIL-2R, atypical lymphocyte, Principle Component Analysis

Introduction

Soluble IL-2R (sIL-2R) is a circulating form of interleukin-2 receptor (IL-2R) released from IL-2R-bearing malignant and normal cells.[1), 2)] IL-2R essentially exists in cell membranes and serves to functionally trigger active signals. This membrane form consists of three different subunits: alpha, beta and gamma. The alpha unit (CD25) was first identified on adult T-cell leukemia (ATL) as Tac antigens.[3)] Since serum sIL-2Rs are substantially overproduced in IL-2R-bearing tumor cells, sIL-2R is used as a surrogate biomarker for assessing the extent of a tumor.[4), 5)]

Interleukin-2 (IL-2) is stimulated mainly by IL-1 and IL-6 via activated mono-macrophages and leads to activation of T-cell immunity, which consequently increases sIL-2R. Thus, serum sIL-2R can similarly be expected to act as a marker for evaluating occult or aberrant immunity.

Atypical lymphocytes were originally defined according to morphologic characteristics, showing an abundant and basophilic cytoplasm with aberrant chromatin and nucleoli. Similarly to sIL-2R, the appearance of atypical lymphocytes in peripheral blood is considered to indicate active immune status.[6)] This phenomena frequently occur with viral infections in children, such as herpes simplex virus, Epstein-Barr virus (EBV), Coxsackie virus, cytomegalovirus and hepatitis A virus (HAV). Furthermore, atypical lymphocytes have been observed in the peripheral blood of patients in a large number of clinical situations, including

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graft-versus-host disease in transplantation, collagen diseases, autoimmune disorders, malignant tumors, and drug reactions. Since atypical lymphocytes can potentially produce various cytokines, it is important to monitor risk for severe conditions caused by cytokine storms. However, the measurement of atypical lymphocytes in an automated complete bood count (CBC) analyzer, which is the most standard device used in hospitals, is often substantially inaccurate. Therefore, we were interested in examining whether serum sIL-2R levels could surrogate the laboratory role of atypical lymphocytes. Data analysis was performed using the method of Principal Component Analysis (PCA), a data projection method which can be helpful in classification.[7] The central idea of PCA is to reduce the dimensionality (number of variables) of a data set but retain most of the original variability in the data.[8),9)] It computes a few linear combinations of the original variables, which can be used to summarize the data with minimal loss of information.[10)]

Subjects and study design

Serum IL-2R levels were measured in a total of 847 blood samples from in- and out-patients as a test mainly for evaluation of malignancy at Nagasaki Harbor Medical Center City Hospital during the period from April to October 2013. The levels were determined by a chemiluminescent enzyme immunoassay (CLEIA) (Siemens Healthcare Diagnostics, Tokyo, Japan), of which the normal range in adults is 152 to 492 U/ml. For samples in which the atypical lymphocyte data as measured by the CBC auto-analyzer Sysmex XE-5000 (Sysmex Corporation, Kobe, Japan) appeared to be incorrect, the samples were flagged for re-evaluating atypical lymphocyte levels by means of microscopic manual examination. As per standard procedure, all samples from patients of Pediatric department were evaluated by microscopic manual examination. Other data included in our analysis were white blood cell (WBC) count, neutrophil (Neu) count, Neu percentage, lymphocyte (Ly) count, Ly percentage, platelet (Plt) count, hemoglobin (Hb) level, alanine aminotransferase (ALT) level, lactate dehydrogenase level (LDH) and serum high-sensitive C-reactive protein (hs-CRP) level. All clinical data were collected from practical examination and saved in the hospital computer system. Surface markers of lymphocytes were not evaluated and the transition of parameters in the each patients were not validated in this study.

Statistical analysis

The Kruskal-Wallis test and the Spearman's rank correlation coefficient test were applied according to the distribution of data. The results were judged by the p-value (significant: < 0.05) and by the boundary value. Correlations of parameters were subjected to Principal Component Analysis (PCA) with Microsoft Excel and add-in software.

Results

1. Serum sIL-2R levels were usually high in patients with atypical lymphocytes.

Serum sIL-2R was detectable in all samples, varying from 122 to 301150 U/mL, thus showing inter-sample variation (Figure 1). Atypical lymphocytes (atyLy) were microscopically identified in 30 patients, including 5 adults with 5% atyLy or more per leukocyte fraction. 25 of the 30 patients were patients of Pediatric department (mean age = 8.4 years, male to female ratio = 0.93). The total serum sIL-2R levels of the pediatric patients were distributed from 200 to 3159 U/mL, as indicated by stars (*) on Figure 1. 4 cases were respectively diagnosed as Adeno virus infection, EB-virus infection, Bartonella infection and familial Mediterranean fever. The chief complaints of other undiagnosed patients were as follows: lymphadenopathy; 7 cases, fever with skin rashes; 3 cases, joint pain; 3 cases, fever alone; 2 cases, skin rushes alone; 1 case, abdominal pain; 1case, general malaise; 1case, listlessness; 1 case, liver disorder; 1 case, fever with neck pain; 1case. The 25 pediatric patients were classified into three groups according to atyLy/Lymphocyte ratio (Class 1: atyLy/Ly of less than 5%, Class 2: 6-49%, Class 3: 50% or more), as summarized in Table 1. In evaluating these 3 groups using the Kruskal-Wallis test, sIL-2R was the parameter which correlated the most closely with the atyLy/Ly ratio (p=3.67e^{-5}<0.05), although LDH (p=0.0166), hs-CRP (p=0.0003) and WBC (p=0.0045) also showed significant correlation with the atyLy/Ly ratio.

2. Correlation and Principal Component Analysis (PCA; a technique for data analysis and processing)

To identify a factor correlating with atyLy/Ly features, we subjected the data of 25 patients to Spearman's rank correlation coefficient test and to PCA. The closest correlation was observed between sIL-2R and atyLy/Ly (Spearman rank correlation coefficient = 0.92, p=6.23e^{-6} <0.05). Figure 2 shows that the atyLy/Ly ratio rose from Class 1 (<5%) to Class 2 (6-49%) across serum sIL-2R levels of 366 to 397 U/ml, and that the atyLy/Ly ratio rose from Class 2 to Class 3 (above

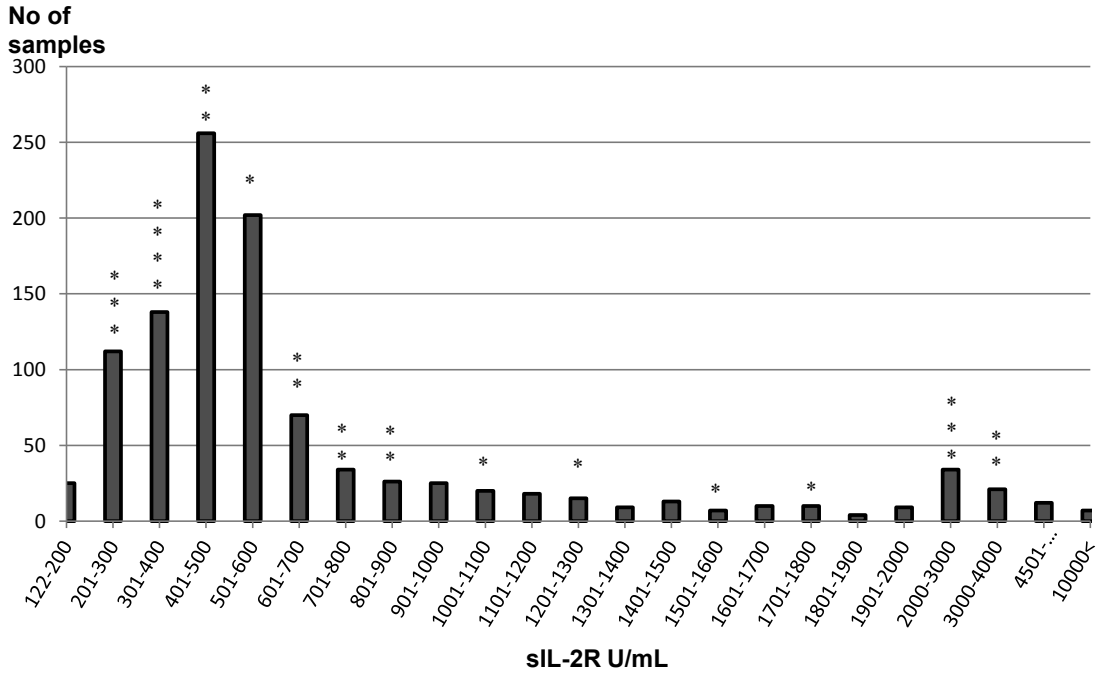

Figure 1. The distribution of the serum sIL-2R levels in 847 patients

50%) across serum sIL-2R levels 799 to 809 U/m. Serum LDH levels (rs=0.57, p=0.006<0.05), hs-CRP (rs=0.79, p=0.0001<0.05) and WBC (rs=0.62, p=0.0022<0.05) were also correlated with atyLy/Ly. However, sIL-2R was more useful for presuming the atyLy/Ly ratio than the other parameters, as clearly illustrated in Figure 2 by the comparatively very low overlap between AtyLy/Ly class distributions.

Next we performed Principal Component Analysis to clarify the relationship between parameters. The results of the PCA are shown in Table 2. The first component increased proportionally with the parameters sIL-2R, LDH, WBC, Ly count, Ly percentile and atyLy/Ly. The first component (contribution ratio (CR) was 0.384) was considered to mainly reflect the extent of lymphocyte activity. Similarly, the second component (CR: 0.258) as represented by Neu count, Neu percentage and hs-CRP was considered to reflect neutrophil-related inflammation. The third component (CR: 0.137) might reflect liver disorders related to ALT and LDH. The cumulative contribution ratio of the first, second and third components was 0.779.

Discussion

Atypical lymphocytes are readily identified by their increased size and the presence of active DNA synthesis. They are considered to be the activated form of lymphocytes. [6] At present, while experimental and clinical analyses of lymphocytes are mainly performed by surface markers or cytokines, morphological assessment of atypical lymphocytes in peripheral blood continues to be essential for evaluating active lymphocytes in vivo.

Although hematology analyzers continue to improve in performance year by year, their ability to classify differing leukocytes remains limited. Sensitivity is especially poor in distinguishing the morphological variations between normal lymphocytes, abnormal lymphocytes, lymphoblasts, and atypical lymphocytes. For example, sensitivity was reported as 51.2% in an efficient analyzer Sysmex XE-5000. [11] With respect to the flagged blood samples from the analyzer in our study, twice as many samples were identified as containing atypical lymphocytes when observed by manual review than when measured by the analyzer alone. In other words, on the basis of the analyzer's results half of all cases of atypical

lymphocytes were overlooked. Therefore, given that the AtyLy/Ly ratio was comparatively high at serum sIL-2R levels of about 400 U/ml and exceeded 50% at serum sIL-2R levels of 1000 U/ml or more, the ratio's close correlation with serum sIL-2R levels is especially of interest. Though we could not find any reports directly describing the relationship between sIL-2R and atypical lymphocytosis, a serum sIL-2R level appears to be applicable as a detective marker for assessing lymphocyte transformation in peripheral blood—similar to its marker role against cancer-derived lymphoid cells. Serum sIL-2R data may therefore prove complementary of automated hematology analyzer data, thereby offsetting analyzer-related inaccuracies in identifying atypical lymphoid cells. Serum sIL-2R is currently recognized as a marker of many cancers, of collagen disease, and of viral infections.[1), 5),12-15)] Serum sIL-2R may therefore additionally prove applicable in treating most diseases as a universal marker, considering that most diseases are substantially accompanied with activation of lymphocytes.

Conclusion

Serum sIL-2R is useful to detect atypical lymphocytosis resulting from lymphoid cell activation. A serum sIL-2R level of approximately 400 U/ml suggests the possibility of detecting atypical lymphocytes. A serum sIL-2R level of 1000 U/ml or more indicates remarkable atypical lymphocytosis.

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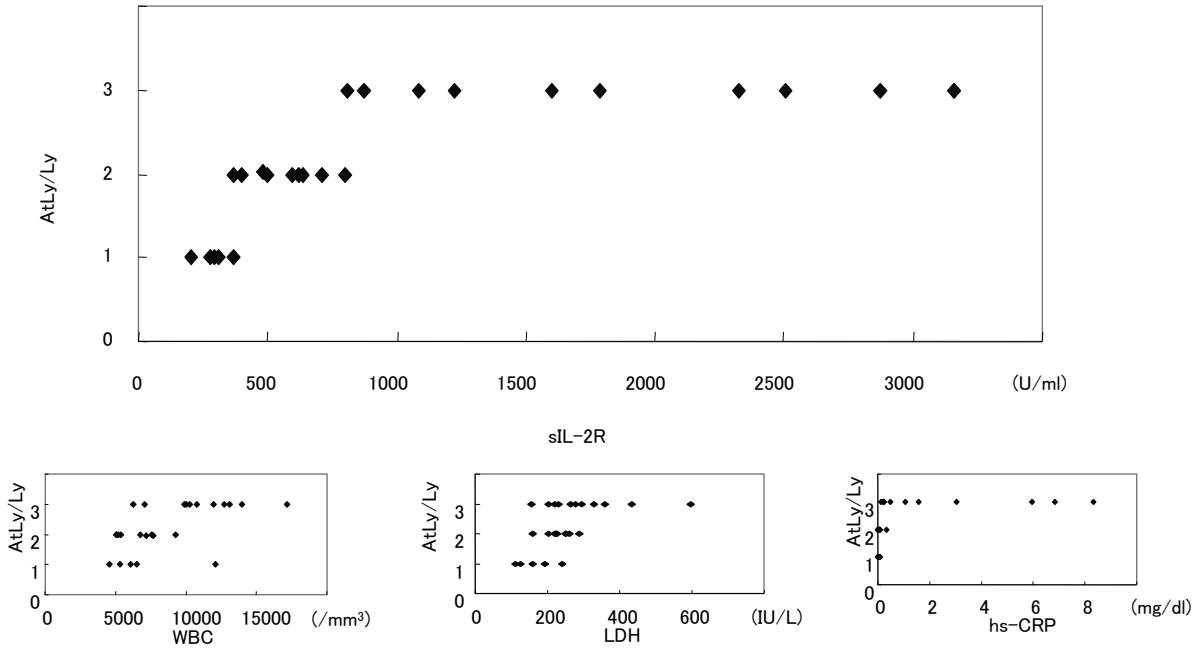
Table 1. Clinical characteristics of the disease status of 3 Groups classified by atypical lymphocyte/total lymphocyte ratio

	Group 1	Group 2	Group 3	*p*-value [a]	
AtyLy/Ly [b]	Class 1 (<5%)	Class 2 (6-49%)	Class 3 (>50%)		
Number of patients	5	9	11		
Male/Female ratio	0.67	1.25	1.75	0.8403	
Mean age (years)	13.6	7.6	6.7	0.0561	
sIL-2R (U/ml) [c]	289.8	565.3	1945.3	$3.67e^{-5}$	<0.05
LDH (IU/L) [d]	166.4	231.4	306.0	0.0166	<0.05
ALT (IU/L) [e]	12.0	15.1	47.8	0.0150	<0.05
hs-CRP (mg/dl) [f]	0.03	0.06	2.56	0.0003	<0.05
Hemoglobin (g/dl)	13.9	13.5	12.7	0.2872	
Platelet ($\times 10^4/mm^3$)	21.5	25.7	35.9	0.0803	
WBC ($/mm^3$) [g]	6920	6644	11218	0.0045	<0.05
Neutrophil (%)	59.8	48.2	52.9	0.4283	
Neutrophil ($/mm^3$)	4280	3318	5791	0.0771	
Lymphoyte (%)	30.6	38.6	38.4	0.4999	
Lymphocyte ($/mm^3$)	2034	2480	4503	0.1564	

[a] *p*-value: *p*-value of Kruskal-Wallis test
[b] atyLy/Ly: atypical lymphocyte/total lymphocyte ratio
[c] sIL-2R: soluble IL-2 receptor
[d] LDH：lactate dehydrogenase
[e] ALT: alanine aminotransferase
[f] hs-CRP: high-sensitive C-reactive protein
[g] WBC: white blood cell



AtyLy/Ly was classified into 3 classes: Class 1; less than 5%, Class 2; 6-49%, Class 3; 50% or more. sIL-2R was the parameter most closely correlated with atyLy/Ly and was useful to presume atyLy/Ly.

Figure 2. Correlation of parameters and atypical lymphocyte/lymphocyte ratio (atyLy/Ly)

Table 2. Principal Component Analysis of laboratory parameters

	Components		
	1	2	3
AtyLy/Ly [a]	0.826	0.331	0.248
sIL-2R (U/ml) [b]	0.685	0.322	0.231
LDH (IU/L) [c]	0.671	-0.158	0.623
ALT (IU/L) [d]	0.467	-0.286	0.727
hs-CRP (mg/dl) [e]	0.215	0.593	0.020
Hemoglobin (g/dl)	-0.595	-0.232	0.491
Platelet ($\times 10^4/mm^3$)	0.715	0.218	-0.468
WBC ($/mm^3$) [f]	0.700	0.498	-0.100
Neutrophil (%)	-0.524	0.802	0.162
Neutrophil ($/mm^3$)	0.038	0.932	0.063
Lymphoyte (%)	0.626	-0.712	-0.225
Lymphocyte ($/mm^3$)	0.851	-0.227	-0.242
Contribution ratio	0.384	0.258	0.137

[a] atyLy/Ly: atypical lymphocyte/total lymphocyte ratio
[b] sIL-2R: soluble IL-2 receptor
[c] LDH：lactate dehydrogenase
[d] ALT: alanine aminotransferase
[e] hs-CRP: high-sensitive C-reactive protein
[f] WBC: white blood cell

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