学位論文

Doctoral Thesis

Clinical application of serum soluble CD30 levels as a biomarker of adult T-cell leukemia/lymphoma

（血清中可溶性CD30レベルの成人T細胞白血病・リンパ腫バイオマーカーとしての臨床応用）

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Contents

1. Abstract

Abstract of the Thesis

Background and Purpose:

Adult T-cell leukemia/lymphoma (ATLL) is an incurable neoplasm of mature T cells with a median survival time of approximately 1 year. The aim of the present study was to investigate the clinical value of soluble CD30 (sCD30) levels as compared with soluble IL-2 receptor α chain (sIL-2R) levels in predicting the response to therapy in ATLL patients.

Methods:

The study subjects were ATLL patients who had been referred to the Department of Hematology of National Hospital Organization Kumamoto Medical Center between September 2005 and December 2010. The levels of sCD30 and sIL-2R in ATLL patients were measured in two different clinical settings: before an initial therapy of chemotherapy or gastric resection (n = 32) and before allogeneic hematopoietic stem cell transplantation (HSCT; n = 24). All patients completed the 2-year follow-up.

Results:

Before the initial therapy, sIL-2R (p = 0.016) and sCD30 (p = 0.030) were significant predictors of overall survival. The number of ATLL cells in peripheral blood (PB) was significantly correlated with sCD30 levels (Spearman correlation coefficient, ρ = 0.46; p = 0.009) but not with sIL-2R levels (ρ = 0.16; P = 0.38). sCD30 levels for long-term survivors (more than 2 years) were relatively low when ATLL cells accounted for <5% of PB, but this tendency was not observed when ATLL cells were more plentiful (≥5%) in PB. Before HSCT, sIL-2R (p = 0.041) and sCD30 (p = 0.0003) were significant predictors of overall survival. Moreover, sCD30 detected more patients with early death

(within 100 days following HSCT) than sIL-2R. A combined test of sCD30 and CRP showed high sensitivity (81.8%) and specificity (84.6%) in detecting early death.

Conclusions:

Our results suggest that sCD30 may be a useful biomarker before HSCT therapy, because a high level of CD30 before HSCT is implicated in early death after HSCT. In cases with sCD30 level ≥ 170 U/ml and/or CRP ≥ 0.15 mg/dL, HSCT may not be suitable for ATLL patients. Early diagnosis and treatment of the proinflammatory state could reduce morbidity and mortality of patients undergoing HSCT.

2. Publication list

1) Clinical Value of Serum Soluble CD30 Levels in Adult T-Cell Leukemia/Lymphoma.

Ratiorn Pornkuna, Shigeki Takemoto, Michihiro Hidaka, Fumio Kawano and Yoshio Haga

J.Hematol. Thrombo. Dis. (in press).

2) A Lack of Cellular Senescence, Formation of Microenvironment, and Role of Soluble CD30 in Development of Adult T-Cell Leukemia/Lymphoma.

Ratiorn Pornkuna and Shigeki Takemoto

J.Hematol. Thrombo. Dis. 2: 151, 2014.

3) Effect of Blood Transfusion on Supportive Therapy of Elderly Patients at Kumamoto, Japan as Compared with Khon Kaen, Thailand.

Ratiorn Pornkuna, Somchai Wongkhantee, Sompong Jinathongthai, Satomi Shimogawa and Shigeki Takemoto

J.Hematol. Thrombo. Dis. 2: 152, 2014.

4) What is the Role of Soluble Cytokine Receptors in Adult T-cell Leukemia/Lymphoma?

Ratiorn Pornkuna, Chie Nishioka and Shigeki Takemoto

J.Hematol. Thrombo. Dis. 2: 154, 2014.

5) Mutualism among HTLV-1-Infected Different Type of Cells or among Other Virus-Infected cells.

Shigeki Takemoto and Ratiorn Pornkuna

J. Hematol. Thrombo. Dis. 2: 164, 2014.

6) Soluble CD30 and peripheral blood or pulmonary involvement of adult T-cell leukemia/lymphoma.

Shigeki Takemoto and Ratiorn Pornkuna

Integr. Cancer. Sci. Therap. 1: 10–11, 2014.

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4. Abbreviations

ATLL: Adult T-cell leukemia/lymphoma

HTLV-1: Human T leukemic virus type 1

STAT: Signal transducers and activators of transcription

Bcl-xL: B-cell lymphoma extra large

HSCT: Allogeneic hematopoietic stem cell transplantation

OS: Overall survival

TRM: Transplantation-related mortality

IL-2: Interleukin-2

sIL-2R: Soluble interleukin-2 receptor α chain

PB: Peripheral blood

MMP: Matrix metalloproteinase

HD: Hodgkin's lymphoma

ALCL: Anaplastic large cell lymphoma

sCD30: Soluble CD30

ADAM: A disintegrin and metalloproteinase

LDH: Lactic acid dehydrogenase

PS: Performance status

ATL-PI: a prognostic index for acute- and lymphoma-type ATL

ELISA: Enzyme-linked immunosorbent assay

TBI: Total body irradiation

BU: Busulfan

GVHD: Graft-versus-host disease

HRs: Hazard ratios

CI: Confidence intervals

PB: Peripheral blood

IP: Interstitial pneumonia

DPI: Diffuse pulmonary infiltrates

MOF: Multiple organ failure

aGVHD: Acute graft versus host disease

PE: Pleural effusion

LN: Lymph node

IPS: Idiopathic pneumonia syndrome

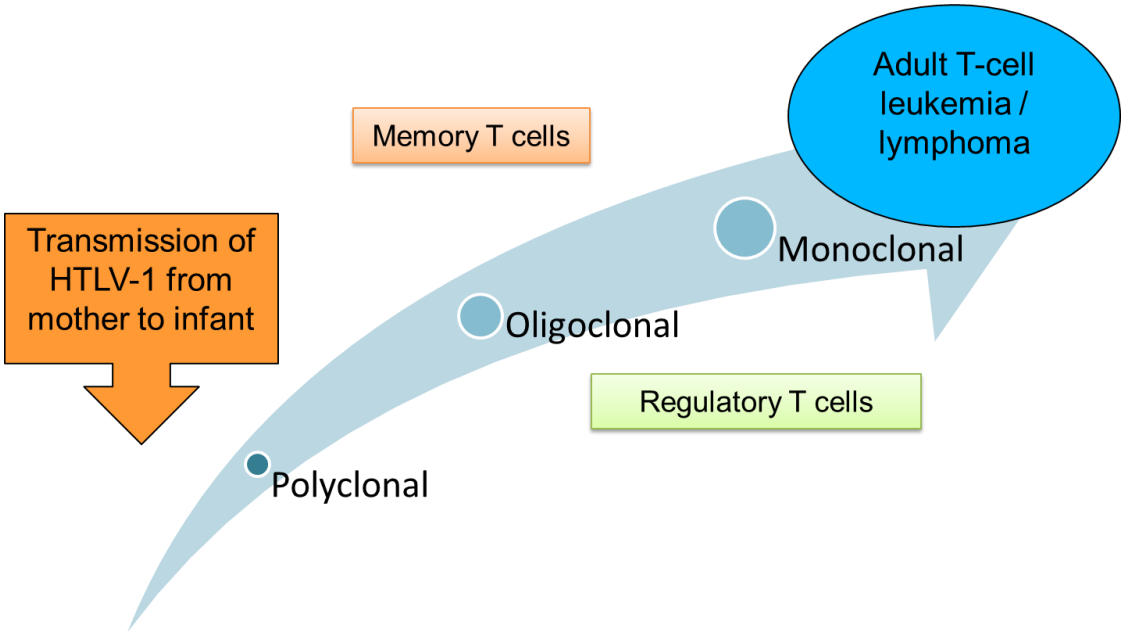
EBV: Epstein-Barr virus

HHV-8: Human herpes virus type 8

5. Background and Purpose

Adult T-cell leukemia/lymphoma (ATLL) is a highly aggressive leukemia/lymphoma which was first proposed as a new disease entity by Takatsuki et al. in Japan [1,2]. Only 2–5% of human T leukemic virus type 1 (HTLV-1) carriers develop ATLL, and the mean latency period is more than 50 years (Figure 1) [3].

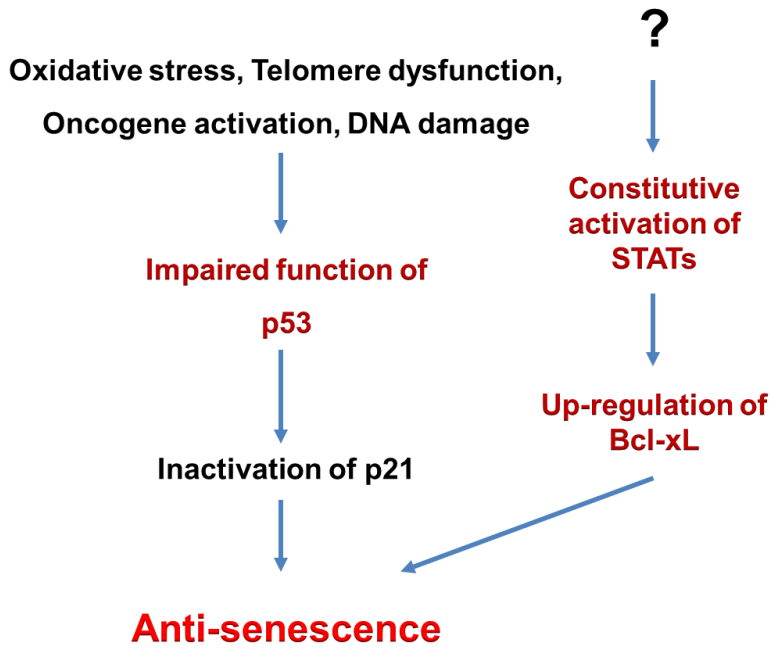
Figure 1 Only a small percentage of HTLV-1 carriers develop leukemia/lymphoma more than 50 years after infection.



HTLV-1 is thought to be mainly transmitted from mother to infant via breast feeding. Although HTLV-1 can infect cells other than T cells, such as B cells and dendritic cells, only CD4+CD25+ T cells are transformed after a long latent period in a small percentage of HTLV-1 carriers.

Approximately 1,000 ATLL patients die in Japan every year [4]. However, the mechanism of ATLL development remains unclear. After World War II, both the per capita Gross Domestic Product and Japanese life expectancy increased dramatically. During that time, ATLL was discovered [5]. It is indicated that ATLL is an age-related disease involving genetic changes prior to malignant transformation and monoclonal expansion of HTLV-1 infected cells [6]. Monoclonal proliferation of HTLV-1–infected cells is observed in patients who ultimately develop ATLL; thus, these patients constitute a high-risk group for ATLL development [7]. The mechanism by which ATLL cells induce monoclonal proliferation rather than undergo senescence is unknown. A series of genetic change were shown to be associated with the development of ATLL. Despite the absence of genetic mutation or deletion, the function of p53 may be impaired in ATLL cells, suggesting inhibition of senescence *in vivo* [8]. ATLL cells have a constitutively activated Janus kinase- signal transducers and activators of transcription (STAT) pathway which is important for the immortalization and transformation mechanisms [9]. Furthermore, aberrant B-cell lymphoma extra large (Bcl-xL) expression may increase ATLL cell survival, contributing to chemotherapy resistance [10]. HTLV-1 infection eventually transforms T cells to ATLL cells by a long process involving inhibition of senescence and apoptosis with immune alteration (Figure 2) [11].

Figure 2 Lack of cellular senescence as a cause of ATLL development.



Cellular proteins, such as p53, STAT and Bcl-xL, contribute to the anti-senescence of ATLL cells.

(Pornkuna R, Takemoto S. *J Hematol Thrombo Dis* 2: 151, 2014)

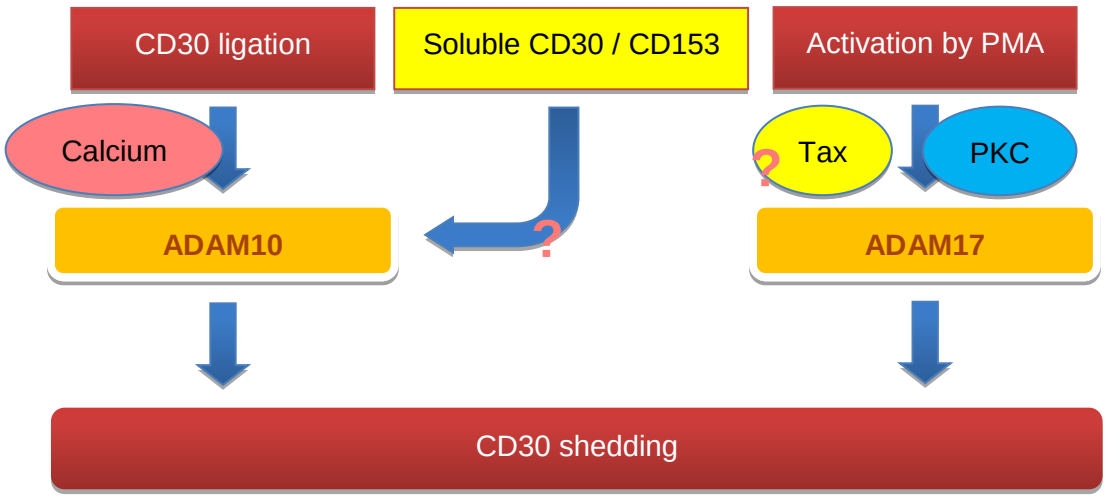
Once the patients are diagnosed as ATLL, they are usually first treated by chemotherapy. In exceptional cases in which the lesions are confined to the stomach, the patients can receive surgical resection, followed by chemotherapy [12,13]. After chemotherapy, the younger patients are candidates for allogeneic hematopoietic stem cell transplantation (HSCT) in Japan [14]. Unfortunately, the unadjusted 3-year probability of overall survival (OS) was only 33% after HSCT. Previous study by multivariable analyses revealed 4 factors that adversely affected OS: older recipient age (>50 years), male recipient, lack of complete remission at transplantation, and transplantation of unrelated cord blood [15]. The reported incidence of transplantation-related mortality (TRM) is between 25%–64% [16].

Several biomarkers have been proposed in ATLL patients [17–19]. Among them, soluble interleukin-2 (IL-2) receptor α chain (sIL-2R) is thought to be the most useful and widely used in clinical practice [20]. IL-2 is a T-cell growth factor and essential in the survival and expansion of $CD4^+CD25^+$ activated T cells in peripheral blood (PB) [21]. IL-2R contains α, β, and γ chains. Matrix metalloproteinase (MMP)-9 mediates cleavage of the IL-2R α chain (IL-2Rα, CD25), which produces sIL-2R [22]. CD25 is strongly expressed on the surface of ATLL cells, and sIL-2R levels are elevated in the sera of ATLL patients [23,24].

CD30 is a cytokine receptor that belongs to tumor necrosis factor superfamily [25]. CD30 is physiologically expressed on activated T cells and B cells, as well as on some tumor virus–infected T cells and B cells [25]. CD30 expression is dependent on mitogen or viral activation and proliferation of B cells and T cells. Tumor cells from Hodgkin's lymphoma (HD) and anaplastic large cell lymphoma (ALCL) strongly express CD30 [26]. HTLV-1–infected cells and some ATLL cells also express CD30 [27,28]. MMP cleaves CD30 in the juxta-membrane region, and soluble CD30 (sCD30) is released as an extracellular region of an 85/90-kDa protein [29].

Interestingly, there are two kinds of MMPs by which CD30 shedding is catalyzed (Figure 3) [30]. In antibody-based immunotherapy, antibody-induced CD30 shedding is catalyzed by a disintegrin and metalloproteinase (ADAM)10 [31]. In contrast, proteasome inhibitor-induced CD30 shedding is catalyzed by ADAM17 [32]. CD30+ cells release sCD30 in vitro and in vivo, and it is detected at low levels in the sera of healthy donors [33].

Figure 3 Two different mechanisms of CD30 shedding.

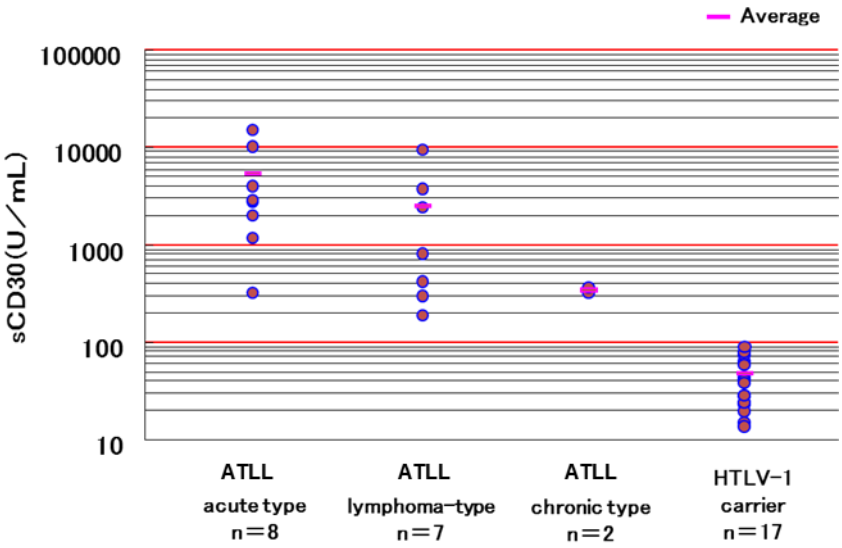


Two matrix metalloproteinases, a disintegrin and metalloproteinase (ADAM)10 and ADAM17, are associated with CD30 shedding.

(Pornkuna R, Nishoka C, Takemoto S. *J Hematol Thrombo Dis* 2: 154, 2014)

Levels of sCD30 are also elevated in the serum of patients with aggressive ATLL (Figure 4) [28,34].

Figure 4 Soluble CD30 (sCD30) levels in the sera of ATLL patients.



Serum levels of sCD30 were plotted in patients with the acute type, lymphoma-type, and chronic type ATLL and HTLV-1 carriers.

(Takemoto S, *et al.* : *BIO Clinica* 27: 75–80, 2012)

Early death after HSCT is a significant problem to be solved. Severe immune suppression and viral infection by treatment and/or relapse of ATLL may be involved in the early death. Recently, the region-wide study in Hokkaido demonstrated for the first time that a high level of sIL-2R at HSCT was a significant risk factor of OS for death following HSCT [35]. In the present study, we assessed and compared the clinical value of sCD30 levels with sIL-2R levels with the aim of predicting the prognosis of ATLL patients before initial therapy as well as before HSCT.

6. Methods

Patients

The present study is a cohort study. The study protocol (No. 77) was approved by the Institutional Review Board of the National Hospital Organization Kumamoto Medical Center on July 4, 2005. Written informed consent was obtained from all patients. This study was conducted in accordance with the Ethical Guidelines on Epidemiological Research jointly designed by the Ministry of Health, Labour and Welfare and the Ministry of Education, Culture, Sports, Sciences and Technology of the Japanese Government.

The study subjects were ATLL patients who had been referred to the Department of Hematology of our hospital between September 2005 and December 2010. ATLL was classified into four clinical subtypes according to the criteria established by the Lymphoma Study Group of the Japan Clinical Oncology Group (Shimoyama's classification): acute, lymphoma, chronic, and smoldering types [36]. These types are determined by the number of abnormal T cells in peripheral blood, serum lactic acid dehydrogenase (LDH) level, tumor lesions in various organs, and clinical course. Venous blood samples to determine sCD30 and sIL-2R levels were collected before the initial therapy or before the conditioning therapy of HSCT.

Data collection

Clinical data including age, sex, clinical subtype, Eastern Cooperative Oncology Group performance status (ECOG PS), Ann Arbor stage, Charlson Comorbidity Index [37], and laboratory findings were recorded before therapy. We also calculated the simplified prognostic index, a prognostic index for

acute type and lymphoma-type ATLL (ATL-PI), as previously reported [38].

Simplified ATL-PI = 2 (if stage = III or IV) + 1 (if ECOG PS >1) + 1 (if age >70 years) + 1 (if albumin <3.5 g/dL) + 1 (if sIL-2R >20000 U/mL).

Endpoint

The endpoint of the study was 2-year OS. The follow-up study was ended on 21st December 2012, so that the last registered patient could be followed up for two years. We analyzed OS at two different phases of ATLL patients, before the initial therapy (chemotherapy or total gastrectomy) and before the HSCT. The starting point for OS before the initial therapy was the starting date of initial therapy (chemotherapy or total gastrectomy), including 4 patients who were successfully treated with HSCT following the initial therapy. One study patient died of an acute crisis before receiving chemotherapy; we included this case in the study, and the starting date for survival was the day of the acute crisis. Before the HSCT, the starting point of OS was the date of transplantation. The cause of death following HSCT was categorized as disease progression or TRM. A death was determined to be caused by disease progression if any evidence of ATLL relapse was detected. TRM was determined to be the cause of death when there were adverse events related to transplantation without evidence of relapse. A 2-year follow-up period was used for all patients.

Measurement of soluble cytokine receptor levels

Sera samples were collected prior to therapy and preserved at −80˚C until the levels of sCD30 and sIL-2R were determined by sandwich enzyme-linked immunosorbent assays (ELISAs). In some

patients, serial blood samples were taken during the course of treatment if they consented to the procedures. Serum sIL-2R concentrations were measured by Cell freeN IL-2R (Kyowa Medex Co., Ltd., Shizuoka, Japan) and Determiner CL IL-2R (Kyowa Medex Co., Ltd.). Serum sCD30 concentrations were measured by Human sCD30 Platinum ELISA (eBioscience, Vienna, Austria) at the Research Laboratories of Kyowa Medex Co., Ltd., Shizuoka, Japan.

Chemotherapy

Conventional chemotherapy for aggressive ATLL was based on the Japan Clinical Oncology Group protocol and first-generation CHOP protocols containing cyclophosphamide, doxorubicin, vincristine, and prednisolone, which is the standard regimen for lymphoma. Until now, ATLL patients have been treated with intensive chemotherapy with G-CSF support, such as LSG15 regimens and CHOP-V-MMV [39,40]. CHOP-V-MMV consists of CHOP plus vindesine, ranimustine, and etoposide. LSG15 is a sequential combination chemotherapy consisting of three regimens: VCAP (vincristine, cyclophosphamide, doxorubicin, and prednisolone), AMP (doxorubicin, ranimustine, and prednisolone) and VECP (vindesine, etoposide, carboplatin, and prednisolone).

Allogeneic hematopoietic stem cell transplantation

In general, relatively younger patients (≤65 years old) who completed chemotherapy were scheduled to receive HSCT unless their general condition worsened or the disease progressed [41]. Most HSCT patients had received chemotherapy in other hospitals.

The most common conditioning regimens for HSCT were: cyclophosphamide (60 mg/kg) with total body irradiation (TBI, 1200 cGy); cyclophosphamide (60 mg/kg) with busulfan (BU) (1 mg/kg) (both myeloablative); and fludarabine (30 mg/m^2) from days −7 to −2 with BU (4 mg/kg) from days −5 to −4 with or without TBI (200 cGy, reduced intensity).

Patients scheduled to receive a sibling- or family-matched HSCT received prophylaxis for graft-versus-host disease (GVHD) comprising cyclosporine (3 mg/kg) and methotrexate (day 1, 10 or 15 mg/m^2; day 3 and 6, 7, or 10 mg/m^2). Cyclosporine doses were adjusted according to renal function and daily cyclosporine levels. Patients receiving matched unrelated donor transplants received tacrolimus (0.03 mg/kg) and methotrexate (day 1, 10 or 15 mg/m^2; days 3 and 6, 7 or 10 mg/m^2).

Statistical analysis

Statistical analysis was performed by using Prism software (GraphPad Prism 5.0, San Diego, CA). OS was plotted by using the Kaplan-Meier method. The log-rank (Mantel-Cox) test was conducted to identify differences in OS. The cut-off points were set at the points in which minimal *P* values were obtained. The effects of clinical factors were evaluated by using hazard ratios (HRs) and the corresponding 95% confidence intervals (CI). Statistical analysis of differences in soluble cytokine receptors between independent groups was performed with a Mann-Whitney U test. Correlation between continuous variables and ordinal variables was analyzed by Spearman's rank correlation (ρ) (IBM SPSS Statistics 20.0, Armonk, NY). Two-tailed *p* values <0.05 were considered statistically significant.

7. Results

Study profile

From September 2005 to December 2010, a total of 52 ATLL patients with aggressive ATLL requiring therapy were enrolled. Table 1 shows the baseline characteristics of 32 patients before the initial therapy. These patients included 29 patients scheduled for chemotherapy, one patient who died before any therapy, and two patients undergoing total gastrectomy followed by chemotherapy. The median age was 70 years old, and the majority of the patients had multiple comorbidities. Acute-type patients had significantly higher levels of sCD30 than lymphoma-type patients (p = 0.025), while there was no difference in the sIL-2R levels between both subtypes (p = 0.24). The 2-year survival rate was 22% after the initial therapy (7 of 32 patients). All patients completed the 2-year follow-up with a median follow-up period of 4.8 months (range, 0.0 to 59 months). Four of these patients underwent HSCT following chemotherapy.

We also analyzed 24 patients before HSCT (Table 1). The 2-year survival rate was 29% (7 of 24 patients) after HSCT. Serum levels of the soluble cytokine receptors were generally low as compared with those before initial therapy, but some patients with lymphoma-type ATLL had high levels of sIL-2R.

Table 1: Clinical characteristics of ATLL patients before initial therapy (n = 32) and HSCT (n = 24)

Characteristics	Before initial therapy [a]	Before HSCT
Median age, y (range)	69.5 (43–86)	54.5 (43–61)
Sex, % men	53	58
Subtype, n, Acute : Lymphoma : Chronic	20 : 10 : 2	14 : 9 : 1
Performance status, n, 0 : 1: 2 : 3	1 : 9 : 9: 7	9 : 12 : 3 : 0
Charlson comorbidity index [b]	2 (0–10)	0 (0–3)
ATLL cells in PB, cells/μL [b]		
Acute	9697 (55–169405)	12 (0–6063)
Lymphoma	0 (0–64)	0 (0–40)
Chronic	2622, 11928	0
Serum level of soluble IL-2R, U/mL [b]	21330 (478–500691)	927 (319–98314)
Acute	23535 (478–500691)	764 (319–27302)
Lymphoma	15511 (1928–104001)	2081 (538–98314)
Chronic	5643, 11368	3270
Serum level of soluble CD30, U/mL [b]	1251 (13–21566)	79 (14–2789)
Acute	1895 (13–21566)	74 (14–2789)
Lymphoma	159 (18–3354)	76 (38–932)
Chronic	313, 1251	720

[a]: The patients included 29 patients who received chemotherapy, one patient who had been scheduled for chemotherapy but died before therapy, and two patients who underwent total gastrectomy followed by chemotherapy. [b]: Data are shown as median (range). HSCT: hematopoietic stem cell transplantation; PB: peripheral blood

Univariate analysis for 2-year overall survival before initial therapy

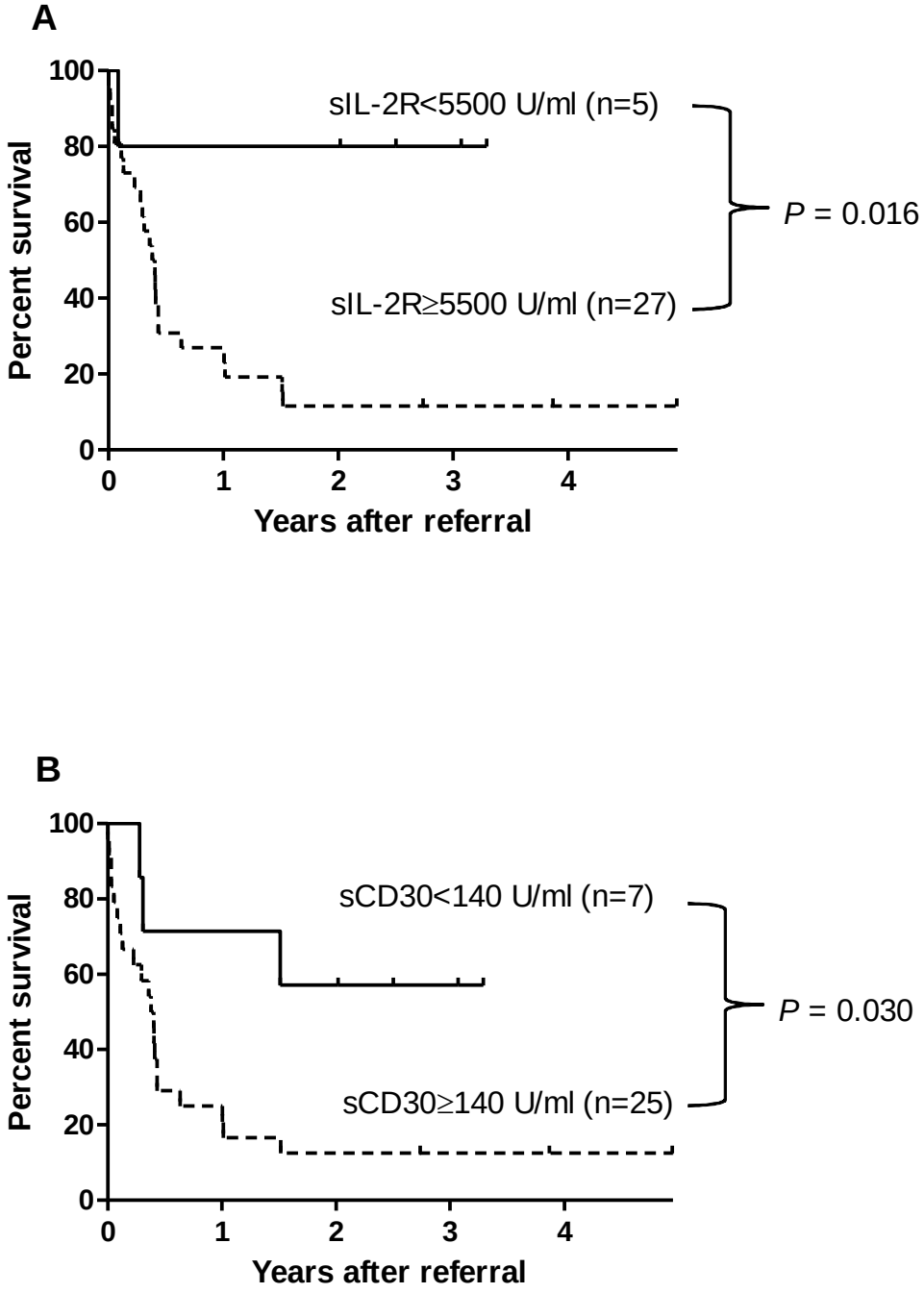
Besides the soluble cytokine receptors, univariate analysis of patients before the initial therapy revealed 11 significant variables for OS as follow:

1. PS ≥ 3 (HR = 8.9, 95% CI 3.2–25, $p < 0.0001$)
2. creatinine ≥ 1.05 mg/dL (HR = 33, 95% CI 5.9–185, $p < 0.0001$)
3. Charlson comorbidity index ≥ 4 (HR = 126, 95% CI 15–1063, $p < 0.0001$)
4. BUN ≥ 45 mg/dL (HR = 56606, 95% CI 1119–2864000, $p < 0.0001$)
5. thrombocyte count ≤ 65000 /μL (HR = 73, 95% CI 7.5–705, $p = 0.0002$)
6. total bilirubin ≥ 3.5 mg/dL (HR = 13, 95% CI 2.0–87, $p = 0.007$)
7. serum calcium level ≥ 10 mg/dL (HR = 3.2, 95% CI 1.4–7.5, $p = 0.008$)
8. AST ≥ 60 IU/L (HR = 3.6, 95% CI 1.3–10, $p = 0.012$)
9. CRP ≥ 2.5 mg/dL (HR = 3.5, 95% CI 1.2–9.9, $p = 0.020$)
10. albumin ≤ 3.2 g/dL (HR = 2.7, 95% CI 1.1–6.6, $p = 0.028$)
11. age ≥ 78 years (HR = 3.5, 95% CI 1.1–11, $p = 0.038$).

Clinical values of sCD30 and sIL-2R before initial therapy

We compared the values of sIL-2R and sCD30 before the initial therapy (Figure 1). The Kaplan-Meier curves showed that patients with sIL-2R ≥ 5500 U/ml (Figure 1A) had a significantly worse OS than those with sIL-2R < 5500 U/ml (HR = 3.2, 95% CI 1.2–8.4, $p = 0.016$). The median survival time was 4.6 months (range, 0 to 59 months) in the high mortality group and 30 months (1 to 39.5 months) in the low mortality group. Similarly, patients with sCD30 ≥ 140 U/ml (Figure 1B) before initial therapy had a significantly worse OS than those with sCD30 < 140 U/ml (HR = 2.6, 95% CI 1.1–6.3, $p = 0.030$). The median survival time was 4.6 months (range, 0 to 59 months) in the high mortality group and 24 months (3.4 to 39.5 months) in the low mortality group.

Figure 5 Pretreatment marker levels and treatment outcome from initial therapy.



Kaplan-Meier analysis of overall survival of patients with high or low pretreatment levels of sIL-2R (A) or sCD30 (B).

We also found that sCD30 levels were correlated with ATL-PI ($\rho = 0.41$; $p = 0.019$) (Table 2).

Table 2 Correlation of serum markers with simplified ATL-PI

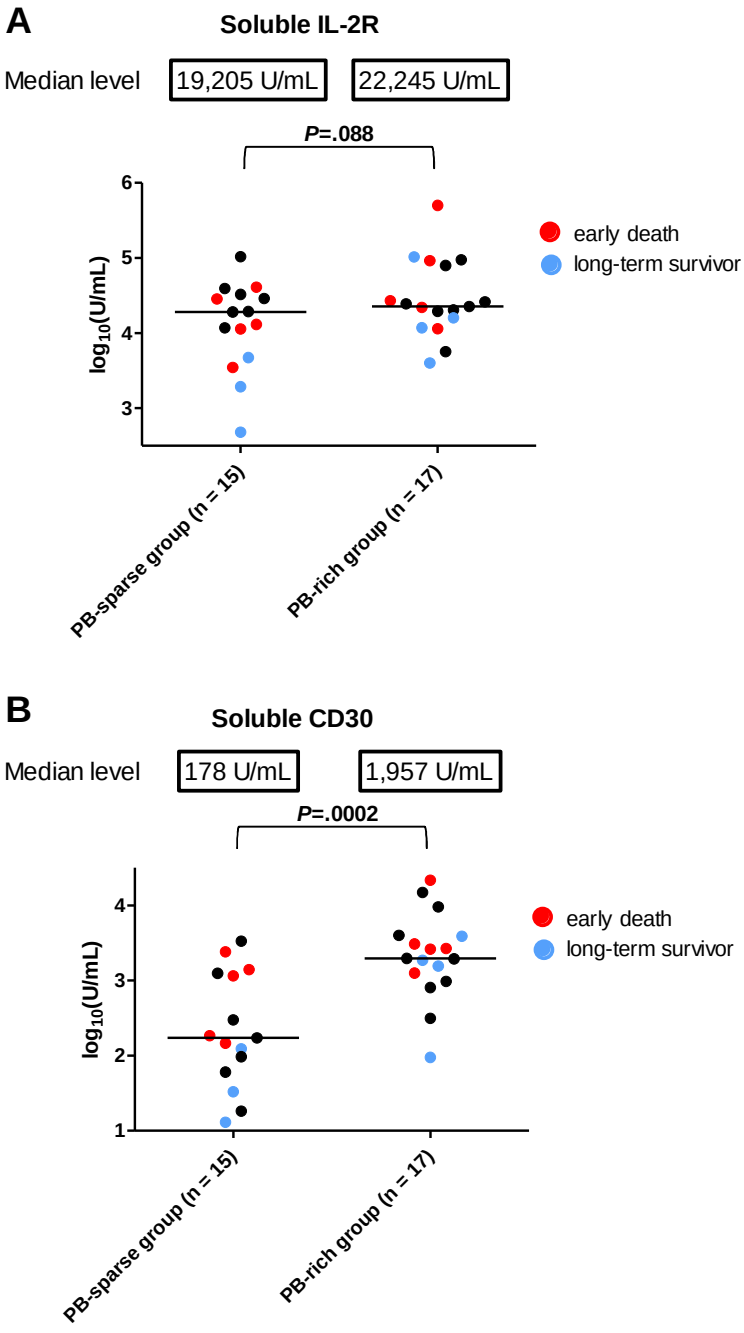
		BUN	AST	LDH	T-Bil	Ca	sCD30
	ρ	0.418*	0.423*	0.364*	0.415*	0.465*	0.413*
simplified ATL-PI	p	0.017	0.016	0.041	0.018	0.011	0.019
	N	32	32	32	32	29	32

ρ: Spearman's correlation coefficient; *Two-tailed *p* values < 0.05.

Serum level of soluble CD30 under influence of the number of ATLL cells in peripheral blood

As shown in Table 1, serum levels of sCD30 in acute-type ATLL were 10-times higher than in lymphoma-type ATLL. Therefore, we hypothesized that the sCD30 level might be associated with the percent of ATLL cells in PB. We used the cutoff value of 5% ATLL cells in PB to differentiate between high and low percentages, because this condition identifies monoclonal proliferation of ATLL cells in PB. Typical smoldering type, chronic type, and acute type show more than 5% ATLL cells in PB. On the other hand, lymphoma-type or other special primary extra-nodal type, such as primary gastric ATLL, usually has less than 5% ATLL in PB. As shown in Figure 6, sIL-2R levels were similar between patients with ≥ 5% ATLL cells in PB (PB-rich group) and <5% ATLL cells in PB (PB-sparse group) (median, range: 22525, 3987–500691 vs. 19062, 478–104001; $p = 0.23$). In contrast, sCD30 levels were significantly different between the two groups (median, range: 1971, 94–21566 vs. 172, 13–3354; $p = 0.001$). When we look at long-term survivors in the PB-sparse group, sIL-2R and sCD30 levels were relatively low. On the other hand, in the PB-rich group, the values for long-term survivors were not always low. Three of 4 cases of long-term survivors in the PB-rich group exceeded 1000 U/mL of sCD30. Furthermore, we found a significant correlation between the number of ATLL cells and sCD30 level ($\rho = 0.46$; $p = 0.009$), but not between the number of ATLL cells and sIL-2R level ($\rho = 0.16$; $p = 0.38$).

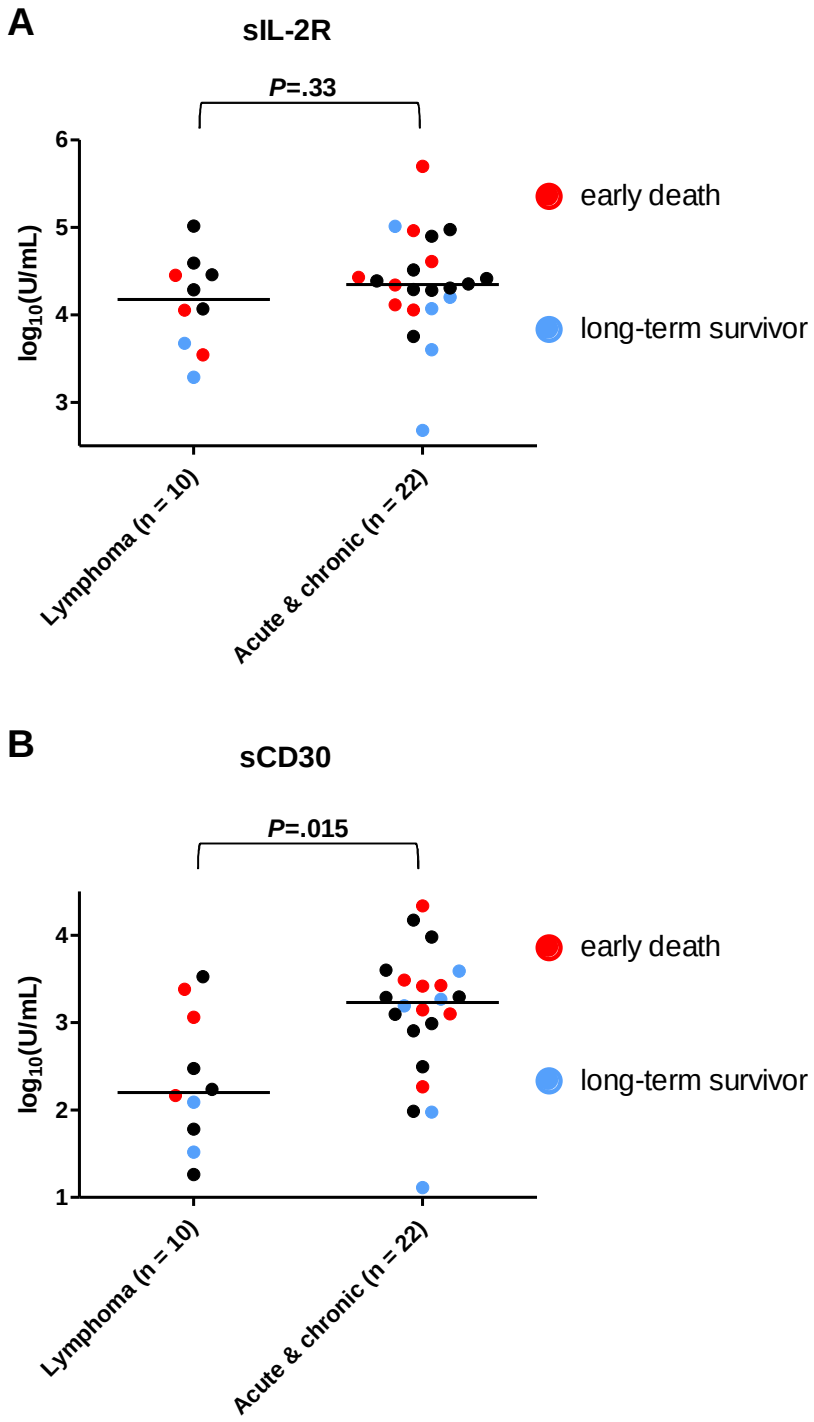
Figure 6 Level of sCD30 under influence of %ATLL cells in peripheral blood**.**



Serum levels of (A) sIL-2R and (B) sCD30 markers were plotted in two groups based on the percentage of ATLL cells in PB. Red closed circles and blue closed circles indicate patients who died within 100 days (early death) and patients who survived for more than 2 years (long-term survivor), respectively.

Subtypes of ATLL (acute and chronic type vs. lymphoma-type) also showed similar results, and sCD30 seems to be a reliable biomarker of lymphoma-type ATLL in a group that underwent initial therapy (Figure 7).

Figure 7 Soluble CD30 is a biomarker of lymphoma-type in ATLL cells.

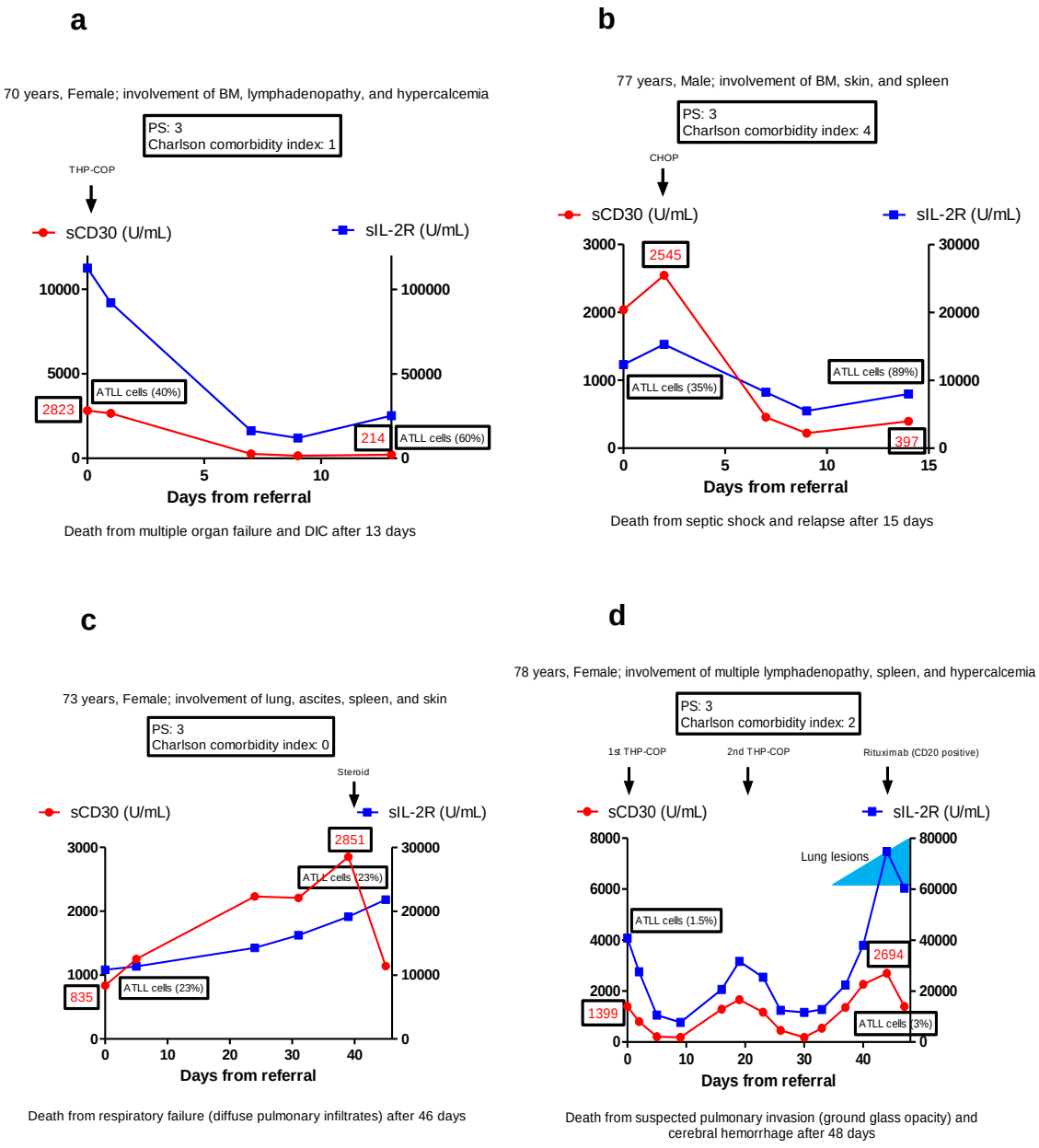


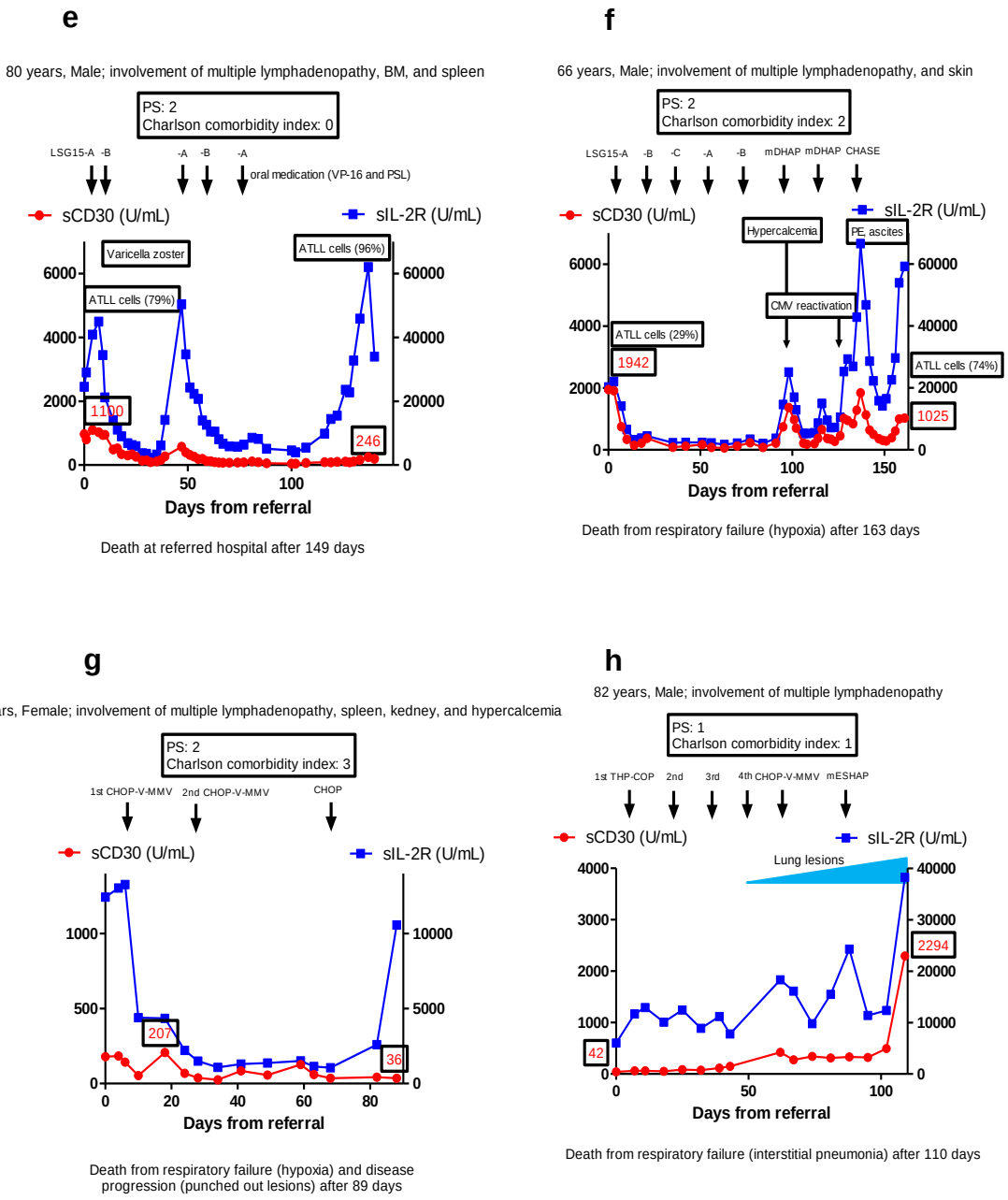
The sCD30 levels have less influence on the number of ATLL cell in lymphoma-type (< 1% of ATLL cells in PB) as compared with acute and chronic types.

Different source of sCD30-producing cells from sIL-2R-producing cells

Subsequently, we tried to evaluate the effectiveness of high soluble cytokine levels in the clinical course of patients with acute type ATLL and lymphoma-type ATLL after initial therapy (Figure 8). Serum levels of sCD30 and sIL-2R decreased after effective therapy; however, these levels were elevated during relapse or during periods without continuous therapy, for example during prolonged bone marrow suppression. The two biomarkers did not always change in parallel with each other. In addition to being produced by ATLL cells in PB, sCD30 was also likely to be produced by ATLL cells in extra-nodal lesions, such as pleural effusion, lung lesions, and ascites, but not in lymph nodes.

Figure 8 Changes in serum levels of soluble cytokines and clinical course of ATLL patients following initial therapy.





In acute type of ATLL (a–f), the number of ATLL cells was also shown in each figure. The different levels of sCD30 from sIL-2R might indicate that sCD30-producing cells are a subset of sIL-2R-producing cells. In lymphoma-type ATLL (g and h), there was a discrepancy between serum levels of sIL-2R and sCD30, suggesting that sCD30 levels are not associated with ATLL cells in lymph nodes.

Univariate analysis for 2-year overall survival in HSCT

Besides sCD30 and sIL-2R, 7 variables significantly differentiated OS as follows:

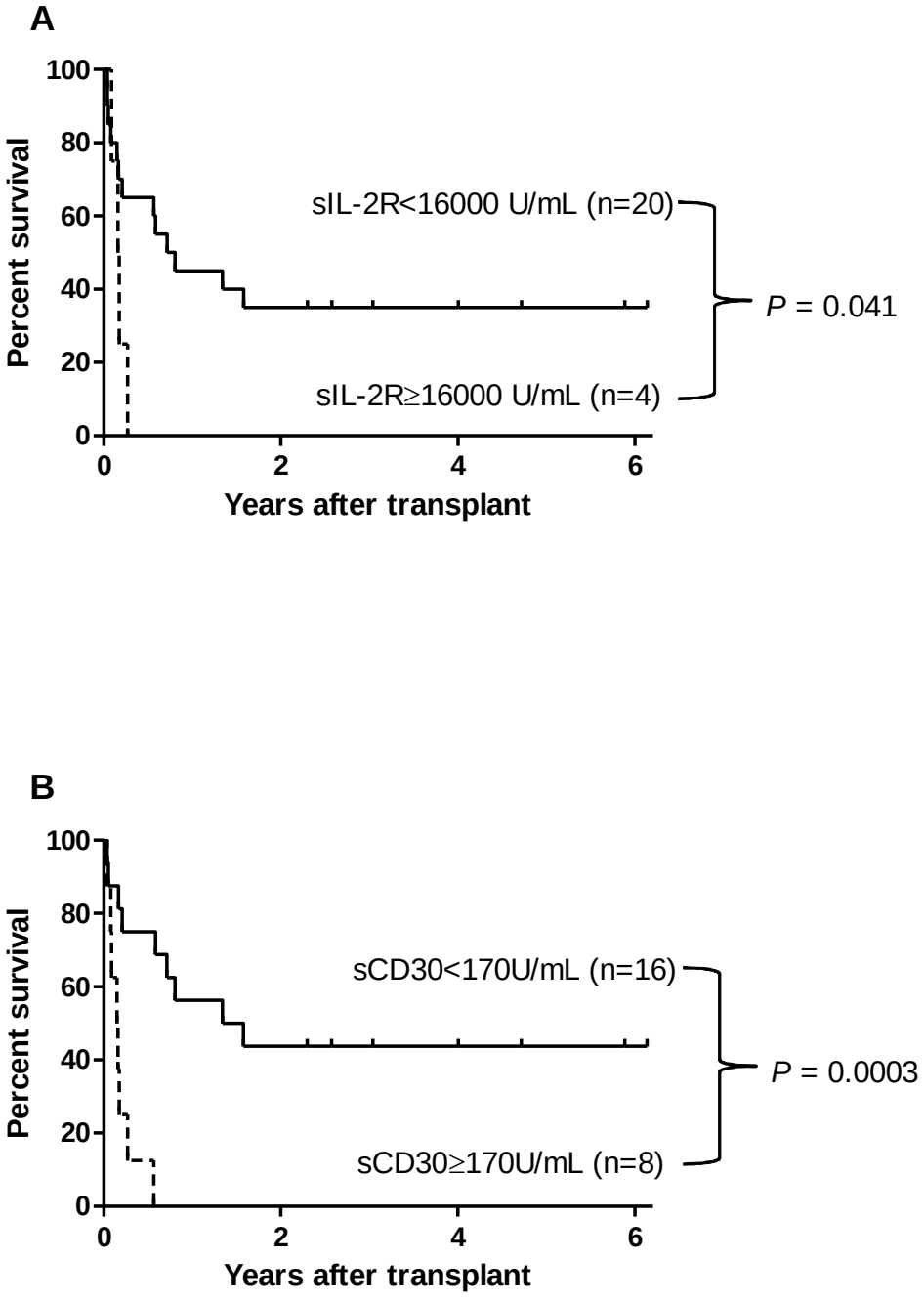
1. LDH ≥ 340 IU/L (HR = 36, 95% CI 5.3–247, $p = 0.0002$)
2. Charlson comorbidity index ≥2 (HR = 142, 95% CI 4.7–4270, $p = 0.0044$)
3. CRP ≥ 0.15 mg/dL (HR = 4.7, 95% CI 1.5–16, $p = 0.0097$)
4. BUN ≥ 17 mg/dL (HR = 65, 95% CI 2.7–1545, $p = 0.01$)
5. HLA- mismatch (HR = 3.6, 95% CI 1.1–12, $p = 0.034$)
6. peripheral blood stem cell transplantation (PBSCT) (HR = 2.9, 95% CI 1.0–8.3, $p = 0.044$)
7. thrombocytopenia (platelet ≤ 50000) (HR = 16, 95% CI 1.1–245, $p = 0.0046$).

Although GVHD occurs after HSCT, no acute GVHD or severe acute GVHD (grades III and IV) was associated with unfavorable prognosis in contrast to grades I and II (HR = 8.2, 95% CI 2.4–28, $p = 0.0007$).

Clinical values of sCD30 and sIL-2R before HSCT

We analyzed OS in patients undergoing HSCT (Figure 9). Patients with sIL-2R ≥ 16000 U/mL (Figure 9A) showed a sharp decline of survivors as compared with sIL-2R < 16000 U/mL (HR = 5.8, 95% CI 1.1–31, $p = 0.041$). Similarly, patients with sCD30 ≥ 170 U/mL (Figure 9B) showed a plummet of survivors as compared with sCD30 < 170 U/mL (HR = 13, 95% CI 3.2–49, $p = 0.0003$). Importantly, a high level of sCD30 (≥ 170 U/mL) was able to detect four more patients with early death as compared with sIL-2R level (≥ 16000 U/mL). The 2-year survival rates were 44% (7/16) for those with sCD30 < 170 U/mL and 35% (7/20) for sIL-2R < 16000 U/mL, respectively.

Figure 9 Pretreatment marker levels and treatment outcome before allogeneic HSCT.



Kaplan-Meier analysis of overall survival of patients with high or low levels of sIL-2R (A) or sCD30 (B) before conditioning regimen of allogeneic HSCT.

Cause of early death within 100 days following HSCT

Table 3 shows the characteristics of patients who died within 100 days of HSCT (n = 11). CRP is included in the table because it showed a high HR in the univariate analysis. Three patients were categorized as having disease progression, since there was evidence of relapse. The other patients did not show any evidence of recurrence and were categorized as TRM. Six cases (Case 1, 4, 5, 6, 8, and 10) died of diffuse pulmonary infiltrates and/or interstitial pneumonia within 76 days. Five of them showed high levels of sCD30 (≥ 170 U/mL) before the conditioning therapy, but only 2 of them showed high sIL-2R levels ≥ 16000 U/mL.

Table 3 Biomarker levels of ATLL patients who died within 100 days of transplantation.

No.	CRP (mg/dL)	sIL-2R (U/mL)	sCD30 (U/mL)	OS (days)	Condition before death (relapse site)	Cause of death
1	2.2	3270	720	14	IP, DPI	TRM
2	0.18	575	43	15	MOF	TRM
3	0.02	713	81	19	MOF	TRM
4	0.1	1943	173	30	IP, DPI, MOF	TRM
5	0.73	19375	2384	32	IP, DPI, MOF	TRM
6	0.32	2630	442	55	IP	TRM
7	0.12	618	66	61	aGVHD	TRM
8	17	98314	932	59	DPI, Relapse (PE)	Disease progression
9	0.16	27302	2789	64	Relapse (LN)	Disease progression
10	0.23	319	14	76	IP, IPS	TRM
11	0.21	16835	808	99	Relapse (LN)	Disease progression

Values above the cutoff levels are underlined. OS: overall survival following transplantation; IP, interstitial pneumonia; DPI, diffuse pulmonary infiltrates; MOF, multiple organ failure; aGVHD, acute graft-versus-host disease; PE, pleural effusion; LN, lymph node; IPS, idiopathic pneumonia syndrome; TRM, transplantation-related mortality

Among patients with sCD30 < 170 U/mL (Cases 2, 3, 7, and 10), two patients (Cases 2 and 3) died from coagulation abnormalities. One patient (Case 2) died of multiple organ failure (MOF) with ascites from disseminated intravascular coagulation, and another patient (Case 3) died of MOF with bone marrow and renal failures from veno-occlusive disease. One patient (Case 7) died from severe acute GVHD (grade III) and exhibited an elevated sCD30 level of 467.4 U/mL at the peak of GVHD. Another patient (Case 10) experienced a complete remission of primary ATLL in the lung but developed idiopathic pneumonia syndrome and died 76 days after HSCT; the patient's sCD30 levels were elevated to 147 U/mL during the conditioning regimen. These cases (Cases 2, 3, 7, and 10) did not have elevated levels of sIL-2R. Cases 3 and 7 did not have elevated levels of CRP, sIL-2R, or sCD30.

Another 3 cases (Cases 1, 4, and 6) were sIL-2R negative and sCD30 positive, in which the causes of death were classified as TRM. In contrast, 4 cases (Cases 5, 8, 9, and 11) showed high levels of sIL-2R (≥16000 U/ml) and sCD30 (≥800 U/mL), and 3 of these patients (Cases 8, 9, and 11) died from relapse (2 lymphoma-type ATLL and 1 acute type ATLL). These 4 patients also expressed high levels of CRP.

Levels of sCD30 ≥ 170 U/mL (sensitivity and specificity, 63.6% and 92.3%) and CRP ≥ 0.15 mg/dL (72.3% and 92.3%) were implicated in early death. Furthermore, the combination of sCD30 ≥ 170 U/mL and/or CRP ≥ 0.15 mg/dL showed more sensitivity in predicting early death (sensitivity 81.8% and specificity 84.6%). The addition of sIL-2R levels to this combination did not improve the sensitivity and specificity.

8. Discussion

In the present study, we evaluated the clinical value of serum levels of sCD30 in comparison with sIL-2R in ATLL patients. Before the initial therapy, both sCD30 and sIL-2R were significant predictors of OS. Nevertheless, the number of ATLL cells was correlated with the sCD30 level in PB. Therefore, sCD30 may be a good marker when the ATLL cells are sparse in PB as well as in lymphoma-type ATLL. Before HSCT, sCD30 showed a better prediction power than sIL-2R because the number of ATLL cells in PB is very low after the completion of chemotherapy. Furthermore, the combination of sCD30 and CRP demonstrated high sensitivity and specificity for early death within 100 days following HSCT. To our knowledge, this is the first report to demonstrate the clinical value of sCD30 as compared with sIL-2R in ATLL patients.

A previous study identified independent prognostic factors for untreated ATLL, including poor ECOG PS, high LDH, age ≥40 years, total number of involved lesions, and hypercalcemia before chemotherapy [42]. Recently, another study identified independent prognostic factors of chemotherapy response such as Ann Arbor stage (III and IV), ECOG PS (2 to 4), age, serum albumin, and sIL-2R [38]. They also proposed a new prognostic index for acute and lymphoma-type ATLL (ATL-PI). We found that sCD30 was significantly correlated with ATL-PI. Therefore, sCD30 might also be a clinical marker in ATLL patients undergoing the initial therapy, as previously reported in B-cell and T-cell lymphomas [43–46]. In addition, we found that the sCD30 levels were higher when more than 5% of PB cells were ATLL cells. ATLL cells in PB might be a strong producer of sCD30 as compared with ATLL cells in lymph nodes or stomach. Furthermore, there were several cases of long-term survivors with elevated sCD30 levels in the PB rich group, group of patients with large percentages of ATLL cells in PB. Therefore, the serum level of sCD30 may be useful for the PB sparse group, patients with fewer ATLL cells in PB, to detect aggressiveness of disease as well as for

other lymphoma patients including those with HD and ALCL.

Some acute-type ATLL patients with high sCD30 and sIL-2R levels before initial therapy survived more than 2 years. Even the ATL-PI could not predict their long-term survival in 2 of the 3 patients. These observations suggest that tumor burden mainly in circulation was not associated with mortality of ATLL patients without severe involvement of other organs. Actually, sCD30 elevation with advanced lung lesions indicated an unfavorable outcome as shown in Figure 8 (c, d, f, and h).

Regarding HSCT, a previous study identified the following five significant variables related to poor OS: advanced age, male sex, failure to attain complete remission, poor ECOG PS, and transplantation from unrelated donors [48]. Furthermore, a high level of sIL-2R was recently demonstrated to predict worse HSCT outcomes in Hokkaido, Japan [35]. In the present study, we compared sCD30 levels with sIL-2R levels before conditioning therapy in patients who underwent HSCT, and our results suggest a better predictive power of sCD30 than sIL-2R in detecting unfavorable prognosis. TRM and relapse also occurred frequently following the HSCT. In the present study, we found that the combination of sCD30 levels and CRP levels may be a powerful tool to predict the early death of patients undergoing HSCT. It is conceivable that elevated sCD30 levels and CRP values are associated with a proinflammatory state before the immunosuppressant therapy. Conditioning therapy probably activates tissue macrophages, leading to cytokine storm and activation of HTLV-1-infected cells. The CRP level is controlled by circulating IL-6 levels, which are elevated in ATLL patients [48,49]. This may be the reason why STAT3 is constitutively activated in ATLL cells *in vivo* [9]. Therefore, HSCT might be ineffective and dangerous for ATLL patients with sCD30 $\geq$ 170 U/ml and/or CRP $\geq$ 0.15 mg/dL before conditioning regimens. Additional therapy including IL-6/jak/STAT3 signaling inhibitor and anti-CD30 monoclonal antibody might be considered for treatment of the proinflammatory state in such patients prior to conditioning regimens [50,51]. Otherwise, alternatives to HSCT must be considered [52,53].

Our data suggest that sCD30 elevation before the conditioning therapy may be associated with diffuse pulmonary lesions in early deceased patients who underwent HSCT. Lung is the preferential site for HTLV-1 infected cells [54,55]. Chest CT scans revealed abnormalities in 98 (30.1%) of 320 HTLV-1 carriers [56], and 13 (44.8%) of 29 ATLL patients had leukemic infiltration [57], suggesting that this peculiar tropism is responsible for the high incidence of pulmonary involvement. HTLV-1–infected T cells probably migrate to the lung due to age-related changes in the pulmonary immune system. Those cells express virus and may induce a strong immune reaction once they invade the lung tissue [58]. Thereafter monoclonal proliferation of infected cells following antigen presentation may form a local network between cytokines and cytokine receptors. This condition may progress to bronchitis and smoldering type ATLL.

For invasion of such tumor virus–infected cells into lung tissue, cleavage of collagen and elastin, which are components of lung tissue, requires the activation of MMPs. The MMPs work as an enzyme for tissue destruction and cell infiltration as well as shedding of the cell surface cytokines and cytokine receptors. The patients who experience pulmonary invasion of ATLL cells prior to conditioning therapy may be susceptible to lung injury caused by the anti-cancer agents, leading to significant ATLL cell proliferation in the lung. The pathophysiology of these pulmonary lesions will be elucidated in the future.

The main limitation of this study is the small number of patients we analyzed. We were unable to perform multivariate analysis. Although HTLV-1 is endemic in Kyushu, the incidence of ATLL is very low and a long latency period exists between HTLV-1 infection and development of ATLL. It is difficult to obtain a sufficient sample size. A multicenter cohort study is needed to confirm our results in the future.

9. Conclusion

We investigated the sCD30 levels in ATLL in 2 different clinical settings, before initial therapy and before HSCT. Our results suggest that sCD30 may be a prognostic factor before the initial therapy. However, the sCD30 levels are correlated with the number of ATLL cells in PB. The sCD30 level may not be useful to predict OS when there are a large percentage of ATLL cells in PB. Our results suggest that sCD30 may be a useful biomarker before HSCT therapy, because a high level of CD30 before HSCT is implicated in early death after HSCT. HSCT may not be suitable for ATLL patients with sCD30 $\geq$ 170 U/ml and/or CRP $\geq$ 0.15 mg/dL. Early diagnosis and treatment of the proinflammatory state could reduce morbidity and mortality of patients undergoing HSCT. Hopefully, these markers can be used to reduce the incidence of early death and TRM after HSCT.

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