Original Article

Clinical features and treatment outcomes of opportunistic infections among human T-lymphotrophic virus type 1 (HTLV-1) carriers and patients with adult T-cell leukemia-lymphoma (ATL) at a single institution from 2006 to 2016

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As opportunistic infections among human T-lymphotrophic virus type 1 (HTLV-1) carriers and patients with adult T-cell leukemia/lymphoma (ATL) pose a serious problem, it is necessary to clarify their clinical characteristics and outcomes in these patients.   We retrospectively analyzed the clinical features and outcomes of opportunistic infections in 127 HTLV-1 carriers and 153 ATL patients between 2006 and 2016.   The cumulative incidence rates of opportunistic infections among HTLV-1 carriers and ATL patients were 1.5% (2/127) and 6.5% (10/153), respectively.   The etiology of opportunistic infections was as follows: fungal infections (3 cases), pneumocystis pneumonia, and cytomegalovirus (CMV) infections.   Even after aggressive treatment, the prognosis of opportunistic infections was poor (50% of overall survival at 28 days).   Regarding prognostic factors affecting the OS of opportunistic infections, higher SOFA scores (especially the respiratory subscore) and higher LDH values were identified by univariate analysis.   Moreover, 3 out of 6 patients achieved spontaneous remission of ATL as the short-term outcome after the development of opportunistic infection.   However, 5 out of 6 surviving patients exhibited ATL progression or relapse after a median of 194 days (133–226) after contracting an opportunistic infection as the long-term outcome of ATL.   In conclusion, opportunistic infections should be carefully followed among HTLV-1 carriers and ATL patients because of their aggressive clinical course and poor outcomes.   Furthermore, early diagnosis and subsequent prompt treatment are necessary in clinical practice.

Keywords: adult T-cell leukemia/lymphoma, HTLV-1 carrier, opportunistic infection, spontaneous remission of ATL, progression of ATL

INTRODUCTION

It is well known that human T-lymphotrophic virus type 1 (HTLV-1) infection causes T-cell dysfunction based on the study by Mituya *et al.* in 1984.[1]   Moreover, Shimoyama reported in 1991 that opportunistic infections, including fungal and viral infections as well as *Pneumocystis* pneumonia, are a major cause of morbidity and mortality among HTLV-1 carriers and patients with adult T-cell leukemia-lymphoma (ATL).[2]   Subsequently, Suzumiya *et al.* published a study on autopsy cases of patients with ATL in Miyazaki prefecture in 1993, demonstrating the high incidence rates, clinical features, and outcomes of opportunistic infection among these patients.[3]   Although the reasons or mechanisms underlying the high incidence of opportunistic infections in HTLV-1 carriers and patients with ATL are not completely understood at

present, one of the mechanisms involved is the reduction of $CD4^+$ T-cell functions due to HTLV-1 infection.[4,5] Recently, Yanagihara *et al*. reported that one of the mechanisms of the susceptibility of HTLV-1–infected patients to opportunistic infection is the expression of PD-1 and PDL-1 on cytotoxic T lymphocytes (CTLs) to suppress host defense against pathogens.[6]

Although there has been marked progress in the discovery of biomarkers (β-D-glucan, Asp Ag, and CMV C7 HRP) for diagnosis, and in antifungal and antiviral treatment [fluconazole (FLCZ), itraconazole (ITCZ) , voriconazole (VRCZ), and liposomal amphotericin B (L-AMPH-B)] of opportunistic infections over the past few decades, opportunistic infections in HTLV-1 carriers and ATL patients still represent a serious problem.[6,7] Furthermore, spontaneous remission of ATL after these patients contract an opportunistic infection has been previously documented in several case reports.[7-24]

However, the incidence, clinical features, and treatment outcomes of opportunistic infections among HTLV-1 carriers and patients with ATL are unclear. Thus, in this study, we elucidated these parameters of opportunistic infections in HTLV-1 carriers and ATL patients. Moreover, we investigated the short- and long-term effects of antimicrobial treatment on opportunistic infection and ATL in clinical practice.

PATIENTS AND METHODS

We retrospectively analyzed 12 patients with opportunistic infections among 153 ATL patients and 127 HTLV-1 carriers during 11 years from January 1, 2006, to December 31, 2016. At present, ATL is diagnosed by Shimoyama's diagnostic criteria (acute, lymphoma, chronic, and smoldering types).[1] Each HTLV-1 carrier in our study was diagnosed by the presence of anti–HTLV-1 antibody and asymptomatic chronic clinical course that did not meet the diagnostic criteria of smoldering ATL type or chronic type according to Shimoyama's guidelines because of the absence of definite diagnostic criteria of HTLV-1.[1] In all patients, HTLV-1 clonality was examined by Southern blot analysis using nonradioactive probes specific for the entire HTLV-I genome.

To clarify the etiology of opportunistic infection among HTLV-1 carriers and ATL patients, we examined the serological markers of β-D glucan, CMV pp65 antigenemia (CMV C7 HRP), *Aspergillus* antigen, *Cryptococcus* antigen, and *Candida* antigen in peripheral blood (PB), computed tomography (CT), cytology, culture, and genetic analysis [polymerase chain reaction (PCR) analysis for *Pneumocystis* jirovecii] of bronchoalveolar lavage fluid (BALF). Furthermore, TBLB was performed to investigate nodular/mass lesions to exclude lung cancer or fungal infection by CT.

In case 4, to clarify the *Aspergillus* species, PCR analysis of BALF was performed.

According to the laboratory findings, radiological findings, and BALF or TBLB findings, we diagnosed the following opportunistic infections: *Pneumocystis* pneumonia, cytomegalovirus (CMV) pneumonia, *Aspergillus* pneumonia, and cryptococcal pneumonia based on the guidelines of the

Infectious Disease Society of America (IDSA), Mycoses Forum, the Japan Society for Hematopoietic Cell Transplantation (JSHCT), and the Japan Society for Medical Mycology.[25-28]

We retrospectively analyzed the etiology, diagnosis, and treatment outcomes of the 12 cases of opportunistic infection. We compared the parameters related to opportunistic infections (β-D-glucan, CMV C7-HRP, and SOFA score) and the parameters related to ATL (WBC, Ab-ly, LDH, and sIL-2R) after treatment of opportunistic infections. Subsequently, we examined the overall survival (OS) in the 12 cases of opportunistic infection. Thus, to improve the poor outcome of opportunistic infections, we compared the profiles of surviving patients and those that died among the 12 cases of opportunistic infection. Lastly, we examined the impact of opportunistic infections on the disease status of patients with ATL and of HTLV-1 carriers.

In case 6, to examine the presence of HTLV-1–specific CTLs and CMV-specific CTLs, we examined the HLA-A24–restricted HTLV-1–specific CTLs and the HLA-A24–restricted CMV-specific CTLs by HLA-A24 flow cytometric (FCM) tetramer assay.

The changes in the parameters related to opportunistic infections and ATL were examined by the Wilcoxon signed-rank test. The parameters between survivors and non-survivors were compared using the Mann–Whitney *U* test. Significance was determined using a 2-sided P-value (should be <0.05).

This retrospective study was performed in compliance with good clinical practices and the ethical principles of the Declaration of Helsinki. We received prior approval from the ethics review board at Miyazaki Prefectural Miyazaki Hospital.

RESULTS

The clinical features and treatment outcomes of 12 HTLV-1 carriers and patients with ATL with opportunistic infections are shown in Table 1.

Among the 12 cases of opportunistic infection, the average age was 66.3 years (range 34–78) and the male/female ratio was 1:1. Patients with the following subtypes of ATL developed opportunistic infections: acute type (5 patients), lymphoma type (0 patients), chronic type (3 patients), smoldering type (2 patients), and HTLV-1 carriage (2 patients). The cumulative incidence rates of opportunistic infections among HTLV-1 carriers and ATL patients were 1.5% (2/127) and 6.5% (10/153), respectively. To clarify the etiology of opportunistic infection among HTLV-1 carriers and ATL patients, we measured the serological markers of β-D glucan, CMV C7HRP, *Aspergillus* antigen, and *Cryptococcus* antigen, and performed cytology, culture, and genetic analysis (PCR for *Pneumocystis* jirovecii) of BALF. Furthermore, focal lesions found on CT underwent TBLB to exclude lung cancer. We empirically treated bacterial, viral, or fungal infections using antibiotics, antifungal agents, or antiviral drugs, including cefepime (CFPM) or meropenem (MEPM),

Table 1. The overview of clinical features and treatment outcomes in 12 immunocompromised patients with infections among HTLV-1 carriers and ATL cases.

Case	Age	Sex	Clinical subtype	Opportunistic infection	Onset of opportunistic infection	βD Glucan (PB)	CMV C7 HRP (PB)	Aspergillus antigen (PB)	Cryptococcus antigen (PB)	The histological Findings (BALF or TBLB)	PS	SOFA	HTLV-1 clonality	Treatment for opportunistic infection	Outcome of opportunistic infection at 28 days	Outcome of ATL at 28 days	Relapse of ATL	Salvage therapy	Final outcome
1	34	M	acute type	Pneumocystis pneumonia	During treatment (LSG 15, PD status)	1278	Negative	negative	negative	Grocott stain+ Pneumocystis PCR+	3	13	Monoclonality	ST, Pentamidine, mPSL	Not improved (4 days)	PD	-	not done	Dead (4 days)
2	71	F	carrier type	Pneumocystis pneumonia	Initial diagnosis	117	negative	negative	negative	Grocott stain+ Pneumocystis PCR+	3	6	n.e.	ST, Pentamidine, mPSL	Not improved (27 days)	SD	-	not done	Dead (27 days)
3	65	M	chronic type	Cryptococcus pneumonia	Initial diagnosis	negative	negative	negative	positive	Grocott stain+ Cryptococcal organism+	3	10	Monoclonality	L-AMPH-B	Improved (55 days needed to remission)	SD	Relapse at 226 days	CHOP	Dead (305 days)
4	73	F	acute type	Aspergillus pneumonia	Initial diagnosis	negative	4	2.2	negative	Grocott stain+ Aspergillus bundles, PCR+	3	8	Monoclonality	L-AMPH-B	Not improved (26 days)	PD	-	not done	Dead (26 days)
5	74	F	smoldering type	Cryptococcus pneumonia	Initial diagnosis	negative	negative	negative	positive	Grocott stain+ Cryptococcal organism+	0	0	Clonality (-)	Surgery FLCZ	Improved (16 days needed to remission)	SD	-	not done	Alive (2689 days)
6	56	F	acute type	CMV pneumonia	During treatment (CHOP-VMMV, PD status)	18	1132	negative	negative	CMV PCR+	3	6	Biclonality	DHPG mPSL	Improved (52 days needed to remission)	CR	Relapse at 213 days	RTx	Dead (240 days)
7	67	M	acute type	CMV pneumonia	During treatment (CHOP-VMMV, PD status)	negative	124	negative	negative	CMV PCR+	1	8	Monoclonality	DHPG mPSL	Improved (51 days needed to remission)	PR	Relapse at 133 days	CHOP	Dead (246 days)
8	78	F	chronic type	CMV pneumonia	Initial diagnosis	negative	360	negative	negative	CMV PCR+	0	6	Monoclonality	DHPG mPSL	Not improved (14 days)	SD	-	not done	Dead (14 days)
9	74	M	acute type	CMV pneumonia	Initial diagnosis	negative	121	negative	negative	CMV PCR+	4	8	Monoclonality	DHPG mPSL	Not improved (15 days)	PD	-	not done	Dead (15 days)
10	72	F	carrier	CMV pneumonia	Initial diagnosis	negative	520	negative	negative	CMV PCR+	4	10	Clonality (-)	DHPG mPSL	Not improved (2 days)	SD	-	not done	Dead (2 days)
11	54	M	chronic type	Pneumocystis pneumonia	Initial diagnosis	353	negative	negative	negative	Grocott stain+ Pneumocystis PCR+	2	2	Monoclonality	ST, mPSL	Improved (18 days needed to remission)	PR	Relapse at 153 days	mLSG 15 →allo-HSCT	Alive (631 days)
12	78	M	smoldering type	Pneumocystis pneumonia	Initial diagnosis	374	negative	negative	negative	Grocott stain+ Pneumocystis PCR+	2	3	Monoclonality	ST, mPSL	Improved (29 days needed to remission)	SD	Relapse at 194 days	BSC	Dead (215 days)

caspofungin, liposomal amphotericin B (L-AMPH-B), ganciclovir (DHPG), and trimethoprim-sulfamethoxazole (ST), respectively, until a definite diagnosis of the opportunistic infection was made.   The identified etiologies of opportunistic infections were *Pneumocystis* pneumonia (4 cases), cryptococcal pneumonia (2 cases), *Aspergillus* pneumonia (1 case), and CMV pneumonia (5 cases).

Pneumocystis pneumonia (cases 1, 2, 11, and 12)

Pneumocystis pneumonia was diagnosed by combination of clinical signs such as progressive respiratory failure, serological findings, CT findings, and cytology and genetic analysis (PCR for *Pneumocystis* jirovecii) of BALF.   Serological findings included high β-D-glucan levels.   In addition, CT demonstrated bilateral diffuse ground-glass opacities with peripheral sparing.[4]   Subsequent cytology analysis of BALF revealed cysts of *Pneumocystis* jirovecii and PCR analysis of

BALF revealed the presence of *Pneumocystis* jirovecii. Regarding treatment, sulfamethoxazole trimethoprim (ST) or pentamidine was administered.   mPSL pulse was performed according to the degree of acute respiratory failure.   A representative clinical course (case 11) is shown in Fig. 1A.

Aspergillus pneumonia (case 4)

Aspergillus pneumonia was diagnosed by the combination of aggressive clinical symptoms, CT findings, *Aspergillus* antigen-positive status, the culture findings of BALF and the histological findings of TBLB.   Chest CT demonstrated right upper lobe cavitary lesions consistent with pulmonary aspergillosis.   Moreover, histopathological findings included marked inflammation with neutrophilic infiltration, coagulation necrosis, and fungal elements.   PCR analysis of BALF revealed the etiology of fungal infection to be *Aspergillus* fumigatus.   L-AMPH-B was administered for treatment.
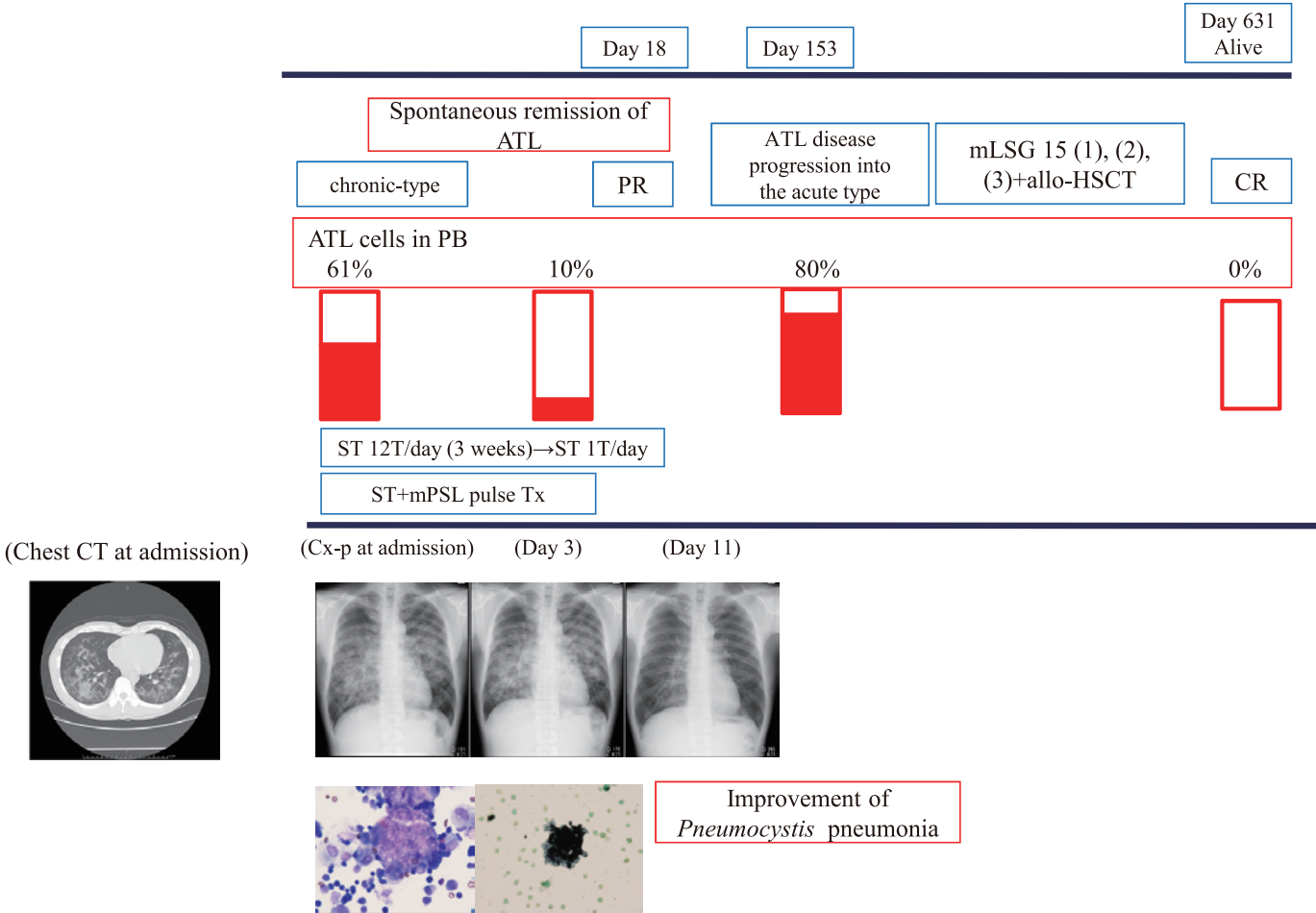

Fig. 1A. Clinical course of case 11. Age 54 years, chronic-type ATL, pneumocystis pneumonia → improvement → the development of acute-type ATL.
A 54-year-old male was followed for chronic-type ATL with the CD3+CD4+CD25+CD5+CD7+ phenotype on FCM analysis and a monoclonal band on Southern blot analysis. The patient presented with acute progressive respiratory failure. Laboratory findings were as follows: WBC 32550/μL (60%), LDH 500 IU/ml, sIL2R 14500 IU/ml, CRP 2 mg/dl, β-D-glucan: 353.7 pg/mL, *Aspergillus* Ag(-), and CMV C7 HRP(-). Furthermore, CT showed bilateral interstitial pneumonia sparing the normal lung field under the pleural membrane. BAL analysis revealed a foamy population after May–Giemsa staining, Papanicolaou staining, and Grocott staining. Furthermore, *Pneumocystis jiroveci* was confirmed by PCR analysis. These findings led to the final diagnosis of *Pneumocystis* pneumonia. Subsequent ST treatment and mPSL pulse therapy led to the cure of *Pneumocystis* pneumonia. During the treatment of *Pneumocystis* pneumonia, the status of ATL was PR. However, chronic-type ATL progressed to acute-type ATL on day 153. Subsequent chemotherapy including mLSG 15 and allo-HSCT led to CR of ATL on day 631.

Cryptococcus pneumonia (cases 3 and 5)

Cryptococcus pneumonia was diagnosed by the subacute clinical course, serum *cryptococcus* antigen-positive status, CT findings, histological findings of TBLB, and culture of BALF. CT demonstrated solitary[1] or multiple nodules[2] requiring differential diagnosis from lung cancer. Moreover, histological findings of the TBLB specimen included histiocytic aggregation with multinuclear giant cells and cryptococcal organisms. Furthermore, to exclude *cryptococcus* meningitis, lumbar puncture was performed. L-AMPH-B was administered for treatment.

CMV pneumonia (cases 6, 7, 8, 9, and 10)

CMV pneumonia was diagnosed by the combination of clinical symptoms, CT findings, CMV pp65 antigenemia, CMV PCR, and cytology of BALF. CT demonstrated ground-glass opacities in all patients, with bilateral patchy (n = 1) and bilateral diffuse (n = 4) distribution, respectively. Serological CMV C7 HRP in PB was positive in all patients. In addition, PCR analysis of BALF for CMV was positive in all patients. Furthermore, cytology analysis of BALF for CMV also revealed the presence of inclusion bodies, suggesting the reactivation of CMV in all patients. Regarding

treatment, ganciclovir (DHPG) was administered. For patients with bilateral diffuse distribution in CT (n = 4), mPSL pulse therapy was performed because of the aggressive respiratory failure mimicking acute interstitial lung disease on CT. A representative clinical course (case 6) is shown in Fig. 1B.

The onset of opportunistic infection was observed at initial diagnosis (9/12) or during the treatment of ATL (3/12). Prophylactic administration of antimicrobial drugs for opportunistic infections was not carried out in the group that developed opportunistic infections at initial diagnosis. For the group that contracted opportunistic infections during the treatment of ATL (cases 1, 6, and 7), ST and ITCZ were administered. Regarding the prophylactic treatment, e.g., ST and FLCZ/ITCZ for opportunistic infections, it was not administered to HTLV-1 carriers. Although prophylactic treatment, such as the administration of ST and ITCZ, was performed for patients with ATL during chemotherapy, opportunistic infections developed in cases 1, 6, and 7.

Next, we compared the changes in parameters related to infection among immunocompromised patients after treatment in the 12 cases of opportunistic infection (Fig. 2). The values of β-D-glucan and CMV C7-HRP decreased after treatment of opportunistic infections (*Pneumocystis* pneumonia

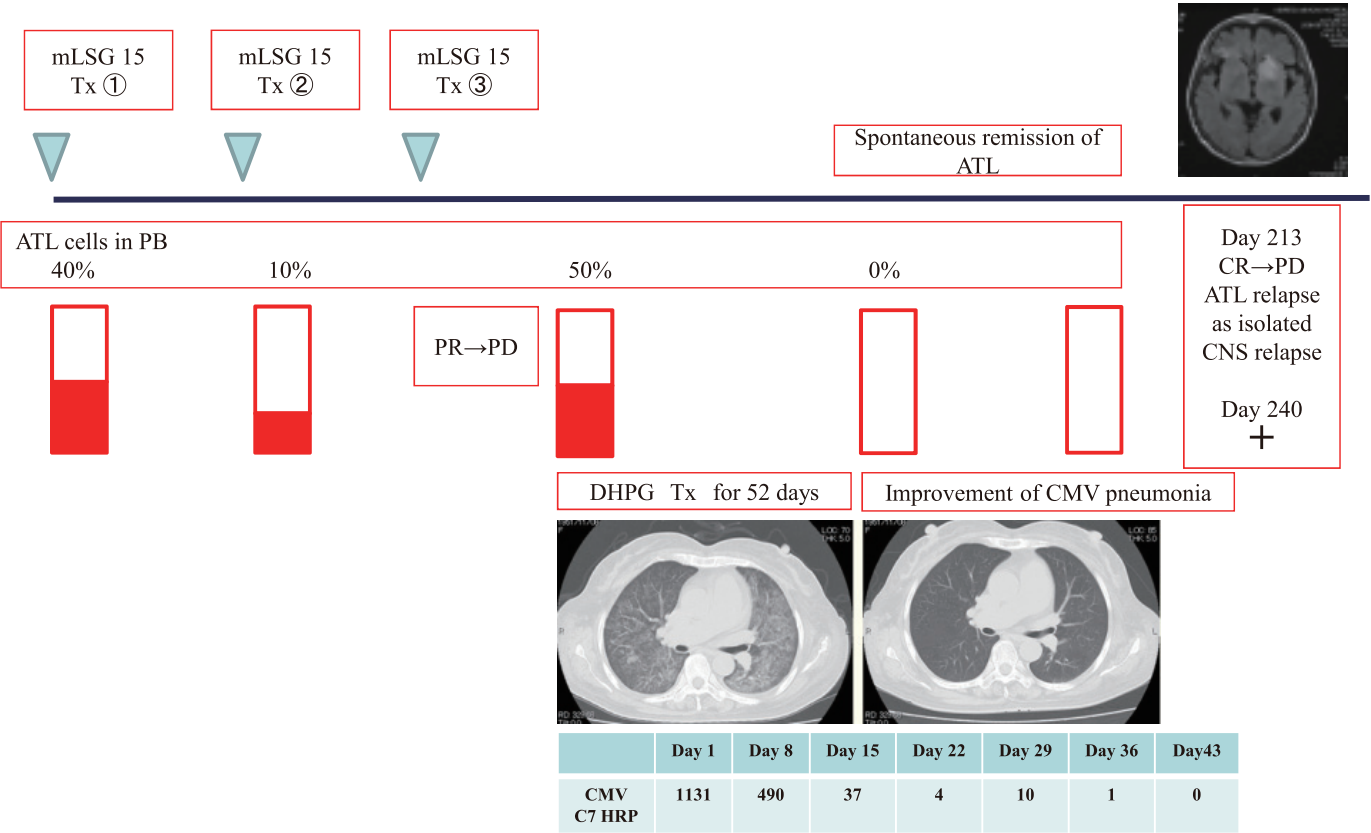|	Day 1	Day 8	Day 15	Day 22	Day 29	Day 36	Day43
CMV C7 HRP	1131	490	37	4	10	1	0

Fig. 1B. Clinical course of case 6. Age 56 years, acute-type ATL, CMV pneumonia → improvement → ATL relapse.
A 56-year-old female was diagnosed with acute-type ATL and treated using the mLSG 15 regimen. However, the disease status of ATL was PD after mLSG 15 therapy. During chemotherapy, the patient developed acute progressive respiratory failure due to opportunistic infection. Subsequent CT revealed bilateral interstitial pneumonia. Based on the positive findings of CMV C7-HRP in PB and CMV PCR in BALF, CMV pneumonia was diagnosed. Subsequent DHPG therapy and mPSL pulse therapy cured CMV pneumonia within 52 days. During the treatment of CMV pneumonia, the disease status of ATL was CR, but she developed isolated CNS relapse on day 213. Subsequent RTx did not affect the CNS lesions of ATL at 240 days.

and CMV pneumonia, respectively). The values of SOFA did not significantly improve and varied from progression to improvement after treatment (Fig. 2). In case 11 (survivor), the β-D-glucan level slowly declined over 2 months despite the improvement of bilateral interstitial pneumonia after 1 month. In case 6 (survivor), the CMV C7-HRP level slowly decreased over 2 months despite the improvement of bilateral interstitial pneumonia after 1 month.

We also examined the changes in parameters related to ATL after treatment (Fig. 3). The values of WBC, absolute numbers of abnormal-lymphocytes, LDH, Ca, and sIL-2R decreased after treatment of opportunistic infections (Fig. 3).

Consequently, we investigated treatment outcomes of the 12 cases of opportunistic infection among ATL patients and HTLV-1 carriers (Fig. 4). The OS of 28 days indicated a poor prognosis (OS rate of 50%; 6/12). Among the 6 surviving patients, 5 developed PD (3 patients) or relapse of ATL (2 patients) at a median of 194 days (range 133–226). Thus, the median OS in this group was 121 days, and OS rate was 16.7% (2/12).

We next compared the physiological, laboratory, and immunological findings of ATL patients who achieved spontaneous remission after the treatment of opportunistic infections (remission group: cases 6, 7, and 11) with those whose disease progressed after the treatment of opportunistic infections (non-remission group; cases 1, 2, 3, 4, 5, 8, 9, 10, and 12; Table 2). Our analysis revealed that the absolute numbers of abnormal lymphocytes in the peripheral blood (PB) of the remission group were slightly higher than those of the

non-remission group (Table 2). Moreover, based on our analysis, the non-remission group had hyperbilirubinemia (Table 2).

Furthermore, we carefully analyzed the non-survivors with opportunistic infections (Table 1). All six non-survivors presented with aggressive acute respiratory failure requiring intensive treatment with intubation and ventilation in an intensive care unit. The patients with *Pneumocystis* pneumonia (cases 1 and 2) died on days 18 and 27, respectively. The patient with *Aspergillus* pneumonia (case 4) died on day 26. The patients with CMV pneumonia (cases 8, 9, and 10) died on days 14, 15, and 2, respectively. Thus, all six non-survivors exhibited aggressive progressive disease within 4 weeks.

To elucidate the factors affecting the poor prognosis, we analyzed the survivors and non-survivors among the 12 cases of opportunistic infection (patients with ATL and HTLV-1 carriers; Table 3). Non-survivors had a shorter OS and higher SOFA score and LDH than survivors. Moreover, all 6 non-survivors presented with aggressive acute respiratory failure requiring intensive treatment with intubation and ventilation in an intensive care unit. Thus, we evaluated each factor composing the SOFA score such as the cardiovascular subscore, renal subscore, respiratory subscore, coagulation subscore, hepatic subscore, and neurological subscore. Among these six subscores, the respiratory subscore was found to be a significant factor affecting OS by univariate analysis, as shown in Table 3. There was no difference in platelet number, serum T-bilirubin, or creatinine levels

Wilcoxon signed-rank test

Fig. 2. The changes in parameters related to opportunistic infection after treatment.
We compared the changes of parameters related to infection in immunocompromised patients after treatment in 12 cases of opportunistic infection. The values of β-D-glucan and CMV C7-HRP decreased after treatment of opportunistic infections (*Pneumocystis* pneumonia and CMV pneumonia, respectively). The SOFA scores decreased in survivors and deteriorated in non-survivors.

Changes in WBC before and after treatment

Changes in absolute abnormal lymphocytes before and after treatment

Changes in LDH before and after treatment

WBC — The p-value is 0.03.

Absolute ab-ly — The p-value is 0.10.

LDH — The p-value is 0.35.

Changes in Ca before and after treatment

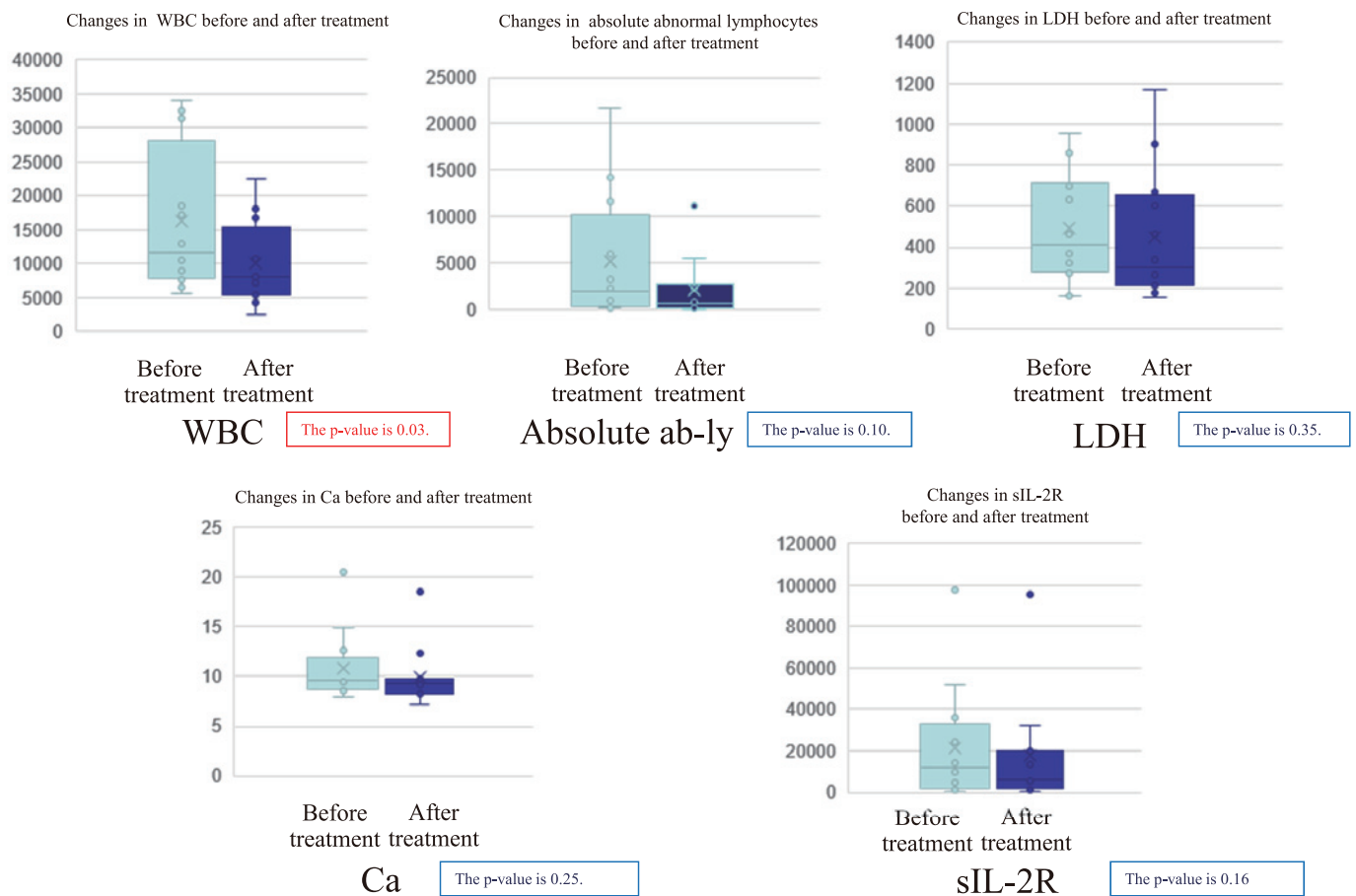Changes in sIL-2R before and after treatment

Ca — The p-value is 0.25.

sIL-2R — The p-value is 0.16

Fig. 3. The changes in parameters related to ATL after treatment.
We examined the changes in parameters related to ATL after treatment. The values of WBC, Ab-ly, LDH, Ca, and sIL-2R decreased after treatment of opportunistic infection.
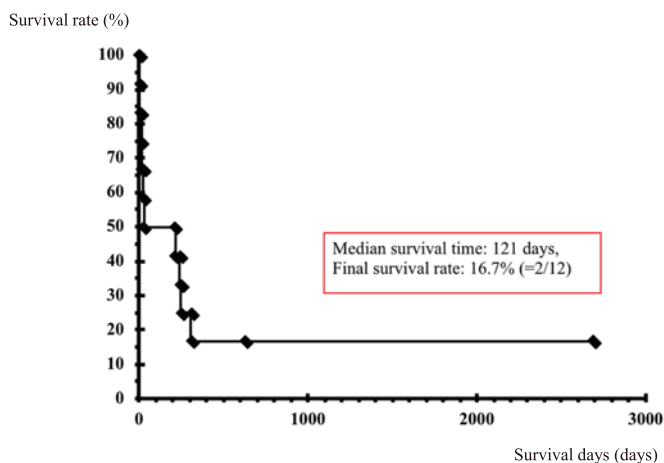
Survival rate (%)

Median survival time: 121 days,
Final survival rate: 16.7% (=2/12)

Survival days (days)

Fig. 4. Treatment outcomes of opportunistic infection among HTLV-1 carriers and ATL patients.
We investigated treatment outcomes of 12 cases of opportunistic infection among ATL patients and HTLV-1 carriers. The prognosis was poor: OS of 28 days and OS rate of 50% (6/12). Among 6 survivors, 5 presented with ATL PD (3 patients) and relapse of ATL (2 patients) at a median of 194 days (range 133–226). Thus, the median OS was 121 days and OS rate was 16.7% (2/12).

between the two groups, as shown in Table 3.

In addition, we examined the short-term outcome of ATL patients who achieved spontaneous remission after the development of opportunistic infection (spontaneous remission; Table 4). In this regard (spontaneous remission), 3 out of 6 survivors (cases 6, 7, and 11) developed spontaneous remission after opportunistic infection (complete response [CR]: 1 patient and partial response [PR]: 2 patients). Of note, 2 of these 3 patients (cases 6 and 7) achieved remission despite PD status during systemic chemotherapy. The etiologies of the opportunistic infections leading to spontaneous remission were CMV pneumonia (2 cases) and *Pneumocystis* pneumonia (1 case). Presented are the CT findings, FCM analysis, southern blot analysis and CTL analysis by a tetramer assay in a representative case of spontaneous remission of ATL (case 6) after treatment of opportunistic infection (Fig. 5A-D). CT findings after treatment of CMV pneumonia demonstrated the improvement of CMV pneumonia (Fig. 5A-a and 5A-b). FCM findings after treatment of opportunistic infection confirmed the improvement of CMV pneumonia (Fig. 5B-a and 5B-b). Southern blot analysis after treatment of opportunistic infection revealed the absence of HTLV-1 clonality in PB (Fig. 5C-a and 5C-b). Thus, CR was achieved in case 6 because of the disappearance of ATL

Table 2. The differences in laboratory markers between responders (n = 3) and non-responders (n = 9).

	All patients (n=12)	Responders (n=3)	Non-responders (n=9)	Statistical analysis
Age	71.5 (34-78)	56 (54-67)	73 (34-78)	0.07
PS	3(0-4)	2 (1-3)	3 (0-4)	0.44
WBC	11695 (5630-34000)	9070 (6440-32500)	12900 (5630-34000)	0.78
Absolute numbers of abnormal lymphocytes	875 (56-19855)	5895 (3220-19855)	979 (56-14280)	1.75
Hb	11.9 (4-15)	7.8 (7.5-15)	12.1 (4-13.9)	0.64
Plt	21.9 (0.6-31.8)	5.7 (0.6-31.8)	22.7 (2-30.6)	0.51
PT-INR	1.3 (1-1.4)	1.1 (1-1.4)	1.3 (1-1.3)	0.60
FIB	257 (77.1-663)	250 (192-663)	262 (77.1-325)	0.92
FDP	8.3 (1.5-14.1)	8.2 (1.5-8.6)	8.4 (3.2-14.1)	0.51
T.Bil	1.15 (0.3-13.1)	0.4 (0.3-0.7)	1.2 (0.5-13.1)	0.03 *
GOT	37 (13-355)	28 (14-58)	38 (13-355)	0.57
GPT	20.5 (8-286)	24 (10-28)	19 (8-286)	0.85
LDH	414.5 (163-956)	368 (285-461)	634 (163-956)	0.64
BUN	18.2 (7.6-97.7)	14.1 (7.6-23.2)	19.1 (9.7-97.7)	0.31
Cr	0.8 (0.6-5.6)	0.8 (0.7-0.8)	0.8 (0.6-5.6)	0.70
CRP	3.3 (0.24-29.2)	5.2 (1.5-6.4)	2.4 (0.24-29.2)	0.51
Ca	9.55 (8-20.5)	8.9 (8-9.5)	9.6 (8.5-20.5)	0.11
sIL-2R	12145 (481-97900)	14500 (9790-36200)	4520 (481-97900)	0.51
SOFA score	7 (0-13)	6 (2-8)	8 (0-13)	0.39
DIC score	2 (1-5)	2 (2-2)	2 (1-5)	0.71

*, Mann-Whitney U test

Table 3. The analysis of surviving patients and those that died among 12 immunocompromised patients with infection among HTLV-1 carriers and ATL cases.

	Improved cases of opportunistic infection	Not improved cases of opportunistic infection	P-value
Age	66 (51-77)	72.5 (34-78)	0.47
WBC	8985 (5630-32550)	17790 (7590-34000)	0.14
Absolute numbers of AB-Ly	2385 (56-19855)	1285 (171-14280)	0.32
Hb	12.5 (7.5-15)	11.6 (4-12.8)	0.33
Plt	24.6 (0.6-31.8)	17.35 (2-30.6)	0.52
PT-INR	1.1 (1.0-1.4)	1.3 (1.3-1.4)	0.06
FIB	271 (192-663)	257 (77.1-325)	1.0
FDP	7.5 (1.5-8.6)	8.8 (5-14.1)	0.05
T.BIL	0.6 (0.3-1.3)	1.2 (0.5-13.1)	0.12
GOT	21 (13-58)	57 (17-355)	0.05
GPT	23 (8-28)	16 (9-286)	0.87
LDH	303.5 (163-461)	707 (275-956)	0.02 *
BUN	12.3 (7.6-28.1)	30.45 (13.3-97.7)	0.07
Cr	0.8 (0.7-0.9)	0.75 (0.6-5.6)	0.86
CRP	2.85 (0.24-6.4)	4 (0.47-29.2)	0.33
Ca	9.25 (8-9.6)	11.15 (8.5-20.5)	0.19
sIL-2R	7155 (481-36200)	20100 (1420-97900)	0.33
SOFA score	4 (0-8)	8 (6-13)	0.03 *
Hepatic subscore	0 (0-1)	0 (0-4)	0.40
Renal subscore	0 (0-0)	0 (0-3)	0.139
Coagulation subscore	0 (0-4)	0.5 (0-3)	0.788
Respiratory subscore	2 (0-3)	3 (2-3)	0.0259*
Cardiovascular subscore	1.5 (0-3)	2.5 (2-3)	0.0749
Neurological subscore	0 (0-4)	0 (0-1)	0.673
DIC score	2 (1-2)	2 (2-5)	0.09
βD glucan	9 (0-374)	0 (0-1278)	0.72
CMV C7 HRP	0 (0-1132)	62.5 (0-520)	0.49
PS	2 (0-3)	3 (0-4)	0.11
OS	275.5 (215-2689)	14.5 (2-27)	0.003 **

*, Mann-Whitney U test   **, Log-rank test

Table 4. The short-term outcomes of ATL patients who developed opportunistic infections (spontaneous remission).

No.	Age/Sex	Subtype	CR/PR	Remission duration	Clonality	Author (year)
1	36/M	ND	CR	4 years	ND	Kimura (1983)
2	54/M	chronic	PR	ND	ND	Kawano (1983)
3	73/M	acute	CR	several weeks	ND	Schnizer (1983)
4	74/M	ND	PR	ND	ND	Tagawa (1984)
5	50/F	ND	PR	ND	ND	Tagawa (1984)
6	41/M	ND	CR	5 years	ND	Mattock (1986)
7	57/F	chronic	CR	1 year	ND	Murakawa (1990)
8	55/F	acute	CR	10 months	(+)	Taniguchi (1993)
9	50/F	acute	CR	11 months	(+)	Shimamoto (1993)
10	64/M	lymphoma	CR	3 months	(+)	Shimamoto (1993)
11	49/F	acute	CR	6 years	(+)	Shimamoto (1993)
12	42/M	acute	CR	2 months	(+)	Suzuki (1995)
13	62/F	lymphoma	CR	2 months	(+)	Jinnohara (1997)
14	70/M	chronic	CR	ND	(+)	Mizumoto (1997)
15	42/M	acute	CR	2 years	(+)	Kawada (1998)
16	76/M	acute	PR	ND	(+)	Matsushita (1999)
17	79/M	acute	CR	1 year	(+)	Takezako (2000)
Present case 6	56/F	acute	CR	213 days	biclonality	The present study
Present case 7	67/M	acute	PR	133 days	monoclonality	The present study
Present case 11	54/M	chronic	PR	194 days	monoclonality	The present study

Abbreviations: Abbreviations: CR, complete remission; PR, partial remission; ND, not described or not done.

Table 5. The long-term outcomes of ATL patients who developed opportunistic infections (ATL progression or ATL relapse).

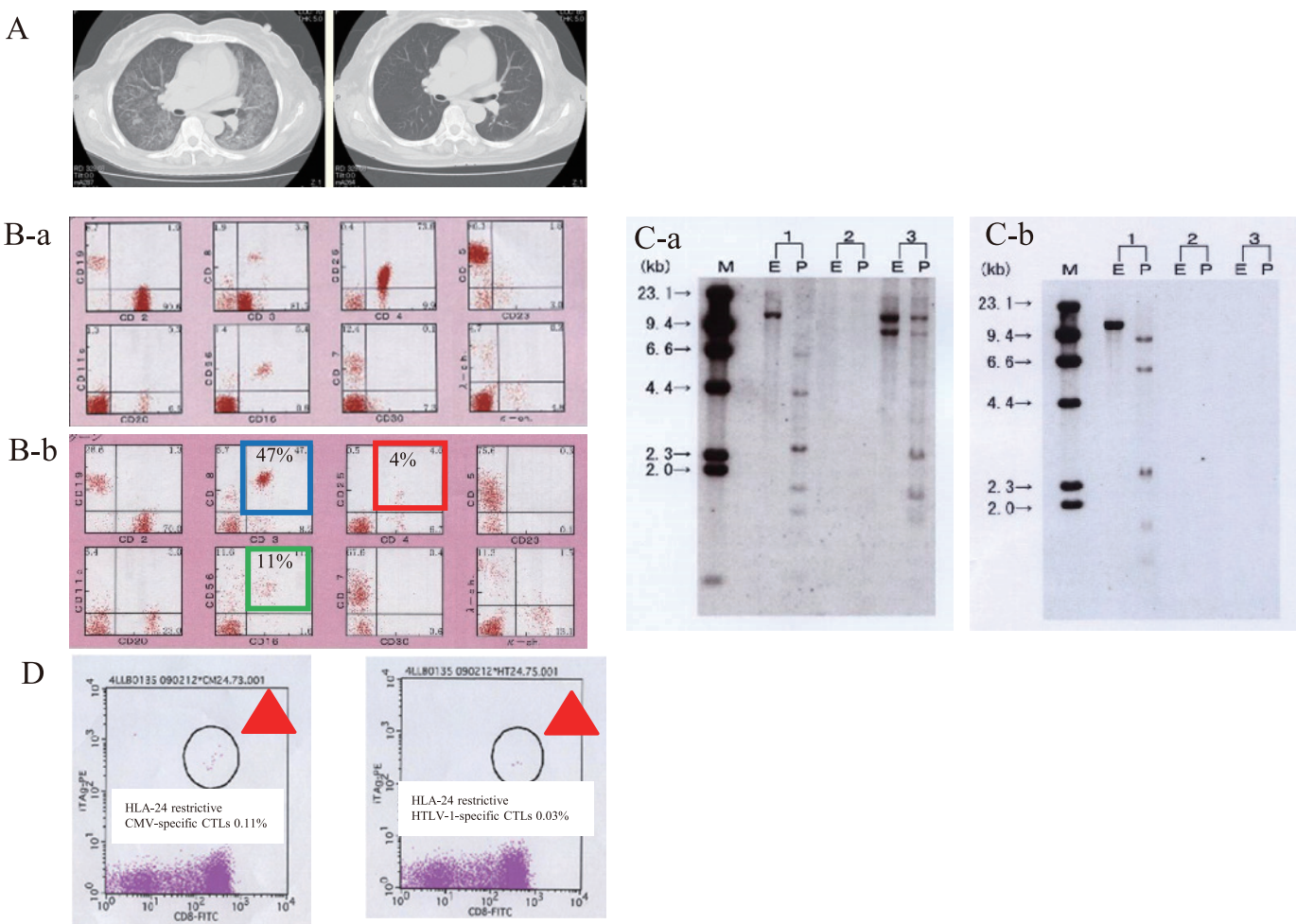|	Age	Sex	Subtype	Infection	Onset	Outcome of opportunistic infection	Response of ATL	Duration of ATL disease progression	ATL progression	Clonality	sIL-2R	Final outcome after ATL development
Previous report's case 1 Tashiro T *et al.* 1992	66	F	smoldering	*Cryptococcus* pneumonia	Initial diagnosis	Alive	SD	16 months	Aggressive Type	n.e.	n.e.	dead (4 months)
Previous report's case 2 Tashiro T *et al.* 1992	46	M	smoldering	*Pneumocystis* pneumonia	Initial diagnosis	Alive	SD	16 months	Aggressive Type	n.e.	n.e.	dead (3 months)
Previous report's case 3 Tashiro T *et al.* 1992	55	F	carrier	*Cryptococcus* pneumonia	Initial diagnosis	Alive	SD	14 months	Aggressive type	n.e.	n.e.	alive (12 months)
Previous report's case 4 Tanaka T *et al.* 2015	67	M	carrier	CMV enteritis	Initial diagnosis	Alive	n.d.	n.d.	Smoldering type	n.e.	4304	n.d.
Previous report's case 5 Tanaka T *et al.* 2015	78	F	carrier	*Pneumocystis* pneumonia, herpes infection	Initial diagnosis	Dead	n.d.	n.d.	Smoldering type	n.e.	14058	dead (4 months)
Present case 3	65	M	Chronic	*Cryptococcus* pneumonia, meningitis	Initial diagnosis	Alive	SD	226 days	Acute type	mono-clonality	2280	dead (79 days)
Present case 6	56	F	acute	CMV pneumonia	During treatment	Alive	CR	213 days	Relapse after self remission	biclonal-ity	9790	dead (27 days)
Present case 7	67	M	acute	CMV pneumonia	During treatment	Alive	PR	133 days	Relapse after self remission	mono-clonality	36200	dead (133 days)
Present case 11	54	M	chronic	*Pneumocystis* pneumonia	Initial diagnosis	Alive	PR	194 days	Acute type	mono-clonality	14500	alive (194 days)
Present case 12	78	M	smoldering	*Pneumocystis* pneumonia	Initial diagnosis	Alive	SD	153 days	Acute type	mono-clonality	4520	dead (153 days)

Fig. 5. Clinical presentation of a spontaneous-remission case before and after treatment according to FCM analysis, Southern blotting, and CTL analysis.
Presented are the CT findings, FCM analysis, southern blot analysis, and CTL analysis by a tetramer assay in a representative case of spontaneous remission of ATL (case 6) after treatment of opportunistic infection (*A-D*). CT findings after treatment of CMV pneumonia showed the improvement of CMV pneumonia (*A-a* and *A-b*). FCM findings after treatment of opportunistic infection demonstrated the improvement of CMV pneumonia (*B-a* and *B-b*). Southern blot analysis after treatment of opportunistic infection showed the absence of HTLV-1 clonality in PB (*C-a* and *C-b*). Thus, CR was achieved because of the disappearance of ATL cells on smears of PB, FCM analysis, and Southern blot analysis after CMV pneumonia (*A–D*). Furthermore, case 6 had 47% CD8+ T cells and 11% NK cells according to FCM analysis of PB. In the HLA-A24 FCM tetramer assay, there were 0.03% HLA-24–restricted HTLV-1–specific CTLs and 0.1% HLA-24–restricted CMV-specific CTLs (*D*). Thus, CTL analysis by HLA 24 restrictive FCM analysis after treatment of opportunistic infection confirmed the presence of CMV-specific CTLs and HTLV-1-specific CTLs.

cells on smears of PB, FCM analysis, and Southern blot analysis after CMV pneumonia (Fig. 5A–D). Furthermore, case 6 had 47% CD8+ T cells and 11% NK cells according to FCM analysis of PB. Furthermore, based on the HLA-A24 FCM tetramer assay, there were 0.03% HLA-24–restricted HTLV-1–specific CTLs and 0.1% HLA-24–restricted CMV-specific CTLs (Fig. 5D-a and 5D-b). Thus, CTL analysis by HLA 24 restrictive FCM analysis after treatment of opportunistic infection demonstrated the presence of CMV-specific CTLs and HTLV-1-specific CTLs.

We next examined the long-term outcomes of ATL patients who achieved spontaneous remission after the development of opportunistic infection (ATL progression or ATL relapse; Table 5). Five out of 6 survivors after opportunistic infection presented with ATL progression (3 patients) or relapse (2 patients). The 3 patients with ATL progression

after opportunistic infection had monoclonal ATL clones and high levels of sIL-2R at the initial diagnosis of opportunistic infection.

DISCUSSION

This study demonstrated that opportunistic infections should be carefully followed among HTLV-1 carriers and ATL patients because of the aggressive clinical course exhibited by the non-survivors; they had a median OS of 14.5 days (range 2–27) and poor outcome–50% OS rate and OS of 28 days–despite the low cumulative incidence rates of opportunistic infections among HTLV-1 carriers (1.5% = 2/127) and ATL patients (6.5% = 10/153). As for the prognostic factors affecting opportunistic infections, our study revealed that higher SOFA scores (especially the respiratory subscore) and

higher LDH values are closely related to the poor outcome of opportunistic infections. Thus, early diagnosis and subsequent prompt treatment are necessary for opportunistic infections among HTLV-1 carriers and ATL patients. Moreover, the detection of opportunistic infections may be predictive of the progression of ATL because of the high incidence rates of ATL progression and relapse in our study (83.3% = 5 out of 6 patients) at a median of 194 days (range 133–226 days). Based on our present study, further studies are needed to clarify the characteristics of opportunistic infections and the relationship between opportunistic infections and ATL, as well as to identify predictive factors for ATL progression in the future.

The principal focus of this manuscript was the (i) clinical characteristics of opportunistic infections (aggressive clinical course and poor outcome) among HTLV-1 carriers and ATL patients, (ii) short-term outcomes of ATL patients who achieved spontaneous remission after the development of opportunistic infection, and (iii) and long-term outcomes of ATL patients who achieved spontaneous remission after the development of opportunistic infection (ATL progression and ATL relapse).

First, regarding the clinical characteristics of opportunistic infections (aggressive clinical course and poor outcome) among HTLV-1 carriers and ATL patients, our present study revealed the cumulative incidence rates of opportunistic infections among HTLV-1 carriers and ATL patients [1.5% (2/127) and 6.5% (10/153), respectively] to be consistent with those reported by Shimoyama in 1991, with an incidence rate of opportunistic infections including bacterial, viral, and *Pneumocystis* pneumonia of 11.9% (98/818) in ATL patients.[2] Opportunistic infections also developed in HTLV-1 carriers. Furthermore, consistent with the report by Suzumiya on the etiology of opportunistic infections among autopsies of ATL patients in 1993,[3] the etiologies of opportunistic infections were diverse, such as *Cryptococcus*, *Aspergillus*, *Pneumocystis*, or CMV, with the main target organ being the lungs (12/12). Consequently, our present study demonstrated that the clinical course and prognosis of opportunistic infections are aggressive and poor, respectively; 50% OS rate, OS of 28 days, and median OS of 121 days. Of note, the 6 non-survivors had an aggressive clinical course in terms of OS; median OS of 14.5 days (range 2–27 days). Regarding the prognostic factors affecting opportunistic infections, a higher SOFA score and higher LDH values were closely related to the poor outcome of opportunistic infections. Among the factors composing the SOFA score, such as cardiovascular subscore, renal subscore, respiratory subscore, coagulation subscore, hepatic subscore, and neurological subscore, the respiratory subscore was identified as a significant factor affecting OS by univariate analysis. This is consistent with all 6 non-survivors presenting with aggressive acute respiratory failure requiring intensive treatment with intubation and ventilation in an intensive care unit. Regarding LDH values as a prognostic factor in opportunistic infections, further accumulation of cases is essential because higher LDH values may affect lung failure due to opportunistic infection and ATL. As such, early diagnosis and subsequent prompt treatment are necessary for opportunistic infections among HTLV-1 carriers and ATL patients.

Second, we focused on the short-term outcomes of ATL patients who developed opportunistic infection (spontaneous remission) and the long-term outcomes of ATL patients who developed opportunistic infection (ATL progression and ATL relapse).

Regarding the short-term outcomes of ATL patients who developed opportunistic infection, half of the survivors (3/6) with opportunistic infections presented with spontaneous remission (1 CR and 2 PR) during relatively short periods (213, 133, and 194 days). Previously, 17 ATL patients who achieved spontaneous remission after the development of opportunistic infection, including fungal infections and CMV infections, were reported (Table 4).[7-24]

Previous reports and our study suggest that the onset of opportunistic infections induces an unknown immune system attack on ATL. In one CR case after opportunistic infection in our study, the patient attained CR according FCM and Southern blot analyses with the presence of HLA-24–restricted CTLs for CMV infection and ATL. Moreover, our analysis revealed more abnormal lymphocytes in the PB of the remission group than in the non-remission group (Table 2). Furthermore, the non-remission group had hyperbilirubinemia (Table 2). Further accumulation of data is needed to identify factors predicting remission, e.g., by multivariate analysis.

As for the long-term outcomes of ATL patients who achieved spontaneous remission after the development of opportunistic infection, there were high incidence rates of ATL progression (3 patients) and relapse (2 patients) after opportunistic infection in our study (83.3% = 5 out of 6 patients) at median 194 days (133–226 days). Previously, 2 ATL patients and 3 HTLV-1 carriers exhibited disease progression after opportunistic infection (Table 5).[23,24] In our study, 3 (cases 3, 6, and 11) out of 5 ATL patients were treated by chemotherapy, radiation therapy, and allogeneic hematopoietic stem cell transplantation (allo-HSCT), respectively. Only one patient (case 11) was successfully treated by allo-HSCT because of good PS and ADL after the treatment of opportunistic infection.

Of note, the 3 patients with ATL PD after opportunistic infections had monoclonality of ATL clones and high levels of sIL-2R. Thus, the detection of opportunistic infections may be predictive of the progression of ATL in clinical practice. In our study, only 1 patient was successfully treated by mLSG 15 and subsequent allo-HSCT among the 6 survivors. The other 5 patients were contraindicated for allo-HSCT because of poor PS and ADL after the treatment of opportunistic infections. For further elucidation, maintenance of the PS and ADL is essential during treatment of opportunistic infections. Allo-HSCT may be necessary to overcome the poor outcome of ATL progression after opportunistic infection.

In conclusion, opportunistic infections should be carefully followed among HTLV-1 carriers and ATL patients

because of their aggressive clinical course and poor outcomes. Thus, early diagnosis and subsequent prompt treatment are important. Our present study demonstrated that further studies are needed to clarify the characteristics of opportunistic infections and the relationship between opportunistic infections and ATL, as well as to identify predictive factors of ATL progression in the future.

CONFLICTS OF INTEREST

The authors state that they have no Conflict of Interest (COI).

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