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Clinical and histopathological significance of PD-1 expression in cutaneous lesions of adult T-cell leukemia–lymphoma

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ABSTRACT

Background: Adult T-cell leukemia–lymphoma (ATL) is a mature T-cell malignancy caused by human T-cell leukemia virus type I infection and is known to exhibit cutaneous involvement in 50% or more patients. Few studies have evaluated the clinicopathological significance of programmed death-1 (PD-1) expression in the cutaneous lesions of ATL.

Methods: Skin biopsy specimens from 29 ATL patients with cutaneous lesions were evaluated regarding the clinicopathological feature, survival outcome, and PD-1 expression level on infilitrated CD3 + CD4 + CD25 + cells. The optimal cut-off point of PD-1 expression for clinicopathological feature and outcome was determined as the value of the maximum Youden index by receiver operating characteristic (ROC) analysis.

Results: PD-1 was expressed broadly from zero to 90% on the skin biopsy specimens of the 29 patints, with the median value of 50%. The PD-1-expression level was significantly higher in the poorer-prognosis eruption group (nodulotumoral, erythrodermic and purpuric types) ($P = 0.003$), in the poorer histopathological infiltration patterns (diffuse and nodular) ($P = 0.007$), and in the poorer infiltrating cell-size group (large-sized cells) ($P = 0.017$) than in the corresponding group. ROC curve analyses showed that the optimal cut-off value for PD-1-expression level to predict the poorer-prognosis eruption, the poorer- histopathological infiltration pattern, the poorer infiltration cell size, and the poorer outcome (death) was 60%, 50%, 50%, and 80%, respectively. Patients with high PD-1 expression had a shorter median survival time than those with low PD-1 expression (18.2 months vs. 26.0 months), but the difference was not statistically significant.

Conclusions: ATL patients with cutaneous lesions in which PD-1 were highly expressed have more advanced dermatological and histopathological patterns and possibly worse survival than those with low PD-1 expression on cutaneous lesions. Further large-scale studies are warranted to verify these findings.

1. Introduction

Adult T-cell leukemia–lymphoma (ATL) is a mature peripheral CD4 + T-cell malignancy caused by human T-cell leukemia virus type I (HTLV-1) infection [1], and is usually classified into four clinical subtypes: acute, lymphoma, chronic and smoldering [2,3]. ATL cells can be involved in many organs and tissues [2]; in particular, more than 50% of ATL patients exhibit cutaneous involvement [4–6]. Therefore, the evaluation of cutaneous lesions in relation to disease severity and prognosis is important.

Previous studies have reported that cutaneous involvement might be an independent risk factor for poor prognosis of ATL, except for the lymphoma subtype [4–9]. Furthermore, when cutaneous lesions of ATL were divided into several histopathological types based on macroscopic evaluation, the prognosis was worse in patients with nodular or diffuse infiltration of medium or large-sized lymphoma cells [6] and in patients with the erythrodermic skin eruption type [10] than in others. Although the previous classification system for cutaneous lesions is very useful for predicting the prognosis of ATL with cutaneous involvement, one or more quantitative indicators that are related to poor cutaneous lesions

Abbreviations: ATL, adult T-cell leukemialymphoma; CI, confidence interval; HTLV-1, human T-cell leukemia virus type I; IQR, interquartile range; LDH, lactate dehydrogenase; MST, median survival time; OS, overall survival; PD-1, programmed death-1; sIL-2R, soluble interleukin-2 receptor; WBC, white blood cell

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would be desirable.

Recently, many studies have examined the expression patterns of programmed death-1 (PD-1) in a variety of T-cell lymphoid malignancies, including ATL [11–13]. Shimauch et al. reported that the levels of PD-1 expression on both CD4 + CD25+ and CD4 + CD25– T-cell populations in peripheral mononuclear cells from ATL patients were increased compared to healthy people [11]. Kozako et al. reported that PD-1 expression on HTLV-1-specific cytotoxic T-lymphocytes was elevated in both HTLV-1 carriers and ATL patients, and suggested a role for PD-1 expression in ATL development [12]. However, few studies have evaluated PD-1 expression patterns in cutaneous lesion of ATL, or the relationship between the degree of PD-1 expression, types of histopathological features, and the clinical course. Therefore, we aimed to examine the role of PD-1 expression in cutaneous lesions of ATL and its consequences for survival by assessing the dermatological and pathological features of the biopsy specimens from cutaneous lesions of ATL patients.

2. Material and methods

2.1. Patients and laboratory data

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Nagasaki University, which granted an exemption from the requirement for written informed consent in accordance with the comprehensive prior consent given to the Dermatology Department (Approval No. 10072370).

We assessed 29 patients (13 male, 16 female) with ATL having cutaneous lesions, who were referred from the Department of Hematology to the Department of Dermatology of the Nagasaki University Hospital to confirm whether they exhibited “cutaneous involvement of ATL,” between January 2011 and September 2015. The definitive diagnosis of ATL and the subtype classification [2] were made by the Department of Hematology, based on clinical features, cytologically-proven mature T-cell malignancy, presence of anti-HTLV-1 antibody, and monoclonal integration of HTLV-1 proviral DNA in peripheral mononuclear cells. We evaluated serum soluble interleukin-2 receptor (sIL-2R) levels (U/ml), lactate dehydrogenase (LDH) levels (IU/L), and white blood cell counts (WBC; $\times 10^4$/L), all of which are known biomarkers of disease aggressiveness in ATL [2]. At the time of skin biopsy in the Dermatology Department, 18 patients had not received any therapy, 10 had received chemotherapy, and one had received both chemotherapy and stem cell transplantation.

2.2. Evaluation of cutaneous lesions

1) Using biopsy specimens from the patients’ cutaneous lesions, we evaluated the following four histomorphometric features and PD-1 expression.
2) Six macroscopic eruption patterns: patch, plaque, multipapular, nodulotumoral, erythrodermic and purpuric, according to Sawada’s report (listed in order of increasing severity) [10]. When two or more eruption types were found in a lesion, we recorded the most severe type. We then dichotomized the six macroscopic patterns into a better-prognosis eruption group (patch, plaque and multipapular types) and a poorer-prognosis eruption group (nodulotumoral, erythrodermic and purpuric types).
3) Three histopathological infiltration patterns of atypical lymphoid cells: perivascular, diffuse and nodular, based on Yamaguchi’s report [6]. We then dichotomized the infiltration patterns into a better-prognosis group (perivascular) and a poorer-prognosis group (diffuse or nodular).
4) Three histopathological cell-size patterns of the infiltrating atypical lymphoid cells: small-sized atypical lymphoid cells with mild to moderate nuclear atypia, medium-sized atypical lymphoid cells with moderate nuclear atypia, and large-sized atypical lymphoid cells with severe nuclear atypia, based on Yamaguchi’s report [6]. We then dichotomized the infiltrating cell-size patterns into a better-prognosis group (small- or medium-sized cells) and a poorer-prognosis group (large-sized cells).
5) Immunohistochemical staining using mouse monoclonal antibodies for CD3 (LN10, Leica), CD4 (1F6, Leica), CD7 (SP94, Ventana), CD8 (4B11, Leica), CD25 (4C9, Leica), CD30 (Ber-H2, DAKO), CCR4 (1G1, BD) and MUM1 (MUM-1p, DAKO): for all of which the staining of greater than 30% of tumor cells was taken as positive according with manufacturer's instructions. We considered cells positive for CD3, CD4 and CD25 to be ATL-specific atypical lymphoid cells.
6) PD-1 expression on CD3 + CD4 + CD25 + cells was assessed by Immunofluorescence staining using mouse monoclonal antibodies for PD-1 (NAT105, Abcam). A variety of cut-off points to divide PD-1-positive and negative cells, ranging from 1% to 60%, have been reported in literature [14,15]. In the present study, we performed a receiver operating characteristic (ROC) curve analysis to determine the best cut-off point for discriminating between positive and negative staining for PD-1 expression for each of the histomorphometric features and survival outcome. We then dichotomized the PD-1 expression pattern into a PD-1-high or a PD-1-low group based on the optimal cut-off point.

Table 1
Summary of the clinical and histopathological features of 29 ATL patients with cutaneous lesions.

Characteristics	$n = 29$
Male sex, n (%)	13 (44.8)
Age at biopsy, y, median (min, max, IQR)	70 (34, 90, 57–77)
Shimoyama classification, n (%)	
Smoldering subtype	6 (20.7)
Favorable Chronic subtype	3 (10.3)
Unfavorable Chronic subtype	3 (10.3)
Acute subtype	17 (58.7)
Previous treatments, n (%)	
None	18 (62.1)
Chemotherapy	10 (34.5)
Chemotherapy + SCT	1 (3.4)
Laboratory examinations, median (min, max, IQR)	
sIL-2R (U/ml) x 1000	4.4 (0.5, 63.4, 2.6–14.7)
LDH (IU/L)	235 (112, 1315, 205–342)
WBC ($\times 10^9$/L)	7.9 (0.7, 32.6, 4.9–10.4)
Skin eruption types, n (%)	
Patch	5 (17.2)
Plaque	8 (27.6)
Multipapular	5 (17.2)
Purpuric	0
Nodulotumoral	9 (31.0)
Erythrodermic	2 (7.0)
Histopathological infiltration pattern, n (%)	
Perivascular	14 (48.2)
Diffuse	11 (38.0)
Nodular	4 (13.8)
Size of infiltrated atypical lymphoid cells, n (%)	
Small	5 (17.3)
Medium	15 (51.7)
Large	9 (31.0)

ATL, adult T-cell leukemia-lymphoma; SCT, stem cell transplantation; sIL-2R, soluble interleukin-2 receptor; LDH, lactate dehydrogenase; WBC, white blood cells; IQR, Interquartile range.

2.3. Statistical analysis

Continuous data other than PD-1-expression are presented as the median value, range, and interquartile range (IQR), and were compared using Wilcoxon rank-sum tests. Categorical data were compared using chi-squared or Fisher's exact tests. Survival durations were calculated

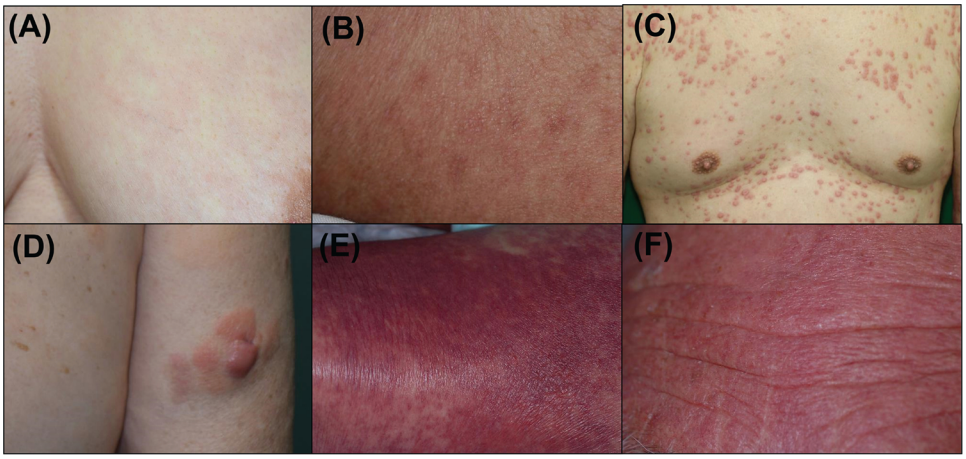


Fig. 1. Typical clinical features of cutaneous lesions of ATL. Low magnification views of (A) patch type, (B) plaque type, (C) multipapular type, (D) nodulotumoral type, (E, F) erythrodermic type. The purpuric type was not seen in this study population. The classification was based on Sawada et al. [10].

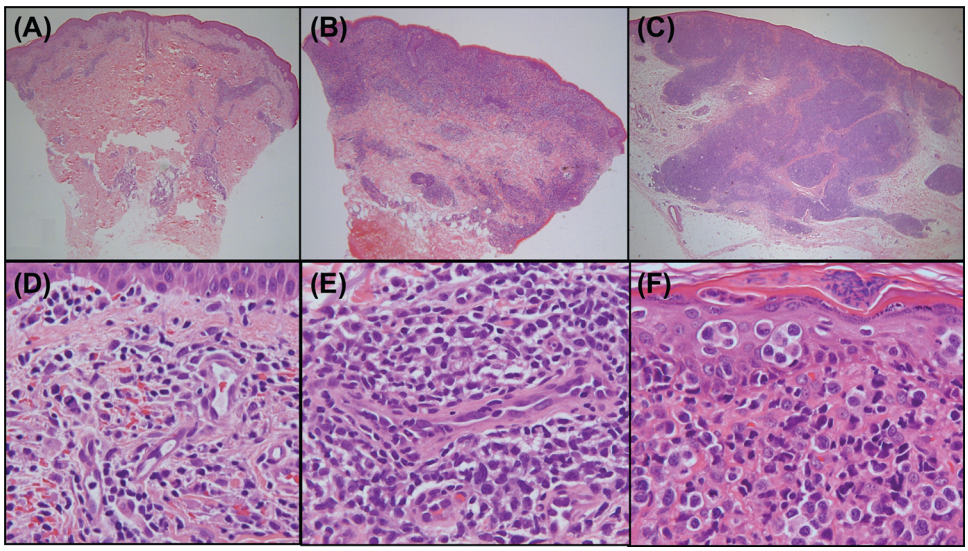


Fig. 2. Typical histopathological features of cutaneous lesions of ATL. High magnification views of (A) perivascular infiltration type, (B) diffuse interstitial infiltration type, (C) nodular infiltration type, (D) small cell-size type, (E) medium cell-size type, and (F) large cell-size type. Original magnification, × 0.10. The classification was based on Yamaguchi et al. [6].

from the date of diagnosis by the Department of Dermatology to the last follow-up or death (the cutoff date was May, 2017). Survival curves were generated by the Kaplan–Meier method using both GraphPad Prism (version 6.0 for Mac, GraphPad Software, La Jolla, CA) and JMP Pro 13.0.0 software (SAS Inc., Cary, NC, USA). The comparisons of survival curves were done using the log-rank test and generalized Wilcoxon test. The 95% confidence intervals (CIs) for overall survival (OS) and the median survival time (MST) were generated by using JMP Pro 13.0.0 software. ROC curve analyses were also performed using JMP Pro 13.0.0 software in which the best cut-off point and the area under the ROC curve (AUC) were determined automatically based on Youden's index. Other statistical analyses were done using JMP Pro 13.0.0 software. All the statistical tests were two-tailed, and a P value of less thasn 0.05 was considered to be statistically significance.

3. Results

3.1. Clinical and histopathological profile

The clinical and histopathological characteristics of the 29 patients in this study are summarized in Table 1. The median age at the time of skin biopsy was 70 years (range, 34–90 years). The most common subtype of ATL based on the Shimoyama classification [2] was acute type (58.7%). Fig. 1 shows typical clinical features for each type of skin eruption. Fig. 2 shows typical findings for each histopathological type (Fig. 2A–C) and cell-size category (Fig. 2D–F). The most common skin eruption type was nodulotumoral (n = 9; 31%) (Table 1 and Fig. 1D). The most common infiltration pattern was perivascular (Table 1 and Fig. 2A), and the most frequent infiltrating cell size was medium-sized (Table 1 and Fig. 2E). In patients with the diffuse or nodular infiltration patterns (Fig. 2B or C), the cells infiltrated all of the layers of the dermis and adipose tissue. Furthermore, the infiltrating large-sized cells showed severe dysplasia (Fig. 2F). Immunohistochemically, infiltrating atypical lymphoid cells showed positivity for CD7 in 14 (48.2%) patients, CD8 in nine (31.0%), CD30 in seven (24.1%), and MUM-1 in six (20.7%).

3.2. Distribution of PD-1-expression on CD3 + CD4 + CD25 + tumor cells

The histogram of the number of patients based on the percentages of PD-1-positive CD3 + CD4 + CD25 + tumor cells on biopsy specimens from the 29 patients was shown in Fig. 3A. The median value of the PD-1-positive cells was 50% (ranges, 0–90%; IQR, 0–75%) and the mean value was 39.6% (SD, 35.5%). Among the 14 patients with the PD-1-expression less than the median value (50%), the distribution was sporadic; four patients showed the PD-1-expression with 20% and the rest of ten patients showed no expression. Among the 15 patients with the PD-1-expression greater than the median value, PD-1- positive cells

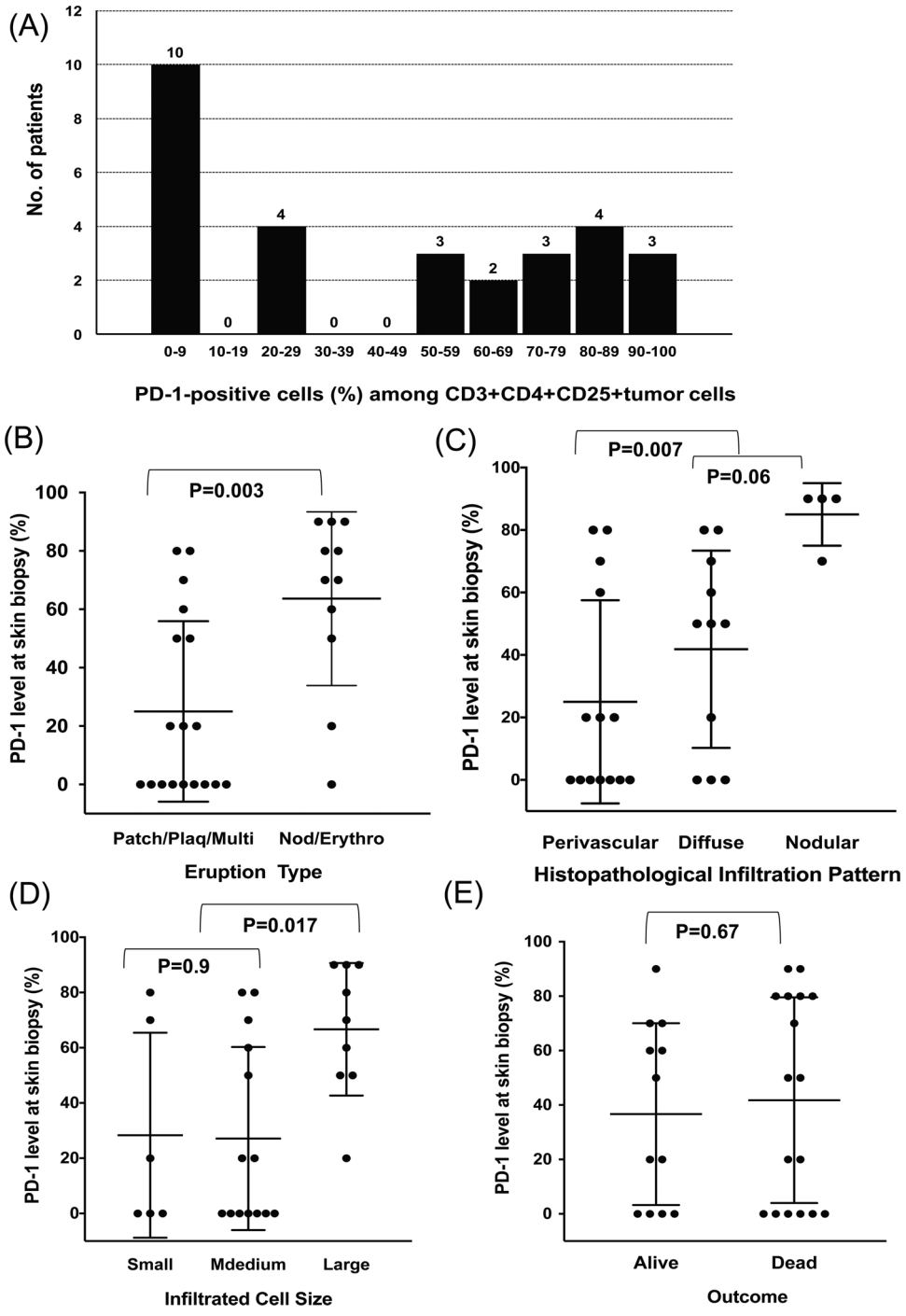


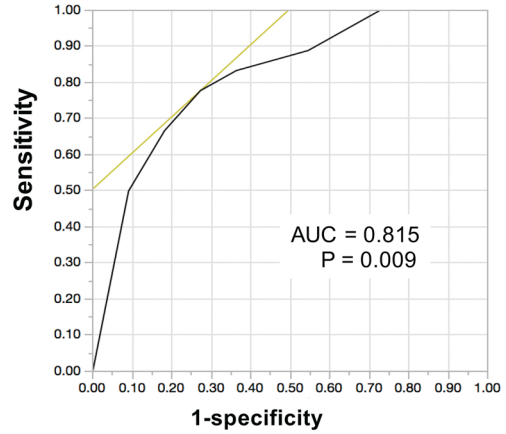
Fig. 3. Distribution of PD-1-expression on CD3 + CD4 + CD25 + tumor cells by clinico-histopathological feature. (A) The histogram of the number of patients based on the percentages of PD-1-positive tumor cells, (B) PD-1-expression level by dichotomized eruption group; (C) PD-1-expression level by histopathological infiltration patterns; (D) PD-1-expression level by infiltrating cell-size category, and (E) PD-1-expression level by outcome status.
Abbreviations: Patch/Plaq/Multi: patch, plaque and multipapular types; Nod/Erythro: nodulotumoral and erythrodermic types.

varied from 50% to 90%, in each of which there were from two to four patients.

When we compared the PD-1-expression level by the respective clinico-histopathological feature, there was a significantly different distribution between the poorer-prognosis eruption group (nodulotumoral, erythrodermic and purpuric types) and the better-prognosis eruption group (patch, plaque and multipapular types) ($P = 0.003$) (Fig. 3B). There were also significant differences in the PD-1-expression level between the poorer histopathological infiltration patterns (diffuse and nodular) and the better infiltration patterns (perivascular) ($P = 0.007$) (Fig. 3C), and between the poorer infiltrating cell-size group (large-sized cells) and the better-prognosis group (small- or medium-sized cells) ($P = 0.017$) (Fig. 3D). However, the PD-1-expression level was not significantly different between outcome status ($P = 0.67$) (Fig. 3E). Linear correlation analyses between the raw value of PD-1-positive tumor cells (%) and both the serum levels of sIL-2R (natural-logarithm-transformed values) and LDH were not statistically significant ($P = 0.30$ and 0.32, respectively) (Figures not shown).

(A)

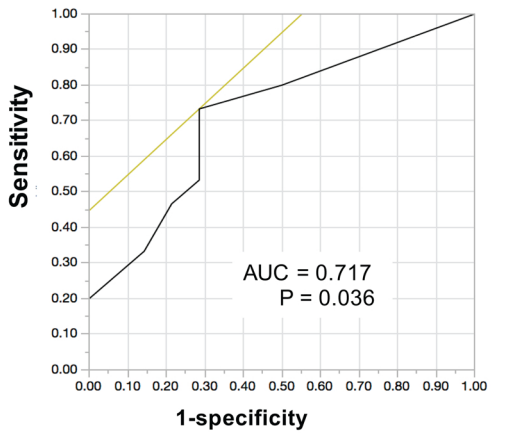
Eruption type
(Nodulo/Erythro vs. Patch/Plaq/Multi)



PD-1	Probability	1-Spec	Sensitivity	Sen-(1-Spec)	
.	.	0.0000	0.0000	0.0000	
90.00000	0.7699	0.0000	0.2727	0.2727	
80.00000	0.6962	0.1111	0.4545	0.3434	
70.00000	0.6108	0.1667	0.6364	0.4697	
60.00000	0.5181	0.2222	0.7273	0.5051	*
50.00000	0.4241	0.3333	0.8182	0.4848	
20.00000	0.1914	0.5000	0.9091	0.4091	
0.00000	0.0999	1.0000	1.0000	0.0000	
0.00000	0.0999	1.0000	1.0000	0.0000	

(B)

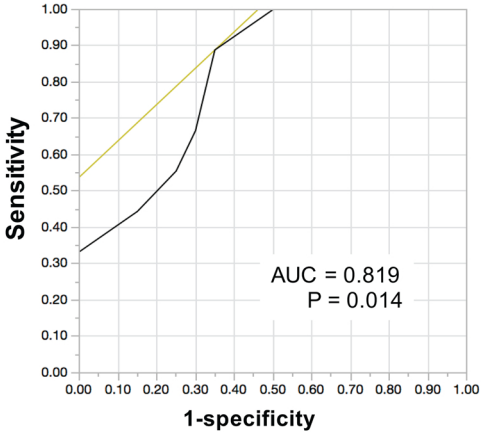
Histopathological Infiltration pattern
(Diffuse/Nodular vs. Perivascular)



PD-1	Probability	1-Spec	Sensitivity	Sen-(1-Spec)	
.	.	0.0000	0.0000	0.0000	
90.00000	0.7958	0.0000	0.2000	0.2000	
80.00000	0.7516	0.1429	0.3333	0.1905	
70.00000	0.7014	0.2143	0.4667	0.2524	
60.00000	0.6459	0.2857	0.5333	0.2476	
50.00000	0.5862	0.2857	0.7333	0.4476	*
20.00000	0.3988	0.5000	0.8000	0.3000	
0.00000	0.2857	1.0000	1.0000	0.0000	
0.00000	0.2857	1.0000	1.0000	0.0000	

(C)

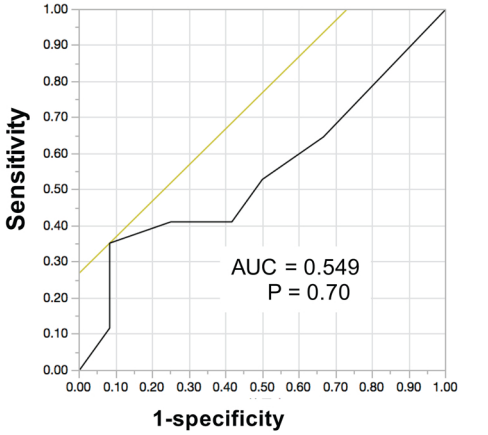
Infiltrated Cell Size
(Large vs. Small/Medium)



PD-1	Probability	1-Spec	Sensitivity	Sen-(1-Spec)	
.	.	0.0000	0.0000	0.0000	
90.00000	0.6991	0.0000	0.3333	0.3333	
80.00000	0.6100	0.1500	0.4444	0.2944	
70.00000	0.5128	0.2500	0.5556	0.3056	
60.00000	0.4147	0.3000	0.6667	0.3667	
50.00000	0.3229	0.3500	0.8889	0.5389	*
20.00000	0.1270	0.5000	1.0000	0.5000	
0.00000	0.0618	1.0000	1.0000	0.0000	
0.00000	0.0618	1.0000	1.0000	0.0000	

(D)

Outcome
(Dead vs. Alive)



PD-1	Probability	1-Spec	Sensitivity	Sen-(1-Spec)	
.	.	0.0000	0.0000	0.0000	
90.00000	0.6369	0.0833	0.1176	0.0343	
80.00000	0.6271	0.0833	0.3529	0.2696	*
70.00000	0.6172	0.2500	0.4118	0.1618	
60.00000	0.6072	0.4167	0.4118	-0.0049	
50.00000	0.5972	0.5000	0.5294	0.0294	
20.00000	0.5665	0.6667	0.6471	-0.0196	
0.00000	0.5457	1.0000	1.0000	0.0000	
0.00000	0.5457	1.0000	1.0000	0.0000	

Fig. 4. Results of ROC curve analysis to identify optimal cut-off point of PD-1-expression level on Clinicohistopathological profile of ATL. (A) to predict a poorer-prognosis eruption type; (B) to predict a poorer-prognosis histopathological infiltration pattern; (C) to predict a poorer-prognosis infiltrating cell-size pattern; (D) to predict death from ATL. The asterisk and underlined PD-1 expression level denotes the row with the highest value of Sensitivity = (1-specificity), which is the point of optimal classification accuracy.

Abbreviations: ROC: receiver-operating characteristic; AUC: Area under the receiver-operating characteristic; Patch/Plaq/Multi: patch, plaque and multipapular types; Nodulo/Erythro: nodulotumoral and erythrodermic types.

Table 2
Comparison of the clinical and histopathological features according to the PD-1-expression level.

Characteristics	PD-1-high (positive cells ≧50%) N = 15	PD-1-low (positive cells < 50%) N = 14	*P* value
Male sex, n (%)	9 (60.0)	4 (28.6)	0.14
Age at biopsy, y, median (min, max, IQR)	72 (34, 90, 56–82)	67 (43, 79, 57–74)	0.31
Shimoyama classification, n (%)			
Smoldering/Favorable Chronic subtypes	7 (46.7)	2 (14.3)	0.11
Unfavorable Chronic/Acute subtypes	8 (53.3)	12 (85.7)	
Previous treatments, n (%)			
None	11 (73.3)	7 (50)	0.27
Chemotherapy/SCT	4 (26.7)	7 (50)	
Laboratory examinations, median (min, max, IQR)			
sIL-2R (U/ml) x 10^3	4.4 (0.5, 63.4, 1.0–15.5)	4.7 (1.7, 34.5, 3.0–15.6)	0.52
LDH (IU/L)	221 (170, 1315, 203–356)	245 (112, 705, 202–343)	0.98
WBC ($\times 10^9$/L)	6.2 (0.7, 32.6, 4.9–10.6)	8.8 (1.5, 14.9, 3.6–10.2)	0.91
Skin eruption types, n (%)			
Patch/Plaque/Multipapular	6 (40.0)	12 (85.7)	0.021
Nodulotumoral/ Erythrodermic	9 (60.0)	2 (14.3)	
Histopathological infiltration pattern, n (%)			
Perivascular	4 (26.7)	10 (71.4)	0.027
Diffuse / Nodular	11 (73.3)	4 (28.6)	
Size of infiltrated atypical lymphoid cells, n (%)			
Small/Medium	7 (46.7)	13 (92.9)	0.014
Large	8 (53.3)	1 (7.1)	

IQR, Interquartile range; PD-1, programmed death 1; SCT, stem cell transplantation; sIL-2R, soluble interleukin-2 receptor; LDH, lactate dehydrogenase; WBC, white blood cells;

3.3. Optimal cut-off point of PD-1-positive tumor cells on Clinicohistopathological profile

Based on the ROC curve analysis, the optimal cut-off point of PD-1-positive tumor cells for predicting a poorer-prognosis eruption type was 60% (AUC = 0.815, P = 0.009, 72.7% sensitivity, and 77.8% specificity) (Fig. 4A), for predicting a poorer-prognosis histopathological infiltration pattern was 50% (AUC = 0.717, P = 0.036, 73.3% sensitivity, and 71.4% specificity) (Fig. 4B), for predicting a poorer-prognosis infiltrating cell-size pattern was 50% (AUC = 0.819, P = 0.014, 88.9% sensitivity, and 65.0% specificity) (Fig. 4C), and for predicting death from ATL was 80% (AUC = 0.549, P = 0.70, 35.3% sensitivity, and 91.7% specificity) (Fig. 4D). The cut-off value of 80% for survival was very skewed toward the higher value and not statistically significant. Based on these results, a value of 50% was chosen as the optimal cut-off value to divide PD-1-high and -low expression on CD3 + CD4 + CD25 + tumor cells.

3.4. Differences in clinicopathological features by the PD-1 expression level

Based on the optimal cut-off value of PD-1 expression, we dichotomized patients into two groups: the PD-1-high group (expressed 50% or more, N = 15) and the PD-1-low group (expressed less than 50%, N = 14), and then compared the clinicopathological characteristics between the two groups (Table 2).

There were no significant differences between the PD-1-high and -low groups in the age or sex distribution of patients, Shimoyama classification, previous treatment status, or laboratory examinations (Table 2). However, the PD-1-high group had significantly higher proportions of the poorer-prognosis eruption types (nodulotumoral/erythrodermic types) ($P = 0.021$), the poorer-prognosis histological infiltration pattern (diffuse/nodular pattern) ($P = 0.027$), and the poorer-prognosis infiltrating cell-size oattern (the large-sized atypical lymphoid cells) ($P = 0.014$) compared with the PD-1-low group.

3.5. Survival according to clinicopathological features and PD-1 expression level

Fig. 5A shows the survival curve for all patients. The OS rate was 26.9% (95%CI, 9.3%–56.9%), and the MST was 24.1 months (95% CI, 13.6 months to upper limit not reached). As expected, the OS was significantly different according to the Shimoyama subtype: the acute subtype was the worst, followed by the chronic and then the smoldering subtype ($P < 0.001$, Fig. 5B). However, the OS was not significantly different between the dichotomized skin eruption groups (Fig. 5C), between the dichotomized atypical lymphoid cell infiltration patterns (Fig. 5D), nor between the dichotomized infiltrating cell size (Fig. 5E). When comparing survival curves based on the cut-off level of 50% PD-1-expression, the MST was shorter for patients with the PD-1-positive greater than 50% (18.2 months, 95% CI, 2.5 months to upper limit not reached) than those less than 50% (26.0 months, 95% CI, 6.4 months to upper limit not reached) (Fig. 5F) but the difference was not statistically significant. However, when the cut-off level of the PD-1-expression was raised to 80%, the MST was significantly shorter for patients with the PD-1-positive greater than 80% (13.7 months, 95% CI, 1.5 months to 18.2 months) than those with less than 80% (26.0 months, 95% CI, 13.6 months to upper limit not reached) (Fig. 5G).

3.6. The effect of the PD-1 expression level on acute transformation from indolent ATL

At the date of skin biopsy, nine patients were classified as indolent ATL (six smoldering and three chronic subtypes without unfavorable prognostic factors) and were receiving no therapy. However, during the follow-up period, five (55.6%) of the nine indolent ATL cases transformed to acute ATL. Among the five patients, four (80%) were PD-1-expression leves were grater than 50%, four (80%) had large-sized infiltrating cells, and four (80%) had the nodulotumoral skin eruption type. More detailed clinical and histopathological characteristics of the five transformed patients were summarized in Table 3. One of these, a 72-year-old woman (patient No. 4 in the Table 3) was referred to the dermatological department to examine the skin eruption on the right dorsal upper arm. At that time, the skin eruption was diagnosed as an erythematous plaque type (Fig. 6A). The pathological findings was diagnosed as a diffuse and large type. There was no Pautrier's microabscess. She was found to have anti-HTLV-1 antibody in peripheral blood and HTLV-1 virus genome in skin biopsy specimen by Southern-blot analysis. The summary of the peripheral blood examination was shown in case No.4 in the Table 3 (sIL-2R, 806/ml; WBC, 5000/μL; LDH, 180IU/L; Ca, 9.1 mg/dl; BUN, 20 mg/dl; Alb, 4.3 g/dl). Based on no abnormality on peripheral blood examination, except for the presence of 6% abnormal lymphocytes, she was diagnosed as the smoldering type of ATL with skin lesion, and clinically followed-up under no therapy. The hematoxylin and eosin stain of the first skin biopsy sction showed dense and diffuse infiltration of large-sized atypical lymphocytes (Fig. 6C). Immunohistochemically, atypical lymphocytes were almost positive for CD4 (Fig. 6D) and CD25 (Fig. 6E), CCR4 was expressed at very high level (80%) (Figure not shown), and PD-1 was expressed on 50% of the CD3 + CD4 + CD25 + cells (Fig. 6F). At that time, however, peripheral blood examination and organs other than skin were clinically as stable as normal. Therefore, the skin eruption alone was treated with steroid ointment, PUVA-bath photochemotherapy, and local irradiation. However, on 30 months after the first skin biopsy, her skin eruption type changed to the nodulotumoral type (Fig. 6B) and a month later the eruption distributed on whole body and bone invasion was found. The patient was diagnosed as

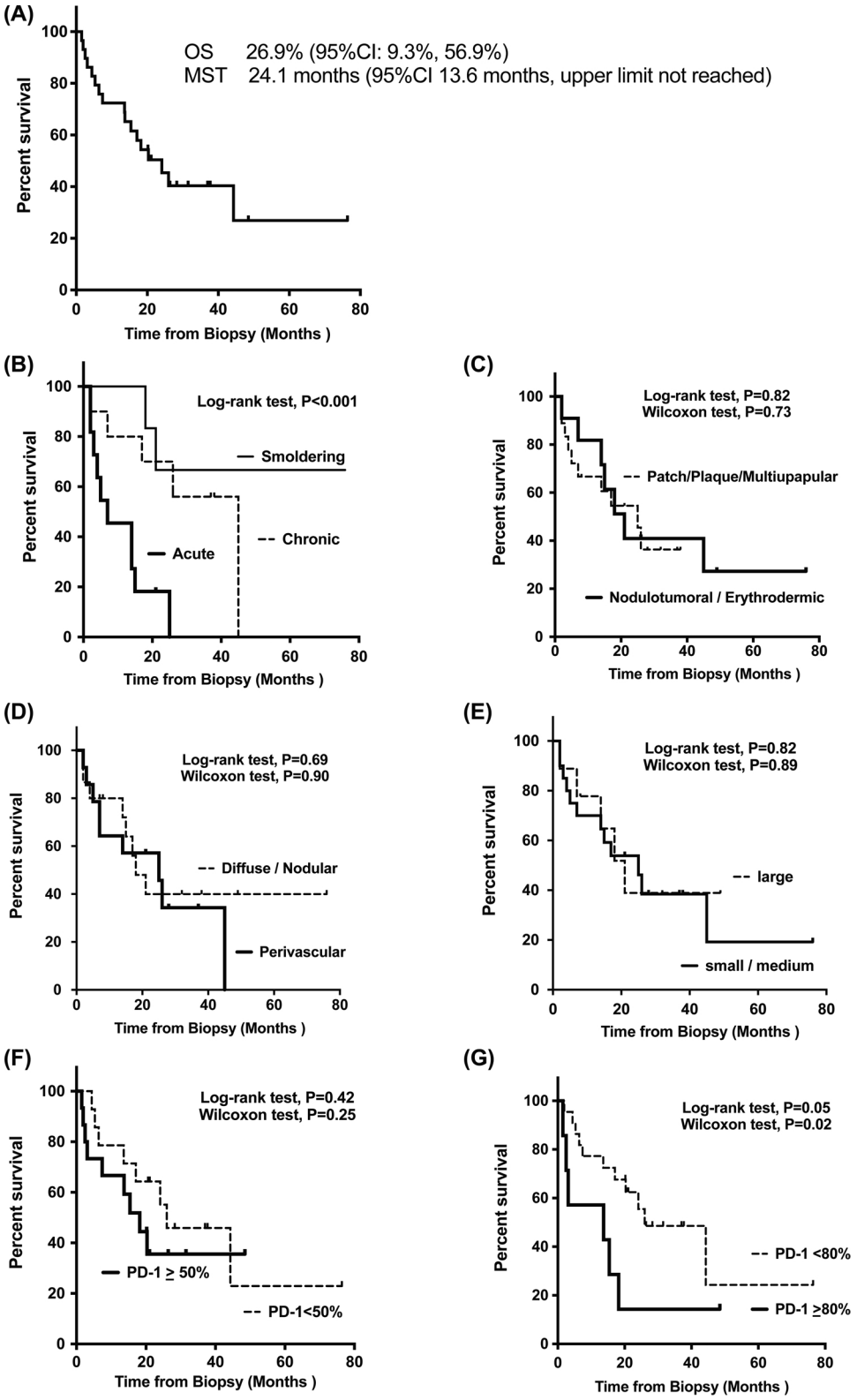


Fig. 5. Overall survival curves for ATL patients with cutaneous lesions. Survival time was calculated in months from the date of skin biopsy to deaths or the last follow-up date. (A) All patients; (B) According to Shimoyama subtypes [2]: acute, chronic and smoldering; (C) According to skin eruption type; (D) According to infiltration pattern of atypical lymphoid cells; (E) According to infiltrating cell size; (F) According to dichotomized PD-1-expression level at 50%; (G) According to dichotomized PD-1-expression level at 80%.

Table 3
Clinical and histopathologic characteristics of 5 patients with indolent ATL who later transformed acute ATL.

	Case No.				
	1	2	3	4	5
At first biopsy					
Sex	M	M	F	F	F
Age at biopsy (y)	82	83	77	72	70
Shimoyama classification at biopsy	Smoldering	Smoldering	Smoldering	Smoldering	Smoldering
Previous treatment	No	No	No	No	No
WBC count at biopsy (/μl)	3900	5400	6400	5000	9800
Atypical lymphocytes (%)	0	0	1	6	27
sIL-2R level at biopsy (U/ml)	858	1051	4412	806	1957
LDH level at biopsy (IU/L)	257	221	248	180	227
Ca level at biopsy (mEq/l)	8.4	8.4	9.3	9.1	9.9
BUN level at biopsy (mg/dl)	15	16	14	20	13
Alb lebel at biopsy (g/dl)	3.6	ND	3.8	4.3	4.6
Skin eruption type	Nodulotumoral	Plaque	Nodulotumoral	Plaque	Plaque
Histopathological ifiltration pattern	Nodular	Diffuse	Diffuse	Diffuse	Perivascular
Size of infiltrated atypical lymphoid cells	Large	Large	Large	Large	Medium
PD-1 expression (%)	90	70	50	50	0
At transformed to Acute ATL					
Age at transfomed to Acute ATL (y)	83	84	77	74	70
Period from first biopsy to transformed to Acute ATL (m)	18	12	5	31	3
Treatment after diagnosis of Acute ATL	Radiation, VCR, Operation	mLSG15, ETP, Moga	mLSG15	Radiation	mLSG15, Moga, Lenalidomide
Outcome	Death	Unknown	Death	Survival	Survival
Period from 1 st biopsy to outcome (m)	18	Unknown	20	32	28
Skin eruption type after first biopsy	No change	Nodulotumoral	No change	Nodulotumoral	Multipapular

ATL, adult T-cell leukemia; M, male; F, female; WBC, white bood cell; LDH, lactate dehydrogenase; sIL-2R, solble interleukin-2 receptor; Ca, calcium; BUN, urea nitrogen; Alb, albumin; PD-1, programmed death-1; ND, not determined; VCR, vincristine; Moga, Mogamulizumab; mLSG15, VCAP-AMP-VECP.

transformation to acute-type ATL, and then transferred to a different hospital.

4. Discussion

In the present study, although the sample size was small, we clearly showed that ATL patients with cutaneous lesions with infiltrating CD3 + CD4 + CD25 + PD-1 + cells had significantly higher proportions of the poorer skin eruption types (nodulotumoral/erythrodermic types), the poorer infiltration patterns (diffuse/nodular patterns), and the poorer cell size characteristics (large-sized atypical lymphoid cells infiltration). Furthermore, we showed that the survival time tended to be the shorter in patients with the higher PD-1 expression on cutaneous lesions, in particular patients with the PD-1 expression over 80% of positive cells were the shortest. Thus, the PD-1-expression level on cutaneous lesions of ATL patients may be an alternative biomarker that would predict a poorer cutaneous lesion of patients with ATL. These results highlight the need for dermatologists and pathologists to recognize the role of PD-1 expression in cutaneous lesions of ATL, particularly in smoldering subtype.

PD-1-positive cells have been published in a variety of cutaneous T-cell lymphomas, such as angioimmunoblastic T-cell lymphoma, Sézary syndrome, primary cutaneous CD4-positive small/medium-sized pleomorphic T-cell lymphoma, and cutaneous extranodal natural killer/T-cell lymphoma [16–22]. However, the clinical significance of the PD-1-positive cells has been debated. A research group from the Netherlands reported that the expression pattern of PD-1 in skin biopsy specimens from patients with Sézary syndrome and mycosis fungoides are different, and a higher PD-1 expression on neoplastic T cells was observed only for Sézary syndrome [20,21]. *Kim et al.* reported that a higher PD-1 expression on tumor cells of cutaneous extranodal NK/T-cell lymphoma was associated with the advanced morphological types (erythematous/purpura patches and small/mixed tumor cells types), but did not affect the difference in OS and progression-free survival [22]. The results of *Kim et al.* were very similar to the present study.

In the present study, we confirmed that cutaneous classification based on the infiltrating ATL cells [6] and detailed dermatological classifications [10] are useful tools for identifying the severity of cutaneous involvement. However, we are also aware that a certain number of ATL patients with aggressive skin manifestations, such as the erythrodermic or purpuric types, are hematologically classified as Shimoyama's smoldering subtype of ATL, which is considered as a type of "indolent" clinical course and is followed-up by watchful-waiting [2]. Therefore, for patients with smoldering subtype of ATL having cutaneous lesions, it is often difficult to predict the prognosis based on dermatological classification alone. To solve this problem, the measurement of PD-1 expression level in the cutaneous lesion may become a useful quantitative indicator for predicting poor prognosis. Recently, *Masaki et al.* reported that there was a significant correlation between higher HTLV-1 provirus load and higher percentage of PD-1-positive Tax-specific cytotoxic T-cells in both ATL patients and asymptomatic HTLV-1 carriers [23]. Therefore, measuring both HTLV-1 provirus load and PD-1-expression on CD3 + CD4 + CD25 + cells in cutaneous regions or among peripheral blood cells may be one of the follow-up strategies for indolent type of ATL with cutaneous involvement.

The principal limitation of this study was that the sample size was too small to perform multivariate analysis of the effect of PD-1-expression level on patient outcome. Although we introduced one patient of indolent ATL with skin eruption having a high PD-1-expression finally transformed to acute-type ATL, the rest of three patients of indolent ATL with skin eruption having PD-1-high expression had no acute transformation. Therefore, the exact effect of PD-1-expression on acute transformation from indolent ATL is unclear from the present study. More large scale studies are needed to further investigate the impact of PD-1-expression level on patient survival and on diagnostic genome profiling to guide clinical decision-making.

In conclusion, we found that ATL patients with PD-1-positive cutaneous lesions have more advanced dermatological and histopathological patterns than those with PD-1-negative cutaneous lesions. Although most of the ATL patients with cutaneous lesions progress relatively slowly and are treated with phototherapy and topical steroids, as shown in the example of Fig. 6, some patients may suddenly

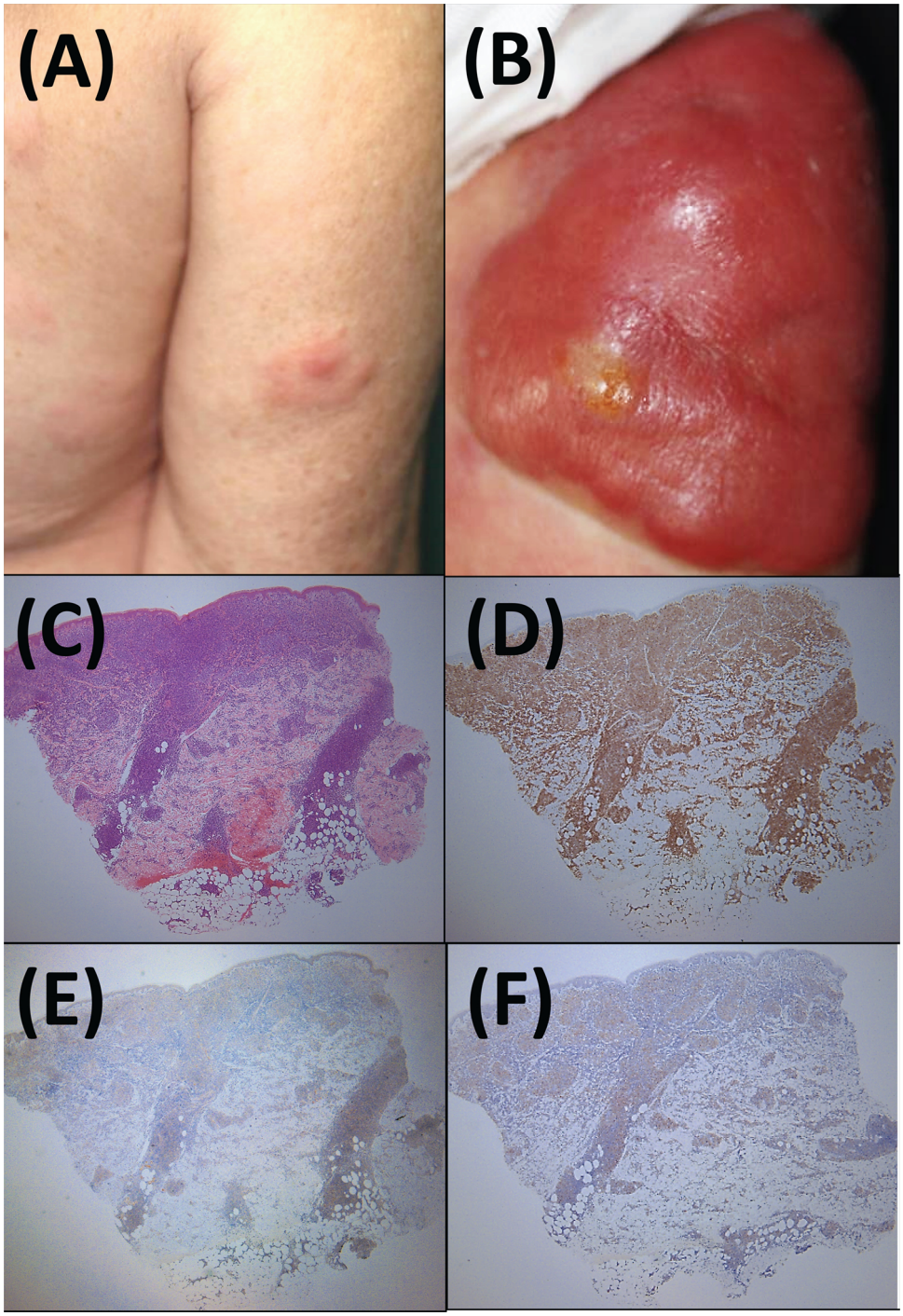


Fig. 6. Dermatological findings of a case of smoldering-type of ATL with a PD-1-positive skin eruption who later transformed to acute-type ATL. (A) Skin eruption: the erythematous plaques were present on the right dorsal upper arm at the first visit to our hospital; (B) Skin eruption: multiple nodules and masses developed all over the body at 30 months after first skin biopsy; (C) Hematoxylin and eosin staining on the first skin biopsy sction: × 20; (D) Immunohistochemical staining on the first skin biopsy sction: CD4 immunostain, × 20, (E) CD25 immunostain, × 20, and (F) PD-1 immunostain, × 20.

transform into acute type of ATL. Therefore, ATL patients with PD-1-positive cutaneous lesions and HTLV-1-infected patients with cutaneous lesions together should be carefully followed by both hematologists and dermatologists.

Authors' contributions

Study conception and design: DN; acquisition of data: MH and YK; data analysis and interpretation: MH and MI; drafting the manuscript: MH, YK, HM, DN and MI.

Declaration of interest

The authors have no competing interests.

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