A Clinical, Pathological and Genetic Characterization of Methotrexate-Associated Lymphoproliferative Disorders

(MTX 関連リンパ増殖性疾患の臨床的、病理学的、遺伝学的特徴の解析)

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Original Article

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Abstract

Objectives Methotrexate–associated lymphoproliferative disorders (MTX-LPD) often regress spontaneously on MTX withdrawal, but the prognostic factors remain unclear. The aim of this study was to clarify the clinical, histological and genetic factors that predict outcomes in patients with MTX-LPD.

Methods Patients with MTX-LPD diagnosed between 2000 and 2012 were analyzed retrospectively regarding their clinical course, site of biopsy, histological typing, Epstein-Barr virus (EBV) in situ hybridization and immunostaining, and human leukocyte antigen (HLA) type.

Results Twenty-one patients, including 20 with rheumatoid arthritis and one with polymyositis, were analyzed. The mean dose of MTX was 6.1 mg/week and the mean duration of treatment was 71.1 months. Clinically, five patients were diagnosed with EBV-positive mucocutaneous ulcer (EBVMCU) and had polymorphic histological findings. The proportion of these patients successfully treated solely by withdrawal of MTX was significantly greater than that of those without EBVMCU (75% versus 7.7%, $P = 0.015$). The HLA-B15:11 haplotype was more frequent in patients with EBV+ RA with MTX-LPD than in healthy Japanese controls ($P = 0.0079$, Bonferroni's method). EBV latency classification and HLA typing were not associated with the prognosis of

MTX-LPD in our cohort.

Conclusions Our data demonstrate that EBVMCU, a specific clinical subgroup of MTX-LPD, had a better clinical outcome when MTX was withdrawn than other patients with MTX-LPD.

Keywords

Rheumatic diseases, Rheumatoid arthritis, Skin manifestations, HLA antigens, Hematopoietic system

Short running footline

MTX-associated lymphoproliferative disorders

Introduction

Methotrexate (MTX)-associated lymphoproliferative disorders (LPD) are a lymphoid proliferation or lymphoma that occur in patients immunosuppressed with MTX, classified as a part of the "other iatrogenic immunodeficiency-associated lymphoproliferative disorders" category by the World Health Organization (WHO) in 2008 (1). As MTX has recently gained acceptance as a first-line therapy for rheumatoid arthritis (RA) (2,3) and other systemic rheumatic diseases (SRD), the incidence of MTX-LPD is expected to increase. A better understanding of this important disease is somewhat limited by its rarity. Epstein-Barr virus (EBV) infection is considered to play an important role in the pathogenesis of MTX-LPD, although EBV can only be detected on histopathologic examination in about half the cases of MTX-LPD (4).

Under normal circumstances, EBV-specific cytotoxic T lymphocytes (EBV-CTLs) act to suppress EBV-infected B cells. However, if the function of EBV-CTLs is impaired by immunosuppressants such as MTX or by aging, EBV-infected B cells are reactivated to induce B-cell proliferation, leading to the development of LPD. There is speculation that MTX could reactivate latent EBV infection, as patients with SRD treated with regimens that include MTX have higher mean EBV loads in their blood than those that are not (5).

EBV-related LPDs (EBV-LPD) can be categorized into three types on the basis of their expression of EBV-encoded small RNA (EBER), EBV latent membrane protein-1 (LMP1), and EBV nuclear antigen-2 (EBNA2): latency I (EBER(+), LMP-1(-), EBNA2(-)) as seen in Burkitt's lymphoma; latency II (EBER(+), LMP-1(+), EBNA-2(-)) as seen in Hodgkin's lymphoma (HL) or nasopharyngeal carcinoma (NPC); and latency III (EBER(+), LMP-1(+), EBNA-2(+)) as seen in post-transplant LPD (PT-LD) (6). EBV-LPD frequently occurs in immunosuppressed patients and its prognosis appears to vary widely.

Recently, LPD with EBV-positive mucocutaneous ulcer (EBVMCU) has been reported as a distinct disease entity with a self-limiting and indolent clinical course (7). EBVMCU is found in various conditions of immunosuppression, including MTX-LPD or in age-related immunosenescence. The latter is characterized by age-related EBV(+) B cell LPDs (Age-LPD) on a background of EBV infection in elderly patients without immunodeficiency (8). Although MTX-LPD often shows spontaneous regression, it is not clear whether MTX-LPD with EBVMCU has a better prognosis. The aim of this study was to clarify the clinical, histological, and genetic factors predictive of a good prognosis in patients with MTX-LPD.

Materials and Methods

Patients

Twenty-one patients with SRD who developed MTX-LPD between 2000 and 2012 were included in this study, consisting of 20 with RA and one with polymyositis (PM). Of the 20 patients with RA, three had RA overlapping with Sjögren's syndrome (SS), one had RA with systemic lupus erythematosus (SLE) and one had RA with polymyalgia rheumatica (PMR). The diagnoses of RA, PM, SS, SLE, and PMR were made according to the American College of Rheumatology classification criteria. The stage of RA was evaluated by Steinbrocker's classification and the stage of LPD by Ann Arbor classification. After the histologic diagnosis of MTX-LPD was made, MTX was withdrawn in all patients. Necessity of chemotherapy was determined according to the histology, karyotypes, stages, or clinical judgment of poor response to MTX withdrawal.

Ethics Statement

The study was conducted in compliance with the Declaration of Helsinki and was approved by the Kyoto University Ethics Committee Review Board; written informed consent was obtained from all patients.

Histological Analysis

Two pathologists performed histological analysis of specimens from each patient. Diagnoses were made in accordance with the criteria specified in the WHO Tumors of Hematopoietic and Lymphoid Tissues, fourth edition (1). Immunostaining of paraffin sections was performed using monoclonal antibodies against LMP1 (Clone CS.1-4, Dako, Glostrup, Denmark) and EBNA2 (M7004, PE2, Dako). The presence of EBER was determined by *in situ* hybridization (ISH) using a peptide nucleic acid (PNA) ISH detection kit (K5201, Dako) and an EBER PNA Probe/Fluorescein kit (Y5200, Dako).

Typing of human leukocyte antigen (HLA)

HLA-A, B and DR typing studies of 16 cases of RA with MTX-LPD and 96 control cases of RA without MTX-LPD diagnosed in our department were undertaken using the polymerase chain reaction (PCR)-Luminex method. The frequency of each HLA allele was analyzed with reference to the Japanese HLA laboratory database (JHD, http://hla.or.jp/haplo/haplonavi.php?type=haplo&lang=ja), which includes over 20,000 cases.

Statistical Analysis

Data are expressed as mean ± standard deviation. Comparisons of HLA and histological data were made using Fisher's exact test. Each allele seen in more than two cases was assessed with significant level corrected P value (Pc) by Bonferroni's method. A Kaplan-Meier plot of the chemotherapy free survival was evaluated by the log-rank test. All analyses were performed with PASW Statistics 18 (18.0.0) and statistical significance was defined as $P < 0.05$.

RESULTS

Clinical and pathological details of patients with MTX-LPD

The clinical, pathological, and genetic characteristics of 21 cases of MTX-LPD are shown in Table 1; 17 (81%) were female; the mean age was 65.8 ± 7.5 years (range 52 to 79 years). The average dose of MTX was 6.1 ± 1.7 mg/week; treatment duration was 71.1 ± 57.8 months. Staging of RA was undertaken using Steinbrocker's classification; one patient fulfilled the criteria for stage I, three for stage II, three for stage III and 13 for stage IV. Three patients (cases 1, 5 and 15) were treated with infliximab. Seventeen out of 20 RA cases (85%) were rheumatoid factor (RF) positive and seven out of 14 cases (50%) were anti-citrullinated protein antibody (ACPA) positive.

The pathological findings were as follows: 10 cases were diagnosed with diffuse large B-cell lymphoma (DLBCL), seven with polymorphic lymphoproliferative disorder (p-LPD), three with HL, and one with small B-cell lymphoma that later transformed into DLBCL. The biopsy site was extra-nodal in 12 cases (57%); all seven cases of p-LPD were extra-nodal, while three cases of HL were nodal. Of the 10 cases of DLBCL, half were extra-nodal and half nodal. Twelve out of 20 cases (60%) were EBER-positive, while eight

cases of 19 (42%) were LMP1-positive and three cases out of 19 (16%) were EBNA2-positive. EBV latency was classified by means of EBER, LMP1 and EBNA2 expression into four groups: EBV-negative and latencies I-III. Representative histological images are shown in Figure 1. The cases diagnosed as p-LPD comprised seven of the 12 patients in latency I-III groups; but there was none among the eight patients in the EBV-negative group (P = 0.012). Extra-nodal involvement was found in eight of 12 patients in the latency I-III groups but only three of eight in the EBV-negative group. EBVMCU was diagnosed in five cases (cases 1, 2, 4, 5 and 21), each characterized by sharply demarcated skin ulcers with an erythematous appearance accompanied by crusting and necrosis (Fig. 2), which, on histological examination, were found to be polymorphic with a mixture of lymphocytes and immunoblasts. Lymphocytic vasculitis was seen in three out of five cases (cases 2, 5, and 21). Four out of five (80%) cases of EBVMCU were seropositive.

HLA typing of patients with MTX-LPD

As shown in Table 2, we found that three cases out of 16 were heterozygous for the HLA-B15:11 allele. The allele frequency of HLA-B15:11 was higher in EBV(+) RA with MTX-LPD, compared with the control JHD group and that of the Japanese RA cohort (Pc = 0.0079 and 0.024, respectively, Table 3). Rheumatoid arthritis-shared epitopes were

observed in 11 of 16 cases (69%), a significantly higher proportion than in healthy Japanese controls (38%, n = 1508) (P = 0.018), but not in Japanese patients with RA (n = 759) (9).

Clinical course of patients with MTX-LPD

MTX was withdrawn in all cases at the time of LPD diagnosis. When examining the need for chemotherapy within 18 months of diagnosis, withdrawal of MTX alone was more successful for those in the EBVMCU group (n = 4, cases 1, 4, 5, and 21) than the other cases (n = 13) (75% *versus* 7.7%, P = 0.015, Fig. 3). As the observation periods were at most 2 years in the majority of cases, we were unable to calculate long-term prognosis. Five patients (cases 2, 7, 8, 9, and 12) died; LPD recurred in two patients at 90 months (case 9) and 21 months (case 12) from the original diagnosis and did not respond to continued chemotherapy. One patient (case 2) died from intercurrent myelitis and sepsis soon after the diagnosis of LPD and one patient (case 7) died from bleomycin-induced pneumonia.

One patient (case 8) was first diagnosed with EBV-positive small B-cell lymphoma (SBL). Although chemotherapy resulted in partial remission, she had an indolent clinical course without chemotherapy until the tumor progressed and histopathological study revealed

EBV-negative DLBCL with the same phenotype as the SBL. A clonal relationship between the two lymphomas was not proven, however, Richter syndrome was suspected clinically. With regard to the predicted unfavorable prognosis, she was treated with autologous peripheral blood stem cell transplantation, but she died of *Pneumocystis* pneumonia under continuous immunosuppression. Although two cases of EBVMCU (cases 2 and 5) were included in Ann Arbor stage IV, each had a favorable clinical outcome.

DISCUSSION

We have shown that the presence of EBVMCU appears to confer a better prognosis in patients with MTX-LPD. Most of our patients with positive outcomes had been diagnosed with EBVMCU. Of the 12 cases of MTX-LPD with mucocutaneous ulcer reported in the literature (7, 10-17) all except one (17) were EBV-positive and all nine cases with available data showed complete remission without chemotherapy.

EBVMCU was first reported to be a favorable prognostic indicator in a case series of 26 patients (consisting of 19 with Age-LPD and seven with iatrogenic immunodeficiency-associated LPD including four with MTX-LPD) (7), but the incidence of EBVMCU in MTX-LPD was not known. Our study shows that the incidence of EBVMCU in EBV(+) MTX-LPD is 42% (five out of 12), which is higher than that of EBVMCU in Age-LPD (13%, 16 cases out of 122) (18).

Age-LPD is believed to be a consequence of an underlying immunological deficit, or immunosenescence of the T cell receptor (TCR) repertoire (19, 20)—a natural degeneration of the immune system that occurs with aging. Considering the high average

age of our cases, age-related immunosenescence might be partly involved in the development of MTX-LPD. This may be revealed by decreases in the TCR repertoire in the future.

Only a few cases of EBV latency in MTX-LPD have been reported; those of EBV latency among MTX-LPD, PT-LD, and Age-LPD are summarized in Table 4 (21-23). Other case series include 53 cases of LPD in a variety of autoimmune diseases, including four cases of MTX-LPD, in which all 16 cases of EBV(+) LPD were in latency II (24). Taken together, these data suggest that EBV(+) MTX-LPD is more likely to be in latency II, followed by latency III. This observation also appears to hold true for PT-LD and Age-LPD. PT-LD has been generally categorized as latency III (25), but are often seen to be in latency II. Cases of Age-LPD were also found to be mainly in latency II. Nonetheless, the latency classification had no value in predicting the prognosis of MTX-LPD in our cohort. The utility of EBV latency in MTX-LPD remains unknown.

Although the pathogenesis of EBVMCU is unclear, lymphocytic vasculitis was observed in more than half our cases of EBVMCU. In EBV-positive LPDs, monokine induced by interferon-γ (Mig) and interferon-γ inducible protein-10 (IP-10)—mainly produced by

reactive cells including endothelial cells—are thought to be powerful instigators of vascular and tissue injuries (26). Thus, we hypothesize that tissue necrosis and the impairment of local blood flow to the area of vascular damage might be pivotal to the pathogenesis of EBVMCU. Although it is unclear why patients with MTX-LPD who developed EBVMCU had a better prognosis, several factors might be responsible. One possibility is that the mucocutaneous ulcer is so conspicuous that patients seek medical help more promptly, when diagnosis is relatively straightforward and the disease is in an earlier, potentially reversible stage. Thus, the prompt cessation of MTX may lead to a good outcome.

Our study is the first to have conducted HLA typing, revealing that HLA-B15:11 could be a risk allele in EBV(+) RA with MTX-LPD. Notably, all three HLA-B15:11-positive cases were EBV-positive and two were polymorphic, indicating that this allele may be linked to the susceptibility to EBV infection and development of LPD. In our study, HLA-B15:11 had no correlation with HLA-shared epitopes, RF or ACPA. To the best of our knowledge, there have been no reports of any relationship between this allele and other diseases. The identification of a genetic risk factor could help to clarify the pathogenesis of LPD and to achieve safer therapy for patients who might be at risk of LPD if their RA

is treated with MTX. As this is a retrospective study, there are some limitations, and a larger scale prospective trial is needed to clarify the pathogenesis of this disease.

In conclusion, we have demonstrated that cases of EBVMCU, a subgroup of MTX-LPD, were all histologically polymorphic and had a more favorable outcome by withdrawing MTX alone. In addition, we found that the frequency of the HLA-B15:11 allele was significantly increased in our cohort, which suggests that it may be a risk factor for EBV(+) RA with MTX-LPD.

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Authors' contributions

N. Yamakawa, M. Fujimoto, C. Terao, M. Nishikori and D. Kawabata were responsible for the study design, acquisition, analysis, interpretation of data and manuscript preparation. R. Nakashima, Y. Imura, N. Yukawa, H. Yoshifuji, K. Ohmura, T. Fujii, T. Kitano, T. Kondo, A. Takaori-Kondo and T. Mimori participated in the clinical investigation and assisted in interpretation of data. M. Fujimoto and H. Haga contributed to the pathological evaluations. K. Yurugi, Y. Miura, T. Maekawa and F. Matsuda undertook DNA purification and preservation and HLA analysis. All authors read and approved the manuscript before publication.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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Figure Legends

Figure 1. Pathological findings in three cases of MTX-LPD classified by EBV latency. Each case was diagnosed as DLBCL (case 10, A-D, latency I), polymorphic LPD (case 4, E-H, latency II) and polymorphic LPD (case 3, I-L, latency III). Pathological findings are shown by HE (A, E, I), EBER (B, F, J), LMP1 (C, G, K) and EBNA2 (D, H, L) staining. For abbreviations see text.

Figure 2. Two representative cases with skin manifestations of EBV-positive mucocutaneous ulcer (EBVMCU). Typical skin manifestations of EBVMCU (A: case 5, B: case 2). Characteristic sharply demarcated skin ulcers with an erythematous appearance accompanied by crusting and necrosis can be seen.

Figure 3. Chemotherapy free survival. Kaplan-Meier curve showing chemotherapy free survival within 18 months, comparing patients in the EBV-positive mucocutaneous ulcer (EBVMCU) group with those with other forms of MTX-LPD. MTX was withdrawn in all cases. *Log-rank test, $P < 0.05$.

Fig.1

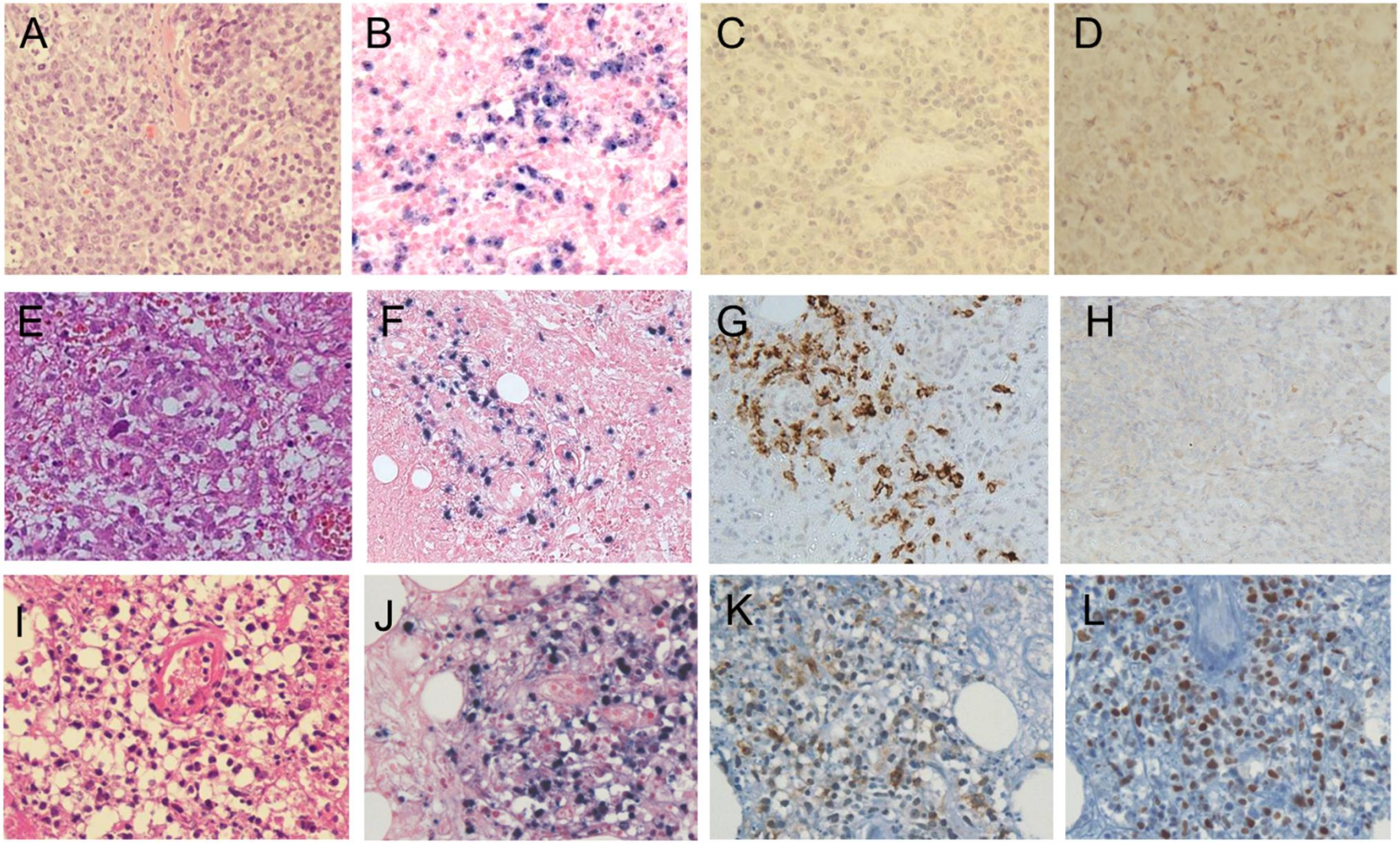
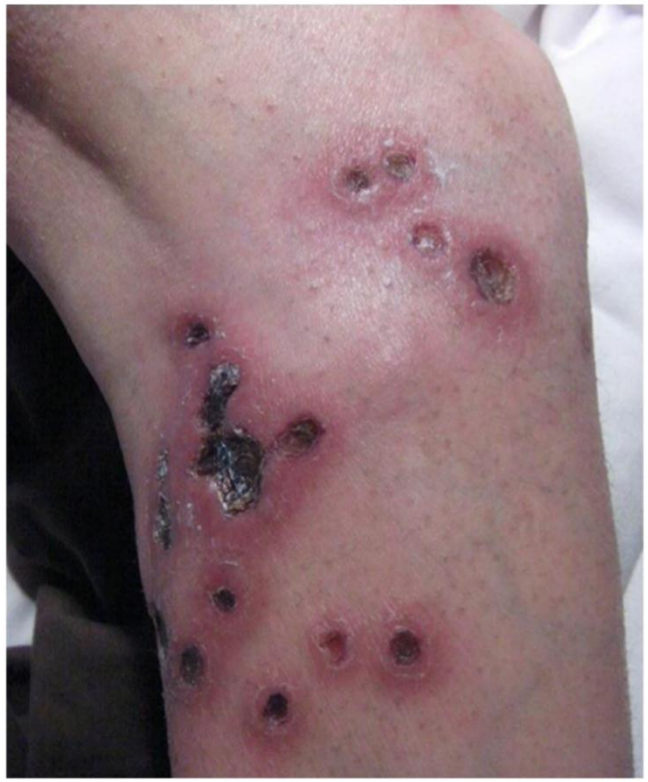


Fig.2

A



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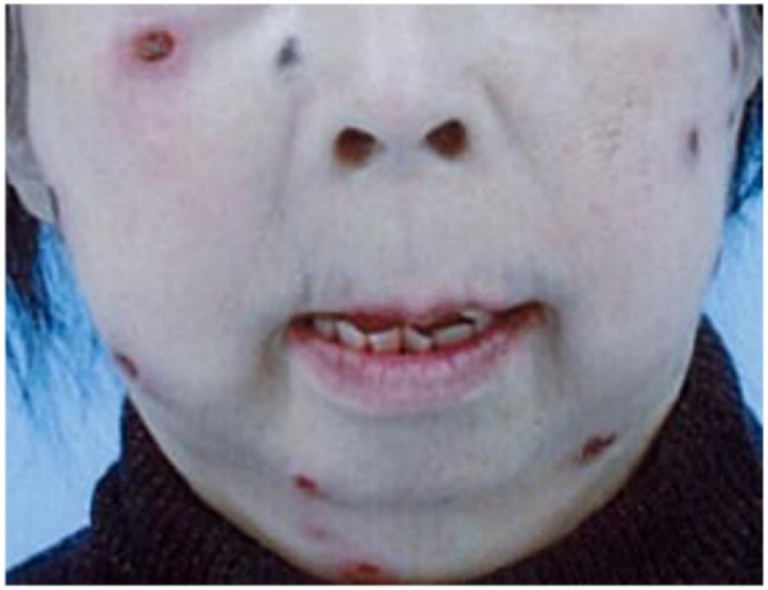

Fig.3

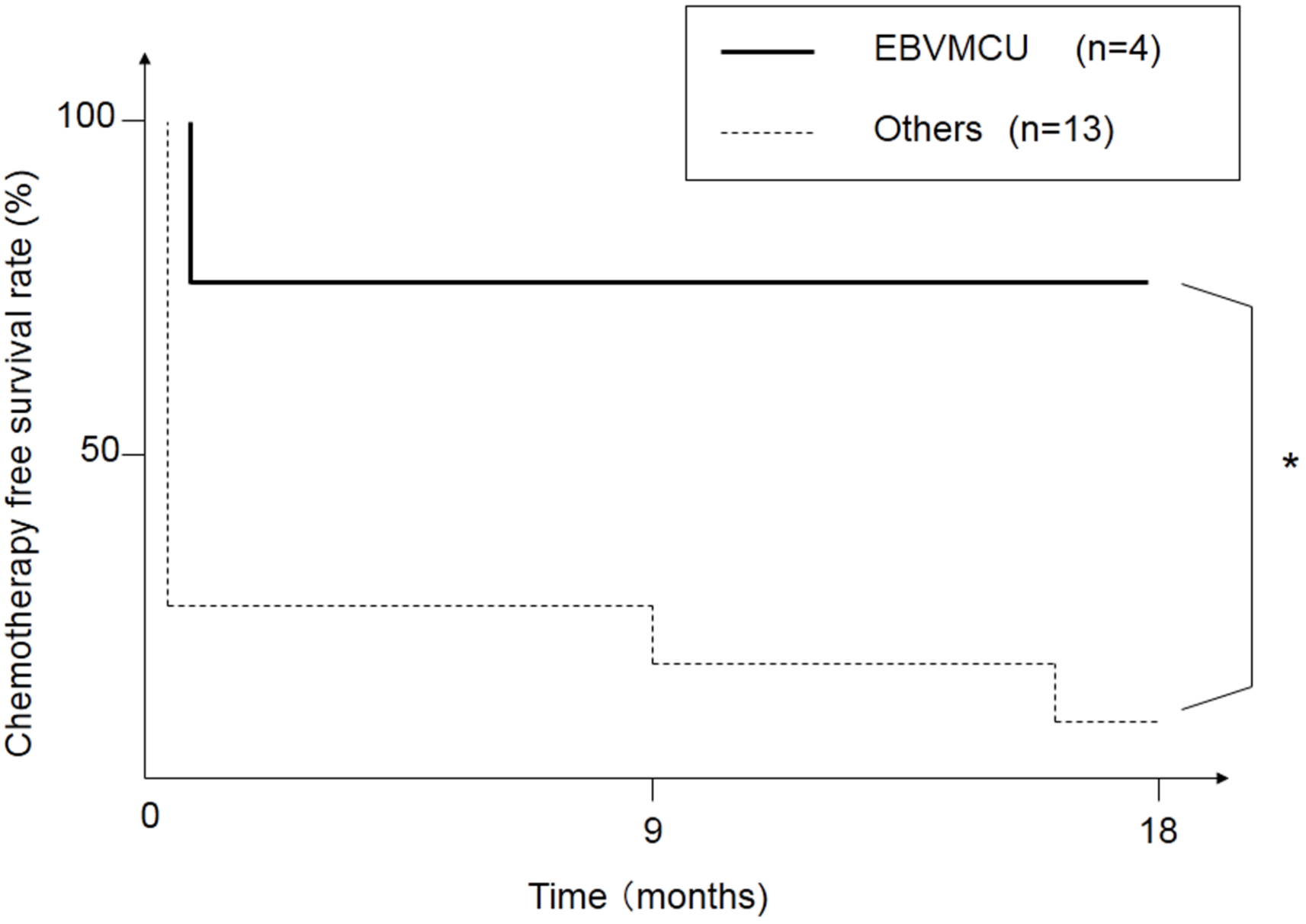
EBVMCU (n=4)
Others (n=13)
100
50
Chemotherapy free survival rate (%)
*
0
9
18
Time（months）

Table 1 Clinical and pathological findings in 21 cases of MTX-LPD

No.	Age	Gender	Disease	Dose of MTX (mg/week)	Duration of MTX(month)	E/N	Site of biopsy	Histological type	EBER	LMP1	EBNA2	EBV Latency	LPD Stage	IPI	Therapy and Response	Response (final state)	Prognosis	Follow up period (month)
1	76	F	RA	8.0	27	E(MCU)	left eyelid	p-LPD	(+)	(+)	(+)	III	I	L	W → CR	CR	A	24
2	64	F	RA	8.0	120	E(MCU)	buccal mucosa	p-LPD	(+)	(+)	(+)	III	IV	HI	W	N.D.	D#1	1
3	71	F	RA	8.0	184	E	left lateroabdominal nodule	p-LPD	(+)	(+)	(+)	III	IV	H	R-CHOP → PR→ oral VP-16 → CR	CR	A	31
4	59	F	RA	5.0	63	E(MCU)	left eyelid	p-LPD	(+)	(+)	(-)	II	I	H	W → CR	CR	A	37
5	61	F	RA	10.0	74	E(MCU)	right lower leg ulcer	p-LPD	(+)	(+)	(-)	II	IV	H	R-CHOP → CR	CR	A	25
6	65	F	RA SS	4.0	27	N	left axillary lymph node	HL	(+)	(+)	(-)	II	II	LI	W → CR → relapse → ABVD, C-MOPP → PR → RT → CR	CR	A	79
7	65	F	RA	5.0	66	N	right axillary lymph node	HL	(+)	(+)	(-)	II	IV	HI	W → CR → relapse → ABVD → CR	CR	D#2	17
8	58	F	RA	4.0	66	N	inguinal lymph node	SBL	(+)	(+)	(-)	II	II	L	CHOP, RT → CR → relapse → observe → R-ICE, CHASER, auto-PBSCT (MEAM) → CR	CR	D#3	92
9	67	F	RA SS	6.0	16	E	parotid gland	DLBCL	(+)	(-)	(-)	I	III	H	R-CHOP → CR → relapse → R-DeVIC → PR → RT, CHASER, R-ESHAP, mini-MEAM, GEM, CPT-11 → PD	PD	D	90
10	61	F	RA	6.0	193	N	right cervical lymph node	DLBCL	(+)	(-)	(-)	I	III	LI	R-CHOP → CR	CR	A	45
11	79	M	RA	8.0	169	E	right forearm	p-LPD	(+)	(-)	(-)	I	I	LI	W → CR	CR	A	9
12	66	M	RA	6.0	46	N	left submandibular lymph node	DLBCL	(-)	(-)	(-)	n	IV	HI	R → CR → relapse → CHOP → PD	PD	D	27
13	72	F	RA SLE	6.0	N.D.	N	right submandibular lymph node	DLBCL	(-)	(-)	(-)	n	II	LI	R-CHOP → CR → relapse → R-DeVIC	PD	A	48
14	74	F	RA	7.0	74	N	right cervical lymph node	DLBCL	(-)	(-)	(-)	n	II	LI	W → CR	CR	A	32
15	52	F	RA SS	5.0	47	E	precordial skin	DLBCL	(-)	(-)	(-)	n	IV	HI	R-CHOP → CR	CR	A	84
16	76	M	RA	4.0	N.D.	N	right axillary lymph node	HL	(-)	(-)	(-)	n	III	HI	W	N.D.	A	1
17	71	M	RA PMR	5.0	7	N	right cervical lymph node	DLBCL	(-)	(-)	(-)	n	II	HI	R-CHOP → PR	PR	A	8
18	62	F	RA	8.0	21	E	right orbital fossa	DLBCL	(-)	(−)	(−)	n	II	LI	R-CHOP → CR	CR	A	15
19	56	F	RA	5.5	98	E	left submandibular skin	DLBCL	(-)	(−)	(−)	n	II	L	W → CR	CR	A	14
20	71	F	RA	6.0	48	E	lumbar mass	DLBCL	N.E.	N.E.	N.E.	N.D.	II	LI	R-CHOP, RT → CR	CR	A	132
21	56	F	PM	4.0	4	E(MCU)	left lower leg ulcer	p-LPD	(+)	N.E.	N.E.	N.D.	I	L	W → CR	CR	A	33

PM=polymyositis, RA=rheumatoid arthritis, SS=Sjögren syndrome, SLE=systemic lupus erythematosus, PMR=polymyalgia rheumatica

E=extranodal, MCU=mucocutaneous ulcer lesion, N=nodal, p-LPD=polymorphic lymphoproliferative disorder, SBL=small B-cell lymphoma, DLBCL=diffuse large B-cell lymphoma, HL=Hodgkin lymphoma

n=EBV-negative, N.E.=not examined, N.D.=not determined, IPI=international prognostic index, L=low risk, LI=low-intermediate risk, HI=high-intermediate risk, H=high risk

W=withdrawal of MTX only, R-CHOP=rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine and prednisolone, ABVD=adriamycin, bleomycin, vinblastine and dacarbazine

C-MOPP=cyclophosphamide, vincristine, prednisolone and procarbazine, RT=radiotherapy, R-ICE=rituximab, ifosfamide, carboplatin and etoposide, auto-PBSCT=autologous peripheral blood stem cell transplantation

MEAM=ranimustine (MCNU), etoposide, cytarabine and melphalan, R-DeVIC=rituximab, dexamethasone, etoposide, ifosfamide and carboplatin, CHASER=cyclophosphamide, cytarabine, etoposide, dexamethasone and rituximab

R-ESHAP=rituximab, etoposide, prednisolone, high-dose cytarabine and cisplatin, GEM=gemcitabine, CPT-11=irinotecan

CR=complete remission, PD=progressive disease, PR=partial remission, A=alive, D-dead

#1: died soon from intercurrent disease (myelitis and sepsis), #2: died from bleomycin-induced interstitial pneumonia, #3: died from pneumocystis pneumonia

Table 2 HLA-A, B and DR alleles of 16 cases of RA with MTX-LPD

No.	**HLA-A**	**HLA-B**	**HLA-DR**
1	11:01/24:02	35:01/48:01	04:05*/09:01
3	02:01/24:02	**15:11**/40:02	09:01/14:02*
4	24:02/26:01	15:07/40:02	04:03/09:01
5	24:02	07:02/52:01	01:01*/15:02
6	02:06/26:01	**15:11**/39:01	04:10*/14:06*
7	02:01/26:01	35:01/55:02	04:05*/04:06
9	24:02	07:02/54:01	04:05*/08:02
10	11:01	48:01/54:01	04:05*/15:01
11	02:01/02:07	**15:11**/46:01	09:01
12	24:02/26:01	40:02/52:01	04:05*/09:01
13	02:01/24:02	40:01/52:01	04:05*/15:02
14	24:02/33:03	44:03/52:01	08:03/15:02
15	24:02	52:01	15:02
18	02:01	07:02/15:01	01:01*/15:01
19	02:01/02:06	48:01/54:01	04:05*/04:07
20	24:02	51:01/59:01	01:01*/04:05*

* Rheumatoid Arthritis Shared Epitope

Table 3 Risk allele of RA with MTX-LPD in Japanese population

	Total allele numbers	(+)	(-)	Frequency of allele in control cases	*P* value*	*Pc* value**	Odds ratio	95% Confidence Interval
HLA-B15:11								
in RA with MTX-LPD (n=16)	32	3	29	0.0096[#1] 0.0052[#2]	0.0036 0.0097	0.061 0.16	10.0 18.5	3.0-32.8 1.9-183.4
in RA with MTX-LPD without EBVMCU (n=13)	26	3	23	0.0096[#1] 0.0052[#2]	0.0020 0.0056	0.032 0.090	13.4 24.9	4.0-45.0 2.5-249.5
in EBV+ RA with MTX-LPD (n=9)	18	3	15	0.0096[#1] 0.0052[#2]	0.00066 0.0020	0.0079 0.024	18.2 38.2	5.3-62.3 3.7-390.0

#1 Japanese healthy control, #2 rheumatoid arthritis control, *2 × 2 Fisher's exact study, **corrected with Bonferroni's method

Table 4 EBV Latency classification among MTX-LPD, PT-LD and Age-LPD

Disease	year	reporter	cases	I	II	III
PT-LD	2003	Birkeland	16	1	8	7
Age-LPD	2009	Asano	26	0	19	7
MTX-LPD	2007	Miyazaki	3	0	1	2
MTX-LPD	**2013**	**present case series**	**11**	**3**	**5**	**3**