Lymphoproliferative Disorders in Rheumatoid Arthritis: Clinicopathological Analysis of 76 Cases in Relation to Methotrexate Medication

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ABSTRACT. ***Objective.*** Individuals with rheumatoid arthritis (RA) with or without methotrexate (MTX) medication occasionally develop lymphoproliferative disorders (MTX-LPD and non-MTX-LPD, respectively). The hyperimmune state of RA itself or the immunosuppressive state induced by MTX administration might contribute to development of LPD. Our objective was to characterize MTX-LPD in comparison to non-MTX-LPD and sporadic LPD in patients with RA.

Methods. We compared MTX-LPD to non-MTX-LPD and sporadic LPD by evaluating 48 cases of MTX-LPD, 28 non-MTX-LPD, and 150 sporadic LPD.

Results. Later onset age of LPD and female predominance were evident in patients with RA-LPD compared to sporadic LPD. The interval between the diagnosis of RA and LPD in MTX-LPD (median 132 mo) was significantly shorter than that in non-MTX-LPD (240 mo). The frequency of diffuse large B cell lymphoma (DLBCL) and positive rate of Epstein-Barr virus (EBV) in RA-LPD was significantly higher than in sporadic LPD (57.9% vs 42.7%, 27.6% vs 9.9%, respectively). After withdrawal of MTX, 11 of the MTX-LPD cases showed a spontaneous regression of tumors. The 5-year survival rate in RA-LPD (59.2%) was significantly worse than that in sporadic LPD (74.6%).

Conclusion. The majority of cases of RA-LPD show similar clinicopathological characteristics irrespective of MTX medication, except for spontaneous regression of LPD after withdrawal of MTX in MTX-LPD, and a shorter interval between the diagnosis of RA and LPD in MTX-LPD than in non-MTX-LPD. RA-LPD cases showed younger age of onset, female predominance, unfavorable prognosis, and higher frequencies of DLBCL and EBV positivity compared to sporadic LPD. (First Release Nov 15 2006; J Rheumatol 2007;34:322–31)

Key Indexing Terms:
RHEUMATOID ARTHRITIS METHOTREXATE LYMPHOPROLIFERATIVE DISORDERS
EPSTEIN-BARR VIRUS HYPERIMMUNE STATE IMMUNOSUPPRESSIVE STATE

Lymphoproliferative disorders (LPD) occasionally develop in individuals with immune deficiencies. These diseases are categorized as immunodeficiency-associated LPD in the recent World Health Organization (WHO) classification for lymphoid neoplasms[1]. Immunosuppressive conditions prior to LPD are categorized into primary immune disorders; human immunodeficiency virus infection; iatrogenic immunosuppression in patients receiving solid organ or bone marrow allografts, i.e., post-transplant LPD; and iatrogenic immunosuppression associated with methotrexate (MTX) administration[1].

MTX is administered to patients with autoimmune diseases, especially rheumatoid arthritis (RA), to suppress the hyperimmune state. This in turn may induce immunosuppression and provide a basis for the development of LPD[2,3]. Since Ellman's first report on lymphoma in a patient with RA who received low-dose MTX[4], a relationship of MTX medication and development of LPD has been discussed[5-11]. Regression of LPD after withdrawal of MTX medication is regarded to be strong evidence for the carcinogenic potential of MTX. On the other hand, patients with RA develop LPD at a frequency 2.0 to 5.5 times higher than the general population[12-14]. These findings raise the question whether the hyperimmune state in RA itself or the immunosuppressive state induced by MTX administration for con-

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trol of RA contributes to the development of LPD. Indeed, Starkebaum has put forward the proposition that whether and how MTX might influence lymphoma development in RA has not been clearly established[15]. Chen and Cronstein suggested that the mechanism of action of MTX might not be one of immunosuppression, but rather an antiinflammatory action[16].

We wished to characterize the development of LPD in patients with RA who were taking MTX (MTX-LPD) or were not (non-MTX-LPD); data on 76 cases of LPD in Japanese patients with RA were collected through a nationwide study. Clinicopathologic features in MTX-LPD and non-MTX-LPD patients were summarized and compared with those in patients with sporadic LPD and post-transplant LPD, one of the immunodeficiency-associated LPD.

MATERIALS AND METHODS

A total of 76 patients with RA in whom LPD developed were selected for study: 28 through a review of Japanese journals, 9 through the *Annual of Pathological Autopsy Cases in Japan (1978-97)* (Japanese Society of Pathology, Tokyo, Japan), 29 from consultation case files of the Department of Pathology, Osaka University, and 10 from case files of the Saitama Medical School. The diagnosis of RA was made according to the American College of Rheumatology (ACR) classification criteria[17,18]. All but 9 autopsy cases were admitted to hospitals during the period 1997–2005. The ACR criteria for RA diagnosis were applied when the patients visited the participating hospitals.

Forty-eight patients had been receiving MTX treatment for RA at the onset of LPD (MTX-LPD). Twenty-eight patients received nonsteroidal antiinflammatory drugs, but not MTX (non-MTX-LPD). Patients of the non-MTX-LPD group never received MTX treatment for RA. Twenty-five patients in the non-MTX group had received prednisolone, bucillamine, sodium aurothiomalate, D-penicillamine, salazosulfapyridine, and cyclophosphamide for the treatment of RA. Clinicopathologic findings in 22 of these cases have been described[19]. For comparison, 150 consecutive cases with sporadic LPD admitted to the Osaka University Hospital during 1997-2005 were analyzed. They included 111 cases of B cell LPD, 16 T cell, 6 natural killer (NK)/T cell LPD, and 16 cases of Hodgkin's lymphoma. The immunophenotype in one case could not be determined. Among the cases of B cell lymphoma, 64 cases were diffuse large B cell lymphoma (DLBCL) and 23 follicular lymphoma.

Histologic specimens obtained by biopsy were fixed in 10% formalin and routinely processed for paraffin-embedding. Histologic sections cut at 4 μm thickness were stained with hematoxylin and eosin and immunoperoxidase procedures. All the histologic sections were reviewed by 2 authors (YH and KA), and classified according to the WHO criteria[1]. Adequate clinical information was available in all cases.

The study was approved by the institutional review board of Osaka University Medical School.

Immunohistochemical staining. Immunoperoxidase procedures (ABC method) were carried out in all cases. The primary antibodies used in the study and dilutions were as follows: CD3€ (1:100; Dakopatts, Glostrup, Denmark), UCHL-1 (CD45RO; 1:100; Dakopatts), L-26 (CD20; 1:200; Dakopatts), JCB117 (CD79a; 1:100; Dakopatts), 123C3 (CD56; 1:40; Zymed, South San Francisco, CA, USA), ZH7 (CD16; 1:200; Novocastra, Newcastle, UK), TIA-1 (1:500; Coulter, Hialeah, FL, USA), and Ber-H2 (1:10; Dakopatts). Before incubation, sections were pretreated with 1% trypsin in Tris buffer (pH 7.8) for CD3€, and heated for 5 min in citrate buffer (pH 6.0) for CD16 and CD56, in a microwave oven.

DNA extraction. DNA was extracted from paraffin-embedded sections using chelating resin (Sigma, St. Louis, MO, USA). Briefly, 5 sections of 5 μm thickness were cut from paraffin blocks, then suspended in 40% resin, boiled for 10 min, and centrifuged for 5 min at 18,000 g. One to 10 microliters of the supernatant was used for each polymerase chain reaction (PCR) analysis. The preservation of DNA was confirmed by PCR amplification with primers specific for a 205 bp segment of the *ß-globin* gene (5'-GGT TGG CCA ATC TAC TCC CAG G-3' and 5'-CAA CTT CAT CCA CGT TCA CC-3').

Detection of human T-lymphotropic virus-1 (HTLV-1) genome. For detection of HTLV-1 proviral DNA, 40 cycles of 94°C/58°C/72°C were used with *tax* primers for a 159 bp segment common to the HTLV-1 and HTLV-2 proviral genome (5'-CGG ATA CCC AGT CTA CGT GT-3' and 5'-GAG CCG ATA ACG CGT CCA TCG -3') and *pol* primers for a 119 bp segment specific to the HTLV-1 proviral genome (5'-CTT CAC AGT CTC TAC TTG TGC-3' and 5'-CGG CAG TTC TGT GAC AGG G-3')[20].

In situ hybridization for Epstein-Barr virus (EBV). EBV RNA *in situ* hybridization was performed in all RA-LPD cases and 111 cases of sporadic LPD as described[21]. The Raji cell line was used as a positive control. As negative controls, the hybridizing mixture was tested with sense probe and antisense probe after RNase treatment (Sigma).

Statistical analysis. To characterize RA-LPD in comparison with sporadic LPD, multivariate analysis was performed by logistic regression analysis[22]. Factors examined were age at the diagnosis of LPD, sex, histology, and EBV positivity of the LPD. The followup period for survivors with RA-LPD and sporadic LPD calculated from the date of diagnosis of LPD ranged from 1 to 288 (median 25) months. Actual survival curves were calculated by the Kaplan-Meier method[23], and differences were examined by the log-rank test to detect significant prognostic factors[24]. Factors examined were age, sex, primary site of LPD lesion, stage of disease, type of histology, immunophenotype of proliferating cells, EBV positivity, and medication for RA. Multivariate analysis was carried out with the Cox proportional-hazards model to identify independent prognostic factors[25]. The 5-year survival rate in sporadic LPD and RA-LPD was adjusted for sex and age. Age adjustment was conducted using categorized dummy variables of < 40, 40–59, and ≥ 60 years. Differences of EBV positivity in the following groups were examined: RA-LPD vs sporadic LPD, sex, stage of disease, histology, and MTX-LPD with or without spontaneous regression after withdrawal of MTX. The Mann-Whitney U-test was used to evaluate the differences in age at the time of development of LPD between patients with and those without RA, and the interval from the onset of RA to development of LPD between patients with and without MTX medication among the patients with RA. A p value of 0.05 was considered significant.

RESULTS

Clinical findings. Clinical features in 76 cases with MTX-LPD and non-MTX-LPD, together with 150 cases of sporadic LPD, are summarized in Table 1. One patient with RA-LPD simultaneously had Hashimoto's thyroiditis, and 4 with RA-LPD had Sjögren's disease. Forty-eight patients had been treated with low-dose MTX (2.5 to 17 mg/wk) until the diagnosis of LPD (MTX-LPD) for periods of 2 to 131 (median 54) months: cumulative doses of the MTX ranged from 24 to 4785 (median 940) mg. One of these patients simultaneously received anti-tumor necrosis factor-α therapy 9 months before the onset of LPD. Details of the clinical and histological information in this case were reported recently[26]. Age at the diagnosis of RA ranged from 3 to 84 (median 51) years. The ages of patients at the diagnosis of RA-LPD and sporadic LPD ranged from 23 to 87 (median 66) years, with a male:female ratio of 21:54, and from 17 to 87 (median 58) years, ratio 93:57, respectively.

Table 1. Clinical findings in MTX-LPD and non-MTX-LPD.

	MTX-LPD	Non-MTX-LPD	RA-LPD	Sporadic LPD
No. of cases	48	28	76	150
Age, yrs				
Median (range)	67 (34–87)	66 (23–77)	66 (23–87)*	58 (17–87)*
Sex				
Male	15	6	21*	93
Female	33	22	55*	57
Interval between onset of RA and LPD, mo				
Median (range)	132 (3–360)*	240 (21–660)*	144 (3–660)	
MTX administration				
Total dose, mg, median (range)	940 (24–4785)			
Duration, mo, median (range)	54 (2–131)			
Immunophenotype				
B cell	38	22	60	111
T cell	3	4	7	16
Natural killer cell	1	0	1	6
Hodgkin's lymphoma	6	2	8	16
Primary site				
Nodal	22	12	34	76
Extranodal	23	12	35	63
Undetermined	3	4	7	11
Stage				
I	10	7	17*	32*
II	7	3	10*	36*
III	15	7	22*	16*
IV	14	8	22*	55*
Undetermined	2	3	5	11
5 yr survival rate, %	58.9	52.8	59.2*	74.6*

* $p < 0.05$. MTX: methotrexate, RA: rheumatoid arthritis, LPD: lymphoproliferative disorder. * $p < 0.05$.

Later-onset age of LPD and female predominance were evident in the patients with RA-LPD ($p < 0.01$ and $p < 0.05$, respectively). The interval between the diagnosis of RA and MTX-LPD (median 132 mo) was significantly shorter than that in non-MTX-LPD (240 mo) ($p < 0.05$). Presenting symptoms included superficial lymphadenopathy (in 33.3%), followed by extranodal mass (27.0%), and much less frequently abdominal pain, bone pain, fever, cough, and thrombocytopenia.

There were no significant differences in the primary sites and distribution of clinical stage between RA-LPD and sporadic LPD. The primary site of RA-LPD was nodal in 34 and extranodal in 35 patients; epipharynx and lung in 4 each; thyroid gland and skin in 3 each; chest wall, spleen, urinary tract, bone, terminal ileum, and bone marrow in 2 each; and mediastinum, gingiva, parotid gland, stomach, brain, tongue, and testis in one each. The remaining 7 patients presented with advanced disease, therefore the primary sites could not be determined. Based on the records of physical examinations, surgical notes, and pathological examinations of the specimens, the Ann Arbor staging system was applied in all patients, with the following results: stage I in 17 patients, stage II in 10, stage III in 22, stage IV in 22, and undetermined in 5 patients. As for 44 patients with DLBCL, 18 presented with localized disease, 21 with advanced disease, and 5 were undetermined.

Histological findings. There were no prominent differences in the histologic findings between MTX-LPD and non-MTX-LPD (Table 2). There were no prominent differences in the distribution of immunophenotype between sporadic LPD and RA-LPD, and between MTX-LPD and non-MTX-LPD. Fifty-nine RA-LPD cases were B cell-type, 7 were T cell-type, one was NK/T cell-type, and 8 were Hodgkin's lymphoma. The frequency of DLBCL in RA-LPD (57.9%) was significantly higher than that in sporadic LPD (42.7%) ($p < 0.05$). Two cases of MTX-LPD and one case of non-MTX-LPD showed a polymorphous appearance consisting of the full range of mature B cells from immunoblasts to plasma cells, small and medium-size lymphocytes, and numerous cells with centrocytic and centroblastic appearance, and they were thus categorized as the diffuse polymorphic type (Figure 1). Another case of MTX-LPD also showed a polymorphous B cell proliferation containing occasional Reed-Sternberg-like cells, and was diagnosed as Hodgkin's lymphoma-like LPD. T cell LPD included peripheral T cell lymphoma, unspecified, and angioimmunoblastic T cell lymphoma.

There was one case of NK/T cell lymphoma in the nasal

Table 2. Histologic classification of MTX-LPD and non-MTX-LPD.

	MTX-LPD (%)	Non-MTX-LPD (%)	RA (%)	Sporadic LPD (%)
Total	48	28	76	150
B cell LPD	38 (79.2)	22 (78.6)	60 (78.9)	111 (74.0)
DLBCL	29 (60.4)	15 (53.6)	44 (57.9)	64 (42.7)
Follicular lymphoma	3	2	5	23
Lymphoplasmacytic	2	0	2	4
Plasmacytoma	0	2	2	1
Mantle cell lymphoma	0	1	1	3
Diffuse polymorphic	2	1	3	0
HL-like LPD	1	0	1	0
Others	1	1	2	16
T cell LPD	3 (6.3)	4 (14.3)	7 (9.2)	16 (10.7)
Natural killer/T cell LPD	1 (2.1)	0 (0)	1 (1.3)	6 (4.0)
HL	6 (12.5)	2 (7.1)	8 (10.5)	16 (10.7)

MTX: methotrexate, DLBCL: diffuse large B cell lymphoma, HL: Hodgkin's lymphoma. * $p < 0.05$.

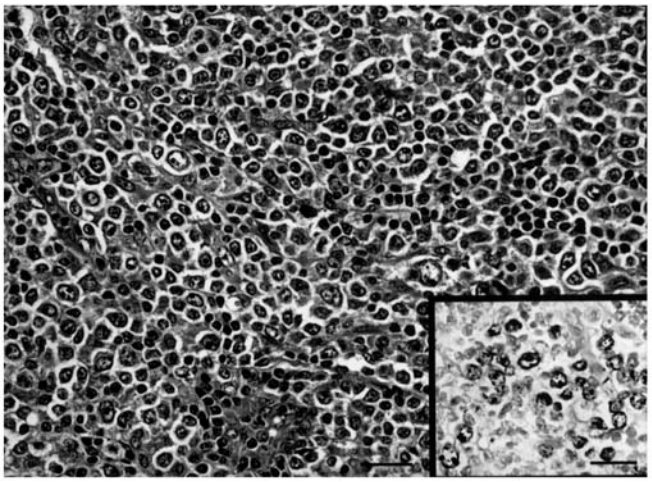

Figure 1. Diffuse polymorphic LPD in RA patient with non-MTX medication. There is a polymorphous pattern of proliferation consisting of the full range of mature B cells from immunoblasts to plasma cells, small and medium-size lymphocytes, and numerous cells (H&E stain). Inset: *In situ* hybridization with EBER-1 probe revealed positive signals in the nucleus of large cells (EBER-1). Scale bar = 200 μm.

cavity in the MTX-LPD group. Hodgkin's lymphoma was subclassified as of mixed cellularity in 7 cases and nodular lymphocyte-predominant in one case.

EBV infection. *In situ* hybridization for EBV revealed positive signals in the nucleus of large atypical cells of non-Hodgkin's lymphoma (NHL) and Reed-Sternberg cells in the cases of Hodgkin's lymphoma: 50%–90% of the large atypical cells in NHL and more than 90% of the Reed-Sternberg cells showed positive signals. There were no differences in the percentages of EBV-positive cells between MTX-LPD and non-MTX-LPD. Among RA-LPD, 21 (27.6%) of 76 cases were judged as EBV-positive (Table 3): 10 of 60 cases of B cell lymphoma, one case of NK/T cell lymphoma, and 7 of 8 Hodgkin's lymphoma cases were EBV-positive. The EBV-positive rate in RA-LPD was significantly higher than that in sporadic LPD. Three of 7 cases of the T cell LPD were EBV-positive. In general, there was no difference in the frequency of EBV positivity between the patients with MTX-LPD and with non-MTX-LPD (Table 4). Three of 4 cases with T cell-type non-MTX-LPD

Table 3. Epstein-Barr virus positivity in MTX-LPD and non-MTX-LPD.

	MTX-LPD (%)	Non-MTX-LPD (%)	RA (%)	Sporadic LPD (%)
Total	13/48 (27.1)	8/28 (28.6)	21/76 (27.6)	11/111 (9.9)
B cell LPD	7/38 (18.4)	3/22 (13.6)	10/60 (16.7)	2/87 (2.3)
DLBCL	4/29 (13.8)	2/15 (13.3)	6/44 (13.6)	2/50 (4.0)
Diffuse polymorphic	2/2 (100)	1/1 (100)	3/3 (100)	0/0 (0)
HL-like LPD	1/1 (100)	0/0 (0)	1/1 (100)	0/0 (0)
Others	0/6 (0)	0/6 (0)	0/12 (0)	0/37 (0)
T cell LPD	0/3 (0)	3/4 (75)	3/7 (42.9)	2/9 (22.2)
Natural killer/T cell LPD	1/1 (100)	0/0 (0)	1/1 (100)	4/5 (80)
HL	5/6 (83.3)	2/2 (100)	7/8 (87.5)	3/8 (37.5)

MTX: methotrexate, DLBCL: diffuse large B cell lymphoma, HL: Hodgkin's lymphoma. * $p < 0.05$.

Table 4. Summary of 11 patients who showed regression of RA-LPD after withdrawal of MTX.

Patient	Age	Sex	Primary Site	Primary Symptom	Histology	Stage	In situ (EBER-1)	RA Duration, mo	MTX Medication Dose, mg/wk	MTX Medication Duration, mo	MTX Medication Total Dose, mg	Regression, mo	Recurrence	Therapy for Recurrence	Effect of Therapy	Followup Periods, mo	Outcome
1	67	F	LN	LN swelling	DLBCL	IV	-	120	5	120	2600	5	Ys	Chemo	PR	46	A
2	62	F	LN	LN swelling	HL-like LPD	III	+	20	5	12	270	2	Yes	Chemo	CR	33	D
3	60	M	LN	LN swelling	AILDT	III	-	12	8	7	228	4	Yes	Chemo	PR	9	A
4	68	M	LN	LN swelling	HL, MC	IV	-	240	6	72	864	1	Yes	Chemo	CR	20	A
5	53	F	LN	LN swelling	HL, MC	IV	+	84	6~12	72	1724	10	Yes	Chemo	CR	42	A
6	62	F	LN	LN swelling	DLBCL	II	-	216	6~10	24	600	64	No	—	—	64	A
7	67	F	LN	Fever, LN swelling	D poly	III	+	8	8	6	192	25	No	—	—	25	DID
8	77	F	Skin	Tumor mass	D poly	II	+	84	8	36	1112	22	No	—	—	22	A
9	70	M	LN	LN mass	FL	III	-	10	12~17	10	584	10	No	—	—	10	A
10	59	F	LN	Fever	HL, MC	III	+	228	5~7.5	123	1076	8	No	—	—	9	A
11	74	F	Nasal cavity	Mass formation	NKTCL	I	+	130	4~8	118	2880	21	No	—	—	21	A

MTX: methotrexate, LN: lymph node, DLBCL: diffuse large B cell lymphoma, HL: Hodgkin's lymphoma, AILDT: angioimmunoblastic T cell lymphoma, MC: mixed cellularity, D poly: diffuse polymorphic, NKTCL: natural killer T cell lymphoma; PR: partial remission, CR: complete remission, A: alive, D: dead, DID: death from intercurrent disease, FL: follicular lymphoma.

were EBV-positive, but none of the T cell LPD in the MTX-LPD was EBV-positive.

HTLV-1 infection. PCR analysis for the HTLV-1 proviral genome was performed in 7 cases with T cell RA-LPD, giving completely negative results.

Clinical outcome. MTX was stopped soon after the appearance of nodal or extranodal masses in patients with MTX-LPD, who subsequently underwent biopsy of the lesional tissues. The masses showed a tendency for regression in 11 patients during the 1 to 2 week periods before definite histologic diagnosis was obtained. Mass lesions regrew 2–10 months after the withdrawal of MTX in 5 cases, and then the chemotherapy was started in these patients. Regression of the lesions continued for 8–64 months without chemotherapy in the remaining 6 cases (Figure 2, Table 4). The EBV-positive rate in these 11 cases, 54.5%, was higher than that in the remaining 38 cases (21.1%; $p < 0.05$).

Another 42 patients (26 MTX-LPD and 16 non-MTX-LPD) received chemotherapy, 3 received radiation therapy, 8 received combined chemo- and radiotherapy, and one received chemotherapy with peripheral blood stem-cell transplant. Two patients with LPD in the testis underwent surgical resection, followed by chemotherapy in one and combined chemo- and radiotherapy in the other. The chemotherapeutic agents included cyclophosphamide, vincristine, doxorubicin, prednisolone, MTX, bleomycin, mitomycin C, mercaptopurine, melphalan, and rituximab. Effects of adjuvant therapy were not recorded in one of these cases, who was lost to followup 6 months after the diagnosis of LPD. As a result, treatment outcome of the adjuvant therapies was evaluated in 46 patients according to the guidelines of the international workshop to standardize response criteria for NHL[27]. After chemo- and/or radiotherapy, 42 (91.3%) of 46 patients showed a complete or partial response. Three patients showed progressive disease. Each patient with lesions in the terminal ileum and testis, respectively, underwent a surgical resection of the lesions, followed by chemotherapy in the latter case. Perforation in the terminal ileum occurred in the former case and the patient died 1 month after the surgery. The latter patient received adjuvant therapy, and entered complete remission, but died of myocardial infarction 39 months after the surgery.

Statistical analysis. To characterize RA-LPD in comparison with sporadic LPD, multivariate logistic regression analysis was performed. Factors examined were age at the diagnosis of LPD, sex, histology, and EBV positivity of LPD. Cases of RA-LPD were found to be older at diagnosis of LPD, and showed female preponderance and a higher positive rate for EBV than sporadic LPD ($p < 0.01$). The frequency of DLBCL in RA-LPD was higher than that in sporadic LPD ($p < 0.064$).

The followup period for survivors calculated from the date of diagnosis of RA-LPD ranged from 1 to 144 (median 22) months. The 1- and 5-year overall survival rate was

80.5% and 58.9%, respectively. Responses to adjuvant therapies and survival rates were rather similar between MTX-LPD and non-MTX-LPD. The 5-year survival rate in sporadic LPD (74.6%) was significantly better than that in RA-LPD (59.2%; $p < 0.05$). Univariate analysis revealed that EBV positivity, stage of disease, and age at diagnosis of LPD were the prognostic factors for survival: EBV-negative LPD, early-stage disease (stage I or II), and younger age were favorable factors. Multivariate analysis showed that younger age at diagnosis and EBV positivity were significant independent factors for overall survival: 5-year survival for EBV-positive and EBV-negative patients and patients aged < 60 and > 60 years was 76.3% vs 54.0% and 76.4% vs 63.9%, respectively ($p < 0.01$). Adjusted for age and sex, RA-LPD data showed worse prognosis than sporadic LPD in those over 60 years of age ($p < 0.01$). This difference was marked among female patients (Figure 3).

DISCUSSION

Our study dealt with the largest number of cases with RA-LPD ever reported from Japan; there might have been an ascertainment bias because this was not a case-controlled study nor were the cases consecutive. The clinicopathologic findings in RA-LPD (MTX-LPD and non-MTX-LPD)

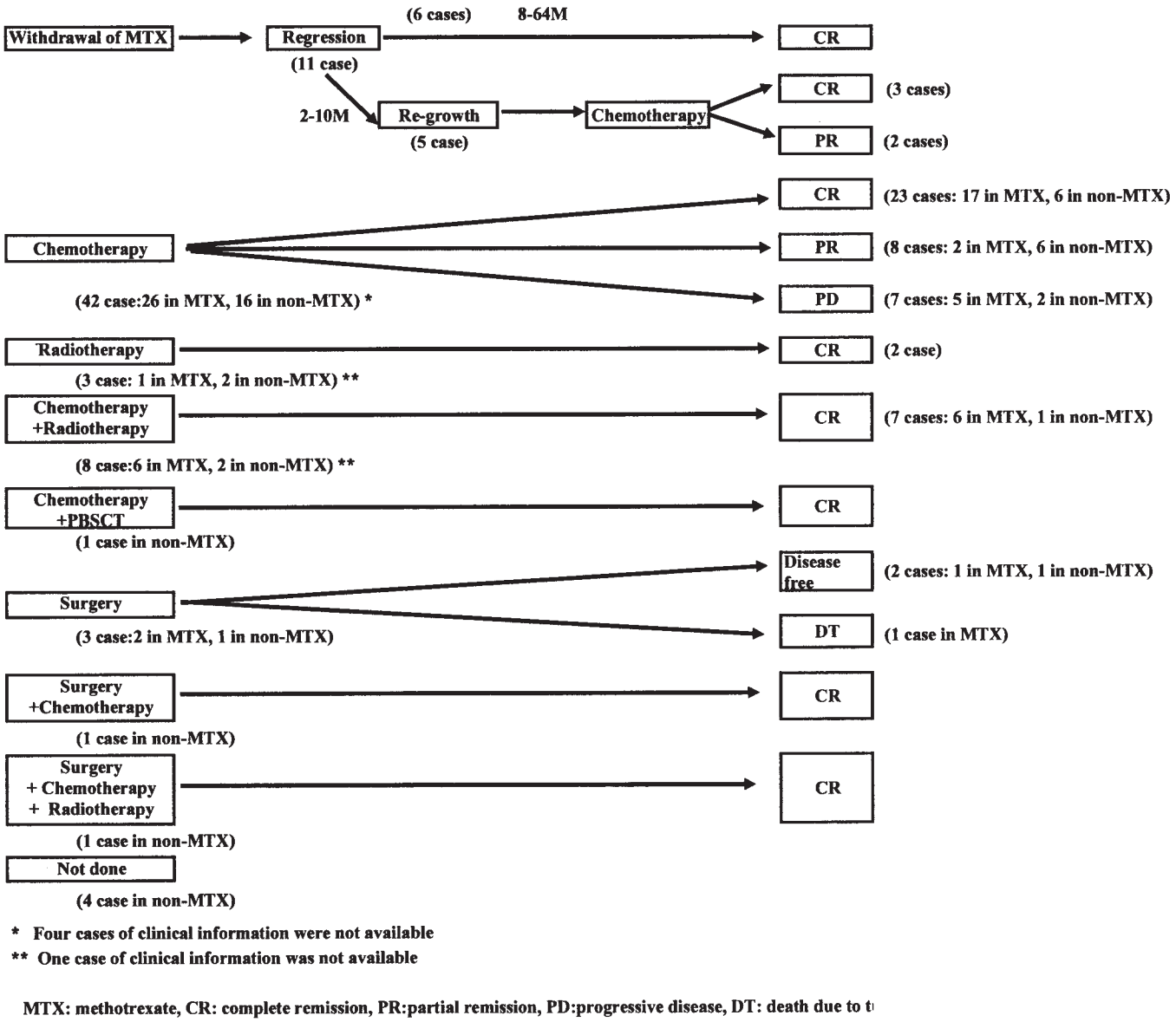

Figure 2. Clinical outcome of MTX-associated lymphoproliferative disorders in Japan. *Four cases of clinical information were not available. **One case of clinical information was not available. CR: complete remission, PR: partial remission, PD: progressive disease, DT: death due to tumor, MTX: methotrexate, PBSCT: peripheral blood stem-cell transplant.

are discussed in comparison with post-transplant-LPD and sporadic LPD (Table 5). MTX-LPD and non-MTX-LPD shared similar clinical findings including age, sex, primary site, and stage of LPD. The median age at the development of LPD in our cases was 66 years, rather higher than that in sporadic LPD, 58 years ($p < 0.05$). Median age at the diagnosis of RA was 51 years. This might result in censorship for the older onset of RA-LPD. There was a female preponderance in the series (M/F ratio of 0.38), in contrast to a male preponderance in LPD developing in immunocompetent (M/F ratio 1.67) and immunocompromised patients (M/F ratio 5 in post-transplant-LPD, and 24 in acquired immunodeficiency disease-related lymphoma)[28-30]. This might be due to the predominance of female patients with RA who develop MTX-LPD and non-MTX-LPD[31]. The female preponderance in our cases of RA-LPD was more marked than that reported from Western countries (0.56 to 0.81)[5,6,32,33]. Another study on RA-LPD from Japan also reported a greater female preponderance than in Western countries[34].

Median duration and total dose of MTX administration in our series was 54 months and 0.940 g, respectively, rather similar to those in reports from Western countries[1,5,6]. Duration between the diagnosis of RA and LPD was shorter (132 mo) in patients with MTX medication than in those without (240 mo; $p < 0.05$). MTX seems to hasten the development of LPD among patients with RA. Alternatively, if MTX is administered in patients with severe RA, a shorter time to development of LPD in these patients might be associated with severity of RA.

The primary site of MTX-LPD was nodal or extranodal in almost equal frequency in this series, similar to reports for MTX-LPD (40%–70%) from Western countries[1,4,5,7] and for sporadic LPD in Japan[8]. In contrast, extranodal disease was much more predominant in post-transplant-LPD and LPD in patients with AIDS[28,35]. About 60% of the current RA-LPD cases presented with advanced disease. Irrespective of receiving or not receiving MTX, LPD in patients with RA usually present as advanced disease[4]. The majority of localized diseases was found in the cases with DLBCL. Post-transplant-LPD is usually diagnosed at an early stage. This might be due to careful monitoring of patients after transplant.

The frequency of DLBCL in our cases of RA-LPD was

Table 5. Clinicopathologic findings of cases of MTX-LPD, non-MTX-LPD, PT-LPD, and sporadic LPD.

				Immunophenotype, %				Clinical Stage, %						Primary Site, %	
	No. of Cases	Age, median yrs	M/F Ratio	B	T	NK/T	HL	I	II	III	IV	5-yr Survival, %	EBV, %	Nodal	Extranodal
RA-LPD	76	66	0.39	79	9	1	11	22	13	29	29	59.2	27.6	49.3	50.7
MTX-LPD	48	67	0.45	79	6	2	13	21	15	31	29	58.9	27.1	48.9	51.1
Non-MTX-LPD	28	66	0.27	79	14	0	7	25	11	25	29	52.8	28.6	50.0	50.0
PT-LPD*	28	40	5.00	57	36	7	0	40	8	20	32	26.7	68.0	22.0	78.0
Sporadic LPD	150	58	1.67	74	11	4	11	21	24	11	37	74.6	9.9	54.7	45.3

LPD: lymphoproliferative disorders, MTX: methotrexate, PT: post-transplant, B: B cell, T: T cell, NK: natural killer cell, HL: Hodgkin's lymphoma, EBV: Epstein-Barr virus. * 24 cases were described[28].

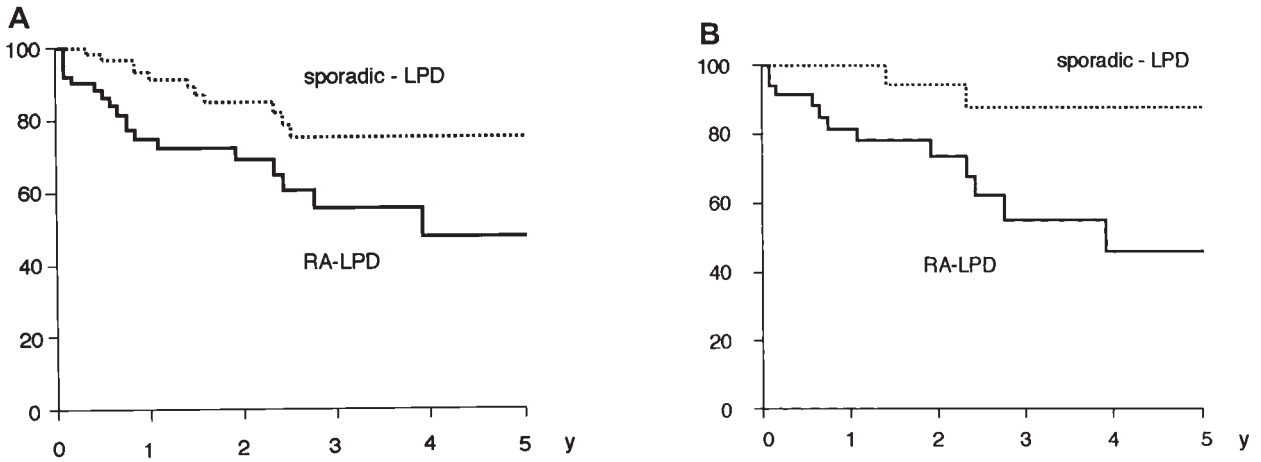

Figure 3. 5-Year survival curves for RA-LPD and sporadic LPD, adjusted for age and sex (%). A. Total. B. Female patients with RA-LPD age ≥ 60 years showed a less favorable prognosis than those with sporadic LPD.

57%–61%, irrespective of MTX medication. This is rather close to the previous report on the frequency of LPD (67%) among RA patients treated in Sweden between 1964 and 1984, an era when aggressive immunosuppressive therapy including MTX was rarely employed[36]. These findings show that the frequency of DLBCL is higher in LPD developing in RA patients than in cases of sporadic LPD (42.7%) and post-transplant-LPD (16%–38%)[28,37,38].

EBV is the well known oncogenic virus engaged in lymphomagenesis. An immunodeficient state is considered to provide a basis for the development of malignant lymphomas, probably through the activation of EBV. The EBV-positive rates in our study cases of RA-LPD (27.6%) and reported cases of post-transplant-LPD (63%–95%)[39,40] are significantly higher than that in sporadic LPD (9.9%; $p < 0.05$). Baecklund, *et al*[32] reported a rather lower frequency of EBV positivity in RA-LPD than that in our cases, although the EBV-positive rates in the DLBCL in their cases and ours were similar: 12% to 13%. There were no differences in clinicopathologic or EBV status between MTX-treated and untreated patients; this may be consistent with the hyperimmune hypothesis for the development of RA-LPD. As stated by Weyand, *et al*[41], the inability to control unwanted inflammation, often referred to as loss of tolerance, may be directly connected to the inability to survey lymphoid and extralymphoid organs for neoplasm and suppress the overgrowth of monoclonal B cell clones.

We found the EBV-positive rate in cases of T cell-type non-MTX-LPD, 75%, was much higher than that reported in sporadic peripheral T cell lymphomas (about 30%)[42] and B cell lymphomas (9.9%). We cannot provide an explanation for this. The presence of T cell LPD and Hodgkin's lymphoma with the higher EBV positivity (Table 3) may in part explain the higher EBV positivity in our non-MTX-LPD cases than, for example, in Kamel, *et al*[43], in which only one (2%) of 42 cases with RA-LPD (the majority being non-MTX-LPD) was EBV-positive. Three cases of polymorphic LPD in patients with and without MTX medication were EBV-positive, as is usually observed in cases of polymorphic post-transplant-LPD[1]. None of 3 cases with T cell MTX-LPD was EBV-positive. One case of Hodgkin's-like LPD was EBV-positive as reported by Kamel, *et al*[43].

Kamel, *et al* reported that some cases of MTX-LPD were associated with EBV and spontaneously regressed after the withdrawal of MTX, as observed in cases of post-transplant-LPD after the withdrawal of immunosuppressive agents[6]. In our series, one-quarter of MTX-LPD (11 cases) regressed spontaneously after withdrawal of MTX. The EBV-positive rate in the cases showing spontaneous regression of LPD (54.5%) was higher than in those without spontaneous regression (21.1%). A recent report by Feng, *et al* showed that MTX directly induced reactivation of EBV infection with release of infectious virions[44]. No case of non-MTX-LPD showed spontaneous regression. On the other hand, it is not clear why MTX-LPD in the patients who were EBV-negative regressed after the withdrawal of MTX. This might also be consistent with the hyperimmune hypothesis in the development of RA-LPD.

Five-year survival rates in MTX-LPD and non-MTX-LPD did not differ. This seems surprising, since 6 cases of MTX-LPD had sustained remissions after stopping MTX. Among MTX-LPD cases, the majority showing regression of LPD were Hodgkin's lymphoma, Hodgkin's-like LPD, and polymorphic LPD with low potential for malignancy. On the other hand, the frequency of DLBCL, an aggressive lymphoma, was higher in the MTX-LPD than non-MTX-LPD cases. As a result, the 5-year survival rates for the 2 groups of RA-LPD patients were not different.

Adult T cell leukemia/lymphoma (ATL), which is rare in Western countries, is common in Japan[45]. Our previous study on LPD in renal transplant revealed that roughly 20% of cases of post-transplant-LPD were ATL[28]. This might be due to transmission of HTLV-1 via blood transfusion during hemodialysis. None of the present MTX-LPD and non-MTX LPD cases were ATL.

The 5-year overall survival rates in cases of MTX-LPD and non-MTX-LPD were 58.9% and 52.8%, respectively, which was significantly worse than that in sporadic LPD ($p < 0.05$). After adjustment for age and sex, this tendency was confirmed in patients over 60 years old, and was more marked in female patients ($p < 0.01$). The 5-year survival rate in cases with post-transplant-LPD was worse than in MTX-LPD and non-MTX-LPD. This might be due to the higher frequency of T cell LPD including ATL and NK/T cell LPD together with the absence of Hodgkin's lymphoma in post-transplant-LPD.

Our study revealed that the majority of cases of RA-LPD showed similar clinicopathological characteristics irrespective of use of MTX medication, except for the spontaneous regression of LPD after withdrawal of MTX in cases of MTX-LPD, and the shorter interval between the diagnosis of RA and LPD in MTX-LPD than in non-MTX-LPD. Cases of RA-LPD showed an older age at onset, a female predominance, an unfavorable prognosis, and higher frequencies of DLBCL and EBV positivity compared to cases of sporadic LPD.

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