Association between mortality and cytomegalovirus reactivation during remission induction therapy in patients with rheumatic diseases

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Abstract

Objective

To elucidate the characteristics of patients with rheumatic diseases with cytomegalovirus (CMV) reactivation.

Methods

In our study, we consecutively reviewed patients with rheumatic diseases who received remission induction therapy in our institution from January 2012 to March 2016 and enrolled the patients who were evaluated about CMV infection. CMV reactivation was characterised by the detection of polymorphonuclear leukocytes with CMV pp65. The characteristics and clinical courses of the patients with CMV reactivation were compared to those without CMV.

Results

We observed CMV reactivation in 71 (39.7%, CMV-positive group) out of 179 patients. Age (odds ratio [OR] 1.023, 95% confidence interval [CI] 1.002–1.044, p=0.03), lymphocyte counts (OR 0.999, 95% CI 0.999–1.000, p=0.03), and initial prednisolone dose (OR 18.596, 95% CI 2.399–144.157, p<0.01) were considered as independent relevant risk factors for CMV reactivation. Patients in the CMV-positive group showed significantly higher incidences of all infections (48%) and severe infections (31%) than those in the CMV-negative group (48% vs .31%, p=0.037; 31% vs. 6%, p<0.001, respectively). Higher mortality was observed in the CMV-positive group than in the CMV-negative group (14.1% vs. 1.9%). The lymphocyte counts were more relevant to CMV infection and mortality than were the serum IgG levels.

Conclusion

Our study revealed that CMV reactivation occurred in one third of all patients with rheumatic diseases who were undergoing intensive remission induction therapy, and it was found to be relevant to other severe infections and infection-related deaths.

Key words

cytomegalovirus reactivation, rheumatic disease

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Introduction

Human cytomegalovirus (CMV) is a type of herpes virus that is found ubiquitously, which infects majority of humans (1). Primary CMV infection occurs in people with normal immune function usually during young age and is frequently asymptomatic. After the primary infection, the virus remains latent in various host cells with occasional sub-clinical reactivations (2). However, when reactivation occurs in immunocompromised patients, the host immune system is sometimes unable to control the viral replication, which causes clinical manifestations resulting in organ damage and fatality (3, 4).

CMV reactivation is a highly problematic opportunistic infection that occurs in patients with rheumatic diseases undergoing intensive immunosuppressive treatment. The incidence of CMV reactivation in immunosuppressed patients with rheumatic diseases is 31–50%, which varies depending on the patient's background, treatment regimen, and diagnostic methods for CMV (5-9). While there is significant evidence on CMV reactivation in transplant patients and patients infected with human immunodeficiency virus, little is known about the risk and optimal management for CMV reactivation and disease in patients with rheumatic disease (7-9).

The aim of this study is to clarify the characteristics of patients with rheumatic diseases with CMV reactivation.

Materials and methods

Patients and data collection

We consecutively reviewed patients who were admitted into the Rheumatology Division of Keio University Hospital from January 2012 to March 2016 retrospectively. We enrolled patients who either received remission induction therapy that included initiation of or an increase in dosage of glucocorticoids with or without other immunosuppressive agents for new-onset or relapsed rheumatic diseases. CMV pp65 antigen tests were performed at the discretion of patients' attending physicians, and we excluded patients whose CMV test results during the admission were not available.

Clinical information, including the patients' characteristics, laboratory data, results of CMV pp65 antigen tests, treatment for underlying rheumatic diseases and CMV, other microbial infections, and clinical courses, was collected from their medical charts. The observation period started when the remission induction treatment was initiated until October 2016.

This study was approved by the ethics committee of Keio University School of Medicine (approval no.: 20130506). Written informed consent from the patients was waived according to the regulations in Japan. All investigations were conducted according to the guidelines in the Declaration of Helsinki.

Definition of CMV reactivation/ disease and other infections

The presence of CMV pp65 antigen was detected in polymorphonuclear leukocytes in the peripheral blood using pp65-specific monoclonal antibodies and was designated as C10/C11 (CMV antigen, Mitsubishi, Mitsubishi Chemical Corporation, Tokyo). CMV reactivation was defined as the detection of at least one positively stained polymorphonuclear leukocyte in the peripheral blood. CMV disease was a symptomatic CMV reactivation in combination with disappearance of symptoms and CMV pp65 antigen by anti-CMV agents such as ganciclovir, valganciclovir, and foscarnet. Great care was taken to ensure that symptoms similar to CMV infections caused by immunosuppressive agents, such as drug-induced cytopenia and liver damage, were not counted as CMV infections. As anti-CMV therapies were performed at the discretion of patients' attending physician, the cases where administration of pre-emptive anti-CMV agents was initiated in the absence of any symptoms were regarded as asymptomatic CMV infection and not CMV disease. Cases with all other infectious diseases besides CMV infection were also counted. Among them, severe infections were defined as infectious events which involved hospitalisation, prolongation of hospitalisation, or death. Those symptoms were carefully assessed and determined due to CMV infection. Cytopenia or hepatic injury caused by other drugs were excluded.

Table I. Baseline clinical characteristics and remission induction therapy.

	All (n=179)	CMV-positive group (n=71)	CMV-negative group (n=108)	*p*-value	Multiple regression analysis	
					Odds ratio	*p*-value
Age (years)*	57.1 ± 17.0	60.7 ± 15.2	54.7 ± 17.7	0.02	1.023	0.03
Female, n (%)	123 (68.7%)	46 (64.8%)	77 (71.3%)	0.36		
Body weight (kg)*	53.9 ± 10.3	54.0 ± 10.6	53.7 ± 10.2	0.84		
Diagnosis						
SLE, n (%)	54 (30.2%)	22 (31.0%)	32 (29.6%)	0.85		
Vaculitides, n (%)	48 (26.8%)	20 (28.2%)	28 (25.9%)	0.74		
PM/DM, n (%)	24 (13.4%)	7 (9.9%)	17 (15.7%)	0.26		
RA, n (%)	21 (11.7%)	8 (11.3%)	13 (12.0%)	0.88		
AOSD, n (%)	10 (5.6%)	5 (7.0%)	5 (4.6%)	0.52		
SSc, n (%)	6 (3.4%)	2 (2.8%)	4 (3.7%)	1.00		
SS, n (%)	6 (3.4%)	4 (5.6%)	2 (1.9%)	0.22		
Others, n (%)	9 (5.0%)	3 (4.2%)	6 (5.6%)	1.00		
New-onset rheumatic disease, n (%)	118 (65.9%)	51 (71.8%)	67 (62.0%)	0.18		
Disease duration (years)*	4.8 ± 9.8	4.8 ± 9.8	4.9 ± 9.0	0.99		
Baseline laboratory findings						
White blood cell count (/μl)*	8208 ± 5562	8133 ± 5400	8257 ± 5690	0.88		
Lymphocyte count (/μl)*	1044 ± 650	896 ± 606	1141 ± 663	0.01	0.999	0.03
Serum total protein (g/dl)*	6.5 ± 1.0	6.3 ± 1.1	6.6 ± 1.0	0.02		
Serum albumin (g/dl)*	3.0 ± 0.7	2.7 ± 0.7	3.1 ± 0.7	<0.01		
Serum IgG (mg/dl)*	1605 ± 675	1667 ± 766	1566 ± 611	0.36		
Serum creatinine (mg/dl)*	0.94 ± 0.82	1.13 ± 1.11	0.81 ± 0.54	0.01	1.522	0.09
Serum CRP (mg/dl)*	4.73 ± 6.71	5.81 ± 7.99	4.02 ± .64	0.08		
Remission induction therapy						
GC use, n (%)	179 (100%)	71 (100%)	108 (100%)	1.00		
Initial GC dose as PSL (mg/kg/day)*	0.94 ± 0.18	0.98 ± 0.16	0.91± 0.18	<0.01	18.596	<0.01
mPSL pulse therapy, n (%)	55 (30.7%)	32 (45.1%)	23 (21.3%)	<0.01		
Concomitant IS use	129 (72.1%)	52 (73.2%)	77 (71.3%)	0.78		
IVCY, n (%)	82 (45.8%)	37 (52.1%)	45 (41.7%)	0.17		
AZP, n (%)	14 (7.8%)	3 (6.5%)	11 (10.2%)	0.15		
CI, n (%)	28 (15.6%)	7 (9.9%)	21 (19.4%)	0.08		
MTX, n (%)	9 (5.00%)	2 (4.3%)	7 (6.5%)	0.32		
MMF, n (%)	7 (3.9%)	3 (4.2%)	4 (3.7%)	1.00		
IFX, n (%)	1 (0.6%)	0 (0.0%)	1 (0.9%)	1.00		
TCZ, n (%)	7 (3.9%)	5 (7.0%)	2 (1.9%)	0.12		
ABT, n (%)	1 (0.6%)	1 (2.2%)	0 (0.0%)	0.43		
RTX, n (%)	1 (0.6%)	0 (0.0%)	1 (0.9%)	1.00		
Two or more ISs use, n (%)	22 (12.3%)	5 (7.0%)	16 (14.8%)	0.16		
Observation period (weeks)*	74.1 ± 61.4	66.5 ± 62.3	79.1 ± 60.6	0.18		

CMV: cytomegalovirus; SLE: systemic lupus erythematosus; PM: polymyositis; DM: dermatomyositis; RA: rheumatoid arthritis; AOSD: adult-onset Still's disease; SSc: systemic sclerosis; SS: Sjögren's syndrome; IgG: immunoglobulin G; CRP: C-reactive protein; GC: glucocorticoid; PSL: prednisolone; mPSL: methylprednisolone; IS: immunosuppressant; IVCY: intravenous cyclophosphamide; AZP: azathioprine; CI: calcineurin inhibitor; MTX: methotrexate; MMF: mycophenolate mofetil; IFX: infliximab; TCZ: tocilizumab; ABT: abatacept; RTX: rituximab. * mean±SD.

Statistical analysis

Continuous values and proportions between both the groups were compared using Student's t-test or Chi-square test, respectively. Comparison of continuous values and proportions between three groups was performed using analysis of variance and Chi-square test, respectively. Multivariate analysis was performed using binary logistic regression with covariates that were identified as potentially significant when the *p*-value was less than 0.1 using forward selection method. Cumulative hazard curves were depicted with Kaplan-Meyer analysis and comparisons were made using Log-rank test. A receiver operating curve (ROC) was depicted to determine the cut-off value of optimal figure. A *p*-value of less than 0.05 was regarded as significant. All statistical analysis was performed by SPSS software (IBM SPSS Statistics, IBM Corporation., Chicago, IL).

Results

Baseline characteristics and regimens of remission induction therapy

Out of a total of 1,253 hospitalised patients, 275 patients received remission induction therapy for rheumatic diseases. CMV pp65 antigen was measured in 179 patients, who were enrolled in the current study. The characteristics of the enrolled patients are shown in Table I. The mean age was 57.1 years, the mean disease duration was 4.8 years and 68.7% of the patients were women. Majority of the underlying rheumatic diseases were systemic lupus erythematosus (n=54), followed by vasculitides (n=48), polymyositis/dermatomyositis (n=24), rheumatoid arthritis (n=21), adult-onset Still's disease (n=10), systemic sclerosis (n=6), and Sjögren's

syndrome (n=6). Other rheumatic diseases included Behçet's disease (n=5), IgG4-related disease (n=2), sarcoidosis (n=1), and spondyloarthritis (n=1). All of the patients were treated with moderate to high dose of glucocorticoids with a mean prednisolone dose of 0.94 mg/kg/day. Of the total number, 129 patients (72.1%) also received concomitant immunosuppressive agents. Ninety-six patients who were not included in the analysis due to lack of detection of CMV antigen received milder treatment compared to the 179 patients who were included in the analyses (Supplementary Table S1).

Incidence and risk factors for CMV reactivation

Among the 179 patients, CMV reactivation was observed in 71 patients (39.7%, CMV-positive group). As shown in Table 1, the patients in the CMV-positive group were older, had higher baseline serum creatinine levels, lower baseline lymphocyte counts, lower serum total protein, and lower albumin levels compared to the CMV-negative group at the beginning of the remission induction therapy. The IgG levels were comparable. The treatment regimens were also significantly different; initial prednisolone dose, and the rate of methylprednisolone pulse therapy (Table I). Multivariate logistic regression analysis revealed that age (odds ratio 1.023, 95% confidence interval [CI] 1.002–1.044, p=0.03), baseline lymphocyte count (odds ratio 0.999, 95%CI 0.999–1.000, p=0.03), and initial prednisolone dose (odds ratio 18.596, 95%CI 2.399–144.157, p<0.01) were independent relevant risk factors for CMV reactivation.

CMV disease in the CMV-positive group

The mean duration from the initiation of induction therapy to CMV reactivation in the CMV-positive group was 23.5±13.3 days, and the mean dose of prednisolone at CMV reactivation was 0.83±0.21 mg/kg/day. The mean maximum number of CMV pp65 antigen positive-polymorphonuclear leukocytes during the clinical course was 9.5 (1–172) cells/2 slides.

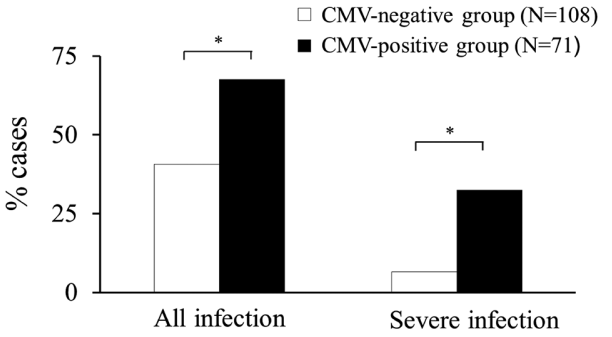
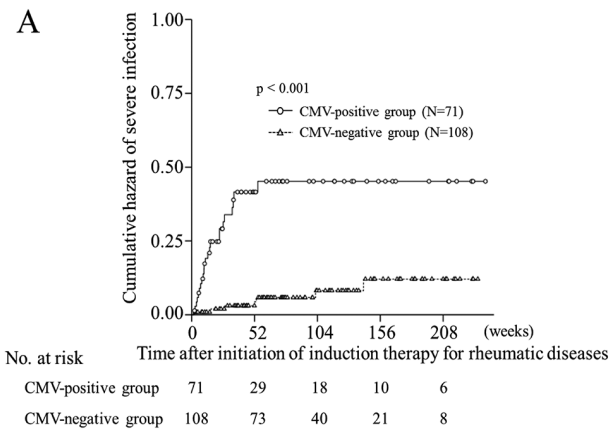


Fig. 1. Incidence rates of infection based on CMV reactivation.
The CMV-positive group showed significantly higher incidence of all infections (**A**) and severe infections (**B**) than the CMV-negative group. CMV, cytomegalovirus; *p<0.001



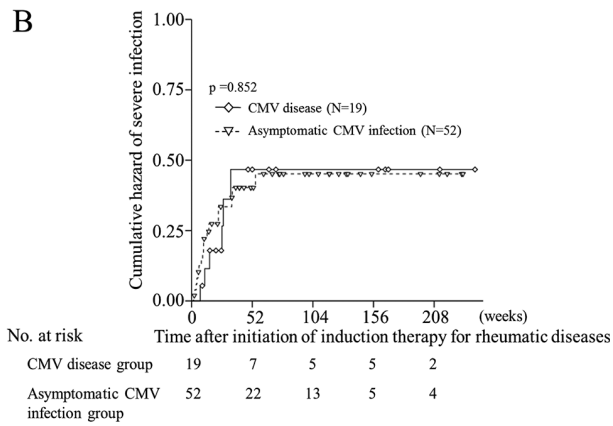


Fig. 2. Cumulative hazard curves in patients with severe infection.
A: Cumulative hazard curves showed significantly higher incidence of severe infection in the CMV-positive group than the CMV-negative group by Log-rank test. Most severe infections in the CMV-positive group occurred within one year from the initiation of induction therapy.

B: In the CMV-positive group, there was no difference in the occurrence of severe infection between the patients with CMV disease and those with asymptomatic CMV infection. CMV, cytomegalovirus.

Among the 71 CMV-positive patients, 52 patients (73.2%) were asymptomatic (26 without CMV treatment and 26 with pre-emptive CMV treatment), and 19 patients (26.8%) developed CMV disease. Although the CMV disease was mainly manifested by cytopenia, some patients developed liver dysfunction or serositis (Suppl. Table S2). Upon comparison of the three patterns of CMV positivity, asymptomatic CMV infection without anti-CMV agents (n=26), asymptomatic CMV infection with pre-emptive anti-CMV agents (n=26), and CMV disease (n=19), we found that the number of CMV pp65 antigen positive-polymorphonuclear leukocytes were significantly different; at reactivation, 1.7±1.3 *vs*. 6.1±9.1 *vs*. 3.8±6.5 cells/2 slides (p=0.05), and at maximum, 2±1.4 *vs*. 8±11.1 *vs*. 21.5±38.1 cells/2 slides (p=0.01), respectively.

Association between CMV reactivation and other infections

Figure 1 shows the incidence of other infections in addition to CMV reactivation. The CMV-positive group showed significantly higher incidence of other infections than the CMV-negative group (48% *vs*. 32%, p=0.037). There was a substantial difference in the incidence of bacterial and fungal infections despite comparable viral infection rates

(Suppl. Table S4). The other infections in the CMV-positive group occurred before CMV reactivation in 19 patients (26.8%), and after CMV reactivation in 41 patients (57.8%).

Association between CMV reactivation and other severe infections

The incidence of severe infection between the CMV-positive and the CMV-negative groups was significantly different (32.4% *vs*. 6.5%, $p<0.001$, Fig. 1), as well as that of infections of major organs such as central nervous system, lungs, and abdomen, which occurred more frequently in the CMV-positive group (Suppl. Table S4). Opportunistic infections such as nocardiosis, aspergillosis, Norway scabies, fungaemia, and bacteraemia were only seen in the CMV-positive group. Moreover, severe infections occurred sooner in the CMV-positive group than the CMV-negative group. The mean duration between initiation of induction therapy and severe infection was 16.6±13.4 weeks in the CMV-positive group and 56.6±49.8 weeks in the CMV-negative group ($p<0.001$). Although the cumulative incidence of severe infection was significantly different between the CMV-positive and the CMV-negative groups as evaluated using Log-rank test ($p<0.001$) (Fig. 2A), the values were comparable between the patients with asymptomatic CMV reactivation and those with CMV disease ($p=0.852$) (Fig. 2B). Notably, the prednisolone dose for patients with severe infection was 0.43±0.27 mg/kg/day in the CMV-positive group and 0.3±0.31 mg/kg/day in the CMV-negative group ($p=0.362$).

Univariate comparisons revealed that higher age, lower body weight, longer disease duration, and CMV reactivation were associated with severe infection. No difference was found in the induction therapy regimens (Table II). Multivariate logistic regression analysis confirmed that age (odds ratio 1.074, 95%CI 1.028–1.121, $p=0.001$), duration of underlying rheumatic diseases (odds ratio 1.080, 95%CI 1.031–1.131, $p=0.001$), and CMV reactivation (odds ratio 8.667, 95%CI 2.834–26.508, $p<0.001$) were independent relevant factors for severe infection (Table II).

Table II. Baseline characteristics and treatment regimens between patients with severe infection and those without.

	Severe infection			Multiple regression analysis	
	(+) n=30	(-) n=149	*p*-value	Odds ratio	*p*-value
Age (years)*	66.2 ± 13.6	55.2 ± 17.0	<0.01	1.074	0.001
Female, n (%)	21 (70.0%)	102 (68.5%)	0.87		
Body weight (kg)*	49.7 ± 10.0	54.7 ± 10.2	0.02		
Diagnosis					
SLE, n (%)	7 (23.3 %)	47 (31.5%)	0.37		
Vaculitides, n (%)	9 (30.0%)	39 (26.2%)	0.67		
PM/DM, n (%)	2 (6.7%)	22 (14.8%)	0.38		
RA, n (%)	6 (20.0%)	15 (10.1%)	0.12		
AOSD, n (%)	0 (0.0%)	10 (6.7%)	0.22		
SSc, n (%)	2 (6.7%)	4 (2.7%)	0.26		
SS, n (%)	3 (10.0%)	3 (2.0%)	0.06	7.379	0.058
Others, n (%)	1 (3.3%)	8 (5.4%)	1.00		
New-onset rheumatic disease, n (%)	17 (56.7%)	101 (67.8%)	0.24		
Disease duration (years)*	9.7 ± 14.5	3.9 ± 7.5	<0.01	1.080	0.001
Baseline laboratory findings					
White blood cell count (/μl)*	8169 ± 5322	8216 ± 5626	0.97		
Lymphocyte count (/μl)*	996 ± 726	1054 ± 637	0.69		
Serum total protein (g/dl)*	6.3 ± 1.2	6.5 ± 1.0	0.43		
Serum albumin (g/dl)*	2.8 ± 0.7	3.0 ± 0.7	0.10		
Serum IgG (mg/dl)*	1611 ± 861	1604 ± 635	0.97		
Serum creatinine (mg/dl)*	1.17 ± 1.21	0.89 ± 0.72	0.23		
Serum CRP (mg/dl)*	6.33 ± 9.34	4.41 ± 6.03	0.29		
Remission induction therapy					
GC use, n (%)	30 (100%)	149 (100%)	1.00		
Initial GC dose as PSL (mg/kg/day)*	0.93 ± 0.25	0.94 ± 0.16	0.78		
mPSL pulse therapy, n (%)	12 (40.0%)	43 (28.9%)	0.23		
Concomitant IS use					
IVCY, n (%)	15 (50.0%)	67 (45.0%)	0.61		
AZP, n (%)	2 (6.7%)	12 (8.1%)	1.00		
CI, n (%)	4 (13.3%)	24 (16.1%)	1.00		
MTX, n (%)	2 (6.7%)	7 (4.7%)	0.65		
MMF, n (%)	1 (3.3%)	6 (4.0%)	1.00		
TCZ, n (%)	0 (0.0%)	7 (4.7%)	0.60		
ABT, n (%)	1 (3.3%)	0 (0.0%)	0.17		
Two or more ISs use, n (%)	3 (10.0%)	19 (12.8%)	1.00		
CMV reactivation, n (%)	23 (76.7%)	48 (32.2%)	<0.01	8.667	<0.001
Observation period (weeks)*	68.0 ± 61.0	75.3 ± 61.6	0.55		

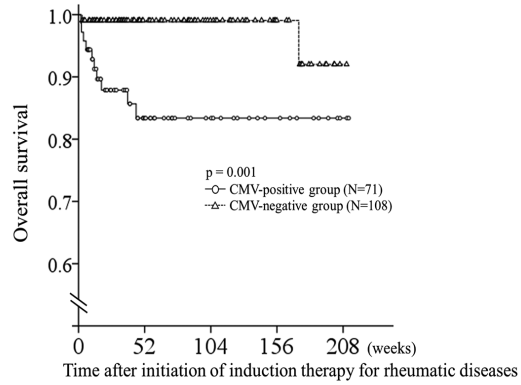
CMV: cytomegalovirus; SLE: systemic lupus erythematosus; AAV: antineutrophil cytoplasmic antibody-associated vasculitis; PM: polymyositis; DM: dermatomyositis; RA: rheumatoid arthritis; AoSD: adult-onset Still's disease; SSc: systemic sclerosis; SS: Sjögren's syndrome; IgG: immunoglobulin G; CRP: C-reactive protein; GC: glucocorticoid; PSL: prednisolone; mPSL: methylprednisolone; IS: immunosuppressant; IVCY: intravenous cyclophosphamide; AZP: azathioprine; CI: calcineurin inhibitor; MTX: methotrexate; MMF: mycophenolate mofetil; TCZ: tocilizumab; ABT: abatacept. * mean±SD.

CMV reactivation and mortality

Ten patients (14.1%) in the CMV-positive group and two patients (1.9%) in the CMV-negative group died during the observation period (Suppl. Table S5). While no patients died of infection in CMV-negative group, eight of the ten patients in the CMV-positive group died of severe infection; sepsis (n=4), bacterial pneumonia (n=3), and pneumocystis pneumonia (n=1). The duration from induction therapy initiation to death was 15.3 (1.9–45.3) weeks in the CMV-positive group, and 87.9 (2.7–173.1) weeks in the CMV-negative group. The overall survival rate was significantly lower in the CMV-positive group than in the CMV-negative group ($p=0.001$) (Fig. 3).

Association between CMV reactivation and lymphocyte counts and serum IgG levels

We specifically investigated the status of the lymphocyte counts and serum IgG levels to understand their associa-



No. at risk

CMV-positive group	71	31	18	10	2
CMV-negative group	108	57	34	16	1

Fig. 3. Overall survival. Overall survival was significantly worse in the CMV-positive group than in the CMV-negative group by Log-rank test. CMV: cytomegalovirus.

tion with CMV reactivation and CMV diseases (Supplementary Table 6). The mean and minimum lymphocyte counts from induction therapy until CMV reactivation in patients with CMV reactivation were significantly lower than those in patients without CMV reactivation during the hospitalisation. However, there was no difference in the mean and minimum serum IgG levels for CMV reactivation and CMV disease. ROC curves of lymphocyte counts revealed that the cut-offs of the mean and minimum lymphocyte counts for CMV reactivation were 1164/μl (sensitivity 69.1%, specificity 67.3%, AUC 0.684) and 590/μl (sensitivity 60.3%, specificity 72%, AUC 0.672).

Discussion

Our study showed that CMV reactivation occurred in patients with rheumatic diseases undergoing intensive remission induction treatment was relevant to other severe infections and infectious death. We have also shown that the infectious events were associated with low lymphocyte counts.

CMV reactivation was common during remission induction treatment for rheumatic diseases when the patients are being administered with moderate to high dose glucocorticoids irrespective of additional immunosuppressive agents. Consistent with previous reports (7, 9), we found that factors such as age, serum creatinine level, initial glucocorticoid dose, steroid pulse, lymphocyte count, serum protein levels, and serum albumin levels were associated with CMV reactivation, suggesting that both the patient's general as well as immunological conditions are relevant. In humans, CMV is neutralised by cellular and humoral immunity. $CD8^+$ and $CD4^+$ T lymphocytes can recognise the CMV antigen peptides with MHC class I and class II restriction, respectively (10, 11), and neutralising antibodies are produced against the envelope proteins of CMV such as glycoprotein B, glycoprotein H, and gH/gL/UL128-130-131A complex, which facilitate the entry of CMV into host cells (12, 13). The current study indicated that lymphocyte count, especially lower than 590/μl, was more important than IgG levels, although the relevance of CMV-specific IgG levels still remains to be elucidated.

We were not able to identify the factors that drive the progression from CMV infection to CMV disease. There was no difference in lymphocyte counts, IgG levels and treatment regimens. However, half of our patients received anti-CMV agents pre-emptively before the appearance of CMV symptoms, which hampered further analyses. In the field of stem cell transplantation, risk factors for CMV reactivation include presence of positive CMV antibodies from the donor or the recipient, pre-transplantation use of anti-thymocyte globulin, acute graft-vs-host disease, and systemic steroid use (14-18). Recently, administration of pre-emptive anti-CMV drugs has been recommended for CMV reactivation depending on these risk factors, which has decreased the incidence rates of CMV disease from 30% to less than 10% (19-21). However, in rheumatic diseases, the exact threshold value of the CMV pp65 antigen required to start pre-emptive anti-CMV treatment as well as the dosage and period of anti-CMV drugs have not been established yet. Hence, it is important to clarify the risk factors for the development of CMV disease and the optimal use of pre-emptive anti-CMV drugs in rheumatic diseases in the future.

Our study also revealed that CMV reactivation was associated with severe infection and related death. The most reasonable explanation for this association is the underlying immune dysfunction caused due to administration of glucocorticoids with or without immunosuppressive agents. Glucocorticoids induce opportunistic infections by suppressing the function of various immunocompetent cells such as neutrophils, monocytes, B cells, T cells, eosinophils, macrophages, and dendritic cells (22-26). In addition, immunosuppressive agents suppress the function of various immune cells via individual mechanisms such as cytotoxicity, metabolic inhibition, and cytokine inhibition. Another interesting possibility is that CMV reactivation itself can promote susceptibility to infection. Some studies have reported a temporary decrease in the number of naïve CD8 positive T cells after CMV infection along with an increase in CD8 T cells against CMV (27-29). Based on these facts, we suggest that care should be taken to prevent bacterial and fungal infections in patients after CMV reactivation.

There are several limitations in this study. Firstly, this is a retrospective observational cohort in which a part of the patients who received induction therapy did not test positively for CMV PP65 antigen. Although we usually examine CMV antigen every one or two weeks in Japan when high dose glucocorticoids are used, it will be tested only when CMV reactivation is suspected if the dose of glucocorticoids is not high. The exclusion of patients who were not evaluated for CMV antigen would have caused selection bias and overinflate the CMV reactivation incidence. Prospective studies with fixed protocol for CMV monitoring and treatment in patients with rheumatic diseases are needed to understand the actual risk of CMV reactivation. Secondly, half of the patients

with CMV reactivation were treated with anti-CMV agents despite lack of symptoms, and anti-CMV therapies were performed at the discretion of patients' attending physician. Pre-emptive anti-CMV treatment has not been established in patients with CMV antigen without symptoms in Japan, however, if the levels of CMV antigen is high and further intensive immunosuppressive therapies are scheduled, pre-emptive anti-CMV treatment would be usually started. This prevented us from identifying the risk factors responsible for the progression of a mere asymptomatic CMV infection to CMV disease. Thirdly, the results were derived from a cohort from a single centre. As the immunosuppressive regimens for remission induction vary among institutions, a single-centered study about rheumatic diseases has a risk of sampling bias. Previously, a questionnaire-based study about CMV infection in patients with rheumatic diseases was conducted in Japan (31), however a multicentre, prospective cohort study has not been conducted. Investigation of CMV reactivation should be performed on a broader scale in multi-centered prospective cohorts.

In conclusion, our study identified the risk factors for CMV reactivation and its association with other severe infections and mortality. To clarify the optimal management of CMV reactivation in patients with rheumatic diseases, larger multi-centre prospective studies are necessary.

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