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Original

Age and High-dose Oral Glucocorticoid Therapy Increase the Relative Risk of Cytomegalovirus Antigenemia in Patients with Systemic Autoimmune Diseases

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Purpose: Cytomegalovirus (CMV) infection commonly occurs in patients undergoing immunosuppressive therapy; however, the incidence has not been well documented. We investigated the incidence and susceptibility factors of CMV antigenemia in immunosuppressed patients in daily clinical care settings for the management of systemic autoimmune diseases.

Methods: Autoimmune patients who were hospitalized in Tokyo Women's Medical University Hospital from April 2010 to March 2012, for CMV infection, defined by positivity with the monoclonal antibody C7-HRP (CMV antigenemia), were retrospectively investigated according to types of autoimmune disease and immunosuppressive treatment strategies.

Results: One-hundred and thirty cases (30 male, 100 female) were included in this study. Patients with systemic lupus erythematosus (n = 37), systemic sclerosis (n = 22), polymyositis/dermatomyositis (n = 21), rheumatoid arthritis with vasculitis (n = 32) and vasculitis syndrome (n = 15), and others (Sjögren's syndrome (n = 1), adult-onset Stills disease (n = 1), eosinophilic fasciitis (n = 1)) were enrolled. The patients received various immunosuppressive treatments, including glucocorticoid (GC) oral therapy (≥40 mg/day)(n = 30), GC pulse therapy (n = 30), and/or intravenous cyclophosphamide pulse therapy (n = 70). 22.7-46.7% of patients were positive for C7-HRP reactivity, and this varied according to autoimmune disease and treatment strategy. Multivariate analyses revealed that higher age and maximum dose of oral GC were the two factors significantly associated with increased risk of CMV infection, while neither autoimmune disease diagnosis nor treatment strategy was a significant factor.

Conclusion: Higher age and high-dose oral GC therapy were associated with increased risk of CMV infection irrespective of autoimmune disease diagnosis or treatment strategy.

Key Words: cytomegalovirus, systemic autoimmune disease, immunosuppressive therapy, risk factor, glucocorticoids

Introduction

Cytomegalovirus (CMV) is one of the major causes of opportunistic infection after organ transplantation[1)~3)], and has been extensively investigated. As a result, guidelines for the management of CMV infection have been established[4)~7)]. CMV infection is also a major issue in patients with systemic autoimmune diseases who have been treated with immunosuppressive drugs. Along with recent advances in immunosuppressive therapy, patients

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have become more susceptible to opportunistic infections, and this has raised concerns over the potential for increased incidence of CMV infection[8)~10)]. However, there has been no extensive study regarding the incidence of CMV infection in systemic autoimmune diseases. Indeed, it is well known that the incidence of CMV infection is generally high among patients receiving immunosuppressive treatment, and that the outcomes for those patients are poor because CMV infection deteriorates various organ functions[11)~13)]. Since systemic autoimmune diseases are essentially multi-organ diseases, and CMV infection after immunosuppressive therapy might be lethal, clearer management guidelines should be established.

In this report we examined the incidence and susceptibility factors for CMV infection in patients undergoing daily care for systemic autoimmune diseases.

Patients and Methods

1. Patients

We investigated patients with systemic autoimmune diseases including systemic lupus erythematosus (SLE, n = 37), systemic sclerosis (SSc, n = 22), polymyositis/dermatomyositis (PM/DM, n = 21), rheumatoid arthritis with vasculitis (RA with vasculitis, n = 32) and vasculitis syndrome (n = 15), and others (n = 3; Sjögren's syndrome, adult-onset Stills disease, and eosinophilic fasciitis), who were hospitalized in the Department of Rheumatology, Tokyo Women's Medical University Hospital and received immunosuppressive treatment including (1) oral glucocorticoid therapy (GC oral therapy)(prednisolone ≥40 mg/day), (2) glucocorticoid pulse therapy (GC pulse therapy) (methylprednisolone 1,000 mg infusion for 3 days) or (3) intravenous cyclophosphamide pulse therapy (IVCY therapy, cyclophosphamide infusion 400-500 mg/m^2) from April, 2010 to March, 2012. Patients who received both GC oral therapy and GC pulse therapy were categorized as GC pulse therapy, and those who received all three were categorized as IVCY therapy in the analysis. In this study, we did not evaluate the medical treatments the patients received prior to the initiation of immunosuppressive therapy.

2. Study protocol

Baseline patient clinical features before each immunosuppressive therapy, including the diagnosis, age, sex, body weight, height, BMI (body mass index) and laboratory data, were retrospectively collected. The ethical committee of The Tokyo Women's Medical University approved the present study (No. 3135). Data regarding the maximum doses of glucocorticoid (mg/day), CMV antigenemia, duration from the start of treatment and CMV antigenemia were investigated during the treatment process. The incidence of CMV antigenemia (%) was calculated based on the treatment strategy or the disease of the patients. All patients were confirmed to be negative for CMV antigenemia before initiating the immunosuppressive therapies described above in the 'Patients' section.

3. CMV antigenemia assay

CMV antigenemia was detected by an antigen assay performed using the monoclonal antibody C7-HRP (Teijin, Tokyo, Japan) raised against CMV immediate early antigen. In this study, CMV antigenemia was defined as the presence of antigen (≥1 antigen-positive cells/50,000 cells)[5)6)8)9)14)].

4. Definition of CMV infection

Positive CMV infection was defined in this study to be blood samples that reacted with the monoclonal antibody C7-HRP, which is different from CMV disease defined as a positive CMV antigenemia assay, and the presence of any of the following related clinical syndromes: fever, increase in atypical lymphocytes, myelosuppression (neutropenia or thrombocytopenia), or detection of CMV from a biopsy specimen.

5. Statistical analysis

Univalent analysis was conducted with CMV infection as a response variable and various baseline characteristics as explanatory variables. Based on this analysis, multivariate analysis was conducted using CMV infection as a response variable. Statistical significance was defined as a p-value$<$0.05. SAS software (Version 9.1.3; SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

Table 1 Characteristics of the patients stratified by immunosuppressive therapy

Mean ± SD

variable	GC oral therapy (PSL≥40 mg/day)	GC pulse therapy	IVCY therapy
	n = 30	n = 30	n = 70
Gender (male/female) [female %]	8/22 [73%]	6/24 [80%]	16/54 [77%]
Age (years)	45.0 ± 16.5	66.1 ± 15.9	49.6 ± 16.5
Number of lymphocytes (/μL)	1,104 ± 497	1,217 ± 796	1,195 ± 670
Hemoglobin (g/dl)	11.8 ± 1.6	11.1 ± 2.3	11.9 ± 2.5
Platelet ($\times 10^4$/μL)	26.9 ± 11.5	20.9 ± 10.2	24.8 ± 9.3
C-reactive protein (mg/dl)	1.8 ± 3.4	7.4 ± 6.2	1.5 ± 3.2
Immunoglobulin G (mg/dl)	1,766 ± 683	1,247 ± 542	1,437 ± 621
maximum dosage of GC (mg/Day)	49.7 ± 9.3	43.0 ± 14.8	38.2 ± 15.7

This table shows the patients' characteristics stratified by immunosuppressive therapy.
GC, glucocorticoid; PSL, prednisolone; IVCY, intravenous cyclophosphamide pulse therapy.

Table 2 Cytomegalovirus infection rate

Mean ± SD (median) [minimum—maximum]

Diagnosis	C7-HRP positive (%)	The days from immunosuppressive therapy to CMV infection (day)
SLE	40.5	31.8 ± 18.6 (31) [7—74]
SSc	22.7	37.8 ± 19.9 (30) [20—71]
PM/DM	47.6	39.1 ± 25.3 (28) [14—102]
RA with vasculitis	37.5	24.8 ± 14.9 (21.5) [4—57]
Vasculitis syndrome	46.7	42.6 ± 15.9 (40) [15—66]
	p = 0.47 (χ^2 test)	

Immunosuppressive therapy	C7-HRP positive (%)	Time from immunosuppressive therapy initiation to CMV infection (day)
GC oral therapy (PSL≥40 mg/Day)	26.7	32.6 ± 13.3 (28.5) [14—57]
GC pulse therapy	43.3	24.8 ± 14.0 (25) [4—57]
IVCY therapy	42.9	35.5 ± 21.3 (32.5) [7—102]
	p = 0.27 (χ^2 test)	

This table shows C7-HRP positive rate (%) and days from immunosuppressive therapy to CMV infection based on systemic autoimmune disease diagnosis or treatment strategy.
The rates of CMV infection were not significant differences among systemic autoimmune diseases or treatment strategy.
CMV, cytomegalovirus; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; PM/DM, polymyositis/dermatomyositis; RA, rheumatoid arthritis; GC, glucocorticoid; IVCY, intravenous cyclophosphamide pulse therapy.

Results

1. Patient profile

One hundred and thirty cases (30 male, 100 female), mean age 52.3 ± 18.0 (range: 18-88) years old, were investigated in this study. Patient diagnoses included SLE, SSc, PM/DM, RA with vasculitis, vasculitis syndrome, Sjögren's syndrome, adult-onset Stills disease and eosinophilic fasciitis. Patient characterizations based on the treatment strategy were analyzed and listed in Table 1. Patients who received GC pulse therapy were older and had a higher CRP level. The maximum GC dose was highest in the oral GC group.

2. CMV infection

The incidence of CMV infection was investigated based on autoimmune disease diagnosis and treatment strategy. C7-HRP positivity was detected in 22.7-46.7% of patients after 4 to 120 days (median

Table 3 Risk factors for cytomegalovirus infection using multivariate analyses

Explanatory variable	OR (95%CI)	p value
Diagnosis* (Model 1)		
Age (year)	1.07 (1.03-1.11)	0.001
Maximum dosage of GC (mg/Day)	1.09 (1.03-1.15)	0.004
SLE (vs SSc)	0.80 (0.10-6.59)	0.84
SLE (vs PM/DM)	1.45 (0.22-9.40)	0.69
SLE (vs RA with vasculitis)	4.89 (0.67-35.9)	0.12
SLE (vs vasculitis)	1.66 (0.47-5.81)	0.43
SSc (vs PM/DM)	1.81 (0.23-14.0)	0.57
SSc (vs RA with vasculitis)	6.08 (0.83-44.4)	0.08
SSc (vs vasculitis)	2.06 (0.51-8.29)	0.31
PM/DM (vs RA with vasculitis)	3.37 (0.58-19.6)	0.18
PM/DM (vs vasculitis)	1.14 (0.35-3.72)	0.83
RA with vasculitis (vs vasculitis)	0.34 (0.10-1.10)	0.07
Immunosuppressive therapy* (Model 2)		
Age (year)	1.06 (1.02-1.09)	0.002
Maximum dosage of GC (mg/Day)	1.09 (1.04-1.15)	0.001
GC oral therapy (vs GC pulse therapy)	0.93 (0.17-5.10)	0.94
GC oral therapy (vs IVCY therapy)	0.48 (0.19-1.21)	0.12
GC pulse therapy (vs IVCY therapy)	0.52 (0.19-1.41)	0.20

This table shows the risk factors for CMV infection, which were analyzed by 2 types of multivariate analyses, including the disease diagnosis (Model 1) or the treatment strategy (Model 2) as explanatory variables. In both models, only two factors, including age and maximum dose of oral glucocorticoid were significantly associated with an increased risk of CMV infection.

*In which model, Gender, BMI, the number of lymphocytes, hemoglobin, platelet, albumin, and LDH were not significant factors.

GC, glucocorticoid; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; PM/DM, polymyositis/dermatomyositis; RA, rheumatoid arthritis; IVCY, intravenous cyclophosphamide pulse therapy.

29.5 days) from the start of immunosuppressive therapy, and no significant difference was noted among the diseases (Table 2). Regarding the treatment strategy, glucocorticoid pulse therapy and IVCY resulted in higher rates of CMV infection, but no statistically significant differences were noted (Table 2).

3. Risk factor for CMV infection

Risk factors for CMV infection were analyzed by univalent and multivariate methods. First, we conducted a univalent analysis and found that CMV infection was significantly associated with older age (odds ratio [OR]: 1.04, 95% confidence intervals [95% CI]: 1.02-1.06, $p<0.01$), lower hemoglobin (OR: 0.84, 95% CI: 0.72-0.99, $p=0.04$), lower platelets (OR: 0.96, 95% CI: 0.93-0.99, $p=0.03$), lower serum albumin level (OR: 0.54, 95% CI: 0.31-0.92, $p=0.02$), higher serum LDH level (OR: 1.00, 95% CI: 1.00-1.01, $p<0.01$), and higher maximum dose of oral GC (OR: 1.06, 95% CI: 1.02-1.09, $p<0.01$).

Based on the results of the univalent analysis, we conducted a multivariate analysis concerning two models, including the disease diagnosis (Model 1) or the treatment strategy (Model 2) as explanatory variables (Table 3). In both models, only two factors, including age (OR: 1.07, 95% CI: 1.03-1.11, $p<0.01$, and OR: 1.06, 95% CI: 1.02-1.09, $p<0.01$, in Model 1 and 2, respectively) and maximum dose of oral glucocorticoid (OR: 1.09, 95% CI: 1.03-1.15, $p<0.01$, and OR: 1.09, 95% CI: 1.04-1.15, $p<0.01$, in Model 1 and 2, respectively) were significantly associated with an increased risk of CMV infection (Table 3). None of the other factors demonstrated to be significantly associated with CMV infection in our univalent analyses were significantly associated with CMV infection in our multivariate analyses.

Discussion

In this retrospective analysis, we demonstrated that CMV infection is common following immunosuppressive therapy for systemic autoimmune diseases, and determined that higher age and higher doses of oral glucocorticoid were associated with increased risk of CMV infection. These findings are consistent with previous reports[9)14)15)]. Although higher age was a common risk factor for CMV infection in patients with systemic autoimmune disease, glucocorticoid treatment is controversial. Takizawa et al reported age greater than 59.3 years and pulsed methylprednisolone (mPSL) therapy were risk factors for poorer outcome for CMV infection[9)]. Hanaoka et al also reported that the risk factors for CMV infection were old age (>65 years) and high-dose oral glucocorticoids (over 50 mg/day) or pulsed mPSL therapy[15)], whereas Yamashita et al reported that only advanced age (>70 years) was a risk factor for CMV infection in patients with systemic autoimmune disease[14)]. In this study, the risk of CMV antigenemia did not vary across different types of systemic autoimmune disease. However, some studies have suggested that systemic lupus erythematosus (SLE) and polymyositis/dermato-

myositis (PM/DM) may increase the risk of CMV disease with lymphopenia[16].

Based on our results, we should emphasize that CMV reactivation occurs in approximately 40% of patients with systemic autoimmune diseases after immunosuppressive therapy, especially in older-aged patients and in patients who have used high-dose oral GC. The soonest that CMV antigens were detected was four days after the start of immunosuppressive treatment; thus, we strongly suggest conducting screening for CMV antigens at least within one week after the initiation of immunosuppressive treatment.

Since there have been reports of systemic autoimmune cases of cytomegalovirus (CMV) infection associated with a poor outcome[13)~15)], preemptive therapy may be helpful for some patients. However, no guidelines exist for the treatment of CMV infection in patients with systemic autoimmune diseases. We believe that ganciclovir therapy for CMV antigenemia should be guided by clinical symptoms, in addition to the number of C7-HRP-positive cells. Of the 130 patients reviewed in this study, 51 were positive for CMV antigenemia, but only 23 were actually treated with ganciclovir. Of them, 17 patients appeared to have experienced clinical symptoms associated with CMV infection, including thrombocytopenia (n = 11), lymphopenia (n = 1), pancytopenia (n = 1), neutropenia (n = 3), and pneumonia (n = 1). The remaining 6 patients treated with ganciclovir were asymptomatic, but all of them had a CMV antigen-positive cell count of 7/50,000 cells or more. According to the guidelines for hematopoietic cell transplantation, the presence of 2 positive cells/50,000 cells or more is an indication for ganciclovir treatment in high-risk patients receiving systemic glucocorticoid therapy, even if asymptomatic. Further study is warranted to identify patients with systemic autoimmune diseases, especially those positive for asymptomatic CMV antigenemia, who should be treated with ganciclovir, and to assess their outcomes.

The major limitation of this report is the retrospective design of the study. CMV antigens were not routinely monitored in all cases, thus the time course of CMV antigenemia was not well demonstrated. Other limitations include the presence of concomitant therapies that might affect the immune status of patients with systemic autoimmune diseases.

Furthermore, we did not evaluate the association of CMV antigenemia and symptoms of CMV infection.

Another limitation of this study is that we did not include the medical treatments the patients may have received before immunosuppressive treatment was initiated, since the patients in this study consisted of a mixture of new-onset and relapsed systemic autoimmune disease patients.

Further study should be conducted using a prospective study design that addresses the concerns we have raised.

Conclusion

Higher age and high-dose oral glucocorticoids were associated with increased risk of CMV infection. We strongly suggest screening for CMV antigenemia at least within one week after the initiation of immunosuppressive treatment, especially in older individuals or those who have received high-dose oral GC.

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膠原病患者に対する免疫抑制療法とサイトメガロウイルス C7-HRP 抗原検査陽性例に対する検討

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〔目的〕自己免疫疾患患者に対して免疫抑制療法を行った場合にサイトメガロウイルス(CMV)感染を生じることは稀ではない．本研究は日常診療において免疫抑制療法を受けた自己免疫疾患患者における CMV 感染(症状を呈し組織から CMV が検出される CMV 感染症とは異なる）の現状を明らかにする．〔方法〕2010 年 4 月 1 日～2012 年 3 月 31 日に東京女子医科大学病院リウマチ科に入院し，①グルココルチコイド (GC) 経口療法 (PSL≧40 mg/日)，②GC パルス療法，③シクロホスファミド静注療法 (IVCY) の治療を行い，治療開始後に抗原血症検査 (C7-HRP) が施行された患者に対し後ろ向きに自己免疫疾患別，免疫抑制療法別の CMV 感染率を算出し，多変量解析にて CMV 感染のリスク因子を検討した．〔結果〕対象患者 130 例(男性 30，女性 100)の平均年齢は 52.3±18.0 歳で，疾患別には全身性エリテマトーデス (n=37)，全身性強皮症 (n=22)，多発性筋炎・皮膚筋炎 (n=21)，悪性関節リウマチ (n=32)，血管炎 (n=15)，その他 (n=3) の症例であり，免疫抑制療法別には GC 経口療法 (PSL≧40 mg) (n=30)，GC パルス療法 (n=30)，IVCY 療法 (n=70) であった．C7-HRP 陽性率は，疾患別または免疫抑制療法別に 22.7～46.7% であり，治療開始から CMV 陽性までの期間は 4～120 日(中央値 29.5 日)であった．多変量解析において，加齢および GC 最大投与量が有意な CMV 感染のリスク因子であったが，各疾患や免疫抑制療法の違いは有意なリスク因子ではなかった．〔結語〕免疫抑制療法の内容や自己免疫疾患の種類に関わらず，加齢および GC 最大投与量が CMV 感染の有意なリスク因子であった．