Incidence and clinical features of cytomegalovirus infection diagnosed by cytomegalovirus pp65 antigenemia assay during high dose corticosteroid therapy for collagen vascular diseases

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Abstract

Objective

To investigate the incidence and clinical features in patients with cytomegalovirus (CMV)-positive antigenemia during high dose corticosteroid therapy for collagen vascular diseases, and risk factors associated with it.

Patients and methods

We examined retrospectively 35 consecutive patients for the presence of CMV-positive pp65 antigenemia. The patients were admitted to Saka General Hospital from 2000 to 2003, and were administered more than 0.5 mg/kg of body weight/day of peroral prednisolone for collagen vascular diseases. Characteristics of patients with and without CMV-positive antigenemia were compared.

Results

CMV-positive antigenemia was detected in 14 patients (40.0%), including six with microscopic polyangitis, three with rheumatoid arthritis, and five with other conditions. Three patients (8.6%) were diagnosed as having a CMV disease: pneumonitis or encephalitis. Symptoms and laboratory findings, including slight fever and a low increase in levels of hepatic enzymes and cytopenia, were observed in 10 of the 14 patients. Two patients died of CMV diseases refractory to ganciclovir. Ages of more than 70 years old were associated with the presence of CMV-positive antigenemia (relative risk = 4.5, 95% confidence interval = 1.14-17.6).

Conclusion

CMV infection diagnosed by CMV pp65 antigenemia assay is not rare during high dose corticosteroid therapy for collagen vascular diseases, and advanced age is considered a risk factor for it. It has a variety of symptoms and laboratory findings, which are mild and nonspecific to this type of infection, and they may not be clearly noted as clinical signs of CMV infection, even in patients with CMV diseases whose prognoses can be unsatisfactory. During high dose corticosteroid therapy for collagen vascular diseases, careful attention should be paid to CMV infection.

Key words

Cytomegalovirus infection, cytomegalovirus pp65 antigenemia, collagen vascular diseases, clinical features, risk factors.

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Introduction

Cytomegalovirus (CMV), a member of the family *Herpesviridae*, is a well-recognized pathogen that causes an opportunistic and potentially life-threatening infection in immunocompromised patients with acquired immunodeficiency syndrome, and after visceral organ and bone marrow transplantation. CMV infection has been associated with morbidity and mortality in these conditions (1, 2).

Collagen vascular diseases (CVDs) involve multiple organs that are affected by immunological mechanisms. CVDs that involve organs, such as the kidney, lung and central nervous system, are often refractory to corticosteroid therapy alone, and their prognoses are not always satisfactory (3-7). Although recent reports have suggested that more aggressive application of high doses of corticosteroids and immunosuppressive agents may improve prognoses of refractory diseases, this strategy has increased the frequency of opportunistic infections, including bacterial, fungal and viral infections, which have been major causes of death in patients with CVDs (8-16). Although CMV infection is a complication of immunosuppressive therapy for CVDs, the incidence, clinical features and prognosis of CMV infection complicated in CVDs have not been clarified (17-21).

In the present study, we investigated incidence and clinical features of CMV infection that developed during high dose corticosteroid therapy for CVDs, and its risk factors by using CMV pp65 antigenemia assay; this assay is the principal method used to diagnose CMV infection, because it is not only very sensitive (91%) and specific (95%), but is also useful to detect CMV infection early, and it also reflects the activity of CMV infection (22).

Patients and methods

Patients

We evaluated retrospectively 35 consecutive patients with CVD who were admitted to Saka General Hospital from 2000 to 2003, and who received an initial dose of more than 0.5 mg/kg of body weight/day of prednisolone (PSL) (Table I). The 35 patients consisted of 11 patients with systemic lupus erythematosus (SLE) (23), 8 with microscopic polyangitis (MPA) (24), 5 with rheumatoid arthritis (RA) (25), 3 with polymyositis/ dermatomyositis (PM/DM) (26), 2 with Sjögren's syndrome (SjS) (27), 2 with Takayasu arteritis (28) and one each with Behçet's disease (BD), Wegener's granulomatosis, giant cell arteritis, and Henoch-Schönlein purpura (HSP) (29-32). As each patient with RA, SjS, BD, and HSP, suffered from severe disorders, such as interstitial pneumonia or IgA nephropathy, high dose corticosteroid therapy was required. The mean initial dose of oral PSL was 0.97 ± 0.22 mg/kg of body weight/day. Almost all patients continued with the initial dose of oral PSL for four weeks, and then by doses decreased by 10% a week. Halfway after beginning PSL treatment, doses of PSL were not increased in any patient. In addition to oral PSL, steroid pulse therapy and immunosuppressive agents were administered as required. Intravenous methylprednisolone used in steroid pulse therapy was administered fortnightly at 1 g for three consecutive days in each course, not exceeding three courses. Also administered were intravenous cyclophospha-

Table I. Profile of 35 patients with corticosteroid therapy for collagen vascular diseases.

Number of patients	35
Male:Female	13:22
Age (yrs)	65.3 ± 18.3* (16-91)
Underlying disease	
SLE	11
MPA	8
RA	5
PM/DM	3
Other conditions	8
Organ affected	
Kidney	15
Lung	12
Initial dose of PSL (mg/kg/day)	0.97 ± 0.22*
Steroid pulse therapy	20
Immunosuppressant agents	11

SLE: systemic lupus erythematosus; MPA: microscopic polyangitis; RA: rheumatoid arthritis; PM: polymyosits; DM: dermatomyositis; PSL: prednisolone; *mean ± SD.

mide (IVCY) monthly at a dose of 0.5 g/m^2 of body surface area, azathioprine (AZA) at a dose of 50-100 mg/day, and cyclosporine A (CsA) at a dose of 150-250 mg/day, maintaining the serum trough level between 100 and 200 ng/ml.

Monitoring CMV antigenemia

Specimens for the detection of CMV antigen-positive blood leukocytes (CMV antigenemia) were obtained biweekly after the beginning of PSL treatment until the PSL dose was decreased to less than 30 mg/day or 0.5 mg/kg of body weight/day, and additionally if clinical signs characteristic of CMV infections, such as fever, cough or abnormal laboratory findings appeared. Detection of CMV antigenemia was performed according to the method described in detail previously (33). In this method, the presence of CMV pp65 antigen-positive leukocytes on each slide stained by using a direct immunoperoxidase technique with the peroxidase-conjugated monoclonal antibody (HRP-C7) was observed by using light microscopy. The degree of CMV antigenemia was expressed as the number of CMV pp65 antigen-positive cells per 50,000 leukocytes.

Definition of CMV diseases

As CMV diseases, CMV pneumonitis was defined as the demonstration of either nuclear or cytoplasmic inclusions in specimens from pulmonary tissue obtained at lung biopsy or autopsy, in addition to clinical signs such as dyspnea, hypoxemia or ground glass opacity detected by chest X-ray film or computed tomography, and CMV encephalitis was defined as the demonstration of CMV DNA in cerebrospinal fluid, in addition to symptoms such as headache.

Treatment for CMV infection diagnosed by using antigenemia assay

To treat CMV infection, ganciclovir was administered to patients who had more than 10 CMV pp65 antigen-positive cells per 50,000 white blood cells, according to the definition by van den Berg *et al*. (34). Even if this number was less than 10 per 50,000 white blood cells, patients with unexplained symptoms or laboratory findings suggestive of CMV infection received ganciclovir. Ganciclovir treatment continued until patients were free from CMV antigenemia.

Statistical analysis

Statistical significance was calculated by using the Fisher's exact test or Student's t test where indicated. A p value of < 0.05 indicated statistical significance. Associations were expressed using relative risk (RR) with 95% confidence intervals (95% CI).

Results

Characteristics of patients with CMV-positive antigenemia

CMV-positive antigenemia was detected in 14 of 35 patients (40.0%) (Table II). The mean age of the 14 patients was 75.1 ± 9.1 (54-91) years, and 12 of the14 patients were more than 70 years old. The mean initial dose of oral PSL was 1.06 ± 0.15 mg/kg of body weight/day; all 14 patients received more than 0.8 mg/kg of body weight/-day. The mean interval from the beginning of corticosteroid therapy until the initial detection of CMV-positive antigenemia was 33.7 ± 15.3 (12-66) days (Fig. 1).

Table II. Characteristics of the 14 patients with CMV-positive antigenemia.

No.	Age/Sex	Disease	Disorders	Treatment for underlying disease			Duration** (days)	CMV antigenemia (per 50.000)	Treatment for CMV infection***	Outcome
				PSL (mg/kg/day)	mPSL*	Immuno-suppressant				
1	57/M	MPA	AH+Crescentic GN	1.2	2	CsA+IVCY	34	279	+	Death†
2	73/M	MPA	AH+Crescentic GN	1	2	IVCY	21	148	+	Survival
3	71/F	MPA	Crescentic GN	1.2	1	-	34	13	+	Death
4	80/F	MPA	Crescentic GN	1.2	0	-	50	10	+	Death
5	77/F	MPA	Crescentic GN	1.2	2	-	14	4	-	Survival
6	73/F	MPA	Crescentic GN	0.8	0	-	51	1	-	Death
7	74/M	RA	Crhonic IP¶	1.2	2	AZA	29	41	+	Death‡
8	80/F	RA	Subacute IP	1	3	-	26	28	+	Survival
9	75/F	RA	IgAN	1	0	-	40	55	+	Survival
10	85/F	SLE	Lupus nephritis	0.8	0	-	48	12	+	Survival
11	73/F	SLE	CNS	1	1	-	26	8	-	Survival
12	91/F	GCA	-	1	1	AZA	66	137	+	Death‡
13	79/F	SjS	Subacute IP	1	0	-	18	2	-	Survival
14	54/M	DM	Acute IP	1.2	0	CsA	12	299	+	Survival

*Values are given as the number of courses.
**Interval from the start of corticosteroid therapy until the initial detection of CMV-positive antigenemia.
***Administration of ganciclovir.
¶Acute exacerbation of chronic IP.
†Death from underlying disease refractory to immunosuppressive therapy.
‡Death from CMV disease refractory to ganciclovir.
GCA: giant cell arteritis; SjS: Sjögren's syndrome; AH: alveolar hemorrhage; GN: glomerulonephritis; IP: interstitial pneumonia; IgAN: IgA nephropathy; CNS: central nervous system; mPSL: methylprednisolone pulsed therapy; CsA: cyclosporine A; IVCY: intravenous cyclophosphamide; AZA: azathioprine.

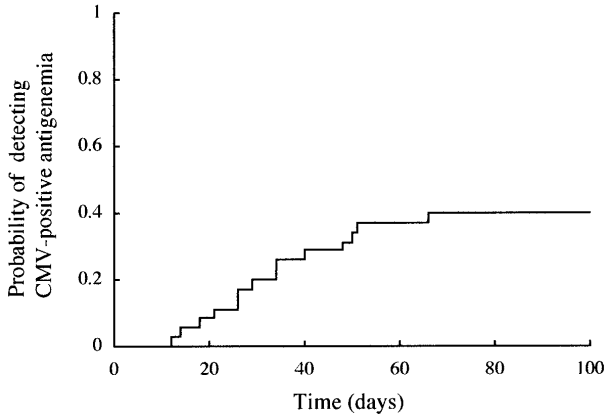

Fig.1. Probability of detecting CMV-positive antigenemia. The time is the interval from the beginning of corticosteroid therapy until the initial detection of CMV-positive antigenemia.

Clinical features and maximum levels of antigenemia

A slight fever was present in 5 of the 14 patients (35.7%), and respiratory symptoms, such as dyspnea and wheezing, were present in 2 patients (14.3%) (Table III). Headache and nausea were each in one patient (7.1%). Mild cytopenia was observed in 6 patients (42.9%): leucopenia in 2 patients, anemia in 2 and thrombocytopenia in 4. Atypical lymphocytes were observed in 2 patients (14.3%). A low increase in levels of hepatic enzymes was observed in 6 patients (42.9%). Deterioration of hypoxemia was observed in 2 patients (14.3%) and of hyponatremia in one (7.1%). Three of 35 patients (8.6%) were diagnosed as having CMV diseases: CMV pneumonitis in 2 patients (Patients 1 and 7), and CMV encephalitis in one (Patient 12). The two patients with CMV pneumonitis suffered from either interstitial pneumonia (IP) or alveolar hemorrhage (AH) as an underlying condition, and CMV pneumonitis developed while they were on a mechanical ventilator. Minor clinical signs, including unexplained fever for three or more days, cytopenia and an increase in levels of hepatic enzymes without CMV diseases, were observed in 7 of the 14 patients with CMV-positive antigenemia. Antigenemia alone without clinical symptoms and with normal laboratory findings was in 4 patients. No patient exhibited CMV-associated gastrointestinal symptoms, retinitis or nephropathy. The number of CMV antigenemia-positive cells per 50,000 white blood cells ranged from one to 299 (Table II).

Table III. Clinical and laboratory features with CMV-positive antigenemia.

Clinical and laboratory features	Number of patients (%)
Fever	5 (35.7)
Respiratory symptoms	2 (14.3)
Headache	1 (7.1)
Nausea	1 (7.1)
Hematological abnormalities	8 (59.1)
Leucopenia	2 (14.3)
Anemia	2 (14.3)
Thrombocytopenia	4 (28.6)
Atypical lymphocyte	2 (14.3)
Hepatic enzyme elevation	6 (42.9)
Hypoxemia	2 (14.3)
Hyponatremia	1 (7.1)
No abnormal findings	4 (28.6)

Treatment and outcomes

Ten of the 14 patients (71.4%) received ganciclovir for CMV infection (Table II). The remaining 4 patients for whom the number of CMV antigenemia-positive cells was less than 10 cells per 50,000 white blood cells did not require anti-CMV agents; the gradual decrease in the dose of PSL resulted in their antigenemia being undetectable. No patient who received ganciclovir exhibited adverse effects, such as cytopenia. Two patients had a recurrence of CMV antigenemia; CMV antigenemia recurred within one week after completion of ganciclovir therapy in Patient 3, and two months after ganciclovir therapy, at 0.4 mg/kg of body weight/day (25 mg) of PSL in Patient 14. Readministration of ganciclovir resolved CMV re-infection in both patients. Twenty-eight of the 35 patients survived (80.0%), and seven patients died (20.0%) of whom two patients died of CMV diseases refractory to ganciclovir: CMV pneumonitis (Patient 7) and encephalitis (Patient 12). Another two patients died of underlying diseases: AH associated with MPA (Patient 1) and acute exacerbation of chronic IP associated with RA (Patient without CMV-positive antigenemia). The remaining three patients died of other causes, including invasive aspergillosis and intestinal perforation; histological examination of surgical specimens obtained from the small intestinal showed that intestinal perforation was not associated with either underlying CVDs or CMV diseases. In four patients, CMV infection occurred on critical conditions resulting in death associated or not with underlying diseases.

Other opportunistic infections

Other infections were detected in 10 of the 35 patients. Seven patients suffered from bacterial pneumonia or bronchitis, the causative organisms of which included *Streptococcus pneumonia*, *Moraxicella catarrhalis*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. Individual patients were affected by invasive aspergillosis, *Pneumocystis carinii* pneumonitis, Herpes zoster, and Herpes simplex-associated pneumonitis. Invasive aspergillosis was uncontrollable and proved fatal, but antibiotics and anti-viral agents resolved the other infections.

Risk factors for CMV-positive antigenemia

The clinical characteristics of patients with and without CMV-positive antigeneima were compared, particularly in terms of age, underlying diseases and disorders, initial dose of oral prednisolone, steroid pulse therapy, and immunosuppressive agents (Table IV). Patients with CMV-positive antigenemia were older than those without it, and the difference was significant (75.1 ± 9.1 vs. 59.1 ± 20.2, $p < 0.05$). The group with CMV-positive antigenemia was given a significantly higher initial dose of PSL than the group without it

Table IV. Comparison of patients with and without CMV-positive antigenemia.

	With	Without
Number of patients	14	21
Male:Female	4:10	9:12
Age (yrs)*	75.1 ± 9.1†	59.1 ± 20.2†
Underlying disease		
MPA	6‡	2‡
RA	3	2
SLE	2	9
PM/DM	1	2
Other conditions	2	6
Involvement and disorder		
Kidney	8	7
Crescentic GN	6‡	2‡
Lung	6	6
IP	4	5
AH	2	1
Initial dose of PSL (mg/kg/day)*	1.06 ± 0.15†	0.91 ± 0.23†
Duration of PSL therapy	33.7 ± 15.3[a]	58.6 ± 18.2[b]
Average dose of PSL (mg/day)	49.4 ± 13.7[a]	41.1 ± 8.1[b]
Steroid pulse therapy (courses)*	1.00 ± 1.00	1.14 ± 1.24
Immunosuppressant agents	5	6
IVCY	2	4
AZA	2	1
CsA	2	1

*mean ± SD, †$p < 0.05$, Student's t test, ‡$p < 0.05$, Fisher's exact test.
[a] Days from the beginning of corticosteroid therapy to the presence of CMV-positive antigenemia.
[b] Days from the beginning of corticosteroid therapy to the time of the reduction of the dose to 0.5 mg/kg of body weight/day.

(1.06 ± 0.15 vs. 0.91 ± 0.25 mg/kg of body weight/day, $p < 0.05$). Steroid pulse therapy and administration of immunosuppressive agents produced no significant differences between the two groups. MPA was more frequently observed in patients with CMV-positive antigenemia, and was statistically significant (42.9% vs. 10.5%, $p < 0.05$). In particular, the age of more than 70 years old was associated with CMV positive antigenemia (RR = 4.5, 95% CI = 1.14-17.6), but no initial dose of PSL was associated with it.

Discussion

CMV not only causes opportunistic infections as a complication of immunosuppressive therapy in CVDs (17-21), but may also have a pathogenic role in the development or exacerbation of CVDs (35-38). In addition, it has been suggested that even without active and systemic CMV infection, local CMV reactivation may directly induce excessive extracellular matrix deposition in cutaneous tissues of patients with systemic sclerosis (39). In the present study, although the negativity for CMV antigenemia before the start of steroid therapy was not confirmed in all patients, the first CMV antigenemia assay after the start of steroid therapy showed negative findings for each patient without prior confirmation of its presence; CMV did not induce the systemic underlying diseases in any of patients with CMV-positive antigenemia.

CMV-positive antigenemia was detected in 14 (40.0%) of 35 patients receiving steroid therapy for CVDs. Almost all our patients were considered to have no primary CMV infection but a reactivation of latent infection, because CMV-IgG is positive in more than 95% and 99% of Japanese who are 50 and 60 years of age or older, respectively (40). Zhang *et al.* reported that 5 out of 21 children (23.8%) on immunosuppressive therapy for SLE were diagnosed as having CMV infection using serological tests (19). Mori *et al.* reported that CMV reactivation was detected in peripheral blood leukocytes in 7 of 17 patients (41%) and in plasma cells in 5 of 17 patients (29%) by using quantitative real-time polymerase chain reaction assay (41). CMV infection is considered to be a common complication in CVDs treated by high doses of corticosteroids. The prevalence of CMV diseases may have been underestimated, because perform a lung biopsy for patients with severe respiratory distress to confirm CMV pneumonitis was difficult.

Symptoms and laboratory findings associated with CMV infection were extremely varied. As many of them were mild and nonspecific to this type of infection, they were not clearly noted as clinical signs of CMV infection and were not always diagnostic, even in the two patients with CMV pneumonitis whose chest X-ray films at diagnosis of CMV pneumonitis showed no remarkable changes compared with the previous those. In some patients, it was also necessary to exclude the possibility that these findings may be of other opportunistic infections, adverse effects of immunosuppressive agents, or an exacerbation of underlying diseases. Symptoms of CMV infection may be too similar for them to be differentiated from symptoms due to the exacerbation of CVDs (17). Identifying and managing CMV infection as early as possible is essential, because prognoses of CMV diseases, such as CMV pneumonitis, can be unsatisfactory even with the administration of anti-CMV agents (42). Therefore, during high dose corticosteroid therapy for collagen vascular diseases, CMV infection should be aggressively diagnosed.

Four patients with antigenemia alone and who had neither symptoms nor abnormal laboratory findings suggestive of CMV infection were monitored without ganciclovir therapy. The gradual decrease in the dose of PSL resulted in a complete resolution of CMV-positive antigenemia so that it was undetectable. The fact seems reasonable that ganciclovir should not be administered at least for patients with less than 10 CMV-positive cells. Administration of ganciclovir based on monitoring CMV

antigenemia may have resulted in overzealous treatment; further control studies are required to clarify if this preemptive treatment is preventive.

CMV-positive antigenemia recurred in 2 of 11 patients who were administered ganciclovir. In Patient 14, CMV-positive antigenemia recurred two months after the completion of ganciclovir therapy at a dose of 0.4 mg/kg of body weight/day (25 mg/day) of PSL, suggesting that a dose reduction to less than 30 mg/day or 0.5 mg/kg of body weight/day of PSL may not necessarily allow for the discontinuation of constant monitoring for the presence of CMV antigenemia in patients after the completion of ganciclovir therapy.

When comparing clinical characteristics of patients with and without CMV-positive antigenemia, the former patients were significantly older, received a higher initial dose of PSL, and more frequently suffered from MPA than those without it. However, whether MPA itself was a risk factor was unclear, because many patients with MPA were of an advanced age. While whether AZA and CsA were risk factors was not conclusive for less frequency of usage, steroid pulse therapy and IVCY were not considered risk factors, as the frequency of uses of steroid pulse therapy and IVCY did not differ statistically significantly between patients with and without CMV-positive antigenemia. It was recently published that steroid-free immunosuppressive agents reduced frequency of CMV infection simultaneous pancreas-kidney transplant recipients (43). Immunosuppressive agents other than corticosteroids may be beneficial also in patients with collagen vascular diseases. Advanced age was considered a risk factor for CMV infection. In particular, the age of more than 70 years old was associated with the presence of CMV-positive antigenemia. Although no initial dose of PSL was associated with it, whether the PSL dose is a risk factor for it should be further investigated. Interstitial lung diseases, including IP and AH, were underlying conditions in the two patients with CMV pneumonitis. The fact should be noted that patients with serious interstitial lung diseases may likely suffer from CMV pneumonitis, although these conditions were not observed more frequently in patients with CMV-positive antigenemia.

In present study, 6 of 14 patients with CMV-positive antigenemia died. Three of the six patients died of opportunistic infections, including the two patients with CMV diseases refractory to ganciclovir; all the 3 patients were more than 70 years old. If a lower initial dose of PSL had been administered, the frequency of death linked to opportunistic infections may have decreased. However, there is no evidence that patients with CVDs who are of an advanced age may be administered a lower dose of PSL than younger patients; in our study, another 2 patients died of their underlying diseases refractory to immunosuppressive therapies.

In conclusion, CMV infection diagnosed by CMV pp65 antigenemia assay is not rare during high dose corticosteroid therapy for collagen vascular diseases, and advanced age is considered a risk factor for it. It has a variety of symptoms and laboratory findings, which are mild and nonspecific to this type of infection, and they may not be clearly noted as clinical signs of CMV infection, even in patients with CMV diseases whose prognoses can be unsatisfactory. During high dose corticosteroid therapy for collagen vascular diseases, careful attention should be paid to CMV infection.

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