



Cytomegalovirus (CMV) infection in HIV/AIDS patients and diagnostic values of CMV-DNA detection across different sample types

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Background: Cytomegalovirus (CMV) is a common herpesvirus that has spread throughout the human population. It can easily cause organ damage in individuals who are immunocompromised. While many studies have examined the virology and immunology of simple CMV infection, few articles have described the unique immunological features of patients with HIV-CMV coinfection.

Methods: CMV infection was detected in 808 HIV/AIDS inpatients in our center from January 2017 to October 2019, and the relevant data from these patients were retrospectively analyzed. HIV-RNA, CD4+ T lymphocyte count, CMV-DNA, and related antibodies were measured in all patients. The positive rates of CMV-DNA in blood, urine, and cerebrospinal fluid (CSF) were compared. The positive rates between anti-CMV-immunoglobulin M (IgM) antibody and CMV-DNA in blood were compared. The correlation between immune status and CMV positive rate was analyzed based on the CD4+ T lymphocyte count.

Results: The overall positive rate of CMV infection in HIV/AIDS patients was 29.05%. The positive rate of CMV-DNA in urine, blood, and CSF samples was 25.27%, 26.01%, and 5.70%, respectively, and showed no significant difference between urine and blood. However, plasma CMV-IgM antibody level was significantly different between the urine and blood. The absolute CD4+ T lymphocyte count and the HIV-RNA level were significantly different between the CMV-infected group and the non-CMV infected group.

Conclusions: Low CD4+ T lymphocyte count and high HIV-1 viral load are risk factors for CMV infection in HIV/AIDS patients. Detection of urine or plasma CMV-DNA by using real-time fluorescence quantitative polymerase chain reaction (PCR) is highly valuable in screening CMV infection in HIV/AIDS patients, while detection of blood CMV IgG and CMV IgM levels has limited clinical value.

Keywords: Cytomegalovirus (CMV); AIDS; CD4+ T lymphocytes; HIV-RNA quantification

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Introduction

Cytomegalovirus (CMV) is a common herpesvirus that is widespread across human populations. CMV infection usually does not cause symptoms and mainly affects the salivary glands, kidneys, and other organs. However, viral

replication can occur in immunocompromised patients and may contribute to the damage to organs and their functions. Opportunistic infections are more frequent and severe in HIV/AIDS patients. CMV virus infection is common in these patients and can affect multiple organs/systems

including the eyes, lungs, nervous system, and digestive system. Many studies have explored the virological and immunological characteristics of simple CMV infection (1), especially those found in women and children. The immunological features of CMV infection, however, are quite unique in already immunocompromised HIV/AIDS patients. There are differences in clinical characteristics, treatment measures, prognostic factors between HIV patients with CMV infection and those with CMV infection alone (2). The guideline of China recommended that immediately antiviral therapy of CMV and ART are needed in those HIV/AIDS patients with complications (retinitis, pneumonia, CMV encephalitis, enteritis, etc.) when CMV infection is identified. Because of the non-specificity of clinical symptoms caused by CMV infection, laboratory examination is the main basis for the diagnosis of CMV infection. The most two widely used clinical methods are CMV virus replication and serum immunological methods including the detection of CMV IgG, CMV IgM and CMV antigen PP65 (2). In patients with impaired immune function, the production of CMV antibody is weakened or delayed, which may lead to false negative and increase the rate of missed diagnosis, therefore PCR detection of CMV-DNA has largely become the standard method for diagnosis of CMV infection and monitoring of treatment (3). Daisuke Mizushima *et al.* reported that plasma CMV-DNA PCR has important value in the diagnosis for both CMV retinitis and all CMV-EOD in patients with advanced HIV-1 infection (4). CMV-DNA PCR can be detected in a variety of sample types such as plasma, urine, cerebrospinal fluid, aqueous humor, feces, etc. (5). However, few articles have analyzed and discussed the significance and value of CMV-DNA PCR detection in samples other than plasma. In the present study we retrospectively analyzed the laboratory tests (including CMV-DNA, CMV antibody, CD4⁺ T lymphocyte count, HIV viral load, etc.) in 808 HIV/AIDS patients who were treated in our center from 2017 to 2019 to understand the CMV coinfection and the strengths and weaknesses of several detection methods. We present the following article/case in accordance with the MDAR reporting checklist (available at <http://dx.doi.org/10.21037/apm-20-1352>).

Methods

Subjects

A total of 808 HIV/AIDS inpatients admitted to our center

between January 2017 and October 2019 were enrolled. HIV-RNA, CD4⁺ T lymphocyte count, CMV-DNA, and related antibodies were measured in each patient. The diagnosis of HIV infection was based on the *HIV/AIDS Diagnosis and Treatment Guidelines* (third edition) (6), and all patients were confirmed as HIV infection by the Fuzhou Municipal Centers for Disease Control (CDC) or the local CDC which treated the patient. Diagnosis of CMV infection was based on the following criteria: (I) CMV-DNA positive in plasma, alveolar lavage fluid, urine, cerebrospinal fluid (CSF), and/or other body fluids; (II) serum CMV-immunoglobulin M (IgM) positive; (III) an ophthalmological diagnosis of CMV retinitis; (IV) CMV pneumonia mainly manifesting as fever, cough, phlegm, and chest tightness; CMV-DNA positive in alveolar lavage fluid; (V) CMV enteritis or esophagitis mainly manifesting as dysphagia/swallowing pain, diarrhea, or abdominal pain, and effective diagnostic anti-CMV treatment; (VI) CMV encephalitis manifesting as nervous system symptoms and CMV-DNA positive in CSF or brain tissue; (VII) CMV hepatitis manifesting as fever, abnormal liver function, and discomfort in the liver area, and CMV-DNA positive in liver biopsy. A diagnosis of CMV infection was made if any of the above criteria except item (II) was met or if item (II) plus any other criterion was met (7).

All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the medical ethics committee of our center (approval No. KS2019-070-01). The data are anonymous, and the requirement for informed consent was therefore waived.

Inclusion and exclusion

Inclusion criteria: patients with HIV/AIDS who first visited Mengchao Hepatobiliary Hospital of Fujian Medical University and completed the detection of HIV-RNA, CMV-DNA, CMV-IgG antibody, CMV-IgM antibody and CD4⁺ T lymphocytes within 14 days. Exclude HIV/AIDS cases that cannot complete the above testing items due to insufficient sample size.

Reagents and equipment

HIV-RNA quantification

Fluorescent quantitative real-time polymerase chain reaction (PCR) (FQ-PCR) was performed on the Mx3000p QPCR system (Agilent, USA) by using the HIV-RNA

quantification kit produced by QiaGen (Shenzhen, China).

CD4⁺ T lymphocyte counting

The instrument used was the FACSCalibur flow cytometer (BD Biosciences, USA), and the reagent kit used was BD Multitest CD3/CD4/CD8/CD45 (BD Biosciences, USA).

CMV-DNA quantification

FQ-PCR was performed on the Mx3000p QPCR system (Agilent, USA) to detect the CMV-DNA levels in plasma, urine, and CSF. The kit used was produced by Sansure Biotech (Hunan, China).

CMV antibody test

Chemiluminescence immunoassay was performed to detect anti-CMV-IgG and IgM antibodies on the Cobas e601 analyzer (Roche Diagnostics, Germany) with the supporting kits (Roche Diagnostics, Germany).

Study methods

Nucleic acid detection

After the HIV/AIDS patients were admitted to our hospital, 3 mL of fasting ethylenediaminetetraacetic acid (EDTA)-anticoagulated whole blood specimen was collected and then centrifuged at 3,000 r/min for 5 minutes to harvest 200 μ L of plasma for HIV-RNA and CMV-DNA detection. In all patients, 5 mL of urine specimen was collected in the morning and mixed thoroughly, and then 200 μ L was harvested for CMV-DNA detection. For patients with neurological symptoms, 1 mL of CSF was obtained for CMV-DNA detection. According to the detection limit and positive judgment standard of CMV-DNA kit and HIV-RNA kit. The specimens were considered positive if the CMV-DNA value was above 400 copies/mL, or if the HIV-RNA level was above 500 copies/mL.

Detection of T-lymphocyte subsets

In the HIV/AIDS patients, 3 mL of EDTA-anticoagulated fasting whole blood specimen was collected at admission and then mixed thoroughly for T-lymphocyte subset detection on a BD FACSCalibur flow cytometer using the ancillary reagents.

Detection of immune antibodies

In the HIV/AIDS patients, 3 mL of fasting blood specimen was collected at admission, centrifuged at 3,000 r/min for 5 minutes in a serum tube with gel separator, and then

tested on the machine.

All the above tests were carried out strictly in accordance with the reagent instructions in the manufacturer's manual and the standard operating procedures of the instruments.

Statistical analysis

Statistical analysis was performed using SPSS 21.0 software. The normally distributed measurement data are presented using mean \pm standard deviation ($\bar{x} \pm SD$), and non-normally distributed data are expressed as median (25–75 percentiles). The count data are expressed in percentages and rates. The comparison of the non-normally distributed measurement data between two groups was performed by using a non-parametric rank sum test, whereas the count data were compared using the Chi-square test. A P value of <0.05 was considered statistically significant.

Results

General data

The general data of 808 HIV/AIDS patients are summarized in *Table 1*. The gender and age showed no significant difference between the CMV group and non-CMV group. The absolute CD4 count in the CMV-infected group was significantly lower than that in the CMV-uninfected group ($Z=-8.87$, $P<0.001$), and the HIV-RNA level was significantly higher in the CMV-infected group than in the non-CMV infected group ($Z=-3.207$, $P<0.05$) (*Table 1*).

Positive rates of CMV-DNA, CMV-IgG, and CMV-IgM

The overall positive rate of CMV-DNA was 29.08% (235/808); specifically, it was 5.70% (13/228) in CSF 26.01% (77/296) in plasma, and 25.75% (180/699) in urine. The positive rate of CMV-IgG was 99.8% (806/808), and the positive rate of CMV-IgM was 10.40% (84/808).

Correlation between CMV infection and CD4⁺ T lymphocyte count

After grouping the CD4⁺ T lymphocyte count, we found that the proportion of patients infected with cytomegalovirus at low frequency of CD4⁺ T lymphocyte was greater than that of non-CMV infected patients. The incidence of CMV infection increased with the decline of

Table 1 General data of 823 HIV/AIDS patients

Clinical features	CMV-infected group	CMV-uninfected group	Statistics	P value
Gender, n (%)			$\chi^2=2.25$	0.143
Male	197 (83.83)	454 (79.23)		
Female	38 (16.17)	119 (20.77)		
Age (years, $\bar{x} \pm SD$)	38.68 \pm 12.04	37.68 \pm 11.76	t=1.08	>0.05
CD4 count (cells/ μ L)	18.00 (7.0–47.00)	65 (16.0–212)	Z=-8.87	<0.001
HIV-RNA level (log 10 HIV RNA copies/mL)	5.73 (4.66–6.51)	5.45 (3.95–6.29)	Z=-3.207	0.001
CMV-IgM positive rate, n (%)	37(15.74)	47(8.20)	$\chi^2=10.18$	0.002

CMV, cytomegalovirus.

Table 2 Distribution of CD4 count in CMV-infected group and the CMV-uninfected group

Group	n	CD4 ⁺ T lymphocyte count (cells/ μ L)				Statistics	P value
		0–50	50–100	100–200	>200		
CMV-infected group	235	181	30	14	10	Z=-8.94	<0.001
CMV-uninfected group	573	265	57	97	154		

CMV, cytomegalovirus.

Table 3 Comparisons of the results of plasma CMV-DNA detection and urine CMV-DNA detection with that of plasma anti-CMV-IgM antibody detection

Item	Plasma CMV-IgM positive	Plasma IgM negative	P value
Plasma CMV-DNA positive	10	46	<0.001
Plasma CMV-DNA negative	13	129	
Urine CMV-DNA positive	5	43	0.002
Urine CMV-DNA negative	18	132	

CMV, cytomegalovirus.

CD4 count (*Table 2*).

Detection of CMV-DNA in different samples

Of these 808 patients, 198 patients also received tests for plasma and urine CMV-DNA and plasma anti-CMV-IgG and CMV-IgM antibodies. By using the Chi-square analysis for paired data, we performed pairwise comparisons of urine CMV-DNA and plasma CMV-DNA with plasma CMV-IgM antibody, respectively. It was found that the results of urine and plasma CMV-DNA detection significantly differed from that of plasma CMV-IgM antibody assay ($P<0.001$), whereas the result of plasma CMV-DNA detection was not significantly different from that of urine

CMV-DNA detection ($P>0.05$) (*Tables 3,4*).

Discussion

CMV is a member of the β -herpes virus group and is characterized by its strict species specificity and widespread incidence in human populations. CMV seroprevalence is about 85% in children and up to 95% in adults. In our current study, the positive rate of CMV-IgG reached 99.8%. CMV is latent in most cases, but in immunocompromised individuals, the virus replicates exponentially and spreads throughout the body with the blood, causing CMV viremia and even organic CMV disease (8). CMV infection is one of the common opportunistic infections in HIV/AIDS

Table 4 Comparisons of the detection results of plasma and urine CMV-DNA

Item	Urine CMV-DNA positive	Urine CMV-DNA negative	P value
Plasma CMV-DNA positive	26	30	0.424
Plasma CMV-DNA negative	22	120	

CMV, cytomegalovirus.

patients. CD4⁺ T lymphocytes are the target cells of HIV virus, and their counts can often reflect the course of AIDS and may also be used as an indicator for evaluating the immune status of AIDS patients. This study analyzed the CMV infection, CD4 count, and HIV-RNA level in 808 patients with HIV/AIDS. We found that the positive rate of CMV in AIDS patients increased with the decrease of CD4 count and with the increase of HIV viral load. Also in our current study, up to 77.02% of CMV-positive patients had a CD4 count of <50 cells/ μ L, suggesting that CD4 <50 cells/ μ L is a risk factor for HIV-CMV coinfection, which is consistent with the finding of Deng *et al.* (9). The quantitative level of HIV-RNA in CMV infected group was higher than that in CMV uninfected group, which indirectly suggested that high levels of HIV-RNA viral load was another risk factor of CMV infection in HIV/AIDS patients. Therefore, It is recommended that patients with HIV/AIDS should initiate ART treatment as soon as possible to promote immune reconstitution to prevent opportunistic infections and screening for HCMV is essential for HIV/AIDS patients with a CD4 count of <50 cells/ μ L (2).

We also evaluated the values of viral nucleic acid assays and immunological methods in the clinical detection of CMV infection. Studies in China and internationally have shown that CMV-DNA appears earlier than the clinical symptoms and serological reactions of CMV infections. FQ-PCR is currently the most accurate and rapid microbial quantification method and is able to overcome some problems of traditional PCR (e.g., amplification product contamination and non-quantification) (10). In our current study, the positive rate of CMV-DNA was 5.70% (13/228) in the CSF samples of 228 patients with neurological symptoms, which was much lower than that of in plasma and urine samples, which might be explained by the blood-brain barrier and organ susceptibility. The anti-CMV-IgM antibody is often used as a marker of active viral infection; however, the positive rate of the anti-CMV-IgM antibody was only 10.40% (84/808) in our HIV/AIDS patients, which was lower than the positive rate of CMV infection reported in the non-HIV/AIDS populations

(11). In addition, its positive rate had poor agreement with the results of nucleic acid detection, which might be due to the weakened immune system or due to the delayed generation of IgM antibodies in HIV/AIDS patients. Therefore, detection of CMV-IgM antibody has a limited role in HCMV screening in HIV/AIDS patients. Moreover, the positive rate of CMV infection in 808 patients was 29.05% (235/808) in our current study, which is slightly higher than the 21.85% reported by Li *et al.*, and may be due to the greater variety of samples (including plasma, urine, and CSF) collected in this study (12). The positive rate of CMV-DNA was 25.57% in urine and 26.01% in plasma, the difference was not statistically significant. Therefore, we recommend that CMV-DNA screening in plasma and urine should be performed when the CD4⁺ T lymphocyte count is less than 200 cells/ μ L, especially when <50 cells/ μ L; in addition, detection of CMV-DNA in CSF is required if obvious neurological symptoms are present.

Conclusions

In conclusion, low CD4⁺ T lymphocyte count and high HIV-1 viral load are risk factors for CMV infection in HIV/AIDS patients. Detection of urine or plasma CMV-DNA by FQ-PCR is highly valuable in screening CMV infection in HIV/AIDS patients, while detection of blood CMV IgG and CMV IgM levels has limited clinical value. Blood or urine CMV-DNA screening is recommended for AIDS patients to prevent and diagnose CMV infection early and improve their quality of life.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/apm-20-1352>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the medical ethics committee of our center (approval No. KS2019-070-01). The data are anonymous, and the requirement for informed consent was therefore waived.

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