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ORIGINAL ARTICLE

Comparison of cytomegalovirus pp-65 antigenemia assay and plasma DNA correlation with the clinical outcome in transplant recipients

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Summary

The relationship between quantitative antigenemia and plasma DNAemia was studied for monitoring cytomegalovirus (CMV) viremia in CMV infection (CMVI) or disease (CMVD), in 20 transplant recipients (13 CMD, seven CMVI). A total of 142 samples of blood were assayed for CMV-DNA and pp-65 antigenemia (CMV-Ag). A quantitative correlation between both markers was found ($P < 0.0001$). First CMV antigenemia as well as first plasma DNA viral load was similar in CMVI and CMVD (29 vs. 24 CMV-Ag+ cells/10^5 PMN; and 7445 vs. 12407 CMV-DNA copies/ml). The maximum antigenemia was higher in CMVD than in CMVI (146 ± 87 vs. 61 ± 54 +cells/10^5 PMN, $P < 0.05$), but the highest CMV plasma viral load was similar in both groups (62592 ± 33000 vs. 42055 ± 38600 copies/ml). In nine patients, maximum antigenemia coincided with highest plasma DNA viral load, but in 10 highest DNAemia occurred 6 days later. On the contrary, antigenemia declined faster than CMV-DNAnemia, after treatment.

Introduction

In transplant patients with severe immunosuppressive therapy, active systemic cytomegalovirus (CMV) infection is the major risk factor for severe CMV disease (CMVD) and mortality [1]. The prevention of CMVD is therefore a major goal in the clinical management of these patients. Three major therapeutic strategies have been developed for this effect: universal administration of ganciclovir (GCV) [2,3], administration of GCV therapy by which only patients in highest risk of developing CMVD (i.e. those patients who receive a organ from an antibody-positive CMV donor) [4], and selective use of GCV for patients displaying viremia before CMV end-stage organ disease occurs (pre-emptive therapy) [5]. The last two approaches are the more commonly used as the indiscriminate use of GCV can result in an increase in resistance [6,7] and predisposes the patient to the development of late CMV end-stage organ disease [8]. The success of prevention during the early post-transplant period and the improvement of CMVD management are partly attributed to the advancement in virological diagnose. In the last decades, rapid tests have been developed. The antigenemia assay is a rapid quantitative method that detects CMV antigens by directly immunostaining peripheral blood leukocytes (PBL) with monoclonal antibodies directed against the CMV lower-matrix protein pp65 (UL83) [9]. Quantitative results are expressed as the number of CMV-infected PBL per number of cells evaluates. The CMV-COBAS Monitor (Roche Diagnostics System, Mannheim, Germany) is an automated quantitative PCR system that amplifies a

sequence of 365 bp within the CMV DNA UL54 polymerase gene [10,11]. The key to evaluating the clinical utility of molecular assays for detection of CMVD is clinical correlation with the laboratory results.

The aim of this study was to compare the correlation with clinical outcome of quantitative antigenemia and plasma DNAemia assay, in samples belonging to 20 transplant recipients with CMV infection (CMVI) or CMVD.

Material and methods

Patients and clinical specimens

Twenty solid organ transplant recipients (15 males and five females) with CMV viremia were studied: 10 heart (HT), seven renal (RT) and three liver (LT) transplant recipients. The mean age was 54.6 ± 11.5 years (range 17–68 years). Immunosuppressive therapy was prednisone, micophenolate mofetil (MMF) and cyclosporine for six RT and eight HT, and prednisone, MMF and tacrolimus for three LT, two HT and one RT. GCV prophylaxis was administered in five of them (D+/R−). From the 20 patients studied, 13 had CMVD and seven had no clinical signs of CMVD, in spite of positive antigenemia, and were defined as having asymptomatic CMVI. The 13 patients with CMVD were treated with IV GCV 5 mg/kg of body weight, twice a day for 14 days. In the other seven patients with asymptomatic active infection CMV (CMVI), pre-emptive GCV was administered in four, and reduced immunosuppressive therapy in the rest.

A total of 284 samples (142 PBL and 142 plasmas) collected sequentially from a weekly viral follow-up during the third month after transplant, were studied. Antigenemia assay was performed in real time, and the results were used for patient management. Plasma samples were frozen at −70 °C and subsequently batch tested using the CMV-COBAS Monitor method (Roche Diagnostics System).

Definition of CMV infection or disease

Cytomegalovirus infection was defined as a positive CMV antigenemia assay. Active CMVD was defined as a positive CMV antigenemia assay and any of appropriate symptoms (fever, malaise, and/or diarrhea) or signs (leukopenia or elevated liver enzymes).

Methods

CMV antigenemia assay

The CMV antigenemia assays were as per standard procedures previously described [12]. Briefly, leukocytes were separated from peripheral blood by incubating for 20 min at 37 °C. The upper phase was collected and centrifuged for 10 min at 370 *g*. The pellet was incubated for 1 min with sterile water to eliminate erythrocytes. Thereafter, 10^5 leukocytes were deposited on a slide, air-dried and fixed with formalin. Indirect immunofluorescence with monoclonal antibodies against CMV-pp65 (Sanofi-Pasteur, Marnes-La Coquette and Argene–Biosoft, Varilhes, France) was performed. Quantitative result was informed as positive cells per 10^5 leukocytes.

Molecular assay

The COBAS Monitor assay is a quantitative CMV-DNA PCR assay in which the target is a 362-bp region of the polymerase gene. The linear range of the assay is 400–100 000 copies/ml of plasma. The assay was performed according to the manufacturer's protocol.

Results

From the 142 PBL, antigenemia was positive in 105 (74%), and from 142 plasma, CMV-DNA was detected in 121 (85%) by PCR. A comparison of antigenemia and COBAS-Monitor results is shown in Fig. 1. All time points, either symptomatic or asymptomatic patients, in which both assays were positive are included in the graph. A correlation between both markers was found ($r = 0.48$; $P < 0.0001$, RR test).

Cytomegalovirus viremia was detected at similar time after transplantation by both assays, CMV-Ag and CMV-DNA PCR, either in CMVI or CMVD (Table 1). Data of first and the higher viral load of both antigenemia and DNAemia are also shown.

First CMV-Ag, as well as first CMV-DNA load in plasma was similar in CMVI and CMVD (29 vs. 24 positive cells per 10^5, and 7445 vs. 12407 DNA copies/ml, respectively). However, whereas the highest CMV load in plasma was similar in both groups (62592 vs. 42055 DNA copies/ml), the maximum antigenemia was higher in CMVD than in CMVI (146 vs. 61 positive cells per 10^5, $P = 0.03$).

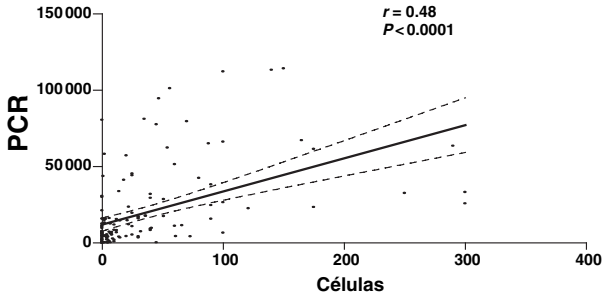

Figure 1 Correlation between positive cells of CMV-Ag and CMV-DNA copies/ml of CMV-COBAS Monitor PCR.

Table 1. Apparition and viral load of CMV antigenemia and DNAemia.

	CMVI ($n = 7$)	CMVD ($n = 13$)	*P*
Days post-transplantation			
Antigenemia	45.7 ± 30.7 (24–113)	44.7 ± 31.5 (19–113)	ns
CMV-DNAenemia	49.6 ± 17.5 (24–85)	51.3 ± 18.6 (24–96)	ns
First detection viral load			
Antigenemia (+cells/10^5)	29 ± 29 (4–90)	24 ± 28 (5–90)	ns
CMV-DNAenemia (copies/ml)	7445 ± 9036 (492–24 400)	12 407 ± 11 751 (969–33 100)	ns
Maximun viral load			
Antigenemia (+cells/10^5)	61 ± 54 (12–165)	146 ± 87 (45–300)	0.03
CMV-DNAenemia (copies/ml)	42 055 ± 3800 (6690–101 000)	62 592 ± 33 000 (17 300–114 000)	ns

In nine patients the maximum antigenemia was simultaneous to with highest DNA load in plasma, but in 10 patients highest DNAemia occurred 6 days after the maximum antigenemia. Only in one patient it appeared before highest DNAemia.

Typical CMVD (fever, leukopenia,) appeared at highest antigenemia time (65 ± 21 days after surgery). Digestive symptoms were also present in four of them. All D+/R− patients had CMVD.

In 11 of 13 CMVD patients, the maximum CMV-Ag preceded clinical symptoms, and highest DNA viral load only in six ($P = 0.09$).

After initiation of therapy, the values of the two assays declined in different ways: whereas in 12 of 13 patients, CMV-Ag decreased to less than five positive cell/10^5 PBL after 15 days of treatment, only in three patients CMV-DNA declined.

Discussion

In transplant recipients, the use of prophylactic and preemptive strategies for the prevention of CMV emphasizes the need for highly sensitive and specific laboratory tests which can identify those patients most likely to benefit from anti-CMV therapy. Several laboratory assays for detecting CMV viremia, including blood cultures, antigenemia assays, molecular amplification assays and Hybrid Capture assay have been evaluated [13–16]. Although these assays have demonstrated some utility in confirming the CMVD or CMVI, and predicting those patients likely to develop disease, the need for improved assays continues. The commercially available CMV DNA assays appear to offer improved sensitivity in a standardized assay, which can be used for comparison between different testing centers.

In order to know the value of quantitative plasma PCR assay, we compared it with the pp-65 antigenemia assay for CMV detection, in organ solid transplant patients with CMV active infection with and without symptoms.

Using the DNA quantitative assay, CMV viremia was detected at a rate similar to that of antigenemia, either in CMVI or in CMVD. Only then can a good correlation between CMV-Ag positive cells and CMV-DNA copies/ml be established. Nevertheless, both assays can detect subclinical CMV viremia in patients who may, or may not, develop CMVD. Furthermore, the onset of CMV viremia was similar in the asymptomatic and symptomatic infection by both tests.

Not all patients with CMV reactivation develop clinical disease. An approach to solve this problem could be the degree and rate of CMV replication that predicts impending CMVD and, thus, the need for specific treatment [17,18]. In addition, higher CMV DNA copy levels [19] or an increasing trend in viral load predicts clinical progression to disease or clinical relapse. Contrary to our results, preliminary data using the COBAS-CMV Monitor assay indicate that the threshold of viral load (around 1000–5000 copies per ml of plasma in solid-organ transplant recipients) predicts the likelihood of CMVD, if untreated [20]. There is need for additional studies to validate the optimal threshold, because there is a wide variability in the laboratory techniques of different centers for the detection of CMV-DNA.

In this study, the number of CMV-Ag-positive cells and plasmatic viral load detected at the first viremia in both assays was similar in CMVI and CMVD. However, whereas the number of copies/ml of highest CMV-DNA load was similar in CMVD and CMVI, the maximum antigenemia correlated with CMVD. These results are consistent with previous studies evaluating the antigenemia assay, in which patients with active CMVD had higher antigenemia levels than those without disease, and an increasing antigenemia level correlates with an increased risk of developing active CMVD [21,22].

In 11 of the 13 episodes of CMVD, the maximum CMV-Ag was positive at or before the development of symptoms, and CMV-DNA in only six patients, because PBL CMV replication precede to virus release in plasma. Moreover, after initiation of therapy, Antigenemia became

faster negative than CMV-DNAnemia, because genome of defective viruses could be detected by PCR.

In summary, there was a good correlation between CMV antigenemia and plasma DNA. Whereas maximum antigenemia correlated with CMVD, the maximum DNA viral load was similar in CMVD and CMVI. Antigenemia was more suitable for distinguishing between infection and disease and for guiding the initiation of pre-emptive therapy, and with antigenemia it is easier to estimate the efficacy and time of antiviral treatment. Nevertheless some drawbacks could be associated with the use of antigenemia: the need for rapid processing in order not to lose sensitivity and the impossibility of using the test during severe neutropenia. Further study will be required to determine the clinical utility of the quantitative CMV-DNA commercial assay in predicting CMVD and monitoring patients' response to anti-CMV therapy.

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