Comparison of the CMV Brite Turbo Assay and the Digene Hybrid Capture CMV DNA (Version 2.0) Assay for Quantitation of Cytomegalovirus in Renal Transplant Recipients

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We compared the CMV Brite Turbo Kit (BT) and the Digene Hybrid Capture CMV DNA (version 2.0) assay (HC2) in the quantitation of pp65 antigenemia and cytomegalovirus (CMV) DNA levels in immunosuppressed renal transplant recipients. Of 123 blood specimens collected from 24 renal transplant recipients, BT and HC2 assays detected 35 and 39 positive samples, respectively. The overall concordance rate between the two assays was 90%. Discordant results were observed at low levels of viremia, so that 8 samples were HC2 positive but BT negative and another 4 were BT positive but HC2 negative. There was good correlation ($R^2 = 0.766$; $P < 0.01$) between the levels of CMV DNA and pp65 antigenemia in the 31 concordant positive samples. Correlation between results obtained with the two assays was confirmed by longitudinal studies for a patient who developed clinical CMV disease. HC2 may be more sensitive at low viremia levels and allow earlier detection of impending CMV disease. The BT assay offered the advantage of a rapid (2-h) turnaround time. We conclude that BT and HC2 assays have similar sensitivity and efficacy in the diagnosis and monitoring of CMV infection and disease in renal transplant recipients. While the HC2 assay would be appropriate for centers that handle a large number of samples, the BT test may be more suitable for small sample numbers or when results are needed urgently.

Cytomegalovirus (CMV) infection is a common complication among immunocompromised hosts. CMV viremia has been shown to be an important marker of disseminated infection and correlates with clinically significant CMV disease (12). Therefore, quantitation of systemic CMV load is useful in the management of patients, especially in monitoring disease progression. Among the investigations which facilitate rapid diagnosis of CMV infection or disease in organ transplant recipients, the detection of CMV antigenemia (13) and viral DNA (5, 7) in peripheral blood have proved most useful clinically. The detection of CMV pp65 antigen in peripheral blood leukocytes has been increasingly utilized to diagnose CMV disease. It is a sensitive and specific test and yields results within 5 h (13). We have reported that leukocyte isolation by direct erythrocyte lysis could reduce the assay time to less than 3 h (4, 9). To date, reports comparing the different CMV pp65 antigenemia assays have focused on in-house assays. There are obvious interlaboratory variations in the choice of antibodies and the performance of the assays (1–3). The development of standardized commercial assays may reduce such variability. The CMV Brite Kit (Immuno Quality Products, Groningen, The Netherlands) is a commercial CMV antigenemia assay which has been validated against conventional culture, shell vial culture, and standard CMV pp65 antigen assay (7). Recently, the CMV Brite Turbo Kit (Immuno Quality Products) has incorporated a direct erythrocyte lysis step to shorten the assay time.

Detecting CMV DNA in peripheral blood is another useful test for the diagnosis and follow-up of CMV infection. The Digene Hybrid Capture CMV DNA assay is a quantitative solution hybridization antibody capture assay for the detection and quantitation of CMV DNA in leukocytes by chemiluminescence. Unlike PCR, it detects CMV DNA directly, without amplification of the genetic material. Recently a second version of the assay was developed, which has shown promise in the detection of CMV viremia in immunocompromised individuals (10, 11).

The aim of this study was to compare the CMV Brite Turbo kit (BT) and the Digene Hybrid Capture CMV DNA (version 2.0) assay (HC2) in the quantitation of CMV viremia in immunosuppressed renal transplant recipients.

MATERIALS AND METHODS

EDTA blood specimens (123) from 24 Chinese renal transplant recipients were included in the study. All specimens were obtained during the first 6 months after transplantation. HC2 and BT assays were performed in duplicate at regular intervals for CMV surveillance. These tests were performed weekly in the first 4 weeks, fortnightly in the second and third month, and monthly thereafter. The frequency was increased to twice weekly when there were clinical signs and symptoms to suggest CMV disease. Blood samples from 22 healthy volunteers were included as controls. The diagnosis of CMV disease was by the International CMV Workshop criteria (8).

BT. The BT assay was performed according to the manufacturer’s instructions (Immuno Quality Products). Briefly, 2 ml of EDTA-blood was mixed with 30 ml of the lysis solution. The retrieved leukocytes were washed and suspended in phosphate-buffered saline. Leukocytes were counted and adjusted to $2 \times 10^9$ cells/ml. Duplicate slides were prepared by cytocentrifugation (Cytospin 2; Shandon Scientific, Pittsburgh, Pa.) of 100 μl of leukocyte suspension per slide. The slides were dried for approximately 5 min and then stained by an indirect immunofluorescence technique with a mouse monoclonal antibody directed to CMV pp65 lower matrix protein and fluorescein isothiocyanate-conjugated sheep anti-mouse immunoglobulin. The number of cells with green fluorescence was scored under a UV microscope at x400 magnification. A positive assay result was defined by the presence of at least 1 stained leukocyte on the slide, and the result was expressed as the number of CMV pp65-positive cells per $4 \times 10^5$ leukocytes.

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TABLE 1. Comparison of results obtained with the HC2 and the BT assays for 123 blood samples from 24 renal transplant recipients and 22 blood samples from 22 healthy controls.

<table>
<thead>
<tr>
<th>Blood sample category and HC2 result</th>
<th>BT result</th>
<th>Concordance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal transplant recipient (n = 123)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC2 positive</td>
<td>31</td>
<td>100</td>
</tr>
<tr>
<td>HC2 negative</td>
<td>8</td>
<td>90</td>
</tr>
<tr>
<td>Healthy controls (n = 22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC2 positive</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>HC2 negative</td>
<td>22</td>
<td>100</td>
</tr>
</tbody>
</table>

* Concordance = (concordant positive + concordant negative)/n.

RESULTS

Among the 123 blood specimens from 24 renal transplant recipients, 39 tested positive by the HC2 assay and 35 were positive by the BT assay. Six of the 24 patients developed clinical manifestations of CMV disease. These six patients provided 32 of the 39 HC2-positive samples and 31 of the 35 BT-positive samples. Three other patients had received preemptive treatment with ganciclovir when they tested positive for pp65 antigenemia, and corticosteroid pulses were administered to treat acute rejections. These three patients accounted for seven HC2-positive samples and four BT-positive samples. Eight samples were HC2 positive (median CMV DNA level, 9.5 pg/ml; range, 2.6 to 37 pg/ml) but BT negative. These samples were obtained from six patients, one of whom subsequently developed CMV disease, with further samples testing positive by both assays. Four samples were BT positive (median pp65 antigenemia level, 2.5 positive cells/4 x 10^5 leukocytes; range, 1 to 7 positive cells per 4 x 10^5 leukocytes) but HC2 negative. None of the four patients who provided these samples had CMV disease. Overall, 43 (35%) samples were positive by either one or both assays. The numbers of concordant positive or negative samples detected by the two assays were 31 and 80, respectively, giving an overall concordance rate of 90%. All the blood samples from 22 healthy controls tested negative by both assays (Table 1).

For the 31 concordant positive samples, the CMV DNA levels measured with the HC2 assay were related to the pp65 antigenemia levels measured with the BT assay (Fig. 1). Results from the two assays demonstrated a linear correlation after logarithmic conversion (correlation coefficient, 0.766; P < 0.01). All the discordant results, i.e., the eight HC2-positive samples and four BT-positive samples, had low levels of CMV DNA (≤37 pg/ml) or pp65 antigenemia (≤7 positive cells per 4 x 10^5 leukocytes).

The relationship of results obtained with the two assays was evaluated further by longitudinal studies of a 41-year-old man who developed CMV disease after renal transplantation. An increase in CMV DNA as well as in the pp65 antigenemia level was noted 2 months after transplantation by HC2 and BT assays, respectively (Fig. 2). Seroconversion of CMV DNA preceded that of CMV pp65 antigenemia by 2 weeks. Abnormalities detected by these serological tests preceded clinical manifestations of fever and leukopenia due to CMV disease by 4 to 6 weeks. Both tests became strongly positive during CMV disease, with a peak CMV DNA level of 1,410 pg/ml and peak pp65 antigenemia of 636 positive cells/4 x 10^5 leukocytes. Serological and clinical manifestations showed rapid normal-
CMV infection is common among renal transplant recipients. Both the CMV pp65 antigenemia test and measurement of CMV DNA have been shown to be useful in the diagnosis and monitoring of CMV disease. The performance of these assays has improved with modifications incorporated in their updated versions. The optimal choice between the current versions of these commercial tests, namely HC2 and BT, remained uncertain since they had not been compared to each other. This study was a direct comparison between HC2 and BT. Since the clinical utility of these tests may vary according to the degree of host immunosuppression, we chose to study a homogeneous population of renal transplant recipients.

Our results showed excellent agreement (90%) between the pp65 antigenemia level measured with the BT kit and the CMV DNA level determined with the HC2 assay. Although initial blood samples may show transient discrepant results with HC2 and BT assays, all the six patients who developed clinical CMV disease had concordant positive samples subsequently. Besides qualitative concordance, data from the 31 concordant positive samples also demonstrated a strong correlation between the levels of antigenemia and viremia measured with the BT and HC2 assays, respectively. Discrepant results were confined to samples with low levels of CMV antigenemia or viremia. Neither test yielded false positive results for the 22 healthy controls. In the longitudinal study, the HC2 assay appeared more sensitive than the BT kit in detecting a low level of CMV viremia, while both tests could detect subclinical progression of CMV disease before the patient developed overt symptoms and signs. The trend towards higher sensitivity may be related to the signal amplification step or the higher number of leukocytes being used in the HC2 assay. The number of leukocytes from 3.5 ml of blood as used in the HC2 assay is in general more than 10 times higher than the 4 × 10^6 leukocytes used in the BT assay.

A technical advantage of the HC2 assay is that it allows blood samples to be stored for up to 6 days at 4°C or for 24 h at room temperature (10, 11), in contrast to the BT assay, for which samples have to be processed within 6 h (1). Each HC2 kit allows for batch testing of up to 48 samples per run and provides results within 6 to 8 h. In contrast to BT, which requires a trained technologist to identify and quantify the pp65-positive cells, the CMV DNA level is measured objectively in HC2 using a luminometer. This has contributed to the low intercenter variability of HC2 (10, 11). On the other hand, it would be less cost-effective to apply the HC2 test to a small number of samples at one time, since several standards must be included each time to generate a calibration curve. The rapid turnaround time of the BT assay is an important advantage when prompt diagnosis is required. In laboratories that handle a small number of samples at one time, the use of the BT test is advisable since, unlike the HC2 assay, multiple standards and controls are not necessary.

We conclude that the HC2 assay and the BT assay have similar efficacy in the diagnosis and monitoring of CMV infection and disease and that results of the two assays correlate with each other. HC2 may be more sensitive at low viremia levels and allow earlier detection of impending CMV disease. While the HC2 assay would be appropriate for centers that handle a large number of samples, the BT test may be more suitable for small sample numbers or when results are needed urgently.

REFERENCES