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Evaluation of a new method for early detection of active cytomegalovirus infections. A study in kidney transplant recipients

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Abstract Early detection of active cytomegalovirus (CMV) infection after organ transplantation is necessary to start effective antiviral treatment. In the present study, blood specimens of kidney transplant recipients (n = 38) were monitored for the expression of CMV immediate early (IE) and late (L) mRNA using nucleic acid sequence-based amplification (NASBA). Results were compared with virus isolation, pp65 antigenemia and serology. In patients developing active CMV infection, pp65 antigen and L mRNA were detected simultaneously. At the same time, positive cell culture results could be reported to the clinic. CMV was detected significantly earlier with IE NASBA than with the other assays. However, the specificity of IE NASBA is lower than that of antigenemia, late NASBA and cell culture. Early detection of IE mRNA is especially useful for patients at high risk of developing symptomatic CMV infection in order that early, adequate antiviral therapy may be started. Late NASBA can be used to monitor further development of CMV infection, comparable to antigenemia.

Key words Human cytomegalovirus · Nucleic acid sequence-based amplification · Kidney transplantation

Introduction

Detection of human cytomegalovirus (CMV) at an early stage of infection is a prerequisite for the initiation of effective antiviral therapy. Nucleic acid sequence-based amplification (NASBA) has been designed for the specific amplification of RNA. NASBA proved to be a highly sensitive method for the detection of human immunodeficiency virus mRNA [4, 5]. The technique has now also been applied to the qualitative detection of CMV. Two different viral mRNAs were chosen as targets for amplification by NASBA, the immediate early (IE) 1 mRNA (UL123) [7] and the late pp67 mRNA (UL65) [2, 3]. These mRNAs are synthesized at different phases in the replication cycle of CMV. IE mRNA is transcribed within a few hours after infection of the cell, while the late phase of transcription is initiated approximately 72 h after infection and requires the active replication of the CMV genome. Blood specimens from patients with a kidney allograft were screened for the presence of IE and late mRNA. Results were compared with virus isolation, pp65 antigenemia and serology, in order to evaluate the diagnostic value of the IE and late NASBA assays.

Materials and methods

A group of 38 kidney transplant recipients was studied, from which 447 blood specimens had been collected after transplantation. The patients were grouped according to the serostatus of the donor (D) and the recipient (R): 10 D +/R +; 9 D +/R −; 10 D −/R +; 9 D −/R −. Samples were tested routinely by pp65 antigenemia [1] and virus isolation (DEAFF and CPE [6]). The results of DEAFF and CPE for one sample were taken together to become one test result. Retrospectively, IE and late NASBA were performed on heparinised whole blood samples, stored at − 70 °C in NASBA lysis buffer

Table 1 Diagnostic parameters of antigenemia, cell culture, immediate early *(IE)* and late nucleic acid sequence-based amplification *(NASBA)* for the detection of active cytomegalovirus infection *(PPV* positive predictive value, *NPV* negative predictive value)

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IE NASBA	95	83	86	94
Late NASBA	60	100	100	69
Cell culture	70	94	93	74
Antigenemia	45	100	100	62

[5]. Amplified RNA was detected using electrochemiluminescent-labelled probes [4]. In addition, anti-CMV IgG and IgM levels were determined using the commercially available AXSym and Im X CMV assays (Abbott Laboratories), respectively. Active CMV infection was diagnosed when there was a minimum of two positive cell culture and/or antigenemia results, and/or seroconversion of IgM and/or a significant rise in IgG levels was found. Statistical analysis was performed with the Wilcoxon matched-pairs signed ranks test.

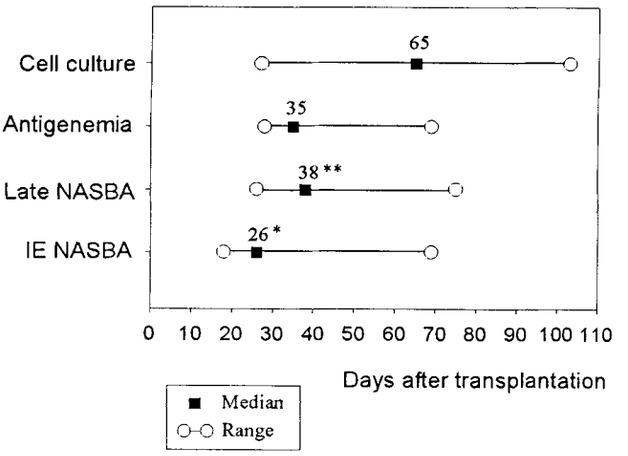

Fig. 1 Detection of the onset of active cytomegalovirus infection by cell culture, antigenemia, late and immediate early *(IE)* nucleic acid sequence-based amplification *(NASBA)* after transplantation. The cell culture data are presented, based on the day on which positive results could be reported to the clinic. (* Significantly earlier than all other assays, $P < 0.05$, ** no significant difference from antigenemia and cell culture)

Results

The incidence of active CMV infection was highest for patients in the serogroup D +/R + (90%). For patients in the serogroups D +/R − and D −/R +, this was 67% and 50%, respectively. CMV was not detected in blood from D −/R − patients. Four of six patients with a primary infection (D +/R −) developed symptomatic CMV infection. Primary infections were detected by all assays, while IE NASBA was the most sensitive assay for the detection of secondary infections (93%), followed by cell culture (57%), late NASBA (43%) and antigenemia (21%). The diagnostic parameters, considering both primary and secondary infections together, are shown in Table 1. The overall sensitivity of the IE NASBA was 95%. This resulted also in a high negative predictive value for the IE NASBA. However, compared to the other assays, the specificity and positive predictive values are lower. Cell culture is less sensitive than IE NASBA (70%), but the specificity is higher (94%). Both late NASBA and antigenemia are very specific and highly predictive for the onset of active CMV infection (100%). However, late NASBA was more sensitive than antigenemia for the detection of active CMV infection (60% versus 45%).

IE NASBA appeared to be the earliest marker indicating the onset of active CMV infection after kidney transplantation. The median for the first positive result after transplantation was day 26 (range 18–69; Fig. 1). For cell culture, late NASBA and antigenemia, this was day 65 (range 27–103), day 38 (range 26–75) and day 35 (range 28–69), respectively. For cell culture it should be noted that these are the data for the day at which a positive result could be reported to the clinic. The median for the day at which the positive blood sample was taken from the patient was 31 (range 19–69). From statistical analysis it can be concluded that the time of detection of CMV with IE NASBA is significantly earlier than with late NASBA ($P < 0.01$; $n = 12$), antigenemia ($P < 0.05$; $n = 9$) and cell culture ($P < 0.01$; $n = 14$, based on the day on which the blood sample was taken). There was no significant difference between detection of CMV by late NASBA and the day on which positive cell cultures could be reported to the clinic, although the cell culture-positive blood samples were taken from the patient before late NASBA was found positive ($P = 0.05$, $n = 11$). Furthermore, positive late NASBA and antigenemia results were also found simultaneously after transplantation.

Discussion

We have evaluated the diagnostic value of monitoring IE and late mRNA expression using NASBA in kidney transplant recipients. We found IE NASBA to be highly sensitive and an early marker for the onset of active CMV infection. For four patients, symptomatic CMV infection was diagnosed after primary infection. Although all assays were found positive for these patients, in particular the early detection of the onset of active CMV infection with IE NASBA can be of great clinical value for those patients at high risk of developing symp-

tomatic infections. In addition, the high negative predictive value of the IE NASBA makes other tests redundant shortly after transplantation, until the first positive IE NASBA result is found. However, the specificity and positive predictive values of IE NASBA for active CMV infection (i.e. viral replication) appeared to be lower. In contrast, late NASBA and antigenemia are both very specific and highly predictive for the onset of active CMV infection. These assays are therefore more suitable to monitor the subsequent development of CMV infection. Late NASBA could be preferred to antigenemia since it is more sensitive than antigenemia for the detection of active CMV infection.

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