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Diagnostic Implications of Human Cytomegalovirus Immediate Early-1 and pp67 mRNA Detection in Whole-Blood Samples from Liver Transplant Patients Using Nucleic Acid Sequence-Based Amplification

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Nucleic acid sequence-based amplification (NASBA) was used for detection of the human cytomegalovirus (CMV) immediate early-1 (IE) and the late pp67 mRNA in 353 blood samples collected from 34 liver transplant patients. The diagnostic value of these assays was compared to that of the pp65 antigenemia assay. Overall, 95 and 42% of the antigenemia-positive samples were IE NASBA and pp67 NASBA positive, respectively. Although the results from pp67 NASBA and the antigenemia assay appeared to correspond poorly, a clear correlation was seen between pp67 NASBA-negative results and low numbers of pp65 antigen-positive cells. Twenty patients (59%) were treated with ganciclovir after the diagnosis of symptomatic CMV infection. Before initiation of the antiviral therapy, the antigenemia assay detected the onset of symptomatic infection in all patients, whereas 95 and 60% of these patients were IE NASBA and pp67 NASBA positive, respectively. Although the sensitivity of IE NASBA was very high, the positive predictive value (PPV) of this assay for the onset of a symptomatic infection was only 63%. The PPV of the antigenemia assay as well as pp67 NASBA was considerably higher (80 and 86%, respectively). Thus, the detection of IE mRNA using NASBA appears to be particularly useful as a marker for early initiation of antiviral therapy in patients at high risk for the development of a symptomatic infection. Also, IE NASBA was found to be more sensitive than the antigenemia assay for monitoring CMV infection during antiviral therapy. On the contrary, pp67 NASBA did not appear to have additional diagnostic value compared to the antigenemia assay.

Although the majority of the adult human population is seropositive for cytomegalovirus (CMV), symptomatic infections are mostly restricted to immunocompromised individuals, such as transplant recipients. Early detection of the virus is necessary to start effective antiviral treatment for the prevention of severe complications and death. The diagnostic value of monitoring the expression of viral immediate early (IE) and late (L) mRNAs in blood as markers for active CMV infection and disease has previously been evaluated in several studies using reverse transcriptase PCR (RT-PCR) (3, 7, 13, 17, 18, 21, 23, 24, 27, 29, 31, 38). However, controversial results were obtained regarding the clinical significance of the detection of IE and L mRNAs, most probably due to varying sensitivities of the different RT-PCR assays. Alternatively, nucleic acid sequence-based amplification (NASBA) has been developed for specific amplification of RNA (19). This technique has recently been adapted for the detection of CMV (2, 4, 5, 15) as well as other microorganisms (8). In previous studies we evaluated the diagnostic value of monitoring the expression of the CMV IE-1 and pp67 mRNA in peripheral blood of kidney transplant patients using NASBA (4, 5). The IE-1 mRNA is expressed directly after entrance of the virus into the cell (34), whereas pp67 mRNA is expressed in the late phase of the replication cycle in vitro (9, 10).

In this study, we present an evaluation of the diagnostic value of IE NASBA and pp67 NASBA for monitoring CMV infection in liver transplant patients. These patients are at higher risk for symptomatic CMV infection than kidney transplant patients (28). Liver transplant patients are usually in a critical clinical condition at the moment of transplantation and frequently require antirejection treatment. As a consequence, these patients are highly susceptible to infections with opportunistic pathogens, such as CMV. It is therefore essential to have a sensitive diagnostic assay that can predict the onset of symptomatic CMV infections at an early stage, such that antiviral therapy can be initiated in a timely fashion.

MATERIALS AND METHODS

Patients. The patient population consisted of 26 adult patients who received a liver allograft at the Helsinki University Hospital in the period from December 1996 to November 1997 and another 10 adult patients who received transplants during the period September to December 1998. The number of blood samples collected after transplantation was used as a selection criterion. Patients from which a minimum of five blood samples were obtained were included in this study ($n = 34$).

As basic immunosuppression, the patients received triple-drug therapy with various combinations of steroids, azathioprine, and oral cyclosporine or tacrolimus. Acute rejections were treated with a high dose of methylprednisolone, and steroid-resistant rejections were treated with OKT3. Diagnosis of rejection was based on histological findings at biopsy (11).

The patients were grouped according to the CMV serostatus (positive [+] or negative [−]) of the transplant donor (D) and recipient (R), which resulted in the following serogroups: D+ R+ ($n = 19$), D+ R− ($n = 4$), D− R+ ($n = 8$), and D− R− ($n = 2$). There was one additional patient who was seropositive at the moment of transplantation, while the serostatus of the donor was unknown. Thus, 82% (28 of 34) of the liver transplant recipients were seropositive at the moment of transplantation.

Antiviral therapy. Symptomatic CMV infections were treated with ganciclovir (intravenously, 5 mg/kg of body weight twice daily) for at least 2 weeks. The

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diagnosis of symptomatic CMV infection was based on positive pp65 antigenemia assay results and clinical signs of CMV infection, mostly fever which could not be explained otherwise. Symptomatic CMV infection was also diagnosed in case of pneumonia with a CMV-positive finding from a bronchoalveolar lavage (BAL). Five patients with a relapse of CMV infection after former antiviral therapy were again treated with intravenous ganciclovir. Furthermore, for five patients the antiviral therapy was switched to orally administered ganciclovir, allowing the patients to be treated at home. In general, the patients received no antiviral prophylaxis. However, eight patients received ganciclovir intravenously during antirejection therapy in order to prevent CMV reactivation due to increased immunosuppression.

Blood samples. A total of 353 whole-blood samples were prospectively collected from 34 liver transplant recipients, including samples which were obtained shortly before the operation. Additional blood samples were collected at least once a week during hospitalization directly after transplantation and on all occasions when viral infection was suspected. Otherwise, blood samples were obtained monthly, or more frequently if the patients were again hospitalized.

pp65 antigenemia. The CMV pp65 antigenemia assay was performed as described previously (36, 37). Briefly, cytospot preparations were made with 1.5×10^5 dextran-isolated leukocytes per spot. The cells were subsequently fixed with a mixture of formaldehyde and NP-40. The pp65 antigen was stained by an indirect immunoperoxidase protocol using the antibodies c10 and c11 (Biotest Pharma, Frankfurt, Germany). The results were expressed as the number of positive cells per 50×10^3 leukocytes.

Shell vial culture. A modification of the rapid shell vial culture (16) was performed on BAL specimens as described previously (22). The BAL samples were collected from the patients when CMV pneumonia was suspected.

Serology. Patient sera were subjected to both the IMx CMV and AxSym CMV assays (Abbott Laboratories, North Chicago, Ill.) in order to detect CMV-specific immunoglobulin M (IgM) and IgG antibodies, respectively. The IgM titers were expressed as index values. Index values ≥0.500 were considered positive. IgG titers were expressed as numbers of antibody units (AU) per milliliter. Sera with IgG levels of ≥15 AU/ml were considered positive. The detection limit of 250 AU/ml could be obtained for sera with high levels of IgG.

NASBA. The isolation of nucleic acids from blood samples and the NASBA procedure for amplification of the CMV IE and pp67 mRNA were carried out as described previously (4, 5). Briefly, nucleic acids were isolated according to the method of Boom et al. (6). First, 100 μl of whole EDTA-treated blood was added to 900 μl of NASBA lysis buffer, containing guanidine thiocyanate (Organon Teknika B.V., Boxtel, The Netherlands). The nucleic acids were subsequently bound to silica and washed with ethanol and acetone to remove residual cell debris. The nucleic acids were eluted from the silica after drying and used as input in the NASBA reaction. This isothermal amplification reaction (41°C, 90 min) depends on the concerted action of three enzymes: T7 RNA polymerase, avian myeloblastosis virus reverse transcriptase, and RNase H. Different primer sets were used for the amplification of either IE mRNA (T7 primer, 5′-AATTCTAATACGACTCACTATAGGGAGACTTAATACAAGCCATCCACA-3′; primer 2, 5′-TAGATAAGGTTCATGAGCCT-3′) or the pp67 mRNA (T7 primer, 5′-AATTCTAATACGACTCACTATAGGGAGAGGGTCGATTCAGACTGA-3′; primer 2, 5′-CTGGAGATATATGTTGACCA-3′). The amplification products were detected using specific ruthenium-labeled probes and electrochemiluminescence. The assays included an internal control RNA, which served as a positive control for the isolation, amplification, and detection procedures.

Statistical analysis. The Student *t* test was used for statistical analysis of the correlation between NASBA results and the number of pp65 antigen-positive cells. The Wilcoxon matched-pair signed-rank test was used to compare the assays with respect to detection of CMV in time. *P* values of ≤0.05 were considered significant.

RESULTS

Interassay correlations. A total of 353 blood samples was prospectively collected from 34 liver transplant patients. The presence of CMV in these blood samples was routinely tested using the antigenemia assay. Part of the blood sample was stored at −70°C in NASBA lysis buffer (Organon Teknika B.V.). Retrospectively, we used NASBA for the detection of IE mRNA and pp67 mRNA in these samples. The antigenemia and NASBA results were subsequently compared for each individual blood sample. Overall, the pp65 antigen was detected in 74 samples (21%). IE mRNA and pp67 mRNA were detected in 175 and 42 samples (50 and 12%), respectively. It was found that 70 (95%) of the antigenemia-positive samples were also IE NASBA positive. However, 105 (38%) of the IE NASBA-positive samples were antigenemia negative. Only 31 (42%) of the antigenemia-positive samples were also pp67 NASBA positive. On the other hand, 11 (26%) of the pp67

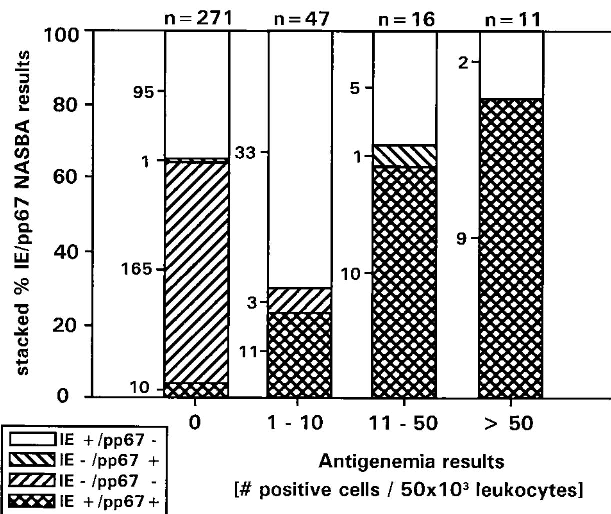

FIG. 1. Correlation between the results of the NASBA assays and the number of pp65 antigen-positive cells. The antigenemia results are divided into four groups according to the amount of pp65 antigen-positive cells/50×10^3 leukocytes: 0, 1 to 10, 11 to 50, and >50. The total number of samples in each of these groups is marked above the graph. The NASBA results are also divided into four groups in which the IE NASBA and pp67 NASBA results are either positive or negative. The NASBA results are expressed as a percentage of the number of antigenemia-positive samples and are represented by a differently hatched bar. The absolute number of NASBA results is indicated on the left side of each bar.

NASBA-positive samples were antigenemia negative. Negative pp67 NASBA results strongly correlated with low numbers of pp65 antigen-positive cells. This is demonstrated in Fig. 1 by the fact that only 23% (11 of 47) of the samples with 1 to 10 pp65 antigen-positive cells per 50×10^3 leukocytes were pp67 NASBA positive. Of the samples with 11 to 50 pp65 antigen-positive cells, 69% (11 of 16) were pp67 NASBA positive. A value of 82% (9 of 11) was found for the group of samples with >50 pp65 antigen-positive cells. In contrast, 94% of the samples with 1 to 10 or 11 to 50 pp65 antigen-positive cells, was also IE NASBA positive (44 of 47 and 15 of 16, respectively). All samples with >50 pp65 antigen-positive cells (n = 11) were IE NASBA positive. Together, the mean number of pp65 antigen-positive cells in samples that were IE NASBA positive and pp67 NASBA negative (n = 135) was four. This was significantly higher for samples that were both IE and pp67 NASBA positive (n = 40) (mean, 36 positive cells; $P < 0.0001$).

Assay results for ganciclovir-treated infections. Of the 34 liver transplant patients, 20 (59%) developed a symptomatic CMV infection during the follow-up (four primary infections and 16 reactivations). The patients with symptomatic infection were treated with ganciclovir. Five of these patients were given ganciclovir more than once to treat a relapse of CMV infection. In the evaluation of the NASBA and antigenemia results we focused on the detection of CMV in the period prior to the first antiviral treatment. In 17 of the symptomatic patients, the first antiviral treatment was initiated on the basis of positive antigenemia results together with clinical signs of CMV infection. For the other three patients, antiviral therapy was initiated after the diagnosis of pneumonia, with concomitant detection of CMV in a BAL sample by the shell vial assay. CMV was also present in the blood of these patients as determined by the antigenemia assay. Thus, all symptomatic infections were detected by the antigenemia assay. However, the positive predictive value (PPV) of antigenemia was 80% (20 of 25), indicating that 5 of 25 antigenemia-positive patients remained asymptomatic. IE NASBA- and pp67 NASBA-positive results

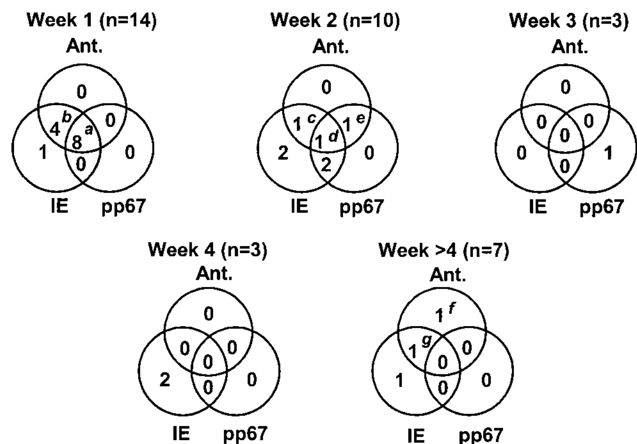

FIG. 2. Venn diagrams for antigenemia, IE, and pp67 NASBA-positive results during the first antiviral therapy after transplantation. The results are shown for 1, 2, 3, 4, and >4 weeks after initiation of therapy. The total number of samples that was collected from different patients in the respective weeks is indicated in parentheses. A total of 37 samples was collected from 13 different patients. Ant., antigenemia assay; IE and pp67, IE and pp67 NASBA assay, respectively. The number of pp65 antigenemia-positive cells/50 $\times$ 10^3 leukocytes for each individual sample was as follows: *a*: 2, 10, 10, 50, 100, 100, 150, and 150; *b*: 1, 2, 2, and 5; *c*: 10; *d*: 40; *e*: 30; *f*: 2; *g*: 5.

were obtained for 19 (95%) and 12 (60%) of the ganciclovir-treated patients before initiation of the first antiviral therapy. The PPV of IE NASBA was only 63% (19 of 30), whereas the PPV of pp67 NASBA was 86% (12 of 14). However, IE NASBA detected the onset of CMV infection significantly earlier than both pp67 NASBA and the antigenemia assay ($P < 0.001$). No significant difference was found between the latter assays with respect to the detection of CMV in time ($P > 0.05$).

Monitoring of CMV during antiviral therapy. Blood samples were collected from patients to monitor the CMV infection during antiviral therapy. In total, 37 samples were obtained from 13 different patients (Fig. 2) during the first antiviral treatment after transplantation. This first treatment was maintained for a minimum of 2 weeks. As shown in Fig. 2, there is a clear correlation between IE NASBA- and antigenemia-positive samples during the first week. Furthermore, 8 of the 12 samples that were both IE NASBA and antigenemia positive were also pp67 NASBA positive. The number of antigenemia-positive cells was much higher in these samples than in samples that were only IE NASBA- and antigenemia-positive. In the second week, the number of samples that were antigenemia, IE NASBA, or pp67 NASBA positive decreased. In the third week, only one sample was pp67 NASBA positive, whereas in the fourth week only IE mRNA was detected in two samples. After more than 4 weeks of treatment, two samples were either IE NASBA positive or antigenemia positive. One sample was positive for both IE NASBA and the antigenemia assay. The antigenemia-positive samples were obtained from patients that were treated orally with ganciclovir. IE mRNA could be detected at a median of 10.5 days after the start of antiviral therapy (range, 0 to 63 days), whereas pp67 mRNA and pp65 antigen were detected at a median of 9 and 3.5 days (range, 0 to 17 and 0 to 63 days), respectively. Compared to the antigenemia assay, IE NASBA detected the presence of CMV significantly longer ($P < 0.05$), whereas no significant difference was found between IE NASBA and pp67 NASBA. Furthermore, there also appeared to be no significant difference between pp67 NASBA and the antigenemia assay.

Patient examples. Figure 3 shows representative examples of assay results obtained for three different liver transplant patients. Patient I suffered from a primary infection that was first detected at day 20 by IE NASBA. The pp65 antigen was detected in the subsequent sample at day 27. The pp67 NASBA test was not positive until day 38. At day 42, symptomatic CMV infection was diagnosed and treatment with ganciclovir was initiated. Ganciclovir was given intravenously until day 51. Subsequently, the treatment was continued by oral administration until day 110, in order to prevent a recurrent CMV infection. However, during this period, positive results were again obtained for both IE NASBA and the antigenemia assay, although only few cells (two to five) were pp65 antigen positive. After antiviral therapy, IE mRNA was still detected in several samples, whereas all other assays remained negative. In the early period after transplantation, i.e., from day 27 to day 57, CMV infection was indicated by a significant rise of IgG and seroconversion of IgM.

Patient I is an example of one of the four patients with a primary infection. However, the majority of the CMV infections were reactivations. In fact, 82% of the 34 patients were seropositive at the moment of transplantation. The results shown for patient II demonstrate the results for a patient with reactivating CMV. Both donor and recipient were CMV seropositive at the moment of transplantation. IE NASBA was the first assay to become positive, at day 15, followed at day 29 by both the antigenemia assay and pp67 NASBA. Then, antiviral therapy with ganciclovir was initiated and maintained for a standard period of approximately 14 days. Antiviral therapy was again initiated after the finding of an antigenemia-positive sample at day 75. However, at this time point, no blood samples were taken that were suitable for performing NASBA assays. The antiviral treatment was continued until day 106. At day 178, the antigenemia assay was found positive, albeit at a low level. Because the patient showed no clinical symptoms of CMV infection, antiviral therapy was not initiated. The overall serological results showed a significant rise of IgG and seroconversion of IgM. Reactivation of CMV was also seen in patient III. Initially, the patient received ganciclovir prophylaxis from day 9 to 22 in order to prevent symptomatic CMV infection during antirejection therapy. Retrospectively, both IE mRNA and pp67 mRNA could be detected during that period, whereas the antigenemia assay became positive at day 26. At days 48 and 50, an increase in the number of pp65 antigen-positive cells was found together with the onset of symptoms of CMV infection, which urged the start of antiviral therapy at day 50. The pp67 NASBA test was also positive at day 50, whereas IE NASBA was positive in every subsequent sample until day 100. At day 123, the patient developed recurrent CMV infection with positive antigenemia results. Consequently, the patient was again treated with ganciclovir. NASBA results are not available for this time point. The IgG titers soon reached the detection limit of the assay (250 AU/ml), and remained high in all subsequent samples. The fluctuation of the IgM titers corresponded well with the course of infection, as indicated by the NASBA and antigenemia results.

DISCUSSION

Severe symptomatic infection after organ transplantation can be prevented by an early start of antiviral therapy with ganciclovir. The adverse effects of ganciclovir (33) and the chance of development of drug-resistant virus strains (1, 20) do not favor prolonged prophylactic use of this antiviral drug in every transplant recipient. Therefore, unnecessary prophylaxis in patients who are not at risk for symptomatic CMV infection

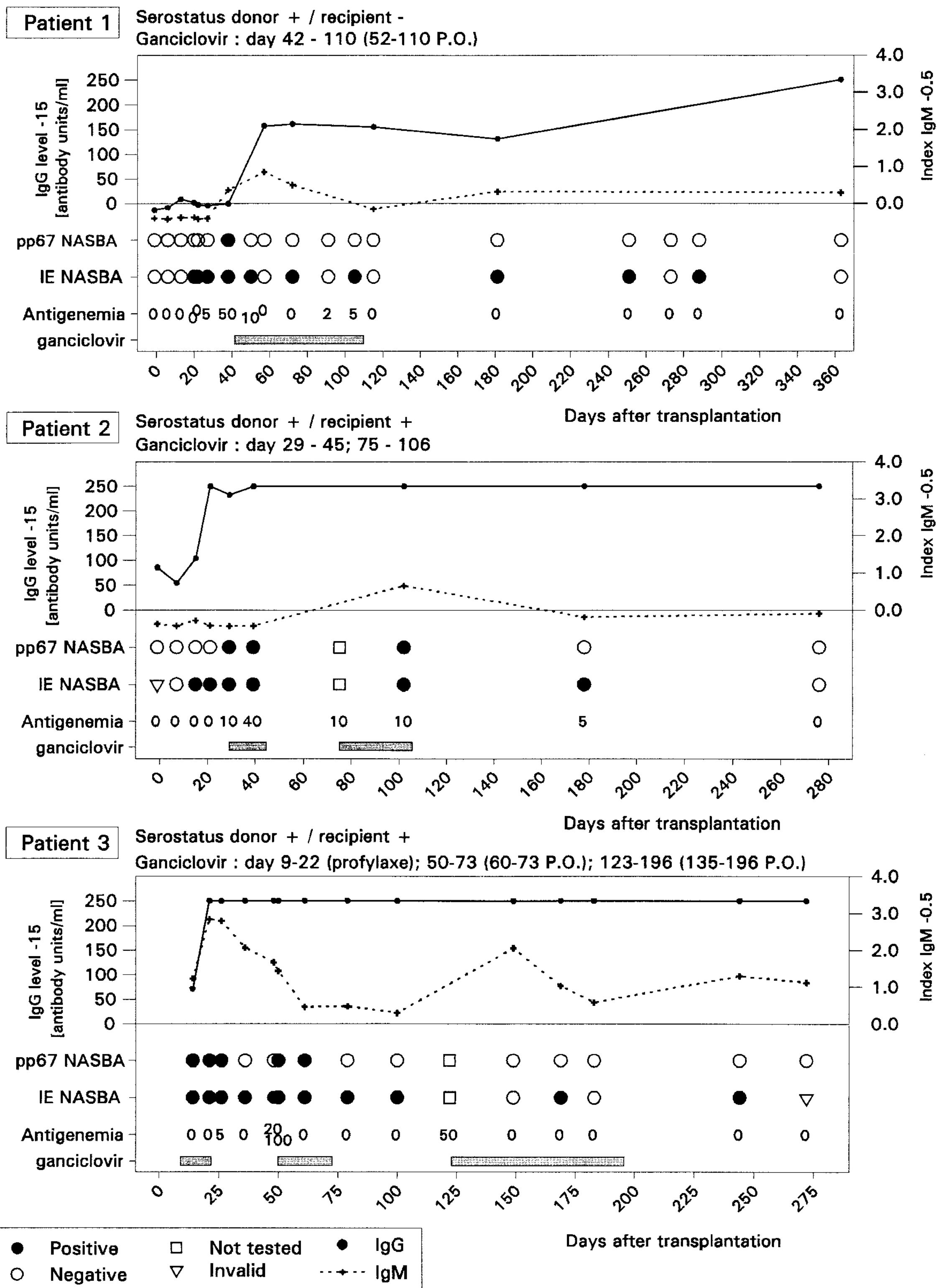

FIG. 3. Representative antigenemia, serology, and IE and pp67 NASBA assay results for three liver transplant patients. Periods of antiviral therapy with ganciclovir are indicated with grey horizontal bars. Antigenemia results are expressed as the number of positive cells/50 × 10^3 leukocytes. The IgG and IgM titers are represented as the assay result minus the cutoff value (15 for IgG and 0.5 for IgM). Thus, titers above the *x* axis are considered positive. P.O., antiviral therapy with orally administered ganciclovir.

should be avoided. Alternatively, preemptive therapy seems to be an effective approach to prevent the onset of a symptomatic infection (32, 33). This approach strongly depends on the availability of an assay which is sensitive enough to detect CMV infections before the actual onset of symptoms. Moreover, the assay should have a high predictive value for the onset of a symptomatic infection.

In this study, we investigated the diagnostic value of monitoring the expression of the CMV IE-1 and pp67 mRNA in whole-blood samples from liver transplant patients using NASBA. The results of these NASBA assays were compared to the results of the pp65 antigenemia assay. This assay is widely used for detection of CMV infections in immunocompromised patients. In general, the antigenemia assay is a sensitive assay. However, for solid-organ transplant recipients, positive antigenemia results do not always correlate with symptomatic infection (33). In this study, the initiation of antiviral therapy in liver transplant patients was guided by both positive antigenemia results and clinical signs of symptomatic CMV infection. In order to investigate the relevance of IE mRNA and pp67 mRNA detection as possible markers for early preemptive therapy, the evaluation of the NASBA results was focused on the period prior to initiation of the first antiviral treatment with ganciclovir

Fifty-nine percent of the liver transplant patients received antiviral therapy with ganciclovir to treat symptomatic CMV infection. IE NASBA detected the onset of symptomatic CMV infection in all but one of these patients. Moreover, IE NASBA was positive significantly earlier than pp67 NASBA and the antigenemia assay. However, the PPV for symptomatic infection of IE NASBA was only 63%. Similar findings were reported for kidney transplant recipients (4). As suggested for those patients, early detection of CMV with IE NASBA would be particularly useful for patients at high risk for a symptomatic CMV infection, e.g., patients with a primary infection or undergoing antirejection treatment. In these patients, the early detection of IE mRNA is likely to be correlated with the onset of a symptomatic infection and could possibly serve as an early marker for initiation of antiviral therapy. However, the majority of the studied population of liver transplant patients was CMV seropositive at the moment of transplantation. Therefore, most CMV infections were reactivations rather than primary infections. In addition, some of the seropositive patients were already IE NASBA positive at the moment of transplantation. This is probably due to the critical condition of most liver transplant patients, which may be accompanied by a degree of immunosuppression that allows CMV to reactivate at an early phase. Furthermore, IE mRNA could be detected in some patients after a period of antiviral treatment, although there was no relapse of a symptomatic infection (Fig. 3). Thus, the detection of IE mRNA does not necessarily predict the onset of a symptomatic infection. In contrast, it was recently reported that IE NASBA could serve as a new parameter for preemptive therapy in allogeneic bone marrow transplant recipients (14). These transplant patients are usually considered a distinct group, because mortality rates due to CMV infections in this group are higher than in other transplant patients (39, 40). Graft-versus-host disease and severe impairment in the development of specific antiviral immunity are important risk factors for development of symptomatic CMV infection in these patients (25, 26). Instant initiation of antiviral therapy upon the first detection of CMV after tranplantation is mandatory to prevent life-threatening disease. Therefore, early detection of the presence of CMV by monitoring the expression of IE mRNA is particularly relevant for allogeneic bone marrow transplant recipients. For kidney and liver transplant recipients, however, IE NASBA results are not sufficient for evaluation of the risk for the onset of a symptomatic infection, in particular for patients with a reactivation.

In this study, it was found that 58% (43 of 74) of the antigenemia-positive samples were pp67 NASBA negative. As a possible explanation for these discrepancies, we hypothesized that samples that were collected during antiviral treatment with ganciclovir are more likely to be positive by the antigenemia assay than by pp67 NASBA. Ganciclovir exerts its antiviral activity by inhibition of the viral DNA polymerase (35). This should result in a block of the synthesis of viral late transcripts, such as the pp67 mRNA. Because, the pp65 protein may be more stable than the pp67 mRNA, it may still be present in the blood at time points at which the pp67 mRNA has already been degraded. In addition, it is possible that pp65 protein is still synthesized during ganciclovir treatment. Unlike the pp67 mRNA, the pp65 mRNA is not a true late transcript (12). Its transcription is therefore not directly affected by inhibitors of viral DNA synthesis. However, of the 43 samples that were antigenemia positive and pp67 NASBA negative, only 7 were obtained during a period of antiviral treatment with ganciclovir. In fact, for eight patients these discrepancies were found before initiation of antiviral therapy. As a consequence, the sensitivity of pp67 NASBA for the detection of a symptomatic CMV infection was low (60%). Nevertheless, for six of these patients, the number of pp65 antigen-positive cells was low, ranging from 2 to 30. The other two patients became pp67 NASBA positive several days after the start of antiviral treatment.

The low sensitivity of pp67 NASBA found in this study is in contrast with the previous findings reported for kidney transplant recipients (5). In these patients, pp67 mRNA could be detected in all patients with a symptomatic infection. A high sensitivity of pp67 NASBA was also reported by Gerna et al. for heart and lung transplant recipients with a reactivated CMV infection (15). A cutoff level of $\geq$100 pp65 antigen-positive cells/2 $\times$ 10^5 leukocytes was used for the initiation of preemptive antiviral therapy in these patients. The pp67 mRNA could be detected in all patients who were treated according to these criteria. In this study, we also found a positive correlation between the number of pp65 antigen-positive cells and the positivity of pp67 NASBA (Fig. 1). However, we did not use a cutoff level for the antigenemia assay. Instead, an antigenemia-positive sample together with clinical symptoms of CMV infection was an indication for the start of antiviral treatment of the liver transplant patients. The discrepancy between the different studies with respect to the sensitivity of pp67 NASBA is probably explained by the difference in timing of the start of antiviral therapy with ganciclovir. As explained above, ganciclovir should inhibit the synthesis of late mRNA. As a consequence, early onset of antiviral therapy could prevent the detection of pp67 mRNA. In fact, in studies in which patients were preemptively treated with ganciclovir, based on the first antigenemia-positive samples after transplantation, the sensitivity of pp67 NASBA for the detection of symptomatic CMV infection was found to be lower than the sensitivity of the antigenemia assay (14, 15, 30). It is difficult to investigate this hypothesis because delayed initiation of antiviral therapy based on pp67 NASBA-positive results in these patients may increase the risk for the development of symptomatic infection to an unacceptable level. Nevertheless, detection of pp67 mRNA appears to have no additional diagnostic value compared to the antigenemia assay as a marker for the early start of preemptive therapy in patients at high risk for the onset of symptomatic CMV infection.

In previous studies, no significant difference was found be-

tween the disappearance of CMV from the blood during antiviral therapy as determined by pp67 NASBA and the antigenemia assay (5, 14, 15). A similar result was found in this study. However, it should be noted that for several patients the pp67 NASBA assay was negative before and during antiviral therapy, whereas the antigenemia assay was found to be positive. In agreement with the results described above, the pp67 NASBA-negative results obtained during antiviral therapy corresponded with low levels of pp65 antigen-positive cells. For IE NASBA, a clear correlation was found with the results of the antigenemia assay, in particular during the first week of antiviral therapy. Thereafter, IE mRNA could still be detected, whereas the antigenemia assay was negative. In addition, it was found that IE mRNA could be detected after termination of therapy (Fig. 3). Similar findings were reported for individual allogeneic bone marrow transplant recipients (14). Nevertheless, for most of these patients, the times to disappearance of CMV during antiviral therapy, as demonstrated by IE NASBA, pp67 NASBA, and the antigenemia assay, were comparable. It should be investigated in more detail whether prolonged IE mRNA detection has clinical implications. As such, this could justify prolonged antiviral treatment with ganciclovir.

In conclusion, the detection of IE mRNA appears to be particularly useful as a marker for early initiation of preemptive antiviral therapy in patients at high risk for the development of a symptomatic infection. In contrast to IE NASBA, pp67 NASBA did not appear to have additional diagnostic value compared to the antigenemia assay.

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