

Surveillance of Cytomegalovirus after Solid-Organ Transplantation: Comparison of pp65 Antigenemia Assay with a Quantitative DNA Hybridization Assay

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In a multicenter study, 113 blood samples from 19 organ transplant patients were analyzed for cytomegalovirus by the pp65 antigenemia assay and a quantitative DNA hybridization assay. Overall, there was 84% agreement among the results obtained by the two tests. Fifteen of 16 episodes of active infection were detected by both assays. One episode was missed by the pp65 assay, and one patient showed significant DNA-emia but only low-level antigenemia.

Cytomegalovirus (CMV) infection is a significant cause of morbidity in transplant recipients (9). The availability of fast and sensitive methods for CMV detection is essential to initiate early antiviral therapy. pp65 antigenemia detection has been accepted as a standard method for making an early diagnosis of active CMV infection (1–5, 8, 10, 12, 13). Recently a luminescence-based DNA hybridization assay (Murex Hybrid-Capture CMV DNA system) has been developed, and limited studies involving this type of assay have been published (6, 7). We have compared in a multicenter study DNA hybridization with the pp65 antigenemia assay for the routine surveillance of CMV after solid organ transplantation. The aim of the study was to detect early infection by demonstrating the presence of CMV pp65 antigen or DNA in peripheral blood but not to diagnose clinical disease.

Blood samples from transplantation patients are routinely collected once or twice a week. A total of 113 blood samples were selected from 19 transplant recipients and tested in parallel by the hybrid capture assay and the antigenemia assay. Multiple (average, 5.9; range, 3 to 12) samples suitable for parallel analysis and at least one positive pp65 result were required to include a patient in the comparison. Eight kidney, three kidney and pancreas, five liver, and three lung transplant recipients were included in the comparison. Routine follow-up for CMV was done by the pp65 antigenemia assay at each of the three centers according to the same protocol with monoclonal antibodies C10 and C11 (Clonab; Biotest AG, Dreieich, Germany) and a peroxidase-based detection system (11). For the DNA hybrid capture assay, EDTA-treated blood was used exclusively, leading to an incomplete follow-up for this test. Leukocyte separation and the hybrid capture assay were done according to the manufacturer's instructions. The leukocyte samples were kept frozen at -20°C and tested batchwise in one center (Zurich, Switzerland). Active infection was assumed when pp65 levels increased to ≥ 5 positive cells per 250,000 leukocytes and/or luminescence was above the cutoff level.

There were 16 episodes (increase and/or decrease of pp65-bearing cells or DNA concentrations in ≥ 2 consecutive blood samples) of active CMV infection detected in the selected

patient group. Overall, there were 95 (84%) convergent and 18 (16%) divergent results between the two assay systems. The divergent results were further analyzed as follows.

Significant viremia (≥ 10 pp65-positive cells and/or ≥ 20 pg of DNA/ml) was unambiguously detected by both assay systems. A total of 10 episodes of CMV infection were detected equally well and at the same time point. In relation to the number of pp65-positive cells, the DNA concentrations typically increased faster, reached maximum levels earlier, and dropped back to zero levels earlier. Discrepant results are preferentially located at both ends of the bell-shaped kinetic curves, but actually the same episode was detected by both assays. Nine of the 18 discrepant results could be explained by the different kinetics of DNA-emia and antigenemia, which increased the number of episodes detected in parallel to 15 of 16. For two patients (patients 3 and 12), DNA-emia was detected 2 to 3 weeks before the onset of significant antigenemia (Table 1). For three further patients, DNA could be detected earlier than pp65; evidence for earlier detection, however, was weak due to an incomplete follow-up with EDTA-treated blood samples.

Low-level antigenemia (≤ 5 pp65-positive cells) usually was paralleled by a negative or grey-zone result in the DNA hybridization assay. For two patients (patients 7 and 11), however, negative or low-level pp65 antigenemia but positive DNA hybridization signals were observed, which accounted for the nine remaining discrepant results. The validity of the DNA hybridization assay was verified by a more detailed case analysis.

Patient 7 (a CMV-negative recipient of a CMV-positive organ) at day 38 after transplantation presented with fever ($\geq 38.5^{\circ}\text{C}$) and showed signs of graft rejection. Ganciclovir was given for 10 days despite the absence of significant pp65 antigenemia. Retrospective analysis of the corresponding blood samples by the DNA hybrid capture assay clearly demonstrated an episode of DNA-emia which was not paralleled by pp65 antigenemia. The reasons for the failure of the antigenemia assay in this episode remained unclear; a higher sensitivity of the DNA detection assay may be a valid explanation. A second episode and a third episode of active CMV infection were clearly detected by the pp65 antigenemia assay; the corresponding blood samples, however, were heparinized and not suitable for parallel analysis by the DNA hybrid capture assay.

Patient 11 (a CMV-positive recipient of a CMV-negative

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TABLE 1. Comparison of results of the CMV pp65 antigenemia assay and the Murex Hybrid Capture assay

Patient no.	No. of days after transplantation	Assay result	
		pp65 antigenemia ^a	Hybrid capture ^b
3	63	2	80 (Pos.)
	84	250	2,525 (Pos.)
	86	325	1,567 (Pos.)
	91	113	308 (Pos.)
	93	2	123 (Pos.)
	98	2	4 (GZ)
	100	1	<1 (Neg.)
	119	61	216 (Pos.)
	126	230	573 (Pos.)
	142	3	<1 (Neg.)
	7	23	0
28		3	27 (Pos.)
37		0	143 (Pos.)
42		3	65 (Pos.)
44		0	43 (Pos.)
49		0	5 (Neg.)
51		0	<1 (Neg.)
56		0	<1 (Neg.)
58		0	1 (Neg.)
94		0	<1 (Neg.)
101		0	12 (Neg.)
106	12	42 (Pos.)	
11	25	3	20 (Pos.)
	32	0	106 (Pos.)
	34	0	113 (Pos.)
	39	2	82 (Pos.)
	41	1	112 (Pos.)
12	82	0	523 (Pos.)
	101	100	489 (Pos.)
	103	60	72 (Pos.)
	108	4	<1 (Neg.)
	115	0	4 (Neg.)
	138	0	<1 (Neg.)
150	2	8 (Neg.)	

^a Number of pp65-positive cells/250,000 leukocytes.

^b Amount of CMV DNA (in picograms per milliliter) and interpretation of the results. Pos., positive; Neg., negative; GZ, grey zone.

organ) did not suffer from problems related to CMV in the posttransplantation period. However, he received high doses of corticosteroids and multiple-agent chemotherapy for reasons not related to the transplantation. Analysis of blood samples of this patient showed no or only low-level pp65 antigenemia but yielded positive DNA hybridization results throughout the observation period. The validity of the positive DNA assay for this patient could not be substantiated because the observation time was short (16 days).

We have compared in a multicenter study the CMV pp65 antigenemia assay with the quantitative DNA hybrid capture assay. The two tests detect different viral components which

show different kinetics. This requires a modified interpretation of the results. From the results we conclude that the hybrid capture assay—if used in the same manner as the antigenemia assay—shows good agreement with and might be superior to the pp65 assay with respect to earlier detection of infection. This finding must be further investigated since the number of samples analyzed in parallel in this study was limited. The DNA hybridization assay is more expensive than the pp65 assay. The requirements of the two tests with regard to processing time and technical expertise are similar. A great advantage of the DNA hybrid capture assay over the pp65 assay is the possibility to store EDTA-treated blood samples as long as 72 h. This makes patient management significantly easier.

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