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Monitoring antigenemia is useful in guiding treatment of severe cytomegalovirus disease after organ transplantation

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Abstract. We investigated the value of monitoring CMV antigenemia during and after antiviral therapy for CMV disease. During the study period, 10 out of 214 renal transplant recipients were treated for CMV disease, receiving a total of 14 courses of treatment. Antigenemia decreased within 7 days after onset of treatment in eight of nine courses associated with a rapid clinical recovery. In three courses with a slow or absent response, antigenemia levels initially increased. Monitoring antigenemia was helpful in differentiating persisting CMV disease from other opportunistic infections and rejection. Relapses of CMV disease were preceded by rises in antigenemia. Viral isolation became negative within 3 days after initiation of ganciclovir, irrespective of the clinical response. Antigenemia is a marker of the effect of ganciclovir on CMV replication in vivo, and its monitoring may be valuable in the management of patients with severe CMV disease.

Key words: CMV, in renal transplantation – CMV antigenemia monitoring – Ganciclovir, in renal transplantation

Cytomegalovirus (CMV) is the most frequent infectious complication after solid organ transplantation and occurs in 50%–60% of all patients. Although asymptomatic or mild in many cases, 5%–20% of these infections cause severe disease manifestations [21, 24, 25]. Uncontrolled studies have suggested that ganciclovir (DHPG) is valuable in the treatment of severe CMV disease [10, 11, 16, 18, 19, 24, 27, 29]. However, insufficient responses to antiviral therapy occurred in about 10%–15% of the patients treated [13]. These failures might have been caused by delayed initiation of therapy, inadequate dosing, or true drug resistance [12]. Alternatively, persisting symptoms could have been a result of coinfection by other opportunistic organisms or by simultaneous allograft rejection. Relapse of symptomatology after cessation of therapy can occur in up to 20% of all patients [10]. In this situation, relapse of CMV activity should again be differentiated from other complications. Clearly, an objective parameter to monitor the effects of antiviral therapy on CMV replication would be very helpful in the management of patients with severe CMV disease. Viral isolation methods have limited value in this regard because processing times may last from 1 day to 1 or more weeks, and cultures often become negative within a few days after the onset of treatment, in patients with both favorable and unfavorable outcomes [11, 16, 20, 29].

Recently, we developed the CMV antigenemia assay: a rapid, sensitive, and quantitative method based on the detection of CMV antigens in peripheral blood leukocytes by immunoperoxidase staining [5]. The number of CMV antigen-positive leukocytes was shown to be closely related to the clinical course of disease in the individual patient. Patients with severe CMV disease had higher maximum numbers of antigen-positive leukocytes than those with asymptomatic or mild infection [1, 3]. Preliminary observations by ourselves and others suggest that antiviral treatment gradually reduces the level of CMV antigenemia [20, 28]. In this report we describe the effect of ganciclovir on CMV antigenemia levels in ten renal allograft recipients treated for severe CMV disease. Our results indicate that monitoring antigenemia may be helpful in detecting insufficient responses, in analyzing the mechanism of treatment failure, and in differentiating CMV relapse from other causes of renewed symptoms and, thus, enable individualization of antiviral therapy.

Materials and methods

Patients and management

Clinical and virological data were reviewed for all patients receiving a renal ($n = 211$) or combined renal-pancreatic ($n = 3$) transplant between September 1987 and January 1991. Details on baseline immunosuppression, diagnosis, and management of rejection have been described elsewhere [1]. No hyperimmune globulin (HIG) or high-dose acyclovir were given as a prophylactic measure against CMV. All patients were monitored for CMV infection during the frist 8–12 weeks after transplantation using the antigenemia assay, viral isolation from blood and urine, and serology.

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Table 1. Baseline characteristics and risk factors in ten patients with 14 episodes of suspected severe CMV disease. C, Cyclosporin A; P, prednisolone; A, azathioprine; MP, methylprednisolone; ATG, antithymocyte globulin

Patient No.	Age/Sex	Serostatus	Immunosuppression	No. of rejections	Antirejection therapy
1	33/M	–	CPA	2	MP; MP→ATG
1[a]			CPA		
2	63/M	–	ATG→CP	–	
3	54/M	–	C→CP	1	MP→ATG
4	52/M	–	CPA	1	MP→ATG
4[a]			CPA	–	
4[b]			CPA	–	
5	47/M	+	CP	2	MP; MP→ATG
6	41/F	–	OKT3→CPA	–	
7	23/F	–	OKT3→CPA	1	MP
8	46/M	–	CP→PA	–	
8[a]			PA	1	MP
9	29/M	–	CPA	1	MP
10	32/M	–	CP→CPA	1	MP→ATG

Generally, no specific measures were taken in asymptomatic CMV infection; immunosuppression was lowered as a first measure in patients with CMV disease.

Ganciclovir was given to 16 patients. Six received pre-emptive antiviral treatment because of simultaneous asymptomatic CMV infection and steroid-resistant rejection; four of these cases have been reported elsewhere and these patients were excluded from the present analysis [4]. Ten patients were treated because of CMV disease that was judged to be life-threatening or severe and that persisted despite reduction of immunosuppression; they form the study population of this report. Two of these patients received HIG (Cytotect, Biotest) as an additional therapeutic measure. A high number of CMV antigen-positive leukocytes per se was not an indication for antiviral therapy. Ganciclovir was administered in a dosage of 5 mg/kg twice daily, adjusted according to the manufacturer's prescription and drug levels (as measured by HPLC) in case of impaired renal function. Duration of treatment was 14 days, although this period could be adjusted according to the clinical status of the patient. During treatment patients were examined daily and had daily monitoring of complete blood count with differential as well as renal and liver function tests.

Virological methods

Tests for CMV antigenemia and antibodies were done once weekly and, during treatment with ganciclovir, twice weekly; viral isolation from blood and urine was done once weekly.

CMV antigenemia was determined as previously described [1]. In short, peripheral blood leukocytes were isolated, cytocentrifuged, and incubated with a mixture of two monoclonal antibodies directed against the 65 kD CMV lower matrix protein, followed by immunoperoxidase staining. The number of antigen-positive cells per 50000 polymorph nuclear leukocytes (PMNs) was counted in duplicate. Presence of at least one CMV antigen-positive leukocyte in one or both slides was regarded as a positive result.

CMV viremia and viruria were determined by the rapid coverslip culture method previously described and by a conventional isolation technique based on the demonstration of a cytopathological effect [22].

Serological diagnosis was based on quantitative determinations of IgM and IgG antibodies against CMV late antigens by an ELISA [14].

Definitions

CMV infection was diagnosed by the presence of antigenemia, viremia, and/or seroconversion (in the case of primary infection) or a significant rise in IgG antibodies (in the case of secondary infection). A positive antigenemia result that was not confirmed by culture or serology was regarded as false-positive.

CMV disease required the presence of CMV infection in combination with compatible clinical symptomatology. Histological evidence of organ involvement was obtained when possible. Other infectious causes were excluded using appropriate procedures.

Statistical methods

The χ^2 test was used to evaluate the data. A level of P less than 0.05 was considered to be statistically significant.

Results

CMV infection and outcome

CMV infection was diagnosed in 114 of the 214 patients studied; 27 were primary and 87 secondary infections. Antigenemia was detected in 99 patients; 96 of these subsequently proved to have CMV infection. In eight patients asymptomatic secondary infection occurred in the absence of antigenemia. The remaining ten infections occurred after discontinuation of antigenemia monitoring and were diagnosed serologically: these were also asymptomatic secondary infections. Antigenemia was generally the first available marker of CMV infection, appearing most often before the onset of symptoms.

CMV disease occurred in 24 of the 27 patients with primary infection and in 12 of the 87 patients with secondary infection. Severe CMV disease prompted treatment with ganciclovir in nine patients with primary and one patient with secondary CMV infection; in addition, four cases of suspected relapse of CMV disease were treated again. The clinical and virological characteristics of these ten patients are shown in Tables 1 and 2.

In the treatment group, one death and one additional case of graft loss occurred in association with CMV disease. No CMV-related death or graft loss occurred in patients in whom treatment of CMV disease was not deemed necessary.

Table 2. Clinical manifestations and duration of therapy in ten patients with 14 episodes of suspected severe CMV disease. F, Fever; A, arthralgia; H, hepatitis; L, leukopenia; Th, thrombocytopenia; P, pulmonary symptoms; p, pericarditis; R, renal dysfunction; G, gastrointestinal symptoms

Patient No.	Period of disease (days after transplantation)	Symptoms	Period of treatment (days after transplantation)	Remarks
1	37–53	F, A, H, Th	45–63	Slow onset of therapeutic response
1[a]	(75–80)	F, R	77–86	Symptoms caused by rejection
2	38–73	F, A, H, Th	49–74	Concomitantly treated with hyperimmune globulin; fatal infection
3	58–72	F, p, H	63–72	Concomitant fungal infection
4	38–53	F, R	43–57	Slow onset of therapeutic response; relapsed after discontinuation of treatment
4[a]	78–81	F, R	80–93	Relapsed after discontinuation of treatment
4[b]	135–136	F	136–150	
5	81–88	F, P	84–96	
6	33–35	F, A	35–47	
7	31–33	F, G	32–46	
8	72–80	F, P	79–107	Relapsed after discontinuation of treatment
8[a]	123–127	F, L, Th	126–140	Concomitantly treated with hyperimmune globulin
9	197–214	F, R, G, H, L, Th	212–225	
10	33–42	F, A, H	41–54	Relapsed after discontinuation of treatment

CMV antigenemia levels in untreated and treated patients

CMV antigenemia levels were generally low (< 10 per 50000 leukocytes) before the onset of symptoms but rose to high levels during the period of CMV disease. In untreated patients with primary infection, maximal antigenemia levels rose to a median of 70 (range 2–437) positive cells per 50000 leukocytes. Clinical improvement, accompanied by decreasing antigenemia levels, occurred simultaneously with the rise in CMV antibodies.

In patients treated for primary CMV infection, mean antigenemia levels were 46 (range 5–921) positive cells at the onset of treatment, comparable to those of untreated patients. However, these levels were still rising in most patients and many of them had no evidence of antibody responses at the onset of treatment.

CMV antigenemia levels in the single patient treated for secondary infection were higher (308 positive cells) than in untreated symptomatic or asymptomatic CMV infection [3 (range < 1–94) positive cells versus < 1 (range 0–282) positive cells, respectively]. No clear influence of donor serostatus on outcome of secondary CMV infection was evident, but donor serology was available for only 32 % of these patients.

Effect of ganciclovir treatment on clinical symptoms

Clinical efficacy of ganciclovir could be evaluated in 12 of the 14 courses of treatment (Table 2). Before treatment, symptoms of disseminated CMV infection were present in all, often accompanied by signs of organ involvement. After the initiation of antiviral therapy, rapid improvement occurred in 9 of the 12 evaluable courses, as evidenced by the disappearance of symptoms and signs of disease within 1–5 days. In 2 courses recovery occurred only slowly (after 9 and 11 days) and response was minimal in 1 course: this patient died from CMV-related gastrointestinal hemorrhage after 25 days of treatment.

The clinical response to treatment could not be completely evaluated in two courses. In one course, symptoms were caused by rejection, while in the other coexistent disseminated fungal infection impeded analysis of the contribution of ganciclovir to recovery. Virological responses during both courses have been included in the analysis, however.

Effect of ganciclovir on CMV antigenemia (Table 3)

At the onset of antiviral treatment, CMV antigenemia levels were mostly high and rapidly rising. During 10 of the 14 courses of treatment, the number of antigen-positive cells dropped sharply within the 1st week, while in the remaining 4 courses (patient nos. 1, 2, 4, and 7) CMV antigenemia continued to rise and decreased only during the 2nd or 3rd week of treatment. CMV antigenemia became undetectable on at least one occasion during 7 of the 14 courses, after a median of 12 (range 7–21) days of therapy. In the remaining 7 courses, generally low levels of antigen-positive cells remained present.

Clinical responses were evaluable in eight of the ten courses associated with a rapidly occurring decrease in CMV antigenemia level. In these courses, recovery occurred a median of 2 (range 1–5) days after initiation of therapy.

In one of the four courses associated with initially rising CMV antigenemia levels (patient no. 7), symptoms had disappeared after the 2nd day of treatment. In the other three courses, symptoms persisted for 9, 11, and 25 days, respectively.

Table 3. Effect of treatment with ganciclovir on CMV antigenemia and viral isolation. A, CMV antigenemia; BC, blood culture; UC, urine culture; ▭ treatment period; ctd, continued

Patient No.	Method	Days before/after onset of treatment																	
		–10		–5	0		5	10				15		20			25	30	
1	A		11		40	77	98	4	0			< 1	0		< 1		< 1		3
	BC		+	+		+	–		–				–						+
	UC		–	–		–	–		+				–						–
1[A]	A			< 1	3	< 1	< 1			4		8	2		1		< 1		
	BC				+		–					+			–		–		
	UC				–		–					–			+		–		
2	A		4		135	263	282			348			66	5			†		
	BC		+		+		–			–				–					
	UC		–							–				–					
3	A		2		921		< 1			< 1				< 1			< 1		< 1
	BC		+				–			–				+					–
	UC		+		+	–	–												+
4	A		59		100	37	139	79		27			110	40		13	97		
	BC		+		+		–			–				–			+		
	UC		–		–		–			–				–			+		
4[A]	A		97		347	206	77	15		2					5	2			
	BC		+		+					–					–				
	UC		+		+					–					–				
4[B]	A	9	45	50	150	37	14	5		1				< 1			< 1		< 1
	BC			+		–								–			–		–
	UC			+		–								–			–		–
5	A		227		308	106	< 1	–		–			4		3	3	8		
	BC		+		+		–			–					+		+		
	UC		–		+		–			–					–		–		
6	A	0			3	5	0	0		0			0		< 1	0		+	
	BC	+				–		–					–		+	+		–	
	UC	–				–							–			–			
7	A		0	3	14	71	154	113		23			0				< 1		2
	BC			+		–		–											
	UC			–		–		–											
8	A				138	37	7	6		5			< 1		0	0			1
	BC				+	–	–	–		–			–		–	–			–
	UC				+		–								–		–		
8[A]	A		1	5	13	12	3			–			–		–	1			
	BC		+	–	+	+	–			–			–		–	–			
	UC			–		+							–						
9	A		4	8	39	12	0	< 1		0									
	BC			+	+	–	–	–			–								
	UC		–		+		–				–								
10	A	< 1	4	17	46	13		1					–		2	1	40		29
	BC	+	+	+		– –		–					+		–				
	UC		–			–	–								–		–		
10 (ctd)	A		123	47	27	7		3		< 1									
	BC		–		+					–									
	UC		–		–					–									

Changes in CMV antigenemia levels during the 1st week of treatment paralleled the clinical response in 11 of 12 evaluable cases ($\chi^2 = 4.5, df = 1, P < 0.05$).

In the patient with combined CMV and fungal infection (no. 3), antigenemia levels rapidly dropped under antiviral therapy. Fever persisted for 10 days after initiation of ganciclovir. This delayed disappearance of symptoms might have been caused by the slow response to antifungal drugs.

Viral isolation during treatment with ganciclovir (Table 3)

Viremia was present before the onset of treatment in 14 and viruria in 6 courses. During treatment, only 2 of 31 blood cultures and 2 of 24 urine cultures were positive: cultures became rapidly negative in patients with both rapidly and slowly occurring clinical responses. Blood samples became culture-negative even when CMV antigenemia levels were still high: 11 of 13 blood cultures from

samples with more than ten antigen-positive cells per 50000 PMNs remained negative, whereas in untreated patients such levels of antigenemia are associated with positive cultures in more than 70% of all cases (data not shown).

Antigenemia viremia after treatment: differentiation of relapse from other infections or rejection

Renewed symptoms after cessation of antiviral therapy occurred in seven courses. Relapse of CMV disease was diagnosed in four of these seven courses (patient nos. 4–twice–8, and 10). All relapses were accompanied by rises in antigenemia levels. Shortly before the onset of symptoms of CMV relapse, antigenemia levels were 97, 50, 13, and 40 positive cells, respectively. In three cases renewed treatment with ganciclovir rapidly resulted in reduced antigenemia levels and clinical recovery. Spontaneous recovery occurred in the remaining case.

Although CMV antigenemia was often present for many weeks or even months after treatment in patients without relapse of CMV disease, the number of antigen-positive cells never rose above 8 per 50000 PMNs in such cases.

Viremia recurred in all four relapsing patients but was also present after six of nine evaluable courses of treatment without clinical relapse.

In three cases, renewed symptoms were accompanied by low numbers of antigen-positive cells. In patient no. 1, fever and declining renal function were initially thought to be caused by relapsing CMV disease. However, treatment with ganciclovir reduced antigenemia levels to even lower levels, while fever continued and renal function declined further. At that moment, renal biopsy showed interstitial rejection, and treatment with methylprednisolone rapidly led to the disappearance of fever and the recovery of graft function. Symptoms were caused by *Staphylococcus aureus* sepsis in patient no. 3 and vascular rejection in patient no. 8, respectively.

Thus, in patients with renewed symptoms after antiviral treatment, CMV antigenemia levels were helpful in differentiating between relapse of CMV disease and other causes of symptoms.

Discussion

In previous studies, we and others have demonstrated that the CMV antigenemia assay is a reliable tool for the early detection of CMV infection and for the identification of patients at risk for severe CMV disease [1–3, 7, 8]. We now report on the value of antigenemia during treatment of severe CMV disease. Although antigenemia ultimately declined in all patients, the kinetics of this response were different in patients with rapid and slow clinical recovery, and changes in the number of antigen-positive leukocytes closely paralleled clinical responses to treatment. Relapses of CMV disease were accompanied – and, in fact, preceded – by increasing antigenemia levels. These data support the notion that antigenemia is a marker of the momentary viral load and suggest that the assay can be used to measure the effect of antiviral treatment.

Management of CMV disease may be relatively straightforward in some patients who show rapid clinical improvement and are cured by a standard 2-week course of treatment. However, as is evident from this and other studies, complicating factors are present in a significant number of patients. These are: (1) slow or absent response, (2) CMV relapse, and (3) superinfection or rejection. Monitoring antigenemia will be valuable in determining optimal management, especially in these "difficult" cases.

When no clinical improvement occurs despite antiviral therapy, rising antigenemia levels would suggest continued CMV disease activity, while decreasing levels would reduce the likelihood of CMV being the cause of symptoms and lead one to search for other infections or allograft rejection. In combination with drug levels, CMV antigenemia might provide insight into the mechanism of treatment failure. High numbers of antigen-positive cells in the presence of low drug levels would suggest underdosage, while high drug levels would be expected in cases of drug resistance. Unfortunately, drug sensitivity could not be determined at the time we treated the patient with fatal CMV infection. However, because viremia disappeared while the patient was under treatment, complete resistance to ganciclovir seems unlikely. Moreover, ganciclovir resistance generally develops only after several months of therapy [9].

Ganciclovir only temporarily interrupts viral replication; it cannot induce latency. Thus, clinical relapse may follow discontinuation of treatment and has been reported to occur in 20% of cases [10]. Monitoring antigenemia after treatment is useful for early detection (and possibly for pre-emptive treatment) of relapsing CMV disease because antigenemia levels higher than 8 per 50000 PMNs occurred only in association with clinical relapse. Conversely, renewed symptoms in the presence of low numbers of positive cells should prompt a search for alternative causes.

Relapses after discontinuation of ganciclovir occurred irrespective of the initial antigenemia response. This is not surprising since the effect of treatment on viral replication is primarily determined by susceptibility of the viral stain in combination with ganciclovir levels, whereas host immunity is necessary to maintain latency after stopping treatment. It is our impression that relapses of primary CMV disease occur in patients having quantitatively low IgG responses against CMV after the initial IgM response. This will be an important subject for further study because it may provide a way to individualize antiviral therapy.

The effects of ganciclovir on CMV antigenemia and viral isolation were quite different. Even in patients with a rapid clinical response to ganciclovir, it took at least 1 week for antigenemia to disappear completely (in fact, CMV remained present during 7 of 14 courses), while 6 of 8 blood cultures were negative on day 3 and 14 of 16 were on day 7, which is in line with observations by Ravello et al. [20].

Negativity of viral isolation under antiviral therapy was an early marker of ultimate recovery from infection, except in the patient with fatal infection. However, close monitoring of CMV antigenemia provided valuable additional information, as discussed earlier.

Detection of viremia in both rapid and conventional cultures relies on complete viral replication, while infec-

tion of PMNs in vivo is limited to the immediate early (IE) stage [6]. Ganciclovir interrupts viral DNA synthesis, which occurs during the late (L) stage, but does not influence the earlier stages of replication. Thus, viral isolation will be inhibited but expression of IE antigens in PMNs will not be directly influenced. The gradually declining number of antigen-positive leukocytes during antiviral treatment probably reflects the reduced amount of infectious virus formed elsewhere in the body. It should be noted that persistence or reappearance of viremia during ganciclovir treatment has been documented to occur: this was associated with poor therapeutic responses and should probably be regarded as a manifestation of high-grade drug resistance [12, 17].

The close correspondence between clinical response and antigenemia pattern, which we also observed in liver and cardiac transplant patients, suggests that in at least these groups of patients, clinical improvement is mediated by the interruption of CMV replication. This is in contrast to observations in allogenic bone marrow transplant patients with CMV pneumonitis, in whom progressive respiratory dysfunction and death occurred despite elimination of CMV from cultures of respiratory secretions and other body fluids [23]. It is tempting to speculate that in these patients CMV triggers immunopathological mechanisms (e.g., recruitment of CD4 + lymphocytes or complement activation) that are relatively independent from active virus replication [15, 26].

No controlled studies on the effect of ganciclovir on severe CMV disease in solid organ transplant recipients have been reported to date and so the efficacy of the drug cannot be regarded as proven. Although this study was not intended to examine the therapeutic usefulness of ganciclovir, our data present new evidence of its efficacy. The close correlation between changes in CMV antigenemia levels and clinical response during antiviral treatment strongly suggests that these improvements were indeed caused by ganciclovir.

In conclusion, monitoring of antigenemia during treatment of severe CMV infection is useful for early identification of patients with insufficient responses, for analysis of the mechanisms of treatment failure, and for detection of relapse. Close monitoring, using both antigenemia assay and host immune responses (e.g., by serology), may prove that a fixed treatment period of 14 days of ganciclovir may be too short for some patients, too long for others (i.e., patients with rapid clearance of CMV and a brisk immune response), and just right for still others. Ultimately, this may lead to "tailor-made" antiviral therapy.

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References

1. Berg AP van den, Bij W van der, Son WJ van, Anema J, Giessen M van der, Schirm J, Tegzess AM, The TH (1989) Cytomegalovirus-antigenemia as a useful marker of symptomatic cytomegalovirus disease after renal transplantation: a report of 130 consecutive patients. Transplantation 48: 991–995
2. Berg AP van den, Bij W van der, Giessen M van der, The TH, Son WJ van (1990) Early identification of patients at risk for severe cytomegalovirus disease after renal transplantation using the immediate early antigenemia assay. Transplant Proc 22: 1800–1802
3. Berg AP van den, Klompmaker IJ, Haagsma EB, Scholten-Sampson A, Bijleveld CMA, Schirm J, Giessen M van der, Slooff MJH, The TH (1991) Antigenemia in the diagnosis and monitoring of active CMV infection after liver transplantation. J Infect Dis 164: 265–270
4. Berg AP van den, Tegzess AM, Scholten-Sampson A, Giessen M van der, The TH, Son WJ van (1991) Quo vadis? The clinical dilemma of simultaneous cytomegalovirus infection and steroid-resistant rejection. Transplantation 52: 1081–1083
5. Bij W van der, Torensma R, Son WJ van, Anema J, Schirm J, Tegzess AM, The TH (1988) Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leukocytes. J Med Virol 25: 179–188
6. Bij W van der, Schirm J, Torensma R, Son WJ van, Tegzess AM, The TH (1989) Comparison between viremia and antigenemia for detection of cytomegalovirus in blood. J Clin Microbiol 26: 2531–2535
7. Boland GJ, Gast GC de, Hene RJ, Janbroes G, Donckerwolcke R, The TH, Mudde GC (1990) Early detection of active cytomegalovirus (CMV) infection after heart and kidney transplantation by testing for immediate early antigenemia and influence of cellular immunity on the occurrence of CMV infection. J Clin Microbiol 28: 2069–2075
8. Dorp WT van, Jonges E, Jiwa NM, Gemert W van, Es LA van, Ploem JS, The TH, Woude FJ van der (1990) Symptomatic cytomegalovirus infections identified by image cytometry and other parameters for CMV infection. Transplant Int 3: 212–216
9. Drew WL, Miner RC, Bush DF, Follansbee SE, Gullett J, Mehalko SG, Gordon SM, Owen WF, Matthews TR, Buhles WC, DeArmond B (1991) Prevalence of resistance in patients receiving ganciclovir for serious cytomegalovirus infection. J Infect Dis 163: 716–719
10. Dunn DL, Mayoral JL, Gillingham KJ, Loeffler CM, Brayman KL, Kramer MA (1991) Treatment of invasive cytomegalovirus disease in solid organ transplant patients with ganciclovir. Transplantation 51: 98–106
11. Enrice A, Jordan MC, Chace BA, Fletcher C, Chinnock BJ, Balfour HH Jr (1987) Ganciclovir treatment of cytomegalovirus disease in transplant recipients and other immunocompromised hosts. JAMA 257: 3082–3087
12. Enrice A, Chou S, Biron KK, Stanat SC, Balfour HH Jr, Jordan MC (1989) Progressive disease due to ganciclovir-resistant cytomegalovirus in immunocompromised patients. N Engl J Med 320: 289–293
13. Faulds D, Heel RC (1990) Ganciclovir. A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in cytomegalovirus infections. Drugs 39: 597–638
14. Giessen M van der, Berg AP van den, Bij W van der, Postma S, Son WJ van, The TH (1990) Quantitative measurement of CMV-specific IgG- and IgM-antibodies in relation to CMV antigenemia and disease activity in kidney transplantation with active CMV infection. Clin Exp Immunol 80: 56–61
15. Grundy JE, Shanley JD, Griffiths PD (1987) Is cytomegalovirus interstitial pneumonitis in transplant recipients an immunopathological condition? Lancet II: 996–999
16. Harbison MA, De Girolani PC, Jenkins RL, Hammer SM (1988) Ganciclovir therapy of severe cytomegalovirus infection in solid-organ transplant recipients. Transplantation 46: 82–88
17. Jennens ID, Lucas CR, Sandland AM, Maclean H, Hayes K (1990) Cytomegalovirus cultures during maintenance DHPG therapy for cytomegalovirus (CMV) retinitis in acquired immunodeficiency syndrome (AIDS). J Med Virol 30: 42–44
18. Metselaar HJ, Weimar W (1989) Cytomegalovirus infection and renal transplantation. J Antimicrob Chemother 23 [Suppl E]: 37–47

19. Paya CV, Hermans PE, Smith TF, Rakela J, Wiesner RH, Krom RAF, Torres VE, Sterioff S, Wilkowske CJ (1988) Efficacy of ganciclovir in liver and kidney transplant recipients with severe cytomegalovirus infection. Transplantation 46: 229–234
20. Ravello MG, Percivale E, Zavattoni M, Parea M, Grossi P, Gerna G (1989) Detection of human cytomegalovirus immediate early antigen in leukocytes as a marker of viremia in immunocompromised patients. J Med Virol 29: 88–93
21. Rubin RH (1988) Infections in the patient after renal and liver transplantation. In: Rubin RH, Young LS (eds) Clinical approach to infections in the immunocompromised patient. Plenum, New York, pp 557–583
22. Schirm J, Timmerije W, Bij W van der, The TH, Wilterdink JB, Tegzess AM, Son WJ van, Schroeder FP (1987) Rapid detection of infectious cytomegalovirus in blood with the aid of monoclonal antibodies. J Med Virol 23: 31–40
23. Shepp DH, Dandliker PS, Miranda P de, Burnette TC, Cederberg DM, Kirk LE, Meyers JD (1985) Activity of 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]guanine in the treatment of cytomegalovirus pneumonia. Ann Intern Med 103: 368–373
24. Snydman DR (1988) Ganciclovir therapy for cytomegalovirus disease associated with renal transplants. Rev Infect Dis 10 [Suppl 3]: 554–562
25. Son WJ van, The TH (1990) Cytomegalovirus infection after transplantation: an update. Transplant Int 2: 147–164
26. Son WJ van, Tegzess AM, The TH, Duipmans J, Slooff MJH, Mark TW van der, Peset R (1987) Pulmonary dysfunction is common during a cytomegalovirus infection after renal transplantation, even in asymptomatic patients; possible relationship with complement activation. Am Rev Respir Dis 136: 580–585
27. Stoffel M, Gianello P, Squifflet JP, Pirson Y, Alexandre GPJ (1988) Effects of 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]-guanine (DHPG) cytomegalovirus pneumonitis after renal transplantation. Transplantation 46: 594–595
28. The TH, Bij W van der, Berg AP van den, Giessen M van der, Weits J, Sprenger HG, Son WJ van (1990) Cytomegalovirus antigenemia. Rev Infect Dis 12: S737–744
29. Thomson MH, Jeffries DJ (1989) Ganciclovir therapy in iatrogenically immunosuppressed patients with cytomegalovirus disease. J Antimicrob Chemother 23 [Suppl E]: 61–70