CHAPTER 2

EFFECTS OF CHANGING IMMUNOSUPPRESSIVE REGIMEN ON THE INCIDENCE, DURATION AND VIRAL LOAD OF CYTOMEGALOVIRUS INFECTION IN RENAL TRANSPLANTATION: A SINGLE CENTER REPORT

E.F. de Maar, E.A.M. Verschuuren, J.J. Homan van der Heide,
D.M. Kas-Deelen, D. Jagernath, T.H. The,
R.J. Ploeg, and W.J. van Son

Transplant Infectious Disease 2002; 4:17-24

ABSTRACT

Background. In this retrospective single center study we have evaluated the relation between the immunosuppressive regimen and the incidence and characteristics of cytomegalovirus (CMV) infection in the setting without CMV prophylaxis from 1989 through 1998.

Methods. All (470) first cadaveric renal transplantations in nonsensitized (PRA < 60%) patients were analyzed. Immunosuppression consisted of cyclosporine A (Sandimmune) and prednisolone from 1989 through 2-1993 (S; 189 patients), of cyclosporine microemulsion (Neoral) and prednisolone from 3-1993 through 5-1997 (N; 200 patients) and of mycophenolate mofetil, Neoral and prednisolone from 5-1997 until 1998 (M; 81 patients). CMV pp65-antigenemia was measured routinely at least once weekly from day 10 till 12 weeks after transplantation or until pp65-antigenemia became negative. No CMV-prophylaxis was given.

Results. By changing from Sandimmune to Neoral and by adding mycophenolate mofetil, respectively, we observed a higher frequency of especially secondary CMV infections (S vs. N vs. M respectively 28 vs. 50 vs. 63%, $P = 0.026$; S vs. N, $P = 0.027$; S vs. M, $P = 0.015$; and N vs. M n.s.). The CMV infections lasted longer (median duration antigenemia S vs. N vs. M respectively 3 vs. 5 vs. 7 weeks, $P = 0.0003$; S vs. N, $P < 0.002$; S vs. M, $P < 0.001$; and N vs. M, $P < 0.05$). Viral load was higher in M (median maximal pp65-antigenemia S vs. N vs. M respectively 19 vs. 14.5 vs. 73, $P < 0.01$, S vs. N, n.s.; S vs. M, $P < 0.001$; and N vs. M, $P < 0.01$).

Conclusions. The use of Neoral and the addition of mycophenolate mofetil caused significant changes in the incidence, duration and viral load of CMV infections.

2.1 INTRODUCTION

Over the past decades the immunosuppressive regimen used in transplantation has changed considerably. In the seventies immunosuppression after renal transplantation consisted of azathioprine and prednisone. The introduction of cyclosporine improved graft survival in the eighties. To date, mycophenolate mofetil and tacrolimus are fully integrated in most immunosuppressive protocols and cyclosporine standard formulation (Sandimmune, Novartis) has been converted to a microemulsion formulation (Neoral, Novartis) in most European centers. Also rapamycin is currently under study in clinical transplantation and a number of new more specific monoclonal agents are replacing older polyclonal antisera.

The availability of all these new immunosuppressive drugs enables us to design rational and perhaps more individualized combinations to prevent acute and chronic rejection and, very important, may minimize the risks of the sequelae of immunosuppression: malignancy and infection. Also, cardiovascular side effects and prednisone-induced bone disease have become distinguished complications in the long term. Until today only few well-controlled trials have compared different immunosuppressive regimens as regards their long-term side effects. Especially no detailed virological data are presented in the literature concerning the different immunosuppressive regimens. In the majority of predominantly multicenter studies cytomegalovirus (CMV) infection is scored as an 'on-off' phenomenon without enumeration of detailed virological data. Furthermore data are lacking for asymptomatic patients and the interpretation of the studies is difficult owing to different prophylactic protocols for CMV.

However, CMV is one of the most frequent infectious complications after kidney transplantation. It causes severe morbidity and even mortality. If CMV infection causes disease most patients have a so-called self-limiting CMV syndrome consisting of spiking fever, arthralgia, leukocytopenia, thrombocytopenia, and elevated serum liver enzymes.

Many CMV infections after kidney transplantation, however, remain asymptomatic and in our center are recognized by routinely performed pp65-antigenemia assay [1, 2] to be confirmed by a serum-response later [3]. Nevertheless, we have demonstrated that in 'asymptomatic' kidney transplant patients subclinical organ dysfunction can be detected during CMV pp65-antigenemia. This dysfunction was associated with an increased intestinal permeability and a decrease in pulmonary diffusion in kidney transplant recipients with positive antigenemia assays but without symptoms [4-6]. The impact of these manifestations of CMV infection especially on long-term function has yet to be established.

Cytomegalovirus is suspected to play a role in the pathogenesis of atherosclerosis also in non-transplanted patients [7]. Endothelial damage caused by the viral infection might be an important factor in the pathophysiology regarding the suggested connection between atherosclerosis and cytomegalovirus infection. In this respect the hypothesis that cytomegalovirus can cause endothelial damage in the transplanted organ leading to chronic transplant dysfunction [8-16] is particularly interesting.

In this retrospective single center study we have evaluated the relation between the immunosuppressive protocol used and incidence, duration and viral load of cytomegalovirus infections in first cadaveric kidney transplant procedures in our institution from 1989 through 1998.

2.2 MATERIALS AND METHODS

2.2.1 Study design

From January 1989 through July 1998, 721 patients were transplanted in our center. Of those, 470 patients received a first transplant and followed our standard immunosuppressive protocol consisting of Cyclosporine standard formulation (Sandimmune, Novartis) and prednisolone from January 1989 through February 1993 (S group). From March 1993 through May 1997 cyclosporine microemulsion formulation (Neoral, Novartis) and prednisolone (N group) were used. From May 1997 until now mycophenolate mofetil (Cellcept, Roche), Neoral, and prednisolone were administered (M group). Patients on different immunosuppressive regimens were excluded. Also excluded were patients with more than 60% panel reactive HLA-antibodies (primary triple therapy or induction therapy with OKT3 or ATG). Living related kidney transplant recipients on primary triple therapy with azathioprine and Sandimmune were excluded. Also excluded were patients participating in the European Mycophenolate Mofetil Cooperative Study [17] (Sandimmune instead of Neoral in combination with mycophenolate mofetil and prednisolone). All 470 patients were divided into three groups according to the three different immunosuppressive regimens used during the past ten years. All pertinent data of the three groups especially those regarding CMV infections were analyzed in this study.

2.2.2 CMV definitions

All primary and secondary CMV infections were studied.

Primary CMV infections were defined as seronegative recipients receiving a transplant from a seropositive donor (pos-neg serology combinations for donor-recipient) and subsequently became positive in the pp65-antigenemia assay and developed an anti-CMV IgM and subsequently IgG response. The incidence of primary infections was defined as the percentage of primary infections in the patients at risk for primary infections: pos-neg combinations.

Secondary CMV infections were defined as seropositive recipients who developed a positive pp65-antigenemia, irrespective of their serological response (pos-pos or neg-pos serology combinations). The incidence of secondary infections was defined as the percentage of secondary infections in the patients at risk for secondary infections: pos-pos and neg-pos combinations. The neg-neg combinations with no risk to develop CMV infection were not included in the analysis. No CMV-prophylaxis with ganciclovir, acyclovir or immunoglobulins was used. Ganciclovir i.v. was given for CMV-disease or preemptively during antirejection therapy when the yet asymptomatic patient was positive in the pp65-antigenemia assay.

2.2.3 CMV diagnostics

Diagnosis of active CMV infection was made using the CMV pp65-antigenemia assay, as described by Van der Bij et al. [1] and reviewed by Chou [18] and by Ljungman and Griffiths [19] during the Fourth International CMV Workshop (Paris, 1993). The quantitative antigenemia assay correlates well with viral load and CMV induced disease [2]. Thus a higher antigenemia reflects the condition of a higher viral load in this report. Recently an attempt to standardize the CMV pp65-antigenemia assay has been proposed by our group [20]. Briefly, peripheral blood leukocytes were isolated, cytocentrifuged and incubated with a mixture of monoclonal antibodies directed against a 65 kDa CMV antigen, followed by immunoperoxidase staining. The number of antigen-positive cells and total number of leukocytes were counted on two different cytospots and results were expressed as number of pp65 positive cells per 50.000 leukocytes. Antigenemia assay was performed at least once weekly starting on postoperative day 10 until 12 weeks after transplantation or until pp65-antigenemia became negative. In all the patients antigenemia was followed by either seroconversion or significant rise in CMV IgG antibodies. The IgM and IgG CMV

antibodies were measured quantitatively by ELISA using late stage CMV-infected fibroblasts as antigens [3].

2.2.4 Rejection treatment

Rejections were diagnosed clinically by a rise in serum creatinine and sodium retention without a clear other cause, and were treated with a pulse therapy of methylprednisolone 1 gram i.v. on three consecutive days. The majority of clinical rejections were confirmed by biopsy and diagnosed according to the Banff criteria [21]. Patients with a steroid-resistant interstitial rejection were treated with antithymocyte immunoglobulin (rabbit-ATG, Merieux, Lyon, France), five times 4 mg/kg, i.v. every other day. When vascular rejection was found plasmapheresis was added to the ATG treatment protocol on alternate days.

2.2.5 Statistics

The distribution of patients among the three different groups was tested using contingency tables (χ^2-test) for multiple groups and χ^2-test for two groups as a post-test if $P < 0.05$. The three groups were analyzed using ANOVA or non-parametric analysis of variance: Kruskal-Wallis test. Tukeys test or Dunn's Multiple Comparison test was used as post-test if $P < 0.05$.

2.3 RESULTS

2.3.1 Patient characteristics, donors, matching per treatment group

Patient and donor characteristics of all evaluated first cadaveric kidney transplant recipients treated with standard immunosuppression in our center from 1989 till 1998 are given in table 2.1. In the N and M group there were more female donors than in the S group (S vs. N vs. M in percentage females respectively, 44, 68 and 62%, $P < 0.0001$; S vs. N, $P = 0.0001$; S vs. M, $P = 0.0073$; and N vs. M, n.s.). The cold ischemia time was shorter changing from S to N to M, respectively (S vs. N vs.

M in median hours, 25 vs. 23 vs. 19h, $P < 0.0001$; S vs. N, $P < 0.01$; S vs. M, $P < 0.001$; and N vs. M, $P < 0.05$). The M group consisted of fewer well-matched kidneys for HLA-DR compared to the N and S group (S vs. N vs. M in mean mismatches for HLA-DR 0.47 vs. 0.28 vs. 0.54, $P < 0.0001$; S vs. N, n.s.; S vs. M, $P < 0.001$; and N vs. M, $P < 0.01$).

2.3.2 CMV infection: incidence, first day, duration and maximal antigenemia

The incidence of positive pp65-antigenemia as indication for active CMV infection in the donor-patient combinations at risk for CMV (pos-neg, pos-pos and neg-pos combinations) was 35% in the S group compared to 53% in the N group and 64% in the M group ($P = 0.041$; S vs. N, $P = 0.049$; S vs. M, $P = 0.020$; N vs. M, n.s., table 2.2, figure 2.1a). The median time between the transplant operation and the first positive antigenemia assay did not differ between the groups (respectively 4, 5 and 4.5 weeks: n.s.).

However, the median duration of positive pp65-antigenemia was found to be significantly longer after the change of the immunosuppressive protocol from S to N and M, respectively (median duration S vs. N vs. M respectively 3, 5 and 7 weeks, $P = 0.0003$; S vs. N, $P < 0.002$; S vs. M, $P < 0.001$; and N vs. M, $P < 0.05$, table 2.2, figure 2.2b). In addition the median maximal viral load measured as the maximal reached CMV pp65-antigenemia level, was higher in the M group compared to the N and S group (S vs. N vs. M respectively 19, 14.5 and 73, $P < 0.01$; S vs. N, n.s.; S vs. M, $P < 0.001$; and N vs. M, $P < 0.01$, table 2.2, figure 2.2a).

2.3.3 Primary versus secondary infection, IgG and IgM response

The distribution of CMV negative recipients at risk for primary infection and of seropositive recipients at risk for secondary infection did not differ significantly between the three groups (table 2.3). The increase in incidence in CMV infection after changing from Sandimmune to Neoral was owing to an increase in secondary infections (S vs. N vs. M respectively 28, 50 and 63%, $P = 0.026$; S vs. N, $P = 0.027$; S vs. M $P = 0.015$; and N vs. M, n.s., table 2.2, figure 2.1c).

Table 2.1 Patient and donor characteristics. Ischemia times, mismatches and antirejection treatment.

	S vs. N vs. M	Sandimmune Pred (S)	S vs. N	Neoral Pred (N)	N vs. M	Neoral Cellcept Pred (M)	S vs. M
number of patients		189		200		81	
patient age (mean years)	ns	44		47		46	
% female patients	ns	38		38		40	
donor age (mean age)	ns	37		40		40	
% female donors	$P = 0.0001$	44	$P = 0.0001$	68	ns	62	$P = 0.0073$
cold ischemia time*	$P < 0.0001$	25	$P < 0.01$	23	$P < 0.05$	19	$P < 0.001$
second warm ischemia time**	ns	39		38		38	
mismatches HLA-AorB***	ns	1.63		1.22		1.32	
mismatches HLA-DR***	$P < 0.0001$	0.47	ns	0.28	$P < 0.01$	0.54	$P < 0.001$
% atg	ns	16		22		16	
% rejection	ns	42		42		41	

* median hours
** mean minutes
*** mean

atg = anti-thymocyte globulin

Table 2.2 Cytomegalovirus infection; incidence, maximal reached pp65-antigenemia, duration, time of appearance and treatment of patients at risk for CMV infections (neg-neg combination excluded).

	S vs. N vs. M	Sandimmune Pred (S)	S vs. N	Neoral Pred (N)	N vs. M	Neoral Cellcept Pred (M)	S vs. M
incidence of CMV infection	$P = 0.041$	35% (53/151)	$P = 0.049$	53% (88/167)	ns	64% (38/59)	$P = 0.020$
incidence of primary CMV infection	ns	51% (23/45)		58% (30/52)		68% (13/19)	
incidence of secondary CMV infection	$P = 0.026$	28% (30/106)	$P = 0.027$	50% (58/115)	ns	63% (25/40)	$P = 0.0147$
incidence of CMV in patients with atg	ns	52% (11/12)		77% (23/30)		100% (11/11)	
incidence of CMV in patients with rejection	ns	49% (30/61)		66% (42/64)		71% (17/24)	
incidence of primary CMV in patients without rejection	ns	44% (11/25)		54% (15/28)		67% (10/15)	
incidence of secondary CMV in patients without rejection	$P = 0.04$	18% (12/65)	$P = 0.031$	41% (31/75)	ns	55% (11/20)	$P = 0.022$
median maximal pp65-antigenemia	$P < 0.01$	19	ns	14.5	$P < 0.01$	73	$P < 0.001$
median duration of antigenemia in weeks	$P = 0.0003$	3	$P < 0.002$	5	$P < 0.05$	7	$P < 0.001$
first positive antigenemia in weeks (median)	ns	4		5		4.5	
percentage of CMV patients treated with ganciclovir	$P = 0.0021$	21	ns	31	$P = 0.0091$	55	$P = 0.0007$
percentage of CMV patients treated with acyclovir	ns	8		9		11	

Figure 2.1a Incidence of CMV infection. Sandimmune and prednisolone (S), vs. Neoral and prednisolone (N) and mycophenolate mofetil, Neoral and prednisolone (M).

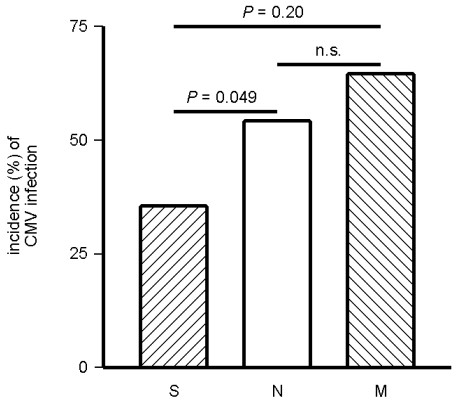

Figure 2.1b Incidence of primary CMV infection. S vs. N vs. M.

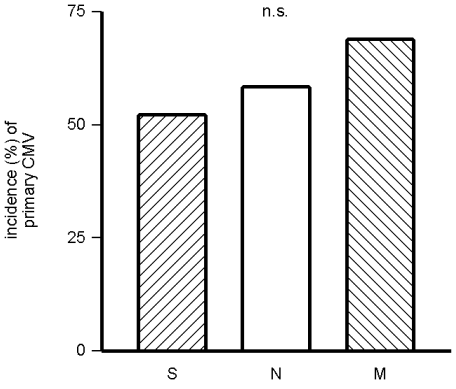

Figure 2.1c Incidence of secondary CMV infection. S vs. N vs. M.

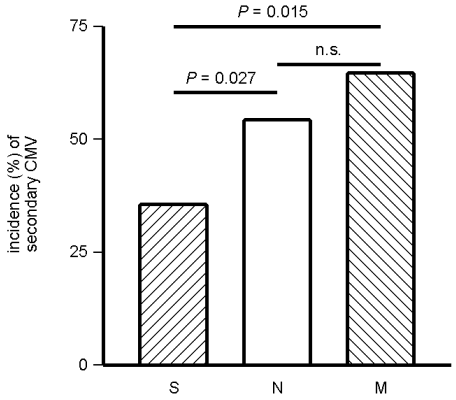

Figure 2.2b Duration antigenemia in weeks. S vs. N vs. M. Horizontal lines are medians. Logarithmic scale.

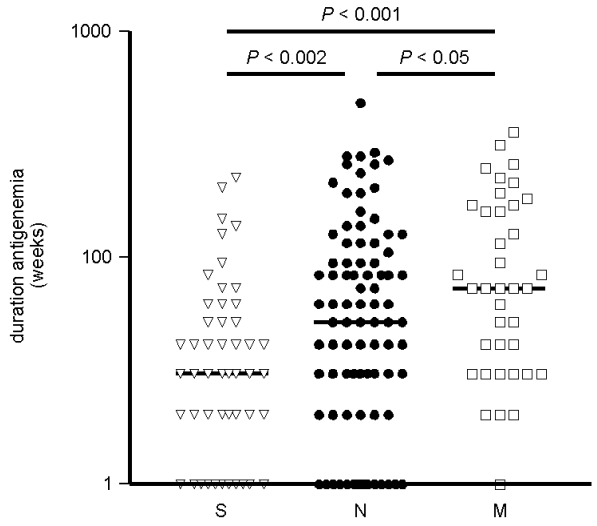

Figure 2.2a Maximal reached CMV pp65-antigenemia. S vs. N vs. M. Horizontal lines are the medians. Logarithmic scale.

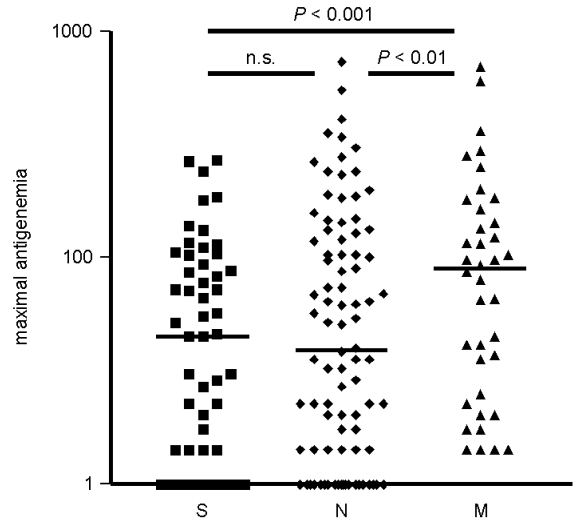

Table 2.3 Cytomegalovirus serology combinations donor-recipient.

	Sandimmune Pred (S)	Neoral Pred (N)	Neoral Cellcept Pred (M)
serology pos-neg	24%	26%	23%
serology neg-pos and pos-pos	56%	58%	49%
serology neg-neg	16%	16%	26%

The median maximal pp65-antigenemia level in the primary infections was significantly higher in the M group than in the S group (S vs. N vs. M respectively 68, 150.5 and 295, $P = 0.007$; S vs. N, n.s.; S vs. M, $P < 0.01$; and N vs. M, n.s., table 2.4). Also the median duration of positive antigenemia was significantly longer in primary infections when protocols changed from S to N and M (S vs. N vs. M respectively 4.5, 9 and 12 weeks, $p = 0.012$; S vs. N, $P < 0.05$; S vs. M, $P < 0.05$; and N vs. M, n.s., table 2.4).

In patients with a secondary infection the median maximal pp65-antigenemia level was significantly higher in the M group compared to the N group (S vs. N vs. M respectively, 3.5, 2 and 16, $P = 0.026$; S vs. N, n.s.; S vs. M, n.s.; and N vs. M, $P < 0.01$). In patients with secondary infections the median duration of positive antigenemia was significantly longer in M compared to S (S vs. N vs. M respectively, 3, 4 and 5 weeks, $P = 0.0017$; S vs. N, ns; S vs. M, $P < 0.01$; and N vs. M, n.s., table 2.4).

In both primary and secondary infections no difference in the time interval between transplant and start of positive antigenemia was seen.

2.3.4 Rejection episodes and CMV infection

The number of antirejection treatments was similar in all three groups. Standard antirejection treatment with methylprednisolone was given in respectively 42, 42 and 41% in the S, N and M group (n.s.). ATG treatment was required in respectively 16, 22 and 16 % of the patients in the three groups (n.s.). When patients without a rejection were analyzed, also a significantly higher incidence of secondary CMV infection, coinciding with the change immunosuppressive protocol was found (S vs. N vs. M respectively, 18, 41 and 55%, $P = 0.04$; S vs. N, $P = 0.031$; S vs. M, $P = 0.022$; and N vs. M n.s.).

Table 2.4 Primary and secondary cytomegalovirus infections; maximal reached pp65-antigenemia, duration and time of appearance.

	P values primary CMV		primary CMV infection	secondary CMV infection	*P* values secondary CMV	
			Sandimmune Pred (S)			
median max antigenemia	*P* = 0.0066*	*P* = ns**	68	3.5	ns**	*P* = 0.026*
median duration antigenemia (weeks)	*P* = 0.0121*	*P* < 0.05	4.5	3	ns	*P* = 0.0017*
median first antigenemia (weeks p.o.)	ns*		4.5	4		ns*
			Neoral Pred (N)			
median max antigenemia		ns^	150.5	2	*P* < 0.01^	
median duration antigenemia (weeks)		ns	9	4	ns	
median first antigenemia (weeks p.o.)			4	5		
			Neoral Cellcept Pred (M)			
median max antigenemia		*P* < 0.01^^	295	16	ns^^	
median duration antigenemia (weeks)		*P* < 0.05	12	5	*P* < 0.01	
median first antigenemia (weeks p.o.)			4	5		

* S vs. N vs. M
** S vs. N
^ N vs. M
^^ S vs. M

2.3.5 Symptomatology

In this study the majority of patients with a positive antigenemia assay were asymptomatic or had a viral syndrome consisting of malaise, fever and arthralgia. Tissue invasive CMV, e.g. clinical CMV pneumonitis or colitis was not seen in this cohort of patients studied and has been a rare observation in our center. This is most likely due to the frequent and routine control of pp65-antigenemia and preemptive use of ganciclovir during rejection treatment in patients with positive antigenemia.

2.3.6 Antiviral medication

The use of ganciclovir during CMV infection was increased significantly in the M group compared to the other two immunosuppressive protocol groups (S vs. N vs. M respectively, 21, 31 and 55 %, $P = 0.0021$; S vs. N, n.s.; S vs. M, $P = 0.0007$; and N vs. M, $P = 0.009$).

No differences were found as regards the treatment, with acyclovir given for other reasons than CMV in patients with CMV infection (respectively, 8%, 9% and 11% for S, N and M, n.s.).

2.4 DISCUSSION

This retrospective single-center analysis reports on the effects of three different immunosuppressive protocols on the incidence, duration and viral load of CMV infection, over a period of almost 10 years, without any form of CMV prophylaxis. This study reveals interesting and sometimes unexpected findings. In contrast to a number of reports, we found that not the introduction of mycophenolate mofetil but the introduction of Neoral, a few years earlier, has been mainly responsible for the increase in CMV infections at our center. Particularly, an increase in secondary CMV infections was observed after introduction of the Neoral formulation of cyclosporine. No significant higher incidence of primary infections was seen although a longer duration of this type of infection was observed. The addition of mycophenolate mofetil (the M group) did not result in a further increase in the number of CMV infections. However, after the introduction of mycophenolate mofetil infections became more prolonged than in patients treated only with Neoral and prednisolone.

Also, a striking increase in viral load was found after mycophenolate mofetil was added to our immunosuppressive protocol. The period between the transplant operation and appearance of the virus did not differ between the three groups. The observed increased incidence of CMV infections, the longer duration of CMV infections, as well as the higher viral load after changing the immunosuppressive protocol was not owing to a more frequent use of potent antirejection treatment with ATG, or owing to differences in cyclosporine trough-levels in the three study groups. In addition an increased incidence of CMV infection was found in patients who did not experience any rejection episode. The increased incidence could not be explained by differences in use of antiviral drugs in the three study groups. In previous studies of Neoral and prednisolone vs. Sandimmune and prednisolone treatment after renal transplantation a reduction of rejection episodes in the Neoral group has been reported. This has been explained by the more stable and effective immunosuppression of Neoral because of better absorption [22-24]. In these studies CMV infections have not been considered in great detail. We speculate that more effective immunosuppression by Neoral compared with Sandimmune can explain the increase in CMV infections. A recent report by Ter Meulen et al. confirms our findings: no increase in the incidence of primary CMV infection was found by adding mycophenolate mofetil to the cyclosporine-based immunosuppressive protocol [25]. More CMV-disease was seen in the patients treated with mycophenolate mofetil. The authors suggest a delayed immune response due to the mycophenolate mofetil treatment that may have caused more symptomatic disease. However no information is given concerning patients with a secondary infection and the diagnosis of CMV infection concerns only serological data and no data of the viral load was presented [25].

In this study we demonstrate that the difference in frequency of CMV pp65-antigenemia is due to an increase especially in the number of secondary infections and not primary infections. The change of our protocol from Sandimmune and prednisolone to Neoral and prednisolone and to Neoral, prednisolone and mycophenolate mofetil, however, has changed the primary infections 'phenotypically' since a longer duration of primary infections was observed.

The majority of the CMV infections observed were asymptomatic or characterized by only mild symptoms such as fever and arthralgia. No severe CMV disease was seen in the patients studied. Nevertheless, even asymptomatic CMV infections can be clinically important long-term owing to their possible association with atherosclerosis and chronic transplant dysfunction [7-16]. Longer duration of this type of smouldering CMV infection could potentially be more atherogenic because of a prolonged endothelialitis and subsequent contribution to chronic transplant dysfunc-

tion. We have previously demonstrated the presence of cytomegalic (CMV-infected) endothelial cells (CEC) and non-CMV infected endothelial cells (EC) in the blood of renal transplant recipients with CMV infection [26]. Furthermore we showed increased levels of Von Willebrand Factor (VWF) and s-VCAM (soluble vascular cell adhesion molecule) to be markers of endothelial damage during CMV infection [27]. In patients with a CMV infection following a rejection episode we found significant more endothelial damage as indicated by higher levels of CEC, EC, VWF and s-VCAM.

Our study was not designed to investigate the impact of the three different immunosuppressive protocols on the incidence of rejection. It was surprising that no difference in the incidence of rejection was found. This contrasts with the literature where a decrease in incidence of rejection was shown by changing from Sandimmune to Neoral and mycophenolate mofetil respectively [17, 23, 28, 29]. However by adding mycophenolate mofetil no changes in graft survival after three years were found by different authors [30, 31]. A possible explanation that correlates with our findings is that more prolonged exposure to CMV might give us a clue. Beneficial effects to reduce the development of chronic transplant dysfunction with fewer rejections might be counteracted by unwanted effects caused by increased immuno-suppression resulting in prolonged CMV viremia. Also mycophenolate mofetil has been claimed to have antiproliferative activity on smooth muscle cells [32] explaining the possible protection against chronic rejection in a rat model for kidney [33] and in heart transplantation in baboons [34]. In human renal transplantation, however, such a positive effect of mycophenolate mofetil has not yet been established. We conclude that the change of the immunosuppressive regimen switching from Sandimmune to Neoral and adding mycophenolate mofetil is accompanied by an increased incidence of secondary CMV infections in the Neoral group. A higher and more prolonged CMV viremia is observed especially in the mycophenolate mofetil group. Although the majority of these infections remain asymptomatic, the perseverance of viral load results in extended endothelial damage and might contribute to the development of chronic transplant failure and atherosclerosis.

2.5 REFERENCES

1. Bij, W. van der, R. Torensma, W.J. van Son, et al. Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leucocytes. *Journal of Medical Virology* 1988; 25:179-188.
2. Bij, W. van der, J. Schirm, R. Torensma et al. Comparison between viremia and antigenemia for detection of cytomegalovirus in blood. *Journal of Clinical Microbiology* 1988; 26:2531-2535.
3. Giessen, M. van der, A.P. van der Berg, W. van der Bij, et al. Quantitative measurement of cytomegalovirus-specific IgG and IgM antibodies in relation to cytomegalovirus antigenaemia and disease activity in kidney recipients with an active cytomegalovirus infection. *Clinical and Expperimental Immunology* 1990; 80:56-61.
4. Maar, E.F. de, J.H. Kleibeuker, W. van Boersma-Ek, et al. Increased intestinal permeability during cytomegalovirus infection in renal transplant recipients. *Transplant International* 1996; 9:576-580.
5. Maar, E.F. de, A.M. Kas-Deelen, Th.W. van der Mark, et al. Cytomegalovirus pneumonitis after kidney transplantation is not caused by plugging of cytomegalic endothelial cells only. *Transplant International* 1999; 12:56-62.
6. Son, W.J. van, R. Peset, J.C. Duipmans, et al. Cytomegalovirus infection after renal transplantation. Pulmonary dysfunction measured by decreased diffusing capacity for carbon monoxide in patients with symptomatic and asymptomatic infection. *Transplantation* 1987; 44:149-150.
7. Nieto, F.J., E. Adam, P. Sorlie, et al. Cohort study of cytomegalovirus infection as a risk factor for carotid intimal-medial thickening, a measure of subclinical atherosclerosis. *Circulation* 1996; 94:922-927.
8. Hayry, P., H. Isoniemi, S. Yilmaz, et al. Chronic graft rejection. *Immunological Reviews* 1993: 134:33-81.
9. Grattan, M.T., C.E. Moreno-Cabral, V.A. Starnes, et al. Cytomegalovirus infection is associated with cardiac allograft rejection and atherosclerosis. *JAMA: The Journal of American Medical Association* 1989; 261:3561-3566.
10. Loebe, M., S. Schueler, O. Zais, et al. Role of cytomegalovirus infection in the development of coronary artery disease in the transplanted heart. *Journal of Heart Transplantation* 1990; 9:707-711.
11. Koskinen, P.K., M.S. Nieminen, L.A. Krogerus, et al. Cytomegalovirus infection and accelerated cardiac allograft vaculopathy in human cardiac allografts. *The Journal of Heart and LungTranplantation: The official publication of the International Society for Heart Transplantation* 1993; 12:724-729.

12. Kroshus, T.J., V.R. Kshettry, K. Savik,et al. Risk factors for the development of bronchiolitis obliterans syndrome after lung transplantation. *The Journal of Thoracic and Cardiovascular Surgery* 1997; 114:195-202.
13. O=Grady, J.G., G.J. Alexander, S. Sutherland, et al. Cytomegalovirus infection and donor/recipient HLA-antigens: interdependent co-factors in pathogenesis of vanishing bile-duct syndrome after liver transplantation. *Lancet* 1988; 302-305.
14. Arnold, J.C., B.C. Portmann, J.G. O'Grady, et al. Cytomegalovirus infection persists in the liver graft in the vanishing bile duct syndrome. *Hepatlogy* 1992; 16:285-292.
15. Lautenschlager, I., K. Hockerstedt, H. Jalanko, et al. Persistent cytomegalovirus infection in liver allografts with chronic rejection. *Hepatology* 1997; 25:190-194.
16. Paul, L.C. Chronic allograft nephropathy: An update. *Kidney International* 1999; 56:783-793.
17. European mycophenolate mofetil cooperative study group. Placebo-controlled study of mycophenolate mofetil combined with cyclosporin and corticosteroids for prevention of acute rejection. *Lancet* 1995; 345:1321-1325.
18. Chou, S. Molecular diagnostic techniques for CMV infection. In: S. Michelson, S.A. Plotkin(eds). *Multidisciplinaryapproach to understanding cytomegalovirus disease.* Excerpta Medica, Elsevier, Amsterdam 1993; 183-194.
19. Ljungman, P., P. Griffiths. Definitions of cytomegalovirus infection and disease. In: S. Michelson, S.A. Plotkin (eds). *Multidisciplinary Approach to Understanding Cytomegalovirus Disease*. Excerpta Medica, Elsevier, Amsterdam, 1993; 233-237.
20. Verschuuren, E.A.M., M.C. Harmsen, P.C. Limburg, et al. Towards standardization of the human cytomegalovirus antigenemia assay. *Intervirology*, 1999; 42:382-389.
21. Solez, K., R.A. Axelsen, H. Benediktsson, J.F. Burdick, et al. International standardization of criteria for the histologic diagnosis of renal allograft rejection: the Banff working classification of kidney transplant pathology. *Kidney International* 1993; 44:411-422.
22. Keown, P., D. Niese. Cyclosporine microemulsion increases drug exposure and reduces acute rejection without incremental toxicity in de novo renal transplantation. International Sandimmun Neoral Study Group. *Kidney International* 1998; 54:938-944.
23. Shah, M.B., J.E. Martin, T.J. Schroeder, et al. The evaluation of the safety and tolerability of two formulations of cyclosporine: neoral and sandimmune. A

meta-analysis. *Transplantation* 1999; 67:1411-1417.
24. Frei, U. Overview of the clinical experience with Neoral in transplantation. *Transplantation Proceedings* 1999; 31:1669-1674.
25. Meulen, C.G. ter, J.F. Wetzels, L.B. Hilbrands. The influence of mycophenolate mofetil on the incidence and severity of primary cytomegalovirus infections ad disease after renal transplantation. *Nephrology, Dialysis and Transplantation: an official publicaition of the European Dialysis and Transplant Association - European Renal Association* 2000; 15:711-714.
26. Kas-Deelen, A.M., E.F. de Maar, M.C. Harmsen, et al. Uninfected and cytomegalic endothelial cells in blood during cytomegalovirus infection: effect of acute rejection. *The Journal of Infectious Diseases* 2000; 181:721-724.
27. Kas-Deelen, A.M., M.C. Harmsen, E.F. de Maar EF, et al. Acute rejection before cytomegalovirus infection enhances von willebrand factor and soluble VCAM-1 in blood. *Kidney International* 2000; 58:2533-2542.
28. Sollinger, H.W. For the U.S. renal transplant mycophenolate mofetil study group. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. *Transplantation* 1995; 60:225-232.
29. The tricontinental mycophenolate mofetil renal transplantation study group. A blinded, randomized clinical trial of mycophenolate mofetil for the prevention of acute rejection in cadaveric renal transplantation. *Transplantation* 1996; 61: 1029-1037.
30. Mathew, T.H. For the tricontinental mycophenolate mofetil renal transplantation study group. A blinded, long term, randomized, multicenter study of mycophenolate mofetil in cadaveric renal transplantation: results at three years. *Transplantation* 1998; 65:1450-1454.
31. European mycophenolate mofetil cooperative study group. Mycophenolate mofetil in renal transplantation: 3-year results from the placebo-controlled trial. *Transplantation* 1999; 68:391-396.
32. Raisainen-Sokolowski, A., P. Vuoristo, M. Myllarniemi, et al. Mycophenolat moefetil (MMF, RS-61443) inhibits inflammation and smooth muscle cell proliferation in rat aortic allografts. *Transplant Immunolology* 1995; 3:342-351.
33. Azuma, H., J. Binder, U. Heemann, et al. Effects of RS61443 on functional and morphological changes in chronically rejecting rat kidney allografts. *Transplantation* 1995; 59:460-466.
34. O=Hair, D.P., R.P. McManus, R. Komorowski. Inhibition of chronic vascular rejection in primate cardiac xenografts using mycophenolate mofetil. *The Annals of Thoracic Surgery* 1994; 58:1311-1315.