CHAPTER 10

DECREASED PLATELET COUNT PRECEDES CMV ANTIGENEMIA AFTER LIVER TRANSPLANTATION

E.F. de Maar, R.J. Porte, M.C. Harmsen, W.J. van Son, A.P. van den Berg, M.J.H. Slooff, T.H. The, and M.W.N. Nijsten

Submitted

ABSTRACT

In 445 consecutive liver transplant recipients we observed lower platelet counts in patients with cytomegalovirus (CMV) infection. This significant difference in platelet counts ($27x10^3/mm^3$, $P < 0.05$) between patients with and without CMV infection was already observed on day 24 postoperatively; 6 days before we observed CMV antigenemia. Leukocytes also were lower in patients with CMV infection (difference 1.7 $x10^3/mm^3$, $P < 0.05$) but this difference was observed later (mean day 36 postoperatively). Apparently the fact that platelet counts diverge so early compared to CMV antigenemia and leukocytes points to early systemic effects of CMV on platelet kinetics. Considering the extensive evidence published on endothelial activation by CMV, we speculate that early relative thrombocytopenia reflects CMV-induced endothelial activation.

10.1 INTRODUCTION

Cytomegalovirus (CMV) infection is a frequent complication after solid organ transplantation. Most patients with symptomatic CMV infection have a self-limiting CMV syndrome consisting of malaise with fever and arthralgia. If no CMV prophylaxis is given CMV infection typically occurs 4 weeks after transplantation [1]. In symptomatic patients leukocytopenia, thrombocytopenia and elevated serum liver enzymes have been described. Due to prophylactic or pre-emptive therapy with ganciclovir lethal CMV infection is rarely seen nowadays. However CMV infection is not without long-term consequences, since CMV is believed to play an important role in the development of atherosclerosis, the most important cause of death in transplant recipients [2, 3, 4]. Although mild thrombocytopenia is well established as a symptom of CMV infection in transplant recipients, there are no studies that have systematically studied the changes in platelet count during CMV infection after liver transplantation. Platelet counts are known to decrease in many acute viral diseases and platelets are known to be involved in diseases characterized by acute and chronic endothelial activation. In the current study the chronology of changes in platelet count, leukocyte count and CMV infection were studied in liver transplant recipients with and without CMV infection.

10.2 METHODS

All orthotopic liver transplantations performed in our center between June 1992 and July 2002 were analyzed. With regard to the incubation period of CMV, retransplant procedures within 1 month of the first transplant were counted as a single transplant episode.

The presence of CMV infection was diagnosed and monitored by the pp65 CMV antigenemia assay [5-9] performed weekly, starting on postoperative day 10 until 12 weeks after transplantation or until CMV antigenemia became negative. Briefly, peripheral blood leukocytes were isolated, cytocentrifuged and incubated with a mixture of monoclonal antibodies directed against a 65 kD 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 the number of pp65 positive cells per 50.000 leukocytes.

Recipient CMV-status before transplantation was assessed with CMV-IgG titers. IgM and IgG CMV antibodies were measured quantitatively by ELISA using late stage

CMV-infected fibroblasts as antigens [9]. Primary CMV infections were defined as infections in seronegative recipients receiving a transplant from a seropositive donor (*pos-neg* donor-recipient serology combinations). Secondary CMV infections were defined as infections in seropositive recipients (*pos-pos* or *neg-pos* serology combinations).

Before 1997 high-risk patients (i.e. pos-neg) received prophylaxis with acyclovir; as off 1997 ganciclovir was given. High-dose ganciclovir or foscavir was started in patients who developed CMV antigenemia.

Table 10.1 Incidence and extent of CMV infection after liver transplantation classified on basis of highest CMV pp65-antigenemia assay value measured.

CMV antigenemia	primary and secondary infections	primary infections rec. seroneg.*	secondary infections rec. seropos.**	serology unknown
0	237	111	119	7
1-10	119	18	100	1
11-100	58	22	35	1
> 100	31	18	13	
total	445	169	267	9

* recipient CMV seronegative
** recipient CMV seropositive

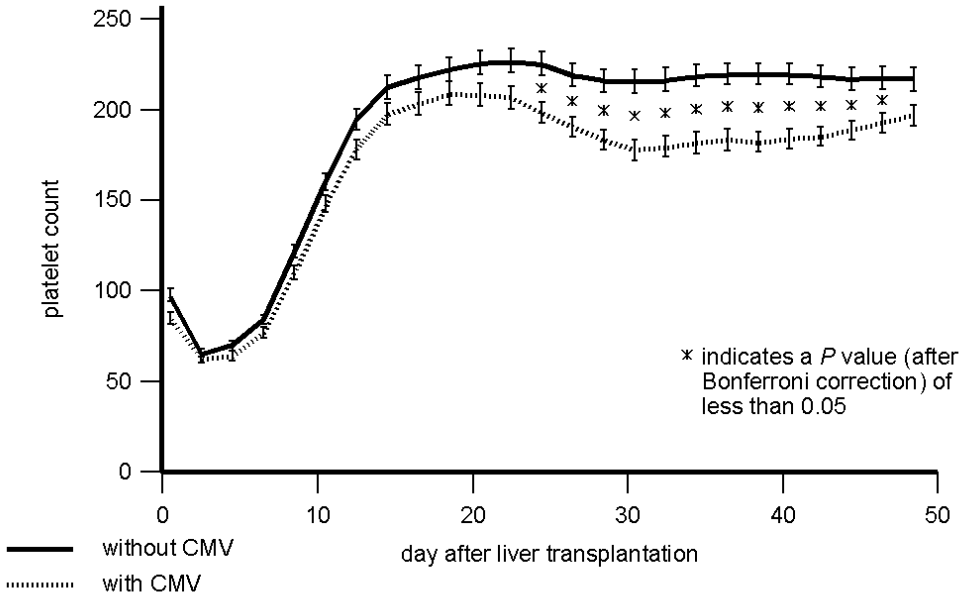
Mean platelet count and leukocyte count were calculated at 2 day intervals for all patients. All CMV antigenemia levels were analyzed between day 0 and day 100, platelet counts and leukocyte counts were analyzed between day 0 and day 50 post-operatively. In order to avoid bias by more frequent determinations of platelet count or leukocyte count in some (e.g. sicker) patients, multiple measurements on the same day were averaged to one value before further analysis. Likewise missing platelet counts or leukocyte counts were interpolated.

Data are expressed as mean ± SEM, unless indicated otherwise. Differences were assessed using Student's *t*-test and were corrected according to Bonferroni in case of multiple comparisons.

10.3 RESULTS

All 461 consecutive liver transplants performed in our center in the study period were analyzed. After combining early retransplants with previous transplants, 445 transplant cases remained. Median age of the recipients was 34 years (range 0-68 years), 52% were males.

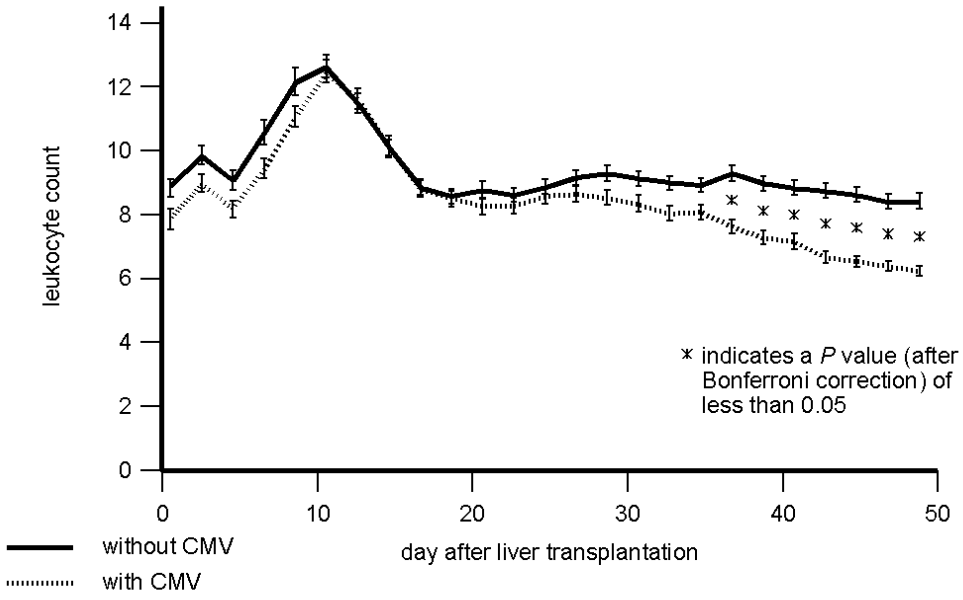
Figure 10.1 Platelet counts in patients with versus patients without CMV infection after liver transplantation.



Of the 445 cases 208 (47%) had CMV infection as diagnosed by a positive CMV pp65-antigenemia assay. Within these 208 patients 119 (57%) had low maximal antigenemia levels (CMV antigenemia < 10 positive granulocytes/50.000 leukocytes), 58 (28%) moderate antigenemia levels (CMV antigenemia between 10 and 100) and 31 (15%) high levels (CMV antigenemia ≥ 100 positive granulocytes/ 50.000 leukocytes) (table 10.1). 28% were primary and 71% were secondary infections. CMV infection was detected at a mean of 30 days after transplantation. A significant difference in the platelet counts was found from day 24 after transplantation, with a platelet count that was $27x10^3/mm^3$ lower in patients with

CMV compared to negative patients ($P < 0.001$, figure 10.1). This difference was due to a lack of further increase in platelet count in patients with CMV infection. In fact, mean platelet count in the CMV antigenemia positive patients remained within the normal range. Absolute thrombocytopenia (i.e. < $50x10^3/mm^3$) was seen in 19% of the CMV antigenemia positive patients compared with 16% of the CMV antigenemia negative patients (n.s.).

Figure 10.2 Leukocyte counts in patients with versus patients without CMV infection after liver transplantation.



Subgroup analysis of the recipient serology status had no effect on the time course of the platelet count for patients with or without CMV-infection. In contrast to the platelet count, the leukocyte count in the two groups did not differ until day 36, after which values became significantly lower in CMV positive patients, with a mean difference of $1.7x10^3/mm^3$ ($P < 0.05$, figure 10.2).

10.4 DISCUSSION

In this study we observed significantly lower platelet counts in liver transplant recipients with CMV infection compared to liver transplant recipients without CMV infection. The difference in platelet counts could already be observed several days before onset of a detectable CMV infection. We also observed lower leukocyte counts, but this was observed 12 days later than the lower platelet counts.
During the first days after liver transplantation early thrombocytopenia can be found in the majority of patients [11]. Rapid increase of platelet count occurs towards the end of the first week and a stable platelet count, within the normal range, is normally achieved within 3-4 weeks [12]. Several factors play a role in the development of this early thrombocytopenia after liver transplantation. Massive blood loss during the operation and disseminated intravascular coagulation probably play a role. An attractive hypothesis for the pathogenesis of early thrombocytopenia is the sequestration of thrombocytes in the sinusoids of the liver by endothelial activation immediately after reperfusion of the liver [13, 14]. Platelets sequester in the liver immediate after reperfusion and induce apoptosis of sinusoidal endothelial cells [14]. Platelet sequestration in the liver correlates with the duration of cold ischemia [15]. Upregulated adhesion molecules such as selectins play an important role in the adhesion of platelets to endothelial cells immediately after reperfusion. For example platelet sticking can be reduced by inhibition of selectin receptor-mediated adhesion by sialyl Lewis X oligosaccharide (sLe^x) [13].

We did not find a relation between CMV infection and early thrombocytopenia. However after postoperative day 24 we observed a difference in platelet counts between patients with and patients without cytomegalovirus infection. This difference is caused by a greater increase in platelet counts in the patients without CMV infection compared with patients with CMV infection. The difference in platelet counts was seen 6 days before the CMV antigenemia assay became positive. Also the leukocyte counts were lower in patients with CMV infection, but this difference appeared later at a mean of 36 days postoperatively.

The kinetics of these events is interesting. Apparently CMV has an effect on thrombocytes before we can detect the virus in blood with the pp65-antigenemia assay. The same may be the case with other assays like nucleic acid sequence-based amplification (IE1-NASBA) [16, 17]. This indicates that CMV infection is an early phenomenon after transplantation, and that dissemination via the blood, and the appearance of antigenemia or viral mRNA in blood, are relatively late. What we can trace back in the blood to an active CMV infection represents only the tip of the ice-

berg, since it is an incomplete reflection of the pathophysiological process that takes place [18, 19].

We can speculate on the pathophysiological mechanisms explaining the lower platelet levels. In our opinion, infection of endothelial cells takes place after reactivation of the virus. Subsequent to infection of endothelial cells, CMV can spread hematogenously to different parts of the body. Circulating cytomegalic endothelial cells probably play an important role in the spreading of the virus. Polymorphonuclear leukocytes take up pp65 from the infected endothelial cells. The pp65 antigenemia assay will become positive and reflects the endothelial viral load. Before appearance of pp65 positive leukocytes in the blood, infection of endothelial cells has already begun and can affect thrombocytes. For example there is evidence that Cytomegalovirus infection can induce tissue factor on the endothelial cell surface [20, 21, 22]. Tissue factor is a main activator of the clotting cascade. Moreover, the viral envelope contains tissue factor and procoagulant phospholipids that cause activation of the clotting cascade even before viral proteins are synthesized [23, 24]. Furthermore CMV induces the release of Von Willebrand factor (VWF) from endothelial cells. VWF is an essential bridging molecule for platelet aggregation and adhesion and acts as a carrier for factor VIII increasing the half-life of factor VIII five-fold [25, 26]. Additionally CMV-induced adhesion molecules can augment the adhesion of platelets to infected endothelial cells very early during CMV infection [27].

Besides sequestration of thrombocytes due to activation of the clotting system or by interaction with endothelial cells, decreased production of platelets is conceivable. For example decreased production of thrombopoetin has been suggested [28, 29]. Thrombocytopenia as shown in our study is a very early phenomenon in CMV infection and a decrease in production of thrombopoetin takes time to have effect in decreasing thrombocyte counts. Thus a CMV-effect on thrombopoiesis would point to an even earlier impact of CMV-infection after transplantation than an effect on platelet clearance.

10.5 CONCLUSIONS

This is the first large clinical study confirming that CMV infection in liver transplant recipients is associated with significantly lower platelet counts. This difference in platelet counts between liver transplant recipients with and without CMV infection was observed even before detectable CMV antigenemia and two weeks before dif-

ferences in leukocyte counts. Apparently CMV infection occurs very early and more widespread than usually realized, and in blood we only recognize the tip of the iceberg relatively late during the infection. Sequestration of platelets caused by increased adhesion to infected endothelial cells and intravascular pooling may be responsible for the early difference in platelet counts.

10.6 REFERENCES

1. Maar, E.F. de, E.A.M. Verschuuren, J.J. Homan vd Heide, A.M. Kas-Deelen, D. Jagernath, T.H. The, R.J. Ploeg, W.J. van Son. Effects of changing immunosuppressive regimen on the incidence, duration and viral load of cytomegalovirus infection in renal transplantation: a single center report. *Transplant Infectious Disease* 2002; 4:17-24.
2. United States Renal Data System 1998 Annual Data Report. *American Journal of Kidney Disease* 1998; 32 (Suppl 1):S91-
3. Briggs, J.D. Causes of death after renal transplantation. *Nephrology, Dialysis, Transplantation* 2001; 16:1545-1549.
4. Bruggeman, C.A. Does Cytomegalovirus Play a Role in Atherosclerosis? *Herpes* 2000; 7:51-54.
5. Bij, W. van der, Torensma R, Son WJ van, et al. Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leucocytes. *Journal of Medical Virology* 1988; 25:179-188.
6. Chou, S. Molecular diagnostic techniques for CMV infection. In: S. Michelson & S.A. Plotkin (eds): *Multidisciplinary Approach to Understanding Cytomegalovirus Disease.* Excerpta Medica, Elsevier, Amsterdam 1993; 183-194.
7. 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.
8. Bij, W. van der, J. Schirm, R. Torensma, W.J. van Son, A.M. Tegzess, T.H. The. Comparison between viremia and antigenemia for detection of cytomegalovirus in blood. *Journal of Clinical Microbiology* 1988; 26:2531-2535.
9. Verschuuren, E.A.M., M.C. Harmsen, P.C. Limburg, W. van der Bij, A.P. van den Berg, A.M. Kas-Deelen, B. Meedendorp, W.J. van Son, T.H. The. Towards standardization of the human cytomegalovirus antigenemia assay. *Intervirology* 1999; 42:382-389.
10. Giessen, M. van der, A.P. van den Berg, W. van der Bij, S. Postma, W.J. van Son, T.H. The. 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 Experimental Immunology* 1990; 80:56-61.
11. Nijsten, M.W.N., H.J. ten Duis, J.G. Zijlstra, R.J. Porte, J.H. Zwaveling, J.C. Paling, T.H. The. Blunted rise in platelet count in critically ill patients is associated with worse outcome. *Critical Care Medicine* 2000; 28:3843-3846.

12. Chatzipetrou, M.A., A.K. Tsaroucha, D. Weppler, P.A. Pappas, N.S. Kenyon, J.R. Nery, M.F. Khan, T. Kato, A.D. Pinna, C. O=Brien, A. Viciana, C. Ricordi, A.G. Tzakis. Thrombocytopenia after liver transplantation. *Transplantation* 1999; 67(5):702-706.
13. Sindram, D., R.J. Porte, M.R. Hoffman, R.C. Bentley, P.A. Clavien. Platelets induce sinusoidal endothelial cell apoptosis upon reperfusion of the cold ischemic rat liver. *Gastroenterology* 2000; 118:183-191.
14. Porte, R.J., E. Blauw, E.A.R. Knot, M.P.M. de Maat, C. de Ruiter, C.M. Bakker, O.T. Terpstra. Role of the donor liver in the origin of platelet disorders and hyperfibrinolysis in liver transplantation. *Journal of Hepatology* 1994; 21:592-600.
15. Cywes, R., M.A. Packham, L. Tietze, J.R. Sanabria, P.R. Harvey, M.J. Phillips, S.M. Strasberg. Role of platelets in hepatic allograft preservation injury in the rat. *Hepatology* 1993; 18:635-647.
16. Blok, M.J., M.H. Christiaans, V.J. Goossens, J.P. van Hooff, P. Sillekens, J.M. Middeldorp, C.A. Bruggeman. Early detection of human cytomegalovirus infection after kidney transplantation by nucleic acid sequence-based amplification. *Transplantation* 1999; 67:1274-1277.
17. Singh, N. Cytomegalovirus infection of liver transplant recipients: comparison of antigenemia and molecular biology assays. *Liver Transplantation* 2001; 7: 1004-1007.
18. Sinzger, C., A. Grefte, B. Plachter, A.S. Gouw, T.H. The, G. Jahn. Fibroblasts, epithelial cells, endothelial cells and smooth muscle cells are major targets of human cytomegalovirus infection in lung and gastrointestinal tissues. *The Journal of General Virology* 1995; 76:741-750.
19. Hendrix, R.M., M. Wagenaar, R.L. Slobbe, C.A. Bruggeman. Widespread presence of cytomegalovirus DNA in tissues of healthy trauma victims. *Journal of Clinical Pathology* 1997; 50:59-63.
20. Nicholson, A.C., D.P. Hajjar. Herpesvirus in atherosclerosis and thrombosis: etiologic agents or ubiquitous bystanders? *Arteriosclerosis, Thrombosis and Vascular Biology* 1998; 18:339-348.
21. Vercellotti, G.M. Effects of viral activation of the vessel wall on inflammation and thrombosis. *Blood Coagulation & Fibrinolysis* 1998; 9(S2):S3-6
22. Dam-Mieras, M.C. van, A.D. Muller, V.W. Hinsbergh, W.J. Mullers, P.H. Bomans, C.A. Bruggeman. The procoagulant response of cytomegalovirus infected endothelial cells. *Thrombosis and Haemostasis* 1992; 68:364-370.

23. Pryzdial, E.L., J.F. Wright. Prothrombinase assembly on an enveloped virus: evidence that the cytomegalovirus surface contains procoagulant phospholipid. Blood 1994; 84(11):3749-3757.
24. Sutherland, M.R., C.M. Raynor, H. Leenknegt, J.F. Wright, E.L. Pryzdial. Coagulation initiated on herpesviruses. *Proceedings of the National Academy of Sciences of the United States of America* 1997; 94:13510-13514.
25. Brinkhous, K.M., H. Sandberg, J.B. Garris, C. Mattsson, M. Palm, T. Griggs, M.S. Read. Purified human factor VIII procoagulant protein: comparative hemostatic response after infusions into hemophilic and von Willebrand disease dogs. *Proceedings of the National Academy of Sciences of the United States of America* 1985; 82(24):8752-8756.
26. Kas-Deelen, A.M., M.C. Harmsen, E.F. de Maar, W.W. Oost-Kort, J.W. CohenTervaert, J. van der Meer, W.J. van Son, T.H. The. Acute rejection before cytomegalovirus infection enhances von Willebrand factor and soluble VCAM-1 in blood. *Kidney International* 2000; 58:2533-2542.
27. Cebulla, C.M., D.M. Miller, D.A. Knight, B.R. Briggs, V McGaughy, D.D. Sedmak. Cytomegalovirus induces sialyl Lewis(x) and Lewis(x) on human endothelial cells. *Transplantation* 2000; 69(6): 1202-1209.
28. Martin, T.G., K.A. Somberg, Y.G. Meng, R.L. Cohen, C.A. Heid, F.J. de Sauvage, M.A. Shuman. Thrombopoietin levels in patients with cirrhosis before and after orthotopic liver transplantation. *Annals of Internal Medicine* 1997; 127:285-288.
29. Peck-Radosavljevic, M., J. Zacherl, Y.G. Meng, J. Pidlich, E. Lipinski, F. Langle, R. Steininger, F. Mühlbacher, A. Gangl. Is inadequate thrombopoietin production a major cause of thrombocytopenia in cirrhosis of the liver? *Journal of Hepatology* 1997; 27:127-131.