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CASE REPORT

Initial cytomegalovirus prophylaxis with ganciclovir: no guarantee for prevention of late serious manifestations of CMV after solid organ transplantation

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ABSTRACT

A 37-year-old woman presented with malaise, upper abdominal pain and fever seven months after renal transplantation. She was seronegative for cytomegalovirus (CMV) and had received a kidney from a seropositive donor. She had received CMV prophylaxis (oral ganciclovir) for three months after transplantation. During this period all tests for CMV remained negative. On admission, she presented with symptoms compatible with an acute abdomen and with deterioration of renal function. On emergency laparotomy a perforation of the ileum was found. The resected specimen showed an ulcer with vasculitis at the site of perforation, with both microscopic (owl's eye inclusion bodies), as well as immunohistochemical evidence for a CMV infection. CMV can reactivate (usually in the first three months) after transplantation, sometimes resulting in serious morbidity. The use of antiviral prophylaxis during and after transplantation has certainly decreased the number and severity of CMV infections. This case illustrates that life-threatening infections such as CMV can still emerge a long time after transplantation. Unrelenting awareness of this condition is mandatory, even after apparently adequate anti-CMV prophylaxis

KEYWORDS

Cytomegalovirus, ganciclovir, late infection, prophylaxis, solid organ transplantation

INTRODUCTION

Cytomegalovirus (CMV) infection is the most important infectious complication after solid organ transplantation.[1] Incidence of CMV infections after solid organ transplantation has been reported to be as high as 43 to 92%, depending on the definition used for active CMV infection as well as on the population studied.[1] In the majority of cases, post-transplant CMV infections will develop shortly after transplantation.[1,2]

Reactivation of CMV after solid organ transplantation can partially be prevented by administration of antiviral prophylaxis to high-risk patients. Aside from prophylactic antiviral therapy, which has been shown to prevent CMV disease during the period that the prophylaxis is given, another approach might be pre-emptive treatment with frequent monitoring of CMV viral load, thereby avoiding unnecessary treatment with antiviral drugs in a substantial number of patients. However, it has been shown that frequent monitoring cannot prevent CMV disease from occurring in all patients and regular measurements of CMV polymerase chain reaction have proved to have only modest value in predicting CMV disease.[3] If a symptomatic CMV infection does develop after solid organ transplantation, the majority of cases can be effectively treated with antiviral medication such as ganciclovir.[1]

A case is presented of a patient who, despite CMV prophylaxis with oral ganciclovir in the first three months after transplantation (during which no signs of CMV infection were present), developed a CMV infection with serious complications late after grafting.

CASE HISTORY

A 37-year-old woman presented to a hospital elsewhere with malaise, nausea and vomiting that had been present for a couple of days. She also had upper abdominal pain as well as frequent green watery diarrhoea accompanied by a fever of up to 39 °C.

Medical history mentioned terminal renal failure of unknown origin in 1992. She was treated with peritoneal dialysis and underwent renal transplantation with a donor kidney from her father in 1994. There was an immediate transplant failure caused by venous thrombosis of the kidney. In 2001 a kidney became available from a CMV IgG seropositive donor; the patient was seronegative for CMV. The patient consented to take part in a multicentre trial in which the effect of a relatively new immunosuppressant rapamycin (Rapamune®, Wyeth) was being investigated. Immunosuppression in the study protocol consisted of cyclosporine A (Neoral®, Novartis) 10 mg/kg once a day adjusted to plasma levels, prednisolone 10 mg once a day and rapamycin 4 mg once a day, adjusted to plasma levels.

The study protocol also contained CMV prophylaxis (oral ganciclovir (Roche)) in every seropositive patient or in case of a CMV seropositive donor. According to the protocol oral ganciclovir was prescribed, 1 g once a day over a total period of three months after transplantation. In our transplant centre CMV pp65 antigenaemia and anti-CMV antibodies are checked in every patient (during hospitalisation, and at every visit to the outpatient clinic) on a routine basis once a week until three months after transplantation, except when the donor and recipient are both seronegative for CMV.[4,5] In our patient, there were no signs of active CMV infection according to these sensitive tests during the first six months after transplantation, both during the prophylaxis period as well as at her visits to the outpatient clinic.

Seven months after the second kidney transplantation, the patient was admitted to a hospital elsewhere, because of vomiting and diarrhoea, which had been present for a couple of days. A diagnosis of gastroenteritis was made on clinical grounds. Renal function deteriorated. Serum creatinine rose from 81 μmol/l to 492 μmol/l. Subsequently the patient was transferred to our hospital for further diagnosis.

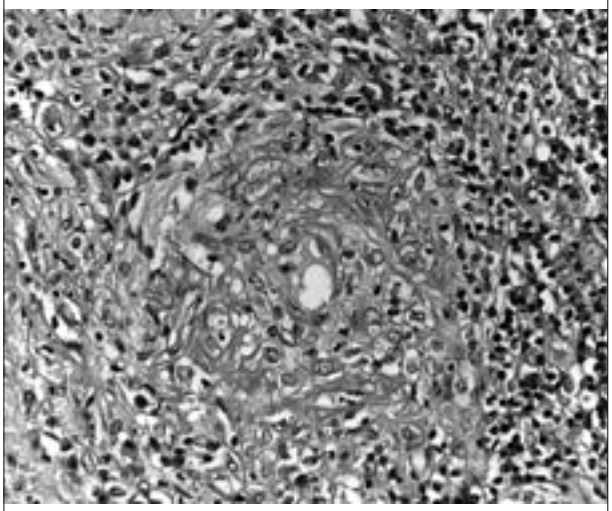
On arrival to our hospital we saw a very ill woman with a blood pressure of 170/95 mmHg, a regular pulse of 119 beats/min and a body temperature of 36.7 °C. She complained of severe pain in her abdomen; transport from the referring hospital to our centre had been very painful. On physical examination of the abdomen overt signs of a generalised peritonitis were found. Laboratory results showed (reference values between brackets): haemoglobin 5.5 mmol/l (7.5-9.9); mean cell volume 72 fl (80-96), leucocytes 18.4 x 10^9/l (4-11), differentiation showed a left shift with 91% neutrophils; thrombocytes 386.0 x 10^9/l (150-350); C-reactive protein 206 mg/l (<3); serum creatinine 492 (mol/l (45-80); urea 21.5 mmol/l (3.0-7.0); aspartate aminotransferase 38 U/l (0-40); alanine aminotransferase 55 U/l (0-30); alkaline phosphatase 102 U/l (13-120); lactate dehydrogenase 415 U/l (114-235); γ-glutamyl transpeptidase 39 U/l (0-65); amylase 51 U/l (70-300). Von Willebrand factor was measured as a part of the study protocol; a high serum value was found in this patient (factor VIII RaG; 537% compared with standard serum). A plain abdominal x-ray in supine position showed marked dilatation of the jejunum with no gas in the other parts of the abdomen, pointing to an obstruction in the proximal small intestine. No free intraperitoneal air could be demonstrated. An ultrasound of the kidney transplant was normal. Furthermore intra-abdominal fluid was found.

An emergency laparotomy was performed, during which a generalised peritonitis was found, with a total of 1.5 litre of purulent fluid in the abdominal cavity. A gram stain of this fluid showed numerous leucocytes; no bacteria could be demonstrated. However, a culture of the fluid revealed coagulase negative *Staphylococci*. A perforation of the ileum was found at 50 cm before the Bauhin valve. An ileocaecal resection was performed: a total of 90 cm of the terminal ileum as well as the caecal pole were resected. An ileostomy and mucous fistula of the ascending colon were created.

In the resected specimen, a marked fibrinous peritonitis was seen, with a transmural inflammatory infiltrate with many neutrophils and lymphoid hyperplasia at the site of the perforation. Staining for Epstein-Barr virus (EBV) was negative as was staining for CD20 (a marker for B cells), excluding the possibility of EBV-induced post-transplant lymphoproliferative disease. Vasculitis was present at the site of perforation (*figure 1*). In the bowel wall and in the blood vessels, owl's eye inclusion bodies could be demonstrated (*figure 2*). This indicates a CMV infection which was confirmed by additional immunohistochemical staining of the material with monoclonal antibody E13 against human CMV (*figure 2*).[6] As mentioned earlier, CMV antigenaemia remained negative during the first three months under CMV prophylaxis, which practically excludes an active CMV infection.[4] This test was still negative at the last regular visit to the outpatient clinic, a month and a half before this episode. On admission the anti-CMV immunoglobulin-M was 2 (% of standard serum); anti-CMV immunoglobulin-G 8 and CMV pp65 antigenaemia 14/50,000 polymorphonuclear neutrophils (PNMs), indicating an active CMV infection with at that time a moderate immune response to the virus.[4,5]

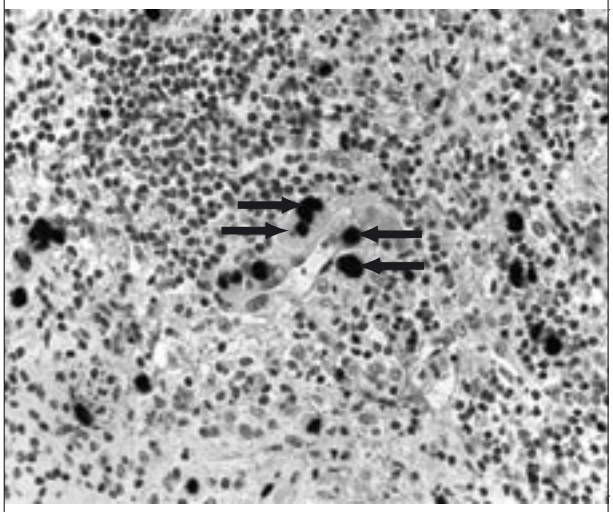
During admission the patient was treated with intravenous ganciclovir 400 mg once a day for four weeks followed

Figure 1 *Vasculitis; a vessel with a narrowed lumen and an inflammatory infiltrate mostly composed of histiocytes, neutrophils and lymphocytes*



Haematoxylin-eosin stain, 40 x.

Figure 2 *Cytomegalic cells containing intranuclear inclusion bodies in the wall of several small blood vessels*



Cytomegalovirus (CMV)-infected cells are coloured by immunohistochemical staining with monoclonal antibody E13 against human CMV (40 x). Arrows point at CMV positive cells.

by oral ganciclovir 2 g once a day for the following three months. Treatment with cyclosporine and prednisolone was continued. Rapamycin was withdrawn. Renal function recovered shortly after the operation. Eventually she made a full recovery with complete restoration of renal function as well as complete resolution of the CMV infection.
More than a year after the event, an operation was carried out to re-establish bowel continuity. At the moment the patient shows a complete recovery and is able to carry out all her daily activities again. Renal function is stable; no (laboratory) signs of reactivation of CMV have occurred so far.

DISCUSSION

In solid organ transplantation a primary CMV infection can occur via the donor organ or, as seen in nonimmunocompromised individuals, by blood transfusions (although this chance is low, since all blood products are filtered before use), sexual intercourse or being in an environment known for its propensity of frequent transmission of highly virulent virus (e.g. day-care centres).[7,8]
A substantial number of especially secondary CMV infections (reactivation of the endogenous virus of the donor) are asymptomatic. When a CMV infection in a transplant patient is symptomatic, most have a self-limiting syndrome consisting of fever (often spiking), arthralgia, leucopenia and/or thrombocytopenia and abnormalities in liver enzymes. The gastrointestinal tract may become involved, with ulceration, gastritis, and pneumatosis intestinalis as a consequence.[1] Ulceration can occur in the whole gastrointestinal tract with the risk of bleeding and even perforation. Well-known gastrointestinal symptoms of CMV are abdominal pain, diarrhoea and rectal bleeding.[9] CMV can be present in the bowel without any other symptoms of CMV or signs of infection, although fever and malaise can be present.[2,10,11]
Although CMV involvement can occur without gastrointestinal symptoms or other signs of CMV infection, monitoring of CMV in blood (either real-time PCR or pp65 antigenaemia) can be of help in diagnosing CMV gastrointestinal disease. However, both have only limited value in predicting or early diagnosing of CMV gastrointestinal disease.[12,13] In our centre pp65 antigenaemia is used. It has shown to be a reliable test with a good correlation with blood culture for CMV.[14]
The severity of CMV infections after transplantation has decreased nowadays for several reasons. Firstly there are better and quicker means of diagnosing infections, implying that treatment with antiviral medication can be started earlier. Secondly, there are now several effective antivirals. Thirdly, patients undergoing transplantation can be treated with antiviral prophylaxis. The theoretical disadvantage of treatment with antiviral prophylaxis might be that a smouldering infection, not detected by the currently available sensitive test, could thus be suppressed. After cessation of the prophylaxis an active CMV infection might emerge in a phase when the doctors taking care of those patients are less aware of the possibility of CMV infection. Cases of late CMV disease may occur even in patients treated with CMV prophylaxis, without known triggering factors such as antirejection treatment and can emerge as late as 18 months after transplantation.[15-17]

Ganciclovir prophylaxis is found to be related to late seroconversion (i.e. 20% after six months) in high-risk patients.[18] The emergence of late ganciclovir-resistant CMV disease among solid organ transplantation has been described by Limaye *et al.*[19]

The patient in this case history developed a perforation of the terminal ileum caused by an active CMV infection. It used to be thought that an ulceration or perforation of the bowel was based on a special affinity of cytomegalovirus for locations in the bowel with pre-existing damage.[1] The current interpretation of the pathophysiology of CMV pathology of the gut is that a CMV vasculitis develops in the small vessels of the mucosa and submucosa, leading to intravascular coagulation, which subsequently gives rise to ischaemic damage, ulceration and even perforation.[20-22] The vasculitis found together with signs of an active CMV infection in the resected specimen correspond with this theory. Furthermore on admission to our hospital a high serum value of Von Willebrand factor was found, which is compatible with endothelial damage as seen in vascular inflammation, i.e. during an active CMV infection.[23,24]

In this case one can only speculate why this patient went through such a serious CMV infection, seven months after transplantation. There is a small chance that the patient had had a primary infection, not transmitted via the donor kidney, but instead contracted as can occur in nonimmunocompromised people (as described earlier in this article). This patient, however, was not in contact with small children, neither professionally, nor personally. She did not receive any blood transfusions and she had not been sexually active in this period.

Another possibility is that a smouldering infection originating from the donor kidney had been present which was not seen by our sensitive tests. But why this infection should reactivate four months after ceasing the CMV prophylaxis remains likewise enigmatic.

A change of the immunosuppressive therapy could be the culprit of the untimely reactivation. However, in this patient only a minor change was made in the dose of the immunosuppressants (cyclosporine went from 200 mg to 250 mg once daily and rapamycin from 3 to 4 mg once daily).[1]

Another means by which CMV could have been reactivated is the production of cytokines. In times of disease (e.g. during myocardial infarction, stress, other infections), cytokines can be produced. For instance tumour necrosis factor α (TNF-α) can cause replication of CMV-IE (immediate early) protein via a signal-transduction cascade, leading to CMV reactivation.[25] In this particular patient, the fever (which was cytokine induced) is not a very likely explanation for the CMV reactivation since it is more conceivable that the fever was caused by peritonitis as a consequence of the perforation by a CMV-induced ulcer and not the other way around. Furthermore HLA-DR matching may play a part. In patients with HLA-DR mismatching, CMV infections appear more often, and with more serious disease.[26] This patient had one HLA-DR mismatch.

In this case history it is not possible to retrieve the exact cause of the CMV infection at this specific moment after transplantation. A primary infection based on a virulent strain after extramural contacts is not very likely but can not, of course, be excluded.

Although there are case reports that show late CMV infections despite initial ganciclovir or acyclovir prophylaxis without any episode of rejection, none of the mentioned cases have data on systematic control of viral parameters during the period of initial prophylaxis. In those cases an early asymptomatic CMV infection cannot be excluded. This is the first report of late CMV infection in which viral parameters have been checked on a routine basis where, even with the sensitive tests used, no active CMV infection could be demonstrated until admission. This applies for both the three months prophylaxis period as for the frequent outpatient clinic visits thereafter.

CONCLUSIONS

With this case report we want to emphasise that despite the use of antiviral prophylaxis after solid organ transplantation, cytomegalovirus can still cause serious disease long after cessation of prophylaxis. Since in this phase after transplantation, active symptomatic CMV infections are not frequently seen, the unawareness of this condition may lead to an unnecessary delay in diagnosis.

REFERENCES

1. Van Son WJ, The TH. Cytomegalovirus infection after organ transplantation: an update with special emphasis on renal transplantation. Transplant Int 1989;2:147-64.
2. Buckner FS, Pomeroy C. Cytomegalovirus disease of the gastrointestinal tract in patients without AIDS. Clin Infect Dis 1993;17:644-56.
3. Humar A, Paya C, Pescovitz MD et al. Clinical utility of CMV viral load testing for predicting CMV disease in D+/R- solid organ transplant recipients. Am J Transplant 2004;4:644-9.
4. Van der Bij W, Torensma R, van Son WJ et al. Rapid immunodiagnosis of active Cytomegalovirus infection by monoclonal antibody staining of blood leukocytes. J Med Virol 1988;25:179-88.
5. Van der Giessen M, van der Berg AP, van der Bij W, Postma S, van Son WJ, The TH. 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. Clin Exp Immunol 1990;80:56-61.
6. Weller TH, Macaulay JC, Craig JM et al. Isolation of intranuclear inclusion producing agents from infants with illnesses resembling cytomegalic inclusion disease. Proc Soc Exper Biol Med 1957;94:4-12.

7. Adler SP. Cytomegalovirus and child day care. Evidence for an increased infection rate among day-care workers. N Engl J Med 1989;321:1290-6.
8. Onorato IM, Morens DM, Martone WJ, Stansfield SK. Epidemiology of Cytomegaloviral infections: recommendations for prevention and control. Rev Infect Dis 1985;7:479-97.
9. Toogood GJ, Gillespie PH, Gujral S et al. Cytomegalovirus infection and colonic perforation in renal transplant patients. Transpl Int 1996;9:248-51.
10. Franzin G, Novelli P, Fratton A. Histologic evidence of Cytomegalovirus in the duodenal and gastric mucosa of patients with renal allograft. Endoscopy 1980;12:117-20.
11. Franzin G, Muolo A, Griminelli T. Cytomegalovirus inclusions in the gastroduodenal mucosa of patients after renal transplantation. Gut 1981;22:698-701.
12. Rubin RH. Infection in the organ transplant recipient. In: Clinical Approach to Infection in the Compromised Host; 4th edition. Robert H Rubin, Lowell S Young (eds). London: Kluwer Academic/Plenum Publishers; 2002. p 573-679.
13. Mori T, Mori S, Kanda Y et al. Clinical significance of Cytomegalovirus (CMV) antigenemia in the prediction and diagnosis of CMV gastrointestinal disease after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2004;33:431-4.
14. The TH, van der Bij W, van der Berg AP, et al. Cytomegalovirus antigenaemia. Rev Infect Dis 1990;12 Suppl 7:734-44.
15. Slifkin M, Tempesti P, Poutsiaka DD, Snydman DR. Late and atypical Cytomegalovirus disease in solid-organ transplant recipients. Clin Infect Dis 2001;33:E62-8.
16. Reinke P, Fietze E, Ode-Hakim S. Late-acute renal allograft rejection and symptomless Cytomegalovirus reactivation. Lancet 1994;344:1737-8.
17. Razonable RR, Rivero A, Rodriguez A et al. Allograft rejection predicts the occurrence of late-onset Cytomegalovirus (CMV) disease among CMV-mismatched solid organ transplant patients receiving prophylaxis with oral ganciclovir. J Infect Dis 2001;184:1461-4.
18. Becker BN, Becker YT, Leverson GE, Simmons WD, Sollinger HW, Pirsch JD. Reassessing the impact of Cytomegalovirus infection in kidney and kidney-pancreas transplantation. Am J Kidney Dis 2002;39:1088-95.
19. Limaye AP, Corey L, Koelle DM, Davis CL, Boeckh M. Emergence of ganciclovir-resistant Cytomegalovirus disease among recipients of solid organ transplants. Lancet 2000;356:645-9.
20. Sackier JM, Kelly SB, Clarke D, Rees AJ, Wood CB. Small bowel haemorrhage due to Cytomegalovirus vasculitis. Gut 1991;32:1419-20.
21. Cheung AN, Ng IO. Cytomegalovirus infection of the gastrointestinal tract in non-AIDS patients. Am J Gastroenterol 1993;88:1882-6.
22. Muldoon J, O'Riordan K, Rao S, Abecassis M. Ischemic colitis secondary to venous thrombosis. Transplantation 1996;61:1651-3.
23. Kas-Deelen AM, Harmsen MC, de Maar EF et al. Acute rejection before Cytomegalovirus enhances von Willebrand factor and soluble VCAM-1 in blood. Kidney Int 2000;58:2533-42.
24. The TH, Kas-Deelen AM, de Maar EF, Driessen C, Harmsen MC, Van Son WJ. Cellular and humoral parameters for vascular damage in blood during Cytomegalovirus infections. Transplant Proc 2001;33:1813.
25. Scholz M, Rabenau HF, Doerr HW, Cinatl J Jr. CMV-related immunopathology. Monogr Virol Basel, Karger 1998,21:29-41.
26. Schnitzler MA, Lowell JA, Hmiel SP et al. Cytomegalovirus disease after prophylaxis with oral ganciclovir in renal transplantation: The importance of HLA-DR matching. J Am Soc Nephrol 2003;14:780-5.