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Surveillance of cytomegalovirus infection in haematopoietic stem cell transplantation patients

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Summary *Objectives.* The aim of this study was to describe our experience in the control of active CMV infection following HSCT using two strategies of CMV infection treatment: ganciclovir universal prophylaxis at low doses and pre-emptive therapy with ganciclovir.

Methods. The surveillance was based on the monitoring of antigenaemia (AGM) and on a nested polymerase chain reaction (N-PCR) for the detection of CMV in both strategies. Forty-five recipients with malignant diseases and with a risk for CMV disease received universal prophylaxis (Group A). The non-treated group consisted of 24 patients, most of them with non-malignant diseases who did not receive universal prophylaxis (Group B).

Results. In Group A, the incidence of positive AGM was 51%, with a positive PCR of 68.9%. In Group B, the AGM positivity was 66.7% and that of N-PCR was 66.7%. CMV disease occurred in 6/55 patients (10.9%), with 2/36 (5.5%) from Group A and 4/19 (21%) from Group B. Two of these six patients (33.3%) died of CMV disease.

Conclusions. Our result suggests that AGM and N-PCR can be used as markers for assessing the monitoring and the introduction pre-emptive therapy. This approach could prove to be more cost-effective than ganciclovir universal prophylaxis for treating CMV infection.

Introduction

Cytomegalovirus (CMV) infection after haematopoietic stem cell transplantation (HSCT) produces significant morbidity and mortality.[1,2] Ganciclovir (GCV) has been established as an effective

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prophylactic agent for infection by CMV.[3,4] Because of the high mortality rate associated with CMV disease, antiviral prophylaxis and pre-emptive therapy have been widely used after HSCT.

The use of GCV universal prophylaxis is very effective in reducing the incidence and severity of CMV disease. However, overall survival is not improved, perhaps because prolonged use of GCV causes neutropenia[4,5] and hence susceptibility to bacterial infection.[5] Another disadvantage is that some patients are exposed to the toxic side effects of GCV, although they will never develop CMV disease.[5–7] Thus, it is important to discriminate patients with active CMV infection from those without such infection. Tests used for this purpose include the pp65 antigenaemia assay and DNA detection methods.[8]

The polymerase chain reaction (PCR) is a sensitive method for the detection of CMV DNA and active CMV infection, but is too sensitive for the exclusion of low-risk patients.[9] The CMV antigenaemia assay is a rapid and quantitative method[10] widely used as a guideline for starting pre-emptive therapy with ganciclovir.[11]

In this study, we describe our experience with the use of low doses of ganciclovir universal prophylaxis and the application of antigenaemia (AGM) and nested polymerase chain reaction (N-PCR) for assessing when to introduce pre-emptive therapy in the control of active CMV infection or disease in HSCT.

Patients and methods

Patients. Sixty-nine myeloablative haematopoietic stem cell transplant recipients with HLA identical siblings, were selected at the Bone Marrow Transplant (BMT) unit of the university hospital. Patients who were positive or negative for IgG-CMV, as well as, positive or negative donors requiring allogeneic HSCT for a variety of conditions, were monitored prospectively for CMV infection from July 1998 to December 2000, using AGM and N-PCR. The patients did not receive CMV hyperimmunoglobulin. The blood products used were neither screened for CMV antibody nor filtered to deplete leukocytes, although all had been irradiated. The conditioning regimens and acute graft versus host disease (GVHD) prophylaxis were selected based on ongoing protocols at the university hospital. Acute GVHD was graded according to published criteria.[12] All patients received GVHD prophylaxis with cyclosporine (CSP) and methotrexate (MTX), and were tested for underlying diseases using established institutional protocols. Two different groups of patients were analyzed. The patients were selected based on ongoing protocols of universal ganciclovir prophylaxis at the referring institution's BMT unit and were not randomized. Group A patients (38 adults and seven children) had haematological malignancies and received ganciclovir universal prophylaxis, whereas Group B consisted of 13 adults with aplastic anaemia and 10 children (seven with malignant disease and three with aplastic anaemia) who did not receive ganciclovir prophylaxis. The patient's characteristics, the pre-transplant conditioning regimens and the prophylaxis for GVHD disease are shown in Table 1. The patients were followed from day 0 until day 150 after the transplant. Blood was collected weekly for AGM and N-PCR. The protocol was designed in accordance with the requirements for research involving human subjects in Brazil, and was approved by the Institutional Ethics Committee.

CMV antigenaemia (AGM) assay. The antigenaemia assay was done at least once a week after engraftment, as previously described,[13] with some modifications.[14,15] Briefly, EDTA-treated blood samples were fractionated by dextran sedimentation followed by erythrocyte lysis. The granulocytes were then centrifuged to prepare Cytospin slides (3×10^5 granulocytes per slide). The slides were air-dried and fixed with formaldehyde, then immunostained with monoclonal antibodies C10 and C11 (Clonab CMV; Biotest, Dreieich, Germany), and reacted with peroxidase-labeled anti-mouse conjugate (HRP, Biotest, Dreieich, Germany). The test was done in duplicate.

Nested polymerase chain reaction (N-PCR). CMV DNA in blood specimens was detected by nested-PCR using primers described by Demmler et al.[16] and Shibata et al.[9] Briefly, leukocytes remaining from the CMV antigenaemia assay were lysed and the DNA was precipitated. The primers were selected from the MIE region of CMV-AD169. The size of the PCR amplification products was 159 base pairs. The same protocol was used to amplify the human β-globin gene sequence to guarantee the quality of the extracted DNA.

Definitions. Active CMV infection was defined based on one or both of the following criteria: (1) one or more positive cells in the AGM assay, and (2) two or more consecutive positive N-PCR results. For the diagnosis of CMV disease, the active infection had to be accompanied by clinical symptoms and histopathological identification of CMV.[17] Recurrence of CMV infection was defined as active CMV infection occurring after negative N-PCR and/or AGM assays following treatment of the initial