Journal of Clinical Virology 68 (2015) 37–42

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Effect of the immunosuppressive regimen on the incidence of cytomegalovirus infection in 378 heart transplant recipients: A single centre, prospective cohort study

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ARTICLE INFO

Article history:
Received 11 March 2015
Received in revised form 17 April 2015
Accepted 21 April 2015

Keywords:
Cytomegalovirus
Immunosuppression
Opportunistic infection
Transplant
Prognosis

ABSTRACT

Background: Cytomegalovirus (CMV) infection is a major complication of immunosuppression after heart transplant. Recent studies suggest the actual immunosuppressive regimen may affect the risk of CMV infection.
Objectives: To evaluate incidence, risk factors and clinical consequences of CMV infection and assess the possible differential effect of distinct immunosuppressive protocols.
Study design: Single centre, prospective cohort study of 378 consecutive heart transplant recipients undergoing CMV monitoring. Preemptive treatment was the standard of care. Patients were grouped as follows: group A, without any CMV infection; group B, with CMV infection not requiring pre-emptive treatment; group C, treated for CMV infection or disease.
Results: Most recipients never required antiviral therapy because of no CMV infection/disease (group A, 31%) or CMV levels below the cut-off for pre-emptive treatment (group B, 28%). Group C recipients (41%) were significantly older than group A patients (49.1 ± 13.2 vs. 44.8 ± 15.1 years; $p = 0.028$). Most cases occurred within the second month post-transplant. CMV viremia was detected in 77% and 62% of patients primed with thymoglobulin or ATG Fresenius, respectively, (OR 2.06, 95% C.I. 1.27–3.34; $p = 0.0034$). Use of everolimus was associated with a significantly lower rate of CMV infection compared to azathioprine or mycophenolate (OR 0.19, 95% C.I. 0.09–0.39; $p < 0.0001$). Major opportunistic infections were significantly more common in groups B and C.
Conclusion: In a large and homogeneous cohort of heart transplant recipients, we observed a strong relationship between the immune suppressive regimen and CMV infection, as well as an increased incidence of other opportunistic infections in recipients with CMV infection/disease.

1. Background

Cytomegalovirus (CMV) infection is a major complication of immune suppression, and occurs commonly following solid organ transplantation. Recipients of a heart transplant (HT) are considered at high risk of CMV infection and disease [1], showing a 9–35% incidence, mostly within the initial sixth months post-transplant.

CMV infection may occur in three different epidemiologic patterns: primary infection (donor- or transfusion-borne transmission); reactivation of a prior latent infection; superinfection or reinfection with a different viral strain [2,3]. The highest risk of CMV infection is associated with the matching of an antibody positive-donor (D+) with a seronegative recipient (R−) [3,4]. The serological status also influences the therapeutic approach, as universal prophylaxis with prolonged use of antiviral agents (ganciclovir or valganciclovir) is strongly recommended in D+/R− cases [5,6]. In contrast, in the seropositive recipient status (R+), two validated strategies for CMV management exist: universal prophylaxis and pre-emptive therapy. The latter requires a consistent monitoring of CMV replication markers and relies on the initiation of antiviral treatment once a threshold level of replication is reached [5,6].

Virologically and clinically, CMV may cause a lone infection, when viremia is not accompanied by any clinical sign, or a viral disease, characterised by either a generalised syndrome (fever, malaise, leukopenia, thrombocytopenia) or target organ damage (pneumonia, hepatitis, retinitis, gastro-enteritis) in the presence of active viremia [4].

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http://dx.doi.org/10.1016/j.jcv.2015.04.017
1386-6532/

Besides virus-related morbidity due to direct viral effects, CMV infection/disease also appears to increase the incidence of other opportunistic infections (bacterial, fungal and viral) and could promote acute rejection, cardiac allograft vasculopathy (CAV) and opportunistic malignancies [3,7–10]. Therefore, effective control of CMV after HT is a major goal of treatment.

Recent data suggest that the actual immune suppressive regimen could affect the incidence of CMV infection/disease. A lower risk has been associated to both induction regimens with ATG Fresenius™ (ATG) anti-thymocyte globulins [11] and sequential oral administration of mTOR-inhibitors (sirolimus and everolimus) [12–14]. However, most of these data were generated in multicentre studies, pooling cases with different donor/recipient statuses, managed with diverse therapeutic strategies (either prophylaxis or pre-emptive therapy), receiving various immune suppressive agents at undefined doses and using heterogeneous laboratory methods of CMV replication detection. Moreover, whether a relationship exists between the immune suppressive regimen, the severity of CMV infection, and the related morbidity and mortality, remains unknown.

2. Objectives

We performed this study in a large, homogeneous cohort of HT recipients, to assess the effect of different immunosuppressive protocols on the incidence of CMV infection/disease and its related morbidity and mortality within the first six months post-transplant.

3. Study design

3.1. Patients enrolled

This was a single centre, prospective, observational study, aimed at assessing predictors of CMV infection/disease after HT. The study protocol and procedures were approved by our institutional review board and complied with the ethics principles of the declaration of Helsinki. Included in this study under informed consent were all consecutive patients who received a HT in our centre between December 1999 and 2011. In our centre, CMV monitoring was started in 1999 and was performed up to 2007 by the detection of pp65 antigenemia. Since 2008, CMV-DNA measurement has replaced antigenemia detection. In parallel, the immunosuppressive protocols have also changed during this time period, allowing us to assess different schedules with both of these two validated laboratory methods.

Three possible outcomes were considered: (i) no evidence of CMV reactivation at any time during follow up; (ii) CMV replication below the threshold used in our centre for pre-emptive antiviral treatment; (iii) CMV replication, with or without clinical symptoms of CMV disease, above the threshold for pre-emptive treatment. Patients who died during the first four weeks post-transplant were assessed separately.

3.1.1. Definitions

CMV infection was defined as any documented viremia in the absence of clinical signs and symptoms. CMV generalised disease was defined as a viral syndrome, occurring in the presence of CMV pp65 antigen or DNA positivity, characterised by fever >38 °C without other causes and at least one of the following conditions: leukopenia (<4000 cells/μL), atypical lymphocytes >3%, thrombocytopenia (<100,000/μL). Tissue-invasive (organ) disease was diagnosed if there was histopathological evidence of CMV infection, except for pneumonia and central nervous system disease where the presence of clinical signs and symptoms coupled with consistent imaging and active viremia sufficed.

3.1.2. Clinical protocol

All patients entered an active screening protocol for CMV replication detection during the first six months post-transplant. Viral load measurement was performed weekly for the first 8 weeks, than twice weekly for the following 8 weeks and then monthly until the sixth month post-transplant. In case of CMV replication or antiviral treatment start, weekly evaluations were performed. Patients were started on pre-emptive antiviral treatment whenever viral load exceeded the predefined threshold, as detailed below. Those who were CMV antibody negative and received the graft from a CMV antibody positive donor were given primary prophylaxis since the fourth post-transplant day. In all cases, treatment was based on either gancyclovir, given intravenously at the dose of 5 mg/kg every 12 h, or valgancyclovir, given orally at the dose of 450–900 mg bid. Dose adjustments were done for patients with glomerular filtration rates below 30 mL/min. In a subset of patients, gancyclovir was given initially after hospital admission and switch to oral valgancyclovir was performed as soon as the patient could be discharged home. The predefined treatment duration was at least 14 days, except for cases of renal function worsening or bone marrow suppression. In most cases, treatment was stopped when antigenemia or viremia was proven undetectable on two occasions.

3.2. CMV replication assays

CMV antigenemia was assessed by means of an indirect immunofluorescence assay (CMV Brite, Immuno Quality Products, Groningen, The Netherlands) using two monoclonal antibodies (C10/C11) directed towards the pp65 protein. Briefly, peripheral blood leukocytes were separated by gradient centrifugation, counted and bound on glass support by cytospin. Cells were then fixed, permeabilised, and labeled with primary antibodies. Secondary antibodies were then added and reading was performed on a fluorescence microscope. Positive cell counts were referred to 2×10^5 leukocytes.

CMV viremia was measured by real time polymerase chain reaction (PCR). CMV DNA was extracted and purified from 100 μL of whole blood by a magnetic silice automated system [NucliSENS EasyMAG, BioMerieux, Marcy l'Etoile, France]. CMV DNA amplification and quantitation was performed by real-time alert Q-PCR [Nanogen Advanced Diagnostics, Turin, Italy] that amplifies the major immediate early antigen coding region. Appropriate controls were run in each assay and results were expressed as genome equivalents per milliliter [gEq/mL].

The cut-off values implying pre-emptive antiviral treatment used in our centre were a number of pp65 positive cells higher than 10 per 2×10^5 leukocytes or a CMV-DNA level of 10,000 gEq/mL of whole blood.

3.3. Statistical evaluation

The analyses were carried out with the aid of the SPSS 16.0 software. The significance level was set at 5% and all tests were two-tailed. The distribution of numerical variables was evaluated by skewness to check for normality and parametric or nonparametric tests were used accordingly. Data distribution was found to be mostly non-normal, thus nonparametric tests were more often employed. When a posteriori between-group studies were needed, analysis of variance with Bonferroni's post-hoc test was performed.

Differences between groups in numerical variables were assessed through Mann–Whitney or Kruskal–Wallis tests for two or multiple independent samples, respectively. Categorical data were compared by the Fisher's exact test. The possible relationship