Journal of Clinical Virology 33 (2005) 35–42

www.elsevier.com/locate/jcv

Follow-up of 28 HCMV seropositive renal-transplant recipients: comparison of clinical, biological and virological parameters in the groups of treated versus untreated infected patients

Nassim Kamar[a,1], Catherine Mengelle[b,*,1], Salah Yahyaoui[a], Karine Sandres-Saunné[b], Dominique Durand[a], Jacques Izopet[b], Lionel Rostaing[a]

[a] *Unité de Transplantation d'Organes, Hôpital Rangueil, CHU Toulouse, Toulouse, France*
[b] *Laboratoire de Virologie, Hôpital Purpan, CHU Toulouse, Toulouse, TSA 40031, 31059 Toulouse Cedex 9, France*

Received 8 January 2004; received in revised form 7 June 2004; accepted 15 June 2004

Abstract

Background: A pre-emptive strategy for prevention of Human Cytomegalovirus (HCMV) disease depends on accurate detection of HCMV infection and clinical and/or biological abnormalities.
Objectives: The aim of our study was to evaluate a therapeutic strategy based on the presence of either minimal clinical and/or biological symptoms or high HCMV viral load assessed by quantitative real-time PCR from whole blood as previously described.
Study design: Between June 2002 and July 2003, 70 HCMV seropositive patients underwent a renal transplantation. Virological monitoring was performed every 2 weeks until day 90 then every 3–4 weeks until day 180. Biochemical and haematological parameters were also prospectively monitored.
Results: Twenty-eight patients (40%) showed at least one positive HCMV DNA in whole blood. Based on the following criteria: HCMV viral load greater than 4 $\log_{10}$/ml, or the persistence of a HCMV DNAemia in two consecutive blood samples, or fever, or leucopenia or neutropenia, or increase in alanine aminotransferase level, 14 of the 28 patients received IV ganciclovir as a pre-emptive treatment. Immunosuppressive therapy and demographical data were comparable in both groups. As far as virological, haematological and biochemical parameters are concerned, no statistical significant difference was observed between treated and untreated patients. Moreover no adverse outcome was observed among untreated patients always having a HCMV viral load below 4 $\log_{10}$/ml.
Conclusions: This study reinforces the need of monitoring of HCMV seropositive renal-transplant recipients based on HCMV diagnosis and quantification by real-time PCR. The results showed that HCMV viraemic patients may benefit of absence of antiviral treatment when they have a low viral load (below 4 $\log_{10}$/ml) and absence of clinical and/or biological abnormalities. Further studies are required now to validate the threshold value at which we should begin pre-emptive therapy.

Keywords: HCMV seropositive renal-transplant recipients; Pre-emptive treatment; Quantitative real-time PCR; Clinical and biological abnormalities; Outcome

1. Introduction

Human Cytomegalovirus (HCMV) remains an opportunistic infection occurring in immunosuppressed patients, such as transplant recipients, and may manifest as symptomatic end-organ dysfunction or HCMV syndrome. Clinical studies have suggested a high link between HCMV infection and allograft rejection, and acceleration of the development of chronic rejection (Humar et al., 1999).

In order to prevent these disorders, transplant recipients may benefit from prophylaxis with anti-HCMV drugs, e.g.

Abbreviations: HCMV, Human Cytomegalovirus; PCR, polymerase chain reaction; WB, whole blood; IV, intravenous; WBC, white blood cell count; PMN, polymorphonuclear cell count; AST, Serum aspartate aminotrasferase; ALT, serum alanine amino transferase; UDG, uracil DNA glycosylase; Hb, hemoglobin; M, male; F, female; PRA, panel reactive anti-HLA alloantibodies; ATG, antithymocyte polyclonal antibodies; S Cr, serum creatinine; Cr Cl, calculated creatinine clearance; RT, renal transplantation; M1, 1 month post-transplantation; Plt, platelet count; cp/ml, copies/ml

* Corresponding author. Tel.: +33 5 61 77 21 13; fax: +33 5 61 77 25 42.
E-mail address: mengelle.c@chu-toulouse.fr (C. Mengelle).

[1] The two first authors equally participated to this work.

1386-6532/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2004.09.028

Valaciclovir, Ganciclovir, and, more recently, Valganciclovir, during the first 3 or 4 months post-transplantation (Humar et al., 2003; Paya et al., 2004; Pescovitz MD et al., 2003a, 2003b, 2003c). However, prophylaxis results in the unnecessary and costly administration of therapy in more than 50% of patients who would have never developed HCMV infection. Moreover, most of the studies addressing this prophylaxis in kidney-transplant recipients have been conducted in a combined population that includes seronegative recipients receiving a seropositive transplant, i.e. the D+/R− patients, and among seropositive recipients receiving either a seropositive or a seronegative transplant. However, HCMV D+/R− recipients are at higher risk of developing HCMV disease than seropositive transplant patients (up to 80%) and, therefore, they benefit most from the anti-HCMV prophylaxis treatment. In this group anti-HCMV treatment, i.e. intravenous (IV) Ganciclovir, is initiated as soon as markers of HCMV infection are positive, even in the absence of clinical manifestations (Humar et al., 2004; Lowance et al., 1999; Razonable et al., 2001).

In contrast, it is believed that prophylaxis is not necessary in seropositive HCMV patients and, in this setting, the strategy is to initiate a pre-emptive treatment. In this case, a regular clinical, biological and virological follow-up during the first months post-transplantation is necessary in order to detect early HCMV infection, i.e., HCMV DNAemia, and to initiate a treatment even in the absence of clinical manifestations. However, although the patients are monitored on a regular basis it is not clear when to initiate the treatment, or even if it has to be initiated. This method means that not all at-risk patients have to be treated; however, its efficacy relies on the early detection of HCMV infection with sensitive and predictive methods. Moreover, accurate diagnosis, i.e., associating good positive and negative predictive values, depends on the kind of organ transplantation as well as the kind of virological test used. Qualitative PCR tests have been proven to be poor indicators of HCMV disease (100% sensitive, but <50% specific) (Aitken et al., 1999; Boeckh et al., 1997; Tong et al., 1998). Therefore, quantitative measurements are required for accurate prediction of HCMV disease (Caliendo et al., 2002; Guiver et al., 2001; Humar et al., 1999, 2002; Li et al., 2003; Norris et al., 2002; Piiparinen et al., 2002; Sanchez et al., 2001; Tong et al., 2000).

We have previously published such monitoring using quantitative real-time PCR (QPCR) from DNA automatically extracted from whole blood (WB) with the MagNA Pure™ instrument followed by quantitative real-time Light Cycler PCR (Mengelle et al., 2003b). We, and others, have demonstrated that there is a good correlation between the HCMV pp65 antigenemia assay and the quantitative real-time PCR test in leukocytes and in whole blood. We have demonstrated that a viral load of $4\log_{10}$/ml corresponded to 50 positive pp65 cells and that this value could be the threshold needed to initiate anti-HCMV preemptive treatment in solid organ transplant recipients (Mengelle et al., 2003a).

The aims of this study were to evaluate, in HCMV seropositive renal-transplant patients during the first months of post-transplantation: (i) a therapeutic strategy based upon the presence of clinical/biological symptoms, and (ii) the relevance of real-time quantitative PCR during this follow-up. For this purpose, we report on and we compared between the two groups the clinical, biological, and virological results obtained during this study period.

2. Patients

Since June 2002, in our department, the monitoring of HCMV infection has relied on the assessment of HCMV DNAemia using quantitative real-time PCR on whole blood. Between June 2002 and July 2003, 121 renal transplantations have been performed at our institution. Of these, 70 patients were HCMV seropositive at the time of transplantation. HCMV DNAemia was assessed every 2 weeks until day 90 and thereafter at every 3 to 4 weeks until day 180. For HCMV infection, reactivation was defined on the basis of at least one DNAemia during the first 6 months post-transplantation. Symptomatic HCMV disease was defined according to agreed criteria (Ljungman, 1995) and included either a fever greater than 38 °C for 48 h in the absence of bacterial or fungal infection or rejection, and/or a progressive falling neutrophil count for 3 days, and/or thrombocytopenia at $<100 \times 10^9\ L^{-1}$ or the involvement of an organ. In the presence of HCVM DNAemia, the decision to treat the patients with IV Ganciclovir 15 days (7–21) (500 mg daily, adapted to renal function) was made on the following criteria: a HCMV viral load greater than 10,000 copies/mL ($4\log_{10}$) associated or not with the presence of fever, or the persistence of a HCMV DNAemia in two consecutive blood samples, or leucopenia (white blood cell count lower than 3000/mm^3), or neutropenia (polymorphonuclear cell count lower than 1500/mm^3), or an increase (twice the normal level) in alanine aminotransferase. In the event of positive HCMV DNAemia, baseline immunosuppression was not modified.

Due to numerous clinical trials conducted in the Transplantation Department, four patients had received anti-HCMV prophylaxis of Ganciclovir IV (Cymevan®) during days 11–15 followed by of Ganciclovir po ($N = 1$) or Valaciclovir (Zélitrex®) ($N = 3$) for 3 months. One patient had received Valaciclovir (Zélitrex®) only, during 128 days. Moreover, trimethoprime/sulfamethoxasole (Bactrim®) at 400 mg every other day was given for the first 6 months after transplantation.

2.1. Immunosuppression

Most patients received induction therapy using either anti-CD25 monoclonal antibodies (basiliximab or daclizumab) or polyclonal antibodies (Thymoglobulins® or ATG Fresinius). All patients received steroids (500 mg IV methylprednisolone) at pre-transplantation and then prednisolone at