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Everolimus-based immunosuppressive regimens in lung transplant recipients: Impact on CMV infection

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ABSTRACT

Cytomegalovirus (CMV) is one of the most important viral pathogen in solid organ transplant (SOT) recipients, with heart and lung transplant patients being at considerably high risk for CMV direct and indirect effects. Prevention strategies have resulted in significant reduction in disease and CMV related morbidity and mortality. Few studies reported a lower incidence of CMV infections in solid organ transplant recipients treated with immunosuppressive protocols including the mTOR inhibitor everolimus (EVR).

Purpose: The aim of the current study was to evaluate the impact of EVR-based immunosuppressive regimens on the occurrence and kinetics of CMV infection in a population of lung transplant recipients, at both systemic and pulmonary level. Thirty-two lung transplants (LT) were investigated; eighteen were on EVR-based immunosuppressive regimens. CMV events occurring in the first two years post-transplantation at both systemic and pulmonary levels were reported.

Principal results: No differences were reported in CMV viraemia occurrence at both one- and two-year follow up between patients undergoing EVR-based and EVR-free immunosuppressive regimens. Considering CMV episodes at pulmonary levels, as determined by routinely performed broncho-alveolar lavages (BALs), during EVR-administration the patients experienced significantly fewer episodes of high-load CMV (as defined by viral loads $\geqslant 10^5$ copies/mL) than during EVR-free immunosuppressive regimens.

Major conclusion: EVR-based immunosuppressive regimens in lung transplantation settings appear to be associated to lower incidence of clinically relevant CMV episodes at pulmonary levels, striking the possibility of extending the use of EVR to such a group of transplant recipients.

1. Introduction

Cytomegalovirus (CMV) is a ubiquitous β-herpesvirus that establishes lifelong latency in host tissues following primary infection. The occurrence of CMV primary infection in seronegative individuals or the capability of viral reactivation in immunocompromised conditions makes CMV one of the most important viral pathogens in solid organ transplantation (SOT), with incidence of infection/disease ranging from 8% to 50% depending on the transplanted organ. Heart and lung transplant (LT) recipients are at particularly high risk for CMV direct and indirect effects and evidence indicates that related morbidity and mortality are greater in LT recipients than in other SOT, as lung is a major site for CMV latency and recurrence. (Zamora et al., 2005; Snydman et al., 2011). Direct effects of CMV infection can manifest as systemic or organ-specific disease, whereas indirect effects reflect altered immune responses associated with infection, resulting in increased incidence of graft dysfunction, acute and chronic rejection, and opportunistic infections (Snydman et al., 2011; Fishman et al., 2007). Acute and chronic graft rejection are relevant determinants of morbidity and mortality, particularly in the first two years post-transplantation and several studies have reported an association between CMV infections and organ rejection, with the CMV donor/recipient (D/R) serological matching D+/R− being at the highest risk,

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although the underlying mechanisms are still unclear (Roux et al., 2013).

Prevention strategies may result in significant reduction in CMV-related morbidity and mortality in SOT recipients. Two main prevention strategies are commonly used: universal prophylaxis with administration of antiviral agents in all the patients and pre-emptive therapy based on virological monitoring (usually by evaluation of CMV-DNA on whole blood) and antiviral administration in the presence of laboratory evidence of infection, with relevant variations in clinical practice in different transplant centers (Costa et al., 2007; Snydman et al., 2011; Eid and Razonable, 2010).

Considering immunosuppressive protocols, few studies on heart and kidney transplantation reported a lower incidence of CMV infections in patients treated with regimens including everolimus (EVR), a proliferation signal inhibitor (PSI)/mammalian target of rapamycin (mTOR). mTOR inhibitors act by leading to inhibition of translational processes depending on mTORC1 activity, preventing cell-cycle progression from G1 to S-phase in T-cells; moreover, a potential antiviral effect through interruption of certain mTORC pathways or immune deviation has been evidenced (Boffini et al., 2009; Brennan et al., 2011; Vigano et al., 2010; Hill et al., 2007; Valantine and Zuckermann 2005; Nashan et al., 2012; Kobashigawa et al., 2013; Vitko et al., 2005; Eisen et al., 2003). The antiproliferative effect of EVR may represent a therapeutic option in immunosuppressive protocols of LT by reducing both the risk of acute rejection and the process of progressive fibrosis that determines chronic graft rejection. However, few data on EVR-based immunosuppression in LT are available and the effectiveness in conferring protection towards CMV infection, along with the specific indications and the most adequate time for its introduction or dosing, are still controversial (de Pablo et al., 2013).

The aim of this study was to prospectively evaluate the impact of EVR-based immunosuppressive regimens on the occurrence and kinetics of CMV infection and CMV-related events at systemic and pulmonary level, in a population of LT recipients.

2. Materials and methods

Thirty-two consecutive patients undergoing LT between 2007 and 2012 (mean age at transplantation ± SD, 49.7 ± 16.2 years; range 17–68.7), with at least one-year follow up, were prospectively studied. The main demographic and clinical features of the study population are summarized in Table 1. Informed consent was obtained from all the patients. The study population was divided in two groups: (1) 18 patients treated with EVR-based immunosuppressive regimens at different times post-transplantation (EVR-group); (2) 14 patients treated with EVR-free immunosuppressive protocols for all the study period (no-EVR-group). The EVR-group included two patients receiving EVR *de novo* within one month post-transplantation (one for gastric intolerance to mycophenolate mofetil, in association with severe relapsing neutropenia flares following introduction of azathioprine; one due to history of breast cancer), four within 6 months, and five within 12 months; four and three patients switched to EVR-based protocols in the second and third year post-transplantation, respectively (mean time at EVR introduction, 14.5 ± 10.9 months). In 17 out of 18 patients EVR was administered for at least six months. EVR was administered twice daily, with a goal trough level of 3–8 ng/dL. The main indications leading to switching to EVR maintenance immunosuppressant are listed in Table 2. In the EVR group, five patients were at high risk for CMV infections (as identified by serological matching D+/R−) and began EVR-administration at 9, 12, 15, 21 and 33 months post transplantation, respectively.

According to our Centre's practice, LT recipients were submitted to surveillance visits, including bronchoscopy with bronchoalveolar lavage (BAL), transbronchial biopsy and whole blood draws, at 1, 3, 6, 9, and 12 months post-transplantation; further visits were performed at 18 and 24 months post-transplantation, and then annually, as well as in the presence of clinical signs and/or symptoms and/or rejection. Further whole blood specimens were collected for CMV-DNA quantitation with trimestral periodicity (Costa et al., 2013).

All LT recipients received a universal, prolonged and combined antiviral prophylaxis for CMV consisting in the administration of ganciclovir or valganciclovir (450 mg bid) from day 21 for 3 weeks associated to CMV-immunoglobulins (Cytotect Biotest™) at days 1, 4, 8, 15, and 30 (1.5 mL/kg body weight) and monthly up to 1 year post-transplantation (1 mL/kg body weight), irrespective of CMV serostatus (Costa et al., 2012). Furthermore, all patients received long-term general antiviral prophylaxis with acyclovir 200 mg bid. Ganciclovir or valganciclovir were further administered based on clinical judgements and/or in case of CMV-DNA viral loads on whole blood and/or BAL greater than 10^4 copies/mL. Long-term immunosuppression was with cyclosporine A or tacrolimus (in patients with cystic fibrosis as underlying disease), mycophenolate mofetil, and prednisone (to be tapered or discontinued). In three patients with cystic fibrosis, also azathioprine was administered (one from the EVR-group, two from no-EVR). Allograft rejection was histopathologically diagnosed and graded on trans-bronchial biopsy specimens, according to the International Society for Heart and Lung transplantation Working Formulation (Stewart et al., 2007).

For CMV-DNA quantitation on whole blood and BAL samples, a real time PCR assay was used. Total DNA was extracted on the automated QIAsymphony® system (Qiagen, Hilden, Germany), according to the manufacturer's instruction. A commercially available real time PCR assay amplifying a region of the exon 4 of MIEA (major immediate early antigen) of CMV (Q-CMV Real Time Complete Kit, Nanogen Advanced Diagnostic, Italy) was performed on a 7500 Real-Time thermo-cycler system (Applied Biosystems,

Table 1
General characteristics of the study population.

Features	Study population total *N* = 32	EVR group *N* = 18	no-EVR group *N* = 14
Age at transplantation	49.7 ± 16.2	52.8 ± 14.8	47.4 ± 17.0
Mean ± SD (range), years	(17–68.7)	(24.2–68.3)	(17–68.7)
M/F	22/10	13/5	9/5
Type of transplant			
Bilateral[a]	17 (53.1%)	11	6
Monolateral	15 (46.9%)	7	8
Donor/recipient CMV matching, *N* (%)			
Low risk (D+/R+ and D−/R+)	26 (81.3%)	12 (66.7)	14 (100)
(D−/R−)	1 (3.1%)	1 (5.6)	0
High risk (D+/R−)	5 (15.6%)	5 (27.7)	0

[a] Including one patient with combined liver–lung transplantation belonging to the no-EVR group.