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Detection of cytomegalovirus drug resistance mutations in solid organ transplant recipients with suspected resistance

Rubén López-Aladid[a], Alba Guiu[a], Gemma Sanclemente[b], Francisco López-Medrano[c], Frederic Cofán[d], M. Mar Mosquera[a], Julián Torre-Cisneros[e], Elisa Vidal[e], Asunción Moreno[b], Jose Maria Aguado[c], Elisa Cordero[f], Cecilia Martin-Gandul[f], Pilar Pérez-Romero[f], Jordi Carratalá[g], Nuria Sabé[g], Jordi Niubó[h], Carlos Cervera[i], Anna Cervilla[a], Marta Bodro[b], Patricia Muñoz[j], Carmen Fariñas[k], M. Gemma Codina[l], Maitane Aranzamendi[m], Miguel Montejo[n], Oscar Len[o], M. Angeles Marcos[a,*], Group for Study of Infection in Transplantation of the Spanish Society of Infectious Diseases Clinical Microbiology GESITRA-SEIMC Spanish Network for Research in Infectious

[a] *Department of Clinical Microbiology, Hospital Clinic, Universidad de Barcelona, Barcelona Institute for Global Health, Barcelona, (ISGlobal), Spain*
[b] *Department of Infectious Diseases, Hospital Clinic, Institut d'Investigacions Biomediques August Pi I Sunyer (IDIBAPS), Universidad de Barcelona, Barcelona, Spain*
[c] *Unit of Infectious Diseases, Instituto de Investigación Hospital 12 Octubre (i + 12) University Hospital 12 de Octubre, Universidad Complutense, Madrid, Spain*
[d] *Nephrology and Renal Transplant Department, Hospital Clinic, Universitat de Barcelona, Barcelona, Spain*
[e] *Clinical Unit of Infectious Diseases, Hospital Universitario Reina Sofia-IMIBIC-UCO, Córdoba, Spain*
[f] *Infectious Diseases Department, Hospital Universitario Virgen del Rocío, Sevilla, Instituto de Biomedicina de Sevilla (IBIS), Unit of Infectious Diseases, Microbiology and Preventive Medicine, University Hospital Virgen del Rocío, Spain*
[g] *Department of Infectious Diseases, Bellvitge University Hospital, IDIBELL, Barcelona, Spain*
[h] *Department of Clinical Microbiology, Bellvitge University Hospital, IDIBELL, Barcelona, Spain*
[i] *Department of Medicine, Division of Infectious Diseases, University of Alberto, Edmonton, Canada*
[j] *Department of Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Instituto de Investigación Sanitario Gregorio Marañón, Madrid, Spain*
[k] *Unidad de Enfermedades Infecciosas, Hospital Universitario Marqués de Valdecilla, Universidad de Cantabria, Santander, Spain*
[l] *Microbiology Service, Hospital Vall d'Hebron, Barcelona, Spain*
[m] *Servicio de Microbiología, Hospital Universitario de Cruces, Bilbao, Spain*
[n] *Unidad de Enfermedades Infecciosas, Hospital Universitario de Cruces, Bilbao, Spain*
[o] *Department of Infectious Diseases, Hospital Universitari Vall d'Hebrón, Uniiversitat Autónoma de Barcelona, Barcelona, Spain*

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ABSTRACT

Background: Current guidelines recommend that treatment of resistant cytomegalovirus (CMV) in solid organ transplant (SOT) recipients must be based on genotypic analysis. However, this recommendation is not systematically followed.
Objectives: To assess the presence of mutations associated with CMV resistance in SOT recipients with suspected resistance, their associated risk factors and the clinical impact of resistance.
Study design: Using Sanger sequencing we prospectively assessed the presence of resistance mutations in a nation-wide prospective study between September 2013-August 2015.
Results: Of 39 patients studied, 9 (23%) showed resistance mutations. All had one mutation in the UL 97 gene and two also had one mutation in the UL54 gene. Resistance mutations were more frequent in lung transplant recipients (44% p = 0.0068) and in patients receiving prophylaxis ≥6 months (57% vs. 17%, p = 0.0180). The mean time between transplantation and suspicion of resistance was longer in patients with mutations (239 vs. 100 days, respectively, p = 0.0046) as was the median treatment duration before suspicion (45 vs. 16 days, p = 0.0081). There were no significant differences according to the treatment strategies or the mean CMV load at the time of suspicion. Of note, resistance-associated mutations appeared in one patient during CMV prophylaxis and also in a seropositive organ recipient. Incomplete suppression of CMV was more frequent in patients with confirmed resistance.

* Corresponding author at: Department of Clinical Microbiology, Hospital Clinic, Universidad de Barcelona, Institute for Global Health, (ISGlobal), Villarroel 170, Barcelona 08036, Spain.
E-mail address: mmarcos@clinic.ub.es (M.A. Marcos).

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Conclusions: Our study confirms the need to assess CMV resistance mutations in any patient with criteria of suspected clinical resistance. Early confirmation of the presence of resistance mutations is essential to optimize the management of these patients.

1. Background

Cytomegalovirus (CMV) is one of the most important pathogens affecting solid organ transplant (SOT) recipients. In these patients, CMV is a significant cause of morbidity and mortality associated with both invasive disease and modulating effects in the host immune system [1,2].

The development of antiviral agents and preventive strategies over the past decades have significantly improved patient outcomes, but they have also promoted the development of antiviral-resistant CMV strains that can significantly contribute to adverse clinical outcomes [3–6].

Antiviral drug resistance should be suspected when CMV viremia or clinical disease persist in spite of prolonged antiviral therapy [7,8]. However, not all such cases are associated with genomic resistance mutations. Genotypic testing is the routine method for detecting drug resistance and the basis for the selection of alternative therapies [9].

Clinical suspicion of the development of resistance should be assessed early for prompt initiation of the most appropriate therapy. However, virologic resistance is likely underdiagnosed since mutation assessment is not systematically performed.

Several risk factors have been associated with CMV resistance thereby conditioning the inclusion criteria of some studies [10]. The present study was prospectively conducted within the context of the Group for the Study of Infection in Transplantation (GESITRA), constituting one of its major lines of clinical research. The nation-wide network of Spanish hospitals has allowed the inclusion of a wide variety of solid organ transplant recipients.

2. Objectives

The aim of this study was to assess the presence of mutations associated with CMV resistance in solid organ transplant recipients with suspected resistance in a nation-wide study, as well as to determine the associated risk factors and the clinical impact of resistance.

3. Study design

3.1. Setting and study population

We conducted a prospective observational study in nine hospitals included in the Spanish Network for Research in Infectious Diseases (REIPI). Adult solid organ transplant patients with suspected resistance to antiviral drugs were included in the study from September 2013 to August 2015. Resistance was suspected on the presence of progressive or stable viral loads or if clinical symptoms persisted despite the use of adequate antiviral treatment for at least 2 weeks [7,8].

The study was approved by the local Ethics Committees of the participating hospitals and was endorsed by GESITRA.

The coordinating center was the Hospital Clinic of Barcelona, which performed the genotypic resistance testing as well as the data analysis. Patient treatment and follow-up were conducted as per the protocol of each center. All patients were treated with standard-dose ganciclovir/valganciclovir (GCV/VGCV) (adjusted to renal function). Monitoring was based on locally quantified plasma CMV PCR tests. No standardization was imposed. On suspicion of resistance, plasma samples were frozen at −80 °C in the respective hospital and sent in batches to the Microbiology Laboratory of Hospital Clínic every 4 months.

Data of interest at the time and beyond for each participant were registered in a clinical database at each participating hospital. The resistance mutations detected were also included in the database CMV infection and disease and acute cellular rejection were defined as previously reported [7,8].

3.2. Microbiological studies

In the coordinating laboratory, extraction of DNA was performed in 500 μl of plasma of each sample using QIAsymphony system (Qiagen, Hilden, Germany). CMV viral load was confirmed by PCR CMV Real Time (Nanogen Advanced Diagnostics, Italy) according to the manufacturerís instructions.

Genotypic antiviral resistance testing was based on PCR amplification of the CMV UL97 protein kinase gene (codons 400–670) in a single fragment and the UL54 DNA polymerase gene (codons 300–1000) in four fragments followed by Sanger nucleotide sequencing (see Supplementary Material).

3.3. Data management and statistical analysis

Data were registered using the program OpenClinica 3.1 program [copyright (C) 2005–2014, by LLC GNU Lesser General Public License (GNU LGPL)]. Data were analyzed using Stata version 14.1 (Stata Corp., College Station, TX). We used the *t*-test or Wilcoxon rank-sum test to compare continuous variables and the Fisher test to compare proportions. Following univariate analysis, a logistic regression model was constructed as an exploratory analysis to identify independent factors significantly associated with the presence of a mutation. We did multivariate analyses by logistic regression with a stepwise forward model ($p_{in} < 0.05$, $p_{out} < 0.10$ in the likelihood ratio test). Odds Ratios (OR) and 95% confidence intervals (CI) were calculated for factors associated with the presence of mutation. The threshold of statistical significance was $p < 0.05$ (see Supplementary material).

4. Results

During the study period, we enrolled 43 adults who had undergone solid organ transplantation and in whom CMV antiviral resistance was suspected. Four were excluded from the analysis because sequencing could not be carried out due to low CMV viral load. Finally, 39 patients were included. Table 1 shows the baseline characteristics of the study population.

The kidney was the most frequent type of organ transplant (44%) followed by liver transplant (21%). Donor/recipient CMV serostatus was mainly D+/R- (62%) with fewer R+ (39%). More than 20% of patients received depleting anti-lymphocyte antibodies as induction therapy. The most common maintenance immunosuppressant regimen consisted of tacrolimus, mycophenolate and steroids. Mammalian target of rapamycin inhibitors (mTORi) were used in 4 patients (11%). Half of the patients had received post-