

Evaluation of Cytomegalovirus Refractoriness and Resistance in Solid Organ Transplantation in Argentina: A Multicenter Study

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Authors: Laura Barcan, Emilio Felipe Huaier Arriazu, Temporiti Elena, Ameri Diego, Giorgio Patricia, Gago Rocío, Niveyro Carla, Villamandos Silvina, Salgueira Claudia, Ajzenszlos Martín, Illán Hector, Natalia Pujato, Astrid Smud.

Affiliation:

Hospital Italiano de Buenos Aires. CABA

CEMIC. CABA

Hospital El Cruce. Pcia Bs As

Hospital Británico. CABA

Hospital Austral. Pcia Bs As

Hospital Madariaga. Posadas, Misiones

ITAC. CABA

Instituto de Cardiología. Corrientes

Sanatorio Trinidad. Mitre

Hospital Cosme Argerich

Servicio de Neurovirosis, INEI. ANLIS Malbrán

1. Abstract

Introduction: Cytomegalovirus (CMV) infection remains one of the most frequent infections after solid organ transplantation (SOT). There are no data in our country regarding its incidence in the different types of organ transplantation, nor regarding its different clinical presentations: asymptomatic infection or disease. There is even greater lack of knowledge about the incidence of refractory and resistant CMV, conditions that generally show poor response to conventional treatments.

Objectives: The objective of this study is to determine the prevalence of refractory CMV in Argentina and the results of genotyping to assess resistance in those cases in which this test was performed.

Methods: A multicenter retrospective cohort study will be conducted including adult patients (≥ 18 years) who are solid organ transplant (SOT) recipients in participating centers in Argentina, during the period between January 1, 2017, and December 31, 2024, who have been followed for at least 18 months after transplantation and for at least 12 months since the onset of infection. Cases of CMV infection meeting criteria for refractory or resistant infection according to the established operational definitions will be identified.

Keywords: solid organ transplantation, CMV, refractory/resistant.

2. Introduction

Cytomegalovirus (CMV) infection remains one of the most frequent infections after solid organ transplantation (SOT).

Its impact on the outcome of transplant recipients is significant since, in addition to its direct effects causing disease, its indirect effects can be associated with graft loss and morbidity and mortality, and it is known that graft survival is better in CMV-negative patients (D⁻ and R⁻) than in any other serological status (1).

Prevention strategies (preemptive therapy or prophylaxis) have reduced CMV disease and associated mortality; both prophylaxis and preemptive therapy have shown benefit (2,3). However, refractory and resistant CMV, particularly in association with high-risk transplants (D⁺/R⁻ and thymoglobulin use in particular), remains a challenge, as until recently appropriate drugs for its treatment were not available. Refractory infection is likely multifactorial, including host-related factors such as excessive immunosuppression, impaired absorption or intolerance to drugs, use of subtherapeutic dosing during prophylaxis, or viral resistance to the administered antiviral agents (4).

Determining the incidence of refractoriness is difficult, as definitions have changed over time. In 2024, Aguado et al. performed a systematic review to assess the incidence of refractoriness in Spain. Nineteen studies including 1,973 solid organ transplant recipients were reviewed. Refractory infection occurred in 3% to 10% of cases, and resistance ranged from 1% to 36% among patients with refractory infection. These heterogeneous results are clearly influenced by the different definitions of refractoriness and resistance used over time (5). In patients with refractory CMV infection, genotyping studies should be performed to evaluate resistance-associated mutations.

Resistance to ganciclovir is mainly due to mutations in the UL97 gene, which encodes the viral protein kinase, and less frequently in the UL54 gene, which encodes the viral DNA polymerase. Mutations in UL54 may also confer resistance to foscarnet and/or cidofovir (6,7).

In the study by Fisher et al. published in 2017, the incidence of ganciclovir-resistant CMV (GAN-R) among all solid organ transplant recipients was 1%, with a higher incidence (4.1%) in D⁺/R⁻ recipients (8). Incidence also varied according to the transplanted organ: 11.9% in lung transplantation, 5.8% in

heart transplantation, 2.4% in kidney transplantation, 7.8% in pancreas transplantation, and 0.4% in liver transplantation. No variation in prevalence was observed between 1993 and 2010.

The objective of this study is to determine the prevalence of refractory CMV in Argentina (number of centers) and the results of genotyping to assess resistance in those cases in which this test was performed.

3. Research Question(s)

What is the incidence and distribution by type of transplant of refractory or resistant cytomegalovirus (CMV) infection in solid organ transplant (SOT) recipients in centers in Argentina during the period 2017–2024, and what factors are associated with its occurrence and clinical outcomes?

What genetic resistance mutations are identified in cases in which genotyping was performed? What relationship was observed in terms of response to antiviral treatment?

4. Objectives

4.1. Primary Objective

To estimate the incidence of refractory or resistant cytomegalovirus infection within 18 months after transplantation in solid organ transplant (SOT) recipients in centers in Argentina during the period 2017–2024.

4.2. Secondary Objectives

1. To estimate the overall incidence of CMV infection within 18 months after transplantation, stratified by type of transplanted organ in the same population.
2. To characterize the epidemiology of CMV infections overall in all solid organ transplants, differentiating their distinct clinical forms: primary infection, reactivation, asymptomatic infection, and CMV disease.
3. To describe the genetic mutations associated with antiviral resistance detected through genotyping studies in cases with clinical suspicion of resistance in those cases that were submitted for testing, and their relationship with antiviral treatment.
4. To analyze the clinical and therapeutic characteristics of refractory or resistant CMV episodes, including treatment received, virological response, and clinical outcomes (90-day and 1-year mortality).
5. To explore the impact of refractory or resistant CMV infection on graft outcomes and overall recipient survival.

5. Purpose

The present study aims to describe and understand the local epidemiology of refractory or resistant CMV in solid organ transplant recipients in Argentina, providing robust multicenter data that will allow:

- to quantify the burden of disease and its clinical impact,
- to identify resistance patterns and potential predictive factors, and
- to strengthen diagnostic and therapeutic strategies in this high-risk population.

In addition, the results will serve as a basis for the development of future prospective and collaborative studies aimed at optimizing CMV management in the context of solid organ transplantation.

6. Materials and Methods

6.1. Study Design

A multicenter retrospective cohort study will be conducted including adult patients (≥ 18 years) who are solid organ transplant (SOT) recipients in participating centers in Argentina, during the period between January 1, 2017, and December 31, 2024.

The analysis will focus on episodes of cytomegalovirus (CMV) infection occurring within 18 months after transplantation, identifying those that meet criteria for refractory or resistant infection according to the established operational definitions.

6.2. Setting

The study will be carried out in solid organ transplant centers located in different provinces of Argentina, all of them with infectious diseases teams integrated into the transplant programs and with diagnostic capacity for quantitative CMV PCR. Each center will report the commercial name of the PCR assay used in its laboratory, the limit of detection, the unit of measurement, and any changes in methodology over time.

The following institutions will participate: Hospital Italiano de Buenos Aires, CEMIC, Hospital El Cruce, Hospital Británico, Hospital Austral, Hospital Madariaga (Posadas), ITAC, Instituto de Cardiología de Corrientes, Sanatorio Trinidad Mitre, and ANLIS–Malbrán.

Each center will have a responsible investigator for the collection of clinical and microbiological data, following a common standardized data collection form and harmonized procedures.

Resistance mutations from submitted strains will be analyzed at the Virology Department of Instituto Malbrán, the national reference center.

The UL97 gene encodes the thymidine kinase required for the initial phosphorylation of ganciclovir (GCV); therefore, mutations in this gene result in inadequate intracellular phosphorylation of the drug, leading to resistance. Sequence analysis of UL97 from clinical CMV isolates resistant to GCV has demonstrated point mutations or deletions in this region of the viral genome. These mutations affect codons 460 (V460, I460), 520 (Q520), and codons 591 to 607. Mutations in this gene do not confer resistance to foscarnet or cidofovir, as these compounds do not require this enzyme.

The technique consists of amplification of DNA corresponding to the UL97 gene of HCMV by PCR. Once DNA amplification is obtained, sequencing is performed using the Sanger method, and the resulting sequence is analyzed and compared with a reference control strain (AD169 strain) using the Ugene software.

6.3. Population

The study population will consist of adult solid organ transplant recipients (kidney, liver, heart, lung, pancreas, or combined transplants) who develop CMV infection in any of its forms within 18 months after transplantation.

Medical records will be reviewed to obtain demographic data and information on the clinical course of infection in recipients of any type of solid organ transplant. From this population, CMV incidence will be calculated, and among these patients, those with refractory forms will be analyzed in greater detail according to the established operational definitions.

6.3.1. Inclusion Criteria

- Patients ≥ 18 years of age.
- Solid organ transplant recipients during the period 2017–2024.
- Documented evidence of CMV infection (according to current consensus definitions).
- Clinical follow-up available for at least 12 months after infection.

6.3.2. Exclusion Criteria

- Incomplete follow-up or loss of relevant clinical information.
- Transplantation or follow-up performed outside the participating centers.
- CMV infections occurring remote from transplantation (beyond 18 months).

6.3.3. Definitions

CMV infection:

Isolation of the virus or detection of viral proteins or DNA/mRNA in any body fluid or tissue.

CMV disease:

Disease is considered present when a patient with CMV infection develops signs and symptoms.

a. Viral syndrome: viremia plus any of the following:

- Nonspecific laboratory abnormalities (e.g., leukopenia, thrombocytopenia, liver function test abnormalities, functional impairment of the transplanted organ).
- Nonspecific clinical manifestations: fever, asthenia, myalgias.

b. Organ disease (invasive or visceral):

- Clinical manifestations with identification of virus in the affected parenchyma/parenchymas, with or without viremia.

Refractory CMV:

Viral load does not decrease or increases (any increase or decrease in value) after 2 weeks of appropriate antiviral treatment.

Resistant CMV:

Refractory infection with documentation of at least one genetic mutation associated with resistance to one or more antivirals.

6.4. Data Collection

Based on the databases of the solid organ transplant (SOT) teams of the participating centers, medical records of all patients who presented CMV infection between January 2017 and December 2024 will be reviewed in order to obtain demographic and clinical data.

Among patients who developed CMV infection, either infection or disease, refractory cases will be analyzed according to the definitions established for the study.

Risk factors for refractoriness will be evaluated.

In cases in which samples were referred for genotyping studies, detected resistance mutations will be analyzed, as well as whether there was correlation with response to treatment.

Samples sent for resistance testing, based on the decision of the infectious diseases specialist at each center, were analyzed by the Virology Department of Instituto Malbrán (ANLIS Malbrán).

Data will be recorded in a unified, anonymized, and coded database. All information will be verified by the principal investigators prior to central analysis.

Initiation of treatment: Each center will define its viral load threshold for initiation of treatment.

6.4.1. Data Collection Plan and Data Confidentiality

In the present study, a meticulous data collection plan will be implemented to ensure accuracy and confidentiality of the collected information. The procedures to be followed are detailed below:

Data source: Data will be obtained from medical records and alerts from the microbiology laboratory.

Data entry: Variables corresponding to each patient will be entered into a centralized REDCap database. Responsibility for data entry will lie with the investigators. To maintain privacy and confidentiality, each patient will be assigned a randomly generated registration number that will identify them in the database.

Data access: Only the principal investigator will have access to the full dataset. Each investigator will access REDCap using their personal username and password, ensuring that only authorized personnel can access data from their institution.

Confidentiality and anonymization: All applicable legal provisions will be strictly followed, including the National Personal Data Protection Law No. 25,326 and the Habeas Data Law, to protect the confidentiality and privacy of patients involved in the study. All collected data will be treated anonymously, without information that directly identifies patients.

Data storage: Once data entry is completed, the full database will be stored under password protection and will be available only to the principal investigators of the study. All necessary precautions will be taken to ensure that data are maintained in a secure and reliable environment.

The research team commits to safeguarding data privacy and confidentiality in accordance with current legal regulations and to conducting the study in an ethical and responsible manner.

6.5. Variables

6.5.1. Demographic Variables

1) Patient characteristics:

- Age: period expressed in years at the time of the study, reported by the patient; expressed as an ordinal quantitative variable.
- Sex: reported by the patient at the time of the study; expressed as a non-ordinal qualitative variable with options such as female / male / not reported.
- Pre-transplant CMV serology: positive or negative.
- CMV reactivation: detectable DNAemia with a value defined by each institution.

6.5.2. Potential Predictors / Explanatory Variables

These variables will be collected in all patients with refractory/resistant (R/R) CMV. In the remaining patients, only some of these variables will be collected.

1. Patient characteristics

Variable	Operational definition	Type / coding
Age	Age at the time of CMV diagnosis (completed years).	Continuous numeric
Sex	Gender recorded in the medical record.	Categorical (F/M/Other)
Comorbidities	Hypertension, diabetes, renal failure, liver disease, neoplasia, COPD, among others.	Multiple categorical (Yes/No per condition)
Baseline immune status	Presence of pre-transplant immunosuppression (chemotherapy, corticosteroids >20 mg/day, etc.).	Categorical (Yes/No)

2. Transplant characteristics

Variable	Operational definition	Type / coding
Type of transplanted organ	Primary organ: kidney, liver, heart, lung, pancreas, combined.	Nominal categorical
Transplant date	Date of the procedure (DD/MM/YYYY).	Date (continuous temporal)
Donor CMV serology	Donor IgG serology (D+ or D-).	Dichotomous categorical
Recipient CMV serology	Recipient IgG serology (R+ or R-).	Dichotomous categorical
Serological risk	Combination D+/R-, D+/R+, D-/R+, D-/R-.	Categorical
Immunosuppressive induction	Drug used: thymoglobulin, basiliximab, rituximab, methylprednisolone.	Multiple categorical
Maintenance immunosuppression	Active regimen at the time of the event (e.g., tacrolimus/mycophenolate/prednisone, cyclosporine, mTOR inhibitors, belatacept, etc.).	Categorical
Previous acute rejection episodes	Number and date of the last episode before CMV.	Numeric / Date
Retransplantation	History of previous transplantation.	Categorical (Yes/No)

3. Characteristics of CMV infection

Variable	Operational definition	Type / coding
Type of CMV infection	Primary (D+/R-), reactivation, asymptomatic infection, or disease.	Categorical
Date of diagnosis	Date of the first positive test (PCR/antigen).	Date
Primary prophylaxis	Antiviral drug received from the time of transplantation.	Categorical
Duration of primary prophylaxis	Duration of prophylaxis in days from transplantation.	Continuous numeric
Initial viral load	IU/mL; if unavailable, copies/mL at the first quantitative determination.	Continuous numeric (log ₁₀)
Peak viral load	Peak value during the episode.	Continuous numeric
Time to negativization	Days from treatment initiation to negative PCR.	Continuous numeric
Affected organ	Gastrointestinal, pulmonary, hepatic, others.	Multiple categorical
Clinical form	Asymptomatic infection / viral syndrome / organ disease.	Categorical
Associated hospitalization	Requires inpatient care or outpatient management.	Categorical (Yes/No)
Initial antiviral	Drug used as first-line therapy (ganciclovir, valganciclovir, foscarnet, others).	Categorical

Variable	Operational definition	Type / coding
Change of antiviral	Need to modify treatment due to failure or toxicity.	Categorical (Yes/No)
Total duration of treatment	Days of effective antiviral therapy.	Continuous numeric
Detected mutation	Presence and type of mutation (UL97, UL54, or others).	Categorical
Antiviral toxicity	Neutropenia, renal failure, or other documented adverse events.	Multiple categorical
Time from transplant to CMV event	Days between transplant date and first CMV infection.	Continuous numeric
Relapse	New CMV infection episode within 6 months after resolution.	Categorical (Yes/No)

Therapeutic and outcome variables

Variable	Operational definition	Type / coding
Refractory CMV infection	Persistence or increase of viral load after ≥ 14 days of appropriate antiviral treatment.	Dichotomous categorical (Yes/No)
Resistant CMV infection	Refractory infection with identification of a genomic mutation associated with antiviral resistance (UL97 or UL54).	Dichotomous categorical (Yes/No)
90-day mortality	Death from any cause within 90 days from CMV infection diagnosis.	Dichotomous categorical (Yes/No)
1-year mortality	Death from any cause within one year from CMV infection.	Dichotomous categorical (Yes/No)
CMV-attributable 90-day mortality	Death attributable to CMV infection within 90 days from diagnosis.	Dichotomous categorical (Yes/No)
CMV-attributable 1-year mortality	Death attributable to CMV infection within one year from diagnosis.	Dichotomous categorical (Yes/No)
Graft loss	Irreversible dysfunction or need for retransplantation during follow-up.	Dichotomous categorical (Yes/No)

6.5.3. Derived and Analysis Variables

For exploratory and multivariable analyses, the following variables will be derived:

- Time to event (T): from transplantation to the first refractory/resistant CMV episode (survival analysis).
- Combined immunological risk score: assigning points according to type of induction, maintenance regimen, and D/R risk.
- Mean weekly logarithmic viral load.
- Therapeutic complexity score: number of antiviral changes or combinations.

6.5.4. Handling of Missing Data

Mandatory variables (age, sex, organ type, D/R serology, type of infection, treatment, and outcomes) must be complete for inclusion in the primary analysis.

Missing data will be evaluated under the missing at random (MAR) assumption, and multiple imputation strategies will be explored for sensitivity analyses in secondary analyses.

6.6. Statistical Considerations

6.6.1. Sampling and Sample Size Calculation

(No sample size calculation is planned.)

6.6.2. Statistical Analysis

Baseline characteristics will be evaluated according to the distribution of observations, which will be assessed graphically using histograms and analytically using the Shapiro–Wilk test. Variables with a normal distribution will be expressed as mean and standard deviation (SD), while variables with a non-normal distribution will be expressed as median and interquartile range (IQR). Categorical variables will be presented as absolute and relative frequencies.

Comparative analyses between groups will be performed using the Student's t test or the Mann–Whitney U test, according to the distribution of quantitative variables, and the Chi-square test for categorical variables, with Fisher's exact test applied when appropriate.

A p-value <0.05 will be considered statistically significant.

Statistical analyses will be performed using STATA version 16 MP – Parallel Edition (Copyright 1985–2017 StataCorp LLC, StataCorp, 4905 Lakeway Drive, College Station, Texas 77845, USA).

6.7. Ethical Considerations

This research will be conducted in accordance with ethical principles and regulatory standards for human health research at both national and international levels, in compliance with national regulations (Resolution of the Ministry of Health of Argentina No. 1480/2011 and Law No. 3301/09 of the Autonomous City of Buenos Aires), the Declaration of Helsinki of the World Medical Association and all its amendments, and the International Conference on Harmonisation Good Clinical Practice guidelines (ICH E6).

All study data will be handled with the utmost confidentiality, with access restricted exclusively to authorized study personnel, in accordance with current legal regulations, including the National Personal Data Protection Law No. 25,326/00 (Habeas Data Law) and Law No. 26,529/09.

The identity of personal data will be kept under absolute confidentiality. Data will be coded using a randomly generated numerical key, which will replace the patient's name and surname. Identifying information will be removed from the database to be analyzed, in accordance with the 18 identifiers detailed in HIPAA regulations. The database will be stored on a password-protected computer with access limited to the principal investigator of the study and will be deleted after completion of the statistical analysis.

Given that this is a minimal-risk study involving the handling of participant data, a waiver of informed consent will be requested, based on CIOMS Guideline 10 (2019): Modifications and Waivers of Informed Consent, as we consider that:

- A. it would not be feasible or viable to conduct the research without such a waiver or modification;
- B. the research has important social value; and
- C. the research entails only minimal risk to participants.

Regarding point A, data will be obtained retrospectively. At the time of data collection, it is likely that hospitalization will have already ended and patients will be receiving outpatient care. Regarding point B, this study has social value because it will allow us to understand the epidemiology of CMV in solid organ transplant recipients in a country from a region with limited epidemiological data. Finally, regarding point

C, this study entails minimal risk for patients, as it is a retrospective observational study. The main risk relates to patient privacy; therefore, all study data will be treated with maximum confidentiality, in coded form, and with access restricted exclusively to authorized personnel, in compliance with current legal regulations.

The research team remains available for any audits, monitoring activities, or reviews of processes, documentation, final or interim versions of datasets or documents that the Ethics Committee may consider necessary for the purposes of the study.

6.8. Funding

This study will be fully funded by the Adult Infectious Diseases Departments of the participating centers.

6.9. Timeline

Activities	Nov 2025	Dec 2025	Jan–Jun 2026	Jul 2026	Aug 2026
Literature review	✓				
Definition of research question and hypothesis	✓	✓			
Protocol writing		✓			
Submission to Ethics Committee		✓			
Database development			✓		
Data entry			✓	✓	
Statistical analysis				✓	
Final report of results					✓

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