transplantology

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A Retrospective Review of Calcineurin Inhibitors' Impact on Cytomegalovirus Infections in Lung Transplant Recipients

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Abstract: Immunosuppressive therapy reduces the risk for allograft rejection but leaves recipients susceptible to infections. Cytomegalovirus (CMV) is one of the most frequent causes for infection after transplantation and increases the risk for allograft rejection. As lung transplant recipients (LTRs) need to be under immunosuppression for life, they are a vulnerable group. To determine the potential association between the development of CMV infection and the calcineurin inhibitor (CNI) blood levels within previous 90 days, a retrospective review of LTRs was performed. Data from recipients who underwent a lung transplantation (LTx) at our center from January 2011 to December 2018 were collected. The studied recipients, after case/control matching, included 128 CMV-infection cases. The median time from the transplant to the first positive CMV viral load was 291.5 days. In our study, more patients were treated with tacrolimus (91.9%) than with cyclosporine (8.1%). Drug blood levels at selected timepoints showed no statistically significant difference between cases and controls. However, we found that CMV infection was more frequent in the donor-seropositive/recipient-seronegative group, interstitial lung disease (ILD) recipients, LTRs who underwent basiliximab induction, cyclosporine treated recipients, and LTRs with lymphopenia (at the time of CMV infection and 90 days before). In this review of LTRs, no association between the CNI blood level and CMV infection was seen, although other immunity-related factors were found to be influencing, i.e., basiliximab induction, cyclosporine treatment, and lymphopenia.

Keywords: immunosuppression; calcineurin inhibitors; transplantation; lung transplantation; therapeutic drug monitoring; cytomegalovirus infections; clinical pharmacology

Citation: Nogueiras-Álvarez, R.; Mora-Cuesta, V.M.; Cifrián Martínez, J.M.; de Cos Cossío, M.Á.; García Sáiz, M.d.M. A Retrospective Review of Calcineurin Inhibitors' Impact on Cytomegalovirus Infections in Lung Transplant Recipients. *Transplantology* **2021**, *2*, 478–490. https://doi.org/10.3390/transplantology2040045

Academic Editor: Pasquale Esposito

Received: 12 October 2021
Accepted: 27 November 2021
Published: 30 November 2021

1. Introduction

Lung transplantation (LTx) has become a well-established therapeutic option for patients with an end-stage pulmonary disease. Patients who undergo an LTx need to be under immunosuppression therapy for life, but this treatment, which is needed to reduce the risk of allograft rejection, involves risks.

1.1. Morbidity and Mortality after LTx

In addition to chronic lung allograft dysfunction (CLAD) which is the main cause for morbidity and the leading cause of death for those lung transplant recipients (LTRs) who survive beyond the first year, infections are another important cause for morbidity and mortality, especially in the first year after transplantation, reaching percentages of around 30% of the total identified causes of death at that time [1]. The most frequent infectious complication in LTRs is bacterial infections (43–63%) [2], followed by those caused by cytomegalovirus (CMV).

CMV, a member of the herpesvirus family, may produce itself an infection, and as it is an immunomodulatory virus, CMV increases the net state of immunosuppression of these patients facilitating the occurrence of opportunistic infections (such as bacterial and fungal infections) and increasing the risk for allograft rejection (a fact that is simultaneously facilitated by inflammation in allografts). CMV, hence, is a virus with an important role in the overall patient and lung allograft survival [3,4].

The data from the International Society for Heart and Lung Transplantation (ISHLT) Registry show there are very few deaths that could directly be related to CMV [1]; nevertheless, side effects from drugs used for the prophylaxis/treatment of CMV infection carry significant morbidity (mainly myelotoxicity and renal dysfunction).

After LTx, CMV viral load testing should be performed on a regular basis for monitoring CMV infection and disease [5].

1.2. Immunosuppression in LTx

Currently, according to the ISHLT Registry [1], the most used combination in LTRs is a triple immunosuppressive therapy, which usually includes a calcineurin inhibitor (CNI; tacrolimus is nowadays more used than cyclosporine), mycophenolate mofetil (or mycophenolic acid), and a corticosteroid. However, the ISHLT Registry also mentions that an induction therapy is frequently added in some centers.

There are different immunosuppression guidelines, but there is no study that is able to prove which of them is better to reduce the incidence of infection. The only facts that have already been proved are as follows: anti-lymphocyte antibodies increase the risk for CMV infection and protocols based on everolimus seem to reduce CMV infection when compared with mycophenolate mofetil [6].

1.3. Objectives

The aim of this study was to determine the potential association between the development of CMV infection and blood concentrations of the CNI within previous 90 days. The influence of other factors such as demographics, donor/recipient (D/R) CMV serostatus, basiliximab induction, and lymphocytes count were also analyzed.

2. Patients and Methods

2.1. Study Population

We initially examined a total number of 339 LTRs who received their lung allografts at the Marqués de Valdecilla University Hospital (HUMV) between 1 January 2011 and 31 December 2018.

The data were analyzed on a retrospective basis from the prospectively compiled LTx database of the center. The following variables relating to the recipients were recorded: gender, age at the transplantation moment, type of LTx, underlying lung disease, pre-transplant serology for CMV, D/R CMV serostatus, induction with basiliximab, and immunosuppressive regimen used. We also reviewed and recollected data from the first CMV-positive viral load (if any), immunosuppressant blood levels, and other analytical data from blood studies.

2.2. Definitions: CMV Infection versus CMV Disease

We studied those patients diagnosed of CMV infection, following the latest definitions published in 2016 [7]. It is important to distinguish between CMV infection, defined as the virus isolation or detection of viral antigens or nucleic acid in any body fluid or tissue specimen, and CMV disease, defined as the presence of appropriate clinical symptoms and/or signs together with the documentation of CMV in tissue from the relevant organ (by histopathology, virus isolation, rapid culture, immunohistochemistry, or DNA hybridization).

2.3. CMV Infection Diagnosis

The criteria for the diagnosis of CMV infection were the detection of CMV DNA by quantitative polymerase chain reaction (PCR). CMV infection data were retrieved from microbiology reports. CMV infection was measured by PCR cobas® CMV for use on the cobas® 6800/8800 Systems (Roche Diagnostics, Basel, Switzerland). The test was considered positive, when the assay reporting value was ≥34.5 copies/mL (1.54 log10) [8].

In cases of recipients with a positive CMV serology, the donor serotype was not collected, as the clinical management in these cases at our institution does not change depending on donor serotype, and it is represented in our text by "D(?)".

2.4. Immunosuppression Therapy and Drug Blood Level Measurement

At the immediate post-transplantation period, the LTRs in our study received a triple immunosuppressive therapy including a CNI (cyclosporine or tacrolimus), mycophenolic acid, and corticosteroids. The protocolary doses administered at the immediate post-transplantation period in our center are shown in Appendix A.

Basiliximab induction was routinely started to be used on 1 April 2016 (before that date, it was just employed in elderly patients or in cases of renal dysfunction or severe pulmonary hypertension). In those patients who received induction therapy, the first 20 mg dose of basiliximab was administered on the same day of the transplantation (just 2 h before declamping the first pulmonary artery), and the second dose was administered 96 h after declamping the first pulmonary artery.

Once the immediate post-transplantation period was overcome, the triple immunosuppression therapy must be continued. Drug dosification was then adjusted following the therapeutic drug monitoring (TDM) for tacrolimus and cyclosporine recommendations for LTRs; see Appendix A and Table A1.

The blood concentrations of the immunosuppressant were measured in a clinical pharmacology laboratory by chemiluminescent microparticle immunoassay in an ARCHITECT i-1000® platform (Abbot Diagnostics, Chicago, IL, USA).

2.5. CMV Prophylaxis in LTx

Until 2010, when the World Health Organization (WHO) published the international consensus guidelines on the management of cytomegalovirus in solid organ transplantation (SOT) [9], there was no international reference standard.

In our center, the prophylaxis in LTx is performed according to the recommendations from the Spanish Consensus document [10] written by the Spanish Transplantation Infection Study Group (GESITRA) of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC), which is based on the CMV serostatus (see Appendix A for more information).

2.6. Case/Control Selection

Those patients with the diagnosis of CMV infection determined by virus quantitative PCR were considered cases, and those who remained negative for CMV were regarded as controls.

According to the time from transplant to the first positive viral load quantification in each case, a control for an equivalent post-transplantation time interval was selected. To reduce the differences between cases and controls in the immunosuppressant and prophylaxis protocols, controls were chosen if their transplant dates were in the range of −365 to 365 days compared with the dates of the transplant from the cases.

We reviewed the immunosuppressant blood concentrations on the same day when the first positive viral load was detected and the last 3 levels prior to CMV-positive results (these three moments matched in time with the 30, 60, and 90 days before the first positive viral load quantification). In controls, the same data from the same timepoints were recorded.

2.7. Data/Statistical Analysis

Qualitative variables were expressed as absolute and relative frequencies (percentages); quantitative variables were summarized as means (with standard deviation) or medians (with the interquartile range), depending on their homogeneity.

The Student's t-test and the Mann–Whitney U-test were used to compare quantitative variables, depending on the distributions of the parameters. The chi-squared test or the Fisher's exact test in turn was applied in categorical variables. A probability value less than 0.05 was considered to be significant. A survival analysis was carried out, considering CMV infection as an event, and survival curves were plotted based on the Kaplan–Meier method.

3. Results

We initially reviewed a total number of 339 LTRs. We identified 128 recipients with a positive viral load for CMV (defined as "cases"). Although we tried to match each case with two controls, we could not achieve it due to difficulties to find two controls that matched in time (as previously explained).

Finally, after case/control matching, we studied 128 recipients (49.2%) with a positive viral load for CMV (defined as "cases") and 132 recipients (50.8%) that were selected as "controls" for them.

The studied LTRs (n = 260) included 165 male patients (63.5%) and 95 female patients (36.5%). Baseline demographic and clinical characteristics are shown in Table 1.

Table 1. Demographic features.

Characteristics	n = 260	Cases (n = 128)	Controls (n = 132)	p ^
Median (IQR) **age at the transplantation** (years)	57.64 (52.02–62.23)	58.07 (53.23–62.74)	56.93 (50.85–61.80)	0.2
Gender				
- Male	165 (63.5%)	85 (66.4%)	80 (60.6%)	0.2
- Female	95 (36.5%)	43 (33.6%)	52 (39.4%)	
Type of lung transplantation				
- Single-lung transplant	89 (34.2%)	46 (35.9%)	43 (32.6%)	0.330
- Double-lung transplant	171 (65.8%)	82 (64.1%)	89 (67.4%)	
Underlying lung disease (ULD)				
- Chronic obstructive pulmonary disease (COPD)	99 (38.1%)	43 (33.6%)	56 (42.4%)	**0.018**
- Interstitial lung disease (ILD)	123 (47.3%)	72 (56.2%)	51 (38.6%)	
- Bronchiectasis/cystic fibrosis (CF)	18 (6.9%)	3 (2.3%)	15 (11.4%)	
- Pulmonary arterial hypertension (PAH)	10 (3.8%)	5 (3.9%)	5 (3.8%)	
- Re-transplantation	3 (1.2%)	1 (1.6%)	1 (0.8%)	
- Others	7 (2.7%)	3 (2.3%)	4 (3.0%)	
Pretransplant serology for cytomegalovirus **(CMV)**				
- IgG (+)	213 (81.9%)	103 (80.5%)	110 (83.3%)	0.330
- IgG (−)	47 (18.1%)	25 (19.5%)	22 (16.7%)	
Basiliximab induction	95 (36.5%)	55 (43%)	40 (30.3%)	**0.023**
Immunosuppressant drug				
- Tacrolimus	239 (91.9%)	112 (87.5%)	127 (96.2%)	**0.009**
- Cyclosporine	21 (8.1%)	16 (12.5%)	5 (3.8%)	

^ The boldface type indicates differences that are statistically significant ($p < 0.05$). IQR: interquartile range.

The study included 239 patients treated with tacrolimus (91.9%) and 21 patients treated with cyclosporine (8.1%). Basiliximab induction was performed in 95 patients (55 cases and 40 controls).

There were 213 patients (81.9%) with a positive serology for CMV before transplantation.

The CMV serostatus information showed 213 D(?)/R(+) (81.9%), 39 D(+)/R(−) (15%), and 8 D(−)/R(−) (3.1%).

The median time from the transplant to the first positive CMV viral load was 291.50 (252.25 to 392.00) days.

No association between the immunosuppressant blood level and CMV infection was detected when analyzing the CNI blood concentration at the day when the first positive viral load was detected or within the previous 90 days (see Table 2).

Table 2. Immunosuppressant blood levels and the first positive CMV viral load [1].

Timepoints	Tacrolimus trough Blood Level (mcg/L)		*p*	Cyclosporine trough Blood Level (mcg/L) (C0)		*p*
	Cases	Controls		Cases	Controls	
Same date as the first positive CMV viral load	11.3 (9.8–13.55)	10.6 (9–12.92)	0.117	205.24 ± 66.37	181.64 ± 40.53	0.466
Previous blood test n°1 (30 days)	11.05 (9.5–12.8)	11 (8.97–12.97)	0.493	215.99 ± 91.39	271.16 ± 40.43	0.960
Previous blood test n°2 (60 days)	11.41 ± 3.22	11.36 ± 3.38	0.906	223.53 ± 92.53	213.26 ± 40.62	0.814
Previous blood test n°3 (90 days)	11.78 ± 3.2	11.41 ± 3.80	0.425	239.13 ± 94.05	211.68 ± 40.42	0.539

In our cohort, CMV infection developed more frequently in the D(+)/R(−) group, in which 25/39 (64.10%) developed CMV (p = 0.004) (see Table 3). We also found that CMV-infection-free survival was significantly different between groups. Figure 1 shows the Kaplan–Meier CMV-infection-free survival according to D/R CMV serostatus.

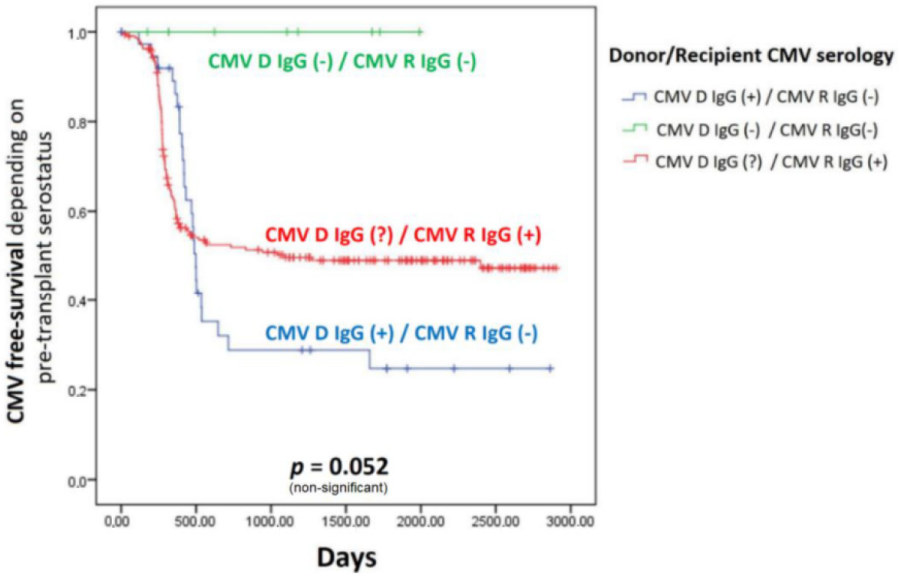

Figure 1. Donor/recipient CMV serology. The CMV serostatus of donor/recipient pairs influences the risk of CMV infection. The CMV-infection-free survival was shorter in the group of patients with CMV serostatus pairing between the donor and the recipient: D IgG (+)/R IgG (−) (p = 0.052, non-significant).

Table 3. CMV serostatus and detection of the first positive CMV viral load.

Characteristics	*n* = 260	Cases (*n* = 128)	Controls (*n* = 132)	*p* ^
CMV serostatus				
CMV D IgG (−)/CMV R IgG (−)	8 (3.1%)	0 (0%)	8 (6.1%)	**0.004**
CMV D IgG (+)/CMV R IgG (−)	39 (15%)	25 (19.5%)	14 (10.6%)	
CMV D IgG (?)/CMV R IgG (+)	213 (81.9%)	103 (80.5%)	110 (83.3%)	

^ The boldface type indicates differences that are statistically significant ($p < 0.05$).

There were included recipients with different underlying lung diseases that motivated LTx (see Table 1). According to underlying lung disease, CMV infection was more frequent between receptors with interstitial lung disease (ILD), as from the total number of cases (*n* = 128), there were 72 (56.2%) recipients with ILD.

We found more cases of CMV infection between patients who underwent basiliximab induction (Figure 2).

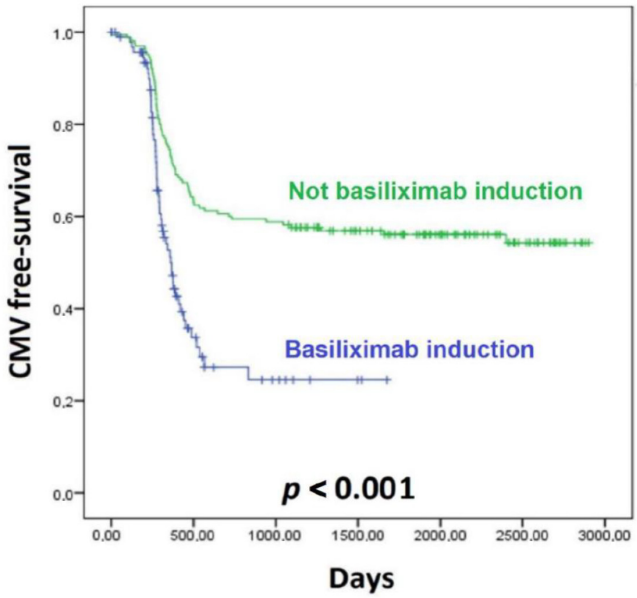

Figure 2. Basiliximab induction. The CMV infection rate was lower in the group of patients with basiliximab induction ($p < 0.001$).

The CMV infection rate was lower in the group of patients treated with tacrolimus as compared with that in the cyclosporine group ($p = 0.021$) (Figure 3).

Those recipients with lymphopenia showed a higher risk for CMV infection (Table 4).

Table 4. Lymphocyte count and detection of the first positive CMV viral load.

Timepoints	Absolute lymphocyte count (10^3/μL)		*p* ^	Lymphocyte percentage (%)		*p* ^
	Cases	Controls		Cases	Controls	
Same date as the first positive CMV viral load	1200 (900–1790)	1745 (1200–2392)	**<0.001**	23.03 ± 12.79	28.01 ± 12.3	**0.002**
Previous blood test n°1 (30 days)	1470 (1000–2000)	1600 (1200–2200)	**0.035**	21.5 (14.3–31)	27.05 (20.07–35.07)	**0.008**
Previous blood test n°2 (60 days)	1400 (1100–2000)	1600 (1200–2400)	**0.044**	24.19 ± 11.90	27.07 ± 11.44	**0.049**
Previous blood test n°3 (90 days)	1600 (1000–2100)	1600 (1187–2300)	0.155	24.01 ± 12.33	27.15 ± 11.44	**0.035**

^ The boldface type indicates differences that are statistically significant ($p < 0.05$).

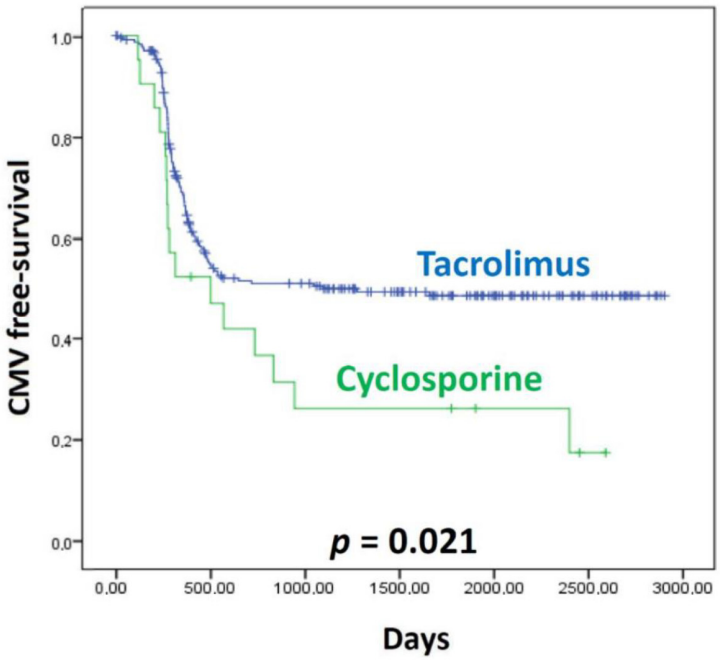

Figure 3. Immunosuppressant drug. The CMV infection rate was lower in the group of patients treated with tacrolimus (p = 0.021).

4. Discussion

To the best of our knowledge, this study is the first of our region to evaluate the association between the development of CMV infection and the CNI blood concentration in LTRs.

In order to prevent graft rejection in LTRs, as well as in other cases of SOT, patients should be pharmacologically immunosuppressed.

However, there are some differences when comparing LTRs with other SOTs: lungs are directly exposed to environmental microbes; after the surgery, there are some modifications that contribute to facilitate pulmonary infections (lung denervation results in impaired cough reflex, dysfunctional mucociliary clearance, and impaired lymphatic drainage), and immunosuppression therapy usually is more intense [11]. As a result, recipients are at high risk for infections [12].

The incidence of CMV infection (and disease), although it depends on the series, it is higher in LTRs (with an incidence of 54–92% in patients without CMV prophylaxis) [13] than other SOT recipients. This is partially explained, not only because they need an intense immunosuppression, but also because CMV has predilection for lung parenchyma [14].

The D/R CMV pre-transplant serostatus is the most important known risk factor associated with CMV infection. The greatest risk occurs, when donor is seropositive and recipient is seronegative (which means the recipient has no pre-existing CMV-specific immunity).

In our cohort, CMV infection developed more frequently in the D(+)/R(−) group, which is an expected finding, as previously mentioned, and agrees with the results of other SOT studies [15].

In the last years, with the widespread use of prophylaxis, the incidence of CMV infection (and disease) has decreased, while the median time to the onset of those CMV-related events has increased [16–18].

When we analyzed the recipients' underlying lung diseases, we found that ILD was the first cause for LTx and chronic obstructive pulmonary disease (COPD) was the second most frequent cause. These results agree with those of the ISHLT Registry (data from January 1995 to June 2018), where ILD is the most frequent cause for LTx in adults (20,192 recipients, 31.8% of the total number of LTRs), followed by COPD (19,152 recipients, 30.1% of the total

number of LTRs) and bronchiectasis/cystic fibrosis (CF; 11,388 recipients, 17.9% of the total number of LTRs) [19].

In our study, CMV infection was more frequent between receptors with underlying ILD. This relationship could be probably due to the fact that ILD was precisely the main reason for the transplantation in the recipients included in our study (however, we did not specifically explore by multivariate study whether ILD was an independent risk factor for developing CMV infection).

Nevertheless, in relation to that point, some recent publications have shown that many patients with ILD have an abnormal short telomere length (TL) [20]. This fact associates with premature lung aging and defects in adaptive immunity in humans and animal models [21,22].

In order to prove if these shortness in TL leads to an increase of CMV viremia among LTRs with ILD, the John Hopkins University and the University of Pittsburgh performed a study [23]. The results showed that LTRs with ILD were at increased risk for CMV relapsing viremia compared to age-matched LTRs without ILD (rate of relapse of LTRs with ILD: 69% vs. rate of relapse of LTRs without ILD: 31%; odds ratio (OR) = 4.98; 95% CI (confidence interval): 1.95–12.50; $p < 0.001$, Fisher's exact test).

Another important factor is the immunosuppressive therapy used.

Induction drugs such as rabbit anti-thymocyte globulin (rATG), alemtuzumab, or anti-interleukin-2 receptor antibodies (basiliximab and daclizumab) have become popular among SOT in the last years, as they seem to reduce acute rejection. Actually, up to 80% of LTx are currently performed under induction protocols, being basiliximab the preferred drug according to the ISHLT data [1]. Basiliximab is related with an improved long-term survival [24]. A study carried out in double LTx [25] showed that induction therapy (both basiliximab and alemtuzumab) appears to increase recipients' survival.

Although induction therapy has not proved to increase CMV infections, we found more cases of CMV infection among patients who underwent basiliximab induction. The Kaplan–Meier survival analysis also demonstrated a shorter CMV-infection-free survival in the basiliximab group (log-rank, $p < 0.001$) (Figure 2).

This is an unexpected result, as basiliximab leads to a decrease in circulating T-cells (by inhibiting lymphocyte proliferation) but does not cause T-cell depletion (versus alemtuzumab, which causes profound and prolonged T and B cell depletion; or rATG, which causes profound T lymphocyte depletion and has shown to increase the risk of infections, particularly CMV infection [26]). To try to explain this finding, we could point basiliximab's modulating effect on the immune system: although its half life is 7–9 days, the immunomodulatory effect seems to be longer. Due to this long-acting effect, basiliximab could improve long-term survival increasing also the risk for CMV infections (however, we do not know whether basiliximab could also increase the risk for other infections, as we did not register that information). In relation to this issue, further studies are needed to establish an association between basiliximab induction and the risk of developing CMV infection.

We found that cyclosporine was associated with more risk for CMV infection, but this could be due to a bigger number of patients taking tacrolimus ($n = 239$, 91.9%) than cyclosporine ($n = 21$, 8.1%). However, there is a Brazilian heart transplantation case–control study [27] with similar results: they found out that positive PCR correlated with higher doses of a CNI in a statistically significant way ($p = 0.002$), as did an elevated serum level of cyclosporine ($p = 0.004$) whereas elevated serum levels of tacrolimus had no such association ($p = 0.17$).

Although we did not register the patients undergoing everolimus therapy, this drug has been associated with less acute rejection and fewer CMV infections. This fact has been incorporated to the latest Spanish guidelines on the management of CMV infection in SOT recipients [10].

Although everolimus was first used in kidney transplantation [28,29], it has demonstrated to be useful in other SOTs (e.g., heart transplantation, where an advantage in the avoidance of allograft vasculopathy has been reported as well as a lesser incidence of CMV-

related events [30]). The reduction in CMV infections with everolimus (versus azathioprine or mycophenolate) [30–35] is independent of CMV prophylaxis and D/R serostatus [36].

In LTx, everolimus is related with a longer CMV-infection-free survival [6], and one of the indications for switching to everolimus as a maintenance immunosuppressor is repeated CMV infections after one year [37].

Among the other factors that we found to have an influence on the development of CMV infection was the total number of lymphocytes. In our study, lymphopenia was related with CMV infection. Absolute lymphopenia (especially CD4+ T cell), is related with a higher risk of opportunistic infections, including CMV, in immunocompromised patients [38]. This fact has already been reported in several types of SOT (p.e., renal transplant [39] and liver transplant [40]), where pre- or post-transplant lymphopenia has been associated with the development of CMV infection.

Some limitations to our study must be noted. As a retrospective study, it is subject to the presence of information biases, because it depends on the information contained in the patients' medical records. Although it is an unicentric study, we would like to point out that our hospital is a reference center for adult LTx in the North of Spain and performs this type of SOT in different kinds of underlying lung diseases. Basiliximab induction was routinely started to be used on 1 April 2016 (before that date, it was just employed in elderly patients or in cases of renal dysfunction or severe pulmonary hypertension). The number of patients undertaking cyclosporine was lower than those with tacrolimus, and this could have an influence on the different CMV infection rates observed between groups. With regard to the use of other immunosuppressive agents, such as mycophenolate mofetil or mTOR inhibitors (such as everolimus), as we focused on calcineurin inhibitors, we did not register the patients undergoing these other treatments, so we could not analyze their impact on our patient's CMV-infection-free survival. Another fact to point out is that other confounding variables that may affect the association between CMV infection and the CNI level was not included (such as renal function, episodes of acute rejection, or other infections).

5. Conclusions

In conclusion, in this review of 260 LTRs treated with a calcineurin inhibitor (tacrolimus/c yclosporine) and dose adjustment based on therapeutic drug monitoring (TDM), no association between the blood level and CMV infection was seen. We found that LTRs with ILD had more CMV infections. CMV infection developed more frequently in the donor-eropositive/recipient-seronegative (D(+)/R(–)) LTR group. Other factors related to immunity were found to influence CMV infection, such as lymphopenia and basiliximab induction.

Further studies are warranted to corroborate these findings.

Author Contributions: M.Á.d.C.C. and V.M.M.-C. conceptualized the study; R.N.-Á. and V.M.M.-C. wrote the manuscript and arranged the tables and figures; V.M.M.-C. analyzed the data; M.Á.d.C.C., V.M.M.-C., J.M.C.M. and M.d.M.G.S. reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki. No formal ethical approval was necessary owing to the retrospective, observational nature of this study.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

List of Non-Standard Abbreviations

CF	cystic fibrosis
CLAD	chronic lung allograft disfunction
CMV	cytomegalovirus
CNI	calcineurin inhibitor
COPD	chronic obstructive pulmonary disease
D	donor
ILD	interstitial lung disease
ISHLT	The International Society for Heart and Lung Transplantation
LTR	lung transplant recipient
LTx	lung transplant
PAH	pulmonary arterial hypertension
PCR	polymerase chain reaction
R	recipient
SOT	solid organ transplantation
TDM	therapeutic drug monitoring
TL	telomere length

Appendix A. Protocol Treatment for Lung Transplant Recipients Used in Our Center (Marqués de Valdecilla University Hospital) at the Immediate Post-Transplantation Period

Appendix A.1. Protocolary Doses of Maintenance Immunosuppression Therapy Used in Our Center (Marqués de Valdecilla University Hospital)

- The tacrolimus starting dose is 0.06 mg/kg by nasogastric tube, divided into two equal doses. If continuous intravenous infusion is needed, the dose is 0.01–0.05 mg/kg/day. After 48–72 h, doses are adjusted, after the first blood level report is performed by the Clinical Pharmacology Department. The tacrolimus dose is titrated to maintain a blood level of 10–15 mcg/L.
- The cyclosporine starting dose is 1–2 mg/kg/day by intravenous continuous infusion, as soon as the recipient is in hemodynamically stable conditions (which is usually 6 to 8 h after transplantation). After 48–72 h, doses are adjusted, after the first blood level report is performed by the Clinical Pharmacology Department. The dose of cyclosporine is titrated to maintain a trough (C0) level of 250–350 mcg/L and a 2-h post-dose (C2) level of 800–1000 mcg/L.
- Corticosteroids. An intraoperative 500 mg intravenous bolus of 6-methylprednisolone is administered just before declamping the pulmonary artery. Usually, on the first day at the Intensive Care Unit (ICU), the recipient receives 125 mg of 6-methylprednisolone every 8 h. Later on, the daily dosage is gradually decreased: 1 mg/kg/day (the first 7 days); after that 0.75 mg/kg/day; at the 14 day post-transplantation, a dose reduction is usually performed to 0.5 mg/kg/day.
- The mycophenolate mofetil starting dose is 1000 mg by intravenous infusion, twice daily (starting in the first 6 h after leaving the operating theatre). On the subsequent days, the same regimen of 1000 mg every 12 h is maintained. Switching to the oral route is recommended, as soon as the patient's oral tolerance is verified. The dosage can be modified in case of toxicity manifestations, such as the appearance of neutropenia. In case of poor digestive tolerance, an alternative is the use of mycophenolate sodium. The recommended dose in this case is 720 mg every 12 h, corresponding to 1 g mycophenolate mofetil twice a day and a daily dose of 2 g in terms of mycophenolic acid content.

Appendix A.2. Protocolary CMV Prophylaxis in Lung Transplantation Used in Our Center (Marqués de Valdecilla University Hospital) Based on CMV Serostatus

- In D(+)/R(−) recipients, prophylaxis consists in anti-CMV gammaglobulin (150 International Units (IU)/kg on day 0 and 100 IU/kg/day on days 2, 7, 14, 22, 35, 56,

and 77) plus intravenous ganciclovir (GCV) at 5 mg/kg each 12 h, starting on day 1 post-transplantation until the resumption of oral intake. Then, GCV is switched to valganciclovir (VGC) at 900 mg/day (dose adjusted to renal function following information from the safety data sheet) up to 12 months post-transplantation.

- In D(−)/R(+) and D(−)/R(−) recipients, prophylaxis consists in intravenous GCV following surgery until the resumption of oral intake. After that, GCV is also switched to VGC at 900 mg/day (dose adjusted to renal function following information from the safety data sheet) up to 6 months post-transplantation. Later, it could be withdrawn, and a weekly CMV viral load detection assay is performed during the next 6 weeks, due to the risk increase for the positive viral load in that time period.

Table A1. Target trough blood levels for calcineurin inhibitors in lung transplantation.

Tacrolimus			Cyclosporine		
1–6 months Post-LTx	**6–12 months Post-LTx**	**>12 months Post-LTx**	**1–6 months Post-LTx**	**6–12 months Post-LTx**	**>12 months Post-LTx**
10–15 mcg/L		8–10 mcg/L	C0: 250–350 mcg/L	C0: 200–250 mcg/L	C0: 125–250 mcg/L
			C2: 800–1000 mcg/L	C2: 600–800 mcg/L	C2: 400–600 mcg/L

C0, trough level; C2, 2 h post-dose level; post-LTx, post-lung-transplantation.

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