

Tailored combined cytomegalovirus management in lung transplantation: a retrospective analysis

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Abstract

Background: There is no univocal prophylactic regimen to prevent cytomegalovirus (CMV) infection/disease in lung transplantation (LT) recipients. The aim of this study is to evaluate short-term clinical outcomes of a tailored combined CMV management approach.

Methods: After 1-year follow up, 43 LT patients receiving combined CMV prophylaxis with antiviral agents and CMV-specific IgG were evaluated in a retrospective observational study. Systemic and lung viral infections were investigated by molecular methods on a total of 1134 whole blood and 167 bronchoalveolar lavage (BAL) and biopsy specimens. CMV immunity was assessed by ELISPOT assay. Clinical and therapeutic data were also evaluated.

Results: We found 2/167 cases of CMV pneumonia (1.2%), both in the donor-positive/recipient-positive (D⁺/R⁺) population, and 51/167 cases of CMV pulmonary infection (BAL positivity 30.5%). However, only 32/167 patients (19.1%) were treated due to their weak immunological response at CMV ELISPOT assay. Viremia $\geq 100,000$ copies/mL occurred in 33/1134 specimens (2.9%). Regarding CMV-serological matching (D/R), the D⁺/R⁻ population had more CMV viremia episodes ($p < 0.05$) and fewer viremia-free days ($p < 0.001$).

Conclusions: Compared to previous findings, our study shows a lower incidence of CMV pneumonia and viremia despite the presence of a substantial CMV load. In addition, our findings further confirm the D⁺/R⁻ group to be a high-risk population for CMV viremia. Overall, a good immunological response seems to protect patients from CMV viremia and pneumonia but not from CMV alveolar replication.

The reviews of this paper are available via the supplemental material section.

Keywords: CMV ELISPOT, CMV pulmonary infection, CMV viremia, cytomegalovirus, lung transplant, prophylaxis

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Introduction

Despite significant advances in prevention, cytomegalovirus (CMV) is one of the most significant opportunistic infections occurring after lung transplantation (LT).¹ It can be asymptomatic or manifest as CMV syndrome or tissue-invasive disease.^{2,3} The incidence of CMV infection and disease is highly variable among studies, ranging from 38% to 75% in the absence of any prophylaxis.⁴ CMV infection is associated with increased susceptibility to various infections, such as bronchiolitis

obliterans syndrome (BOS), increased risk of acute allograft rejection and diminished graft and patient survival.^{5–11} The increased risk of CMV disease and CMV-related mortality appears to be dependent on the CMV status of the donor (D) and/or recipient (R). In this regard, seropositive donor (D⁺) and seronegative recipient (R⁻) have the highest risk of developing these complications.^{12,13}

Universal prophylaxis involves the administration of antiviral medication to all patients or to a subset

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of at-risk patients.^{14,15} Randomized controlled trials and other prospective studies have proposed specific prophylaxis schemes for routine CMV management, with encouraging results in terms of incidence and severity of CMV disease. However, many questions remain unanswered, and a correct strategy to prevent CMV infection/disease in LT has yet to be defined.¹⁶

Even though most anti-CMV therapies rely on intravenous or oral ganciclovir administration, valganciclovir has recently become the first-line therapy due to its excellent oral bioavailability.^{17–21} Indeed, valganciclovir has proven to be far more effective in preventing CMV infection/disease when given on a long-term basis (i.e. 6–12 rather than ≤ 3 months).^{8,22,23} However, the high variability among LT centers has led to a lack of consensus.²⁴ In particular, a recent survey has revealed that the majority of LT centers administer CMV-specific hyperimmune globulin in combination with antiviral therapy solely in D⁺/R⁻ patients instead of using it as part of universal prophylaxis.²⁴

Recently, the quantification of the CMV-specific cellular immune response through T-cell ELISPOT has allowed more precise prediction of the individual risk of post-transplantation CMV disease and optimization of prophylaxis.¹⁵ However, only a few studies have assessed the CMV-specific response in LT recipients. In particular, one study has shown a significant correlation between low levels of CMV-specific T-cells and higher frequencies of infectious episodes.²⁵ Furthermore, others have proposed that an earlier recovery of the immune response may prevent and reduce the duration of CMV infection, avoiding the occurrence of overt disease or its recurrence.^{26–29}

The aim of our study was to assess the occurrence and outcomes of CMV infections among LT recipients receiving a combined universal CMV prophylaxis for 12 months post-transplant.

Patients and methods

Study population

All patients receiving LT over a 2-year period (from 1 January 2014 to 31 December 2015) at the Lung Transplant Centre of Turin (Città della Salute e della Scienza di Torino, Italy) were evaluated in a retrospective, observational cohort

study. The study was conducted in accordance with the STROBE (strengthening the reporting of observational studies in epidemiology) statement for observational studies.³⁰

Inclusion and exclusion criteria for study participants were those established by the International Society of Heart and Lung Transplantation (ISHLT) expert panel.³¹ This study was approved by our institutional review board (Protocol No. 0004577 – CS/416).

Variables

Patients' were classified into three different LT phases as follows:

- (1) *Pre-transplant*: age, underlying disease, smoking status, comorbidities and CMV serology.
- (2) *Transplant* (data collected during hospital stay for LT procedure): age, type of LT procedure, *ex-vivo* lung perfusion reconditioning (EVLP), number of in-hospital days, number of intensive care unit (ICU) days, radiological images on chest x-ray (CXR) and thorax CT scan, CMV D/R serostatus, CMV serology, CMV ELISPOT, CMV-DNA load in whole blood and bronchoalveolar lavage (BAL), CMV isolation from BAL³² and presence of CMV infection in transbronchial lung biopsies (TBLBs).
- (3) *Post-transplant follow up*: in accordance with Turin Lung Transplant Centre practices, the same data collected during hospital stay for TBLB procedures were evaluated at 4, 8 and 12 months post-LT. Moreover, data were collected and recorded between ambulatory evaluations (i.e. CMV-DNA load in whole blood and ongoing antiviral treatment). Each antiviral treatment was also recorded.

Definitions

CMV systemic or local infection/disease and proven and probable CMV pneumonia were diagnosed according to international guidelines.^{14,15} A diagnosis of asymptomatic pulmonary infection was made in the presence of viral inclusion bodies, also known as owl's eyes, or positive immunohistochemistry of TBLB and/or BAL specimens, together with parenchymal diffuse or perivascular inflammation or CMV-DNA viremia detected in whole blood. A CMV-DNA viral load of $\geq 10^4$ copies/ml in BAL specimens³³ or $\geq 10^5$

copies/ml in whole blood samples (conversion factor to UI/ml only for whole blood samples: 0.39)³⁴ was deemed significant.

The immune response against CMV was assessed by ELISPOT, as described elsewhere. Patients were classified as responders or nonresponders in the presence of ≥ 20 spot-forming units (SFUs) or < 20 SFUs, respectively.³⁵

Prophylaxis scheme

According to our center practices, the universal combined prophylaxis scheme consists of intravenous administration of ganciclovir, followed by oral valganciclovir at prophylactic dosage. During follow up, we administered oral valganciclovir at prophylactic dosage.^{36–41} The sequential administration scheme was as follows:

- acyclovir (400 mg) twice per day from post-operative day (POD) 5 to POD 14;
- intravenous ganciclovir (5 mg/kg) twice per day or valganciclovir (450 mg) twice per day from the POD 15 to POD 45;
- CMVIG (0.75 ml/kg) (Cytotect® Biotest 500 U with the following composition: IgG1 62%, IgG2 34%, IgG3 0.5%, IgG4 3.5%, IgA 5 mg) at PODs 1, 4, 8, 15 and 30 and then monthly at a dose of 0.5 ml/kg;
- acyclovir (400 mg) twice per day from POD 46.

Treatment of CMV disease, pneumonia, pulmonary infection and viremia was administered according to international guidelines and recommendations.¹⁴

ELISPOT evaluation played a crucial role in the decision to start the anti-CMV regimen, according to the following criteria:

- CMV pneumonia was always treated regardless of ELISPOT status.
- CMV pulmonary asymptomatic infection: no treatment in cases of patient responders with ≤ 10 CMV cells isolated from BAL or with $\leq 10,000$ CMV-DNA copies/ml BAL; treatment in cases of patient responders with increased number of CMV cells isolated from BAL or increased CMV-DNA copies in BAL with respect to a previous untreated infection; treatment in the absence of ELISPOT evaluation.

- CMV viremia: no treatment in responders with CMV-DNA viremia $\leq 100,000$ /ml (i.e. 39,000 UI/ml).

Immunosuppressive scheme

For induction of immunosuppression, we used antithymocyte globulins (Fresenius, Munich, Germany). The immunosuppressive regimen consisted of a triple-drug therapy with one calcineurin inhibitor (i.e. cyclosporine or tacrolimus), one antiproliferative agent (i.e. azathioprine, mycophenolate or everolimus) and corticosteroids. In cases of first asymptomatic pulmonary infection or CMV viremia, the immunosuppressive regimen was maintained. In cases of recurrence of CMV infections, we preferentially used everolimus due to its effects on CMV.^{39,40}

Statistical analysis

For statistical analysis, we used Chi-square test for categorical data and two-sided Student's *t* test and ANOVA for continuous variables. The Kaplan–Meier curves test was used to compare disease-free days among different patient groups. Statistical analysis was performed using Prism 7.0 (GraphPad, La Jolla, CA, USA).

Results

Baseline characteristics

Forty-three patients were included in this study. Demographic and clinical features are shown in Table 1. Considering all the different study phases, we collected a total of 167 BAL and 167 TBLB specimens and 1134 whole blood samples. Overall 1-year survival was 90.7%.

With regard to the humoral response against CMV, 33/43 (77%) patients exhibited IgG positivity with a mean titer of 215 AU/ml. In our cohort, 27/43 (63%) of patients were D⁺/R⁺, 9/43 (21%) D⁺/R⁻, 6/43 (14%) D⁻/R⁺ and 1/43 (2%) D⁻/R⁻ (Table 1).

CMV pneumonia

Overall, we recorded two cases of CMV pneumonia, accounting for an incidence of 1.2% (2/167) and a prevalence of 4.7% among patients (2/43). Both cases were D⁺/R⁺ patients.

Table 1. Patient baseline demographics and characteristics.

	Number of patients	Percentage
Total patients included	43	
Gender (female)	21	49%
Mean \pm SD age at transplant (years)	48.2 \pm 15.4	
Mean \pm SD length of ICU stay (days)	9.76 \pm 12.2	
Mean \pm SD length of in-hospital stay (days)	44.2 \pm 25.0	
Mean \pm SD survival (days)	365.5 \pm 29.8	
Transplant type		
Bilateral lung transplant	37	86%
Single lung transplant	4	10%
Heart/lung transplant	1	2%
Liver/lung transplant	1	2%
Indication for transplant		
IPF	8	19%
COPD	14	33%
CF	9	21%
Pulmonary arterial hypertension	3	7%
Lymphangiomyomatosis	2	4%
A1AT deficiency	2	4%
Other	5	12%
CMV serostatus		
D ⁺ /R ⁺	27	63%
D ⁺ /R ⁻	9	21%
D ⁻ /R ⁺	6	14%
D ⁻ /R ⁻	1	2%
R ⁺ mean \pm SD titer of IgG (AU/ml)	215 \pm 49.2	
CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; D ⁺ , seropositive donor; D ⁻ , seronegative donor; IPF, idiopathic pulmonary fibrosis; R ⁺ , seropositive recipient; R ⁻ , seronegative recipient; SD, standard deviation.		

CMV asymptomatic pulmonary infections

A total of 51/167 (30.5%) episodes of asymptomatic pulmonary infection were observed, occurring in 30/43 patients (69.8%, incidence 1.18 \pm 1.1 episodes/patient/year). The number of episodes in the first month of observation was the highest of

the study period, though not statistically significant (observation period, mean number of episodes \pm standard deviation (SD): first month, 0.42 \pm 0.50; fourth month 0.35 \pm 0.84; eighth month, 0.27 \pm 0.45; twelfth month, 0.16 \pm 0.37; $p > 0.05$). The median viral load in BAL

specimens was $270,794 \pm 21,057$ copies/ml (mean \pm SD). In the two pneumonia cases, BAL CMV-DNAs were 219,100 and 530,700 copies/ml. Most infections occurred during treatment with acyclovir (34/51, 66.6%), whereas 19.6% (10/51) and 13.7% (7/51) of infections arose during treatment with valganciclovir or in the absence of any antiviral treatment, respectively, albeit the difference was not statistically significant. Furthermore, no significant difference in terms of pulmonary infection occurrence was found among the four D/R serogroups. Lastly, even though the number of asymptomatic pulmonary infections was higher in D⁺/R⁺ patients (17/27, 63.0%), all 9 D⁺/R⁻ patients developed a primary infection, with at least one episode of infection occurring during the observation period (Table 2).

CMV viremia

Significant viremia was observed in 33/1134 specimens (2.9%, mean 0.76 ± 1.1 episodes/patient/year). Overall, 19/43 (44.2%) patients experienced at least one episode of significant viremia during follow up. As for asymptomatic pulmonary infections, the occurrence of significant viremia was higher in the first part of the follow up, albeit not statistically significant ($p > 0.05$). The majority of CMV viremia episodes occurred during acyclovir treatment compared to valganciclovir or no treatment (28/33, 85.0% *versus* 3/33, 9.1% or 2/33, 6.1%, respectively, $p < 0.001$). Of note, D⁺/R⁻ patients had a higher prevalence of significant viremia than D⁺/R⁺ patients (8/9, 88.8% *versus* 9/27, 33.3%, respectively). Furthermore, of all groups, the D⁺/R⁻ group had the highest risk of developing CMV viremia during the first year (mean prevalence 0.88 ± 0.3 patient/year and mean incidence 1.77 ± 1.1 episodes/patient/year, both with $p < 0.05$) and the lowest number of viremia-free days post-LT (51.3 ± 20.8 days, $p < 0.05$) (Table 2).

Treatment

All different clinical CMV manifestations were managed according to international guidelines as described in the Methods section. Both CMV pneumonia cases were treated with valganciclovir for at least 30 days. Only 62.7% of CMV asymptomatic pulmonary infections were treated (32/51), with a mean of $0.74 (\pm 0.9)$ of treatment/patient/year. All untreated patients had a good immunological response to CMV, as judged by

ELISPOT assay. Most cases of significant CMV viremia received treatment (32/33, 97.0%) (Table 2). Among the untreated cases of asymptomatic pulmonary infection, 31.6% had a confirmed infection at BAL/TBLB follow-up sampling (6/19). However, only two of these were treated due to increased CMV positivity in BAL isolates.

The mean treatment duration of patients with CMV viremia was higher than that of patients with asymptomatic pulmonary infection (mean number of days \pm SD: 43.3 ± 20.9 *versus* 33.5 ± 13.5 , respectively, $p < 0.05$). Seven out of 51 cases of pulmonary infection relapsed during the following 120 days, and one case of viremia relapsed despite being treated.

In our cohort, patients took valganciclovir 66 times, with only 6/66 (9%) discontinuing the treatment due to adverse drug reactions. In this regard, it should be pointed out that all these cases occurred after more than 30 days of continuous treatment. No cases of relapse occurred following the discontinuation of therapy. Therapy discontinuation in four patients was due to leukopenia, while in two others it was caused by renal dysfunction despite dosage reduction.

CMV ELISPOT assay

Changes in the immune response to CMV during the first year after LT were measured through CMV ELISPOT assay. Specifically, at 1 month post-LT we classified 22 patients as being responders (52.3%), whereas 13 were deemed nonresponders (30.9%). Unfortunately, in 8/42 (19%) cases, specimens were not suitable for ELISPOT assay due to low cell viability. All IgG seropositive patients were responders (22/22, 100%), while among nonresponders IgG seropositivity was generally lower (8/13, 61.5%). During the first month, 22% of responders suffered from pulmonary infection (including the two cases of pneumonia), whereas only one non-responder contracted this disease (1/8, 12.5%, $p > 0.05$).

All 22 responders maintained their CMV response status over the whole observation period. In contrast, only one nonresponder remained as such, while all the others became responders. Among those switching their status, at least one episode of pulmonary infection or viremia had occurred during the previous observation period.

Table 2. Episodes of CMV infections and D/R serostatus.

	D ⁺ /R ⁺	D ⁺ /R ⁻	D ⁻ /R ⁺	D ⁻ /R ⁻	Total	p value
Patients	27 (63%)	9 (21%)	6 (14%)	1 (2%)	43	
Survival (days)						
Mean	353.8 (±35)	365 (±0)	354.1 (±26)	365 (±0)	356.5 (±29)	ns
Median – IQR	365–0	365–0	365–0	365–0	365–0	
Pulmonary asymptomatic infections						
Patients	17/27 (63%)	9/9 (100%)	4/6 (66%)	0/1 (0%)	30/43 (69.8%)	
Mean/patient/year	0.62 (±0.5)	1.00 (±0.0)	0.66 (±0.5)	0	0.69 (±0.4)	ns
Median – IQR	1–1	1–0	1–1	0–0	1–1	
Episodes	32/167 (19%)	13/167 (7%)	6/167 (3%)	0/167 (0%)	51/167 (30.5%)	
Mean incidence/patient/year	1.18 (±1.1)	1.44 (±0.7)	1.00 (±0.9)	0	1.18 (±1.1)	ns
Median – IQR	1–2	1–1	1–2	0–0	1–2	
Treated pulmonary						
Asymptomatic infections	19/32 (59%)	10/13 (77%)	3/6 (50%)	0/0 (0%)	32/51 (62%)	
Mean treatment/patient/year	0.70 (±0.9)	1.1 (±1.0)	0.5 (±0.5)	0	0.74 (±0.9)	ns
Median – IQR	0–1	2–2	0–1	0–0	0–1	
CMV pneumonia						
Patients	2/27 (7%)	0/9 (0%)	0/6 (0%)	0/1 (0%)	2/43 (4.7%)	
Mean/patient/year	0.07 (±0.2)	0	0	0	0.04 (±0.2)	ns
Median – IQR	0–0	0–0	0–0	0–0	0–0	
Episodes	2/167 (1%)	0/167 (0%)	0/167 (0%)	0/167 (0%)	2/167 (1%)	ns
Mean incidence/patient/year	0.07 (±0.2)	0	0	0	0.04 (±0.2)	
Median – IQR	0–0	0–0	0–0	0–0	0–0	
Treated CMV pneumonia	2/2 (100%)	0 (0%)	0 (0%)	0 (0%)	2/2 (100%)	
Mean treatment/patient/year	0.07 (±0.2)	0	0	0	0.04 (±0.2)	ns
CMV viremia						
Patients	9/27 (33%)	8/9 (88%)	2/6 (33%)	0/1 (0%)	19/43 (44%)	
Mean/patient/year	0.33 (±0.4)	0.88 (±0.3)	0.33 (±0.5)	0	0.44 (±0.5)	0.018
Median – IQR	0–1	1–0	0–1	0–0	0–1	

(Continued)

Table 2. (Continued)

	D ⁺ /R ⁺	D ⁺ /R ⁻	D ⁻ /R ⁺	D ⁻ /R ⁻	Total	<i>p</i> value
Episodes	13 (39%)	16 (49%)	4 (12%)	0 (0%)	33/1134 (2.9%)	
Mean incidence/patient/year	0.48 (±0.8)	1.77 (±1.1)	0.66 (±1.2)	0	0.76 (±1.1)	0.009
Median – IQR	0–1	2–2	0–1	0–0	0–1	
Treated CMV viremia	12/13 (92%)	16/16 (100%)	4/4 (100%)	0/0 (0%)	32/33 (97%)	
Mean treatment/patient/year	0.44 (±0.7)	1.77 (±1.1)	0.66 (±1.2)	0	0.74 (±1.02)	0.004
Median – IQR	0–1	2–2	0–1	0–0	0–1	
Viremia-free days	164.1 (±140)	51.3 (±20.8)	177.8 (±166)	365	147.1 (±137)	0.048

Data are presented as number (percentage), means (± standard deviations), medians and interquartile ranges (IQR). Significant variable was marked with bold numbers.
D⁺, seropositive donor; D⁻, seronegative donor; ns, not significant; R⁺, seropositive recipient; R⁻, seronegative recipient.

Table 3. CMV ELISPOT assay among patients.

	Responders	Nonresponders	OR, 95% CI	<i>p</i> value
Number of patients (%)	22 (52.3%)	13 (30.9%)		
Pulmonary infections episodes/episodes (%)	42/120 (35%)	3/17 (17.6%)	2.51, 0.76–8.55	>0.05
Significant viremia episodes/episodes (%)	16/88 (18.2%)	7/15 (46.7%)	0.25, 0.08–0.76	<0.05

Percentages of patients refers to all patients included in the study; data of patients without a valid ELISPOT assay are not represented in this table.
CI, confidence interval; OR, odds ratio.

Responders experienced 42 episodes of pulmonary infections (42/120, 35.0%), while we observed only three cases among nonresponders (3/17, 17.6%). The presence of CMV response at ELISPOT assay did not correlate with protection from asymptomatic CMV pulmonary infection (OR 2.51, 95% CI 0.76–8.55, $p > 0.05$). Furthermore, responders had fewer significant viremia episodes than nonresponders (16/88, 18.2% *versus* 7/15, 46.7% respectively, $p < 0.05$), and they seemed to be protected against CMV viremia in the presence of a CMV response (OR 0.25, 95% CI 0.08–0.76, $p < 0.05$) (Table 3).

Discussion

Cytomegalovirus infection can have negative health consequences for LT recipients, for whom in the past decades a number of prophylactic schemes based on valganciclovir have been developed.^{42–44}

In addition to valganciclovir, current prophylactic regimens include ganciclovir and CMV hyperimmune globulin given for different lengths of time, with variable efficacy rates against CMV infection and disease.^{14,24}

In this study, we have evaluated the results of a universal combined prophylaxis scheme for CMV in LT patients. In our cohort, incidence of CMV pneumonia, infection and asymptomatic viremia were similar to or even lower than those recorded in studies addressing longer courses of antiviral drugs, but with our protocol we administered a much lower drug load.²³ We also observed fewer ganciclovir/valganciclovir therapy discontinuation episodes due to adverse drug effects. D⁺/R⁺ patients had a higher incidence of pulmonary infection, albeit not significant, whereas the D⁺/R⁻ population displayed a higher risk of developing significant CMV viremia.

High-titer CMV IgG provided a passive CMV-specific immunity and seemed to play an important role in immunomodulation of specific responses, showing an antiviral effect similar to that of antivirals.⁴⁵ Although the use of these preparations seems to be effective in reducing CMV pneumonia and to have some effect on acute rejection, the high variability in dosages and administration schedules has undoubtedly contributed to downplaying their evidence-based effectiveness despite the existence of many single-center or anecdotal studies.^{36,37}

The main point of discussion of our results are the following

CMV pneumonia

The incidence of CMV disease and pneumonia is highly variable among studies, ranging from 4% to 32%, and it is related to the duration of prophylaxis.^{13,23} Other LT centers adopted schemes with different treatment durations, depending on the D/R status.¹³ In Schoeppler and colleagues' study,⁴⁶ the authors reported an almost doubled risk of CMV disease in D⁺/R⁻ versus D⁺/R⁺ recipients (19.5% versus 10.7%, respectively). In our scheme, given a lower incidence of CMV pneumonia reported in previous studies,^{38,39} CMV-specific hyperimmune globulins were administered regardless of D/R status. Fittingly, our incidence of CMV pneumonia was 1% on follow-up BAL and TBLB specimens, which was even lower than that reported in the literature despite the shorter antiviral regimen. Both of our pneumonia cases were among the most represented D⁺/R⁺ population and occurred within 30 days of transplant. Even though the D⁺/R⁺ population has been shown to have a lower risk of CMV disease and pneumonia compared to the D⁺/R⁻ one, it is likely that the strong immunosuppressive regimen in the first post-LT month might have exposed the patients to a higher risk, regardless of their CMV serostatus.

CMV pulmonary infections

In the literature, the incidence of pulmonary infection is highly variable, depending on which of the various definitions is being adopted. In our BAL specimens we found a 30% incidence of pulmonary infection, which is higher than that reported in other studies. In particular, we show that 69% of these patients experienced at least one episode during the first year, recorded over five follow-up BAL samplings per patient. The

D⁺/R⁺ group, the most represented population in our study, had the highest number of asymptomatic infections. On the other hand, D⁺/R⁻ patients showed the highest prevalence (100%) among all groups, which makes this population the 'at-risk population'. These findings differ from those reported by previous studies, where the incidence of asymptomatic infection was significantly lower, especially in the presence of antiviral prophylaxis.^{11,23} Conversely, the incidence increased (ranging from 10% to 64%) when a shorter prophylaxis course was carried out,^{11,23} and the survival rate was lower compared to those patients who never experienced a CMV infection (55% versus 84%).¹¹ In our study, D⁺/R⁻ patients had a higher incidence of asymptomatic pulmonary infection, albeit not statistically significant, but none of them exhibited invasive disease (e.g. CMV pneumonia), indicating the appropriateness and effectiveness of the prophylactic strategy. Incidence was higher during the first month after LT, slowly decreasing during the subsequent months. This phenomenon might be in part due to the immunosuppressive load received by the patient during the first period.

CMV viremia

From our data, the incidence of CMV viremia is considerably lower than that of pulmonary infection, as the CMV monitoring strategy is expressly aimed at limiting the infection to the alveolar environment, the natural replication domain of the virus, thereby preventing the development of systemic (blood) disease. Although we found a lower number of cases of CMV viremia compared to those of pulmonary infection, 44% of patients of our population experienced at least one episode of significant CMV viremia, whereas only 30% of patients had asymptomatic pulmonary infection. Once again, the incidence reported in the literature is lower, ranging from 21%, in the case of 6–12 months of prophylaxis,¹¹ to 12% in the case of indefinite prophylaxis,¹⁶ even though the CMV-DNA copy threshold is variable among studies. Nevertheless, our data confirm the D⁺/R⁻ population to be at higher risk of developing CMV viremia during the first year, with a lower number of viremia-free days.^{11,16}

Antiviral treatment

In our cohort, all patients with CMV pneumonia received antiviral treatment, whereas only

62% of cases with asymptomatic CMV pulmonary infection were treated. The therapeutic management was based on the recipient's serostatus, immunological response at ELISPOT assay, ongoing prophylactic treatment and previous episodes of CMV infection. If we only take into account the treated patients, because of an unfavorable balance load/immunity, the incidence of asymptomatic CMV pulmonary infections was 19% (32/167), in good agreement with previous studies assessing LT recipients treated with longer ganciclovir/valganciclovir prophylactic regimens.^{11,16,23} Due to the shorter duration of the ganciclovir/valganciclovir prophylactic scheme, the discontinuation rate in our cohort was only 9%, which was significantly lower than what has been previously reported.¹⁶ Moreover, 19% of our patients had asymptomatic pulmonary infection during the first (and unique) month of valganciclovir therapy, confirming the relative inefficacy of valganciclovir prophylaxis in preventing alveolar viral replication.

CMV ELISPOT in clinical practice

The CMV ELISPOT assay can predict the protection from CMV disease and viremia by estimating T-cell responsiveness,^{33,34,47,48} thus, the evaluation of the CMV-specific T-cell response by ELISPOT has played a key role in our tailored approach. Indeed, the detection of an immunological response determined our decision to monitor CMV replication.

ELISPOT assay quantifies T-cells producing IFN- γ in response to CMV, albeit not distinguishing the CD4⁺ T-cell response from that of CD8⁺ T-cells.¹⁴ High dosages of immunosuppressive drugs administered after LT can result in dysfunction and progressive loss of the CD4⁺-specific response to CMV,⁴⁹ which in turn favors the recurrence of infections and clinical manifestations of CMV replication.⁴⁹ This appears to be critical during the first months after LT when the susceptibility to CMV infections is higher and the immune response can be measured. Our study showing two cases of pneumonia occurring during the first month after LT in two D⁺/R⁺ responders (notably low-risk patients) further corroborates the higher susceptibility to CMV infections soon after LT. Consistently, after this critical period we did not record any CMV pneumonia episodes in our cohort.

Remarkably, all responders at ELISPOT assay maintained their status throughout the whole observation period. Most nonresponders (12/13, 92.3%) switched to a responder status because of either immunosuppression modulation or, in case of the R⁻ group, new infection. In this regard it is important to mention that constant circulating levels of CMV-DNA are necessary for the correct stimulation of an immunological response. Thus, immediate antiviral therapy, knocking down CMV-DNA circulating levels, would hamper the CD4⁺-specific response, explaining the higher incidence of infective episodes and viral reactivations in nonresponders.^{34,36} In our cohort, the presence of a CMV-specific immune response appeared to protect patients from significant CMV viremia but not from CMV asymptomatic pulmonary infections in natural CMV replication sites.

In conclusion, our data give rise to some reasonable clinical interpretations. First and foremost, our 'low drug load combined prophylaxis' resulted in a low number of CMV pneumonia cases and treatments for both asymptomatic CMV pulmonary infections and significant viremia. In addition, our prophylaxis allows the development of natural immunity thanks to a low continuous exposure to CMV, while simultaneously preventing CMV invasive disease. Lastly, ELISPOT CMV assay is an essential tool for making the correct therapeutic choice against CMV infection, especially when dealing with an antiviral approach, given that positivity is related to lower CMV blood replication.

Further randomized studies exploring different prophylactic schemes and alternative DNA cut-off levels for preemptive therapy are clearly needed to evaluate the best balance between CMV infection and treatment costs, in terms of clinical outcome, drug-related side effects and economical load.

Author contributions

SP and PF share first authorship. Design of the study: PF, SP, LD, CC, BM, RM, CR; acquisition of data: PF, VG, CA, SE; interpretation of data: PF, SP, LD, CC, BM, RM; drafting the manuscript: PF, SP, CC, CA; critical revision of the manuscript: PF, SP, LD, SF, CC, BM, RM, CR.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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Supplemental material

The reviews of this paper are available via the supplemental material section.


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