Incidence of post-transplant cytomegalovirus viremia in patients receiving lungs after ex vivo lung perfusion

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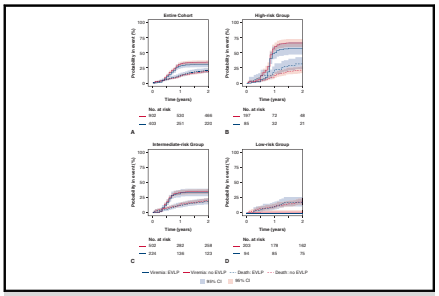
ABSTRACT

Objectives: Cytomegalovirus infection after lung transplant is associated with increased morbidity and mortality. Inflammation, infection, and longer ischemic times are important risk factors for cytomegalovirus infection. Ex vivo lung perfusion has helped to successfully increase the use of high-risk donors over the last decade. However, the impact of ex vivo lung perfusion on post-transplant cytomegalovirus infection is unknown.

Methods: We performed a retrospective analysis of all adult lung transplant recipients from 2010 to 2020. The primary end point was comparison of cytomegalovirus viremia between patients who received ex vivo lung perfusion donor lungs and patients who received non–ex vivo lung perfusion donor lungs. Cytomegalovirus viremia was defined as cytomegalovirus viral load greater than 1000 IU/mL within 2 years post-transplant. Secondary end points were the time from lung transplant to cytomegalovirus viremia, peak cytomegalovirus viral load, and survival. Outcomes were also compared between the different donor recipient cytomegalovirus serostatus matching groups.

Results: Included were 902 recipients of non–ex vivo lung perfusion lungs and 403 recipients of ex vivo lung perfusion lungs. There was no significant difference in the distribution of the cytomegalovirus serostatus matching groups. A total of 34.6% of patients in the non–ex vivo lung perfusion group developed cytomegalovirus viremia, as did 30.8% in the ex vivo lung perfusion group ($P = .17$). There was no difference in time to viremia, peak viral loads, or survival when comparing both groups. Likewise, all outcomes were comparable in the non–ex vivo lung perfusion and ex vivo lung perfusion groups within each serostatus matching group.

Conclusions: The practice of using more injured donor organs via ex vivo lung perfusion has not affected cytomegalovirus viremia rates and severity in lung transplant recipients in our center. (JTCVS Open 2023;14:590-601)



Cumulative incidence of CMV after LTx.

CENTRAL MESSAGE

The practice of using high-risk donor lungs via EVLP has not affected CMV viremia rates and severity in lung transplant recipients.

PERSPECTIVE

Ex vivo lung perfusion has enabled the assessment and transplantation of higher risk donor lungs without compromising long-term outcomes and CMV viremia rates in transplant recipients. However, our study confirms that rates of viremia remain high, which highlights the importance of developing novel strategies to avoid this important infection in transplant recipients.

Human cytomegalovirus (CMV) is the leading cause of viral infectious complications after solid-organ transplantation.[1] CMV viremia in solid organ transplant recipients is most commonly acquired via reactivation of the latent virus from the seropositive donor or seropositive recipient. Primary infection after transplantation also can occur, but it is less common. The development of CMV is associated with an important impact on patient outcomes, including direct and indirect effects of the virus. The direct effects can manifest as CMV syndrome (fever, leukopenia, thrombocytopenia) or, more important, as tissue invasive viral disease, with end-organ damage such as enteritis, pneumonitis,

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Abbreviations and Acronyms

CLAD = chronic lung allograft dysfunction
CMV = cytomegalovirus
D− = cytomegalovirus seronegative donor
D+ = cytomegalovirus seropositive donor
DCD = donor after cardiac death
EVLP = ex vivo lung perfusion
FiO_2 = fraction of inspired oxygen
IL = interleukin
IQR = interquartile range
LTx = lung transplantation
PCR = polymerase chain reaction
R− = cytomegalovirus seronegative recipient
R+ = cytomegalovirus seropositive recipient

colitis, and hepatitis.[2] Indirectly, CMV viremia has been associated with allograft rejection and added immunosuppression, resulting in increased risk of bacterial and fungal infections.[3,4]

Approximately 70% of adults are latently infected with CMV, which makes their exclusion from the organ donation pool unfeasible.[5] As a result of CMV positivity in the recipient or donor, CMV infection poses a substantial burden on post-transplant care for many lung transplant recipients. In fact, studies have shown that CMV infection can occur in 30% to 86% of lung transplant recipients and can increase the costs of care by approximately 50%.[6] CMV disease after lung transplantation (LTx) has been associated with chronic lung allograft dysfunction (CLAD) and has a significant impact on survival when compared with patients who do not develop CMV disease.[7] Most of the pathogenesis associated with CMV infection is due to the virus's ability to establish lifelong latency infection within the host, which cannot be targeted or cleared by current antiviral therapies.[8]

To meet the growing demand for LTx procedures and to decrease waitlist mortality, normothermic ex vivo lung perfusion (EVLP) has allowed for the increased use of lungs from high-risk extended criteria donors across the world over the last decade.[9] EVLP is a well-established clinical method of donor lung assessment and reconditioning before transplantation, which attempts to simulate an in vivo environment by ventilating and perfusing the donor allograft at 37 °C ex vivo. High-risk donor lungs are defined by specific criteria, including PaO_2:fraction of inspired oxygen (FiO_2) ratio less than 300 mm Hg, smoking history, pulmonary edema, bronchopulmonary secretions, and concerning chest radiographic findings.[9] Long-term outcomes of LTx recipients of lungs treated with EVLP in our institution show comparable results to those recipients of donor lungs without EVLP.[10] The use of these higher-risk donors has increased transplant activity significantly without compromising outcomes. However, inflammation and tissue damage that are associated with extended criteria donors may be an additional trigger of CMV reactivation from latency into a lytic and infectious state.[11-14]

This raises the question of whether the increased inflammation that may be associated with the more damaged high-risk donor lungs used in EVLP leads to greater CMV reactivation compared with non-EVLP lungs. In addition, it is conceivable that the process of ex vivo perfusion itself may have unforeseen effects upon CMV reactivation. Thus, the objective of this study was to compare CMV viremia outcomes between transplant recipients of EVLP and non-EVLP lungs.

MATERIALS AND METHODS

Population

This is a single-center retrospective cohort study. The study cohort consists of all adult patients who underwent LTx between October 1, 2010, and January 31, 2020 at our institution, Toronto General Hospital. Pediatric patients and those who underwent retransplantation were excluded from the analysis. This study was approved by the institutional research ethics board (#20-5597, approved on November 22, 2020), and informed consent was waived because of the retrospective nature of this study.

All patients included in the study received similar immunosuppression therapy as per protocol, which consists of triple-drug therapy with a calcineurin inhibitor (cyclosporine or tacrolimus), cell-cycle inhibitor (azathioprine, mycophenolate mofetil, or mycophenolate acid), and prednisone. Our institutional CMV prophylaxis protocol for recipients who are CMV-positive or received CMV-positive lungs is ganciclovir 5 mg/kg intravenously daily until patients can tolerate enteral or oral therapy, at which point they are switched to valganciclovir 900 mg enterally once daily. Cytomegalovirus seronegative donor (D−)/cytomegalovirus seronegative recipient (R−) patients receive no prophylaxis. Duration of prophylaxis depends on risk of CMV infection, and doses are adjusted to renal function. Prophylaxis duration changed in our center during the study period. For cytomegalovirus seropositive donor (D+)/cytomegalovirus seronegative recipient (R−) patients, the prophylaxis duration was 6 months until May 2015, after which it was increased to 9 months. For D+ cytomegalovirus seropositive recipient (R+) and D− R+ patients, the prophylaxis duration was 3 months until May 2015, and it was increased to 6 months thereafter.

Ex Vivo Lung Perfusion Indication and Protocol

Donor and EVLP selection criteria were as per our standard clinical practice, and have been reviewed.[10] After the donor lung is retrieved, the decision whether the allograft meets standard criteria for transplantation or needs to undergo further EVLP assessment is made at the discretion of the surgeon on call. In general, high-risk donor lungs (PaO_2:FiO_2 ratio <300 mm Hg, pulmonary edema, high suspicion for aspiration and pneumonia, donor after cardiac death [DCD] donors taking more than 60 minutes to arrest, uncontrolled DCD) require EVLP assessment for a final decision.

For the evaluation of these high-risk donor lungs, the Toronto EVLP technique was used.[9] Cannulation of the left atrium and main pulmonary artery is performed, and an endotracheal tube is placed. The lungs are connected to the EVLP circuit through the cannulas, and over the first hour of perfusion, normothermia is slowly achieved with a targeted flow of 40% of donor predicted cardiac output. Protective ventilation is applied during the perfusion (7 mL/kg of donor ideal body weight). Hourly lung function evaluation is performed measuring PaO_2:FiO_2 ratio, pulmonary artery pressure, peak airway pressures, and lung compliances. Lung

x-rays are performed at the first and third hours of perfusion to help guide the surgeon's final decision. General acceptance criteria are PaO_2:FiO_2 ratio greater than 400 mm Hg and stable or improved lung function.

Cytomegalovirus Monitoring Protocol and Definitions

Polymerase chain reaction (PCR)-based CMV testing was adopted at our center as of October 1, 2010, which is why we chose 2010 as the start of the study period. Two different plasma CMV-PCR assays were used during the study period. Before March 2012, CMV PCR kit 1.0 from Astra Diagnostics (Berlin, Germany) was used and results reported in copies/mL. After March 2012, reports changed to IU/mL because the assay used was Roche (Basel, Switzerland) Cobas AmpliPrep TaqMan CMV assay. To standardize the analysis, we converted all CMV-PCR reports to IU/mL by multiplying copies/mL results by 0.9 as per our laboratory recommendation.

Blood CMV testing was done according to our lung transplant program protocol. After prophylaxis cessation, D+ R− patients are screened weekly by PCR for 3 months, and D+ R+ and D− R+ patients are screened every 2 weeks for 3 months. CMV viremia was defined as a CMV viral load greater than or equal to 1000 IU/mL at any time up to 2 years post-LTx, regardless of symptoms.

Ex Vivo Lung Perfusion Perfusate Protein Measurement

Frozen clinical perfusate samples from CMV seropositive donors included in this cohort were thawed overnight at 4 °C and spun down at 4 °C, 3000*g* for 4 minutes as per manufacturer's protocol. The samples were run on MAGPIX multiplex cytokine assay (Milliplex, Millipore-Sigma, Canada) by EVE Technologies Corporation (Calgary, Canada). Proinflammatory markers associated with lung injury such as interleukin (IL)-6, IL-8, and IL-1β were measured. All standards and samples were run in duplicate and are reported as pg/mL after adjusting for the effects of perfusate volume exchanges during EVLP.

Study End Points

The primary end point was cumulative incidence of CMV viremia over time, up to 2 years post-transplant, in recipients who received EVLP donor lungs compared with recipients of non-EVLP lungs. Competing risk for CMV viremia included death from any cause. For secondary end points, we analyzed peak CMV viral load within the first 2 years post-transplant in both groups, and overall survival and survival specifically of those patients who developed CMV viremia post-transplant. All outcomes were also assessed separately in the different donor recipient CMV serostatus matching groups.

Statistical Analyses

Continuous variables are presented as mean ± standard deviations or median and interquartile range (IQR) and were analyzed by the Student *t* test or Mann–Whitney *U* test as appropriate. Categorical variables are presented as absolute frequency with percentage. The chi-square test and Fisher exact test were used.

The difference in time to event was compared between EVLP and non-EVLP recipients using a competing risk model. With death as the competing risk, the cumulative incidence of CMV viremia over time was visualized using Kaplan–Meier curves and compared between the groups by using a Cox proportional hazard model. The analysis started from the date of transplant to 2 years post-transplant. The cumulative incidence of CMV viremia at different time points (ie, 6 months, 1 year, and 2 years) was compared using log-rank test. Additionally, the 2 different eras of prophylaxis duration were considered for the study end points, and a separate subanalysis was performed to exclude bias related to different prophylactic regimens. The Mann–Whitney *U* test was used to compare cytokine levels during EVLP of patients who become viremic and those who do not after transplantation.

The Kaplan–Meier method and log-rank test were used to calculate survival probabilities during the study period and compare survival curves between the groups, respectively. GraphPad (Boston, Mass) Prism software version 9.3.1 and R version 4.1.0 were used to perform the analyses.

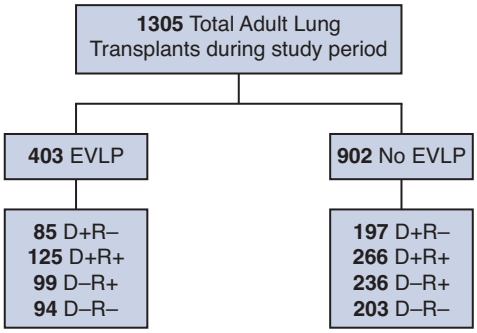

FIGURE 1. Study flow chart. Of a total of 1338 lung transplant recipients, 33 met exclusion criteria. Of the 1305 patients included in the study, 403 were in the EVLP group and 902 were in the non-EVLP group. *EVLP*, Ex vivo lung perfusion; *D+*, seropositive donor; *R−*, seronegative recipient; *R+*, seropositive recipient; *D−*, seronegative donor.

RESULTS

Recipient and Donor Characteristics

A total of 1305 patients were included in this study, of whom 902 were recipients of non-EVLP lungs and 403 were recipients of EVLP lungs (Figure 1). Compared with the non-EVLP group, the EVLP group had a significantly higher percentage of DCD donors (48% vs 10%, $P < .0001$), lower PaO_2:FiO_2 ratio at time of retrieval (mean of 357 mm Hg vs 430 mm Hg, $P < .0001$), higher proportion of donors with positive smoking history (62% vs 51%, $P = .0004$), and longer total preservation time (median, 961 minutes [IQR, 874-1097] vs 535 [444-632], $P < .0001$). Table 1 shows donor and recipient baseline characteristics. Single-lung transplants were more frequent in recipients in the EVLP group when compared with non-EVLP (28.2% vs 10.9%, $P < .0001$). There was also a significant difference in age and sex between EVLP and non-EVLP lung recipients. Of note, there was no difference in the distribution of CMV serostatus matching groups between the study groups.

Cumulative Incidence of Cytomegalovirus Viremia

Within the entire cohort, 33.4% (n = 436) developed CMV viremia post-transplant (CMV viral load ≥1000 IU/mL). The 2-year cumulative incidence of CMV viremia did not differ between the EVLP and non-EVLP groups and was also similar when stratifying the patients by the different CMV serostatus matching groups (Figure 2). In a univariable competing risk model analyses where CMV viremia was set as outcome of interest and death as a competing risk event,

TABLE 1. Donor and recipient baseline characteristics

	No EVLP (n = 902)	EVLP (n = 403)	*P* value
Donor characteristics			
Age, median [IQR], y	50 [32-62]	47 [32-56]	.002
Male sex	474 (52.5)	262 (65.0)	<.0001
Donor type (%)			
DBD	814 (90.2)	210 (52.1)	<.0001
DCD	88 (9.8)	193 (47.9)	
Preprocurement PaO2:FiO2 ratio, mean (SD), mm Hg	430 (96)	357 (106)	<.0001
Smoking history (%)	430 (51)	235 (62)	.0004
Total preservation time, median [IQR], min*	534 [444-632]	961 [873-1068]	<.0001
Recipient characteristics			
Age, median [IQR], y	58 [45-65]	60 [50-66]	.001
Male sex (%)	478 (53.0)	257 (63.8)	.001
Diagnosis (%)			
COPD	249 (27.6)	122 (30.3)	.4
CF	134 (14.9)	38 (9.4)	
ILD	422 (46.8)	206 (51.1)	
Pulmonary hypertension	32 (3.5)	13 (3.2)	
Other	27 (3.0)	4 (1.0)	
Retransplant	38 (4.2)	20 (5.0)	
CMV serostatus (%)			
D+ R−	197 (21.8)	85 (21.1)	.7
D+ R+	266 (29.5)	125 (31.0)	
D− R+	236 (26.2)	99 (24.6)	
D− R−	203 (22.5)	94 (23.3)	
Type of transplant (%)			
Single	99 (11.0)	114 (28.3)	<.0001
Double	803 (89.0)	289 (71.7)	

EVLP, Ex vivo lung perfusion; *IQR*, interquartile range; *DBD*, donation after brain death; *DCD*, donation after cardiac death; *SD*, standard deviation; *COPD*, chronic obstructive pulmonary disease; *CF*, cystic fibrosis; *ILD*, interstitial lung disease; *CMV*, cytomegalovirus. *Cold ischemic time 1 + EVLP time + cold ischemic time 2 + implantation time.

there was no difference when comparing EVLP and non-EVLP recipients (Table 2).

The median time from LTx to CMV viremia was 235 days (IQR, 179-319) in the non-EVLP group and 249 days (IQR, 186-313) in the EVLP group ($P = .65$). Cumulative incidence of CMV between the EVLP and non-EVLP groups was 6.9% versus 9.1% at 6 months, 27.8% versus 34.4% at 1 year, and 30.8% versus 34.5% at 2 years after transplant (Figure 2, *A*). For the D+ R− subgroup, the cumulative incidence of CMV between the EVLP and non-EVLP groups was 2.4% versus 6.1% at 6 months, 49.4% versus 56.9% at 1 year, and 57.6% versus 66.5% at 2 years after transplant (Figure 2, *B*). For the intermediate-risk group of patients, cumulative incidence of CMV between the EVLP and non-EVLP groups was 11.6% versus 13.5% at 6 months, 31.2% versus 33.5% at 1 year, and 33.5% versus 35.3% at 2 years after transplant (Figure 2, *C*). Finally, in the low-risk group of patients, CMV incidence only occurred in the non-EVLP patients with a cumulative incidence of 1% at 6 months and 1.5% at 1 year after transplant (Figure 2, *D*).

We also compared whether the 2015 change in prophylaxis protocols had any effect on the incidence of viremia. Until May 2015 (era 1), a total of 505 patients underwent LTx in our institution, of whom 395 were recipients of non-EVLP lungs and 110 were recipients of EVLP lungs. After May 2015 (era 2), of the 800 patients who received LTx in our center, 507 were recipients of non-EVLP lungs and 293 were recipients of EVLP lungs. Table E1 and Figure E1 show the overall incidence of CMV viremia in both eras comparing EVLP and non-EVLP groups (with analyses of the different CMV serostatus matching groups), and cumulative incidence of viremia over 2 years, respectively. During era 1, 35.2% of patients (n = 178) developed viremia. In the EVLP group, 30.0% of patients (n = 33) became viremic, as did 36.7% (n = 145) in the non-EVLP group ($P = .21$). Likewise, during era 2, 31.0% of patients (n = 91) developed viremia in the EVLP group, as did 32.9% (n = 167) in the non-EVLP group, $P = .63$. Because the CMV viremia outcome was similar comparing the different eras of prophylaxis, the secondary outcomes analyses were carried out as 1 combined cohort.

Last, specifically for the EVLP group, we measured traditional inflammatory markers of lung injury in donors at higher risk of future CMV development (CMV

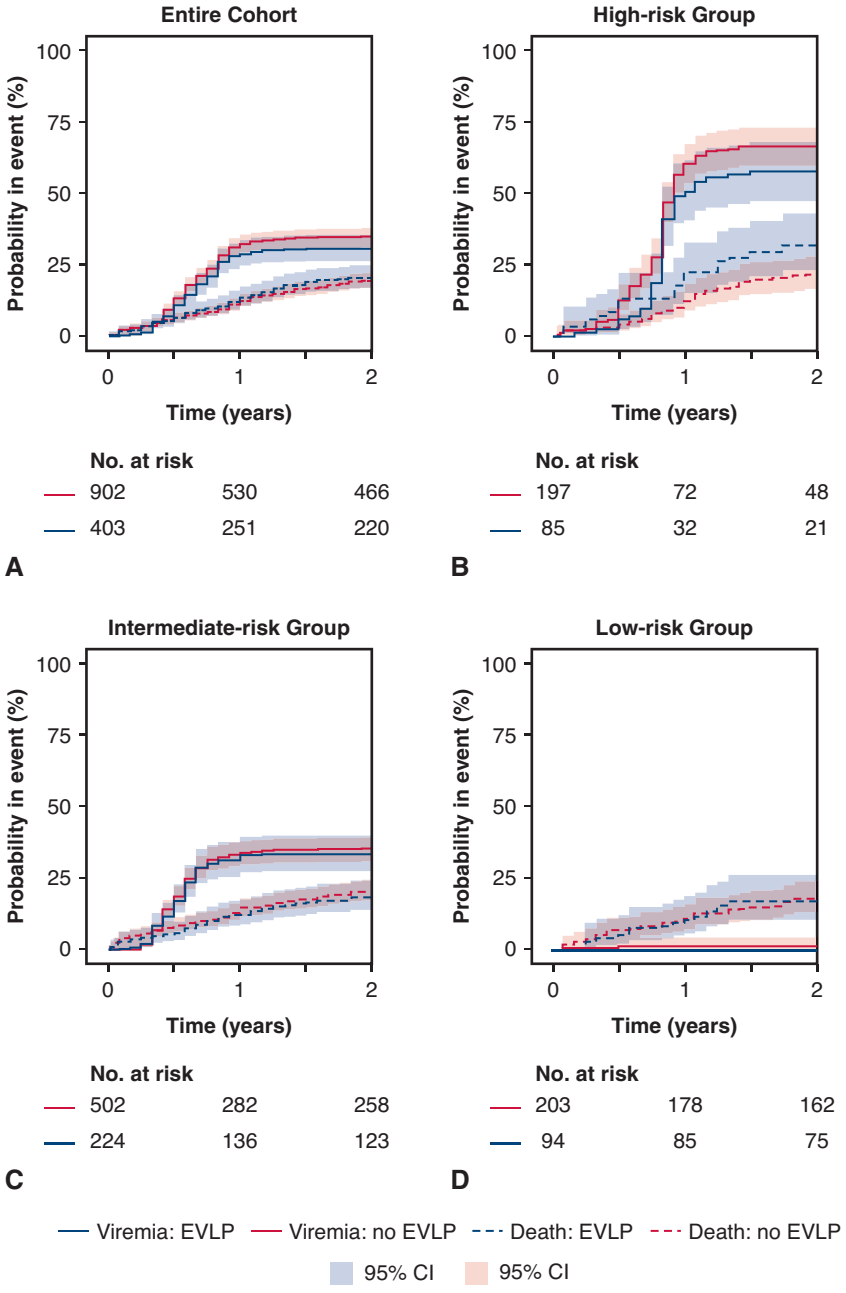

FIGURE 2. Cumulative incidence of CMV viremia after LTx: (A) entire cohort, (B) high-risk patients (D+ R−), (C) intermediate-risk patients (D+ R+ and D− R+), and (D) low-risk patients (D− R−). The *shaded areas* represent 95% CI for each outcome. *EVLP*, Ex vivo lung perfusion; *CI*, confidence interval; *D+*, seropositive donor; *R−*, seronegative recipient; *R+*, seropositive recipient; *D−*, seronegative donor.

seropositive lungs) to elucidate if patients who developed viremia after transplantation were associated with donors with a higher inflammatory burden at the time of transplantation. The median levels of IL-6 in lungs not associated with future CMV development was 774 pg/mL (IQR, 115-3781) and 1097 pg/mL (IQR, 360-3848) in lungs associated with CMV viremia ($P = .98$). Likewise, there was no difference in levels of IL-8 and IL-1β when comparing patients who do or do not develop viremia (Figure 3).

Peak Cytomegalovirus Viral Loads

We also sought to investigate whether EVLP had any impact on the peak viral loads post-LTx for those patients who developed viremia. The median peak viremia was 8060 IU/mL (IQR, 2939-36,075) in the non-EVLP group

TABLE 2. Univariable competing risk model

	Hazard ratio (95% CI)	*P* value
Entire cohort	0.86 (0.70-1.06)	.16
High-risk group	0.86 (0.62-1.19)	.36
Intermediate-risk group	0.91 (0.69-1.19)	.52
Low-risk group	0.00 (0.00-0.00)	-

Cox regression model with CMV viremia as outcome of interest and death as a competing risk event for the entire cohort and different subgroups. Hazard ratios are for all of the EVLP groups to develop CMV viremia. *CI*, Confidence interval.

and 10,650 IU/mL (IQR, 3157.5-47,425) in the EVLP group ($P = .40$). Median viral loads were also similar between the non-EVLP and EVLP groups within all serostatus matching groups (Figure 4): D+ R−, 22,320 IU/mL (IQR, 5220-130,000) versus 24,300 IU/mL (IQR, 8430-138,000) ($P = .68$); D+ R+, 4800 IU/mL (IQR, 2385-23,190) versus 7580 IU/mL (IQR, 2480-40,900) ($P = .28$); and D−R+ 3570 IU/mL (IQR, 2215-7321) versus 3525 IU/mL (IQR, 1885-7625) ($P = .69$). In the D− R− group, the median viral load in the non-EVLP group was 22,770 IU/mL (IQR, 3030-30,900).

Survival Analyses

Overall survival was similar among the groups (Figure 5, *A*). Median survival was 113 months post-LTx for the EVLP group and 98 months for the non-EVLP group. Estimated graft survival between EVLP and non-EVLP groups was 73% versus 74% at 3 years, 63% versus 62% at 5 years, and 48% versus 42% at 10 years after transplantation (log-rank $P = .97$). Likewise, there was no difference in survival when analyzing the different serostatus matching groups (Figure 5, *B-D*). A summary of the study design and main results is provided in Figure 6.

Additional survival subanalysis specifically for the viremic patients after transplantation was performed. When comparing EVLP and non-EVLP donors, there was no difference in survival (Figure E2).

DISCUSSION

This study evaluates the use of higher-risk donors during EVLP and the potential effect on CMV viremia after LTx in a large single-center cohort of patients. We show that the clinical use of EVLP and extended criteria donors has not affected CMV viremia rates and severity in lung transplant recipients. To our knowledge, this is the first large cohort study investigating post-LTx effects of EVLP on CMV outcomes in lung transplant recipients.

For the past 13 years, EVLP has allowed for the increased use of lungs from high-risk extended criteria donors across the world. Since the initiation of the EVLP program at our institution in 2008, the number of lung transplants has steadily increased.[15] From 2012 to 2017, there has been a 70% increase in transplant activities compared with the previous eras.[10] Specifically in this study, EVLP was used to facilitate the transplant of 403 lungs. Compared with non-EVLP donor lungs, these lungs, on average, had significantly worse pretransplant measures, including lower PaO_2:FiO_2 ratio, higher abnormal radiographic findings, higher proportion of smoking history, and higher use of DCD type of donors.[16,17] Despite the higher risk of donor lungs, lung transplant activities have successfully increased with similar short- and long-term outcomes, such as primary graft dysfunction, overall graft survival, and CLAD.[10]

Latent CMV in donor organs plays a key role in disease development after transplantation.[2] Rates of CMV viremia and disease are higher in LTx recipients than in all other solid-organ transplants and account for 2% to 12% of post-lung transplant mortality.[6] Additionally, donor lungs undergoing EVLP assessment have a higher degree of inflammation and longer preservation times, and both factors have been associated with CMV reactivation and infection in transplant recipients in non-EVLP studies.[18,19] The strategy adopted to reduce the burden of CMV post-LTx is the use of antiviral prophylaxis for long periods.[20] Most recent guidelines recommend 6 to 12 months of prophylaxis depending on the patient's risk

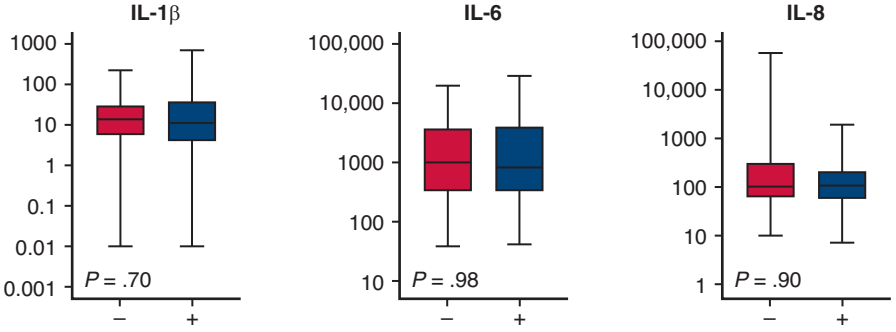

FIGURE 3. Levels of inflammatory proteins during EVLP comparing patients who developed CMV viremia and those who did not. Box-and-whisker plots comparing levels of IL-1b, IL-6, and IL-8. The *lower* and *upper borders* of the box represent the *lower* and *upper* quartiles (25th percentile and 75th percentile). The *middle horizontal line* represents the median, and the *lower* and *upper* whiskers represent the minimum and maximum values. Y axis shows levels of the protein in pg/mL and in log scale. On the x axis, – represents no CMV viremia and D+ represents CMV viremia after transplantation. *IL*, Interleukin.

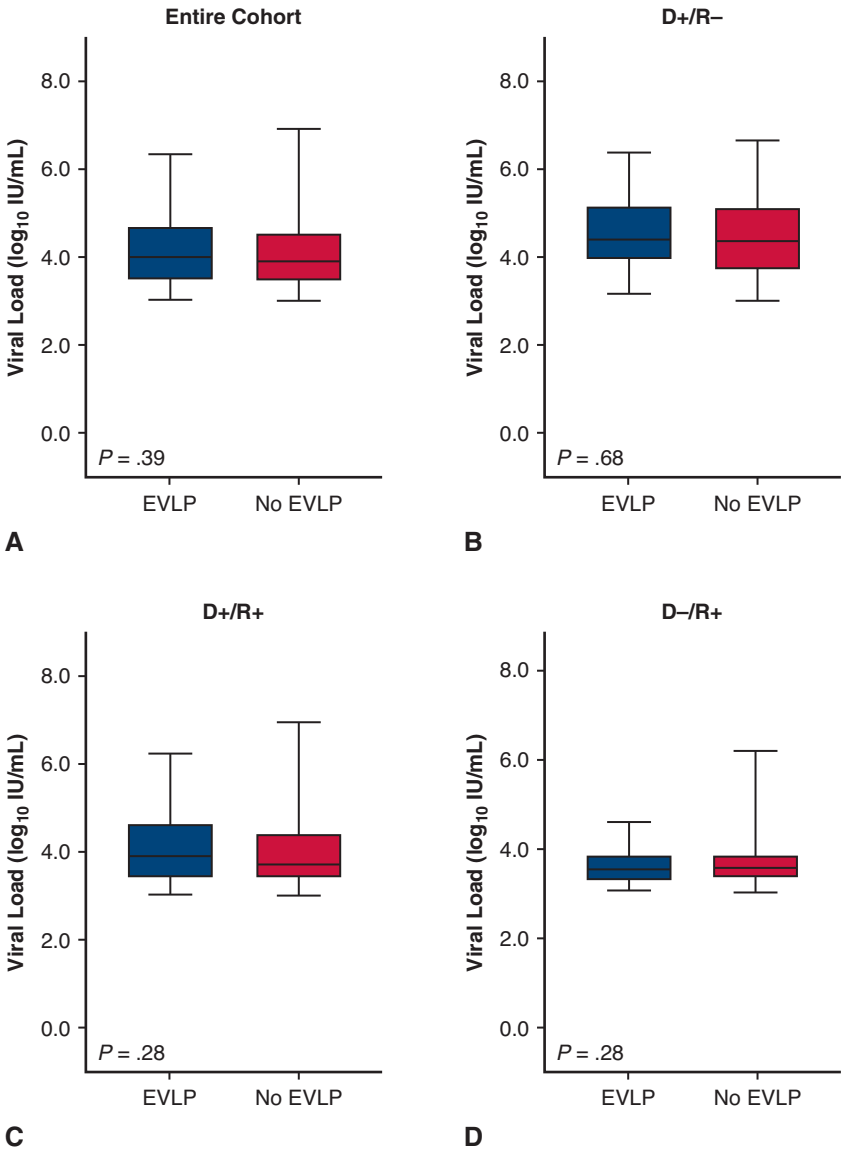

FIGURE 4. Peak CMV viral loads. Box-and-whisker plots comparing peak viral loads between EVLP and no EVLP in the different at-risk groups (A-D). The *lower* and *upper borders* of the box represent the *lower* and *upper* quartiles (25th percentile and 75th percentile). The *middle horizontal line* represents the median, and the *lower* and *upper* whiskers represent the minimum and maximum values. *EVLP*, Ex vivo lung perfusion; *D*+, Seropositive donor; *R*−, seronegative recipient; *R*+, seropositive recipient; *D*−, seronegative donor.

for CMV development. At our institution, for the intermediate-risk groups of patients (D+/R+ and D−/R+), prophylaxis lasts 6 months. For the high-risk group of patients (D+/R−), this period is 9 months. In our study, we observed that despite these extensive prophylactic periods, CMV viremia was still common. Overall, there was an approximately 34% incidence of CMV within the entire cohort in the first 2 years post-transplant, and for the higher risk patients, the incidence was as high as 64%. In the absence of preventive therapy, prior reports have shown that up to 92% of the high-risk patients (D+/R−) develop viremia and 50% to 65% develop symptomatic disease before 90 days post-LTx.[21,22] Overall, we did not observe any difference in viremia incidence comparing EVLP and non-EVLP donor lungs. However, it is important to point out that the majority of CMV viremia development happened after cessation of prophylaxis. The current prophylactic approach might be masking an earlier impact of EVLP on CMV viremia. Another important aspect that could influence the results is that EVLP is performed for different reasons in our institution, ranging from those true high-risk donors who need EVLP assessment for a safer decision to proceed with transplantation to sometimes being performed only for logistic reasons. However, during the

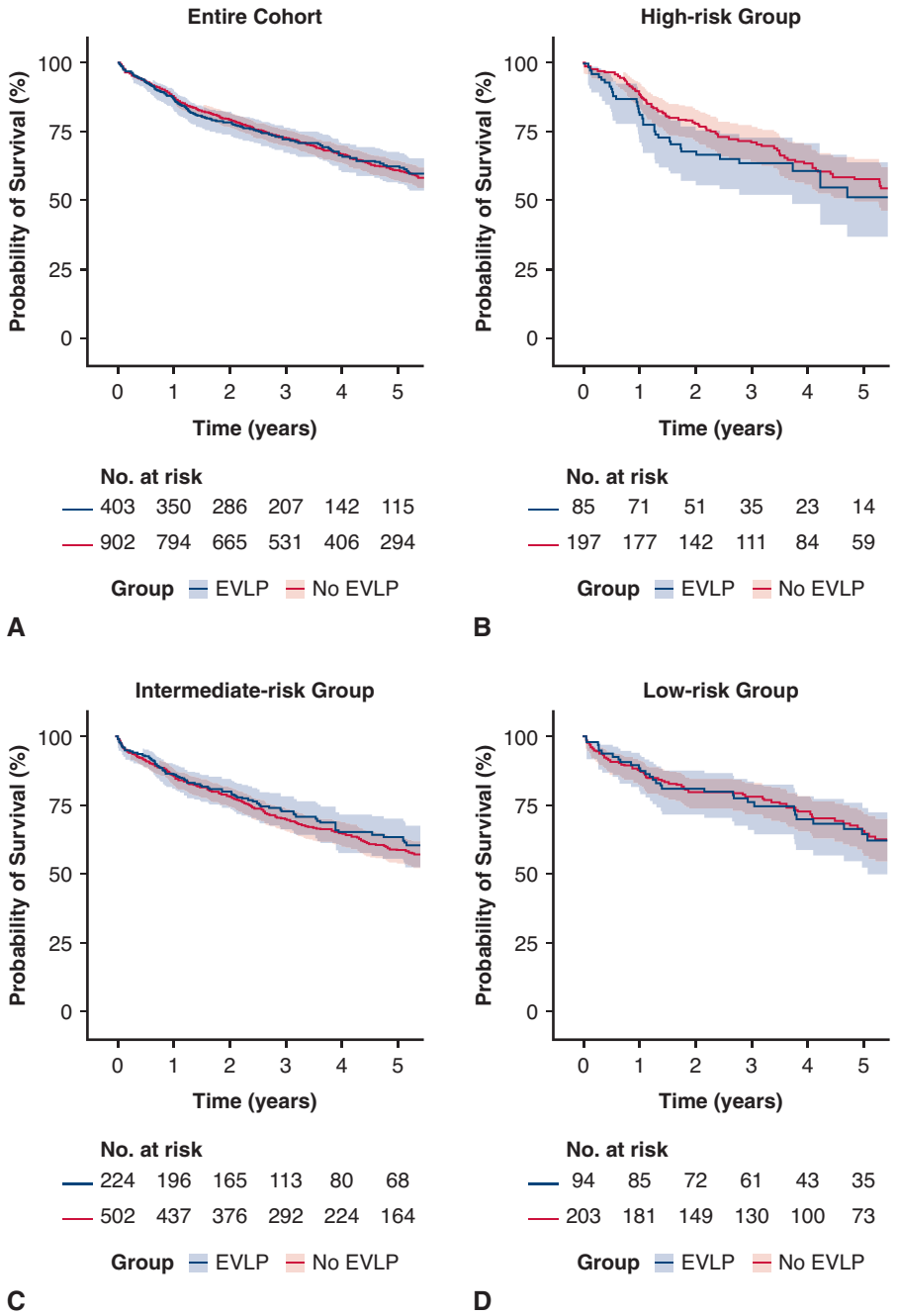

FIGURE 5. Survival curves. Kaplan–Meier curves comparing overall survival of EVLP and no-EVLP of the (A) entire cohort, (B) high-risk patients (D+ R−), (C) intermediate-risk patients (D+ R+ and D− R+), and (D) low-risk patients (D− R−). The *shaded areas* represent 95% CI. *EVLP*, Ex vivo lung perfusion; *D*+, seropositive donor; *R*−, seronegative recipient; *R*+, seropositive recipient; *D*−, seronegative donor.

period of this study, EVLP was rarely done for logistic reasons, which is why we did not pursue further stratified analysis.

The greatest risk of CMV disease is among CMV-seronegative patients without preexisting CMV-specific immunity who receive a latently infected organ from a CMV-seropositive donor.[23] Interestingly, our study shows an approximately 10% higher incidence of CMV viremia in recipients of non-EVLP donor lungs compared with EVLP lungs for this higher-risk group of patients, despite not reaching significance. A small study by Koch and colleagues[24] suggested a similar finding of lower CMV reactivation rates after transplantation of donor lungs that underwent ex vivo perfusion. It is known that the CMV virus has a predilection for lung parenchyma due to the greater amount of lymphatic tissue present in lungs compared with

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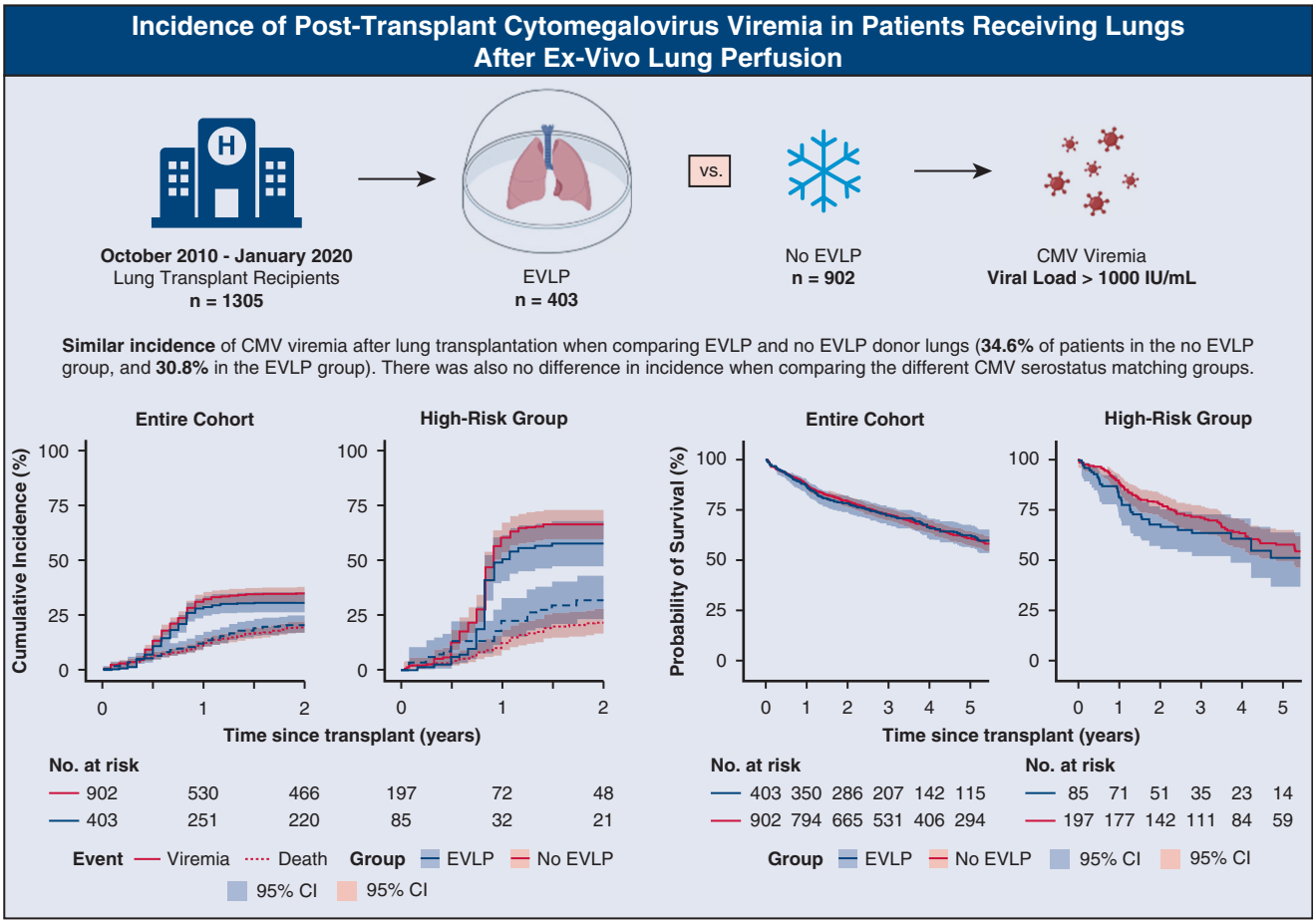

FIGURE 6. Incidence of post-transplant CMV viremia in patients receiving lungs after ex vivo lung perfusion. When comparing lungs from EVLP donors with lungs from no EVLP donors, there was a similar incidence of CMV viremia and also no difference in survival. The *shaded areas* represent 95% CI. *EVLP*, Ex vivo lung perfusion; *CMV*, cytomegalovirus; *CI*, confidence interval.

other transplantable organs,[25] and perhaps perfusion of donor lungs via EVLP results in reduction of the latent viral load within the graft by washing out cells harboring latent CMV and, therefore, diluting the overall latent burden. Of note, this observation requires a larger cohort of D+ R− patients comparing EVLP and non-EVLP donors and preclinical experimental studies to elucidate this finding. The high rates of viremia observed in our cohort underscores the need for additional preventive strategies, and EVLP is the ideal platform for treatment pretransplantation. We have previously studied this concept of targeting latent CMV in donor lungs before transplantation during EVLP.[26] We perfused human donor lungs for 6 hours with a novel highly specific immunotoxin resulting in less reactivation of CMV. This could be a promising strategy to mitigate post-transplant CMV viremia for this high-risk set of patients.

Study Limitations

Our study is limited by a few aspects. First, this was a retrospective, single-center cohort study. Second, we purposefully chose the cutoff value of 1000 IU/mL of viral load to define viremia across risk groups, although the treatment thresholds may be higher for some of the lower risk groups. Third, we did not perform an analysis of incidence of CMV disease; because of the retrospective nature of the study, we did not think we could accurately capture disease development. For example, many cases of CMV viral syndrome present with nonspecific symptomatology that is difficult to distinguish from other etiologies. However, the large EVLP database in our center allowed for the first large cohort study looking into CMV outcomes post-LTx after the adoption of EVLP into clinical practice and did not show any increase in CMV viremia in

the group of patients receiving lungs after EVLP assessment.

CONCLUSIONS

The increased use of high-risk donor lungs and EVLP has successfully increased transplant activities without compromising CMV viremia rates in transplant recipients. The rates of viremia are still impressive, highlighting the importance of more efficient detection and preventative strategies even before transplantation.

Conflict of Interest Statement

A.H. has received honoraria or grant support from Takeda, Merck, and Astellas. D.K. has received a research grant from Qiagen and Takeda, and consulting fees from Roche, Takeda, and Merck. T.M. has received a research grant from Sanofi, Inc, received research material from APCBio Innovations, Inc, and collaborates with Trove Therapeutics, Inc. M.C. and S.K. are founders of Traferox Technologies Inc. Traferox devices were not used for the performance of this study. M.C. and S.K. are consultants for Lung Bioengineering. This study was approved by the Toronto General Hospital institutional research ethics board (20-5597). Informed consent was waived because of the retrospective nature of this study. This work was supported by internal funding at University Health Network from the Toronto General and Western Foundation. All other authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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Key Words: CMV viremia, ex vivo lung perfusion, freedom from CMV, lung transplantation

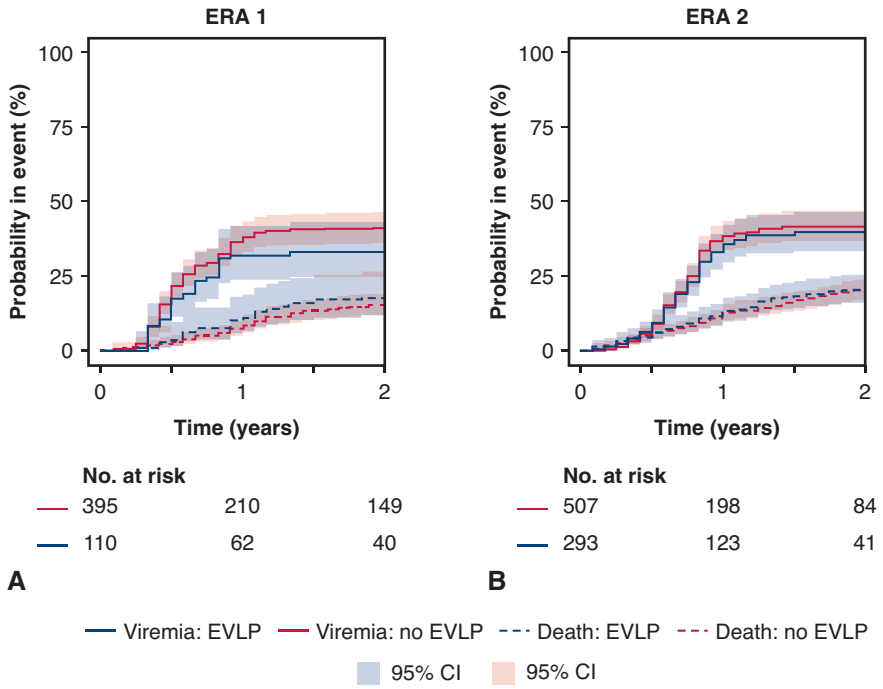

FIGURE E1. Cumulative incidence of CMV viremia after LTx stratified by prophylaxis eras. A, represents Era 1, which was until May 2015, and (B) represents era 2, which was after May 2015. The *shaded areas* represent 95% CI. *EVLP*, Ex vivo lung perfusion; *CI*, confidence interval.

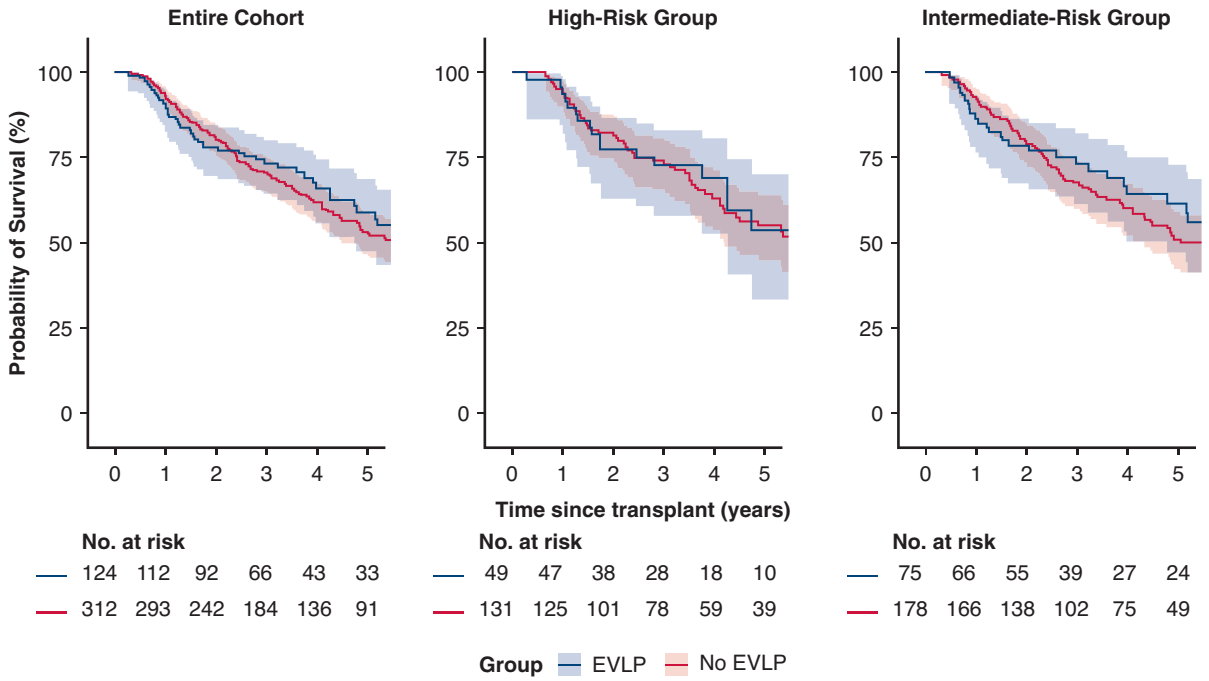

FIGURE E2. Survival curves for viremic patients. Kaplan–Meier curves comparing overall survival of EVLP and no-EVLP of the entire cohort, high-risk patients (D+ R−) and intermediate-risk patients (D+ R+ and D− R+). The *shaded areas* represent 95% CI. *EVLP*, Ex vivo lung perfusion; *D+*, Seropositive donor; *R−*, seronegative recipient; *R+*, seropositive recipient; *D−*, seronegative donor.

TABLE E1. Incidence of cytomegalovirus viremia after lung transplantation in the different prophylaxis eras and stratified by the different cytomegalovirus serostatus matching groups

	Era 1 (until May 2015)		
	No EVLP (n = 395)	**EVLP (n = 110)**	***P* value**
CMV viremia	145 (36.7%)	33 (30.0%)	.21
D+ R−	65 (73.8%)	11 (57.9%)	.16
D+ R+	55 (50.0%)	17 (54.8%)	.68
D− R+	22 (19.8%)	5 (15.1%)	.62
D− R−	3 (3.5%)	0 (0.0%)	>.999
	Era 2 (after May 2015)		
	No EVLP (n = 507)	**EVLP (n = 293)**	***P* value**
CMV viremia	167 (32.9%)	91 (31.0%)	.63
D+ R−	66 (57.6%)	38 (60.0%)	.75
D+ R+	74 (47.4%)	42 (44.7%)	.69
D− R+	27 (21.6%)	11 (16.7%)	.45
D− R−	0 (0.0%)	0 (0.0%)	>.999

EVLP, Ex vivo lung perfusion; *CMV*, Cytomegalovirus; *D+*, seropositive donor; *R−*, seronegative recipient; *R+*, seropositive recipient; *D−*, seronegative donor.