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Molecular detection of human herpesviruses in pleural effusions after lung transplantion

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ABSTRACT

Background: Lymphocytic pleural effusions of unclear etiology are frequent after lung transplantation and are associated with adverse outcomes. Reactivation of human herpesviruses, in particular Epstein-Barr Virus (EBV), has been demonstrated to account for a share of unclear pleural effusions in immunocompetent and -compromised patients. Here, we assessed the detection of human herpesviruses in a large lung transplant pleural effusion cohort.

Methods: A prospectively sampled cohort of 99 pleural effusions of 67 lung transplant recipients was analyzed for HSV-1, HSV-2, EBV, CMV, HHV-6 and HHV-7 by qPCR. Clinical characteristics were compared between virus-positive versus negative pleural effusions.

RESULTS: EBV could be detected in 35 %, HHV-6 in 19 %, CMV in 4 % and HHV-7 in 1 % of assessed pleural effusion samples, whereas HSV-1 and HSV-2 could not be detected. Median viral load for EBV was 1500 copies/ml, for HHV-6 1300 copies/ml and for CMV 163 copies/ml. Overall, no relevant differences were found comparing the characteristics of EBV-, HHV6- and CMV-positive versus negative pleural effusions.

Conclusions: Herpesviruses can be detected in low copy numbers in a share of lung transplant pleural effusions. However, they do not seem to account for the high rate of unclear lymphocytic effusions after lung transplantation.

Abbreviations: BLAD, baseline lung allograft dysfunction; CMV, cytomegalovirus, human herpesvirus 5; EBV, epstein-Barr virus, human herpesvirus 4; eGFR, estimated glomerular filtration rate; HSV-1, herpes simplex virus 1; HSV-2, herpes simplex virus 2; HHV-6, human herpesvirus 6; HHV-7, human herpesvirus 7; ILD, interstitial lung disease; IQR, interquartile range; KDIGO, kidney disease improving global outcomes; LTX, lung transplantation; MMF, mycophenolate mofetil; PCR, polymerase chain reaction; PTLD, post-transplant lymphoproliferative disorder; SOT, solid organ transplant; TBC, tuberculosis.

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1. Introduction

Pleural effusions are fluid accumulations in the pleural cavity that can lead to dyspnea and cough [1]. Pleural effusions after LTX are not merely incidental findings: they have been associated with impaired lung function, an increased probability of baseline lung allograft dysfunction (BLAD) and higher mortality [2–4]. Early pleural effusions are usually related to surgical factors, are highly exudative and have a high neutrophil count [5]. Pleural effusions that occur at later timepoints may have a wider variety of etiologies, e.g. renal failure, malignancy or infection [2]. A large share of late pleural effusions after LTX, however, cannot be attributed to a specific etiology despite extensive evaluation and remain unclear [2]. These effusions are frequently lymphocytic exudates and represent a major diagnostic and pathophysiological challenge [2]. To improve lung transplant outcomes, it is imperative to develop a better understanding of their etiology paving a way for potential therapeutic strategies.

Reactivation of herpesviruses under immunosuppressive therapy is an important cause of morbidity and mortality in solid organ transplant recipients with lung transplant recipients being particularly vulnerable. Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are among the most clinically relevant herpesviruses in organ recipients and are known to cause severe systemic and organ-specific complications [6]. Earlier observations suggested a possible link between herpesviruses and pleural effusions: a study by Takei and Mody investigating EBV-positive pleural effusions reported that all examined cases occurred in lung transplant recipients [7]. However, systematic and prospective data addressing the prevalence, viral burden, and clinical relevance of herpesviruses in pleural effusions after lung transplantation are lacking.

To address the hypothesis that herpesviruses might be associated with pleural effusions after lung transplantation, we examined the prevalence, virus load and associated clinical characteristics of herpesviruses in pleural effusions derived from the largest prospectively biobanked cohort of LTX pleural effusions to date. By sytematically analyzing pleural fluid samples, this study aims to clarify the clinical relevance of herpesvirus detection in pleural effusions and to provide a more robust evidence base for future diagnostic and therapeutic approaches.

2. Materials and methods

2.1. Ethics

This study was approved by the local ethics committee of the Ludwig-Maximilians-University (LMU) Munich, Ethic vote #19-629. Written informed consent to participate in this study was obtained from all patients, in accordance with the local ethics committee of LMU, Munich, Germany.

2.2. Patient cohort

The prospective pleural effusion biobanking included 99 pleural effusion samples from 67 LTX recipients obtained between March 2021 and June 2024 from the CPC-M bioArchive at the Comprehensive Pneumology Center (CPC Munich, Germany).

2.3. Clinical data

Clinical data were collected from the CPC-M bioArchive. Serology testing for EBV and CMV was performed in the evaluation of the recipient before lung transplantation. Microbiological examination including bacterial cultures, acid-fast bacilli (AFB) staining, tuberculosis polymerase chain reaction (TB PCR), tuberculosis cultures as well as histopathological examination was performed for every pleural effusion. Classification into trans- and exudates was performed according to Light's criteria [1] extended by the protein gradient criterion by Romero et al [7]. Pleural effusions were considered lymphocytic when containing more than 50 % lymphocytes, neutrophilic when containing more than 50 % neutrophils, and in other cases classified as mixed effusions [8]. Renal function was categorized by the estimated glomerular filtration rate (eGFR) at the time of pleural effusion. For repeated pleural effusion samples, the eGFR at the time of the first thoracentesis was included in the study when demographics were demonstrated. Underlying lung diseases were classified functionally into interstitial lung disease, obstructive lung disease, vascular lung disease and others.

2.4. Categorization of etiology of pleural effusions

Etiologies of pleural effusions were assigned by a transplant physician based on clinical context, laboratory findings and pleural fluid findings, including cytology and microbiology. Detailed definitions for etiologies of pleural effusions can be found in the Supplement.

2.5. Nucleic acid extraction

Nucleic acids from patient material were extracted in the accredited routine diagnostics laboratory of the Max von Pettenkofer institute virology department according to established standard operating procedures using a QIAsymphony SP instrument in combination with the QIAsymphony DSP Virus/Pathogen Mini Kit (Qiagen, Hilden, Germany; art. No. 937036).

2.6. Quantitative polymerase chain reaction

Quantitative real-time PCR (qPCR) was performed to detect and quantify herpesviral DNA in patient samples. Reactions were run according to established and validated protocols in the accredited routine diagnostics laboratory. In-house PCRs were used to quantitatively assess herpesviral loads using LightCycler 480 II (Roche Diagnostics, Mannheim, Germany) or 7500 Fast Real-Time PCR (Applied Biosystems, MA, USA) cyclers. Serial dilutions of plasmid DNA were used to generate standard curves for quantitative analysis. Validated LOD95 were 300 copies/ml for EBV, 1000 copies/ml for HHV-6, 800 copies/ml for CMV, 900 copies/ml for HHV-7 and 700 copies/ml for HSV1 and 2.

2.7. Statistical analysis

Mean and standard deviations (SD) were used for parametric data. Median and interquartile range (IQR) was used for non-parametric data. SPSS was used to calculate statistics. For the comparison of continuous variables, the Mann-Whitney U test was used. For comparison of categorical variables, the Chi-squared test was applied. For comparisons, where multiple pleural effusion samples of the identical study individual were analyzed, linear mixed-effects models were used to account for the repeated measurements. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics

Between March 2021 and June 2024, 67 LTX patients were diagnosed with pleural effusions and thoracentesis was performed. In total, 99 pleural effusion samples from these 67 LTX recipients were analyzed. 35 patients (52 %) contributed more than one pleural effusion sample. Median age was 60 years, 30 % of the patients were female. Reasons for lung transplantation in this cohort were interstitial lung disease (66 %), obstructive lung disease (27 %), vascular lung disease (4 %) and other causes (3 %). At the time of effusion, the majority (87 %) of LTX recipients received triple immunosuppressive therapy with tacrolimus, mycophenolate mofetil (MMF) and corticosteroids. According to KDIGO guidelines, 21 % of the recipients had a normal kidney function (G1) at

time of first thoracocentesis, while 19 % had a mild reduction (G2), 33 % a moderate reduction (G3), 16 % a severe reduction (G4) and 10 % a very severe reduction of kidney function (G5). Only 1 of the recipients had a history of PTLD and 2 recipients a history of other malignancies prior to thoracentesis. None of the patients developed PTLD (post-transplant lymphoproliferative disorder) after thoracentesis. Other malignancies after thoracocentesis were detected in 6 LTX recipients. Donor and recipient serostatus were assessed for EBV and CMV before transplantation. Most frequent combination of EBV serostatus was donor and recipient positive (D+R+, 69 %). 34 % of the study cohort had a CMV high-risk constellation (D+R-), 46 % an intermediate risk constellation (D+R+ or D-R+) and 19 % a CMV low risk constellation (D-R-) (Table 1).

3.2. Pleural effusion characteristics

Median time from LTX to thoracocentesis was 326 days with a wide distribution (IQR: 51 -1518). For almost half of the pleural effusions (43 %) the etiology was unclear. Second and third most common causes of pleural effusions were post-operative (24 %) and renal failure (16 %). Malignancy (5 %), heart failure (4 %), infection (2 %) and other causes (3 %) were less frequent. 70 % of the effusions were exudates and 28 % transudates. More than half of the pleural effusions (55 %) were documented as lymphocytic. Mixed effusions (26 %) were more common compared to neutrophilic effusions (13 %). Pleural effusions had a median pleural protein of 2,9 g/dl (IQR: 2,2-3,55 g/dl) and median LDH of 179,5 U/I (IQR: 139-272 U/I). Median serum CRP levels were mildly elevated with 2,3 mg/dl (IQR: 0,7-7,4 mg/dl). Microbiology results revealed that 7 % of the pleural effusions were aerobic and/or anaerobic bacteria positive. None (0 %) tested positive for mycobacterium tuberculosis (Table 2).

3.3. Prevalence of Herpesviruses in pleural effusions

EBV could be detected in 35/99 (35 %), HHV-6 in 19/99 (19 %), CMV in 4/99 (4 %) of lung transplant pleural effusions. HHV-7 was detected in only 1/99 (1 %) pleural effusions while HSV-1, HSV-2 were not detected in any sample (Table S1). Pleural effusion viral loads and their relation to time after lung transplantation are depicted in Figure S1.

3.4. EBV positive versus negative pleural effusions

Median EBV copy number in EBV-positive effusions was 1500 copies/ml (IQR 435-10450). No relevant differences were observed

Table 1
Demographics of the study cohort consisting of lung transplantation (LTX) patients.

Demographics	LTX Recipients (n= 67)
Age at LTX (years) (median [IQR])	60 [54-69]
% female patients	30 % (20/67)
Patients with Multiple Effusions (n)	52 % (35/67)
Underlying Lung Disease	
Interstitial Lung Disease (n)	66 % (44/67)
Obstructive Lung Disease (n)	27 % (18/67)
Vascular Lung Disease (n)	4 % (3/67)
Other (n)	3 % (2/67)
Immunosuppressive Regimen	
Tacrolimus+ Steroids (n)	87 % (58/67)
Tacrolimus+ Everolimus+ Steroids (n)	4 % (3/67)
Tacrolimus+ Sirolimus+ Steroids (n)	4 % (3/67)
Ciclosporin+ Steroids (n)	4 % (3/67)
Renal Function at the Time of Pleural Effusion*	
G1 (n)	21 % (14/67)
G2 (n)	19 % (13/67)
G3 (n)	33 % (22/67)
G4 (n)	16 % (11/67)
G5 (n)	10 % (7/67)
History of Malignacy Pre-effusion**	
PTLD (n)	1 % (1/67)
Other (n)	3 % (2/67)
History of Malignacy Post-effusion**	
PTLD (n)	0
Other (n)	9 % (6/67)
EBV serostatus pre-LTX ***	
D+R+ (n)	69 % (46/67)
D+R- (n)	4 % (3/67)
D-R+ (n)	4 % (3/67)
D-R- (n)	0
CMV serostatus pre-LTX	
D+R+ (n)	25 % (17/67)
D+R- (n)	34 % (23/67)
D-R+ (n)	21 % (14/67)
D-R- (n)	19 % (13/67)

* Renal function classified based on eGFR-value at the time of thoracentesis, in accordance with KDIGO guidelines.

** Pre-effusion malignancy: any malignancy diagnosed before the thoracentesis. Post-effusion malignancy: any new or newly detected malignancy diagnosed after the thoracentesis.

*** Donor or recipient EBV serostatus was missing in 15 patients.

Table 2
Characteristics of the cohort of pleural effusions.

Characteristics	Pleural Effusion (n= 99)
LTX to PE time (in days) (median [IQR])	326 [51-1518]
Etiology of Effusion	
Unclear (n)	43 % (43/99)
Post-operative (n)	24 % (24/99)
Renal Failure (n)	16 % (16/99)
Malign (n)	5 % (5/99)
Heart Failure (n)	4 % (4/99)
Infection (n)	2 % (2/99)
Other (n)	5 % (5/99)
Type of Effusion (Transudate/ Exudate)	
Exudate (n)	70 % (70/99)
Transudate (n)	28 % (28/99)
Unknown (n)	1 % (1/99)
Type of Effusion (Lymphocytic/Neutrophilic)	
Lymphocytic (n)	55 % (54/99)
Neutrophilic (n)	13 % (13/99)
Mixed Effusion (n)	26 % (26/99)
Unknown (n)	6 % (6/99)
Pleura Differential Cell Count #	
Total Cell Count (/µl) (median [IQR])	757 [379-1732]
Lymphocyte (/µl) (median [IQR])	334 [129-830]
Neutrophil Granulocyte (/µl) (median [IQR])	52 [11-240]
Eosinophil Granulocyte (/µl) (median [IQR])	0 [0-3]
Macrophages (/µl) (median [IQR])	8 [0-67]
Monocytes (/µl) (median [IQR])	89 [30-208]
Mesothelial Cell (/µl) (median [IQR])	3 [0-27]
Serum laboratory chemistry results	
CRP (mg/dl) (median [IQR])	2,3 [0,7-7,4]
Creatinine (mg/dl) (median [IQR])	1,5 [1,1-2,3]
LDH (U/I) (median [IQR])	257 [230-323]
Protein (g/dl) (median [IQR])	5,8 [5,2-6,4]
Albumin (g/dl) (median [IQR])	3,4 [3-3,9]
Pleural laboratory chemistry results*	
LDH (U/I) (median [IQR])	179,5 [139-272]
Protein (g/dl) (median [IQR])	2,9 [2,2-3,55]
Albumin (g/dl) (median [IQR])	1,75 [1,3-2,2]
Cholesterol (mg/dl) (median [IQR])	55,5 [32-71]
Triglycerides (mg/dl) (median [IQR])	19 [14-28]
Microbiology results	
aerobic and/or anaerobic bacteria positive	7 % (7/99)
Fungal pathogen detected	5 % (5/99)
TBC PCR positive	0

Pleura total cell counts were unavailable for two pleural effusion samples and differential cell counts were missing for six pleural effusion samples; these were therefore excluded.

* Some lab results on pleural fluid protein (2 samples), albumin (5 samples), LDH (3 samples), Cholesterol (7 samples), and triglyceride (7 samples) were absent and thus omitted from the analysis.

between the characteristics and demographics of EBV-positive and EBV-negative pleural effusions (Table 3, Table S2).

3.5. CMV positive versus negative pleural effusions

4 pleural effusions were CMV positive (4 %) with a median CMV copy number of 163 (IQR 18-111.448) (Table 4, Table S3). One pleural effusion had an exceptionally high CMV virus load (4.600.000 copies/ml). This effusion was from a patient who was an inward patient at that time for antiviral treatment for systemic CMV infection. 3 CMV positive pleural effusions (75 %) occurred in patients with a CMV high risk constellation (D+R-). 2 of the 4 CMV-positive pleural effusions occurred in patients who had systemic CMV reactivation at the time of thoracocentesis.

3.6. HHV-6 positive versus negative pleural effusions

19 pleural effusions tested positive for HHV-6 (19 %) with a median copy number of 1300 (IQR: 550-13000) (Table 5, Table S4). The etiology of the majority of HHV-6 positive samples was post-operative (58 %). HHV6-positive pleural effusions had higher amounts of neutrophil granulocytes (107/µl vs. 48/µl, p=0.049) and higher amounts of monocytes (91/µl vs. 86/µl, p=0.024).

3.7. HHV-positivity and pleural effusion etiology

When stratified by EBV-, HHV6- and CMV-positivity, it was noted that EBV-positive pleural effusions represented a similar share of unclear (37 %), post-operative (33 %) and renal failure effusions (44 %). In contrast, HHV-6 positivity was predominantly noted in post-operative (46 %) and only occurred in a minority of unclear (9 %) or renal failure (6 %) pleural effusions (Table S5).

4. Discussion

Pleural effusions represent a frequent problem after lung transplantation and often remain unclear despite thorough diagnostics. Previous studies have demonstrated associations between HHV infections and lymphocytic effusions [9,10]. Yet, evidence of HHV presence in pleural effusions of lung allograft recipients remains sparse. Here, we analyzed presence, viral load and clinical correlates of human herpesviruses in a large prospectively sampled pleural effusion cohort of LTX recipients.

Prevalence of EBV in pleural effusions has been controversially discussed in previous publications [8–10]. We could demonstrate that EBV is detectable in 35 % of LTX pleural effusions, however with low virus load. The median copy number of 1500 copies/ml was slightly higher compared with previous studies, where a median EBV viral load of around 450 copies/ml was found in EBV-positive pleural effusions in immunocompetent patients and a median EBV viral load of 530 copies/ml was found in EBV-positive effusions in lung transplant recipients [9,10]. Post-transplant lymphoproliferative disorder (PTLD) is a malignancy that occurs in SOT recipients and arises from an unrestrained proliferation of lymphocytes that is frequently triggered by EBV reactivation. LTX recipients have a moderate to high incidence of PTLD compared to other solid organ transplants [11]. In our study, no association of EBV-positive effusion and PTLD before or after thoracocentesis was found. When comparing clinical and biochemical characteristics between EBV-positive and negative groups, we were not able to demonstrate any differences between these groups. Analogous to Arnold et al., EBV prevalence was not different between pleural effusions of various etiologies in lung allograft recipients [8]. This pattern might be compatible with bystander shedding or passive diffusion of virus material of adjacent compartments and thus does not indicate a causative role of EBV in unclear pleural effusions in lung allograft recipients.

Table 3
Demographics of lung transplantation (LTX) patients by pleural effusion EBV (Epstein-Barr virus) status (EBV- vs. EBV+).

Demographics	EBV Negative n=64	EBV Positive n=35	p-value
Age at LTX (years) (median [IQR])	60 [55-64]	59 [57-62]	0,489
Age at PE (years) (median [IQR])	61 [56-68]	63 [60-68]	0,168
LTX to PE time (in days) (median [IQR])	238 [50-1388]	419 [73-1747]	0,137
% female patients	30 % (19/64)	37 % (13/35)	0,453
Patients with multiple effusions (n)	50 % (33/64)	67 % (24/35)	0,104
Underlying Lung Disease			0,133
Interstitial Lung Disease (n)	55 % (35/64)	74 % (26/35)	
Obstructive Lung Disease (n)	36 % (23/64)	14 % (5/35)	
Vascular Lung Disease (n)	6 % (4/64)	11 % (4/35)	
Other (n)	3 % (2/64)	0	
History of Malignancy Pre-effusion*			0,137
PTLD (n)	0	3 % (1/35)	
Other (n)	5 % (3/64)	9 % (4/35)	
History of Malignancy Post-effusion*			0,749
PTLD (n)	0	0	
Other (n)	9 % (6/64)	11 % (4/35)	
Etiology of Effusion			0,360
Unclear (n)	42 % (27/64)	46 % (16/35)	
Post-operative (n)	25 % (16/64)	23 % (8/35)	
Renal Failure (n)	14 % (9/64)	20 % (7/35)	
Heart Failure (n)	5 % (3/64)	3 % (1/35)	
Malign (n)	5 % (3/64)	6 % (2/35)	
Infection (n)	3 % (2/64)	0	
Other (n)	6 % (4/64)	3 % (1/35)	
Type of Effusion (Transudate/Exudate)			0,840
Transudate (n)	27 % (17/64)	31 % (11/35)	
Exudate (n)	74 % (47/64)	66 % (23/35)	
Unknown (n)	0	3 % (1/35)	
Type of Effusion (Lymphocytic/Neutrophilic)			0,272
Lymphocytic (n)	53 % (34/64)	57 % (20/35)	
Neutrophilic (n)	8 % (5/64)	23 % (8/35)	
Mixed Effusion (n)	33 %(21/64)	14 % (5/35)	
Unknown (n)	6 % (4/64)	6 % (2/35)	
Pleura Differential Cell Count#			
Total Cell Count (/µl) (median [IQR])	708 [357-1592]	843 [491-2296]	0,502
Lymphocyte (/µl) (median [IQR])	46 [19-74]	49 [24-62]	0,665
Neutrophil Granulocyte (/µl) (median [IQR])	27 [8-56]	38 [18-57]	0,680
Eosinophil Granulocyte (/µl) (median [IQR])	0 [0-0]	0 [0-6]	0,600
Macrophages (/µl) (median [IQR])	8 [0-75]	8 [0-45]	0,278
Monocytes (/µl) (median [IQR])	85 [29-275]	89 [32-192]	0,280
Mesothelial Cell (/µl) (median [IQR])	6 [0-50]	0 [0-11]	**0,038**
Pleura laboratory chemistry results**			
LDH (U/l) (median [IQR])	184 [134-310]	182 [145-291]	0,663
Protein (g/dl) (median [IQR])	2,9 [2,2-3,6]	2,7 [2,2-3,5]	0,827
Albumin (g/dl) (median [IQR])	1,7 [1,4-2,2]	1,6 [1,1-2,3]	0,236
Cholesterol (mg/dl) (median [IQR])	57 [33-69]	53 [30-73]	0,683
Triglycerides (mg/dl) (median [IQR])	17 [13-27]	22 [16-31]	0,512
Microbiological results			0,203
aerobic and/or anaerobic bacteria positive	9 % (6/64)	3 % (1/35)	
Fungal pathogen detected	2 % (1/64)	11 % (4/35)	
Unknown (n)	2 % (1/64)	3 % (1/35)	
EBV Serostatus pre LTX			0,105

(*continued on next page*)

Table 3 (*continued*)

Demographics	EBV Negative n=64	EBV Positive n=35	p-value
D+R+	59 % (38/64)	74 % (26/35)	
D+R-	3 % (2/64)	3 % (1/35)	
D-R+	5 % (3/64)	11 %(4/35)	
D-R-	0	0	
D?R+	28 % (18/64)	6 %(2/35)	
D?R-	0	3 % (1/35)	
D+R?	5 % (3/64)	0	
D-R?	0	0	
D?R?	0	3 % (1/35)	
EBV Median Viral load in Pleural Effusion (copies/ml) [IQR]	not detectable	1500 [435-10450]	**0,05**

All the analyses were performed using linear mixed-effects models (LMMs) to account for repeated measurements within patients. A p-value of <0.05 was considered statistically significant.

* Pre-effusion malignancy: any malignancy diagnosed before the thoracentesis. Post-effusion malignancy: any new or newly detected malignancy diagnosed after the thoracentesis. #Pleura total cell counts were unavailable for two pleural effusion samples and differential cell counts were missing for six pleural effusion samples; these were therefore excluded.

** Some lab results on pleural fluid protein (2 samples), albumin (5 samples), LDH (3 samples), Cholesterol (7 samples), and triglyceride (7 samples) were absent and thus omitted from the analysis.

HHV-6 is a herpesvirus that frequently reactivates upon immunosuppression and that can be detected after various solid organ transplantations such as lung, kidney, liver and heart [12]. Usually HHV-6 reactivation is asymptomatic and only leads to clinically apparent disease in a minority of patients. HHV6 viral load is frequently an indicator of high levels of immunosuppression and is indirectly linked to other opportunistic infections such as CMV-reactivation or fungal diseases [12]. To date, only a few case reports exist about HHV-6 detection in pleural fluid and both originate from severely immunocompromised patients [13,14]. In this study, we could detect HHV-6 in 19 pleural effusion samples (19 %) with a rather low median copy number (1300 copies/ml). HHV-6 positive effusions predominantly originated from patients that only recently had transplantation, and the adjudicated etiology of the pleural effusion was most often post-operative. This finding is likely attributable to high-intensity immunosuppression in the early post-transplant period and therefore predominantly observed in early postoperative effusions.

In our cohort consisting of 99 pleural effusions, only 4 tested for CMV positive. Due to this small number, a meaningful interpretation of treatment outcomes and trends remain limited. The high viral load in one CMV-positive effusion (4.6 million copies/ml) under antiviral therapy demonstrates that high CMV viremia can also lead to high viral loads in the pleural space, which warrants further investigation. In our study, however, we could not differentiate between active replication in the pleural space or passive shedding of viral DNA.

To our knowledge, there are only very limited reports about HSV-1, HSV-2 and HHV-7 in pleural effusions in the literature [15,16]. In our study, we could not detect HSV-1, HSV-2 in LTX pleural effusions, and only one effusion tested positive for HHV-7 thus suggesting that these are unlikely to contribute to pleural effusion pathogenesis in LTX recipients.

It is remarkable that the predominant cell type in (unclear) post-transplant effusions are lymphocytes, which was not only noted in our study, but also in previously published studies by other transplant centers [10,2,17]. In the Takei study, lymphocytosis could be detected in more than half of pleural effusions collected from LTX recipients (65 %, 13/20) [10]. In our study neither EBV-positive, HHV-6-positive or CMV-positive effusions constituted a distinct subgroup. Only minimal differences were observed between virus-positive and virus-negative

Table 4
Demographics of lung transplantation (LTX) patients by pleural effusion CMV (Cytomegalovirus) status (CMV- vs. CMV+).

Demographics	CMV Negative (n=95)	CMV Positive (n=4)	p-value
Age at LTX (years) (median [IQR])	59 [56-63]	59 [57-61]	0,919
Age at PE (years) (median [IQR])	61 [57-68]	59 [58-62]	0,705
LTX to PE time (in days) (median [IQR])	326 [49-1524]	247 [94-643]	0,332
% female patients	34 % (32/95)	0	0,161
Patients with Multiple Effusions (n)	58 % (55/95)	50 % (2/4)	0,757
Underlying Lung Disease			0,29
Obstructive Lung Diseases (n)	27 % (26/95)	50 % (2/4)	
Interstitial Lung Diseases (n)	62 % (59/95)	50 % (2/4)	
Vascular Lung Diseases (n)	8 % (8/95)	0	
Other (n)	2 % (2/95)	0	
CMV specific prophylaxis/ therapy			
Val-/Aciclovir (n)	20 % (19/95)	0	0,325
Valganciclovir (n)	35 % (33/95)	0	0,152
Letermovir (n)	3 % (3/95)	50 % (2/4)	**<0,001**
History of Malignacy Pre-effusion*			0,702
PTLD (n)	0	25 % (1/4)	
Other (n)	7 % (7/95)	0	
History of Malignacy Post-effusion*			0,499
PTLD (n)	0	0	
Other (n)	11 % (10/95)	0	
Etiology of Effusion			0,843
Unclear (n)	43 % (41/95)	50 % (2/4)	
Post-operative (n)	25 % (24/95)	0	
Renal Failure (n)	16 % (15/95)	25 % (1/4)	
Malign (n)	5 % (5/95)	0	
Heart Failure (n)	4 % (4/95)	0	
Infection (n)	1 % (1/95)	25 % (1/4)	
Other (n)	5 % (5/95)	0	
Type of Effusion (Transudate/ Exudate)			0,922
Exudate (n)	71 % (67/95)	75 % (3/4)	
Transudate (n)	28 % (27/95)	25 % (1/4)	
Unknown (n)	1 % (1/95)	0	
Type of Effusion (Lymphocytic/ Neutrophilic)			0,186
Lymphocytic (n)	56 % (53/95)	25 % (1/4)	
Neutrophilic (n)	14 % (13/95)	0	
Mixed Effusion (n)	24 % (23/95)	75 % (3/4)	
Unknown (n)	6 % (6/95)	0	
Pleura Differential Cell Count#			
Total Cell Count (/µl) (median [IQR])	757 [368-1792]	744 [464-1535]	0,575
Lymphocyte (/µl) (median [IQR])	45 [21-71]	55 [31-76]	0,981
Neutrophil Granulocyte (/µl) (median [IQR])	33 [10-56]	32 [8-46]	0,631
Eosinophil Granulocyte (/µl) (median [IQR])	0 [0-3]	0 [0-4)	0,602
Macrophages (/µl) (median [IQR])	7 (0-54]	74 [25-168]	0,668
Monocytes (/µl) (median [IQR])	91 [30-208]	52 [9-494]	0,921
Mesothelial Cell (/µl) (median [IQR])	2 [0-27]	73 [5-133]	0,135
Pleura laboratory chemistry results**			
LDH (U/l) (median [IQR])	185 [140-306]	181 [98-181]	0,508
Protein (g/dl) (median [IQR])	2,8 [2,2-3,6]	2,6 [1,7-2,6]	0,783
Albumin (g/dl) (median [IQR])	1,7 [1,3-2,3]	1,6 [1,2-1,6]	0,952
Cholesterol (mg/dl) (median [IQR])	56 [32-73]	39 [30-39]	0,249
Triglycerides (mg/dl) (median [IQR])	18,5 [14-30]	22 [10-22]	0,699
Microbiological results			0,459
aerobic and/or anaerobic bacteria positive (n)	7 % (7/95)	0	
Fungal pathogen detected (n)	5 % (5/95)	0	

(*continued on next page*)

Table 4 (*continued*)

Demographics	CMV Negative (n=95)	CMV Positive (n=4)	p-value
Unknown (n)	2 % (2/95)	0	
CMV Serostatus pre LTX			0,222
D+R+ (n)	24 % (23/95)	25 % (1/4)	
D+R- (n)	31 % (29/95)	75 % (3/4)	
D-R+ (n)	25 % (24/95)	0	
D-R- (n)	20 % (19/95)	0	
D?R+(n)	0	0	
CMV Median Viral load in Pleural Effusion (copies/ml) [IQR]	not detectable	163 [18-111448]	<0,001
CMV Viral load in Blood			
Median (copies/ml) [IQR]	0 [35-0]	6694[159-3453300]	**<0,001**
>200 copies/ml (n)	3 % (3/95)	50 % (2/4)	**<0,001**

All the analyses were performed using linear mixed-effects models (LMMs) to account for repeated measurements within patients. A p-value of <0.05 was considered statistically significant.

[*] Pre-effusion malignancy: any malignancy diagnosed before the thoracentesis. Post-effusion malignancy: any new or newly detected malignancy diagnosed after the thoracentesis.

[#] Pleura total cell counts were unavailable for two pleural effusion samples and differential cell counts were missing for six pleural effusion samples; these were therefore excluded.

[**] Some lab results on pleural fluid protein (2 samples), albumin (5 samples), LDH (3 samples), Cholesterol (7 samples), and triglyceride (7 samples) were absent and thus omitted from the analysis.

pleural effusions, and overall copy numbers were low. In particular, the proportion of lymphocytic effusions as well as the share of pleural effusions of unclear etiology did not differ significantly between HHV-positive and HHV-negative cases. These findings suggest that HHV-positive effusions do not represent a distinct pleural effusion etiology after transplantation and instead point toward alternative causes of lymphocytic pleural effusions.

Advantages of the study include the use of a large, prospectively collected and representative cohort of LTX recipients from a high-volume center, in which every available pleural effusion sample undergoing thoracentesis underwent a standardized clinical, biochemical, microbiological, and cytological evaluation as well as a consistent clinical annotation. The inclusion of both early and late post-transplant pleural effusions allows for temporal analysis of viral prevalence patterns, while the parallel evaluation of multiple herpesviruses using accredited clinical-grade assays provides a more complete virological assessment than in previous studies. However, our study beholds a number of limitations: PCR-based viral load quantification cannot differentiate between replicating (viable) virus and ambient viral DNA or viral shedding, limiting the interpretation of pathogenic relevance in virus-positive samples. Undirected transport of virus particles or viral DNA from adjacent tissue compartments or localized reactivation within the pleural space cannot be definitively differentiated without matched serum or tissue-based viral quantification. The pathogenic significance of the low-copy viral detection thus remains unclear. Further, serum EBV and HHV-6 viral loads were not routinely monitored at our center, prohibiting a correlation between systemic and pleural levels.

5. Conclusions

Overall, herpesviruses, especially EBV, HHV6 and CMV were detectable in pleural effusions of lung allograft recipients however with rather low copy numbers. HHV-positive pleural effusions did not form a distinct entity. Altogether, the data argues against a potential viral origin in unexplained pleural effusions after lung transplantation and promotes an alternative hypothesis of unclear, lymphocytic effusions, e.g. an alloimmune process between the visceral (donor) and parietal (recipient) pleural sheets.

Table 5
Demographics of lung transplantation (LTX) patients by pleural effusion HHV-6 status (HHV-6- vs.HHV-6+).

Demographics	HHV-6 Negative n=80	HHV-6 Positive n=19	p-value
Age at LTX (years) (median [IQR])	59 [56-62]	61 [59-65]	0,079
Age at PE (years) (median [IQR])	62 [56-68]	64 [59-68]	0,285
LTX to PE time (in days) (median [IQR])	463 [88-1559]	24 [20-419]	0,249
% female patients	34 % (27/80)	26 % (5/19)	0,538
Patients with Multiple Effusions (n)	63 % (50/80)	37 % (7/19)	0,219
Underlying Lung Disease			0,252
Obstructive Lung Diseases (n)	28 % (22/80)	32 % (6/19)	
Interstitial Lung Diseases (n)	69 % (48/80)	68 % (13/19)	
Vascular Lung Diseases (n)	10 % (8/80)	0	
Other (n)	3 % (2/80)	0	
History of Malignacy Pre-effusion*			0,586
PTLD (n)	1 % (1/80)	0	
Other (n)	8 % (6/80)	11 % (2/19)	
History of Malignacy Post-effusion*			0,441
PTLD (n)	0	0	
Other (n)	11 % (9/80)	5 % (1/19)	
Etiology of Effusion			0,068
Heart Failure (n)	4 % (3/80)	5 % (1/19)	
Renal Failure (n)	19 % (15/80)	5 %(1/19)	
Malign (n)	4 % (3/80)	11 % (2/19)	
Post-operative (n)	16 % (13/80)	58 % (11/19)	
Infection (n)	3 % (2/80)	0	
Other (n)	6 % (5/80)	0	
Unclear (n)	49 % (39/80)	21 % (4/19)	
Type of Effusion (Transudate/Exudate)			0,238
Exudate (n)	68 % (54/80)	84 % (16/19)	
Transsudate (n)	31 % (25/80)	16 % (3/19)	
Unknown (n)	1 % (1/80)	0	
Type of Effusion (Lymphocytic/Neutrophilic)			0,986
Lymphocytic (n)	58 % (46/80)	42 % (8/19)	
Neutrophilic (n)	9 % (7/80)	32 % (6/19)	
Mixed Effusion (n)	26 % (21/80)	26 % (5/19)	
Unknown (n)	8 % (6/80)	0	
Pleura Differential Cell Count#			
Total Cell Count (/µl) (median [IQR])	794 [386-1592]	718 [206-3577]	0,14
Lymphocyte (/µl) (median [IQR])	354 [179-848]	192 [71-513]	0,216
Neutrophil Granulocyte (/µl) (median [IQR])	48 [10-142]	107 [25-2862]	**0,049**
Eosinophil Granulocyte (/µl) (median [IQR])	0 [0-5]	0 [0-0]	0,542
Macrophages (/µl) (median [IQR])	10 [0-76]	2 [0-20]	0,157
Monocytes (/µl) (median [IQR])	86 [29-199]	91 [29-428]	**0,024**
Mesothelial Cell (/µl) (median [IQR])	3 [0-32]	3 [0-14]	0,163
Pleura lab results**			
LDH (U/l) (median [IQR])	176 [132-255]	479 [188-1195]	0,113
Protein (g/dl) (median [IQR])	2,9 [2,4-3,6]	2,6 [1,9-2,8]	0,055
Albumin (g/dl) (median [IQR])	1,8 [1,3-2,3]	1,6 [1,1-1,7]	**0,039**
Cholesterol (mg/dl) (median [IQR])	56 [33-69]	54 [31-83]	0,718
Triglycerides (mg/dl) (median [IQR])	19 [13-27]	18 [15-30]	0,472
Microbiological results			0,151
aerobic and/or anaerobic bacteria positive	4 % (3/80)	21 % (4/19)	
Fungal pathogen detected	4 % (3/80)	11 % (2/19)	
Unknown (n)	3 % (2/80)	0	
HHV-6 Median Viral load in Pleural Effusion (copies/ml) [IQR]	not detectable	1300 [550-13000]	**0,006**

All the analyses were performed using linear mixed-effects models (LMMs) to account for repeated measurements within patients. A p-value of <0.05 was considered statistically significant. Pre-effusion malignancy: any malignancy diagnosed before the thoracentesis.

[*] Post-effusion malignancy: any new or newly detected malignancy diagnosed after the thoracentesis. [#]Pleura total cell counts were unavailable for two pleural

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** Some lab results on pleural fluid protein (2 samples), albumin (5 samples), LDH (3 samples), Cholesterol (7 samples), and triglyceride (7 samples) were absent and thus omitted from the analysis.

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In the development of this paper ChatGPT v5.2 was used as a basic writing assistant for refinement, correction and editing of text passages.

CRediT authorship contribution statement

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Declaration of competing interest

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcvp.2026.100245.

References

[1] R.W. Light, M.I. Macgregor, P.C. Luchsinger, WC. Ball, Pleural effusions: the diagnostic separation of transudates and exudates, Ann. Intern. Med. 77 (4) (1972) 507–513, https://doi.org/10.7326/0003-4819-77-4-507.

[2] O. Joean, M.Z. Kayser, C. Valtin, R. Ewen, J. Gottlieb, Characteristics and clinical implications of pleural effusions after lung transplantation: A retrospective analysis of 195 thoracocenteses in 113 patients, Clin Transpl. 35 (5) (2021) e14267, https://doi.org/10.1111/ctr.14267.

[3] A. Tang, H.U. Siddiqui, L. Thuita, J. Rappaport, A.C. Bribriesco, K.R. McCurry, et al., Natural history of pleural complications after lung transplantation, Ann. Thorac. Surg. 111 (2) (2021) 407–415, https://doi.org/10.1016/j.athoracsur.2020.06.052.

[4] M. Gerckens, N. Weiss, D. Khmelovska, A. Richard, M. Klemm, P. Plohmann, et al., Pleural effusions requiring thoracocentesis are associated with baseline lung allograft dysfunction and mortality in lung transplant recipients, Clin Transpl. 39 (8) (2025) e70234, https://doi.org/10.1111/ctr.70234.

[5] M.A. Judson, J.R. Handy, SA. Sahn, Pleural effusions following lung transplantation. Time course, characteristics, and clinical implications, Chest 109 (5) (1996) 1190–1194, https://doi.org/10.1378/chest.109.5.1190.

[6] F. Patrucco, A. Curtoni, F. Sidoti, E. Zanotto, A. Bondi, C. Albera, et al., Herpes virus infection in lung transplantation: diagnosis, treatment and prevention strategies, Viruses 15 (12) (2023), https://doi.org/10.3390/v15122326.

[7] S. Romero-Candeira, L. Hernández, The separation of transudates and exudates with particular reference to the protein gradient, Curr. Opin. Pulm. Med. 10 (4) (2004) 294–298, https://doi.org/10.1097/01.mcp.0000128430.34150.80.

[8] D.T. Arnold, T. Suri, F. Hamilton, A. Morley, A. Medford, I.B. Vipond, et al., Epstein-Barr virus in pleural effusions: protagonist or pretender? Eur. Respir. J. 54 (4) (2019) https://doi.org/10.1183/13993003.00825-2019.

[9] S.F.T. Thijsen, R. Luderer, J.M.H. van Gorp, S.J.G. Oudejans, AWJ. Bossink, A possible role for Epstein-Barr virus in the pathogenesis of pleural effusion, Eur. Respir. J. 26 (4) (2005) 662–666, https://doi.org/10.1183/09031936.05.00131204.

[10] H. Takei, D. Mody, Epstein-Barr virus-positive pleural effusion: clinical features, cytomorphologic characteristics, and flow cytometric immunophenotyping, Am. J. Clin. Pathol. 142 (6) (2014) 788–794, https://doi.org/10.1309/AJCP3C3BVARTZZWX.

[11] L. Zaffiri, A. Long, M.L. Neely, W.S. Cherikh, D.C. Chambers, LD. Snyder, Incidence and outcome of post-transplant lymphoproliferative disorders in lung transplant patients: analysis of ISHLT Registry, J Heart Lung Transpl. 39 (10) (2020) 1089–1099, https://doi.org/10.1016/j.healun.2020.06.010.

[12] I. Lautenschlager, RR. Razonable, Human herpesvirus-6 infections in kidney, liver, lung, and heart transplantation: review, Transpl. Int. 25 (5) (2012) 493–502, https://doi.org/10.1111/j.1432-2277.2012.01443.x.

[13] H. Arima, T. Kondo, N. Takahashi, T. Kitano, N. Kadowaki, A. Takaori-Kondo, Pleurisy as a novel clinical manifestation associated with human herpesvirus 6 after unrelated cord blood transplantation, J. Hematop. Cell Transplant. 3 (2) (2014) 59–63, https://doi.org/10.7889/hct.3.59.

[14] A. Suminoe, A. Matsuzaki, Y. Koga, K. Kusuhara, T. Hara, Human herpesvirus 6 (HHV-6)-associated pleurisy after unrelated cord blood transplantation in children with chemotherapy-resistant malignant lymphoma, J. Pediatr. Hematol. Oncol. 29 (10) (2007) 709–712, https://doi.org/10.1097/MPH.0b013e318142b50d.

[15] F.J. Trudo, E.V. Gopez, P.K. Gupta, M.G. Schuster, G. Tino, Pleural effusion due to herpes simplex type II infection in an immunocompromised host, Am. J. Respir. Crit. Care Med. 155 (1) (1997) 371–373, https://doi.org/10.1164/ajrccm.155.1.9001338.

[16] C. Langlet, C. Gaugler, M. Castaing, D. Astruc, A. Falkenrodt, A. Neuville, et al., An uncommon case of disseminated neonatal herpes simplex infection presenting with pneumonia and pleural effusions, Eur. J. Pediatr. 162 (7-8) (2003) 532–533, https://doi.org/10.1007/s00431-003-1218-7.

[17] D. Shitrit, G. Izbicki, G. Fink, D. Bendayan, D. Aravot, M. Saute, et al., Late postoperative pleural effusion following lung transplantation: characteristics and clinical implications, Eur. J. Cardio-Thorac. Surg. 23 (4) (2003) 494–496, https://doi.org/10.1016/s1010-7940(03)00020-4.