Transplant Infectious Disease, ISSN 1398-2273

Significance of new lung infiltrates in outpatients after lung and heart–lung transplantation

V. Díaz-Ravetllat, M. Greer, A. Haverich, G. Warnecke, M. Dierich, T. Welte, J. Gottlieb. Significance of new lung infiltrates in outpatients after lung and heart–lung transplantation.
Transpl Infect Dis 2014: **16**: 359–368. All rights reserved

Abstract: *Background.* Infection and rejection represent major complications following lung transplantation and are often associated with pulmonary infiltrates. The differential diagnosis of these infiltrates depends on their timing after transplantation. The aim of this study was to characterize lung transplant recipients (LTR) presenting with new pulmonary infiltrates.
Methods. A retrospective analysis of all LTR and heart–lung transplant recipients attending outpatient follow-up at our institution between September 1, 2006 and October 14, 2011 was performed. All patients presenting with new pulmonary infiltrates on chest x-ray who underwent bronchoscopy were included.
Results. A total of 913 patients accounted for 13,156 attendances, with 3,912 bronchoscopies being performed. Seventy-eight patients (9%) exhibited new pulmonary infiltrates and proceeded to bronchoscopy. Infiltrates occurred at a median 15 (interquartile range [IQR] 5–39) months after transplantation. Forty-eight patients (62%) were male, and median patient age was 47 (IQR 29–57) years. Subsequent investigation revealed pneumonia to be the underlying cause in 63 patients (81%). In the remaining patients, chronic lung allograft dysfunction (CLAD) was responsible in 6 (8%), acute rejection in 5 (6%), and toxic pneumonitis in 4 (5%) patients. Overall 1-year survival in LTR presenting with new infiltrates was 97%, compared with 96% for all LTR attending our Outpatient Department.
Conclusions. New pulmonary infiltrates occurring after the first month in LTR are most likely due to infection. Through prompt diagnosis and treatment, early mortality appears unaffected. Late mortality remains attributable to CLAD.

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Key words: bronchoalveolar lavage; bronchoscopy; immunocompromised host; lung transplantation; pneumonia

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Received 1 March 2013, revised 4 July 2013, 26 September 2013, accepted for publication 23 November 2013

DOI: 10.1111/tid.12209
Transpl Infect Dis 2014: **16:** 359–368

Pulmonary infections are common in all solid organ transplant (SOT) recipient because of their immunocompromised state (1). Diagnosis in lung transplant recipients (LTR) is difficult, as organ rejection can mimic many of the symptoms and signs of pulmonary infection. Together, infection and acute rejection (AR) represent the commonest and most serious complications after lung transplantation (LTx).

The etiology of pulmonary infiltrates appears dependent on the timing of their presentation after transplant surgery. Infection represents a major cause in the early postoperative period, with bacterial (1) and fungal (2) infections being commonest in the first month after transplantation. During the subsequent 3 months, viral and fungal infections prevail.

Other causes of pulmonary infiltrates include acute cellular rejection, chronic lung allograft dysfunction

Abbreviations: AR, acute rejection; BAL, bronchoalveolar lavage; BOS, bronchiolitis obliterans syndrome; CFU, colony-forming units; CLAD, chronic lung allograft dysfunction; CMV, cytomegalovirus; CRP, C-reactive protein; FEV1, forced expiratory volume in the first second; IQR, interquartile range; ISHLT, International Society of Heart and Lung Transplantation; LTR, lung transplant recipients; LTx, lung transplantation; SOT, solid organ transplant; TBB, transbronchial biopsy

(CLAD), and drug toxicity. AR remains common after LTx, with an estimated incidence of up to 55% in the first postoperative year (3). Interstitial infiltration, occasionally in conjunction with a pleural effusion on chest x-ray, may suggest AR. Confirmation, however, requires fiberoptic bronchoscopy and transbronchial biopsy (TBB), with a bronchoalveolar lavage (BAL) being performed to exclude concomitant infection. Incidence of AR appears greatest in the first 6 months, declining markedly thereafter (3). CLAD appears histologically as bronchiolitis obliterans and is functionally defined as bronchiolitis obliterans syndrome (BOS) by loss in expiratory lung volumes.

Drug-related pulmonary toxicity is a known complication of amiodarone (4), sirolimus (5), and mitomycin (6), all of which have treatment indications in LTR. Drug toxicity therefore requires consideration, particularly if pulmonary infiltrates develop early after drug initiation.

Chest x-ray, although representing a sensitive and readily available diagnostic tool, offers little specificity in identifying the underlying cause. Bronchoscopy represents the mainstay of diagnosis, with sampling from the lower respiratory tract (e.g., BAL and TBB) being essential to differentiate infectious and non-infectious causes of pulmonary infiltrate.

The aim of this study was to analyze the etiology of new lung infiltrates on chest x-ray in LTR who subsequently underwent bronchoscopy.

Materials and methods

Patients

A retrospective analysis was performed of all LTR and heart–lung transplant recipients attending the outpatient clinic at our institution between September 1, 2006 and October 14, 2011. Data were retrieved from our transplant database, as well as by reviewing outpatient charts.

This retrospective observational study was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. According to the principles of the Ethics Committee of the Hannover Medical School, neither ethical approval nor informed consent was necessary, as (i) data acquisition was retrospective observational within our clinic; (ii) data were anonymized; and (iii) the study relied on measurements and rescue therapies applied as part of routine care. In addition, personal data were encrypted.

Criteria for inclusion

A chest x-ray was performed in all patients at each outpatient visit. Patients with x-rays demonstrating new infiltrates, who subsequently underwent bronchoscopy within 24 h, were included. All x-rays were independently evaluated by experienced radiologists, with routine comparison to previous radiographs. The radiologists re-reviewed the films. New pulmonary infiltrates were defined as any nodular, alveolar, or interstitial change not identifiable on previous films. Computed tomography scans were performed only if infiltrates were non-resolving, or a non-infectious etiology was suspected. If patients had persistent or recurrent infiltrates, they were included only on the first occasion of a new infiltrate.

Follow-up protocol

All patients participated in our scheduled surveillance program, with additional urgent visits if new symptoms occurred. The initial outpatient attendance was scheduled 4 weeks after surgery. Thereafter, patients returned on average 7–8 times in the first year, reducing to 5–6 visits in the second year, and 3–4 visits in subsequent years.

Standard triple-drug immunosuppression consisting of a calcineurin-inhibitor, prednisolone, and either a cell-cycle inhibitor or a mammalian target of rapamycin inhibitor was used in all patients. Comprehensive anti-infective prophylaxis consisting of cotrimoxazole, along with either voriconazole or itraconazole and valganciclovir (within the first 3 months in patients with high and intermediate risk) was administered.

Along with chest x-rays, pulmonary function testing, capillary blood gas analysis, and routine blood tests were performed at each visit. Clinical examination and review of results were performed by experienced physicians, and an immediate decision regarding bronchoscopy was reached. Spirometry was performed according to guidelines provided by the American Thoracic Society and European Respiratory Society (7). BOS staging was performed according to the International Society of Heart and Lung Transplantation (ISHLT) criteria (8). Baseline forced expiratory volume in the first second (FEV1) was calculated as the mean of the best 2 postoperative measurements taken at least 3 weeks apart.

Within the surveillance program, scheduled fiberoptic bronchoscopy with BAL and TBB are performed at 1, 3, 6, and 12 months post LTx. Thereafter scheduled

bronchoscopies with BAL were performed on an annual basis. Additional bronchoscopies were performed in response to new respiratory symptoms, unexplained deterioration in graft function, or new radiological changes. Purulent secretions, when present, were directly aspirated and sent for microbiological assessment.

Quantitative cultures, Gram staining, and antigen testing (for respiratory viruses, *Legionella*, pneumococci, and *Aspergillus* galactomannan) were performed on all samples. Both acid-fast staining and cultures were used.

Pleural effusions were assessed by thoracocentesis under ultrasound or computed tomography control, if possible. Pleural fluid samples underwent direct microscopy, culture, and biochemical and immunocytological examination.

Definitions

All new infiltrates were retrospectively reviewed and their underlying cause determined by results from blood tests, microbiology, and histopathology findings, as well as the subsequent response to treatment. Pneumonia was defined as the presence of new pulmonary infiltrates, plus ≥2 of the following criteria: body temperature <36°C or >38°C, leukocyte counts <4,000 or >12,000 cells/mm^3, or purulent tracheobronchial secretions (9).

Microbiological confirmation was defined as the presence of ≥1 pathogenic microorganisms in the respective samples at the following thresholds: $>10^3$ colony-forming units (CFU)/mL in bronchial brushings, $>10^4$ CFU/mL in BAL, and/or $>10^5$ CFU/mL in sputum or tracheobronchial aspirates. Organism identification and antibiotic susceptibility testing were performed using standard methods (10). Invasive fungal infections were defined in accordance with European Organization for Research and Treatment of Cancer criteria (11). Community-acquired respiratory viruses were considered pathogenic if detected on antigen testing or polymerase chain reaction. Cytomegalovirus (CMV) was considered as causative in case of virus detection in BAL in combination with histopathological confirmation.

Choice of anti-infective treatment was taken from recent recommendations (12), the locally most frequently isolated pathogens, their antimicrobial sensitivity patterns in our institution, and previous microbiological results, which were available in all patients in our follow-up clinic. Certain bacteria and fungi isolated from respiratory samples were not considered pathogenic and not considered as causative agents (*Staphylococcus epidermidis* and *Streptococcus viridans*, *Enterococcus* species, *Candida* species).

Definition of AR was made by TBB and graded according to ISHLT criteria (13). Restoration of graft function following a steroid pulse was assumed to represent AR in cases where biopsies were not available. Standard treatment of AR consisted of 15 mg/kg methylprednisolone, administered intravenously daily for 3 days, followed by augmented oral prednisolone.

Toxic pneumonitis is the result of a causative agent, the discontinuation of which leads to resolution. Recurrence of the original lung disease or malignancy in the allograft in the post-transplantation period was histologically confirmed by lung biopsy.

Statistical analysis

Data are presented in numbers (percentages) and median (interquartile range [IQR]). Continuous variables were compared using the *t*-test and Mann–Whitney *U*-test. Categorical variables were compared using the chi-square test or the Fisher exact test. Results were considered significant for values of $P < 0.05$. The multivariate analysis was carried out by logistic regression model, using the step-wise forward method. Survival analysis after transplantation was compared using the Kaplan–Meier method with comparisons using the log-rank (Mantel–Cox) test.

Results

Patients

The follow-up period was 5 years (2006–2011) (Fig. 1). Over the period of observation, a total of 913 patients participated in 13,156 outpatient visits, resulting in 3,912 bronchoscopies being performed. Seventy-eight patients (9%) presented with new pulmonary infiltrates and subsequently underwent fiberoptic bronchoscopy. Data relating to patient demographics are displayed in Table 1. Median time to new pulmonary infiltrate was 15 months (IQR 5–39) following transplantation. In the most recent period (2011), the median time after transplantation of patients without infiltrates was 30 months (range 9–66). The commonest underlying diagnosis in the infiltrate group was cystic fibrosis. Fifty percent of these cystic fibrosis patients were colonized, with 85% colonized in the immediate postoperative period, following the results of a previous study by our group (14).

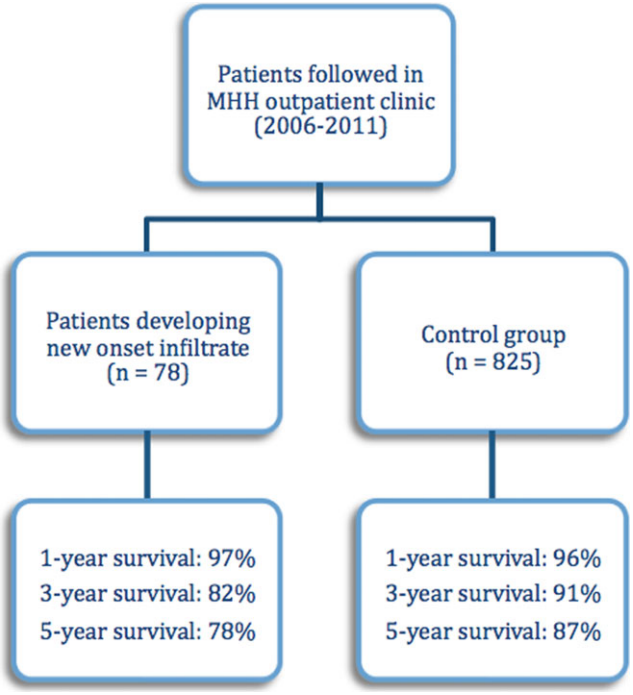

Fig. 1. Flowchart of included patients.

Factors demonstrating statistically significant differences ($P < 0.05$) between infiltrate and non-infiltrate group included pulse, temperature (<36°C and >38°C), new hypoxemia, recent loss in FEV1, leukocyte count, C-reactive protein (CRP), previous diagnosis of BOS, and type of immunosuppression (Table 1).

Characteristics of new lung infiltrates

A summary of the different etiologies of lung infiltrates are represented in Figure 2. Infection accounted for 81% of cases, with the right lower lobe being most frequently involved (Fig. 3). Thirty patients (38%) exhibited a para-pneumonic pleural effusion, 16 of which occurred in the right hemithorax. Resolution of infiltrates was common with infectious etiologies, but most patients with non-infectious etiologies had persistent infiltrates. Six patients in the infiltrate group had repeated infiltrates. Two of 4 recipients of single-lung transplant demonstrated infiltrate in the transplanted lung.

Diagnostic results

Bronchoscopy identified purulent secretions in 15 patients (19.2%), white viscous secretions in a further 4 patients (5.1%), with other secretions being recorded in an additional 15 patients (19.2%). No secretions were evident in 44 patients (56%) with reported x-ray changes. BALs were performed in 64 patients (82%), aspirated secretions in 47 (60%), and protected brush specimens in 25 (32%). From patients with new lung infiltrates of infectious origin, BAL isolation was achieved in 34/51 (67%), aspirates in 28/38 (74%), and protected brush specimens in 18/25 (72%).

TBBs were performed in 19/78 patients (24%), thoracocentesis in 2/78 patients (3%), and blood culture in 6/78 (8%). Biopsy results were grade A0 (no cellular rejection) in 12 patients, A1 (minimal acute cellular rejection) in 4, and Ax in a further 3 patients. No biopsy findings ≥A2 were recorded. With regard to B-grades, 5 patients exhibited B0, 9 were B1R, and 3 patients were B2R and Bx.

During the study period, endoscopic nebulization of mitomycin was part of the treatment algorithm for patients with bronchial stenoses. In approximately 20% of these patients, a clear time correlation (7–14 days) was noticed with a new infiltrate after topical mitomycin spray. Most of these infiltrates resolved spontaneously and other etiologies were excluded in these patients, being attributed to toxic pneumonitis.

From those patients diagnosed with pneumonia, 7 patients (11%) had leukocyte counts <4000 cells/mm^3, and 15 patients (24%) had >12,000 cells/mm^3.

The microorganisms isolated are represented in Table 2. No case of *Pneumocystis jirovecii* was recorded.

Outcome

Initial treatment of the identified infiltrates consisted of antibiotics (73%), systemic steroids (18%), or antiviral therapy (13%). Pulsed corticosteroids were commonly used in patients with clinically suspected AR. Typically, patients were treated with empiric antibiotics but failed to improve after 3–7 days; this was the case in 18% of patients. A final diagnosis of AR by chart review was made if there was a prompt time-related response (clearing on chest x-ray and clinical improvement) to pulse-dose steroids; this was the case in a third of these patients (6%). Thirty-eight (48%) patients required hospitalization.

Quinolones (28%) were the most commonly used antibiotics, followed by carbapenems (14%), acyl-aminopenicillin/beta lactamase inhibitors (11%), and cephalosporins (6%). In 5% of cases, inhaled antibiotics were given.

Twelve patients presenting with new lung infiltration fulfilled criteria for respiratory failure (partial pressure

Demographics of the study population

	Infiltrates group (n = 78)	Total cohort (n = 780)	P-value
General characteristics			
Gender, male/female	48/30	443/337	0.588
Age at transplantation, years	44 (28–54)	46 (33–55)	0.275
Age at presentation, years	47 (29–57)	Not applicable	
Months between transplantation and infiltrate onset	15 (5–39)	Not applicable	
Pulse, bpm	91 (81–103)	76 (67–89)	<0.001
Temperature >38°C	4 (5)	4 (1)	<0.001
Temperature <36°C	15 (19)	361 (46)	0.002
New hypoxemia	12 (15)	31 (4)	<0.001
ΔFEV1 in % previous	−9 (−20 to −2)	−1 (−7 to +4)	<0.001
Leukocyte count (Tsd/μL)	9 (6–12)	7 (5–9)	<0.001
CRP (mg/L)	60 (15–130)	2 (1–8)	<0.001
Previous diagnosis of BOS	55 (71)	634 (81)	0.023
BOS 0	18 (23)	360 (46)	
BOS 1	14 (18)	112 (14)	
BOS 2	17 (22)	67 (9)	
BOS 3	6 (8)	95 (12)	
Indication for transplantation			0.009
CF	29 (37)	171 (22)	
Emphysema	14 (18)	184 (24)	
IPF	4 (5)	103 (13)	
PH/Eisenmenger's syndrome	3 (4)	68 (9)	
Alpha-1 antitrypsin deficiency	10 (13)	65 (8)	
Other etiologies	18 (23)	189 (24)	
Type of transplantation			0.189
Single lung transplantation	4 (5)	89 (11)	
Bilateral lung transplantation	69 (89)	629 (81)	
Heart–lung transplantation	5 (6)	62 (8)	
Type of immunosuppression			<0.001
Tacrolimus	49 (63)	395 (51)	
Cyclosporine	28 (36)	371 (48)	
MMF	65 (83)	607 (78)	
Azathioprine	7 (9)	67 (9)	
Everolimus/sirolimus	6 (8)	144 (18)	
MTX	1 (1)	4 (1)	

Data are presented as number (%) or median (IQR). Percentages were based on the number of patients with non-missing information.
bpm, beats per minute; Δ, change; FEV1, forced expiratory volume in the first second; Tsd, thousand; CRP, C-reactive protein; BOS, bronchiolitis obliterans syndrome; CF, cystic fibrosis; IPF, idiopathic pulmonary fibrosis; PH, pulmonary hypertension; MMF, mycophenolate mofetil; MTX, methotrexate.

Table 1

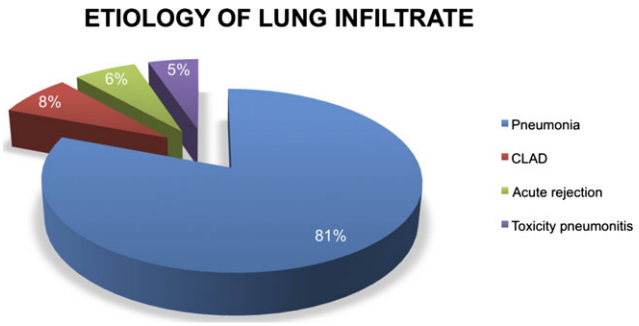

Fig. 2. Final etiology of infiltrate. CLAD, chronic lung allograft dysfunction.

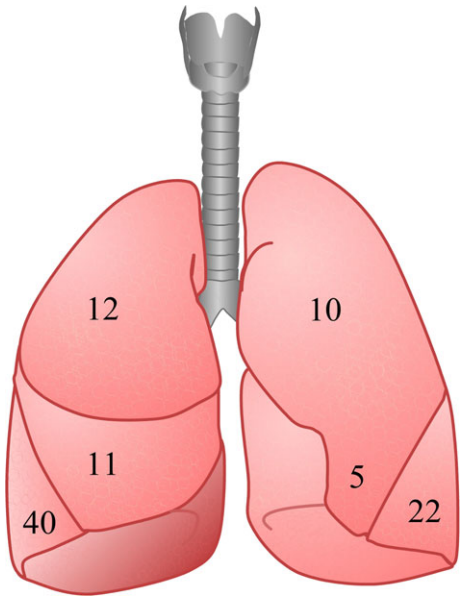

Fig. 3. Lobar distribution of infiltrates. Graphic representation of the lobar distribution of new lung infiltrates in percentages. The most frequent lobe involved was right lower lobe in 40%, followed by left lower lobe in 22%, right upper lobe in 12%, middle lobe in 11%, left upper lobe in 10%, and lingula in 5%.

of oxygen < 60 mmHg). Of the CLAD-naive patients presenting with pulmonary infiltrates, 7 (9%) developed CLAD in the subsequent 12 months, compared with 33 (4%) patients in the non-infiltrate group, with a statistically significant difference ($P = 0.007$). However, overall 1-year survival was similar in both groups, with 96% of patients in the control group and 97% of those with pulmonary infiltration surviving. Subsequent 3- and 5-year survival figures did demonstrate significance in favor of patients without infiltrates compared with those with infiltrates, with 82% vs. 91% and 78% vs. 87% survival, respectively ($P = 0.002$) (Fig. 4).

Characteristics of isolated microorganisms

Isolated microorganisms	Infiltrates group ($n = 78$) No. (%)
Bacterial	
Pseudomonas aeruginosa	18 (16)
Staphylococcus aureus	9 (8)
Streptococcus pneumoniae	5 (4)
Streptococcus coagulase negative	4 (4)
Achromobacter	4 (4)
Serratia marcescens	3 (3)
Streptococcus viridans	3 (3)
Haemophilus influenzae	2 (2)
Stenotrophomonas maltophilia	2 (2)
Enterococcus species	2 (2)
Proteus mirabilis	2 (2)
Viral	
Cytomegalovirus	11 (10)
Coronavirus	3 (3)
Fungal	
Aspergillus fumigatus	5 (4)
Polymicrobial	9 (8)
Other	27 (25)

Percentages are proportional.

Table 2

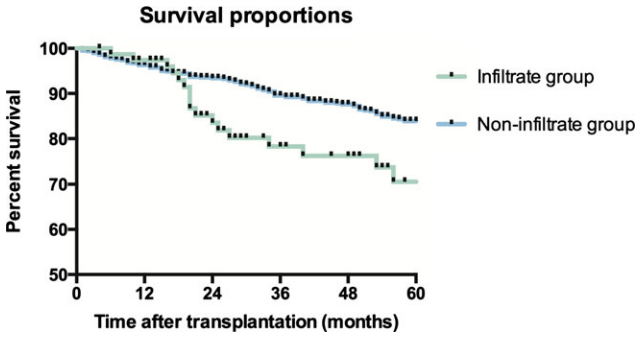

Fig. 4. Survival for outpatients with and without lung infiltrates.

Regarding outcomes based on underlying cause, patients exhibiting pulmonary infiltrates from infection had marginally worse 1-year survival (97% vs. 100%) compared with those with a non-infective etiology. Subsequently, at 3 and 5 years (83% vs. 80%, and 78% vs. 80%, respectively), no differences in survival were observed.

Causes of death

CLAD represented the principal cause of death in patients exhibiting new pulmonary infiltrates (n = 12, 52%). The remaining causes of death included sepsis (n = 2, 9%), neoplasm (n = 1, 4%), and pulmonary embolism (n = 1, 4%). Two patients died within 1 year of transplantation because of sepsis. In patients demonstrating pulmonary infiltrates caused by infection, 8/18 deaths (44%) were attributable to CLAD.

The causes of death from all outpatients are shown in Table 3. Two patients died early (<1 year after presentation) from sepsis; all other causes were attributed to late mortality. CLAD was the cause of death in 8/18 patients with an infectious origin, 0/1 patient with AR, 3/3 patients with CLAD, and 1/1 patient with pulmonary toxicity.

Risk factors: univariate and multivariate analyses

In the multivariate analysis, a CRP ≥90 mg/L proved the sole statistically significant independent risk factor for pneumonia (Table 4). The sensitivity of elevated CRP (≥90 mg/L) for an infectious etiology was 32% (20/63), whereas the specificity was 40% (6/15).

Causes of death

Causes of death	Infiltrates group (n = 78) No. (%)	Non-infiltrates group (n = 825) No. (%)
BOS	12 (52.2)	67 (38.3)
Unknown	3 (13)	27 (15.4)
Sepsis	2 (8.7)	15 (8.6)
Pneumonia	2 (8.7)	14 (8)
Neoplasic	1 (4.3)	15 (8.6)
Cardiovascular failure	1 (4.3)	9 (5.1)
Multiple organ failure	1 (4.3)	6 (3.4)
Pulmonary embolism/ hemorrhage	1 (4.3)	5 (2.9)
ARDS	0 (0)	4 (2.3)
Renal failure	0 (0)	4 (2.3)
Cerebral	0 (0)	3 (1.7)
Primary graft dysfunction	0 (0)	2 (1.1)
Hematemesis/hemoptysis	0 (0)	2 (1.1)
Liver cirrhosis	0 (0)	1 (0.6)
Traumatic	0 (0)	1 (0.6)

BOS, bronchiolitis obliterans syndrome; ARDS, acute respiratory distress syndrome.

Table 3

Univariate and multivariate analyses

	Univariate					Multivariate		
Pneumonia as etiology of new lung infiltrate	Yes (%)	No (%)	HR	95% CI	P-value	HR	95% CI	P-value
CRP (≥90 mg/L)	20 (32)	9 (60)	3.225	1.010–10.30	0.043	3.474	1.084–11.133	0.036
PCT (≥0.1 μg/L)	34 (54)	13 (87)	5.544	1.155–26.621	0.018			
Neutrophils in BAL (>50%)	23 (37)	4 (27)			0.472			
BAL cell count (>600 cells/μL)	27 (43)	3 (20)			0.5			
Fever (>38°C)	4 (6)	0 (0)			0.263			
Hypothermia (<36°C)	12 (19)	3 (20)			0.933			
Multilobar	26 (41)	7 (47)			0.704			
Bilateral infiltrates	26 (41)	7 (47)			0.704			
Purulent secretions	11 (17)	4 (27)			0.416			
Pleural effusion	23 (37)	7 (47)			0.467			
Time between transplantation and infiltrate onset >1 year	36 (57)	10 (67)			0.5			
FEV1 drop >20% with regard to previous one	17 (27)	3 (20)			0.578			

HR, hazard ratio; CI, confidence interval; CRP, C-reactive protein; PCT, procalcitonin; BAL, bronchoalveolar lavage; FEV1, forced expiratory volume in the first second.

Table 4

Discussion

To our knowledge, this is the first study investigating the incidence and etiology of pulmonary infiltrates in LTR. The foremost findings of our study are that infiltrates were identified in 9% of our outpatient attendees, and infections were identified as the primary cause. The primary pathogens responsible for pneumonia were bacterial (50%), fungal (37%), and viral (13%). Non-infectious origin, especially CLAD, is an important differential diagnosis.

As reported in the literature (15), the most frequent lobes involved in the infiltrates group were middle and lower lobes (78% in total), associated with pleural effusion in 38% of cases.

To date, the largest study examining post-LTx pneumonia was published by the Spanish Research Network of Transplantation (RESITRA) (16). In contrast to our data, their cohort of 236 patients focused mainly on the early postoperative period, with median follow-up being 180 days and a median time to diagnosis of 34 days post transplantation. Nonetheless, they identified a similar pathogen profile with bacterial predominance and *Pseudomonas aeruginosa*, CMV, and *Aspergillus* species again proving common. In contrast to our findings that infiltrates had no bearing on survival, early postoperative pneumonia detrimentally affected 1-year survival (74% vs. 99%) in their study (16). Our results corroborate several previous studies that identified bacterial pneumonia, due to *Staphylococcus* species and *P. aeruginosa*, as the foremost post-transplantation infection (17). As we observed, *Aspergillus fumigatus* has been previously reported as the commonest fungal organism.

In a retrospective study by Joos et al. (18), 1066 immunocompromised patients with pneumonia were included (among whom 173 were SOT recipients, including heart and lung transplants, but the exact number of them was not specified). In SOT recipients, the main pathogens isolated by BAL were also bacteria (26%) and CMV (27%) (18).

The reason for the lower rates of non-bacterial infections might be that definitions of CMV and invasive fungi were stricter in our study, and fungal prophylaxis more intense, than in other studies. No case of *P. jirovecii* was recorded in our study, which reflects the effect of strict prophylaxis in all patients.

Aspergillus infection (colonization or invasion [19]), bacterial colonization or infection (15, 20, 21), and respiratory viruses (22) increase the risk of graft failure and may impact survival. According to previous studies, symptomatic viral infection increases the risk for new onset of CLAD. Risk to develop BOS was especially increased after paramyxovirus infection (22). The overall long-term survival was significantly lower in those outpatients diagnosed with new lung infiltrates compared with those who did not present with new infiltrates.

As colonization was more frequent in recipients with infiltrates, and colonization is a risk factor for CLAD (14, 20, 21), these patients might have an increased risk as well.

CLAD is an important differential diagnosis for pulmonary infiltrates following LTx. Restrictive allograft syndrome is a recently recognized phenotype, defined by Sato et al. (23) as CLAD with an irreversible decline in total lung capacity (which is considered to be more accurate to distinguish restrictive physiology) to <90% of baseline. Patients with restrictive allograft syndrome typically demonstrated significant imaging findings of peripheral interstitial lung disease and lung infiltrates (23).

The spectrum of non-infectious causes in LTR is broad. Pulmonary infiltrates on postoperative chest x-rays are common in lung vascular injury of the donor, re-implantation disease (24), reperfusion edema (25), or primary graft dysfunction (26), vascular obstruction, adult respiratory distress syndrome (27), infection, atelectasis (26), rejection, pharmacological toxicity, and post-transplantation lymphoproliferative disorders (28). Congestive heart failure and malignancy, among others, remain causative explanations.

The cause of an early radiographic infiltrate is frequently determined using standard tools of clinical assessment, and judicious use of BAL and TBB (27). Cultures of BAL fluid are useful to detect pneumonia caused by bacteria, virus, or fungi (27). In fact, the use of protected BAL has been shown to be more effective than the use of protected brush specimen in the diagnosis of bacterial pneumonia in immunocompromised patients after bone marrow transplantation (29).

Although TBB may have a 15–28% false-negative rate for rejection, it remains the "gold standard" in practical terms for the diagnosis of AR (30). On biopsy samples, perivascular mononuclear infiltrates remain the cornerstone for AR diagnosis, but are only valid in the absence of infection (31). According to the histopathological definition of acute cellular rejection (13), it requires the exclusion of acute infection. Following our database, TBBs were performed in 24% of patients. Only patients with adequate tissue material were included (ISHLT criteria) and counted as diagnostic. Some patients with respiratory failure were excluded. This might have resulted in a lower-than-expected number of biopsies. Nevertheless, according to ISHLT criteria (13), it was also noted that "infection/rejection often occur

together, they can be confused histologically, and infection needs to be rigorously excluded for the accurate and reproducible interpretation of pulmonary allograft biopsies." Certainly, this problem in definition limits the proportion of true rejection episodes.

According to a prospective study by Stolz et al. (32), in a heterogeneous population of immunocompromised patients, neutrophilia in the BAL fluid and increased serum levels of CRP and procalcitonin were significantly associated with bacterial infection. Infiltrates were documented in most cases (71%), and they were present significantly more often in patients with proven or possible bacterial infection compared with non-bacterial conditions (32). In our study, a statistically significant difference in CRP and procalcitonin values was also found between infectious and non-infectious etiology, whereas BAL neutrophilia did not achieve statistical significance. This difference may possibly be a result of non-infectious BAL neutrophilia, often observed in LTR, especially in those with CLAD (33). Significant predictors of infectious origin in univariate analysis were CRP and procalcitonin, whereas the only factor that appeared in the multivariate analysis as an independent risk factor for pneumonia was CRP. Owing to smaller number of study subjects, we were unable to evaluate other independent predictors.

A major limitation of our study is the retrospective design and being a single-center analysis. The defined sample size in the infiltrates group is small, which makes a robust analysis of all related questions difficult.

The development of pulmonary infiltrates is a frequent life-threatening complication in immunocompromised patients, requiring early diagnosis and specific treatment. Further multicenter prospective studies are needed to determine the potential benefit of combined diagnostic approaches in outpatients, in terms of improving surveillance, early diagnosis, and treatment of the primary causes of these infiltrates. Outpatient LTR with new infiltrates should raise a high suspicion of infection and need an invasive workup with lower respiratory tract sampling including TBB. Patients with infiltrates should be followed closely and monitored for signs of chronic graft dysfunction.

Acknowledgements:

Author contributions: V.D.R. had full access to all of the data in the study, took responsibility for the integrity of the data, contributed to the data collection and analyses, and drafted the paper. M.G. contributed to review and writing of the paper. J.G. and T.W. contributed to the design of the study, data collection, analyses and review, and writing of the paper. A.H., G.W., and M.D. contributed to review of the paper.

References

1. Dauber JH, Paradis IL, Dummer JS. Infectious complications in pulmonary allograft recipients. Clin Chest Med 1990; 11 (2): 291–308.
2. Alexander BD, Tapson VF. Infectious complications of lung transplantation. Transpl Infect Dis 2001; 3 (3): 128–137.
3. Martinu T, Chen DF, Palmer SM. Acute rejection and humoral sensitization in lung transplant recipients. Proc Am Thorac Soc 2009; 6 (1): 54–65.
4. Ashrafian H, Davey P. Is amiodarone an underrecognized cause of acute respiratory failure in the ICU? Chest 2001; 120 (1): 275–282.
5. Seethamraju H, Kaleekal TS, Bag R. Pulmonary toxicity of sirolimus in lung transplant patients. Chest 2003; 124 (4 Meeting Abstracts): 101S.
6. Okuno SH, Frytak S. Mitomycin lung toxicity. Acute and chronic phases. Am J Clin Oncol 1997; 20 (3): 282–284.
7. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J 2005; 26 (2): 319–338.
8. Estenne M, Maurer JR, Boehler A, et al. Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. J Heart Lung Transplant 2002; 21 (3): 297–310.
9. Woodhead MA, Torres A. Definition and classification of community-acquired and nosocomial pneumonias. In: Torres A, Woodhead MA, eds. Pneumonia. Sheffield, UK: European Respiratory Society Journals Ltd., 1997: 1–12.
10. Murray PRBE, Pfaller MA, Tenover FC, Yolken RH. Manual of Clinical Microbiology (6th edn). Washington, DC: American Society for Microbiology, 1995.
11. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 2008; 46 (12): 1813–1821.
12. Kothe H, Dalhoff K. Pneumonia in the immunocompromised patient. European Respiratory Monograph: Respiratory Emergencies: European Respiratory Society Journals Ltd, 2006: 200–213.
13. Stewart S, Fishbein MC, Snell GI, et al. Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. J Heart Lung Transplant 2007; 26 (12): 1229–1242.
14. Gottlieb J, Mattner F, Weissbrodt H, et al. Impact of graft colonization with gram-negative bacteria after lung transplantation on the development of bronchiolitis obliterans syndrome in recipients with cystic fibrosis. Respir Med 2009; 103 (5): 743–749.
15. Collins J. Imaging of the chest after lung transplantation. J Thorac Imaging 2002; 17 (2): 102–112.
16. Aguilar-Guisado M, Givalda J, Ussetti P, et al. Pneumonia after lung transplantation in the RESITRA Cohort: a multicenter prospective study. Am J Transplant 2007; 7 (8): 1989–1996.
17. Remund KF, Best M, Egan JJ. Infections relevant to lung transplantation. Proc Am Thorac Soc 2009; 6 (1): 94–100.

18. Joos L, Chhajed PN, Wallner J, et al. Pulmonary infections diagnosed by BAL: a 12-year experience in 1066 immunocompromised patients. Respir Med 2007; 101 (1): 93–97.
19. Iversen M, Burton CM, Vand S, et al. *Aspergillus* infection in lung transplant patients: incidence and prognosis. Eur J Clin Microbiol Infect Dis 2007; 26 (12): 879–886.
20. Vos R, Vanaudenaerde BM, De Vleeschauwer SI, et al. Circulating and intrapulmonary C-reactive protein: a predictor of bronchiolitis obliterans syndrome and pulmonary allograft outcome. J Heart Lung Transplant 2009; 28 (8): 799–807.
21. Botha P, Archer L, Anderson RL, et al. *Pseudomonas aeruginosa* colonization of the allograft after lung transplantation and the risk of bronchiolitis obliterans syndrome. Transplantation 2008; 85 (5): 771–774.
22. Gottlieb J, Schulz TF, Welte T, et al. Community-acquired respiratory viral infections in lung transplant recipients: a single season cohort study. Transplantation 2009; 87 (10): 1530–1537.
23. Sato M, Waddell TK, Wagnetz U, et al. Restrictive allograft syndrome (RAS): a novel form of chronic lung allograft dysfunction. J Heart Lung Transplant 2011; 30 (7): 735–742.
24. Angles R, Tenorio L, Bravo C, Teixidor J, Rochera M, de Latorre FJ. [Reimplantation lesion in the postoperative period of lung transplantation. Incidence, predictive factors, prognosis and outcome. The Lung Transplantation Group of Vall d'Hebron University General Hospital.] Med Clin (Barc) 1999; 113 (3): 81–84.
25. Anderson DC, Glazer HS, Semenkovich JW, et al. Lung transplant edema: chest radiography after lung transplantation–the first 10 days. Radiology 1995; 195 (1): 275–281.
26. Oto T, Griffiths AP, Levvey BJ, Williams TJ, Snell GI. Unilateral radiographic abnormalities after bilateral lung transplantation: exclusion from the definition of primary graft dysfunction? J Thorac Cardiovasc Surg 2006; 132 (6): 1441–1446.
27. Paradis IL, Duncan SR, Dauber JH, Yousem S, Hardesty R, Griffith B. Distinguishing between infection, rejection, and the adult respiratory distress syndrome after human lung transplantation. J Heart Lung Transplant 1992; 11 (4 Pt 2): S232–S236.
28. Pickhardt PJ, Siegel MJ, Anderson DC, Hayashi R, DeBaun MR. Chest radiography as a predictor of outcome in posttransplantation lymphoproliferative disorder in lung allograft recipients. AJR Am J Roentgenol 1998; 171 (2): 375–382.
29. Dunagan DP, Baker AM, Hurd DD, Haponik EF. Bronchoscopic evaluation of pulmonary infiltrates following bone marrow transplantation. Chest 1997; 111 (1): 135–141.
30. Guilinger RA, Paradis IL, Dauber JH, et al. The importance of bronchoscopy with transbronchial biopsy and bronchoalveolar lavage in the management of lung transplant recipients. Am J Respir Crit Care Med 1995; 152 (6 Pt 1): 2037–2043.
31. Colombat M, Groussard O, Lautrette A, et al. Analysis of the different histologic lesions observed in transbronchial biopsy for the diagnosis of acute rejection. Clinicopathologic correlations during the first 6 months after lung transplantation. Hum Pathol 2005; 36 (4): 387–394.
32. Stolz D, Stulz A, Muller B, Gratwohl A, Tamm M. BAL neutrophils, serum procalcitonin, and C-reactive protein to predict bacterial infection in the immunocompromised host. Chest 2007; 132 (2): 504–514.
33. Verleden GM, Vanaudenaerde BM, Dupont LJ, Van Raemdonck DE. Azithromycin reduces airway neutrophilia and interleukin-8 in patients with bronchiolitis obliterans syndrome. Am J Respir Crit Care Med 2006; 174 (5): 566–570.