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T-lymphocyte subsets in lung transplant recipients: association between nadir CD4 T-cell count and viral infections after transplantation

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ABSTRACT

Background: Little is known about the kinetics of T-cell subsets in lung transplant recipients (LTR) and their association with the occurrence of opportunistic infections (OI).
Objectives: To analyze the kinetics of T-lymphocyte subsets in LTR and the association between nadir CD4 T-cell count and viral infections after transplantation.
Study design: Serial measurements of peripheral blood CD4 and CD8 T-cell counts obtained during the first year post-transplantation from 83 consecutive LTR and their correlation with both viral OI and community-acquired infections post-transplantation were retrospectively analyzed.
Results: LTR with a nadir CD4 T-cell count <200 cells/μl had consistently lower CD4 and CD8 T-cell counts than LTR with a nadir CD4 T-cell count >200 cells/μl ($p < 0.001$). In LTR with a nadir CD4 T-cell count <200 cells/μl, the cumulative incidence of viral infections detected in peripheral blood and in bronchoalveolar lavage (BAL) samples was higher than in LTR with a nadir CD4 T-cell count >200 cells/μl ($p = 0.0012$ and $p = 0.0058$, respectively). A nadir CD4 T-cell count <200 cells/μl within the first three months post-transplantation predicted a higher frequency of viral infectious episodes in BAL samples within the subsequent six month period ($p = 0.0066$).
Conclusions: Stratification of patients according to nadir CD4 T-cell count may represent a new and simple approach for early identification of patients at risk for subsequent virus infections.

1. Background

Lung transplantation is the last treatment option for advanced stage lung disease. Despite advances in immunosuppressive regimens and management after transplantation, infections and rejection remain significant complications in lung transplant recipients (LTR). Compared with other solid organ transplant recipients, LTR are more vulnerable to infections. This is related to intensive immunosuppressive treatments as well as to exposure of the allograft to environmental agents [1]. Thus, both opportunistic (OI) and community-acquired (CAI) infections remain a major cause of morbidity and mortality in LTR. In addition, the role of viral infections in favoring the occurrence of chronic lung allograft dysfunction has been suggested [2,3]. Therefore, early detection and appropriate treatment of infections is mandatory to improve the outcome in these patients. While microbial diagnostic techniques have been developed and/or improved over the years, markers of immunological recovery predicting the risk of infection after transplantation are still not routinely applied during the follow-up period.

Abbreviations: LTR, lung transplant recipients; OI, opportunistic infections; CAI, community acquired infections; HCMV, human cytomegalovirus; BAL, bronchoalveolar lavage; ATG, antithymocyte globulin; EBV, Epstein-Barr virus; HSV, herpes simplex virus; VZV, varicella zoster virus; DFA, direct fluorescence antibody; ROC, receiver operating characteristic; SD, standard deviation; IQR, interquartile range; CI, confidence interval.

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Several studies have demonstrated that a low CD4 T-cell count represents a major risk factor for development of opportunistic complications [4,5], malignancies [6] and poor long-term outcome [7,8] in individuals with human immunodeficiency virus type-1 (HIV-1) infection. Additionally, it has been shown that a low nadir CD4 T-cell count predicts limited immune reconstitution [9,10] and poor CD4 T-cell recovery [11] in HIV-1-infected patients who were receiving antiretroviral therapy. Recently, we have shown that patients developing OI after heart transplantation had low nadir CD4 T-cell counts, while low CD8 T-cell counts were associated with the risk of OI following kidney transplantation [12]. Similarly, a more recent study confirmed that low T-cell counts at month 1 post-transplant predicts the subsequent occurrence of OI after kidney transplantation [13]. However, limited data are available in LTR [14,15].

2. Objectives

To analyze the kinetics of CD4 and CD8 T-cell counts in peripheral blood obtained from LTR during the first year after transplantation and evaluated the association between nadir CD4 T-cell count with the development of viral infections post-transplantation.

3. Study design

3.1. Patients and samples

Eighty-three consecutive patients who received a lung transplantation at the Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, between August 2003 and January 2011, with at least one-year follow-up, were retrospectively analyzed. Demographic characteristics, donor and recipient human cytomegalovirus (HCMV) serostatus, transplant indication, type of lung transplant, induction therapy and immunosuppressive treatment, acute rejection episodes and cause of death were obtained from the patient's medical records and are presented in Table 1. Peripheral blood and bronchoalveolar lavage (BAL) samples were obtained as part of routine post-transplant monitoring of infectious complications.

Table 1
Characteristics of the study population ($n = 83$).

Characteristic	Median [interquartile range] or n (%)
Age (years) at transplantation	51.6 [37.2–58.8]
Gender	
Male	63 (75.9)
Female	20 (24.1)
Donor (D)/Recipient (R) human cytomegalovirus serostatus	
D+/R+	66 (79.5)
D+/R−	8 (9.6)
D−/R+	7 (8.4)
D−/R−	2 (2.4)
Transplant indication	
Idiopathic pulmonary fibrosis	34 (41.0)
Cystic fibrosis	14 (16.9)
Pulmonary emphysema	13 (15.7)
Pulmonary hypertension	9 (10.8)
Bronchiectasis	5 (6.0)
Eisenmenger syndrome	2 (2.4)
Histiocytosis X	1 (1.2)
Bullous dystrophy	1 (1.2)
Nonspecific interstitial pneumonia	1 (1.2)
Ebstein's anomaly	1 (1.2)
Acute respiratory distress syndrome	1 (1.2)
Lymphangioleiomyomatosis	1 (1.2)
Type of lung transplant	
Single	28 (33.7)
Double	48 (57.8)
Heart-lung	7 (8.4)
Induction therapy	
Antithymocyte globulin	24 (28.9)
No	59 (71.1)
Immunosuppressive regimen	
CsA or tacrolimus/AZA/steroids	40 (48.2)
CsA or tacrolimus/MMF/steroids	43 (51.8)
Acute rejection (AR) ≥A2	44 (53)
AR episodes per patient	2 [1–3]
Deceased patients	14 (16.9)
Time (months) to death after transplant	16.8 [14-21.6]
Infections	7 (50.0)
Bronchiolitis obliterans syndrome	3 (21.4)
Neoplasia	3 (21.4)
Hepatic failure	1 (7.1)

CsA, cyclosporine A; AZA, azathioprine; MMF, mycophenolate mofetil.

3.2. Immunosuppressive therapy

Induction therapy with antithymocyte globulin (ATG) was used in 28.9% of LTR. All LTR were receiving a standard triple immunosuppressive regimen including calcineurin inhibitors (cyclosporine A or tacrolimus), azathioprine or mycophenolate mofetil, and steroids and were treated with steroid pulses when an episode of acute rejection was diagnosed; grade ≥ A2 rejection were treated with a steroid bolus. No patient received antiviral drugs (ganciclovir or valganciclovir) for HCMV prophylaxis, while pre-emptive antiviral therapy was initiated when the HCMV DNA level exceeded 3×10^5 copies/ml blood or 1×10^5 copies/ml BAL [16,17].

3.3. Viral infections

In the OI group were considered those infections related to cellular and humoral immunosuppression, which included in our study HCMV, Epstein-Barr virus (EBV), herpes simplex virus (HSV), varicella zoster virus (VZV), human herpesvirus 6 (HHV-6) and polyomavirus [12,13,18]. Infections were defined as the presence of viral DNA in blood or BAL samples, determined using quantitative real-time PCR [16,19,20], in the absence of symptoms. Viral syndrome and disease were defined according to established criteria, which included quantification of viral DNA in blood as well as body fluids or biopsies in the presence of symptoms [21]. In the CAI group were included infections that could be acquired in the community or during hospitalization (nosocomial infection) independently from the patient's immune status, which include in our study parvovirus B19, rhinovirus, coronavirus, parainfluenza virus, adenovirus, influenza A and B. Parvovirus B19, rhinovirus and coronavirus were quantified by real-time PCR as described [20,22,23]. Samples were tested for parainfluenza virus, adenovirus, influenza A and B by direct fluorescence antibody (DFA) staining as described [24]. Samples positive by DFA were quantified by real-time PCR for adenovirus [25] and influenza A and B [26,27].

3.4. T-cell subsets

Peripheral whole blood was stained with anti-CD3 (FITC-conjugated), anti-CD45 (APC-Alexa Fluor 750-conjugated), anti-CD4 (APC-conjugated) and anti-CD8 (PE-conjugated) monoclonal antibodies (Beckman Coulter, Milan, Italy). After lysis of red cells, the percentage of CD4 and CD8 T lymphocytes was determined using a Navios™ Flow Cytometer System (Beckman Coulter). Absolute CD4 and CD8 T-cell counts (cells/μl) were calculated taking into account the total (WBC) and differential lymphocyte counts estimated by an automated hematology analyzer used in our institution's clinical laboratory. Measurements were performed in a

total of 703 serial samples obtained during the first post-transplant year; the median number of samples per patient was 8 (range 3–16). Since in HIV-1-infected individuals [6] and heart transplant recipients [12] the risk of OI increases below the threshold CD4 T-cell count of circa 200 cells/μl, the LTR analyzed in this study were stratified in relation to an arbitrary CD4 T-cell count of 200 cells/μl (referred to as the nadir CD4 T-cell count).

3.5. Statistical analysis

CD4 T-cell count, CD8 T-cell count and CD4/CD8 T-cell ratio were compared over time using a longitudinal data analysis method. Population-average generalized estimating equation models with an autocorrelation of order 1 were fitted considering CD4 T-cell count or CD8 T-cell count as dependent variables and the presence of infections, gender, age, nadir CD4 T-cell count (<200 cells/μl or >200 cells/μl), induction therapy, type of immunosuppressive regimen and acute rejection episodes as independent variables. Data analysis was performed with the STATA statistical package (version 11, Stata Corporation, College Station, 2010, Texas, USA). The Kaplan-Meier method and the receiver operating characteristic (ROC) analysis were performed with GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA). All tests were two-sided. A $p < 0.05$ was considered statistically significant.

4. Results

4.1. Kinetics of T-cell subsets

At baseline, the mean (± standard deviation [SD]) CD4 T-cell count was 331.1 ± 71.9 cells/μl and thereafter increased with time reaching 616.3 ± 52.9 cells/μl at month 12 (Fig. 1A). The mean CD8 T-cell count at baseline was 449.1 ± 57.3 cells/μl, this level dropped to 392.2 ± 43.6 cells/μl at month one and then improved over the follow-up period reaching 893.8 ± 129.4 cells/μl at month 12 (Fig. 1A). The mean CD4/CD8 T-cell ratio at baseline was 0.7 ± 0.1 and the highest level was observed at month one (1.7 ± 0.2), followed by a slight progressive decline over time reaching 0.9 ± 0.1 at month 12 (Fig. 1B).

4.2. Kinetics of T-cell subsets based on nadir CD4 T-cell count

Twenty-five (30.1%) LTR had a nadir CD4 T-cell count <200 cells/μl (median 120, interquartile range [IQR] 76.5–167 cells/μl) reached over a median of 3.9 (IQR 1.3–6.2) months post-transplant, whereas the remaining 58 (69.9%) LTR had a nadir CD4 T-cell count >200 cells/μl (median 407, IQR 290–537 cells/μl) reached over a median of 3.8 (IQR 1.5–6.9) months post-transplant. Patients with a nadir CD4 T-cell count <200 cells/μl maintained significantly lower CD4 T-cell counts (coefficient −427.9, 95% confidence interval [CI] −518.0 to −337.8, $p < 0.001$) up to month 12 post-transplant than patients with a nadir CD4 T-cell count >200 cells/μl (Fig. 1C). In fact, while patients with a nadir CD4 T-cell count >200 cells/μl maintained high CD4 T-cell counts during the follow-up period (mean ± SD, 506.4 ± 53.3 cells/μl at baseline vs. 754.9 ± 67.5 cells/μl at month 12), patients with a nadir CD4 T-cell count <200 cells/μl showed a limited increase over time 155.8 ± 29.2 cells/μl at baseline vs. 362.1 ± 42.1 cells/μl at month 12, (Fig. 1C). Additionally, patients with a nadir CD4 T-cell count <200 cells/μl had significantly lower CD8 T-cell counts (coefficient −319.7, 95% CI −458.9 to −180.4, $p < 0.001$) than patients with a nadir CD4 T-cell count >200 cells/μl (Fig. 1D).

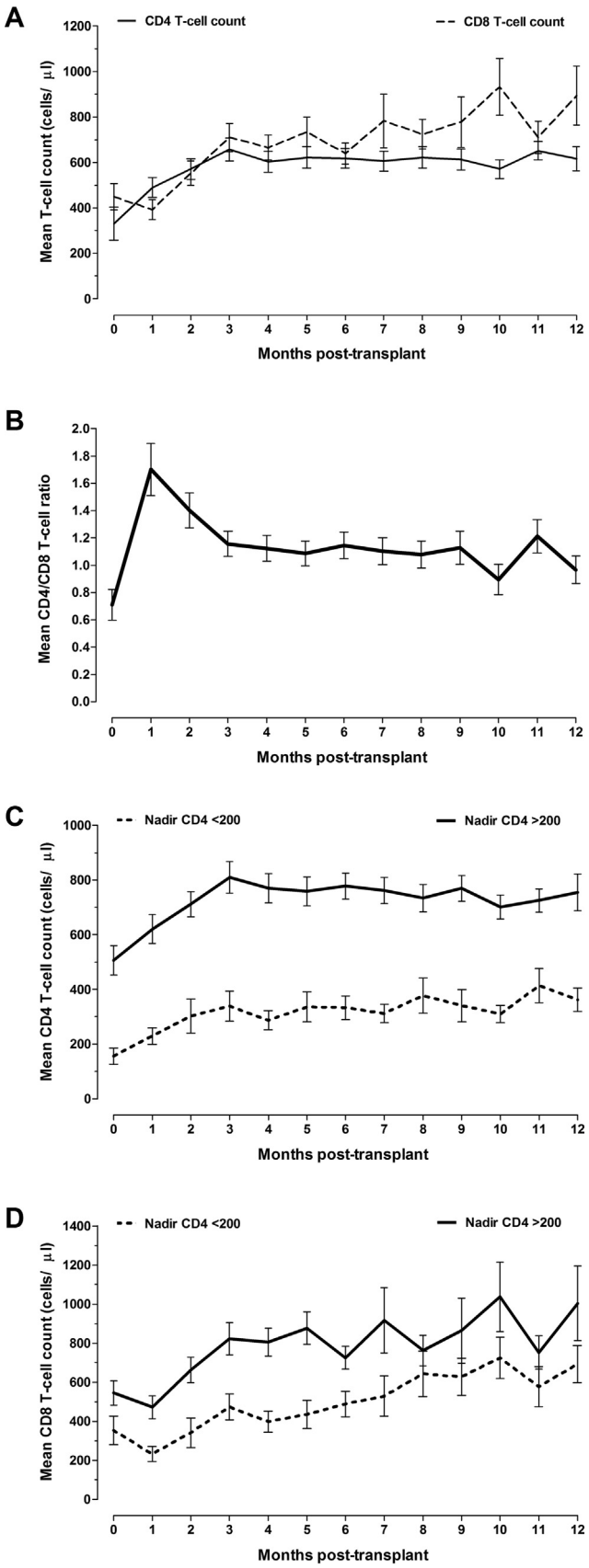

Fig. 1. Kinetics of T-cell subsets in 83 lung transplant recipients within the first 12 months post-transplant. Mean (±SD) CD4 and CD8 T-cell counts (A). Mean (±SD) CD4/CD8 T-cell ratio (B). Mean (±SD) CD4 T-cell count based on nadir CD4 T-cell count <200 cells/μl or >200 cells/μl (C). Mean (±SD) CD8 T-cell count based on nadir CD4 T-cell count <200 cells/μl or >200 cells/μl (D).

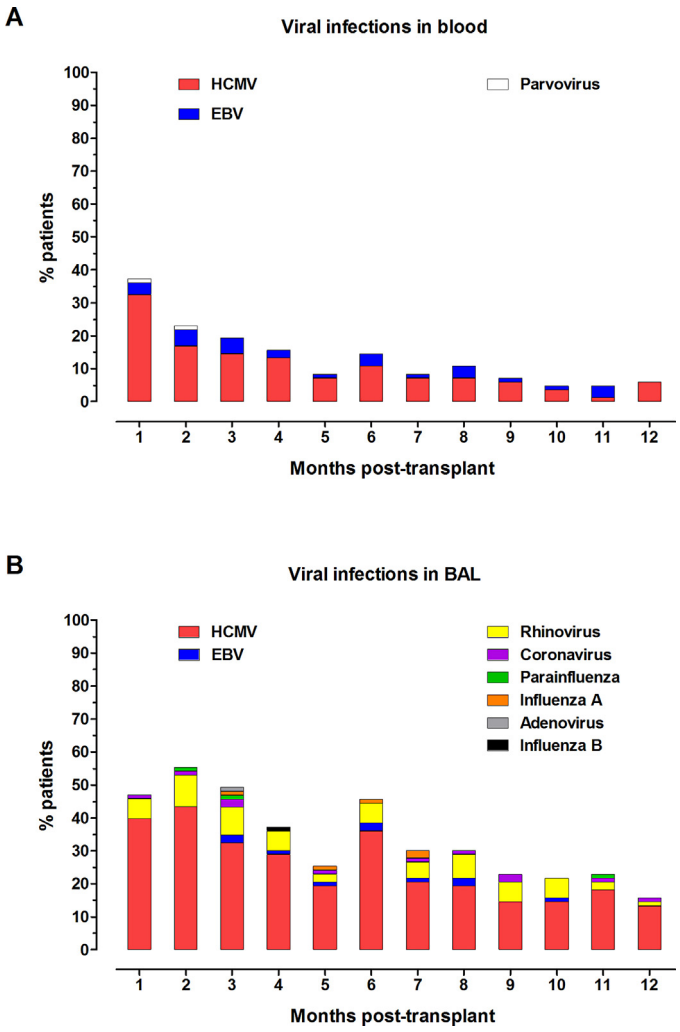

Fig. 2. Occurrence of viral infections in 83 lung transplant recipients within the first 12 months post-transplant. Percentage of patients developing viral infections detected in peripheral blood samples (A). Percentage of patients developing viral infections detected in BAL samples (B). Opportunistic and community-acquired infections are listed on the left and right sides, respectively, of each graph.

4.3. Occurrence of viral infections

HCMV was the most common OI detected in blood (36 patients, 43.4%) followed by EBV (12 patients, 14.5%). HCMV was also the most frequently detected OI in BAL samples (73 patients, 88%) followed by EBV (four patients, 4.8%). No HSV or VZV or HHV-6 or polyomavirus were observed during the study period. Among CAI, rhinovirus was the most common (27 patients, 32.5%) followed by coronavirus (nine patients, 10.8%), parainfluenza (three patients, 3.6%), influenza A (three patients, 3.6%), adenovirus (one patient, 1.2%) and influenza B (one patient, 1.2%). Parvovirus B19 was detected in blood of one patient (1.2%). The highest proportion of patients with systemic infections was observed at month 1 (Fig. 2A), while the highest proportion of patients with infections in BAL was observed at month 2 after transplantation (Fig. 2B).

4.4. Variables affecting T-cell subsets

As summarized in Table 2, no significant differences in CD4 and CD8 T-cell counts were observed between LTR with or without viral infections in blood. However, LTR developing viral infections in BAL had significantly lower CD4 T-cell counts compared to those without infections ($p = 0.036$). No differences were observed regarding

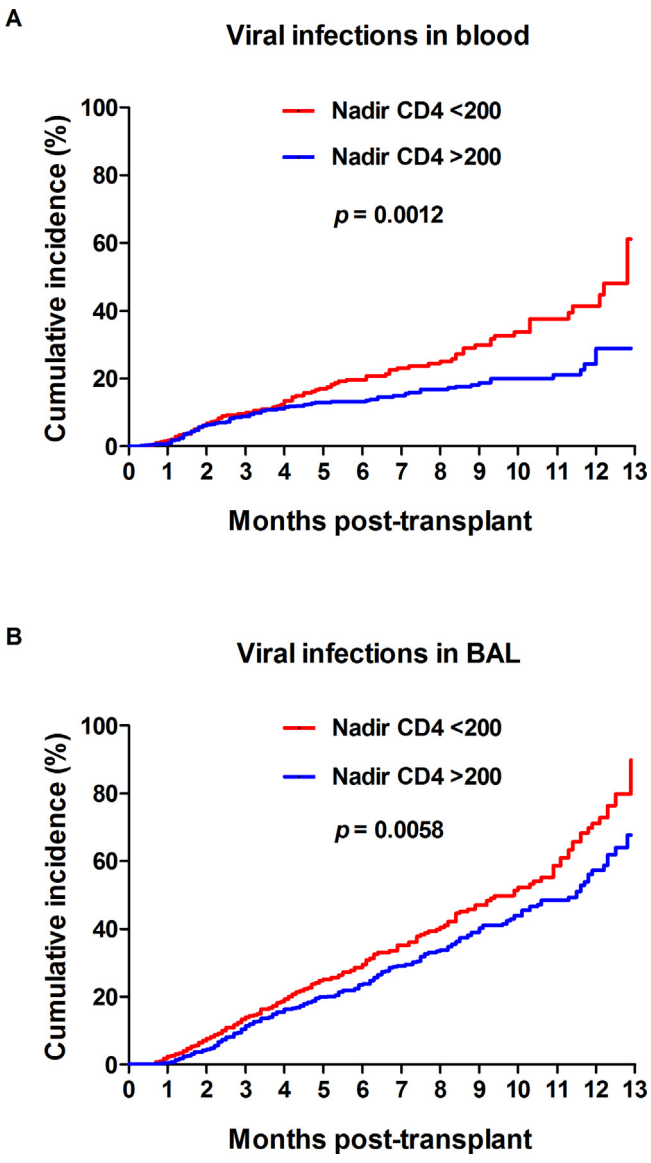

Fig. 3. Cumulative incidence of viral infections in 83 lung transplant recipients in the first 12–13 months post-transplant stratified by nadir CD4 T-cell count (<200 cells/μl or >200 cells/μl). Viral infections detected in peripheral blood samples (A). Viral infections detected in BAL samples (B). *P* values (log-rank test) are depicted for each graph; $p < 0.05$ was considered statistically significant.

CD8 T-cell counts when comparing LTR with or without viral infections in BAL. Remarkably, both CD4 and CD8 T-cell counts in LTR were dependent on nadir CD4 T-cell count ($p < 0.001$). Additionally, CD8 T-cell counts were higher in men than women and, without reaching statistical significance, LTR receiving ATG tended to have lower CD4 T-cell counts, LTR with acute rejection episodes tended to have higher CD8 T-cell counts and CD8 T-cell counts appears to be dependent on age.

4.5. Cumulative incidence of viral infections stratified by nadir CD4 T-cell count

In LTR with a nadir CD4 T-cell count <200 cells/μl, the cumulative incidence of infections in blood was 61%, while in LTR with a nadir CD4 T-cell count >200 cells/μl it was 29% ($p = 0.0012$, Fig. 3A). The cumulative incidence of infections in BAL was significantly higher in LTR with a nadir CD4 T-cell count <200 cells/μl than in LTR

Table 2
Multivariate analysis in 83 lung transplant recipients.

	Coefficient	95% CI	*p* value
CD4 T-cell count[a]			
Viral infections in blood (yes vs. no)[b]	−11.1	−50.0 to 27.9	0.578
Gender (males vs. females)	45.7	−60.5 to 151.9	0.399
Age (per year)	1.3	−2.3 to 4.9	0.494
Nadir CD4 T-cell (< 200 vs. > 200 cells/μl)	−423.4	−516.8 to −329.9	<0.001
Induction therapy (ATG vs. no)	−95.9	−203.1 to 11.1	0.079
Immunosuppressive therapy[c]	−45.1	−138.7 to 48.6	0.345
Rejection episodes (yes vs. no)	−20.2	−65.3 to 24.9	0.380
Viral infections in BAL (yes vs. no)[b]	−28.4	−-54.8 to −1.9	0.036
Gender (males vs. females)	45.1	−61.9 to 152.1	0.409
Age (per year)	1.3	−2.3 to 4.9	0.473
Nadir CD4 T-cell (< 200 vs. > 200 cells/μl)	−421.7	−515.9 to −327.5	<0.001
Induction therapy (ATG vs. no)	−93.9	−201.7 to 13.8	0.088
Immunosuppressive therapy[c]	−43.1	−137.3 to 51.1	0.370
Rejection episodes (yes vs. no)	−10.6	−56.3 to 35.1	0.649
CD8 T-cell count[a]			
Viral infections in blood (yes vs. no)[b]	−36.2	−101.8 to 29.4	0.279
Gender (males vs. females)	206.8	44.1 to 369.6	0.013
Age (per year)	−4.7	−10.2 to 0.8	0.096
Nadir CD4 T-cell (< 200 vs. > 200 cells/μl)	−300.6	−443.1 to −158.1	<0.001
Induction therapy (ATG vs. no)	−58.9	−223.3 to 105.3	0.482
Immunosuppressive therapy[c]	44.7	−98.9 to 188.4	0.542
Rejection episodes (yes vs. no)	64.6	−11.2 to 140.4	0.095
Viral infections in BAL (yes vs. no)[b]	−12.1	−56.9 to 32.7	0.596
Gender (males vs. females)	208.6	45.1 to 372.0	0.012
Age (per year)	−4.7	−10.2 to 0.9	0.098
Nadir CD4 T-cell (< 200 vs. > 200 cells/μl)	−303.2	−446.2 to −160.1	<0.001
Induction therapy (ATG vs. no)	−53.9	−218.6 to 110.9	0.522
Immunosuppressive therapy[c]	39.9	−103.9 to 183.8	0.587
Rejection episodes (yes vs. no)	69.9	−7.2 to 147.2	0.076

CI, confidence interval; ATG, antithymocyte globulin; BAL, bronchoalveolar lavage.
[a] Dependent variable.
[b] Both opportunistic and community-acquired viral infections.
[c] Cyclosporine A or tacrolimus/azathioprine/steroids vs. Cyclosporine A or tacrolimus/mycophenolate mofetil/steroids.

with a nadir CD4 T-cell count >200 cells/μl (90% vs. 68%, $p = 0.0058$, Fig. 3B).

4.6. Association between nadir CD4 T-cell count and subsequent infectious episodes

A ROC analysis was used to study the association between nadir CD4 T-cell count within the first three months post-transplantation and the frequency of infectious episodes within the successive six month period. Although no significant differences in the number of infectious episodes (detected in blood) were observed between LTR with a nadir CD4 T-cell count <200 cells/μl and those with a nadir CD4 T-cell count >200 cells/μl (area under the ROC curve 0.5972, 95% CI 0.4537 to 0.7407; Fig. 4A), the number of infectious episodes (detected in BAL samples) in LTR with a nadir CD4 T-cell count <200 cells/μl was significantly higher than in LTR with a nadir CD4 T-cell count >200 cells/μl (area under the ROC curve 0.6982, 95% CI 0.5671–0.8293; Fig. 4B).

5. Discussion

The present study provides a detailed analysis of T-cell subsets in peripheral blood during the first 12 months after lung transplantation and correlates these immunological data with infectious complications. Patients with a nadir CD4 T-cell count <200 cells/μl had significantly lower CD4 and CD8 T-cell counts as well as significantly higher cumulative incidence of viral infections than LTR with a nadir CD4 T-cell count >200 cells/μl. Furthermore, a nadir CD4 T-cell count <200 cells/μl within the first three months post-transplantation was associated with a high frequency of infectious episodes in BAL within the subsequent six-month period.

Few studies on T-cell subsets in peripheral blood obtained from LTR have been published [14,15] and, when evaluating their association with infections, they were restricted to HCMV pulmonary infection. No difference was observed for CD4 or CD8 T cells in 13 LTR with or without HCMV pulmonary infection [14]. In another study, a decrease in the percentage of CD4 T cells was observed at one year post-transplant in 17 LTR compared with normal ranges; a decrease in the percentage of CD8 T cells in patients with HCMV infection was also observed [15]. Another study evaluated the differences between lymphocyte subsets both in peripheral blood and BAL in 24 LTR [28]. While the percentage of CD4 T-cells decreased, CD8 T-cells increased in BAL compared with blood. However, associations of T-cell subsets in peripheral blood with pulmonary infections were not fully addressed [28]. In the present study, peripheral T-cell subsets were analyzed in relation to both OI and CAI in LTR. We found that patients developing infections in BAL had significantly lower CD4 T-cell counts than those without infections.

BAL specimens have increasingly been used for the diagnosis of infections after lung transplantation and also for the investigation of cell subsets [14,29–31]. Even though BAL provides information on the cellular component present in the alveolar space, the number of lymphocytes comprise a small percentage of total cells making BAL analysis difficult to standardize, thus results regarding the phenotypic analysis of lymphocytes may be contradictory [28,31]. Therefore, based on our findings, the measurement of T-cell subsets in peripheral blood could represent a simple, less invasive tool to identify LTR at risk of developing infections.

In our study, HCMV was the most frequent infection detected after lung transplantation, as was reported in other studies [1,32]. CAI can cause severe lower respiratory tract infections in LTR, resulting in important morbidity and mortality [1,33,34]. In agree-

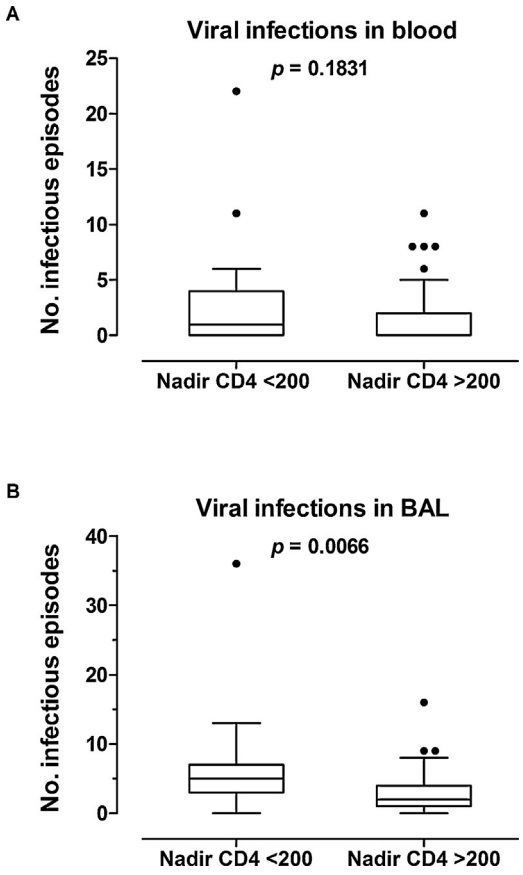

Fig. 4. Association between nadir CD4 T-cell count and subsequent infectious episodes. Relationship between lung transplant recipients with a nadir CD4 T-cell count <200 cells/μl or >200 cells/μl within the first three months post-transplantation and the number of infectious episodes detected in blood (A) or BAL samples (B) within the successive six month period. *P* values (ROC analysis) are depicted for each graph; $p < 0.05$ was considered statistically significant.

ment with other studies [35], rhinovirus was the most frequently isolated in our LTR cohort.

In this study, almost 29% of LTR received ATG. A well-documented effect of ATG treatments is T-cell depletion [36]. In keeping with a recent study [13], our data revealed that LTR receiving ATG tended to have lower CD4 T-cell counts than those that did not. However, CD4 as well as CD8 T-cell counts were dependent on the nadir CD4 T-cell count. Some limitations of this single-center study deserve mention, particularly those associated with the retrospective nature of the data, such as the nonsystematic T-cell measurements and the relatively small number of observations. Although the study has limitations, our findings reveal an association between nadir CD4 T-cell count and incidence of infectious episodes in LTR, suggesting that, particularly in patients with low CD4 T-cell numbers, monitoring of infections should be intensified to improve early detection and treatment.

In conclusion, stratification of patients according to nadir CD4 T-cell count may represent a simple and feasible approach for identification of patients at risk for infectious viral complications and adoption of tailored monitoring and treatment schedules.

Author contributions

Sandra A. Calarota participated in study design, in data analysis and in the writing of the paper. Antonella Chiesa collected the data and participated in the analysis. Annalisa De Silvestri performed the statistical analysis. Monica Morosini participated in the performance of the study and collected the data. Tiberio Oggionni, Piero Marone and Federica Meloni participated in clinical data collection and in the writing of the paper. Fausto Baldanti participated in the study design, in data analysis and in the writing of the paper. All authors have approved the final manuscript.

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Competing interests

None declared.

Ethical approval

This retrospective study was performed according to the guidelines of the Institutional Review Board on the use of biological specimens for scientific purposes in keeping with Italian law (art.13 D.Lgs 196/2003).

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