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# Pulmonary safety and clinical outcomes of inhaled colistin after lung transplantation: a retrospective cohort study

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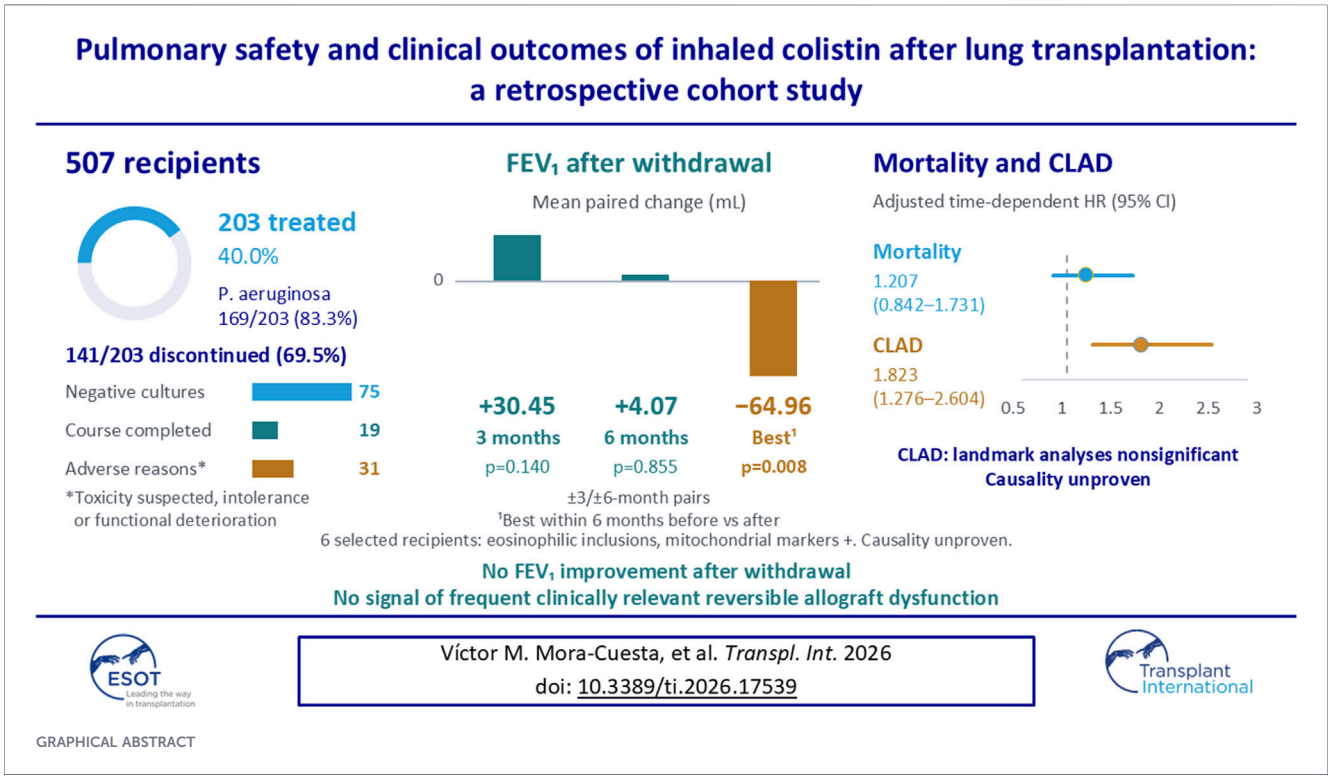
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Pulmonary safety of inhaled colistin after lung transplantation remains uncertain. We retrospectively studied 507 lung transplant recipients transplanted between 2015 and 2026, evaluating treatment patterns, reasons for discontinuation, changes in forced expiratory volume in 1 s ($FEV_1$) after withdrawal, allograft biopsies, mortality, and chronic lung allograft dysfunction (CLAD). Inhaled colistin was administered to 203 recipients (40.0%), mainly for *Pseudomonas aeruginosa* (83.3%); 141 discontinued treatment after a median of 23.90 months. Suspected toxicity, poor tolerance, or functional deterioration was recorded in 31 recipients. Mean $FEV_1$ changes at 3 and 6 months after withdrawal were +30.45 mL (p = 0.140) and +4.07 mL (p = 0.855), whereas the best $FEV_1$ in the 6 months after withdrawal was 64.96 mL lower than before withdrawal (p = 0.008). Biopsies from six recipients showed eosinophilic intracellular inclusions in hyperplastic pneumocytes with mitochondrial-marker positivity. In time-dependent analyses, follow-up after colistin initiation was not associated with mortality (adjusted HR 1.207, 95% CI 0.842–1.731), but was associated with higher CLAD hazard (adjusted HR 1.823, 95% CI 1.276–2.604); landmark analyses did not reproduce this association. Withdrawal did not improve $FEV_1$, providing no signal of frequent clinically relevant reversible allograft dysfunction. The histopathological findings require validation, and the CLAD association should not be interpreted causally.

KEYWORDS
**chronic lung allograft dysfunction, colistin, drug safety, inhaled antibiotics, lung transplantation**

## Introduction

Lung transplant recipients have a high risk of respiratory infection because of direct environmental exposure, impaired airway clearance, altered anatomy, immunosuppression, and repeated healthcare exposure [1, 2]. Gram-negative bacilli are prominent pathogens, and *Pseudomonas aeruginosa* is among the microorganisms most frequently recovered from respiratory samples and a leading cause of post-transplant bacterial pneumonia [2].




These infections may also be relevant beyond the acute episode. Post-transplant *P. aeruginosa* isolation, infection, or colonization has been associated with bronchiolitis obliterans syndrome and chronic lung allograft dysfunction (CLAD), although the direction of this relationship is difficult to establish because a damaged graft is also more susceptible to persistent airway infection [3, 4]. CLAD remains the principal limitation to long-term graft survival [1, 5]. Experimental work provides a plausible link through epithelial injury, inflammation, and fibroblast activation [6].

Inhaled colistin, administered as colistimethate sodium, is commonly used in some lung-transplant programmes to treat or suppress Gram-negative airway infection. Pulmonary delivery achieves high local concentrations while limiting systemic exposure [7]. However, evidence for long-term clinical benefit is inconsistent. Successful *P. aeruginosa* eradication has been associated with improved CLAD-free and graft survival, whereas other cohorts have found no overall reduction in recurrent *Pseudomonas*, rejection, or bronchiolitis obliterans syndrome with inhaled antipseudomonal therapy [4, 8–10]. Short-term data are also reassuring regarding the emergence of colistin resistance [11].

Safety has received much less attention. Inhaled colistin is generally considered well tolerated, although bronchospasm and local intolerance may occur [7]. Experimental studies have shown concentration-dependent toxicity of polymyxins in human lung epithelial cells and have implicated mitochondrial localization, oxidative stress, membrane dysfunction, and apoptosis in pulmonary and renal models [12–14]. However, the histopathological correlates of prolonged inhaled-colistin exposure in human lung tissue remain poorly characterized, and clinical studies have not specifically examined whether prolonged exposure is associated with reversible loss of allograft function. The study was prompted by clinical observations of functional deterioration and unusual histopathological findings in some recipients receiving prolonged inhaled colistin. These observations raised a question about possible treatment-related injury and prompted an internal review, without establishing a causal relationship. We therefore described real-world inhaled-colistin use after lung transplantation, examined documented reasons for discontinuation and changes in forced expiratory volume in 1 s ($FEV_1$) after withdrawal, performed an exploratory histopathological assessment of selected allograft biopsies, and evaluated survival and CLAD using analyses that accounted for the time-varying nature of treatment.

## Materials and methods

### Study design and population

We conducted a retrospective single-centre cohort study at the Lung Transplant Unit of Hospital Universitario Marqués de Valdecilla, Santander, Spain. Consecutive adults who underwent single or bilateral lung transplantation between January 1, 2015, and January 31, 2026, were eligible if post-transplant exposure and

**Abbreviations:** AI, artificial intelligence; CI, confidence interval; CLAD, chronic lung allograft dysfunction; COPD, chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation; $FEV_1$, forced expiratory volume in 1 s; HR, hazard ratio; IQR, interquartile range; ISHLT, International Society for Heart and Lung Transplantation; MU, million international units; SMD, standardized mean difference.

outcomes could be established from the institutional database and electronic medical record. Follow-up began at transplantation and ended at death, retransplantation, the last available clinical assessment, or April 15, 2026 (the administrative data cut-off), whichever occurred first. Potential follow-up was estimated with the reverse Kaplan-Meier method.

## Exposure and outcomes

For each recipient, we recorded baseline and transplant characteristics, inhaled-colistin indication, product/formulation, dose and schedule, use of an alternative inhaled antibiotic during off-colistin cycles, treatment dates, discontinuation and its recorded reason, and subsequent restart. Administration was classified as continuous or cyclic. Cyclic schedules comprised 28 days of inhaled colistin followed by 28 days without colistin, during which recipients either received no inhaled antibiotic or an alternative inhaled antibiotic. Treatment decisions were made during routine care. All inhaled-colistin formulations were administered every 12 h during treatment periods. The dosing approach did not differ between recipients with and without cystic fibrosis. In recipients without cystic fibrosis, inhaled colistin was prescribed off-label. The choice of colistin formulation was primarily determined by local availability of products and delivery devices across the referring hospitals, rather than by antimicrobial susceptibility results or patient characteristics. For descriptive tables and curves, recipients were classified as ever or never exposed during follow-up.

In the primary models, treatment initiation was modelled as a non-reversible time-dependent exposure: recipients contributed unexposed follow-up until first initiation and post-initiation follow-up thereafter, irrespective of subsequent discontinuation. Pre-transplant use was recorded separately; for these recipients, post-transplant exposure began at the first documented administration after transplantation. A sensitivity model for mortality separated untreated, on-treatment, post-discontinuation, and post-restart periods.

The safety evaluation comprised documented reasons for discontinuation and pulmonary-function changes after withdrawal. Suspected toxicity, poor tolerance, and functional deterioration were analysed as recorded; adverse events were not prospectively collected or formally adjudicated. Poor tolerance referred to patient-reported symptoms, primarily cough and dyspnoea, documented during routine follow-up visits and prompting dose reduction or treatment interruption. This category reflected subjective intolerance rather than spirometric deterioration or documented bronchospasm. Overall survival was measured from transplantation to death from any cause. CLAD was defined according to the 2019 International Society for Heart and Lung Transplantation (ISHLT) consensus as a persistent $FEV_1$ decline of at least 20% from the mean of the two best postoperative values obtained at least 3 weeks apart, after exclusion of reversible causes [5]. Death without prior CLAD was treated as a competing event. Primary graft dysfunction and acute cellular rejection were classified using international consensus criteria [15, 16].

## Pulmonary function after discontinuation

Spirometry was performed as part of routine post-transplant follow-up, rather than specifically to assess tolerance to inhaled colistin. No spirometric tolerance testing before and immediately after colistin administration was performed, including at treatment initiation. No minimum $FEV_1$ threshold was used to determine eligibility for inhaled colistin treatment. The discontinuation date was used as the index date. Three separate within-recipient comparisons were performed. For each fixed-time comparison, the available spirometry closest to the target date was selected. The $FEV_1$ closest to 3 months before discontinuation was paired with the value closest to 3 months after discontinuation; the same procedure was used for the 6-month comparison. Separately, the highest $FEV_1$ recorded during the 6 months before discontinuation was paired with the highest value during the subsequent 6 months. A paired comparison was excluded when no corresponding pre- or post-discontinuation spirometry was available. Recipients who restarted inhaled colistin within 90 days of discontinuation were excluded from all paired analyses. An exploratory analysis was restricted to recipients who stopped because of suspected toxicity, poor tolerance, or functional deterioration.

## Histopathological review

As an exploratory ancillary analysis, we retrospectively selected the six most recent recipients in whom pulmonary toxicity from inhaled colistin had been clinically suspected and for whom archived transbronchial-biopsy material was available. Specimens were reviewed by an experienced pulmonary pathologist who was aware of the clinical data; all had been obtained during ongoing inhaled-colistin treatment. Formalin-fixed, paraffin-embedded sections were examined using hematoxylin and eosin and Masson's trichrome stains. Intracellular inclusions were further characterized using mitochondrial immunohistochemistry. Acute cellular rejection was graded according to ISHLT criteria [16], and specimens were examined for histologic features of antibody-mediated injury according to the ISHLT consensus framework [17], as well as for histologic evidence of infection.

## Statistical analysis

Categorical variables are reported as n (%) and continuous variables as mean ± standard deviation or median (interquartile range). Ever-versus never-exposed groups were compared descriptively with chi-square or Fisher exact tests and Student t or Mann–Whitney tests, as appropriate; absolute standardized mean differences (SMDs) describe imbalance. Paired $FEV_1$ changes were tested with paired t tests.

Overall survival was described with Kaplan–Meier estimates. A conventional fixed ever/never Cox model was retained only to illustrate immortal-time bias [18]. The principal mortality analysis used a Cox model with time-dependent treatment. CLAD cumulative incidence was estimated with the Aalen–Johansen method, considering death without prior CLAD as a competing event; associations were estimated with cause-specific Cox models using time-dependent treatment. Adjusted models included age, sex, underlying disease, bilateral transplantation, transplant year, urgent transplantation, intraoperative extracorporeal membrane oxygenation, grade 3 primary graft dysfunction, and surgical reintervention.

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TABLE 1 Selected cohort characteristics according to cumulative inhaled-colistin exposure.

| Variable | Category | Overall (N = 507) | Ever exposed (n = 203) | Never exposed (n = 304) | p value | Absolute SMD |
|---|---|---|---|---|---|---|
| Sex | Male | 314 (61.9%) | 139 (68.5%) | 175 (57.6%) | 0.013 | 0.23 |
| Age at transplantation, years | | 60.56 (54.93–63.82) | 60.37 (54.15–63.82) | 60.69 (55.11–63.83) | 0.376 | 0.19 |
| Underlying disease | | | | | <0.001 | |
| | COPD | 180 (35.5%) | 71 (35.0%) | 109 (35.9%) | | 0.02 |
| | Interstitial lung disease | 236 (46.5%) | 84 (41.4%) | 152 (50.0%) | | 0.17 |
| | Bronchiectasis | 40 (7.9%) | 32 (15.8%) | 8 (2.6%) | | 0.47 |
| | Other* | 51 (10.1%) | 16 (7.9%) | 35 (11.5%) | | — |
| Transplant era | | | | | 0.023 | |
| | 2015–2018 | 175 (34.5%) | 77 (37.9%) | 98 (32.2%) | | 0.12 |
| | 2019–2022 | 172 (33.9%) | 76 (37.4%) | 96 (31.6%) | | 0.12 |
| | 2023–2026 | 160 (31.6%) | 50 (24.6%) | 110 (36.2%) | | 0.25 |
| Bilateral transplantation | | 440 (86.8%) | 179 (88.2%) | 261 (85.9%) | 0.449 | 0.07 |
| Intraoperative ECMO | | 63 (12.4%) | 22 (10.8%) | 41 (13.5%) | 0.376 | 0.08 |
| Surgical reintervention | | 40 (7.9%) | 15 (7.4%) | 25 (8.3%) | 0.725 | 0.03 |
| Primary graft dysfunction grade 3 | | 63/504 (12.5%) | 24/202 (11.9%) | 39/300 (13.0%) | 0.711 | 0.03 |
| Mechanical ventilation, days | | 1.0 (1.0–2.0) | 1.0 (1.0–2.0) | 1.0 (1.0–1.6) | 0.043 | 0.05 |
| Hospital stay, days | | 23.0 (19.8–29.8) | 24.0 (21.0–31.0) | 22.0 (19.0–28.0) | 0.002 | 0.15 |
| Acute cellular rejection | | 213/484 (44.0%) | 92/200 (46.0%) | 121/284 (42.6%) | 0.459 | 0.07 |
| Antibody-mediated rejection | | 43 (8.5%) | 26 (12.8%) | 17 (5.6%) | 0.004 | 0.25 |

Data are n/N (%) when observations were missing and n (%) otherwise, or median (IQR). Denominators vary according to data availability. Exposure groups are defined by treatment received at any time during follow-up and are descriptive, not baseline treatment groups. *Other includes pulmonary hypertension, other indications, and retransplantation. COPD, chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation; IQR, interquartile range; SMD, standardized mean difference.

As sensitivity analyses, landmark models were fitted at 3, 6, and 12 months among recipients alive—and, for the CLAD analysis, alive and CLAD-free—at each time point. Exposure status was fixed at the landmark according to prior colistin initiation, and follow-up began from that date. Multivariable analyses used complete cases, without imputation. Sex was included as an adjustment covariate; sex-stratified associations were not estimated.

Tests were two-sided, with $p \leq 0.05$. Analyses were performed in IBM SPSS Statistics version 20 and Python version 3.12.

## Ethics

The Drug Research Ethics Committee of Cantabria approved the study (protocol 2026.153) and waived individual informed consent because the study was retrospective and non-interventional. The exploratory histopathological analysis was performed using archived diagnostic material obtained during routine clinical care. Data were processed in accordance with European and Spanish data-protection legislation.

# Results

## Study population and treatment

The cohort included 507 recipients; 314 (61.9%) were male, median age was 60.56 years (interquartile range [IQR], 54.93–63.82), and 440 (86.8%) received bilateral grafts. Median potential follow-up was 4.95 years. During follow-up, 203 recipients (40.0%) received inhaled colistin and 304 (60.0%) never received it. The ever-exposed group included more recipients with bronchiectasis (15.8% vs. 2.6%; SMD, 0.47), more males (68.5% vs. 57.6%; $p = 0.013$), and more antibody-mediated rejection during follow-up (12.8% vs. 5.6%; $p = 0.004$) (Table 1).

Pre-transplant inhaled-colistin use was documented in 23 of 197 recipients (11.7%). Treatment began a median of 2.05 months (IQR, 0.06–8.57) after transplantation. The indication was *P. aeruginosa* in 169 recipients (83.3%), *Stenotrophomonas maltophilia* in 17 (8.4%), and another indication in 17 (8.4%). Treatment was continuous in 177 recipients (87.2%), whereas 26 (12.8%) received cyclic treatment; 13 of the latter (50.0%) alternated

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TABLE 2 Characteristics of inhaled-colistin treatment.

| Variable | Category | Value | Denominator |
|---|---|---|---|
| Colistin use before transplantation | Yes | 23 (11.7%) | 197 |
| Time to initiation, months | | 2.05 (0.06–8.57) | 203 |
| Indication | *P. aeruginosa* | 169 (83.3%) | 203 |
| | *S. maltophilia* | 17 (8.4%) | 203 |
| | Other | 17 (8.4%) | 203 |
| Formulation | Generic injectable formulation (administered by nebulisation) | 168 (82.8%) | 203 |
| | Promixin® (powder for nebuliser solution) | 32 (15.8%) | 203 |
| | Colobreathe® (dry powder for inhalation) | 3 (1.5%) | 203 |
| Dose per administration (excluding Colobreathe) | 1 MU | 53 (26.5%) | 200 |
| | 2 MU | 147 (73.5%) | 200 |
| Administration schedule | Continuous | 177 (87.2%) | 203 |
| | Cyclic (28 days on/28 days off colistin) | 26 (12.8%) | 203 |
| Alternative inhaled antibiotic during off-colistin cycles | Yes | 13 (50.0%) | 26 |
| Bronchodilator before inhaled colistin | Yes | 67 (33.0%) | 203 |
| Treatment discontinued | Yes | 141 (69.5%) | 203 |
| Reason for discontinuation | Negative cultures | 75 (53.2%) | 141 |
| | End of planned treatment | 19 (13.5%) | 141 |
| | Poor tolerance | 14 (9.9%) | 141 |
| | Functional deterioration | 10 (7.1%) | 141 |
| | Suspected toxicity | 7 (5.0%) | 141 |
| | Switch to another inhaled antibiotic | 5 (3.5%) | 141 |
| | Lack of response | 1 (0.7%) | 141 |
| | Unknown | 10 (7.1%) | 141 |
| Duration among discontinuers, months | | 23.90 (9.07–47.13) | 141 |
| Restart after discontinuation | | 21 (14.9%) | 141 |
| Time to restart, months | | 9.56 (6.47–21.45) | 21 |

All products contained colistimethate sodium. The generic injectable formulation was administered by nebulisation. In cyclic schedules, 28 days of inhaled colistin were followed by 28 days without colistin; during the off-colistin period, recipients either received no inhaled antibiotic or an alternative inhaled antibiotic. Data are n (%) or median (IQR). Denominators vary according to availability and eligibility. MU, million international units. In recipients receiving a bronchodilator, it was consistently administered before inhaled colistin to improve tolerability. All formulations were administered every 12 h during treatment periods, irrespective of underlying cystic fibrosis.

colistin with another inhaled antibiotic during the off-colistin cycles (Table 2).

## Discontinuation and pulmonary safety

Treatment was discontinued in 141 of 203 recipients (69.5%), after a median of 23.90 months (IQR, 9.07–47.13). The most frequent reasons were negative respiratory cultures (75/141; 53.2%) and completion of the planned course (19/141; 13.5%). Thirty-one recipients (22.0% of those who stopped) had a recorded reason of suspected toxicity, poor tolerance, or functional deterioration. Twenty-one recipients (14.9%) subsequently restarted treatment (Table 2).

Mean within-recipient $FEV_1$ changes were +30.45 ± 221.78 mL at 3 months (n = 117; p = 0.140) and +4.07 ± 234.97 mL at 6 months (n = 112; p = 0.855). The maximum-value analysis showed a mean change of −64.96 ± 256.97 mL (n = 115; p = 0.008), and the adverse-reason subgroup a mean change of −189.60 ± 357.19 mL (n = 25; p = 0.014) (Table 3; Figure 1).

## Exploratory histopathological findings

Transbronchial-biopsy specimens from six recipients, all obtained during ongoing inhaled-colistin treatment, showed intracellular eosinophilic granular inclusions, approximately 1–2 μm in diameter, within hyperplastic pneumocytes. The inclusions stained bright red with Masson's trichrome and were positive on mitochondrial immunohistochemistry, consistent with a mitochondrial origin (Figure 2). No histologic evidence of acute

TABLE 3 Paired $FEV_1$ comparisons before and after inhaled-colistin discontinuation.

| Analysis | n | Before discontinuation, mL | After discontinuation, mL | Paired change (after–before), mL | p value |
|---|---|---|---|---|---|
| Fixed-time assessment: 3 months before vs. 3 months after | 117 | 2,291.68 ± 878.58 | 2,322.13 ± 921.60 | +30.45 ± 221.78 | 0.140 |
| Fixed-time assessment: 6 months before vs. 6 months after | 112 | 2,335.35 ± 861.12 | 2,339.42 ± 912.56 | +4.07 ± 234.97 | 0.855 |
| Maximum-value assessment: Best $FEV_1$ in each 6-month period | 115 | 2,511.04 ± 877.84 | 2,446.09 ± 921.15 | −64.96 ± 256.97 | 0.008 |
| Maximum-value assessment: adverse-reason subgroup | 25 | 2,397.20 ± 815.50 | 2,207.60 ± 920.91 | −189.60 ± 357.19 | 0.014 |

Values are mean ± standard deviation. For the fixed-time analyses, before and after refer to $FEV_1$ recorded approximately 3 or 6 months on either side of the discontinuation date. For the maximum-value analyses, they refer to the highest $FEV_1$ recorded during the 6 months before and the 6 months after discontinuation. Paired change is after minus before. Each row includes the available paired observations and therefore has its own denominator. p values are from paired t tests. The adverse-reason subgroup comprised suspected toxicity, poor tolerance, or functional deterioration.

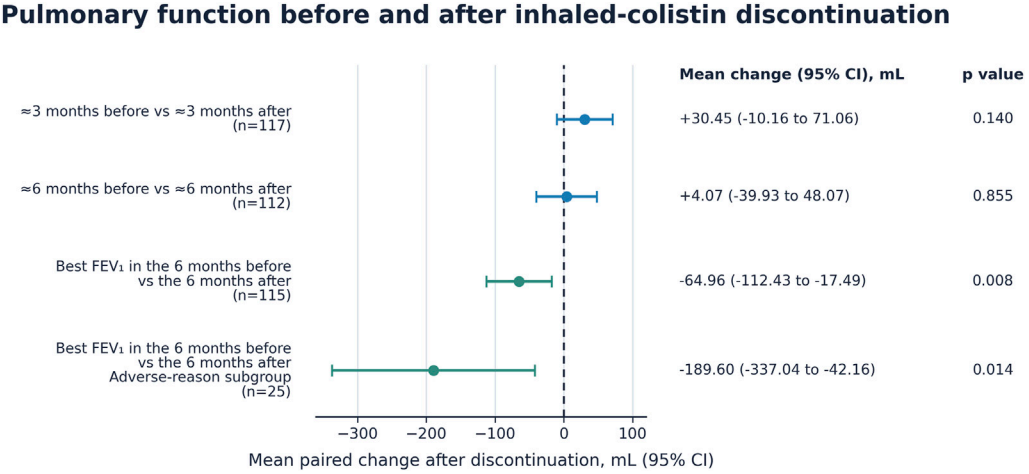



FIGURE 1
Paired $FEV_1$ change after inhaled-colistin discontinuation. Points show mean within-recipient change (after minus before withdrawal); bars show 95% confidence intervals. The dashed line denotes no change.

cellular rejection, antibody-mediated injury, exudative change, or infection was identified.

## Overall survival

Descriptive ever/never survival estimates and fixed-exposure models are shown in Supplementary Figure S1 and Supplementary Table S1.

In the principal non-reversible time-dependent analysis, follow-up after inhaled-colistin initiation was not associated with mortality (adjusted hazard ratio [HR], 1.207; 95% confidence interval [CI], 0.842–1.731; p = 0.305; 139 deaths among 501 complete cases). The treatment-state model and adjusted landmark analyses at 3, 6, and 12 months also showed no statistically significant associations (Supplementary Table S1).

## CLAD and competing death

In the principal cause-specific model, follow-up after inhaled-colistin initiation was associated with a higher CLAD hazard (adjusted HR, 1.823; 95% CI, 1.276–2.604; p < 0.001; 126 CLAD events among 500 complete cases). Follow-up after initiation was not associated with death without prior CLAD (adjusted HR, 0.770; 95% CI, 0.459–1.290; p = 0.320). Adjusted landmark estimates for CLAD at 3, 6, and 12 months were not statistically significant (Supplementary Table S1).

In the descriptive ever/never grouping, Aalen–Johansen cumulative incidence of CLAD at 1, 3, 5, and 10 years was 2.7%, 23.3%, 42.1%, and 70.7% among ever-exposed recipients and 1.1%, 7.1%, 16.1%, and 38.2% among never-exposed recipients (Figure 3).

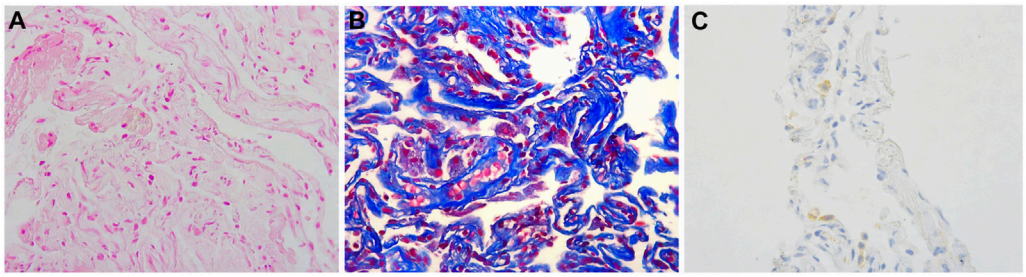



FIGURE 2
Histopathological findings in transbronchial-biopsy specimens obtained during ongoing inhaled-colistin treatment in lung transplant recipients. **(A)** Hematoxylin and eosin staining showing intracellular eosinophilic granular inclusions within hyperplastic pneumocytes. **(B)** Masson's trichrome highlighting the inclusions in bright red. **(C)** Mitochondrial immunohistochemistry showing positivity within the inclusions, supporting a mitochondrial origin.

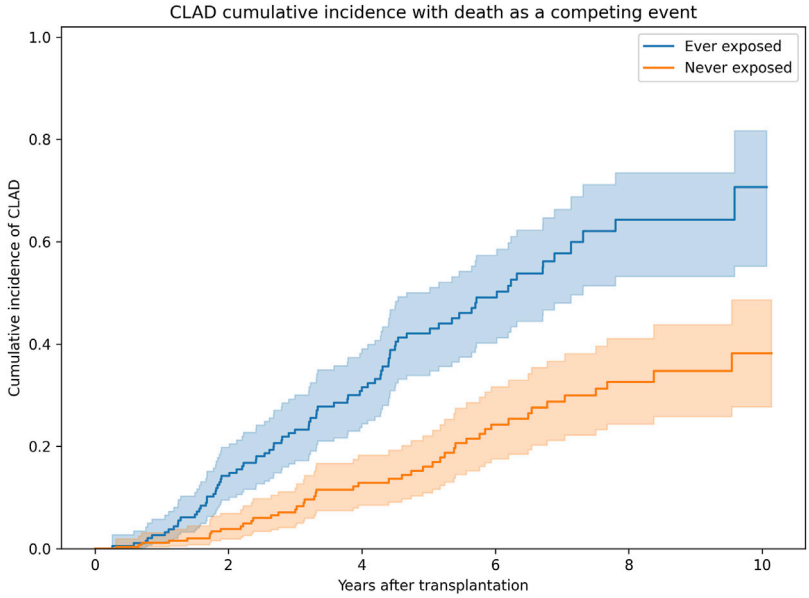



FIGURE 3
Aalen–Johansen cumulative incidence of CLAD, treating death without prior CLAD as a competing event, according to cumulative ever/never inhaled-colistin exposure. Grouping is descriptive because it uses future treatment information.

## Discussion

In this large single-centre cohort, inhaled colistin was used in 40% of lung transplant recipients, predominantly for *Pseudomonas aeruginosa*, and treatment frequently continued for prolonged periods. Four findings are particularly relevant. First, although suspected toxicity, poor tolerance, or functional deterioration was recorded as the reason for discontinuation in 31 recipients, withdrawal was not followed by an improvement in $FEV_1$ at approximately 3 or 6 months. Second, mitochondrial-immunohistochemistry–positive eosinophilic inclusions were identified in hyperplastic pneumocytes in selected allograft biopsies obtained during ongoing treatment, although their relationship to colistin exposure remains uncertain. Third, inhaled-colistin exposure was not associated with mortality when the timing of treatment initiation was appropriately considered. Fourth, follow-up after inhaled-colistin initiation was associated with a higher cause-specific hazard of CLAD, but this association was not reproduced in landmark analyses and cannot be interpreted as a causal effect of colistin.

Pulmonary safety data for inhaled colistin after lung transplantation remain limited. Most transplant studies have focused on microbiological eradication, prevention of recurrent pneumonia, resistance, or long-term graft outcomes rather than on treatment-related changes in allograft function [4, 8–11]. Evidence from bronchiectasis is broadly reassuring: inhaled colistimethate has generally been well tolerated, although cough, bronchospasm, dyspnoea, and local intolerance are recognised [7]. Formulation physicochemical properties may affect local tolerability, while the PROMIS-I and PROMIS-II trials found no major safety concerns over 12 months [19, 20]. However, these findings cannot be directly extrapolated to lung transplant

recipients, whose denervated allografts, airway complications, immunosuppression, and cumulative exposure to other potentially toxic treatments create a different clinical setting. Experimental evidence of concentration-dependent epithelial injury also supports continued clinical surveillance [12, 13].

The paired spirometric analyses provide a complementary and clinically relevant safety assessment. Mean $FEV_1$ changes at approximately 3 and 6 months after discontinuation were small and not statistically significant. If inhaled colistin were commonly causing a clinically relevant and reversible loss of allograft function, a measurable improvement after withdrawal would be expected; this pattern was not observed. Conversely, the best $FEV_1$ recorded during the 6 months after discontinuation was modestly lower than the best value in the preceding 6 months, and the reduction was greater among recipients who stopped because of suspected toxicity, poor tolerance, or functional deterioration. These findings should not be interpreted as evidence that discontinuation is harmful. The subgroup was selected partly because functional deterioration had already occurred, and subsequent $FEV_1$ could have been influenced by persistent infection, rejection, evolving CLAD, airway complications, changes in other treatments, or natural longitudinal variability. Nevertheless, the direction of change does not support recovery from a frequent reversible toxic effect. Our analysis cannot exclude acute bronchospasm, transient symptoms, subclinical epithelial injury, or irreversible toxicity, none of which is adequately captured by spirometry performed months after withdrawal.

The exploratory histopathological findings add a different dimension to the safety assessment. The eosinophilic inclusions identified in hyperplastic pneumocytes stained bright red with Masson's trichrome and were positive on mitochondrial immunohistochemistry, supporting—but not proving—a mitochondrial origin. Experimental evidence provides biological plausibility for a potential relationship with polymyxin exposure. Experimental studies have implicated megalin-mediated pulmonary uptake and mitochondrial injury after polymyxin exposure [14, 21–25]. Nevertheless, the inclusions observed in the present study cannot be attributed to inhaled colistin. The specimens were retrospectively selected on the basis of recency and tissue availability and represented the six most recent cases with suspected inhaled-colistin pulmonary toxicity and available archived biopsy material; no unexposed histological comparator was available, and mitochondrial ultrastructure was not assessed by electron microscopy. These findings should therefore be considered hypothesis-generating and do not conflict with the absence of a detectable reversible functional effect after treatment withdrawal.

The association between follow-up after inhaled-colistin initiation and CLAD requires particular caution. *P. aeruginosa* accounted for more than 80% of treatment indications and is itself associated with CLAD, either as a contributor to epithelial injury and alloimmune activation or as a marker of an already vulnerable graft [3, 4, 6]. Recipients with progressive allograft dysfunction may also develop persistent airway infection and consequently start or continue inhaled colistin. Thus, treatment exposure may identify periods of greater infectious burden or incipient graft dysfunction rather than act as their cause. The greater prevalence of bronchiectasis and antibody-mediated rejection among ever-exposed recipients further illustrates the different underlying risk profiles.

The apparent survival advantage in the descriptive ever/never comparison disappeared in the time-dependent, treatment-state, and landmark analyses, consistent with immortal-time bias from treating post-baseline exposure as fixed [18]. These data provide no evidence that inhaled colistin improves or worsens survival.

From a clinical perspective, these findings provide some reassurance regarding the pulmonary tolerability of inhaled colistin when prescribed for a microbiological indication, but they do not justify indiscriminate or indefinite treatment. Symptoms, acute bronchospasm, adherence, microbiology, and serial allograft function should be monitored, and the ongoing indication should be reassessed. Prospective comparative studies should use standardised safety assessments and account for time-varying infection and allograft status; systematic biopsy studies should include unexposed comparators and electron microscopy.

## Strengths and limitations

The principal strengths of this study are its relatively large contemporary cohort, long follow-up, detailed reconstruction of treatment initiation, discontinuation and restart, and the within-recipient assessment of pulmonary function around withdrawal. The use of time-dependent exposure, competing-risk methods for CLAD, and landmark sensitivity analyses addressed important biases present in simpler ever/never comparisons.

Several limitations should be acknowledged. The retrospective, single-centre design limits causal inference and generalisability. Adverse events were not prospectively collected or adjudicated, and recorded reasons for discontinuation cannot be used to estimate the true incidence or severity of toxicity. The $FEV_1$ analyses included only recipients who discontinued treatment and had paired spirometry, introducing potential selection, survivor, and informative-missingness biases; test timing was approximate, and no untreated microbiologically comparable control group was available. Spirometric changes could not be separated from concurrent infection, rejection, CLAD, airway complications, or changes in antimicrobial and immunosuppressive treatment. The histopathological analysis was exploratory and restricted to the six most recent recipients in whom inhaled-colistin pulmonary toxicity had been suspected and for whom archived transbronchial-biopsy material was available, without systematic review of all available biopsies or an unexposed comparator; consequently, neither the prevalence nor the specificity of the inclusions for colistin exposure can be established. Mitochondrial immunoreactivity supports a mitochondrial origin but does not establish drug causality, and electron microscopy was not performed. Adherence, nebuliser performance, formulation-specific physicochemical characteristics, pathogen burden, antimicrobial susceptibility, and systemic adverse effects were not comprehensively assessed. Tolerability was not analysed by formulation or delivery device, and the small, unevenly distributed formulation groups limit meaningful comparisons. Finally, despite multivariable adjustment, residual and time-varying confounding by indication remain likely, particularly for the CLAD analysis. Sex-specific treatment associations were not evaluated because subgroup effects were outside the study objectives.

## Conclusion

Inhaled colistin was frequently used after lung transplantation, mainly for *P. aeruginosa*. Withdrawal was not followed by an improvement in $FEV_1$, providing no signal of frequent clinically relevant reversible allograft dysfunction. In selected allograft biopsies obtained during ongoing treatment, eosinophilic inclusions positive on mitochondrial immunohistochemistry were identified in hyperplastic pneumocytes; this exploratory finding cannot establish causality but warrants systematic investigation. Follow-up after colistin initiation was not associated with mortality, and the observed CLAD association should not be interpreted causally.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The study involving humans was approved by the Drug Research Ethics Committee of Cantabria (protocol 2026.153). The study was conducted in accordance with local legislation and institutional requirements. The committee waived the requirement for individual informed consent because of the retrospective, non-interventional design.

## Author contributions

Conceptualization: VM-C, JG-R; Methodology: VM-C, JG-R; Investigation: JG-R, BS-F, PÁ-V, ST-M, SI-C, VC-L; Data curation: VM-C, JG-R, BS-F, PÁ-V, ST-M, SI-C; Formal analysis: VM-C; Writing – original draft: VM-C; Writing - review and editing: All authors; Supervision: JG-R; Project administration: PA-L; Visualization: VM-C, JG-R. All authors reviewed and approved the final manuscript.

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## Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Generative AI statement

The author(s) declared that generative AI was used in the creation of this manuscript. During the preparation of this work, the authors used OpenAI ChatGPT for language editing and translation. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2026.17539/full#supplementary-material

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