Impact of Cold Ischemic Time on Airway Complications After Lung Transplantation: A Single-center Cohort Study

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ABSTRACT

Background. Despite significant improvements in lung transplantation procedures, the incidence of airway complications (ACs) remains high (2%-18%); these complications are associated with high costs, great morbidities, and a decreased quality of life. There is general disagreement over potential risk factors determining ACs, including graft cold ischemic time (CIT). The aim of this study was to evaluate the association between CIT and ACs.

Methods. All patients undergoing lung transplantation between January 2011 and December 2017 were evaluated. We excluded retransplantations and patients with 90-day mortality. Demographic and clinical data regarding donors, recipients, and surgical procedures were analyzed using propensity score weighted marginal Cox regression model.

Results. Out of the 161 lung transplantations performed in the study timeframe, 147 fulfilled the inclusion criteria and supplied complete data to be analyzed. Median follow-up was 25.5 months (interquartile range = 35.2). Ten patients (6.8%) had late ACs; out of the 260 anastomoses considered, 14 proved to be complicated (5.4%). Median time to event was 5.5 months (range, 3-15). ACs were classified as bronchial stenosis (12) and malacia (2). Mean CIT was 446.6 minutes (range, 117-1200). Without considering time-to-event data, CIT was significantly higher in complicated anastomoses ($P = .002$). The unweighted marginal univariate Cox model showed a significant association between ACs and CIT ($P < .001$). The propensity score weighted marginal univariable Cox model confirmed this significant association ($P < .001$).

Conclusions. The prolonged CIT time seems to be a risk factor for the development of late ACs; we endorse any measure that could limit CIT within 600 minutes.

IT is well known that early experience in lung transplantation (LuTx) was characterized by high mortality rate owing to bronchial anastomotic dehiscence [1]. Since then, significant improvements were made in terms of organ procurement, graft preservation, surgical technique and post-transplant medical treatment. This has led to considering lung transplantation an established procedure for end-stage respiratory insufficiency in selected patients. Nevertheless, the incidence of airway complications (ACs) remains rather high, ranging from 2% to 18% [2]; these dreaded complications are associated with high costs, great morbidities, and a decreased quality of life.

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The ischemia of the graft airway during the first 2 to 4 weeks after transplantation appears to be a relevant risk factor for impaired healing of the bronchial anastomosis [2]. In that time frame, bronchial supply depends on the retrograde flow from the pulmonary arterial system, a low-pressure system that is faintly oxygenated. There is general disagreement on potential risk factors determining postoperative bronchial ischemia; several original articles and reviews have been published; nevertheless, a pooled quantitative analysis is still needed. Many factors could actually contribute to developing ACs such as cold ischemic time (CIT), length of the graft bronchus, bronchial diameter mismatch, airway infection, acute rejection, and prolonged post-transplant mechanical ventilation [3-5]. In particular, there is disagreement over the association between CIT and ACs.

The aim of this study was to evaluate the association between CIT and ACs after lung transplantation.

METHODS

The study design was single-center retrospective cohort research. The inclusion criterion was having undergone lung transplantation from January 2011 to December 2017; exclusion criteria were retransplantation and 90-day mortality. Data regarding donors and recipients were downloaded from our lung transplant database, according to a protocol approved by the institutional ethical committee (N. 749_2016bis).

Donors' data, including age, sex, height, days of mechanical ventilation, body mass index (BMI), Oto score, ex vivo lung perfusion (EVLP), and colonization of the airway were extracted and tabulated. The collected recipients' variables were as follows: age, sex, height, diabetes, coronary artery disease, BMI, underlying disease, single or bilateral LuTx, lobar LuTx, days of mechanical ventilation after LuTx, overall use of any extracorporeal life support device, use of pre-, intra- and postoperative extracorporeal membrane oxygenation support, CIT calculated for single anastomosis, pre-LuTx airway colonization, postoperative airway colonization at days 1 to 14, and treatment for acute rejection within 6 months after LuTx.

ORGAN PRESERVATION

Grafts received anterograde and retrograde flushes with a low-potassium dextran glucose solution. During transport, grafts were stored on ice. Lungs evaluated with EVLP received another flush with the low-potassium dextran glucose solution at the end of the procedure and were stored on ice until transplantation. The protocol for donation after circulatory death donors has been previously outlined [6].

Surgical Technique

The surgical technique, as well as the surgical staff, did not change during the study period. Five surgeons performed all transplantations. Main bronchi were cut as proximally as possible to the first bifurcation; bronchial anastomoses were performed with an "end-to-end" approach whenever possible. A telescopic suture was reserved to selected cases when bronchial lumen mismatch did not permit direct apposition of graft and recipient bronchial stumps. The bronchial suture was always performed with 2 running absorbable monofilament sutures (4/0 Poly-p-dioxanone) and covered with native peribronchial tissue. It is worth mentioning that the surgical team has wide experience in lobar lung transplantation and lung graft downsizing [7,8].

Bronchoscopic and Radiological Evaluations

Bronchoscopic surveillance routinely included flexible bronchoscopy at 1st postoperative day followed by one examination a week until the first postoperative month; therefore, bronchoscopy is planned at 3, 6, and 12 months after transplantation. Thorax computed tomography scan is routinely obtained at 3, 6, and 12 months after transplantation. In addition, clinically driven thorax computed tomography scans and/or bronchoscopies are carried out in case of signs and symptoms of rejection, pneumonia, or suspected bronchial complication. Any bronchoscopic and/or radiologic abnormalities diagnosed during follow-up and recorded on our dedicated database were considered. Ischemia, necrosis, dehiscence, stenosis, and malacia were reclassified following the International Society for Heart and Lung Transplantation Consensus Statement on airway complications [2].

Statistical Analysis

Data were described with median and interquartile range (IQR), mean and range, or absolute frequencies and percentages. Mann-Whitney or χ^2 test was performed as appropriate. Propensity score (PS) techniques were used to balance the distributions of measured potentially confounding covariates. In particular, we used the PS weighting for continuous exposure, represented by CIT, with multilevel data, to account for the clustered nature of anastomosis related to each patient. Specifically, we adopted a random effect model using weights stabilized by the cluster-specific exposure to estimate the average treatment effect, using a bootstrap Pearson correlation between the CIT and each covariate [9]. The covariates included in PS are the following: 1. recipient-related covariates: sex, underlying disease, right side of anastomosis, bilateral LuTx, recipient BMI, Lobar LuTx, days of post LuTx mechanical ventilation, use of intraoperative or postoperative extracorporeal membrane oxygenation, height, treated acute rejection within 6 months after LuTx, pre-LuTx airway colonization, post-LuTx airway colonization, coronary artery disease, diabetes; 2. donor-related covariates: age, sex, days of mechanical ventilation, use of EVLP, donation after cardiac death donors, Oto score, size mismatch (ratio of donor/recipient height). These covariates were selected according to our expertise and according to Brookhart et al [10]. The association with airway complication and CIT was evaluated using the PS weighted marginal Cox model with penalized splines for the exposure variable. Proportional hazard and linearity assumptions were checked [11]. The pointwise

nonparametric hazard ratio curves were plotted [12]. This analysis considered each performed anastomosis as one statistical unit. Two-sided P values and 95% confidence intervals were computed. Statistical significance was considered when the P value was equal to or less than .05. All analyses were performed using the R 3.5.1 software (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

From January 2011 to December 2017 a total of 161 LuTxs were performed in our center. Fourteen patients were excluded from the study population for the following reasons: incomplete data (11 patients), death within 90 days after LuTx (1 patient), and retransplantation (2 patients). Lastly, 147 patients were selected and analyzed. One hundred and thirteen patients received bilateral LuTx, whereas 34 had single LuTx (16 right; 18 left). Median follow-up was 25.5 months (IQR = 35.2 months).

The main recipients, donors, and anastomoses characteristics were described in Table 1. None of the patients developed early ACs. Ten patients (6.8%) had late ACs; out of the 260 anastomoses considered, 14 proved to be complicated (5.4%). These ACs were classified as stenosis in 12 cases (any grade) and 2 as bronchial malacia. ACs were diagnosed between 3 and 13 months after LuTx (median = 5.5 months). Mean CIT was 446.6 minutes (range, 117-1200). Without considering time-to-event data, CIT was significantly higher in complicated anastomoses ($P = .002$).

Propensity Score Analysis

Table 2 shows the covariance balance across clusters after weighting with propensity score analysis; the correlation coefficient ranged from 0.001 to 1.060, indicating that confounding bias could be considered as reasonably controlled. The unweighted marginal univariable Cox model showed a significant association between ACs and CIT ($P < .001$). The propensity score weighted marginal univariable Cox model confirmed this significant association ($P < .001$). In particular, the log hazard ratio increased over time (Fig 1). Cox diagnostic tools indicated that the proportional hazard assumption is valid ($P = .996$) but not the linearity assumption ($P < .001$); therefore, we used penalized splines.

Table 1. Recipients, Donors, and Anastomoses Characteristics

Patients	No Airway Complications (n = 137)	Airway Complications (n = 10)	P Value
Recipient			
Age, y, mean (SD)	44 (15)	51 (17)	.183
Male, n (%)	70 (51.1)	7 (70.0)	.408
Underlying disease, n (%):			
cystic fibrosis	67 (48.9)	4 (40.0)	.829
obstructive disease	12 (8.8)	1 (10.0)	.999
restrictive disease	58 (42.3)	5 (50.0)	.887
Bilateral LuTx, n (%)	107 (78.1)	6 (60.0)	.356
Lobar LuTx, n (%)	5 (3.6)	0 (0.0)	.999
Post-LuTx MV, d, median (IQR)	2.0 (4.0)	2.0 (3.3)	.414
ECLS, n (%)	59 (43.1)	4 (40.0)	.999
Pre-LuTx ECMO, n (%)	19 (13.7)	2 (20.0)	.947
Intraoperative ECMO, n (%)	57 (41.6)	3 (30.0)	.698
Post-LuTx ECMO, n (%)	25 (18.2)	2 (20.0)	.999
Height, cm, median (IQR)	165 (13)	173 (17.8)	.105
BMI, median (IQR)	21.3 (5.8)	23.0 (4.1)	.161
Pre-LuTx airway colonization, n (%)	77 (56.2)	4 (40.0)	.506
Post-LuTx airway colonization (POD 1-14), n (%)	93 (67.8)	7 (70.0)	.998
Treatment for AR within 6 months, n (%)	28 (20.4)	2 (20.0)	.998
CAD, n (%)	8 (5.8)	1 (10.0)	.998
Diabetes, n (%)	49 (35.7)	5 (50.0)	.574
Donor			
Age, y, median (IQR)	49.0 (19.0)	43.0 (7.5)	.315
Male sex, n (%)	82 (79.8)	7 (70.0)	.765
MV, d, median (IQR)	2.0 (3.0)	1.5 (4.3)	.683
BMI, median (IQR)	24.5 (5.3)	25.9 (2.5)	.250
Oto score, median (IQR)	3 (3)	5 (5)	.201
EVLP	15 (10.9)	4 (40.0)	.031
DCD	4 (2.9)	0 (0.0)	.998
Airway colonization	58 (42.3)	3 (30.0)	.665
Mismatch D/R (height), median (IQR)	1.04 (0.10)	1.04 (0.05)	.594

Anastomosis	No Airway Complications (n = 246)	Airway Complications (n = 14)	
Right, n (%)	123 (50.0)	6 (42.9)	.806
CIT, min, median (IQR)	427.5 (252.8)	603.5 (397.5)	.002

BMI, body mass index; CAD, coronary artery disease; CIT, cold ischemia time; DCD, donation after circulatory death; ECLS, extracorporeal life support; ECMO, extracorporeal membrane oxygenation; EVLP: ex vivo lung perfusion; IQR, interquartile range; LuTx, lung transplant; MV, mechanical ventilation; POD, postoperative day.

DISCUSSION

Airway anastomotic complications are still regarded as a major problem in lung transplantation. Despite decades of progress, this problem is far to be overcome. The reduction of proximal blood flow that occurs immediately after transplantation in the bronchial graft is a fact. Moreover, the donor side of anastomosis receives only the retrograde revascularization from low-pressure and low-oxygenated pulmonary circulation. Several factors have been deemed responsible for slowing down revascularization: length of donor bronchus, size discrepancy, infection, primary graft dysfunction, prolonged positive pressure airway ventilation, and donation after circulatory death. Among these, the length of graft CIT is discussed. Papers that directly addressed this issue failed to show the association between CIT and ACs [13,14]. Notably, the mean CIT reported in literature ranged from 90 to 340 minutes [13-16]; in contrast, CIT was consistently higher in our cohort (mean 433 minutes), essentially because of logistical management. This time discrepancy was probably one of the main

Table 2. Absolute Pearson Correlation Coefficient Between Cold Ischemic Time and Baseline Covariates

Variable	Absolute Pearson Correlation Coefficient
Recipient age	0.020
Recipient sex	0.098
Underlying disease	0.001
Right side	0.102
Bilateral LuTx	0.067
Recipient BMI	0.034
Lobar LuTx	0.101
Post LuTx MV	1.060
Intraoperative ECMO	0.033
Post LuTx ECMO	0.032
Recipient height	0.088
Treatment of AR within 6 months	0.107
Pre-LuTx airway colonization	0.048
Post-LuTx airway colonization (POD 1-14)	0.018
CAD	0.061
Diabetes	0.020
Donor age	0.044
Donor sex	0.077
Donor MV (days)	0.088
EVLP	0.110
DCD	0.044
Oto score	0.106
Height mismatch D/R	0.077

AR, acute rejection, BMI, body mass index; CAD, coronary artery disease; DCD, donation after circulatory death; ECMO, extracorporeal membrane oxygenation; EVLP, ex vivo lung perfusion; LuTx, lung transplant; MV, mechanical ventilation; POD, postoperative day.

elements that allowed us to observe the association between CIT and ACs. In particular, looking at Chart B in Figure 1, a significant risk of ACs is evident when the CIT exceeds 600 minutes. This relationship seems nonlinear thus, the outcome could be substantially different when considering the same time intervals at different time points. It is well known that mitochondria play a central role in determining cell death by apoptosis and necrosis. After 60 minutes of warm ischemia, the electron transport complexes show a reduction in their activity with structural damage [17]; damage to ATP synthase (complex V) has been showing also after cold ischemic injury [18]. Those damages persist for an extended period after reperfusion; in addition, bronchial mucosa takes approximately 4 weeks to regain adequate vascularization. It could be speculated that these mechanisms cause excessive cell loss through apoptosis leading to fibrotic rearrangement and late bronchial stenosis.

The association between ACs and EVLP could be partially explained with the sum of the CITs before and after the procedure. On the other hand, the EVLP introduces several donor and procedural variability components, thus calling for further dedicated studies.

There were several limitations to this study worth noting: the retrospective nature of the design and the relatively small number of events were among the major problems. In particular, the number of events yielded wide confidence limits and restricted the possibility to estimate the precise hazard function of ACs and precluded multivariable analysis.

Timing of ACs development and their diagnosis did not always match owing to the lack of symptoms. The very nature of surgical techniques imposed another limitation: slight differences among surgeons' methodologies might occur and cannot be assessed. Despite bronchoscopies being frequently recorded and discussed among the surgical team members, an interobserver variation could not be excluded.

The association between CIT and ACs estimated with PS weighted Cox model could be spurious. Moreover, the residual confounding bias owing to unmeasured and unmeasurable confounders could not be excluded. For this reason,

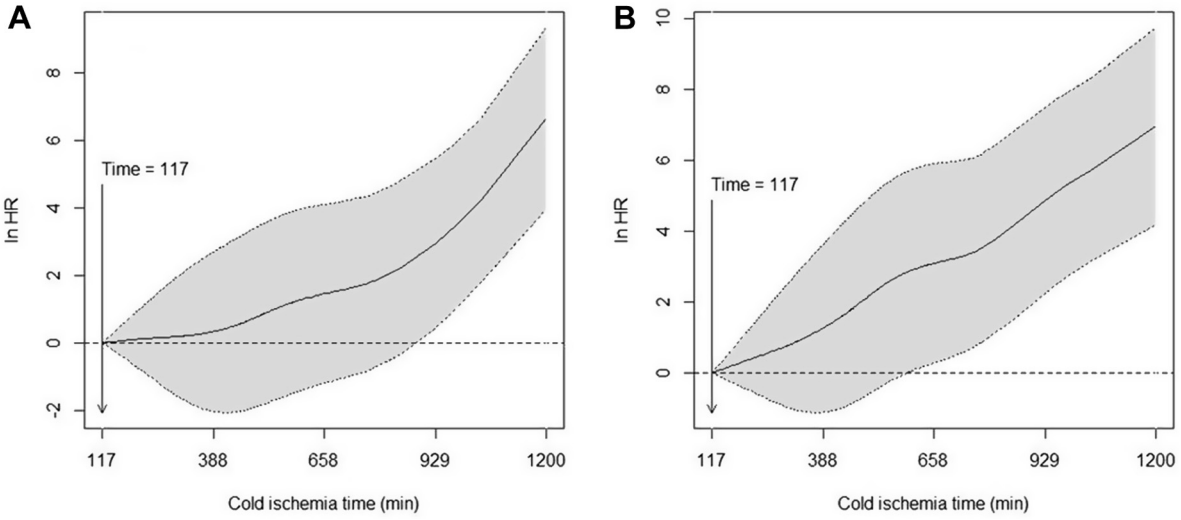

Fig 1. Nonparametrics estimates for the relation between ln hazard ratio and cold ischemic time with 95% confidence limits. **(A)** Unadjusted model; **(B)** Propensity score weighted model. A value of 117 minutes is the minimum cold ischemic time measured.

a large-scale study should be carried out to confirm our findings and add more precise estimations.

In conclusion, considering univariable analysis, the prolonged CIT seems to be a risk factor for the development of late ACs; we endorse any action that could limit CIT within 600 minutes. Further research is needed to confirm this result and to clarify the impact of EVLP on ACs.

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