Epithelial Cell Death Markers in Bronchoalveolar Lavage Correlate with Chronic Lung Allograft Dysfunction Phenotypes

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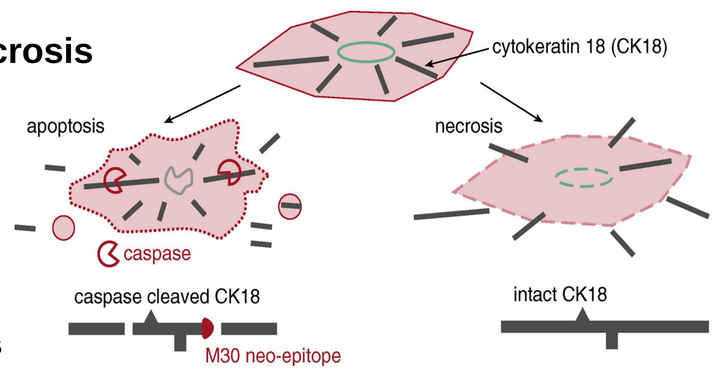
Introduction

- ❑ Chronic lung allograft dysfunction (CLAD) remains the main hurdle for the survival after lung transplantation (LTx). Two phenotypes of CLAD have been defined:
  - Bronchiolitis obliterans syndrome (BOS) is characterized by an obstructive physiology, air trapping on CT and obliterative bronchiolitis on histopathology
  - Restrictive allograft syndrome (RAS) is characterized by a restrictive physiology and parenchymal fibrosis on CT and histopathology
- ❑ Chronic epithelial injury is thought to be a key event in the pathogenesis of CLAD
- ❑ Cytokeratin 18 (CK18) is a component of the cytoskeleton of epithelial cells that are released during epithelial cell death
- ❑ CK18 fragments may serve as a marker of epithelial cell death:

M30 → apoptosis

M65 → apoptosis plus necrosis



Hashimoto et al., JRCCM. 2016;194(1):97-105

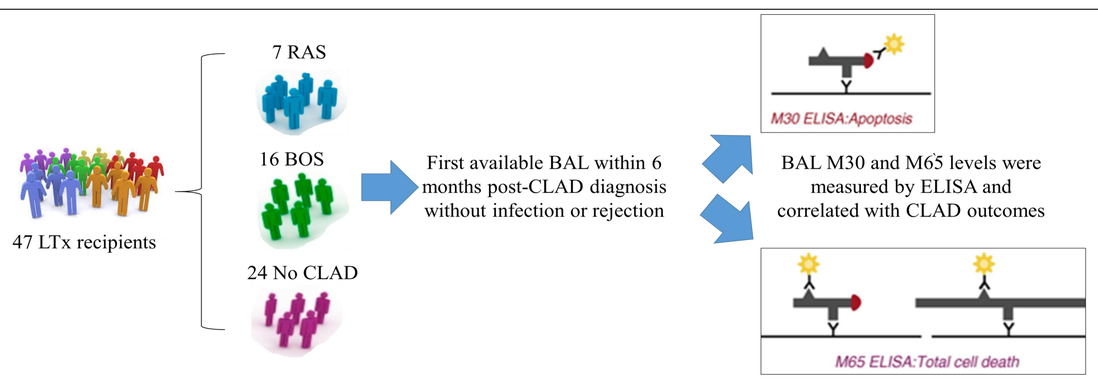
Hypothesis

CLAD phenotypes are associated with differential degrees and types of lung epithelial cell death

Objective

To determine whether markers of epithelial cell apoptosis and necrosis are elevated in the bronchoalveolar lavage (BAL) of patients with CLAD, and specifically in RAS vs. BOS

Materials and Methods



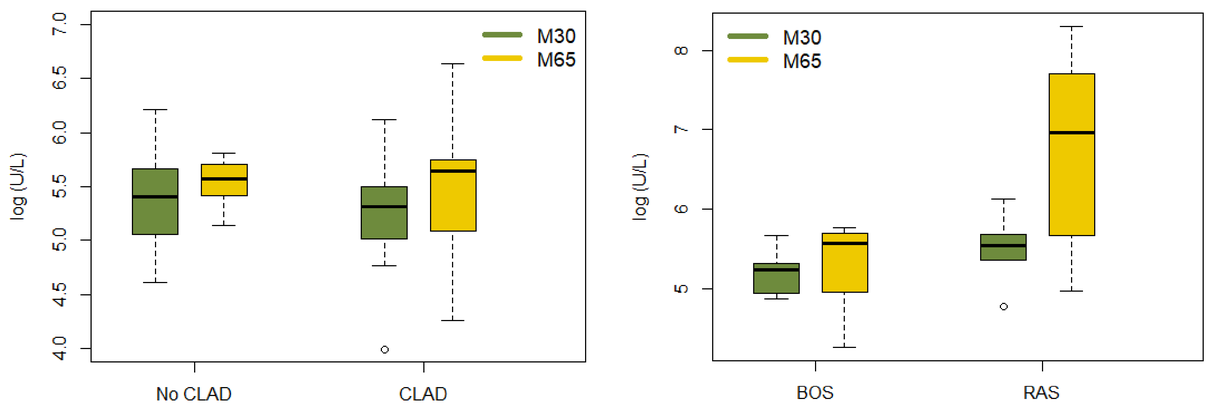
- ❑ Variables were compared between groups using Kruskal-Wallis and Chi-squared tests
- ❑ Association of M30 and M65 levels with survival was assessed using univariable and multivariable Cox Proportional Hazard models
- ❑ Survival curves for patients high and low M65 levels were compared using the Log Rank Test

Results

Table 1. Patient characteristics

		Control (n=24)	BOS (n=16)	RAS (n=7)	P value
Recipient age at transplant, year (median ± IQR)		51.5 (46,61.5)	48.5 (30.8, 53.8)	43.0 (31.5, 55)	0.18
Male Sex, n (%)		14 (58.3)	10(62.5)	4 (57.1)	0.96
Donor-recipient sex mismatch		7 (29.2)	4 (25)	1 (14.3)	0.73
Primary diagnosis, n (%)					0.20
Pulmonary fibrosis		9 (37.5)	9 (56.3)	1 (14.3)	
Chronic obstructive pulmonary disease		5 (20.8)	2 (12.5)	0	
Cystic fibrosis		3 (12.5)	3 (18.8)	3 (42.9)	
Other		7 (29.2)	2 (12.5)	3 (42.9)	
CMV Serology, n (%)					0.0002
D+/R-		2 (8.3)	4 (25)	6 (85.7)	
D+/R+, D-/R+		14 (58.3)	5 (31.3)	1 (14.3)	
D-/R-		8 (33.3)	7 (43.8)	0	
Transplant type					0.85
Bilateral lung transplant, n (%)		22 (91.7)	16 (100)	7 (100)	
Heart-Lung transplant, n (%)		2 (8.3)	0	0	
Time from transplant to bronchoscopy, days	median (IQR)	734.5 (727,	737 (555, 1271.5)	380 (373, 540.5)	0.015
Time from transplant to CLAD onset, days	median (IQR)	741.8)	683 (508, 1254)	380 (342, 473)	0.019
Time from CLAD onset to bronchoscopy, days	median (IQR)	N/A	54 (21.8, 124.3)	21 (9.5, 78.5)	0.19

Figure 1. M30 and M65 levels do not differ between CLAD and No CLAD but are significantly elevated in RAS compared with BOS



	No CLAD	CLAD	P value
M30	223.4±101.7 U/L	201.4±94.6 U/L	0.49
M65	262.6±49.4 U/L	619.7±985.2 U/L	0.43

	BOS	RAS	P value
M30	172.3±72.5 U/L	264.4±112.3 U/L	0.045
M65	219.8±88.2 U/L	1486±1469.5 U/L	0.002

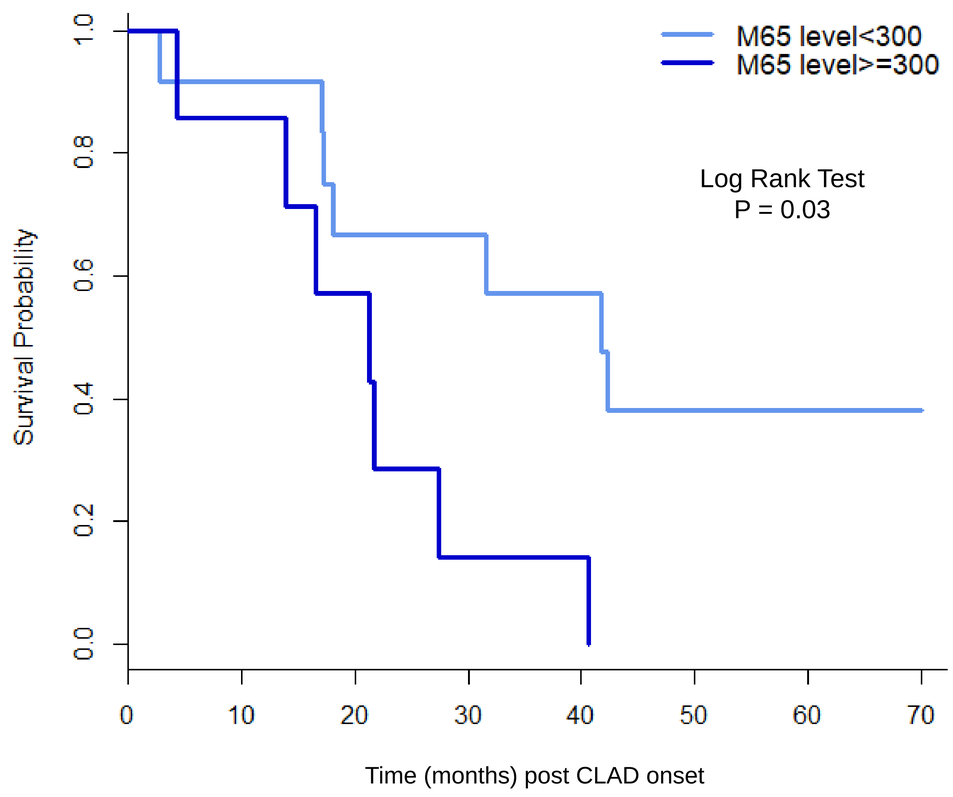
Table 2. Association of M30 and M65 levels with survival after CLAD onset, using Cox proportional hazards model

	Univariable analysis		
	Hazard ratio	95% CI	p-value
M65	2.37	1.27-4.43	0.007 *
M30	1.74	0.92-3.29	0.09

* The association remained significant after adjusting the models for age, sex, primary diagnosis, or CMV mismatch status

* After adjusting for CLAD phenotype, the association was no longer significant, due to the relationship between CLAD phenotype and M65 levels

Figure 2. Kaplan–Meier curves showing reduced survival after CLAD onset for patients with higher M65 levels



Conclusions

- ❑ Markers of epithelial cell apoptosis and necrosis are elevated in the BAL of patients with RAS early after CLAD onset and may be useful to differentiate CLAD subtypes
- ❑ The marker of total epithelial cell death M65 may be used as a potential prognostic biomarker, especially early after CLAD onset

References

1. Beers et al., J Clin Invest. 2011;121(6):2065-2073
2. Hashimoto et al., AJRCCM. 2016;194(1):97-105
3. Borthwick et al., Thorax. 2009;64(9):770-777.

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Disclosures

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