SUPPLEMENTARY MATERIAL

Title: **Role of Soluble T-Cell Immunoglobulin Mucin Domain-3 in Differentiating Nontuberculous Mycobacterial Lung Disease from Pulmonary Colonization**

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Running Title: Soluble TIM-3 and NTM lung disease

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Supplementary material

Appendix 1. Diagnostic Criteria for Nontuberculous Mycobacterial Lung Disease (NTM-LD)

The American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) guideline defines diagnostic criteria for NTM-LD [1]. The criteria were as follows: 1) compatible respiratory symptoms; 2) chest radiography or computed tomography findings compatible with NTM-LD (ie, tree-in-bud patterns, nodular bronchiectasis, or fibrocavitary lesions); 3) culture positivity for NTM in at least 2 sputum samples, one bronchoalveolar lavage, or one lung tissue; and 4) exclusion of alternative diagnosis.

Appendix 2. Factors Associated with Soluble T-Cell Immunoglobulin Mucin Domain-3 (sTIM-3) Level

Pearson's correlation coefficients, r, were calculated to assess the associations between continuous variables. The correlation analyses revealed that sTIM-3 levels were positively correlated with advanced age (r = .264, P = .019), male sex (r = .299, P = .007), malignancy history (r = .247, P = .028), prior tuberculosis (r = .266, P = .018), soluble programmed cell death-1 (sPD-1) (r = .511, P < .001), and soluble programmed cell death ligand-1 (sPD-L1) levels (r = .335, P= .003). The sTIM-3 levels were not associated with body mass index, acid-fast bacilli smear status, or radiographic score and patterns.

Supplemental Reference

1. Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med*. 2007;175(4):367-416.

Supplemental Table S1-S3

Supplemental Table S1. Immune checkpoint protein expression in lymphocytes and levels of their soluble forms in patients with flow cytometry data in 2019 (n = 37)

Variables	Healthy control (n=11)	NTM-Col (n=11)	NTM-LD (n=15)	P value (ANOVA)	P value for trend	P value (NTM-LD vs NTM-Col)	P value (NTM-LD vs healthy)
Tim-3 expression (%)							
CD4+ lymphocyte	11.6 ± 12.8	13.0 ± 9.6	28.6 ± 24.0	0.032	0.017	0.034	0.130
CD8+ lymphocyte	28.3 ± 12.9	36.9 ± 23.1	48.3 ± 20.9	0.040	0.011	0.188	0.013
sTim-3 levels (pg/ml)	534.4 ± 351.9	706.7 ± 347.8	476.0 ± 302.7	0.218		0.084	0.751
PD-1 expression (%)							
CD4+ lymphocyte	32.8 ± 24.8	27.7 ± 12.0	34.1 ± 20.0	0.704	0.813	0.353	0.867
CD8+ lymphocyte	25.9 ± 23.7	28.6 ± 12.7	32.1 ± 20.2	0.719	0.414	0.616	0.497
sPD-1 levels (pg/ml)	51.6 ± 34.6	65.7 ± 57.2	45.9 ± 27.2	0.461		0.304	0.642
PD-L1 expression (%)							
CD4+ lymphocyte	9.8 ± 9.2	12.9± 11.5	11.4 ± 8.5	0.750	0.702	0.711	0.637
CD8+ lymphocyte	7.0 ± 7.8	11.0 ± 9.8	9.4 ± 7.0	0.528	0.520	0.634	0.429
sPD-L1 levels (pg/ml)	6.2 ± 7.2	7.3 ± 12.1	10.7 ± 30.0	0.846		0.726	0.635

Continuous data are expressed as mean ± standard deviation (SD).

Abbreviation: ANOVA, analysis of variance; NTM-Col, nontuberculous mycobacterium (NTM) colonization; NTM-LD, NTM lung disease; sPD-1, soluble programmed cell death-1 (PD-1); sPD-L1, soluble programmed cell death ligand-1 (PD-L1); sTIM-3, soluble T-cell immunoglobulin mucin domain-3 (TIM-3).

Supplemental Table S2. Subgroup analysis of factors associated with NTM-LD in patients without cavitary lesions (n = 69)

Variables	Univariate Crude OR (95% CI)	p value	Multivariate, forward selection Adjusted OR (95% CI)	p value	Multivariate, fully adjusted Adjusted OR (95% CI)	p value
Age (years)	0.988 (0.955-1.022)	0.474			0.988 (0.934-1.044)	0.663
Male sex	0.200 (0.070-0.568)	0.002	-		0.161 (0.021-1.214)	0.076
BMI (Kg/m^2)	0.790 (0.681-0.916)	0.002	0.771 (0.632-0.940)	0.010	0.837 (0.661-1.059)	0.139
Ever-smoker	1.969 (0.464-8.361)	0.359				
sTim-3 (100-pg/ml increment)	0.805 (0.689-0.940)	0.006	0.712 (0.581-0.873)	0.001	0.618 (0.450-0.847)	0.003
Bilateral lung disease	5.224 (1.760-15.505)	0.003	-			
Radiographic score ≥5	5.500 (1.929-15.684)	0.001	5.383 (1.317-22.005)	0.019	8.144 (1.075-61.709)	0.042
Sputum AFB-smear positivity	1.000 (0.364-2.745)	1.000			2.207 (0.318-15.301)	0.423
No. of NTM-positive sputum[a]	1.639 (0.971-2.767)	0.064	-			
MAC subspecies (n=60)						
Other species	1.0 (reference)				1.0 (reference)	
M. avium complex	2.917 (0.954-14.327)	0.187			3.060 (0.305-30.674)	0.342
M. abscessus complex	5.714 (1.532-21.317)	0.009			31.946 (1.737-555.612)	0.017

[a] The number of NTM-positive sputum samples was not included in the fully adjusted model because it was highly linearly correlated with NTM subspecies.

Abbreviation: AFB, acid-fast bacilli; BMI, body mass index; NTM-LD, nontuberculous mycobacterium (NTM) lung disease; sTIM-3, soluble T-cell immunoglobulin mucin domain-3.

Supplemental Table S3. Characteristics of patients classified by number of factors associated with NTM-LD (n = 79)

Variables	0 factor (n=10)	1 factor (n=16)	2 factor (n=34)	3 factor (n=19)	P values
Criteria-meeting NTM-LD	0	5 (31%)	26 (76%)	18 (95%)	<0.001
Age, years	72.6 ± 17.3	67.9 ± 8.9	70.6 ± 16.5	61.4 ± 10.1	0.102
Female sex	8 (80%)	7 (44%)	12 (35%)	4 (21%)	0.018
BMI ≤22.5 Kg/m2	0	7 (44%)	31 (91%)	19 (100%)	<0.001
sTim-3 ≤530 pg/ml	0	7 (44%)	14 (41%)	19 (100%)	<0.001
Radiographic score ≥5	0	2 (13%)	23 (68%)	19 (100%)	<0.001
Sputum AFB-smear positivity	4 (40%)	7 (44%)	10 (29%)	10 (53%)	0.404
No. of NTM-positive sputum	2 [1-2.25]	2 [1.25-2.75]	3 [2-3]	3 [3-3]	0.018
NTM-species (n=68)					0.043
Mycobacterium avium complex	1/8	5/10	22/32	8/18	
Mycobacterium abscessus	2/8	1/10	5/32	6/18	
Others	5/8	4/10	10/32	4/18	

Continuous and categorical data are expressed as mean ± standard deviation (SD) and number (%), respectively.

Abbreviation: AFB, acid-fast bacilli; BMI, body mass index; NTM-LD, nontuberculous mycobacterium (NTM) lung disease; sTIM-3, soluble T-cell immunoglobulin mucin domain-3.