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Preprocurement *In Situ* Donor Lung Tissue Gene Expression Classifies Primary Graft Dysfunction Risk

To the Editor:

Primary graft dysfunction (PGD) is a form of acute lung injury following lung transplantation that is responsible for 50% of all 30-day mortality experienced and has an attributable risk of death of 23% at 1 year (1). PGD is the primary driver of morbidity after transplant and predisposes patients to death from other causes (2). Given that donors are often assessed over a period of days prior to transplantation, prediction of recipient PGD risk based on donor characteristics is an attractive concept. Thus far, reliable clinical PGD risk classification has

been elusive. We previously showed that upregulation of innate immune pathways in recipient blood and lung lavage fluid occurs soon after transplantation (3, 4). We therefore hypothesized that innate immune transcripts from donor lung tissue obtained prior to transplantation could be used to predict recipient PGD risk.

We conducted a prospective cohort study of matched donor/recipient pairs transplanted at the University of Pennsylvania between October 2011 and December 2017. Institutional review board approval and patient written informed consent were collected prior to enrollment. Those not included in the analysis had insufficient sample, did not consent for participation, or failed to be collected by the procurement surgeon owing to circumstance (e.g., donation after circulatory death donor or time limitations). A 1 × 1 cm lung tissue biopsy was collected *in situ* in the donor prior to cross clamp and stored immediately in RNAlater. Gene expression profiles were obtained using the Affymetrix Human ST 2.1 (ThermoFisher Scientific) oligonucleotide microarray. Raw expression data were corrected for background, quantile normalized using the robust multiarray average method, and summarized at 23,819 gene levels (5).

We focused on TLR (Toll-like receptor) and NLR (Nod-like receptor) signaling pathways from the Kyoto Encyclopedia of Genes and Genomes Pathway Database because our prior work demonstrated association with PGD in bronchial wash and blood (3, 4). We applied a feed-forward deep learning method for PGD classification (6). Recipient body mass index, diagnosis, pulmonary artery pressure, and donor smoking status were considered to be included in the model as previously identified (7). A set of covariates, including body mass index, cytomegalovirus status, cardiopulmonary bypass use, transplant type, and total ischemic time, were subsequently selected to optimize Akaike's information criterion in a logistic regression model. We used cross entropy as the objective loss function, the hyperbolic tangent function as the activation function, two hidden layers with sizes 200 and 100, a learning rate of 0.001, and an epoch of 300. We used five-fold cross-validation to avoid overfitting. We report the receiver operating characteristic curve, the area under the receiver operating characteristic curve, and prediction accuracy at a cutoff probability optimized for sensitivity and specificity. The results were also compared with a deep learning model that used the five covariates as the only predictors.

We enrolled 113 subjects, of whom 28 (24.8% [95% confidence interval, 17.1–33.8]) developed grade 3 PGD defined at 48–72 hours. Differences in donor and recipient characteristics or operative factors between subjects with and without PGD appear in Table 1. Table 2 summarizes the predictive classifier performance between clinical variables alone or in combination with our identified pathways. The TLR signaling pathway demonstrated superior discriminant ability to covariates alone ($P=0.014$) in contrast to the NLR signaling pathway ($P=0.296$). The TLR-based classifier improved PGD prediction by 0.20 over the model using clinical factors alone. With respect to diagnostic test performance, TLR signaling demonstrated superior performance to covariates alone and not NLR (Table 2).

The clinical implications of this TLR classifier depend on PGD prevalence and context of use. For enrichment purposes using the values from Table 2, the predicted PGD probability could be enriched two-fold over baseline (20% PGD pretest probability [odds of 0.25]; 2.673 likelihood ratio [+]; the *post hoc* test odds would be 0.67 [0.25 × 2.673], corresponding to a *post hoc* test probability of 40% [0.67/(1 + 0.67)]). Conversely, for efforts focused on PGD risk reduction, this classifier could reduce the predicted probability of PGD by more than 60% (*post*

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Table 1. Patient Characteristics

Characteristics	No PGD (n = 85)	PGD (n = 28)	P Value
Sex, M	50 (58.8)	15 (53.6)	0.626
Age, yr	56.6 ± 12.8	57.0 ± 10.0	0.884
Race			0.521
White	69 (81.2)	19 (67.9)	
Black	5 (5.9)	3 (10.7)	
Asian	2 (2.4)	1 (3.6)	
Hispanic	2 (2.4)	1 (3.6)	
Other or unknown	7 (8.2)	4 (14.3)	
Diagnosis			0.187
COPD	18 (21.2)	5 (17.9)	
IPF	30 (35.3)	6 (21.4)	
Non-IPF ILD	18 (21.2)	10 (35.7)	
Cystic fibrosis	10 (11.8)	2 (7.1)	
Bronchiectasis	4 (4.7)	0	
Sarcoidosis	3 (3.5)	4 (14.3)	
PAH	1 (1.2)	1 (3.6)	
Bronchiolitis obliterans	1 (1.2)	0	
CMV positive	41 (48.2)	10 (35.7)	0.248
BMI	24.8 ± 4.7	26.5 ± 4.7	0.109
Pulmonary artery pressure	29.5 ± 15.4	31.2 ± 10.7	0.595
LAS	45.0 ± 15.7	47.3 ± 14.0	0.504
Bilateral transplant	55 (64.7)	24 (85.7)	0.056
Total ischemic time			
Single	234.9 ± 52.3	241.0 ± 126.6	0.841
Bilateral	334.8 ± 77.2	364.5 ± 95.5	0.149
Donor mode of death			0.675
Head trauma	29 (34.1)	9 (32.1)	
Stroke/cerebrovascular	27 (31.8)	12 (42.9)	
Anoxia	28 (32.9)	7 (25.0)	
Other	1 (1.2)	0 (0.0)	
Donor PaO ₂	477.2 ± 80.4	475.9 ± 79.7	0.940
Donor sex, M	43 (71.7)	17 (28.3)	0.352
Donor smoking	40 (47.1)	15 (53.6)	0.550

Definition of abbreviations: BMI = body mass index; CMV = cytomegalovirus; COPD = chronic obstructive pulmonary disease; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; LAS = Lung Allocation Score; PAH = pulmonary arterial hypertension; PGD = primary graft dysfunction. Data are shown as mean ± SD or n (%).

Table 2. Diagnostic Performance

Pathway	AUC	Sensitivity	Specificity	PPV	NPV	LR+	LR–
TLR signaling	0.776 (0.671–0.867)	0.786 (0.643–0.929)	0.706 (0.565–0.847)	0.471 (0.375–0.618)	0.910 (0.860–0.966)	2.673	0.303
NLR signaling	0.674 (0.547–0.790)	0.679 (0.536–0.857)	0.682 (0.529–0.812)	0.396 (0.316–0.539)	0.862 (0.810–0.928)	2.135	0.471
Clinical covariates only	0.574 (0.445–0.684)	0.607 (0.464–0.786)	0.624 (0.447–0.730)	0.347 (0.262–0.450)	0.828 (0.767–0.900)	1.614	0.630

Definition of abbreviations: AUC = area under the receiver operating characteristic curve; LR– = likelihood ratio negative; LR+ = likelihood ratio positive; NLR = Nod-like receptor; NPV = negative predictive value; PPV = positive predictive value; TLR = Toll-like receptor.

This table includes common classifier performance metrics. The AUC is also known as a C-statistic and often used as a global measure of test accuracy, with higher values signifying greater test accuracy. Comparison between TLR signaling and covariates alone ($P=0.0137$), NLR signaling and covariates alone ($P=0.296$), and TLR and NLR ($P=0.216$) are detailed. Also included are test characteristics of PPV and NPV, as well as LR+ and LR–, which are independent of primary graft dysfunction prevalence. Parentheses define 95% confidence intervals.

hoc test odds would be 0.076 [0.25×0.303] and corresponding to a *post hoc* test probability of 7.6% [$0.076/(1 + 0.076)$] (8).

We designed the classifier to assess organ quality for donor pool expansion strategies, to identify organs for possible rehabilitation on *ex vivo* lung perfusion, and to identify potential targets for directed therapies in clinical trials. The validation of the pathways identified in this transcript panel highlights the importance of the innate immune system in the development of PGD and identifies its constituent genes as potential therapeutic targets. This work builds on previous work identifying associated pathways and developing clinical predictors important in PGD to assess risk prior to procurement to facilitate decision making for potential therapeutics and advanced surgical therapies (3, 4, 7).

Although these results are promising, there are limitations to consider. The cohort sample size was small, therefore reducing overall power. Nonetheless, this study represents a temporal validation in tissue of prior findings in blood and BAL using a conventional machine learning approach. Tissue biopsy is an invasive procedure with associated risks. We validated our classifier in tissue to ensure that all lung compartments were sampled (endothelial, epithelial, and lymphoid), and we acknowledge that further refinement will be necessary to scale to clinical practice. Additionally, translating gene expression prediction to the bedside will require development of point-of-care technologies using abbreviated gene sets, such as those using microfluidics (9). As this study cohort did not overlap with our prior cohorts, we were unable to assess interactions between blood, BAL, and tissue compartments. Although this work is supported by several other studies that show association (3, 4, 10), additional validation will be necessary to confirm discriminant and diagnostic validity and generalizability.

In summary, we have demonstrated that transcript analysis of donor lung tissue, using an innate immunity pathway classifier, can be used in conjunction with clinical variables to predict PGD with excellent discrimination and precision. With the ability to identify organ risk, this panel has the potential to alter future PGD clinical trial designs and lead to the development of precision medical approaches. As PGD drives morbidity and mortality associated with lung transplant, further research in this area has the potential to improve outcomes following transplantation. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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Smoking Pattern in Men and Women: A Possible Contributor to Sex Differences in Smoke-related Lung Diseases

To the Editor:

Chronic obstructive pulmonary disease (COPD) due to cigarette smoking is a major cause of morbidity and mortality worldwide. Given

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