

**Peripheral Blood Monuclear Cells Gene Expression Patterns Predict Mortality In Patients With Idiopathic Pulmonary Fibrosis, [Publication Page: A5306]**

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**Background:** Idiopathic Pulmonary Fibrosis (IPF) is a chronic and fatal lung disease with lung transplant as the only available therapy. The course of IPF is variable and unpredictable. In this study we aimed to identify and validate a PBMC gene expression signature indicative of outcome in IPF.

**Methods:** Microarray analysis of PBMC from patients with IPF was performed in two independent cohorts and two different microarray technologies: a derivation cohort of 75 patients at the University of Pittsburgh and a replication cohort of 45 patients at the University of Chicago. Mortality was the major outcome studied. After blood draw and PBMC isolation, RNA was extracted, labeled and hybridized to human gene expression microarrays (Agilent – Pittsburgh, Affymetrix – Chicago). Data was analyzed using BRB Array Tools. A Cox regression model was used to evaluate individual gene association with survival followed by Hierarchical clustering of these genes. Survival gene set analysis was performed to identify pathways associated with survival in both cohorts. For PCR validation of prognostic genes we designed a multi-sample high-throughput qRT-PCR SmarChip (Wafergen) that allowed us to measure their transcripts in the combined cohort of 139 patients. PCR results were adjusted to age, sex and FVC.

**Results:** 2595 genes were associated with survival in the derivation cohort ( $p < 0.05$ ); Hierarchical clustering of these genes identified 2 major clusters of IPF patients with a significant difference in mortality ( $p = 0.003$ ), without a significant difference in other variables. The CTLA4 pathway was associated with survival in the derivation ( $p = 0.0008$ ) and replication cohorts ( $p = 0.0001$ ). SmartChip qRT-PCR confirmed that a decrease in PBMC expression of CD28, ITK, ICOS or LCK was predictive of earlier mortality in patients with IPF. While this prediction was significant in both genders it was dramatic in males. The male median survival adjusted to age, gender and FVC at baseline was 1.16, 1.16, 1.37 and 1.2 years for low CD28, ITK, ICOS and LCK respectively compared to 3.0, 2.38, 3.47 and 3.0 years for patients expressing high levels with hazard ratios of 2.51, 2.14, 2.44 and 2.16 respectively.

**Conclusions:** This is the first identification of a prognosis predictive peripheral blood gene expression signature in IPF. The replication of the arrays in two cohorts despite different platforms and the SmartChip validation of the results suggest that qRT-PCR measurement of CD28, ICOS, ITK and LCK in PBMC, should be evaluated and used for risk stratification of patients with IPF.

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