FINAL REPORT
DATE: 10 October 2023
GRANT: Conquer Cystic Fibrosis Lung Health Transplant Grant

RECIPIENT: Herbert Ludewick

PROJECT TITLE: Transforming diagnosis of lung transplant rejection.

Overview

Lung transplantation is the only treatment for many end-stage lung diseases, but it often results in poor outcomes and challenges in diagnosing rejection episodes. Liquid biopsy is emerging as a minimally invasive technology that can detect subtle changes in peripheral blood indicative of early disease, progression, and treatment response. This study aims to evaluate whether a non-invasive blood test could replace the current invasive lung biopsy methods used for diagnosing lung rejection.

Outcomes of the Research

The primary objective is to investigate if circulating cell-free DNA (cfDNA) can serve as a non-invasive biomarker for early post-transplant lung rejection. Key objectives achieved include:

1. Recruitment of 20 lung transplant patients at Fiona Stanley Hospital.
2. Development of assays to quantify cell-free circulating mitochondrial and nuclear DNA.
3. Establishment of a panel of 12 lung-specific methylation markers via multiplex PCR and next-generation sequencing to assess lung pathology in transplant patients.

Research Summary

We recruited 20 patients who underwent lung transplants at Fiona Stanley Hospital: 14 recent recipients and 6 patients with declining lung function. Preliminary analyses revealed elevated cfDNA levels in transplant recipients compared to healthy controls. One patient with exceptionally high cfDNA levels post-transplant unfortunately passed away.

Circulating mitochondrial DNA (cmtDNA) has shown potential as an indicator of tissue damage. We developed an assay to quantify cmtDNA in plasma, finding higher levels in lung transplant recipients than in healthy controls, suggesting tissue damage that may not be solely due to lung injury.

In some patients, high cfDNA levels appeared as early as three weeks post-transplant, potentially correlating with future exacerbations or mortality. DNA methylation, a common epigenetic marker, can indicate tissue origins. We optimized a multiplex PCR and next-generation sequencing approach for a panel of 12 lung-specific DNA methylation markers. These markers are hypomethylated in lung tissue and methylated elsewhere and if present in the plasma, indicate lung pathology.

In conclusion, circulating mitochondrial DNA and lung-specific methylation markers in plasma could be valuable for monitoring lung damage post-transplant. Further research is needed to fully realize cfDNA's potential for detecting acute lung rejection, particularly acute lung allograft dysfunction (ALAD), a potential precursor to chronic allograft dysfunction (CLAD).

Next Steps

1. Complete patient recruitment.
2. Analyze NGS data to determine cfDNA tissue origin.
3. Investigate cfDNA as a diagnostic biomarker for ALAD.
4. Examine the relationship between bronchoalveolar lavage fluid (BALF) mitochondrial DNA and clinical variables for predicting lung rejection.
5. Understand cfDNA release dynamics during declining lung function.
6. Expand recruitment to all lung transplant patients at the Advanced Lung Disease Unit at FSH.
7. Establish the first lung transplant biobank in Western Australia.

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