A Novel, Quantitative, Multi-analyte Immunoassay to Detect Biomarkers Involved in Lung Disease

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Abstract

Lung disorders affect 5–10% of the world's population, and account for 1/6 of deaths worldwide. Pulmonary insult can arise from a multitude of factors, including those that are extrinsic, intrinsic, or iatrogenic-related. Manifestations of pulmonary disease include asthma, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), interstitial pneumonia (UIP), lung cancer, and tuberculosis (TB). The array of symptoms associated with these lung conditions involve shortness of breath, cough, chest pain, fever, pleural effusion, pleural thickening pulmonary fibrosis, necrobiotic nodules, and bronchiolitis obliterans organizing pneumonia (BOOP). A decline in the quality of life in patients with lung disease is experienced not only physically, but also mentally due to the severity of the symptoms. One example is lung cancer, which is the most common cause of death in developed countries. Patients diagnosed with stage III or IV lung cancer have a survival rate of only 15% with current therapies.

The study presented here looked at several cytokine markers involved in airway constriction including IL-13, IL-5, and IL-4 as well as select biomarkers known to be involved in the regulatory pathways for IFN-γ and TNF-α. The Simple Plex™ assay that was employed is a novel, quantitative, multi-analyte immunoassay platform that delivers high precision and accuracy from only 25 µL of sample. This platform measures up to four analytes simultaneously from a single sample with very high sensitivity. It is a microfluidic-based system that allows parallel single analyte detection, and reduces non-specific antibody interactions often observed in other traditional multiplex platforms. The assay is a closed system, removing potential user variability. This is accomplished by enclosing the entire assay process within a single cartridge that provides results in just over one hour. Our preliminary studies suggest this platform may represent a highly efficient method for the quantitation of select biomarkers known to be involved in lung disease.

Introduction

According to the National Heart, Lung, and Blood Institute, asthma affects approximately 25 million people in the United States, including about 7 million children. Chronic lower respiratory diseases include chronic obstructive pulmonary disease (COPD), which is the third leading cause of death in the United States. Globally, the prevalence of COPD, a common inflammatory disease of the airways, is also on the rise worldwide.[1] Coupled with the fact that lung cancer is the leading cause of cancer death among both men and women in the United States, these diseases, which have very poor prognoses, demonstrate an urgent need for further research to understand the underlying mechanisms and factors that regulate the immune response in the respiratory system.[2]

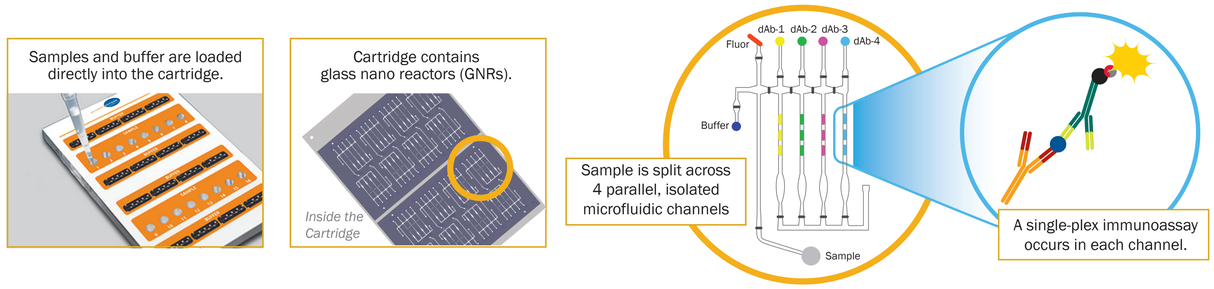
Pulmonary inflammation is a physiological response to tissue injury and insult. It can facilitate repair after injury, and set in motion a cascade of events to deal with both infectious and noninfectious agents. But prolonged inflammation, in response to chronic infection or repeated antigenic challenge, can also serve as a source of further lung injury and dysfunction.[3] The study presented here focuses on the molecular markers involved in both the pro-inflammatory and anti-inflammatory responses associated with pulmonary injury. Pro-inflammatory markers selected include IL-6, IL-8, and TNF-α. Anti-inflammatory markers investigated include IL-4, IL-10 and IL-13.[3,4] While these markers may be prevalent in general respiratory diseases, the levels may vary by disease state. In addition to these well-established pro- and anti-inflammatory markers, the levels of a number of other markers that may be relevant to pulmonary injury were also investigated, with the hope of identifying potential new targets of interest in the different disease states.

Methods

Serum samples from individuals diagnosed with asthma, pneumonia, lung cancer, or COPD were purchased from BioreclamationIVT. Severity of the condition, time since diagnosis, and treatments varied between samples, as did the age, gender, and race of all donors. Additional serum and plasma samples were obtained from apparently healthy in-house donors; no medical information was available.

All serum and plasma samples were evaluated on the Simple Plex™ multi-analyte immunoassay platform for the analytes specified in the table below. Disease state and normal samples were diluted according to each respective Simple Plex™ kit specification in sample diluent, with mixing prior to assaying. Data analysis excluded all values outside the dynamic range of the standard curve (Lower Limit of Quantification, LLOQ, and Upper Limit of Quantification, ULOQ). The concentration of each biomarker, in each sample, was quantified by comparison to the standard curves which are pre-loaded onto the cartridge bar code.

Assay Principle



Analytical Performance Characteristics for the Simple Plex™ Platform

Analyte Name	LLOQ (pg/mL)	ULOQ (pg/mL)	Average Intra-Assay	Average Inter-Assay	Average Linearity
Very High Abundance					
Total Adiponectin	20.9	51540	8.9%	6.5%	99%
CRP	4.55	50550	3.1%	3.7%	102%
High Abundance					
CD14	16.7	2830	9.7%	7.3%	105%
Chitinase-3 Like 1	5.10	52610	6.6%	10.0%	90%
MPO	1.57	18530	6.9%	9.2%	102%
Medium-High Abundance					
E-Cadherin	25.4	14860	3.1%	10.3%	101%
E-Selectin	3.80	14020	6.2%	9.0%	93%
MMP-8	37.2	9950	9.3%	12.0%	109%
ST2	39.10	28180	2.4%	7.7%	98%
Medium Abundance					
BAFF	1.05	9970	7.2%	7.9%	91%
HGF	5.69	15160	7.1%	5.4%	102%
IL-2 Rα	3.14	3920	5.6%	7.9%	86%

Analyte Name	LLOQ (pg/mL)	ULOQ (pg/mL)	Average Intra-Assay	Average Inter-Assay	Average Linearity
RAGE	3.37	6180	7.0%	9.0%	95%
TNF RI	0.80	2980	5.2%	10.9%	99%
TNF RII	1.41	2260	4.5%	7.7%	98%
Low-Medium Abundance					
MIG	13.5	15590	8.2%	10.7%	101%
VEGF A	4.84	4490	5.5%	6.7%	107%
Low Abundance					
IL-6	0.41	3850	3.2%	7.7%	115%
IL-8	0.80	2470	5.8%	6.9%	104%
IL-13	4.13	13720	3.6%	6.5%	92%
TNF-α	1.14	2900	5.0%	4.7%	111%
Very Low Abundance					
IL-4	0.32	1290	7.5%	11.0%	109%
IL-5	0.07	3120	6.5%	9.2%	98%
IL-10	0.46	5530	5.3%	7.1%	111%
IL-15	0.46	1950	6.9%	5.6%	90%

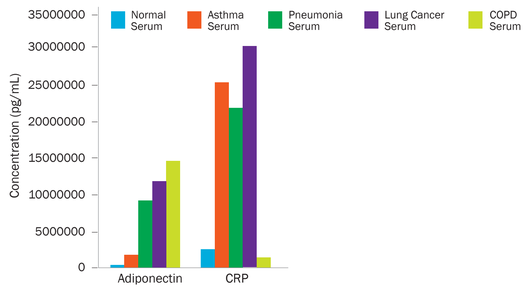
LLOQ= Lower Limit of Quantification ULOQ= Upper Limit of Quantification

References

1. Eagan, T.M. *et al.* (2010) Eur. Respir. J. **35**:540.
2. Bordon, J. *et al.* (2013) Int. J. Infect. Dis. **17**:e76.
3. Lian, X. *et al.* (2004) Am. J. Physiol. Lung Cell Mol. Physiol. **286**: L801.
4. Cuneo, A.A. & M.V. Autieri (2009) Curr. Vasc. Pharmacol. **7**:267.

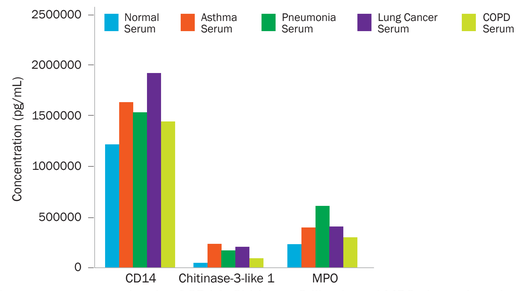
Results

Very High Abundance



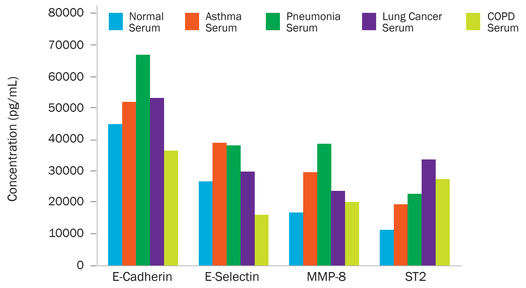
Total Adiponectin levels showed a mean level increase in each disease state serum population when compared with normal serum. CRP levels increased in each disease state, except in the COPD serum, where values showed a net decrease.

High Abundance



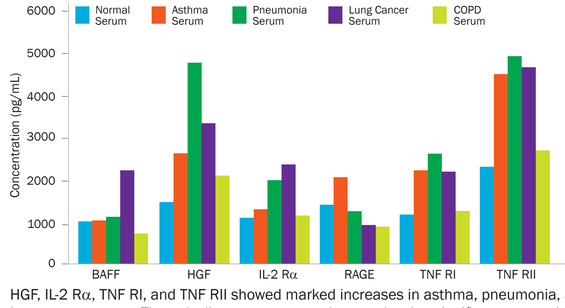
Disease-state serum levels of CD14, Chitinase 3-like 1, and MPO showed moderate to high mean level increases when compared with the normal serum levels of each marker.

Medium-High Abundance



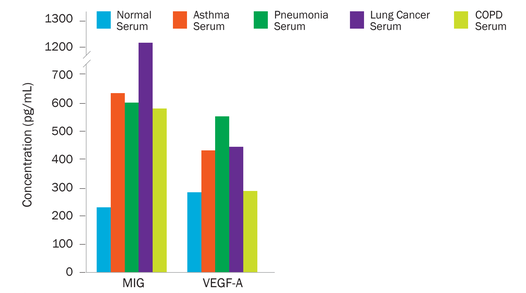
All markers in this category showed marked increases in asthma, pneumonia, and lung cancer serum. COPD samples expressed decreased levels of E-Cadherin and E-Selectin and increased levels of MMP-8 and ST2 when compared with normal serum.

Medium Abundance



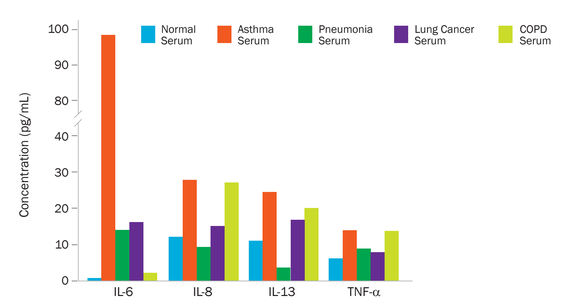
HGF, IL-2 Rα, TNF RI, and TNF RII showed marked increases in asthma, pneumonia, and lung cancer serum. The only disease-state sample type showing significant up-regulation of BAFF levels was the lung cancer serum, and the only disease-state sample showing significant up-regulation of RAGE was the asthma serum. COPD serum expressed mildly-increased or decreased levels of each analyte shown above when compared with normal serum.

Low-Medium Abundance



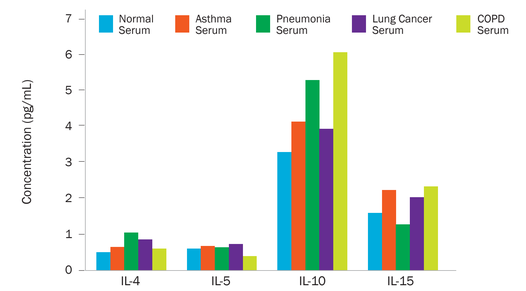
MIG levels increased significantly in all disease state serum evaluated. VEGF-A levels increased in asthma, pneumonia, and lung cancer serum, and were unchanged in COPD serum.

Low Abundance



All analytes in this graph showed increased levels in asthma serum. Pneumonia serum showed increased levels of IL-6 and TNF-α, and decreased levels of IL-8 and IL-13. Lung cancer samples displayed increased levels of IL-6, IL-8, and IL-13, while TNF-α levels were essentially unchanged. COPD caused minimal IL-6 up-regulation, but led to significant increases in IL-8 and IL-13, and to a lesser degree, TNF-α.

Very Low Abundance



The levels of IL-4 and IL-5 showed slight increases or decreases, depending on the disease-state serum evaluated, while the levels of IL-10 increased in all of the disease-state serum populations. IL-15 showed slight increases in asthma, lung cancer, and COPD serum samples, and a slight decrease in pneumonia serum.

Summary

An important area of interest to researchers investigating pathological respiratory conditions involves an understanding of the dynamic changes in biomarkers that accompany pulmonary disease. The purpose of our study was to determine if the Simple Plex™ multianalyte immunoassay platform could be utilized by researchers to efficiently and effectively identify respiratory disease-associated biomarkers in blood. Our results suggest that this is the case.

We compared the levels of 25 proteins in serum samples from control individuals and individuals diagnosed with asthma, pneumonia, lung cancer, and COPD using the Simple Plex™ platform. The results led to the classifications of very low, low, low-medium, medium, medium-high, high, and very-high abundance analytes. The conclusions obtained from this study can be found below the respective graph for each analyte set. By allowing the comparison of multiple biomarkers simultaneously from a single small sample volume, our results demonstrate the broad utility of the Simple Plex™ platform as a tool for discovery. This study is just a small part of a growing portfolio of research that is attempting to identify biomarkers involved in respiratory pathological processes.