Project Title: Immune Profiles in CF Fungal Infection

Study Protocol and Plan

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Project Title: Immune Profiles in CF Fungal Infections
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Hypothesis and Specific Aims: While bacteria are predominant agents in cystic fibrosis (CF) lung disease, fungi are often isolated in both sputum and bronchoalveolar lavage fluid (BAL), yet their role in CF is not fully understood. Fungal infection in CF has a wide spectrum of presentations, varying from transient detection to persistent infection, acute fungal bronchitis with resulting pulmonary exacerbation, sensitization to fungal allergens, and allergic bronchopulmonary aspergillosis (ABPA).[1-5] While there are clinical guidelines for the diagnosis and treatment of ABPA in patients with CF, there are few guidelines and recommendations regarding monitoring, clinical care, and antifungal treatment in the various presentations of CF fungal disease; further, the clinical impact of fungi without ABPA is poorly understood.[1, 6] While inflammation in CF is classically considered a neutrophil and macrophage driven process (Th1), eosinophils and allergic cytokines (Th2) have been shown to be elevated in the presence of fungal disease in both clinical and animal CF studies.[5, 7-12] Studies have also shown decreased lung function (e.g. percent predicted forced expiratory volume in one second, ppFEV1) in individuals with frequent fungal detection in their sputum, and associations with *Pseudomonas* infection and use of antimicrobials.[2, 5, 13] Adults with CF are also more prone to fungal sensitization when compared to non-CF patients as well as having higher rates of allergic rhinitis and atopy.[14-16] In a retrospective study performed at our center, we found that children with intermittent or chronic fungal infections experienced more rapid decline in lung function compared to those without fungal infections regardless of ABPA status (see preliminary data). Understanding whether fungal infections are driven by an allergic (Th2) inflammatory process may alter treatment approaches by utilizing steroids and antifungal therapies more readily in patients with fungal infection to combat lung function decline.[2, 4, 5] Limited studies have investigated the frequency of allergic sensitization to fungal pathogens in individuals with CF, the frequency of allergic sensitization in the pediatric CF population in general, and the unique inflammatory profiles and phenotypes of CF fungal infections, both in the sputum and the serum.

Hypothesis: We hypothesize that children with CF and fungal infections without ABPA will have elevated allergic inflammatory profiles and increased sensitization to fungal elements compared to those without fungal infection.

Specific Aims:
- Specific Aim 1: Compare Th2 inflammation in patients with and without fungal infections in patients with CF and to those with ABPA.
  - Approach: We will obtain serum and sputum samples from 25 patients with either (1) fungal infection without ABPA, n=10 (defined as ≥ two positive fungal cultures in prior 18 months), (2) no fungal infection, n=10, and (3) ABPA, n=5, and measure specific sputum and serum cytokine measurements to evaluate Th2 and Th1 inflammatory pathways.
  - Biomarkers measured: Eosinophilic cationic protein (ECP), Interleukin-4 (IL4), Interleukin-5 (IL5), Interleukin-10 (IL10), Interleukin-13 (IL13), and eosinophil count in both serum and sputum.
  - Expected outcome: Individuals with fungal infection without APBA will have elevated Th2 markers of inflammation compared to those with no fungal infection. We will also compare to those with ABPA as a separate control group. We will also measure Th1 markers to be used as a reference to previously established inflammatory profiles seen in CF, seeing if those with fungal disease deviate towards a more Th2 driven process.
- Specific Aim 2: Investigate allergic sensitization to fungal elements in patients with CF fungal infection without ABPA compared to those without fungal infection and to those with ABPA.

- Approach: We will measure total IgE and serum ImmunoCAP testing of specific IgE levels to fungal proteins of various species (*Aspergillus, Candida,* etc). to determine fungal sensitization. We will also survey patients regarding environmental exposures to explore relationships with allergic sensitization.
- Expected outcome: Individuals with fungal infection without ABPA will be more likely to show allergic sensitization to fungal elements when compared to those with no fungal infection.

The results of this study will help characterize the inflammatory profile associated with CF fungal infections contributing to the understanding of both the infectious and allergic nature of disease in the CF population. From this, the contribution to both the pathophysiology and clinical characteristics of CF fungal infections will serve as a step towards understanding management options, care guidelines, and disease progression for this unique set of hard to treat organisms.

Experimental Design and Methods

Specific Aim 1: Compare Th2 inflammation in patients with and without fungal infections in patients with CF and to those with ABPA.

Rationale: Studies have shown associations with fungal infection and worse clinical outcomes in CF, yet the data is limited and conflicting. It is unclear whether fungus is a driver of inflammatory change, a co-traveler with other infections, or the sequelae of microbiota shifts from antibiotic use. Furthermore, ABPA is a known condition that individuals with CF are at increased risk for, yet there is limited data on both serum and sputum immune profiles in patients with both CF and ABPA, as well as fungal infections in general. Given the findings in our retrospective study, we have seen greater decline in lung function in CF individuals with fungal infection, even when excluding ABPA diagnoses. Understanding whether fungal infections are driven by an allergic (Th2) inflammatory process may alter treatment approaches by utilizing steroids and antifungal therapies more readily in patients with fungal infection to combat lung function decline. [2, 4, 5]

Participant details:
- Gender: Male, female
- Ethnicity/Race: White, Hispanic, African-American, Native American, Other
- Age: 8-25 years old

Inclusion:
- Diagnosis of CF per CFF guidelines and followed at Children's Hospital Colorado (CHCO) CF Center
- Meets criteria of only one fungal group (described below)
- Clinical stability without any change acute antibiotic regimen in the past 14 days
- Clinical stability without any use for acute NSAID or oral steroids in past 14 days
- Individuals with other co-morbid conditions related to and unrelated to CF, including but not limited to CF related diabetes, CF related liver disease, asthma, etc.

Exclusion:
- History of *Burkholderia sp*. or Non-tuberculosis Mycobacterium
- Comorbid or health contraindication to induced sputum treatment or blood draw

Fungal Infection group: Have had a fungal species isolated from sputum and/or BAL culture on >= 2 separate occasions in the 18 months preceding study visit and do not have a diagnosis of ABPA (N=10).
Control group: Have never previously isolated fungus from sputum, BAL, or OP swab (N=10).
ABPA group: Previous diagnosis of ABPA as defined by CFF guidelines, regardless of the amount of fungal infection or history thereof.
- ABPA Minimum diagnostic criteria per CFF: Acute or subacute deterioration, total serum IgE > 500 IU per mL, immediate cutaneous reactivity to Aspergillus or in vitro IgE antibody to A. fumigatus, and either a new or recent chest imaging change that has not responded to antibiotics and standard physiotherapy OR precipitin to A. fumigatus or IgG antibody to A. fumigatus[1] (N=5). Culture positive sputum is not required for ABPA diagnosis and is not taken into account for the diagnosis per CFF guidelines.

Recruitment: We propose to recruit 25 patients with CF aged between 8 and 25 years who agree to participate in this cross-sectional study looking at the immune system characteristics of fungal infection and ABPA. We plan to screen individuals coming to their routine CF clinic and approach them regarding their interest in participating in this study. From this, we will explain the concept, hypothesis, and procedures involved with intent to schedule them for a research visit.

Patient Selection: Patients will be recruited from the CF Center at CHCO by study investigators or qualified research coordinators at the time of a routine clinic visit.

Sample Size Estimates: Power and sample size for the study proposal is fixed due to the expected number of eligible patients to be recruited into this pilot study during the study window. Based on preliminary data and the described definitions of types of infection, investigators anticipate that over a 1-year recruitment window they will be able to enroll approximately 10 individuals with no fungal infection, 10 patients with a fungal infection but not ABPA and 5 with ABPA. The primary analysis plan focuses on the description and comparison of key plasma and sputum measures described in Table 3. Power analysis provides the detectable effect sizes when comparing the control arm (no fungal infection) to either the non-ABPA group or the ABPA group. Calculations are based on a power of 80% and Type I error rate of 0.05 for two-sided two-sample equal-variance t-test conducted in PASS version 15 statistical software (2). Group sample sizes of 10 and 5 in the fungal infection groups achieve 80% power to reject the null hypothesis of zero effect size when the population effect size is 1.36 and 1.66 respectively. Thus, the study is powered at 80% to detect a difference in the mean markers to be measured greater than 1.66 and 1.32 standard deviations. Given limited data in the literature regarding Th2 markers in CF, we are basing these calculations off of deviations from a mean for the multiple Th2 markers we are testing.

Table 2. Study Activities During Visit

Study Activities	Perform at Visit
Informed consent	x
History and physical	x
BMI Measurement	x
Environmental Questionnaire	x
Spirometry	x
Induced Sputum Collection	x
Blood Draw	x

Table 3. Biomarkers to be studied. IL4, IL5, and ECP measurements are priority for Th2 assessment. NE, IL8, IL-1B are priority for Th1 assessment.

Sputum	Serum
• Culture for CF pathogens • Culture for fungi • Cell count with differential **Th1 pathway** • IL8, IFNY, IL-1B • NE **Th2 pathway** • IL4, IL5, IL10, IL13 • ECP Aliquot frozen and banked	**Th1 pathway** • IL8, IFNY • NE-APC **Th2 pathway** • IL4, IL5, IL10, IL13 • ECP • Total IgE • Fungal panel (Table 4) **Other** • CBC with diff • Vitamin D • Calprotectin • High-sens CRP Aliquot frozen and banked

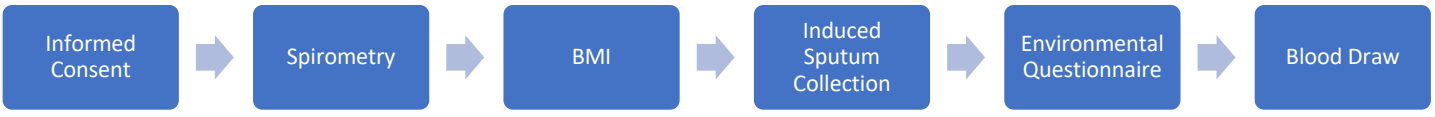
Study Visit: We plan to prospectively recruit 25 subjects with CF during a period of clinical stability. Subjects will have one research clinic visit that will coincide with their clinic visit at CHCO Pulmonary Clinic facilities. The following diagnostic information will be entered onto Case Report Forms: demographic information, diagnostic history and mutations, CF-related co-morbidities, historic microbiology results including fungal culture results, medications including modulator therapy and long-term antibiotic regimens, previous allergy testing, diagnostic criteria for ABPA (if applicable). We will also utilize historical clinical data from the medical record to establish other co-morbidities, lung function trends and baseline, history of other bacterial infections, history of allergy diagnoses, and other pertinent medical history and diagnostic testing (ie previous CT scan results, previous blood work, etc.). The following evaluations will be performed (Table 2, Figure 2):

- Historical data in the medical record
  - Examples include Co-morbid diagnoses related to CF (such as but not limited to CF related diabetes, CF liver disease, asthma, etc), previous blood work (such as but not limited to serum IgE levels, previous allergy testing, bronchoscopy data and lavage studies, routine and urgent/admission CF labs, etc), previous spirometery and lung function data, previous nutritional and growth data (BMI, vitamin levels, etc), previous microbiology and culture data, etc.
- BMI measurements obtained from regular CF clinic visit

- Height and weight to be obtained and subsequently calculated.
- Pulmonary function testing obtained from regular CF clinic visit
  - Spirometry will be performed according to American Thoracic Society criteria, while absolute values will be converted to percent predicted using the Global Lung Function initiative reference equations[17].
- Sputum collection
  - Spontaneously expectorated sputum will be used as possible
    - This will be collected at home by the patient following a routine respiratory airway clearance regimen within 12 hours of clinic visit. The sample will be placed in a mailed specimen cup and placed on ice and brought to the clinic visit
    - If sputum is not able to be expectorated prior to visit, we will attempt to utilize remaining sputum expectorated that is not used for clinical needs for further analysis and storage
  - Sputum may be collected by sputum induction according to the current CFF Therapeutics Development Network standard operating procedure if 1) unable to expectorate, (2) patient meets clinical criteria for sputum induction and (3) clinic is able to perform research induced sputum based on current COVID-19 guidelines to ensure safety of team member and adequate PPE supply.
    - For safety reasons, the induction procedures will only be performed for subjects who meet the following criteria ***on the day of the induction***:
      - FEV1 ≥ 30% predicted.
      - No history of > 5 mL hemoptysis within 48 hours prior to the visit
      - Able to tolerate the sputum induction procedure.
  - Of note, there is little significant difference in specimen quality for analysis between expectorated and induced sputum.[18]
  - Markers to be tested described in Table 3.
- Environmental fungal exposure questionnaire
  - Will be performed prior to finish of clinic visit. This questionnaire in entirety is a validated questionnaire used by Dr. Andy Liu. Given time constraints for study participants, we are using a modified questionnaire focused on questions of interested to this study (e.g. pertaining to fungal exposure). This has not been validated but may provide important information in this pilot study for future research
- Serum collection
  - Peripheral venous blood draw will be performed by certified individual using appropriate sterile technique by the CTRC. Samples will be labeled per research protocol standards.
  - Markers to be tested described in Table 3.

*All sputum samples to be banked and saved for potential microbiome, mycobiome and transcriptome analysis, assessing gene expressions unique to various inflammatory pathways.

Figure 2. Research Visit Timeline for Each Subject



Specific Aim 2: Investigate allergic sensitization to fungal elements in patients with CF fungal infection without ABPA compared to those without fungal infection and those with ABPA.

Table 4. Specific fungal panel to be run

Sensitization
• Serum ImmunoCAP Testing
• m13 Aspergillus fumigatus
• m207 Aspergillus niger
• m14 Epicoccum
• m8 Helminthosporium
• m12 Aureobasidium pullulans
• m5 Candida albicans
• m16 Curvularia species
• m6 Alternaria alternata
• m9 Fusarium vasinfectum
• m1 Penicillium species,
• m2 Cladosporium cladosporioides
• m10 Stemphylium languinosum
• m11 Rhizopus oryzae
• m4 Mucor

Rationale: Studies have shown increased rates of atopy, asthma, and fungal allergen sensitization in adults with CF, yet the impact of this is not clearly understood[14, 15]. There have been few studies in pediatrics with little understanding of when this sensitization develops and the changes in outcome measures that may be the result of a more allergic airway in the setting of CF. Furthermore, ABPA is a known severe condition that individuals with CF can develop, yet the timeline and predisposition that patients face is unclear. Given this, allergic sensitization to fungal elements in the environment may be playing a reactive role in the CF airway and provide clues to the development of ABPA, asthma, and other pulmonary sequelae.

Study Design and Visit: Described fully under specific aim 1, discussing patient criteria, inclusion, and study operations. Serum ImmunoCAP samples will be collected under the same blood draw as described in the study design. Specific panel to be collected described in Table 4.

Statistical Analysis: Will utilize descriptive statistics and associations of each group as well as differences between groups. Will utilize a biostatistician to help with data analysis, organization, and reporting.

Specific Aim 1: Compare Th2 inflammation in patients with and without fungal infections in patients with CF.

- Individual markers: We will use descriptive statistics to measure the differences between each individual marker in both the sputum and serum between groups. We will further look at associations with serum and sputum measurements in each group.
- Clinical and Environmental characteristics: We will use descriptive statistics to account for differences and associations in lung function and BMI between groups. We will also account for differences in individuals by other co-infections (*Pseudomonas*, MRSA, etc.) in our analysis and study these associations as well. Given that ABPA individuals will be harder to recruit, we will do our best to control based upon individuals recruited into this group.

  This is a pilot and discovery study searching for possible Th2 markers to further study and use clinically. Given this, our goal is to detect as many possible markers as we can feasibly study and thus why we will not initially be correcting for multiple comparisons.

Specific Aim 2: Investigate allergic sensitization to fungal elements in patients with CF fungal infection without ABPA compared to those without fungal infection and those with ABPA.

- Serum ImmunoCAP measurements: We will use descriptive statistics to measure the differences and associations between each individual mold reaction between groups.
- Immune Profile: After each individual marker is analyzed, the questionnaire is assessed, and clinical characteristics are described, we will attempt to use this information develop a “profile” describing a panel of serum/sputum markers, allergic sensitization measurements, clinical characteristics, and other findings consistent with each group of subjects in association with their type of fungal disease.

Shapiro-Wilks tests will be used to assess for normality of the distributions of measured outcomes. Basic descriptive statistics will be presented in tabular form as mean ± standard deviation, median [inner-quartile range; min and max] and frequency (percent). ANOVA regression models will assess for overall between group differences and estimate the least-squared differences for all pairwise comparisons after evaluating the jackknife studentized residual plots. If residuals suggest violations to modeling assumptions, namely heteroscedasticity of variance, Breslow-Day tests will be conducted to confirm unequal variance across groups. Welch’s T-test will be used in the presences of un-equal variance. If evidence of non-normality of residuals exists, either Box-Cox transformations of the outcomes or non-parametric approaches such as Wilcoxson rank sum will be used to compare groups when evidence of non-normally distributed outcomes exists. For dichotomous outcomes, Fisher’s Exact tests will be used to compare groups. As the study is

hypothesis generating, statistical significance will be set at an alpha 0.05 level for both overall and pairwise comparisons. All analyses will be conducted in either R studio or SAS statistical software 9.4 (1).

Outcome Measures:
Primary outcome: Difference in sputum Th2 biomarkers (ECP, IL4, IL5, IL10, IL13, and eosinophil count) in patients with CF with fungal infection with expected elevation of sputum Th2 biomarkers in patients with CF and ABPA compared to those without fungal infection and without ABPA (Table 3).
Secondary outcomes:
- Serum Th2 biomarkers in patients with fungal infection and ABPA (Table 3).
- Serum Th1 biomarkers in patients with fungal infection and ABPA (Table 3).
- Serum sensitization markers to fungal allergens in patients with fungal infection and ABPA (Table 4).
- Baseline and historic lung function, historical comorbid diagnoses and BMI measurements in patients with fungal infection and ABPA.
- Environmental factors that are possibly related to fungal infection and ABPA in patients with CF.
- Immune profile: A profile of each group will be based upon their findings of each set of biomarkers: Th1, Th2, mold allergy panel, and systemic markers of inflammation. Based upon findings in each of these categories (elevated, depressed), we will be able to formulate a profile based upon the type of marker/inflammatory pathway.

Tertiary outcomes: Banking of both sputum and serum to potentially utilize microbiome and transcriptome techniques for further immunotyping and infection characterization.

Data Collection and Storage: All subjects will be de-identified upon study sample collection and stored with a master key. There will be limited access to this information with the PI and only essential research staff able to monitor and view the data. All hardcopy source documents will be kept in a locked file cabinet in the CF research team's storage facilities. Additionally, to further ensure data integrity, data for this study will be stored in Research Electronic Data Capture system (REDCap), which will allow limited access to only essential personal. REDCap enables in-line validation to minimize transcription errors and provides real-time notifications of data submission and allows immediate central monitoring and feedback. Access to data requires an encrypted secure socket layer (SSL) connection and changes are logged by user ID, time stamp and project. Databases are backed up frequently.

Description, Risks, and Justification of Procedures and Data Collection Tools.

Assessment of Risk:
The level of risk is determined to be minimal given its observational nature. There will be a blood draw, induced sputum utilizing hypertonic saline (HTS) nebulization administration, spirometry, and a questionnaire on an electronic tablet. All of these interventions are common, routine practices in regular clinical visits for both pediatric and adult patients with CF. Patients with CF have blood drawn at minimum annually, if not more often. Many patients with CF utilize HTS daily as a part of their treatment regimen. Spirometry is a benign, patient dependent measurement that patients with CF perform multiple times per year starting at the age of 4 years old. All procedures and subsequent risks associated in this study will be done for research purposes only.

Induced Sputum: If needed, sputum will be induced using 3% hypertonic saline. Sputum induction is performed according to a standard operating procedure. We have extensive experience with this procedure [12]. After each inhalation period subjects expectorate saliva into a cup, perform forced expiratory techniques and coughing and then expectorate sputum into a sterile specimen container. On average sputum induction requires 12-15 minutes to complete but may be terminated early if an adequate sample is provided. The procedure will also be terminated if the subject's peak flow decreases by 20% or more from baseline. Sputum will be then be sent to the Microbiology Lab and processed as described above for expectorated sputum. The anticipated risks with this procedure include cough, wheeze, chest-tightness, and a decrease in pulmonary function test values. In order to minimize these, a physician or respiratory therapist will collect each sputum sample and subjects will be monitored closely for any adverse events. Prior sputum induction, pre-treatment with albuterol will be performed to minimize the risk of bronchospasm. Peak flow will be monitored during sputum induction and a decrease of 20% or greater from baseline will result in termination of the procedure

and administration of additional albuterol. The use of hypertonic saline is often a routine part of respiratory treatments for patients with CF.

The following testing will be performed on all sputum samples:

- **Standard microbiologic culture:** Expectorated and induced sputum specimens will undergo standard microbiological analyses including gram stain, bacterial culture and fungal culture in the clinical microbiology laboratory according to a standard CF protocol. No risks are associated with this procedure. These results will be made available to the clinical care team if they are positive for CF pathogens. Given that CF cultures (including fungal cultures) are obtained at least quarterly for clinical surveillance, our CF center has standard guidelines for approaching positive cultures and decisions regarding treatment that will be made by the primary CF provider.
- **Inflammatory Indices:** Expectorated and induced sputum samples will be sent to the Core Laboratory to measure various markers (Table 3) which are thought to reflect clinical parameters in CF. The main markers of Th2 inflammation to investigate are: IL-4, IL-5, and ECP. The main markers of Th1 inflammation to investigate are: IL8, Il-1B, NE. Total white blood cell and neutrophil counts will also be obtained via cell counts and differentials performed on sputum samples.
- **Banking of samples to be used for molecular identification of bacteria/fungus and Molecular identification of transcription factors/gene signaling:** All remaining serum and sputum samples will be stored in the appropriate storage device and temperature in the CF research teams designated sample storage area for later analysis utilizing transcriptomics, proteomics, and/or microbiome analysis.

Environmental Exposure to Fungus Questionnaire: This will be performed on an electronic tablet device during the clinic visit and has little to no risk associated with administration.

Pulmonary Function Testing: Spirometry will be performed according to American Thoracic Society Guidelines [19]. The indices measured during the testing include forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), FEV1/FVC ratio and mid-volume forced expiratory flow (FEF25-75). Our pulmonary function laboratory has been used in a number of studies of CF [12, 20, 21]. The anticipated risks involved with this procedure are a temporary increase in cough and shortness of breath. To minimize this risk, we will encourage the subject to take big breaths and take breaks between coughing to reduce the tendency to develop gagging or violent coughing. Pulmonary function testing is a part of the standard clinical care for patients with cystic fibrosis.

Blood draw: This will be performed per standard operating procedure of the CHCO clinical and research laboratory sites utilizing patient samples and body fluids. Sterile technique with fresh needles and devices will used in to minimize infection risk. Trained nursing staff and/or phlebotomists will be the ones performing the blood draw in a CHCO approved environment for proper drawing and handling of specimens. Using these individuals will also minimize the risk for local trauma, bruising, and/or injury that is possible with routine blood draws.

The following testing will be performed on all serum samples:

- **Inflammatory Indices:** Serum samples will be sent to the Core and Clinical Laboratory to measure various systemic markers of inflammation (Table 3) which are thought to reflect clinical parameters in CF. The main markers of Th2 inflammation to investigate are: IL4, IL5, and ECP. The main markers of Th1 inflammation to investigate are: IL8, IFNY, NE-APC.
- **Mold Panel:** A panel of allergic sensitization (IgE levels) to various environmental fungi and molds will be sent on serum samples, along with a total IgE sample (Table 4).
- **Banking of samples to be used for molecular identification of bacteria/fungus and Molecular identification of transcription factors/gene signaling:** All remaining serum and sputum samples will be stored in the appropriate storage device and temperature in the CF research teams designated sample storage area for later analysis utilizing transcriptomics, proteomics, and/or microbiome analysis.

Storage of Data: Data will be entered from the CRF's and stored in electronic format using REDCap (Research Electronic Data Capture). Hard copies of the CRF will be kept in a secure location at the site of collection for data entry verification. REDCap is a secure, web-based application designed exclusively to support data capture for research studies. REDCap provides: 1) an intuitive interface for data entry with data validation, 2) audit trails for tracking data manipulation and export procedures, 3) automated export procedures for seamless data downloads to common statistical programs (SPSS, SAS, Stata, R), 4) procedures for importing data from external sources, and 5) advanced features such as branching logic and calculated fields.

Potential Scientific Problems.
Limitations: This is a single center cross sectional pilot study, thus our findings will be preliminary but may inform future larger studies. All patients will likely continue to follow clinically at the CHCO CF center, however this is a one-time study visit with one time point of data collection. We will do our best to control for age, disease severity (BMI, lung function), sex, and other demographic factors between groups, however it has been shown that individuals with ABPA and fungal infection CF are often older than those without fungal infection. This study is being performed in patients with clinical stability. Inflammatory changes may be more dynamic and evident during acute illness and undetectable during baseline status. We will attempt to account and analyze the effects of other bacteria or infectious organisms to the best of our ability. This study will mainly involve pediatric patients of various age groups, which may be difficult to translate to adult outcomes and disease. We may find that fungal infection without ABPA induces a Th1 response rather than Th2 contrary to our hypothesis; however this will still provide important information regarding the role of fungi in CF lung disease.

Data Analysis Plan.

Analysis of Study Results: Will utilize descriptive statistics and associations of each group as well as differences between groups. Will utilize a biostatistician to help with further analysis and data analysis, organization, and reporting.

- Individual markers: We will use descriptive statistics measure the differences and associations between each individual marker between groups.
- Clinical and Environmental Characteristics: We will use descriptive statistics to measure the differences and associations of lung function, BMI, environmental questionnaire score, and other clinical measurements between each group.
- Immune Profile: After each individual marker is analyzed, the questionnaire is assessed and clinical characteristics are described, we will attempt to use this information develop a "profile" describing a panel of serum/sputum markers, allergic sensitization measurements, clinical characteristics, and other findings consistent with each group of subjects in association with their type of fungal disease.

G. Summarize Knowledge to be Gained. This study is in effort to better understand the clinical course and underlying pathophysiology of fungal infections and ABPA in patients with CF. By investigating the inflammatory markers and pathways unique to fungus, we hope to develop a better understanding of how this group of organisms play a role in pulmonary disease in CF. We also intend to broach unique diagnostic methods and biomarkers for fungal infection in patients with CF to help with the eventual development of care algorithms and surveillance. From this initial study, we hope to construct a foundation in which further information can be gathered and built upon to understand clinical outcomes unique to fungal infection, predictors of fungal infection, and eventually the appropriate screening, treatment, and guideline development of this hard to treat infection. Furthermore, these findings may easily segue into further investigations in individuals with non-CF ABPA, applying the knowledge gained to an even large population I need of further diagnostic and treatment guidelines.

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