Microbiota-associated Nonspecific Interstitial Pneumonia Severity & Treatment Response

Rakin Nasar, David Welsh, Amita Krishnan

Affiliations.

Introduction

The role of the gut microbiome in the modulation of the immune system and inflammation is an increasingly popular area of research, but little is known about its association with interstitial lung diseases (ILD), especially in the case of Non-Specific interstitial pneumonia (NSIP). There are many genetic, environmental, and molecular drivers for ILDs, and NSIP is a subset from ILDs which is characterized through clinical, histological, and radiological features. NSIP is often associated with specific conditions, such as connective tissue diseases and autoimmune diseases, or may be categorized as idiopathic. The unique cytokine profile of NSIP patients compared to patients with other ILDs indicates an inflammatory dysregulation; however, the treatment is primarily limited to non-targeted approaches to reduce inflammation such as steroids and other immunosuppressive medications. While current treatment options are often, but not always, effective in reducing morbidity and mortality, they are associated with significant side effects. Since the gut microbiome is known to program immune and inflammatory responses, we **hypothesize that changes in the intestinal microbiota are associated with disease severity and treatment responsiveness in patients with NSIP.** Establishing these underlying associations can help develop new and personalized treatment strategies to reduce inflammation and immune system overactivation in patients with NSIP.

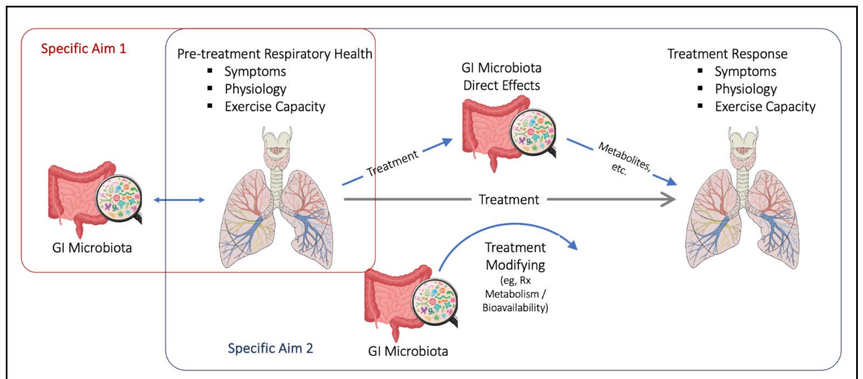
The microbiome plays an important role in the maintenance of regular bodily functions across many organ systems, and its dysregulation is associated with many diseases. However, the role of the gut microbiome has not been elucidated in the case of NSIP. The gut microbiota also plays an important role in affecting inflammation systemically through the gut microbiota's key role in programming host immune mechanisms. We have previously demonstrated the role the gut microbiota plays in programming host defense in preclinical models of lung infection and T cell function and in humans[1], [2]. The presence of filamentous segmented bacteria protects against Staphylococcus aureus pneumonia through IL-17 producing T helper cells [3]–[5]. The gut microbiota drives the differentiation of these cells through specific microbiota including segmented filamentous bacteria [4], [6], [7]. However, in NSIP the lymphocytic infiltrates commonly associated with the histological features of the disease are composed mainly of B-cell lymphocytes, greater CD4+/CD8+ ratio, and predominantly Th1 type [8]. It stands to reason that the host defense mechanisms are dysregulated in NSIP differently compared to pneumonia, IPF or ILDs, which is why it is important to understand the regulatory potential of host immune dysregulation in NSIP. Determining the changes in the gut microbiome in patients with NSIP would be a valuable step towards understanding the mechanisms behind NSIP and, potentially, the response to therapy, because of the gut microbiome's multifaceted involvement in host lung responses.

Studies in patients and animal models of COPD, asthma, and other lung diseases show that corticosteroid treatment can change the microbiota composition. There are only a handful of studies that characterize the change of the gut microbiota as a result of therapy in patients with lung diseases, and very few of those studies look at NSIP patients. However, the studies that look at patients with COPD show that there is an increase in the diversity and density of specific taxa after treatment with corticosteroids [9]–[11]. In asthma, some studies show that inhaled corticosteroids and oral glucocorticoids are associated with an increase in, proteobacteria and Pseudomonas and a decrease in Bacteroidetes, Fusobacteria, and Prevotella [12], [13]. In mice and rats, prednisone usage has been shown to change metabolites that are associated with the gut microbiome such as decreases in the abundances of valeric acid, propanoic acid, isobutyric acid, isovaleric acid, and caproic acid. These changes can affect the differentiation of various immune cells such as how propanoic acid and butyrate promote differentiation of Treg cells by inhibiting histone deacetylase. Also, caproic acid is involved in the proinflammatory states of TH1 and TH17 cells [17]–[19]. Treatment efficacy and microbiota composition have a bi-directional relationship in lung disease in which treatment with steroids can alter the microbiota composition in organisms and the microbiota can modify the immune system to control inflammation which modulates the effectiveness of treatments with steroids.

Methods

This project will perform 16s rDNA sequencing based phylogenetic analyses and metabolomic analyses of stool samples of patients with NSIP. Samples will be collected from NSIP patients when the patients will receive standard treatment and again after 3 months of treatment. Respiratory and health statuses will be assessed through K-BILD (ILD specific questionnaire), a EuroQuol-5D (quality-of-life questionnaire), ASA24 (dietary assessment tool), AUDIT-C (alcohol use questionnaire), and the MRC Dyspnea Score at each time point. Data will also be extracted from the patients' medical records including 6-minute walk, pulmonary function testing, radiographic findings on high resolution computed tomography, and histological results from samples of lung tissue. These findings will be used to characterize disease severity and assess response to therapy.

Conceptual Model



Our conceptual model proposes that the microbiota of the gut and lungs cause a pro-inflammatory state and immune system hyper activation which contributes to NSIP symptoms, physiological conditions of the lungs, and exercise capacity. The microbiota may modulate treatment responses through drug metabolism and/or bioavailability. Alternatively, treatment may directly affect the microbiota composition and metabolites produced by microbes in the gut. These may then contribute to NSIP symptoms, lung physiology, and exercise capacity.

Schedule of Activities

Table 1: Schedule of Activities	Screening Visit	Full Testing Visit Timepoint 0	Full Testing Visit Timepoint 3 Months	Data Collection from EHR, up to 3 years
Invitation to Participate	X			
Informed consent	X			
Demographics	X			
Medical history (questionnaire + medical record review)	X			
Distribute Stool Collection Kit	X			
K-BILD Questionnaire		X	X	
MRC Dyspnea Scale		X	X	
EuroQol-5D		X	X	
Vital Signs		X	X	
Stool Collection		X	X	
Medication Review & Adherence		X	X	
Medical Chart Abstraction*		X	X	X

Timeline

Informed Consent Visit → Timepoint 0 Full Testing Visit → 3 Months Full Testing Visit → Data collection from EHR for up to 3 years

Significance

We have not collected any data yet, so there are no conclusions to be drawn. However, the goal of the study is to elucidate the potential underlying causes that mediate disease severity and responsiveness to therapy in NSIP. The incidence and prevalence of NSIP have not been definitively defined; however, the prevalence of NSIP is estimated to be 1 to 9 per 100,000 people, and the incidence has been estimated at around 3 per one million people [14]–[16]. Considering that patients with severe NSIP are frequently hospitalized and the treatment options are limited to steroids and immunosuppressive agents, which can be accompanied with serious side effects, it is imperative to elucidate the mechanisms in which NSIP develops and responsiveness to treatment. NSIP can either be idiopathic or associated with connective tissue disease, HIV infection, toxins, or numerous other causes [17]. Even though many associated diseases and causes of NSIP are known, little is known about the mechanisms by which these causes lead to the harmful inflammation and fibrosis that debilitate patients with NSIP. Furthermore, since NSIP is defined by a constellation of clinical symptoms, radiographic findings, and histological findings it is difficult to pinpoint mechanisms that explain the wide array of potential causes for NSIP.

The presence of specific bacterial and viral organisms in the airways is associated with IPF [18]–[20]. We and other groups have demonstrated that the gut microbiome is involved in programming host defense mechanisms against bacteria in preclinical models of pneumonia and in humans [2]–[5]. However, no data exists on characterizing the microbiota of the gut in patients with NSIP. Understanding changes in the gut and lung microbiota of patients with NSIP can help identify how the interactions between the respiratory microbiota and the gut microbiota inform host immune response. This will allow for new targeted therapies with fewer side-effects which can improve symptoms and quality of life for patients with NSIP.

Based on research on the role of the gut microbiome in other ILDs and pneumonia, we expect that there will be differences in specific taxa and broader changes in the overall composition and function of the gut microbiomes of patients with NSIP. With our multi-omics and sequencing methodologies which we implemented in previous studies, we hope to characterize the microbiome of the gut with enough depth and dimensionality to portray differences in specific taxa and broader functional deficits of the microbiomes of the gut and lungs of patients with NSIP. Tracking changes in the microbiomes of NSIP patients as they receive steroidal treatments which will lower the activity of their immune systems and reduce inflammation. Following these changes will help identify how the gut and lung microbiomes respond to dampened immune systems, which will further our understanding of the role of the gut and lung microbiomes in host immune programming and modulation. The end goal is to use knowledge gleamed from our study to inspire the development of novel, specific, and targeted treatment options for NSIP patients that improve their quality of life and reduce harm from non-specific treatments.

Bibliography

[1] V. J. Maffei *et al.*, "Alcohol Use Is Associated With Intestinal Dysbiosis and Dysfunctional CD8+ T-Cell Phenotypes in Persons With Human Immunodeficiency Virus," *J Infect Dis*, vol. 223, no. 6, pp. 1029–1039, Mar. 2021, doi: 10.1093/infdis/jiaa461.

[2] D. R. Samuelson *et al.*, "Pulmonary immune cell trafficking promotes host defense against alcohol-associated Klebsiella pneumonia," *Commun Biol*, vol. 4, no. 1, Art. no. 1, Aug. 2021, doi: 10.1038/s42003-021-02524-0.

[3] S. Gauguet *et al.*, "Intestinal Microbiota of Mice Influences Resistance to Staphylococcus aureus Pneumonia," *Infect Immun*, vol. 83, no. 10, pp. 4003–4014, Oct. 2015, doi: 10.1128/IAI.00037-15.

[4] J. P. McAleer *et al.*, "Pulmonary Th17 Antifungal Immunity Is Regulated by the Gut Microbiome," *The Journal of Immunology*, vol. 197, no. 1, pp. 97–107, Jul. 2016, doi: 10.4049/jimmunol.1502566.

[5] C. T. Weaver, C. O. Elson, L. A. Fouser, and J. K. Kolls, "The Th17 Pathway and Inflammatory Diseases of the Intestines, Lungs, and Skin," *Annual Review of Pathology: Mechanisms of Disease*, vol. 8, no. 1, pp. 477–512, 2013, doi: 10.1146/annurev-pathol-011110-130318.

[6] I. I. Ivanov *et al.*, "Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine," *Cell Host Microbe*, vol. 4, no. 4, pp. 337–349, Oct. 2008, doi: 10.1016/j.chom.2008.09.009.

[7] I. I. Ivanov *et al.*, "Induction of intestinal Th17 cells by segmented filamentous bacteria," *Cell*, vol. 139, no. 3, pp. 485–498, Oct. 2009, doi: 10.1016/j.cell.2009.09.033.

[8] K. A. Keogh and A. H. Limper, "Characterization of lymphocyte populations in nonspecific interstitial pneumonia*," *Respir Res*, vol. 6, no. 1, p. 137, 2005, doi: 10.1186/1465-9921-6-137.

[9] R. P. Dickson and A. Morris, "Macrolides, inflammation and the lung microbiome: untangling the web of causality," *Thorax*, vol. 72, no. 1, pp. 10–12, Jan. 2017, doi: 10.1136/thoraxjnl-2016-209180.

[10] Y. J. Huang, S. Sethi, T. Murphy, S. Nariya, H. A. Boushey, and S. V. Lynch, "Airway Microbiome Dynamics in Exacerbations of Chronic Obstructive Pulmonary Disease," *Journal of Clinical Microbiology*, vol. 52, no. 8, pp. 2813–2823, Dec. 2020, doi: 10.1128/jcm.00035-14.

[11] E. Karakasidis, O. S. Kotsiou, and K. I. Gourgoulianis, "Lung and Gut Microbiome in COPD," *Journal of Personalized Medicine*, vol. 13, no. 5, Art. no. 5, May 2023, doi: 10.3390/jpm13050804.

[12] W. Barcik, R. C. T. Boutin, M. Sokolowska, and B. B. Finlay, "The Role of Lung and Gut Microbiota in the Pathology of Asthma," *Immunity*, vol. 52, no. 2, pp. 241–255, Feb. 2020, doi: 10.1016/j.immuni.2020.01.007.

[13] D. R. Denner *et al.*, "Corticosteroid therapy and airflow obstruction influence the bronchial microbiome, which is distinct from that of bronchoalveolar lavage in asthmatic airways," *J Allergy Clin Immunol*, vol. 137, no. 5, pp. 1398-1405.e3, May 2016, doi: 10.1016/j.jaci.2015.10.017.

[14] K. R. Flaherty and F. J. Martinez, "Nonspecific Interstitial Pneumonia," *Semin Respir Crit Care Med*, vol. 27, no. 6, pp. 652–658, Dec. 2006, doi: 10.1055/s-2006-957336.

[15] C. Hyldgaard, O. Hilberg, A. Muller, and E. Bendstrup, "A cohort study of interstitial lung diseases in central Denmark," *Respir Med*, vol. 108, no. 5, pp. 793–799, May 2014, doi: 10.1016/j.rmed.2013.09.002.

[16] J. Lee, Y. H. Kim, J. Y. Kang, Y. Jegal, and S. Y. Park, "Korean Guidelines for Diagnosis and Management of Interstitial Lung Diseases: Part 3. Idiopathic Nonspecific Interstitial Pneumonia," *Tuberc Respir Dis*, vol. 82, no. 4, pp. 277–284, May 2019, doi: 10.4046/trd.2018.0092.

[17] A. S. Nayfeh, V. Chippa, and D. R. Moore, "Nonspecific Interstitial Pneumonitis," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2023. Accessed: Jul. 09, 2023. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK518974/

[18] F. Amati *et al.*, "Lung Microbiome in Idiopathic Pulmonary Fibrosis and Other Interstitial Lung Diseases," *Int J Mol Sci*, vol. 23, no. 2, p. 977, Jan. 2022, doi: 10.3390/ijms23020977.

[19] M. K. Han *et al.*, "Association Between Lung Microbiome and Disease Progression in IPF: A Prospective Cohort Study," *Lancet Respir Med*, vol. 2, no. 7, pp. 548–556, Jul. 2014, doi: 10.1016/S2213-2600(14)70069-4.

[20] P. Spagnolo *et al.*, "The Role of the Lung's Microbiome in the Pathogenesis and Progression of Idiopathic Pulmonary Fibrosis," *Int J Mol Sci*, vol. 20, no. 22, p. 5618, Nov. 2019, doi: 10.3390/ijms20225618.