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Original research article

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Please cite this article as: Biblowitz K, Lee C, Zhu D, *et al*. Association of antinuclear antibody seropositivity with inhaled environmental exposures in patients with interstitial lung disease. *ERJ Open Res* 2021; in press (https://doi.org/10.1183/23120541.00254-2021).

Association of Antinuclear Antibody Seropositivity with Inhaled Environmental Exposures in Patients with Interstitial Lung Disease

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Funding:
NIH K23HL146942

Conflict of Interest Disclosures: MES has received institutional support to conduct ILD clinical trials for Boehringer Ingelheim and Galapagos, fees for clinical trial adjudication commiittee service from Fibrogen and editorial support from Boehringer Ingelheim. SKB has received speaking and advisory board fees from Genentech. AA has received speaking and advisory board fees from Genentech and Boehringer Ingelheim and is supported by a career development award from the National Heart, Lung, and Blood Institute (NHLBI K23HL146942).

Keywords: Interstitial Lung Disease, Antinuclear antibody, Environmental exposures, Inhalational toxins, Autoimmunity.

Author's contributions: Conception and design (KB, SKB, AA), acquisition of data for the work (KB, CL, RV, SKB, AA), analysis and interpretation (KB, CL, IN, RV, MES, SKB, AA), Drafting the manuscript for important intellectual content (KB, CL, IN, RV, MES, SKB, AA), and critical revision for important intellectual content: All authors (KB, CL, IN, RV, MES, SKB, AA). Final approval of the submitted manuscript and accountability for all aspects of the work: All authors (KB, CL, IN, RV, MES, SKB, AA)

ABSTRACT

Background

Interstitial lung diseases (ILD) are diffuse parenchymal lung disorders that cause substantial morbidity and mortality. In patients with ILD, elevated antinuclear antibody (ANA) titers may be a sign of an autoimmune process. Inhalational exposures contribute to ILD pathogenesis and affect prognosis and may trigger autoimmune disease. The association of inhalational exposures with ANA seropositivity in ILD patients is unknown.

Methods

This was a retrospective cohort study of adult ILD patients from five US centers. Exposures to tobacco, inhaled organic antigens, and inhaled inorganic particles were extracted from medical records. A multivariable logistic regression model was used to analyze the effects of confounders including age, ILD diagnosis, gender, and exposure type on ANA seropositivity.

Results

Among 1,265 patients with ILD, there were more ANA seropositive (58.6%, n=741) than ANA seronegative patients (41.4%, n=524). ANA seropositive patients had lower total lung capacity (69% vs 75%, $P<0.001$) and forced vital capacity (64% vs 70%, $P<0.001$) than patients who were ANA seronegative. Amongst patients with tobacco exposure, 61.4% (n=449) were ANA positive compared to 54.7% (n=292) of those without tobacco exposure. In multivariable analysis tobacco exposure remained independently associated with increased ANA seropositivity (OR=1.38, 95%CI=1.12-1.71). This significant difference was similarly demonstrated among patients with and without a history of inorganic exposures (OR=1.52, 95%CI=1.12-2.07).

Conclusion

Patients with ILD and inhalational exposure had significantly higher prevalence of ANA seropositivity than those without reported exposures across ILD diagnoses. Environmental and occupational exposures should be systematically reviewed in patients with ILD, particularly those with ANA seropositivity.

BACKGROUND

Interstitial lung diseases (ILD) are diffuse parenchymal lung disorders characterized by cellular proliferation, inflammation, and fibrosis or a combination of these within the alveolar wall, not due to cancer or infection.[1] ILD is associated with substantial morbidity and mortality.[2]

Autoimmunity has an established connection to ILD, which is considered the primary pulmonary manifestation of multiple autoimmune diseases, and the diagnosis of ILD requires evaluation for underlying connective tissue disease (CTD).[3,4] The presence of antinuclear antibodies (ANA) has been independently associated with ILD in previous studies as well, with ILD subtypes characterized by the presence of ANA serology.[5,6] Furthermore, inhalational exposures including smoking are known contributors to ILD and to the development of CTD.[7,8,9,10,11]

There is a gap in knowledge, however, in the link between ANA serology and inhalational exposures in ILD. In this study, we systematically assessed a cohort of ILD patients with documented ANA serology to identify prior or current exposure to inhalational antigens. We categorized the exposures into tobacco-related, organic antigens, or inorganic antigens and determined if there was an association between documented ANA serology and inhalational exposure in these patients.

MATERIAL AND METHODS

Study population

This retrospective analysis was conducted at NorthShore University HealthSystem and University of Chicago. The study was approved by the respective local IRB ethics committees (#16-1062; #17-025). Patients aged ≥18 years old from five US hospital centers with a diagnosis of ILD were identified and included. ILD diagnosis was multidisciplinary and based on clinical, pulmonary, radiologic, and/or histopathologic evaluation conducted between January 2006 and July 2016.

Data was extracted from the electronic medical record (EMR) of both institutions as documented at the time of initial ILD diagnosis. Laboratory data collected included ANA titer or immunoassay screen value depending on the institution's standard. Data from pulmonary function tests (PFTs) were also assessed at baseline evaluation for diagnosis of ILD, including percent predicted forced vital capacity (FVC), total lung capacity (TLC), and diffusion capacity of the lung for carbon monoxide (DLCO).

Additional variables included demographic data (age, gender, race/ethnicity), history of exposure to tobacco (with pack years), inhaled organic antigens (such as mold, avian dander, hot tubs, or paint), and inhaled inorganic particles (such as asbestos, silica, or metal particles).

Enrollment criteria

All patients were from within the University of Chicago ILD registry or the NorthShore University HealthSystem and had an ICD-9 or ICD-10 billing code for ILD. Patients were included in the study if they had an available quantitative ANA titer value drawn from within the institution within three months of the date of diagnosis. Patients with a diagnosis of obstructive lung disease or sarcoidosis were excluded.

Patients with an ICD-9 or ICD-10 code-based ILD diagnosis at the tertiary center (University of Chicago Hospital) underwent a multidisciplinary evaluation using available clinical data, PFTs, high-resolution computed tomography (HRCT) scans and surgical lung biopsies according to current American Thoracic Society/European Respiratory Society criteria. Pulmonologists, rheumatologists, dedicated chest radiologists, and a thoracic pathologist did an assessment for the multidisciplinary diagnosis of ILD. The four non-tertiary hospitals are suburban community hospitals that do not routinely perform multidisciplinary discussions for ILD diagnosis. As such, an independent "adjudication" panel of two academic pulmonologists with expertise in ILD evaluated clinical data including PFTs and HRCT scans to confirm the diagnosis of ILD in all patients who received an ICD-9 code-based ILD diagnosis from a pulmonologist at that center.[12]

Autoimmune serology

Patients with a quantitative ANA titer value were included in the study. An ANA titer value ≥1:160 (University of Chicago) or an immunoassay screen value ≥0.90 (NorthShore University HealthSystem) was considered positive. One study comparing healthy patients to those with known autoimmune disease found that 5.0% of the healthy population was ANA seropositive at 1:160 and a slightly lower 3.3% were seropositive at 1:320.[13] Pathology guidelines published in 1999 also noted that while the use of ANA titers above 160 to define a positive test result may lead to better specificity for the diagnosis of systemic lupus erythematosus (SLE), this practice will decrease diagnostic sensitivity of the ANA test.[14] A more recent study evaluating the characteristics that distinguish autoimmune-featured ILD (AIF-ILD) from IPF and CTD-ILD also considered ANA titers ≥1:160 as positive.[5]

Although the sensitivity of enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody (IFA) are similar (>80%), ANA IFA measured on Hep-2 cells is considered the gold standard for measuring ANA given much higher specificity (99% vs. 36-94%).[15] Therefore, because ELISA testing has lower specificity than IFA testing, it may have more false positive tests.

Exposure history

The exposure history for patients at all five hospitals was systematically extracted from the EMR. Data on environmental exposures for patients at the University of Chicago was obtained from the ILD registry. Data for patients at the NorthShore University HealthSystem were pulled from the EMR social and occupational history. If not listed

explicitly in these sections, the pulmonary physician notes were reviewed as were notes of other internal medicine physicians.

Statistical analysis

Continuous variables were reported as means with standard deviation and compared using a two-tailed student's t-test. Categorical variables were reported as counts and percentages; comparisons were made using the Chi-square test. A *P*-value of <0.05 was considered to be statistically significant. All multivariable data analyses were performed in Stata v.15 (StataCorp; College Station, Texas). A multivariable logistic regression model was used to analyze the effects of confounders including age, ILD diagnosis, gender, and exposure type on ANA seropositivity.

As age has been variably suggested to demonstrate a linear correlation with ANA seropositivity,[16,17] subject ages were stratified into deciles to evaluate the relationship between age and ANA seropositivity. A multivariable logistic regression model was used to analyze the effects of race, age, ILD diagnosis, gender, and exposure type (tobacco, organic and inorganic matter) on ANA seropositivity.

RESULTS

Of the initial cohort of ILD patients (n=2,128) obtained from the University of Chicago and Northshore Hospitals, 863 were excluded due to lack of ANA serology data leaving 1,265 patients in the study. Baseline demographics revealed the mean age at time of diagnosis was 64.7±12.3 years old. The average BMI was 29.8±6.6 kg/m^2, and the sex distribution of subjects was near equal (females 50.8%; and males 49.2%) (**Table 1**).

Pulmonary function testing

The majority of subjects (77.1%, n=975) had full pulmonary function testing performed. The calculated mean values were total lung capacity (TLC)(71.3%±18.6), forced vital capacity (FVC)(66.3%±19.0), and diffusing capacity for carbon monoxide (DLCO)(51.0%±21.7) (**Table 1**). ANA seropositive patients had lower TLC (69% vs 75%, *P*<0.001) and FVC (64% vs 70%, *P*<0.001) than patients who were ANA seronegative (**Table 1**).

Autoimmune serology and inhalational exposures

Among the 1,265 ILD patients in the study, there were more ANA seropositive (58.6%, n=741) than ANA seronegative patients (41.4%, n=524) based on titer or immunoassay screen. An ANA-enzyme immunoassay (ANA-EIA) of 0.90 was considered an approximate equivalent to an ANA-indirect fluorescent antibody (ANA-IFA) of 1:160. Of note, an ANA-EIA with a reference range of 0.9 demonstrates equivalent sensitivity and somewhat higher specificity compared to ANA-IFA (**Supplementary Figure 1**).[18]

The majority of patients reported at least one inhalational exposure (73.6%, n=931). A significant majority of patients reported a history of cigarette smoking (57.8% n=731). Fewer subjects reported either organic exposure (36.7%, n=465) or inorganic exposure (16.4%, n=207).

Using a univariate analysis, an assessment of ILD patients with an exposure history revealed a higher prevalence of ANA seropositivity for all exposure subtypes (Figure 1). Of the patients with tobacco exposure, 61.4% (n=449) was ANA seropositive compared to 54.7% (n=292) of those without tobacco exposure (OR=1.32, 95%CI = 1.05-1.66). This significant difference was similarly demonstrated among patients with and without a history of organic exposure (OR=1.32, 95%CI=1.04-1.67); and among subjects with and without inorganic exposures (OR=1.47, 95%CI=1.08-2.01) (**Figure 2**).

These results remained consistent upon multivariable analysis for tobacco (OR=1.38; 95%CI=1.12-1.71), and inorganic antigen exposure (OR=1.52; 95%CI=1.12-2.07) (**Table 2**). However, when using the multivariable logistic regression model, organic exposure was not predictive of a positive ANA *(*OR=1.11, 95%CI=0.89-1.40) (**Table 2**).

ANA seropositivity in HP was 58.2%, which was greater than the prevalence of positive ANA titers found in those with other ILD (30.2%), IPF (52.7%), and unclassifiable ILD (47.8%)(**Table 1**). Of those with HP, 67.4% (n=95) had a history of organic exposure compared to those who did not have a history of organic exposures (32.6%, n=46). This is significant when compared to patients without HP of whom only 32.9% (n=370) had organic exposures compared to those without organic exposures (67.1%, n=754; p<0.001)(**e-supplementary Table 3**).

Race/Ethnicity

Subjects were predominantly White (72.6%) followed by Black (16.0%), Hispanic (5.1%), and Asian (3.7%) (**Table 1**). A small percentage of ILD patients (2.5%) identified as other. A multivariable logistic regression showed that being Black *(*OR=1.78 95%CI=1.30-2.45) or Hispanic (OR=2.23, 95%CI=1.34-3.71) is highly predictive of having positive ANA. Identifying as “other” was associated with not having a positive ANA (OR=0.14, 95%CI=0.058-0.36). The null hypothesis could not be rejected for Asian ethnicity (**Table 2**).

ILD Diagnosis

The prevalence of ANA seropositivity in IPF patients was 52.7% (n=212) compared to ANA negative patients (47.3% n=190, *P*=0.003). We performed a multivariable logistic regression comparatively evaluating risk of ANA seropositivity in subjects with various ILD diagnoses against a benchmark diagnosis of IPF. The regression demonstrated that the diagnosis of IPAF and CTD-ILD were associated with increased risk of ANA seropositivity, while a diagnosis of “other ILD” was protective. IPAF carried a higher odds ratio of 3.56 (95%CI=2.48-5.13) compared to the lower odds ratio of 2.56 for CTD-ILD (95%CI=1.81-3.60). A diagnosis of other ILD was associated with lower odds of having a positive ANA serology (OR 0.44; 95%CI=0.29-0.67). Amongst those categorized as unclassifiable ILD who had combined pulmonary fibrosis and emphysema (CPFE), patients with a significant smoking history of more than ten pack years had numerically higher prevalence of ANA seropositivity (n=16, 50.0%) than ANA seronegativity (n=4, 23.5%; *P*=0.073) but this was not statistically significant (**e-supplementary table 4**).

Pneumoconiosis
Electronic medical record documentation regarding a secondary diagnosis of pneumoconiosis was available for review in 1,045 patients of which 8.1% (n=85) had pneumoconiosis. Among these patients with a secondary diagnosis of pneumoconiosis, more patients had ANA seropositivity (n=64, 9.1%) than those having ANA seronegativity (n=21, 6.2%; *P*=0.12) (**e-supplementary Table 5**). Amongst the subset classified as having "other ILD" we identified six patients who also had a secondary diagnosis of pneumoconiosis. Five of these six patients had ANA seropositivity (n=5, 83.3%). Amongst the remaining 30 patients with "other ILD" without pneumoconiosis, fewer had ANA seropositivity (n=13, 72.2%) than those with ANA seronegativity (n=17, 94.4%; *P*=0.074) (**e-supplementary Table 5**).

Discussion

In this study, we report that ILD patients with a history of inhalational exposure had a significantly higher prevalence of ANA seropositivity than those without reported exposures. Demographics of the cohort revealed mean pulmonary function test values consistent with moderate lung dysfunction. To our knowledge, this is the first multicenter demonstration of an association between ANA serology and inhalational exposures in ILD.

African American and Hispanic race within the entire ILD cohort were both highly predictive of having a positive ANA, consistent with recent retrospective cohort studies showing patients of African ancestry have the greatest prevalence of seropositive ANA titers and CTD-ILD compared to other racial groups.[19,20] There may also be a role for structural racism in this discrepancy, in that patients who are of African American and Hispanic race are more likely to live in areas where they may be exposed to ambient pollution, which could then increase the risk of autoimmunity.[21,22]

The majority of patients in this study, all of whom carried a diagnosis of ILD, were ANA seropositive. ANA serology was used as a marker of a systemic autoimmune response and an ANA titer of 1:160 or higher was considered seropositive.[23]

ILD has an established association with autoimmunity. This is evident in ILD patients diagnosed with CTD or presenting with autoimmune features, classified as having subtypes of CTD-ILD, AIF-ILD, or IPAF. In addition, ILD is the primary pulmonary manifestation of multiple autoimmune conditions including rheumatoid arthritis, scleroderma (systemic sclerosis), inflammatory myositis (polymyositis and dermatomyositis), Sjögren's syndrome, and undifferentiated connective tissue disease (UCTD).[3]

The majority of patients in our cohort had at least one reported exposure. This is not a novel finding, however, because numerous ILDs have been linked to environmental exposures. Hypersensitivity pneumonitis (HP) can manifest from exposure to a variety of inhaled antigens which results in diffuse inflammation of small airways and pulmonary parenchyma.[8] This study demonstrated that ANA seropositivity in HP was higher than

those with other ILD, IPF, and unclassifiable ILD. However, ILDs such as IPAF and CTD-ILD had higher ANA seropositive prevalence because they required an ANA seropositive value for diagnosis. Notably, this study showed that patients with HP have a higher prevalence of inhaled organic particles than patients with another ILD diagnosis. As with HP, pneumoconiosis are lung diseases related to inhalational exposures, specifically mineral dusts like silicosis, coal mining dust, asbestosis, and beryllium.[8] Our study demonstrated that ILD patients with a history of one or more inhalational exposures were more likely to be ANA seropositive than those without a reported exposure. This was true for the univariate analysis of tobacco, organic, and inorganic exposures and ANA. The association, however, was not present for organic exposures in our multivariable analysis. This might suggest a weaker association between organic antigen exposures such as mold and ANA serology.[23,31]

This study also identified patients with a secondary diagnosis of pneumoconiosis and found that among those with pneumoconiosis, there was a higher prevalence of ANA seropositivity than ANA seronegativity, although this was not statistically significant. The disproportionately higher prevalence of ANA seropositivity further supports the idea that ANA titers are associated with environmental exposures in patients with ILD. In addition, we demonstrated an association between tobacco exposure and ANA seropositivity in the entire cohort. Interestingly, amongst those with unclassifiable ILD, patients with CPFE and a heavy smoking history had a high prevalence of ANA seropositivity compared to ANA seronegativity, further strengthening the relationship with tobacco exposure.

There are several studies that corroborate an association between the presence of autoimmune serology and inhalational exposures, but not exclusively in patients with a diagnosis of ILD. A case report of patients in Libby, Montana with a history of mining asbestos-contaminated vermiculite reported a higher frequency of ANA seropositivity in miners compared to healthy patients. In addition, it demonstrated a positive correlation between ANA titers, lung disease severity and extent of exposure, suggesting a relationship between autoimmune response and asbestos-related disease processes.[24] A retrospective study of Brazilian silica-exposed workers found that silica may nonspecifically enhance the immune response, supporting the association between silica exposure and autoimmune disorders.[25] The diagnosis of silicosis in these workers was based on confirmation of exposure and the presence of pulmonary parenchyma alterations, but not a multidisciplinary diagnosis of ILD. Furthermore, multiple epidemiological studies including meta-analyses have identified cigarette smoking as an important risk factor for rheumatoid arthritis (RA).[26,27] RA is serologically characterized by the presence of autoantibodies against factors like rheumatoid factor and cyclic citrullinated peptide. Cigarette smoking is a known trigger of immune reactions to autoantigen modified by citrullination.[28] Additionally, animal model studies have shown administration of various heavy metals induce production of autoantibodies and polyclonal activation of the immune system in genetically susceptible rats.[29]

The association between autoimmunity and exposure in ILD patients raises questions about the pathophysiology of ILD. Specifically, it remains unknown whether the presence of autoantibodies in ILD patients represents a heightened immune response and is a protective influence or if it represents a dysregulated immune response. Interestingly, the presence of autoantibodies in patients with ILD of known etiology, including inhalational exposure (tobacco, organic, and inorganic), CTD, and drug exposure (e.g. amiodarone, methotrexate) has been associated with worse clinical outcomes.[30] In this study by *Lee et al*, unadjusted transplant-free survival was significantly worse in patients who had ILD with a history of inhalational exposure compared to those without. However, this difference did not remain significant after adjustment for gender, age, lung function indices, and pack-years of smoking, which might suggest that in addition to these prognostic indices, smoking may independently influence outcomes in the population of exposure-related ILDs. Notably, in that investigation, patients with autoimmune-related ILD subtypes such as IPAF and CTD-ILD also had a high prevalence of inhalational exposures, further supporting the link between autoimmunity and environmental exposures.[30] It has previously been shown in a cohort of 120 patients with HP that the presence of clinical and serologic autoimmunity may portend a poorer prognosis.[31] Another study of 71 patients with coal worker's pneumoconiosis concluded that high-titer rheumatoid factor in these patients may be associated with more severe disease, extra-articular features, and rheumatoid nodules.[32]

Conversely, autoantibodies have been associated with better clinical outcomes in patients with idiopathic interstitial pneumonia. One study demonstrated that in patients with AIF-ILD a higher ANA titer conferred a better prognosis.[5] Another study examining autoantibodies in patients with IPF found no significant difference between the number of IPF patients and healthy controls in the number of circulating autoantibodies. The presence of autoantibodies was associated with a longer transplant-free survival in IPF patients, although the significance depended on the statistical model used.[33]

Our study has several limitations, including the retrospective nature of the study. There is no established time course between the exposure, ANA serology, and ILD diagnosis. This, as well as the small sample size and observational design, do not allow for conclusions about causality, although they do allow for an association. There is a selection bias in that only patients with serologic testing were included. Although smoking status was noted in most medical records, not all charts documented the presence or absence of other exposures. Because ELISA testing has lower specificity than IFA testing, it may have more false positive tests.

CONCLUSION

This study shows that ANA seropositivity is associated with inhalational exposure to environmental antigens in patients with ILD. Our findings suggest that ANA, and by extension, autoimmunity likely plays a significant role in the pathophysiology of ILD. While we cannot demonstrate causality in this retrospective analysis, these associations provide additional insights into the underlying mechanisms of disease and may offer guidance for future testing and therapies.

Acknowledgements:

Our profound appreciation goes to the support staff of the University of Chicago Respiratory Clinical Research Unit, the Interstitial Lung Disease Program (Nancy Trojan, Catherine Brown and Spring Maleckar) and NorthShore University HealthSystem (Evanston, IL, USA) (Daniel Chertok, Mohammad Imran Beig, Paul Chung, and Naomi BenIsrael Olive), for their assistance with this study. We also extend our gratitude to the patients with ILD who made these research endeavors possible.

Table 1. Baseline Demographics of Patients with ILD

Characteristic	Combined	ANA seropositive	ANA seronegative	*P*-value*
Age, years (SD)	64.7 (12.3)	64.1 (12.4)	65.5 (12.1)	0.045
Male, n (%)	622 (49.2)	362 (58.2)	260 (41.8)	0.789
BMI, mean (SD)	29.8 (6.6)	29.6 (6.7)	30.1 (6.5)	0.251
Caucasian, n (%)	918 (72.6)	515 (56.1)	403 (43.9)	0.004
African American, n (%)	203 (16.0)	147 (72.4)	56 (27.6)	<0.001
Hispanic, n (%)	65 (5.1)	50 (76.9)	15 (23.1)	0.002
Asian, n (%)	47 (3.7)	25 (53.1)	22 (46.8)	0.445
Other, n (%)	32 (2.5)	4 (12.5)	28 (87.5)	<0.001
Lung Function				
TLC %, mean (SD)	71.3 (18.6)	69.0 (17.2)	74.8 (20.0)	<0.001
FVC %, mean (SD)	66.3 (19.0)	64.1 (18.4)	69.5 (19.4)	<0.001
DLCO %, mean (SD)	51.0 (21.7)	50.0 (21.8)	52.5 (21.6)	0.065
ILD Subtype				
IPF, n (%)	402 (31.8)	212 (52.7)	190 (47.3)	0.003
IPAF, n (%)	177 (14.0)	141 (79.7)	36 (20.3)	<0.001
CTD-ILD, n (%)	240 (19.0)	177 (73.8)	63 (26.3)	<0.001
HP, n (%)	141 (11.2)	82 (58.2)	59 (41.8)	0.914
Other ILD, n (%)	96 (7.5)	29 (30.2)	67 (69.8)	<0.001
Unclassifiable ILD, n (%)	209 (16.5)	100 (47.8)	109 (52.2)	0.001

TLC = total lung capacity; FVC = forced vital capacity; DLCO = diffusion capacity for carbon monoxide
IPF = idiopathic pulmonary fibrosis; IPAF = interstitial pneumonia with autoimmune features; CTD-ILD = Connective tissue disease associated ILD; HP = hypersensitivity pneumonitis; ILD = interstitial lung disease. Other ILD include pneumoconiosis, lymphocytic interstitial pneumonia, Langerhans cell histiocytosis, cryptogenic organizing pneumonia, lymphangioleiomyomatosis, and other less common ILDs with small sample sizes.
**P value based on univariate analysis for categorical variables. For example, Caucasian compared to non-Caucasian, IPF compared to non-IPF patients, etc. P value based on two-tailed student t test for continuous variables. For example, age, BMI.*
Exception for participants: Age, n=1265; gender, n=1265; ANA serologies, n=1265; BMI, n=1122; TLC, n=989; FVC, n=1162; DLCO, n=1115; and other ILD, n=95.

Table 2. Association with ANA seropositivity

	Odds Ratio*	95% CI	Std Error	*P*-Value
Diagnosis				
IPAF	3.56	2.48 - 5.13	0.22	<0.0001
CTD-ILD	2.56	1.82 - 3.60	0.21	<0.0001
HP	1.39	0.97 - 1.98	0.22	0.13
Other ILD	0.44	0.29 - 0.67	0.25	0.0014
Unclassifiable ILD	0.92	0.68 - 1.23	0.18	0.63
Age	1.00	0.99 - 1.01	0.01	0.46
Male Gender	1.05	0.84 - 1.31	0.14	0.73
Race				
African American	1.78	1.30 - 2.45	0.19	0.003
Hispanic	2.23	1.34 - 3.71	0.31	0.01
Asian	1.04	0.62 - 1.75	0.32	0.89
Other Race	0.14	0.06 - 0.36	0.55	0.0004
Exposure				
Tobacco	1.38	1.12 - 1.71	0.13	0.01
Organic Antigen	1.11	0.89 - 1.40	0.14	0.44
Inorganic Antigen	1.52	1.12 - 2.07	0.19	0.02

** Odds ratio (OR) analyzed using multivariable logistic regression with adjustments for age, gender, race. OR comparisons were made for every increase of one year for age, male gender in comparison to female gender, diagnosis compared to IPF, race compared to Caucasians, tobacco compared to non-tobacco users, exposure compared to those without identified environmental exposure*

IPAF = Interstitial Pneumonia with Autoimmune Features; CTD-ILD = connective tissue disease-associated interstitial lung disease; HP = hypersensitivity pneumonitis; ILD = interstitial lung disease

e-Supplementary Table 1. Baseline Demographics of Patients with ILD by Center

	University of Chicago			Northshore University HealthSystem		
Characteristic	**ANA Positive**	**ANA Negative**	***P*-value**	**ANA Positive**	**ANA Negative**	***P*-value**
Age, years (SD)	64.2 (12.3)	54.2 (11.8)	0.966	62.4 (12.6)	68.0 (12.2)	0.015
Male, n (%)	357 (50.6)	187 (55.0)	0.187	5 (13.9)	73 (39.7)	0.004
BMI, mean (SD)	29.6 (6.6)	30.2 (6.8)	0.015	29.5 (5.7)	29.0 (5.8)	0.691
Caucasian, n (%)	490 (69.5)	274 (80.6)	<0.001	25 (69.4)	129 (70.1)	1.000
African American, n (%)	141 (20.0)	41 (12.1)	0.002	6 (16.7)	15 (8.2)	0.124
Hispanic, n (%)	50 (7.1)	15 (4.4)	0.093	0 (0.0)	0 (0.0)	N/A
Asian, n (%)	24 (3.4)	10 (2.9)	0.693	1 (2.8)	12 (6.5)	0.699
Other, n (%)	0 (0.0)	0 (0.0)	N/A	4 (11.1)	28 (15.2)	0.615
Lung Function						
TLC %, mean (SD)	68.6 (17.0)	69.9 (17.0)	0.306	76.4 (18.3)	83.7 (22.0)	0.096
FVC %, mean (SD)	63.9 (18.5)	65.5 (18.0)	0.209	67.7 (15.8)	76.8 (19.8)	0.013
DLCO %, mean (SD)	50.1 (21.9)	51.8 (20.9)	0.265	48.7 (18.8)	54.0 (23.0)	0.239
ILD Subtype						
IPF, n (%)	202 (28.7)	126 (37.1)	0.007	10 (27.8)	64 (34.8)	0.448
IPAF, n (%)	140 (19.9)	31 (9.1)	<0.001	1 (2.8)	5 (2.7)	1.000
CTD-ILD, n (%)	172 (24.4)	58 (17.1)	0.008	5 (13.9)	5 (2.7)	0.012
HP, n (%)	82 (11.6)	49 (14.4)	0.231	0 (0.0)	10 (5.4)	0.374
Other ILD, n (%)	18 (2.6)	18 (5.3)	0.029	11 (30.5)	49 (26.6)	0.683
Unclassifiable ILD, n (%)	91 (12.8)	58 (17.0)	0.074	9 (25.0)	51 (27.8)	0.839

TLC = total lung capacity; FVC = forced vital capacity; DLCO = diffusion capacity for carbon monoxide
IPF = idiopathic pulmonary fibrosis; IPAF = interstitial pneumonia with autoimmune features; CTD-ILD = Connective tissue disease associated ILD; HP = hypersensitivity pneumonitis; ILD = interstitial lung disease. Other ILD include pneumoconiosis, lymphocytic interstitial pneumonia, Langerhans cell histiocytosis, cryptogenic organizing pneumonia, lymphangioleiomyomatosis, and other less common ILDs with small sample sizes.
**P value based on univariate analysis for categorical variables. For example, Caucasian compared to non-Caucasian, IPF compared to non-IPF patients, etc. P value based on two-tailed student t test for continuous variables. For example, age, BMI.*
Exception for participants: Age, n=1265; gender, n=1265; ANA serologies, n=1265; BMI, n=1122; TLC, n=989; FVC, n=1162; DLCO, n=1115; and other ILD, n=95.

e-Supplementary Table 2. Variable Count for Baseline Demographics of ILD Cohort by Center and ANA Seropositivity

	University of Chicago (n = 1045)	**Northshore University HealthSystem (n = 220)**	***P*-value**
TLC, n(%)	818 (78.3)	171 (77.7)	0.858
FVC, n(%)	958 (91.7)	204 (92.7)	0.685
DLCO, n(%)	940 (90.0)	175 (79.5)	<0.001
Age, n(%)	1045 (100.0)	220 (100.0)	1.000
Gender, n(%)	1045 (100.0)	220 (100.0)	1.000
BMI, n(%)	902 (86.3)	220 (100.0)	<0.001
ANA serology, n(%)	1045 (100.0)	220 (100.0)	1.000
	ANA Seropositive (n = 741)	**ANA Seronegative (n= 524)**	***P*-value**
TLC, n(%)	594 (80.2)	395 (75.4)	0.045
FVC, n(%)	684 (92.3)	478 (91.2)	0.531
DLCO, n(%)	668 (90.1)	447 (85.3)	0.010
Age, n(%)	741 (100.0)	524 (100.0)	1.000
Gender, n(%)	741 (100.0)	524 (100.0)	1.000
BMI, n(%)	661 (89.2)	459 (87.6)	0.420
ANA serology, n(%)	741 (100.0)	524 (100.0)	1.000

TLC = total lung capacity; FVC = forced vital capacity; DLCO = diffusion capacity for carbon monoxide

e-supplementary Table 3. Prevalence of organic exposures among patients with and without a diagnosis of HP.

Exposure	HP	Non-HP	Total	*P*-value
Organic Exposure, n (%)	95 (67.4)	370 (32.9)	465	<0.001
No Organic Exposure, n (%)	46 (32.6)	754 (67.1)	800	
Total, n (%)	141 (100.0)	1124 (100.0)	1265	

HP = hypersensitivity pneumonitis

e-Supplementary Table 4. Prevalence of patients with available documentation of radiographic emphysema and smoking history amongst individuals with unclassifiable ILD*

All Unclassifiable ILD	ANA Seropositive	ANA Seronegative	Total	*P*- value
Emphysema, n (%)	24 (31.6)	12 (26.1)	36 (29.5)	0.519
No emphysema, n (%)	52 (68.4)	34 (73.9)	86 (70.5)	
Total, n (%)	76 (100.0)	46 (100.0)	122 (100.0)	
Unclassifiable ILD with significant smoking history				
Emphysema, n (%)	16 (50.0)	4 (23.5)	20 (40.8)	0.073
No emphysema, n (%)	16 (50.0)	13 (76.5)	29 (59.2)	
Total, n (%)	32 (100.0)	17 (100.0)	49 (100.0)	
Unclassifiable ILD without significant smoking history				
Emphysema, n (%)	8 (18.2)	8 (27.6)	16 (21.9)	0.342
No emphysema, n (%)	36 (81.8)	21 (72.4)	57 (78.1)	
Total, n (%)	44 (100.0)	29 (100.0)	73 (100.0)	

**Significant smoking history was regarded as patients with ≥ 10 pack year history of tobacco use.*
ILD = interstitial lung disease

e-Supplementary Table 5. Prevalence of ANA seropositivity in patients with available documentation for a secondary diagnosis of pneumoconiosis

All ILD	ANA Seropositive	ANA Seronegative	Total	*P*- value
Pneumoconiosis, n (%)	64 (9.1)	21 (6.2)	85 (8.1)	0.120
No pneumoconiosis, n (%)	641 (90.9)	319 (93.8)	960 (91.9)	
Total, n (%)	705 (100.0)	340 (100.0)	1045 (100.0)	
Other ILD				
Pneumoconiosis, n (%)	5 (27.8)	1 (5.6)	6 (16.7)	0.074
No pneumoconiosis, n (%)	13 (72.2)	17 (94.4)	30 (83.3)	
Total, n (%)	18 (100.0)	18 (100.0)	36 (100.0)	

ILD = interstitial lung disease

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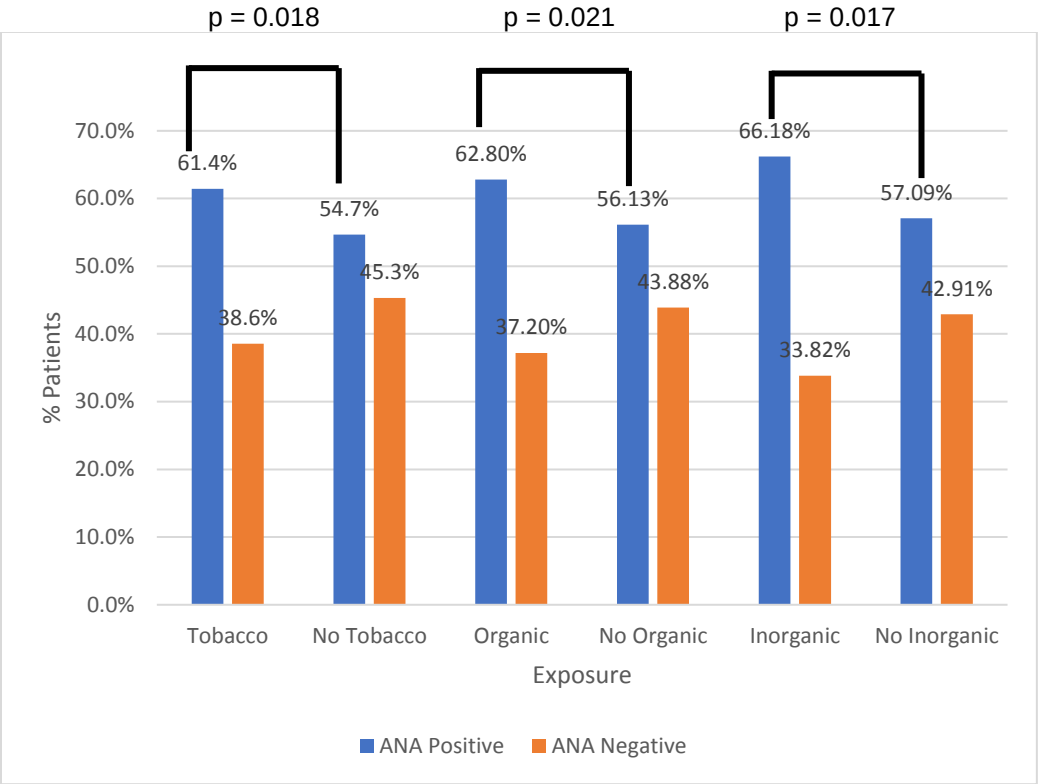
Figure 1. Prevalence of ANA seropositivity in ILD patients with inhalational exposures.



Supplementary Figure 1. Error! Bookmark not defined.

ROC curves for ANA-IFA and ANA-EIA for the common period between 5 January and 23 February 1995, to truly reflect the patient population at University of California at San Francisco (*P* = not significant)