Early View

Original research article

Antigen Identification and Avoidance on Outcomes in Fibrotic Hypersensitivity Pneumonitis

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Take Home Message: Antigen identification and antigen avoidance appear to improve outcomes even in patients with fibrotic disease. Comprehensive ascertainment of causative antigen in fibrotic hypersensitivity pneumonitis should be encouraged in clinical practice.

Abbreviations

CT: computed tomography

DLCO%: percent predicted diffusion capacity for carbon monoxide

EMR: electronic medical record

f-HP: fibrotic hypersensitivity pneumonitis

FVC%: percent predicted forced vital capacity

HP: hypersensitivity pneumonitis

HR: hazard ratio

HRCT: high resolution chest computed tomography

ILD: interstitial lung disease

IPF: idiopathic pulmonary fibrosis

IQR: interquartile range

LT: lung transplant

PFT: pulmonary function test

USSDI: United States Social Security Death Index

ABSTRACT

Suspected causative antigens may be unidentified in 30-50% of patients with fibrotic hypersensitivity pneumonitis (f-HP). It is unclear if antigen identification and avoidance in this setting offer any additional clinical benefit. We hypothesized that antigen identification and avoidance may improve the clinical course of patients with fibrotic disease.

Patients meeting recent international practice guidance for f-HP diagnosis evaluated at Mayo Clinic Rochester from January 2005 to December 2018 were included. Causative antigen and antigen avoidance were specifically defined and ascertained through review of the medical records. Cox proportional-hazards regression was performed to assess antigen identification and avoidance as predictors of either all-cause mortality or lung transplantation.

A total of 377 patients were included. Of these, suspected causative antigen was identified in 225 (60%). Identification of a suspected antigen (adjusted HR of 0.69 [95% CI, 0.48-0.99]; $P = 0.04$) and subsequent antigen avoidance (adjusted HR of 0.47 [95% CI, 0.31-0.71]; $P < 0.001$) were associated with decreased all-cause mortality and transplantation. Both those with suspected antigen identification but non-avoidance and those with unidentifiable antigen had increased risk of all-cause mortality or transplantation (adjusted HR 2.22 [95% CI, 1.34-3.69]; $P = 0.002$ vs. adjusted HR 2.09 [95% CI, 1.34-3.26]; $P = 0.001$, respectively). Exposure to avian antigen was associated with better outcome compared to other antigen subtypes (adjusted HR 0.63 [95% CI 0.43-0.93]; $P = 0.02$).

Our findings suggest antigen identification and antigen avoidance remain relevant even in patients with fibrotic disease, where both appear to be associated with improved outcomes.

Introduction

Hypersensitivity pneumonitis (HP) is an immune-mediated interstitial lung disease (ILD) characterized by injury from chronic or recurrent inhalational exposure to sensitizing environmental antigens (e.g., avian, mold, bacterial, and other organic and inorganic compounds). Chronic exposure is believed to result in persistent inflammation and eventual fibrosis [1]. A recent international consensus guideline for the diagnosis of HP [2] highlights classification of disease into fibrotic vs non-fibrotic subtypes, with fibrotic subtypes often having poorer outcomes. One-year cumulative incidence rates of fibrotic hypersensitivity pneumonitis (f-HP) range from 0.29 to 0.43 per 100,000 persons, with an all-cause mortality rate of 67.5 per 1,000 person-years [3].

Antigen avoidance is recommended as a first step in the management of all patients with HP. However, suspected antigens may be unidentified in approximately 30-50% of those with fibrotic disease [4-7]. The timing of exposures and immediate clinical findings is often diagnostic in acute presentations, but more difficult to ascertain and interpret in those with suspected chronic exposure and more subclinical presentation. Limited evidence supports the importance of antigen identification and avoidance in those with suspected chronic exposure [5, 6], however, data specific to those with fibrotic disease has not been systematically and separately pursued, particularly in the setting of more recently unified diagnostic criteria. Other studies looking at the question in mixed cohorts (fibrotic and non-fibrotic) found little to no effect on outcome with either antigen identification or avoidance [8, 9]. We conducted a large retrospective study of patients with f-HP (using recently published international diagnostic guidance) to clarify the association of antigen identification, avoidance, and subtype, with all-cause mortality or lung transplantation (LT) as a combined endpoint. We hypothesized that successful antigen identification and avoidance may still have impact on short and long-term outcomes in those with fibrotic disease.

Materials and methods

Setting and subject selection

Our study is a large single-center retrospective cohort approved by Mayo Clinic Institutional Review Board (approval no. 20-000211) before patient inclusion and data abstraction. A computer-assisted search using the term "hypersensitivity pneumonitis" was performed for adult patients ($\geq$ 18 years of age) seen at Mayo Clinic from January 2005 to December 2018. This was applied to multiple search fields, including clinical notes, radiology and histopathology reports, and ICD-9/ICD-10 billing databases. Individual records of screened patients were comprehensively reviewed by study members for ascertainment of exposure history, serum IgG precipitin testing, imaging, and histopathology. After exclusion of other causes of ILD, computed tomography (CT) imaging and pathology reports were individually reviewed and adjudicated by the authors as 'typical', 'compatible '(for CT imaging)'/'probable' (for histopathology criteria), or 'indeterminate' for HP, according to the recent 2020 ATS/JRS/ALAT clinical practice guideline [2]. Only patients with radiologic fibrosis (reticulation with or without architectural distortion/traction bronchiectasis or honeycombing) were included. Combined clinical, radiologic, and histopathologic findings provided a diagnosis of f-HP according to levels of confidence, categorized as low (51-69%), moderate (70-79%), high (80-89%), or definite ($\geq$90%) (Figure 6 of Reference 3). Patients with low or moderate diagnostic confidence levels (diagnostic confidence levels <80%) were included in our study if no other ILD diagnoses were made on clinical follow-up. All patients with diagnostic confidence levels less than 50% were considered incompatible with HP and excluded.

Identification of causative antigens and antigen avoidance

Individual patient records were reviewed by study members for suspected causative antigens, defined as documented environmental exposure to avian antigen (bird owner, feather or down-containing items, and bird droppings), prior or active exposure to mold or bacterial antigen in the farm environment (moldy hay, visible mold or water damage), home, or workplace, or other specifically identifiable exposure item or work history (hot tub use,

humidifiers, metal-work fluid, isocyanates, other occupation-related, etc.). Exposure history was obtained and reported by clinicians with the use of institutional questionnaires or review of systems according to personal preference. All patients required documentation of exposure ascertainment either in the clinical note or review of systems template for study inclusion. Those with specific documentation of an unidentifiable or unknown exposure after solicitation were categorized as 'unidentifiable causative antigen'. Patients with positive serum IgG against specific antigens without relevant environmental exposure identification were also defined as 'unidentifiable causative antigen'. To address the concern that elevated serum IgG to precipitin without solicited exposure history may still represent antigen sensitization but not necessarily to the related culprit exposure, prior sensitization but without active exposure, or even false positive values, sensitivity analyses were performed with reclassification of those with only positive serology but no solicited exposures to the 'identifiable causative antigen' group or exclusion from the primary analysis, with comparison of these results to the original classification.

Antigen avoidance was defined by reported antigen removal or abatement of the contaminating agent, including removal of avian-antigen containing items (pet birds, feather-containing items), standing or aerosolized water (indoor hot tub, humidifier, de-humidifiers, welding fluid, etc.), removal of inorganic materials such as isocyanates in paints or plastics material in the home or work environment, comprehensive cleaning or abatement of homes or workplaces for mold or water damage, or abstaining entirely from suspected home or work environments after diagnosis (selling or moving out of a home or changing occupation). If a suspected environmental exposure was documented but no abatement or avoidance effort was reported, this was classified as 'non-avoidance'. For patients with multiple identifiable causative antigens, documentation of avoidance effort for all suspected antigens was required for categorization as completing 'antigen avoidance'. We categorized all documented efforts at antigen avoidance as 'positive' rather than reclassifying as non-avoidance (or misclassified) if disease continued to progress despite avoidance effort. Our intent was to assess as much as possible the separate effects of suspected antigen

identification (solicited present or not) and potential antigen avoidance (efforts pursued with or without clinical improvement when indicated vs not pursued or unable to pursue) on outcome.

Baseline characteristics, follow-up, and outcome identification

Index date was defined as the date of initial clinical presentation for diagnostic evaluation. Baseline characteristics included age at diagnosis, sex, smoker status, CT findings, and baseline pulmonary function testing (PFT). PFT data included presenting percent predicted forced vital capacity (FVC%) and diffusion capacity for carbon monoxide (DLCO%). Specific abstracted CT findings at the time of diagnosis included mosaic attenuation, reticular opacities, honeycombing, and/or findings supporting probable or consistent usual interstitial pneumonia (UIP) radiologic pattern. Treatment type and duration were collated with chronic treatment defined as any immunosuppression (corticosteroids and/or steroid-sparing agent (azathioprine or mycophenolate) for greater than six months cumulatively. The primary outcome was either all-cause mortality or LT, combined as a single composite endpoint. These outcomes were abstracted through comprehensive medical record review and cross-matched with a United States Social Security Death Index Search (USSDI) search. All subjects were censored on the date of study query (April 20, 2020) if death or LT was not found in the EMR or by USSDI search.

Statistical analysis

Summary statistics were presented as mean and SD for normally distributed continuous variables, median and 25-75% interquartile range (IQR) for non-normally distributed variables, and number and percent for categorical variables. Baseline characteristics of patients with identifiable and unidentifiable causative antigens were compared using independent t, Wilcoxon rank-sum, and Chi-square test, as appropriate.

Cox proportional-hazards regression modeling was used to assess the association of antigen avoidance and antigen subtype (avian vs all others) with risk of death or LT. All models were adjusted for a priori covariables of age, sex, radiologic honeycombing, baseline FVC%, and DLCO%. Multicollinearity of independent variables for all regression models was

assessed with the variance inflation factor (VIF) test, noting values between 1 and 5 as having mild and > 5 as moderate or high collinearity. Survival curves were generated using Kaplan-Meier estimator, assessing comparative survival with Log-rank. If Kaplan-Meier curves demonstrated violation of a constant hazard ratio (HR) over time, a time-varying covariate Cox model was used based on the timing of survival curve separation. Statistical significance was defined as a conventional two-tailed α level < 0.05.

Results

Subject selection

From January 2005 through December 2018, 779 suspected HP patients were identified by computer-assisted search of the EMR. Of these, 448 were considered possible f-HP according to 2020 ATS/JRS/ALAT diagnostic guideline and exclusion of other ILD. After removing non-fibrotic patients and those with diagnostic confidence levels less than 50%, 377 were included in the final study cohort. Two hundred twenty-five patients (59.7%) had identifiable suspected causative antigens with antigen avoidance documented in 124 (55.1% of those with identifiable antigen, 32.9% of the total cohort). Avoidance status was unknown in 21 patients with suspected antigen due to incomplete follow-up, and 37 patients had positive serum IgG without identifiable environmental exposure (23 with single positive serum IgG and 14 with multiple positive serum IgG) (Figure 1). Excluded patients were compared to those enrolled based on recent diagnostic guidance, and found to be predominantly male with greater honeycombing and UIP pattern on CT, suggesting possible atypical IPF or other fibrotic ILD (Table E1).

Clinical characteristics

Of 377 patients, 180 were male (47.7%) and 166 had a history of smoking (44.0%) (Table1). Serum specific IgG precipitin testing was positive in 161 (42.7%) with IgG precipitin for avian antigen positive in 110 of these. Environmental exposure by history was documented in 225 patients. Exposure to avian antigen was the most commonly found

subtype (N=129), followed by exposure to mold or bacterial contamination in the farm environment (N=53), home, or workplace (N=55). Fifty patients were categorized as having multiple potential environmental exposures (Table 2).

Patients with identifiable and unidentifiable causative antigens had similar baseline demographics, including age and smoking status (Table 1). Those with unidentifiable causative antigens had lower DLCO% (47.4% vs 52.5%, P = 0.005) and significantly higher long-term corticosteroid (94.1% vs 82.2%, P = 0.001) and/or steroid-sparing agent use (49.3% vs 31.6%, P <0.001). Histopathology was obtained more frequently in those with unidentifiable causative antigen, resulting in similar numbers of 'definite' diagnoses but more varied frequency of other diagnostic confidence levels (Table 1). All-cause mortality and LT was 37.1% (N= 140) and 6.9% (N = 26), respectively, for the whole cohort.

Impact of identifying suspected causative antigens and antigen avoidance

Identification of suspected causative antigens was associated with decreased risk of all-cause mortality and LT, after adjustment for a priori covariables (adjusted HR of 0.69 [95% CI 0.48-0.99], P = 0.04). Median survival was 8.39 years for patients with identifiable causative antigen compared to 5.93 years for those with unidentifiable causative antigen. Association of an identifiable causative antigen with improved survival was not seen in the first three years after diagnosis (Table 3 and Figure 2A) when stratified by identifiable antigen alone, with short-term (≤ 3 years) adjusted HR of 0.97 ([95% CI, 0.58-1.64]; P = 0.92) vs longer-term (> 3 years) adjusted HR of 0.51 ([95% CI, 0.31-0.83]; P = 0.006). Identification of multiple potential antigens was not associated with increased risk of mortality or LT (adjusted HR 1.20 [95% CI, 0.74-1.93]; P = 0.46) (Table 4) but was associated with lower likelihood of reported antigen avoidance (odds ratio (OR) 0.27 [95% CI, 0.13-0.55]; P <0.001) (Table E2). Overall decreased risk of all-cause mortality associated with causative antigen identification was still found on sensitivity analyses with reclassification of those with only positive serologic testing but no exposure history to the 'identifiable antigen' group or with their exclusion from the analysis (Tables E3-4 and Figures E1-4).

Documented approaches to antigen avoidance and frequency of reported antigen avoidance for each type of antigen exposure are presented in Table 5 and Table E2, respectively. Of 124 patients reporting antigen avoidance, 25 (20%) ceased exposure to causative antigen prior to f-HP diagnosis. Among those with antigen avoidance after f-HP diagnosis, median time to exposure cessation was 3.4 months (25%-75% IQR, 0.36-12.89). Reported antigen avoidance was associated with decreased all-cause mortality and LT (adjusted HR 0.47 [95% CI, 0.31-0.71]; P <0.001), as presented in Table 3 and Figure 2B. Patients with identifiable causative antigen but non-avoidance and those with unidentifiable antigen had comparable increased risk of death or LT, with adjusted HR of 2.22 ([95% CI, 1.34-3.69], P = 0.002) and 2.09 ([95% CI, 1.34-3.26]; P = 0.001), respectively (Table 3 and Figure 2C). As multicollinearity may be of concern when adjusting for a priori covariables of FVC, DLCO, and honeycombing, specific testing with VIF was performed for all comparisons and found to range from 1 to 1.4, suggesting minimal to low collinearity between these variables.

Among antigen subtypes, suspected exposure to bird or avian antigen was associated with lower all-cause mortality and risk of LT, as defined by patients with positive serum IgG precipitin against avian antigens (adjusted HR of 0.63 [95% CI, 0.43-0.93]; P = 0.02) or solicited history of environmental bird or feather exposure (adjusted HR 0.61 [95% CI, 0.43-0.88]; P = 0.008) (Table 4). History of bird or avian antigen exposure was also associated with greater likelihood of successful antigen avoidance (OR 8.11 [95% CI, 3.99-16.50]; P <0.001). History of mold and bacterial exposure in the farm environment had lower likelihood of reported antigen avoidance (OR 0.14 [95% CI, 0.06-0.32]; P <0.001) (Table E2).

Discussion

The current study further highlights the impact of antigen identification and subsequent antigen avoidance in a well-described cohort of HP patients with specific fibrotic presentations. We found that approximately 60% of patients in our cohort had identifiable causative antigens. Prior rates of identifiable antigen in f-HP have ranged from 50% to 70%

[4-7], depending on study inclusion and descriptors of chronic vs fibrotic classification. The higher proportion of identifiable causative antigens in our study may be explained by higher rates of serum specific IgG testing, occurring in 87% of patients in our cohort. Positive serum IgG antibody testing may better guide clinicians in ascertaining relevant environmental exposures, resulting in higher rates of suspected causative antigen identification. Positive serum IgG against avian antigen or history of environmental avian exposure was also the most commonly solicited antigen subtype in our cohort. A recent systematic review also found avian exposure or positive avian antigen testing to be the most commonly reported or published exposure subtype [10]. Similarity in exposure frequency or subtype with prior studies might suggest better generalizability of our assessment approach and further strengthen arguments for the role of antigen identification or avoidance on outcomes.

Indeed, the impact of identifying causative antigens on short and long-term outcomes in f-HP remains disputed. Among patients with readily identifiable antigens, some with fibrotic disease may continue to progress or decline despite efforts at antigen avoidance [1]. It is unclear whether occult antigen(s) in the environment continues to propagate disease or if unrelated inflammatory or autoimmune processes, perhaps towards the later stages of fibrotic disease, lead to ongoing disease progression even in the absence of antigen exposure [11]. Fernandez Perez et al. and De Sadeleer et al. reported decreased mortality in patients with identifiable causative antigens in their cohorts of fibrotic and non-fibrotic HP [5, 6]. Chronic though radiologically non-fibrotic patients made up 63% of one study, with survival appearing to be affected by radiologic fibrosis as much as antigen identification. Additional studies with varying methodologies further contrast our findings and those of Fernandez Perez and De Sadeleer [8, 9, 12, 13]. The recent guideline from the American College of Chest Physicians systematically reviewed the impact of antigen identification on outcome and support antigen identification for the possible improvement of survival outcomes [14].

Our study extends these findings with a more systematic focus on well-defined fibrotic patients using recently established diagnostic criteria and specific antigen

identification and avoidance documentation to assess their individual effects, noting positive outcome benefit for both parameters. A prior study by Gimenez et al. demonstrated clinical improvement after causative antigen avoidance was associated with favorable long-term outcomes [15]. De Sadeleer and colleagues reviewed the effects of corticosteroid therapy and antigen avoidance in a cohort of combined fibrotic and non-fibrotic patients with HP [5]. In contrast to our study, exposure cessation occurred in 72% of patients with suspected antigens, noting though no long-term survival difference. While our study included patients with only f-HP, De Sadeleer and colleagues included patients with and without fibrosis and involved those with potentially less disease severity, as highlighted by a mean FVC% of 70-80% compared to 60% in our study. This may have led to a comparatively lower mortality rate of 30% compared to 50% in our study. Our findings suggest that patients with lower FVC may still benefit from antigen avoidance, particularly if comprehensive or standardized approaches to antigen identification are pursued.

While we found antigen identification and avoidance may reduce all-cause mortality or LT risk, this effect was not seen in the first 3 years after diagnosis when stratified by antigen identification alone. Explanations for this may include already advanced fibrotic disease perhaps no longer responsive to medical treatment or antigen avoidance in some patients, or the extended effects of antigen exposure with delayed resolution due to chronicity or dosing of antigen exposure, requiring additional time before recovery or stability [16, 17]. Antigen misidentification with insufficient or incorrect avoidance may also be relevant. We did find that when survival was stratified by antigen avoidance (any effort) vs non-avoidance (no effort), survival differences were seen early on and those not pursuing antigen avoidance had similar long-term outcomes to those with unidentified antigens.

Association of antigen subtype with outcome has been previously reported. A prior study by Okamoto et al. from Japan, where summer-type HP is more common than other geographic areas, found similar outcomes for all antigen subtypes [18]. In contrast, De Sadeleer et al. reported that patients with avian-associated HP had better survival compared to mold-related HP or those with unidentified antigen source [5]. We found similarly

exposure to avian antigen was associated with better outcome compared to other subtypes. This might be explained by avian antigen exposure being more easily recognized and avoided in the environment (OR of 8.11; P <0.001).

Our study has several limitations. First, there is no widely accepted questionnaire or review of systems to externally validate the ascertainment of causative antigens, particularly in f-HP. Even if an acceptable assessment tool were developed [19], practice variation among individual clinicians or institutions remain confounding as a result of incompletely solicited patient recall or reporting. Additionally, serum IgG panels are not specific for causative antigen identification. Positive serum IgG testing may represent active but occult exposure or prior sensitization at any point in the past [2]. Positive antigen-specific inhalation challenge testing or isolation of potential antigens from environment may assure that those antigens are more likely to be potential causes [2], however, these investigations are not generally available in real-world practice with varied standardization. Such challenges in causative antigen identification are not unexpected or unique to our study or those previously published on the topic of HP as an important limitation. However, we demonstrated similar or even a higher rate of identifying suspected causative antigens and antigen subtypes compared to prior studies, which likely reflects a similar degree of clinical engagement and solicitation. A second limitation is confirmation of antigen avoidance which may be confounded by antigen misidentification, disease duration, severity, and response to medical treatment. Despite exhaustive avoidance, new or residual causative antigens may continue to contaminate abated environments and contribute to disease progression [10, 16, 17]. However, a review of simple avoidance approaches as documented here in our study demonstrates its potential independent impact on outcomes in f-HP and warrants further investigation, including documenting positive change in short-term pulmonary function or clinical symptoms. Our study was also not able to assess respiratory-related cause of death in many which might be more informative in terms of the specific impact of antigen avoidance. Finally, our study suffers from referral bias as patients seen at our institution may have greater severity or complexity than local or community practices. Patients seeking

tertiary center referral may also be those with greater interest and/or capacity (financial resource, for example) to pursue and achieve antigen avoidance.

Conclusion

Identification of suspected causative antigens and related antigen avoidance appears to be associated with decreased risk of all-cause mortality and LT in patients with f-HP. Those with identifiable causative antigens who did not pursue antigen avoidance had similar outcomes to those with unknown antigen source. Ongoing efforts to standardize and systematically identify potential causative antigens and pursue antigen avoidance remain relevant towards impacting disease course, even in those with fibrotic presentations as our study suggests.

Author contribution

TP, CT, JHR, MB, and TM contributed to the conception, design, data abstraction, analysis, and writing of the manuscript. TP and TM are guarantors of this work.

Conflicts of interest: The authors have no conflicts of interest to disclose

References

1.   Vasakova M, Morell F, Walsh S, Leslie K, Raghu G. Hypersensitivity Pneumonitis: Perspectives in Diagnosis and Management. *Am J Respir Crit Care Med* 2017: 196(6): 680-689.
2.   Raghu G, Remy-Jardin M, Ryerson CJ, Myers JL, Kreuter M, Vasakova M, Bargagli E, Chung JH, Collins BF, Bendstrup E, Chami HA, Chua AT, Corte TJ, Dalphin JC, Danoff SK, Diaz-Mendoza J, Duggal A, Egashira R, Ewing T, Gulati M, Inoue Y, Jenkins AR, Johannson KA, Johkoh T, Tamae-Kakazu M, Kitaichi M, Knight SL, Koschel D, Lederer DJ, Mageto Y, Maier LA, Matiz C, Morell F, Nicholson AG, Patolia S, Pereira CA, Renzoni EA, Salisbury ML, Selman M, Walsh SLF, Wuyts WA, Wilson KC. Diagnosis of Hypersensitivity Pneumonitis in Adults. An Official ATS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med* 2020: 202(3): e36-e69.
3.   Fernandez Perez ER, Kong AM, Raimundo K, Koelsch TL, Kulkarni R, Cole AL. Epidemiology of Hypersensitivity Pneumonitis among an Insured Population in the United States: A Claims-based Cohort Analysis. *Ann Am Thorac Soc* 2018: 15(4): 460-469.
4.   Adegunsoye A, Oldham JM, Fernandez Perez ER, Hamblin M, Patel N, Tener M, Bhanot D, Robinson L, Bullick S, Chen L, Hsu S, Churpek M, Hedeker D, Montner S, Chung JH, Husain AN, Noth I, Strek ME, Vij R. Outcomes of immunosuppressive therapy in chronic hypersensitivity pneumonitis. *ERJ Open Res* 2017: 3(3).

5.   De Sadeleer LJ, Hermans F, De Dycker E, Yserbyt J, Verschakelen JA, Verbeken EK, Verleden GM, Wuyts WA. Effects of Corticosteroid Treatment and Antigen Avoidance in a Large Hypersensitivity Pneumonitis Cohort: A Single-Centre Cohort Study. *J Clin Med* 2018: 8(1).

6.   Fernandez Perez ER, Swigris JJ, Forssen AV, Tourin O, Solomon JJ, Huie TJ, Olson AL, Brown KK. Identifying an inciting antigen is associated with improved survival in patients with chronic hypersensitivity pneumonitis. *Chest* 2013: 144(5): 1644-1651.

7.   Morisset J, Johannson KA, Vittinghoff E, Aravena C, Elicker BM, Jones KD, Fell CD, Manganas H, Dube BP, Wolters PJ, Collard HR, Ryerson CJ, Ley B. Use of Mycophenolate Mofetil or Azathioprine for the Management of Chronic Hypersensitivity Pneumonitis. *Chest* 2017: 151(3): 619-625.

8.   Vourlekis JS, Schwarz MI, Cherniack RM, Curran-Everett D, Cool CD, Tuder RM, King TE, Jr., Brown KK. The effect of pulmonary fibrosis on survival in patients with hypersensitivity pneumonitis. *Am J Med* 2004: 116(10): 662-668.

9.   Adams TN, Newton CA, Glazer CS. Role of Antigen Type in Survival in Chronic Hypersensitivity Pneumonitis. *Lung* 2019: 197(1): 113-114.

10.   Petnak T, Moua T. Exposure assessment in hypersensitivity pneumonitis: a comprehensive review and proposed screening questionnaire. *ERJ Open Res* 2020: 6(3).

11.   Woda BA. Hypersensitivity pneumonitis: an immunopathology review. *Arch Pathol Lab Med* 2008: 132(2): 204-205.

12.   Hanak V, Golbin JM, Hartman TE, Ryu JH. High-resolution CT findings of parenchymal fibrosis correlate with prognosis in hypersensitivity pneumonitis. *Chest* 2008: 134(1): 133-138.

13.   Ojanguren I, Morell F, Ramon MA, Villar A, Romero C, Cruz MJ, Munoz X. Long-term outcomes in chronic hypersensitivity pneumonitis. *Allergy* 2019: 74(5): 944-952.

14.   Fernandez Perez ER, Travis WD, Lynch DA, Brown KK, Johannson KA, Selman M, Ryu JH, Wells AU, Tony Huang YC, Pereira CAC, Scholand MB, Villar A, Inase N, Evans RB, Mette SA, Frazer-Green L. Diagnosis and Evaluation of Hypersensitivity Pneumonitis: CHEST Guideline and Expert Panel Report. *Chest* 2021: 160(2): e97-e156.

15.   Gimenez A, Storrer K, Kuranishi L, Soares MR, Ferreira RG, Pereira CAC. Change in FVC and survival in chronic fibrotic hypersensitivity pneumonitis. *Thorax* 2018: 73(4): 391-392.

16.   Sema M, Miyazaki Y, Tsutsui T, Tomita M, Eishi Y, Inase N. Environmental levels of avian antigen are relevant to the progression of chronic hypersensitivity pneumonitis during antigen avoidance. *Immun Inflamm Dis* 2018: 6(1): 154-162.

17.   Tsutsui T, Miyazaki Y, Kuramochi J, Uchida K, Eishi Y, Inase N. The amount of avian antigen in household dust predicts the prognosis of chronic bird-related hypersensitivity pneumonitis. *Ann Am Thorac Soc* 2015: 12(7): 1013-1021.

18.   Okamoto T, Miyazaki Y, Ogura T, Chida K, Kohno N, Kohno S, Taniguchi H, Akagawa S, Mochizuki Y, Yamauchi K, Takahashi H, Johkoh T, Homma S, Kishi K, Ikushima S, Konno S, Mishima M, Ohta K, Nishioka Y, Yoshimura N, Munakata M, Watanabe K, Miyashita Y, Inase N. Nationwide epidemiological survey of chronic hypersensitivity pneumonitis in Japan. *Respir Investig* 2013: 51(3): 191-199.

19.   Barnes H, Morisset J, Molyneaux P, Westall G, Glaspole I, Collard HR, Collaborators CHPEA. A Systematically Derived Exposure Assessment Instrument for Chronic Hypersensitivity Pneumonitis. *Chest* 2020: 157(6): 1506-1512.

Table 1: Clinical characteristics of fibrotic hypersensitivity pneumonitis stratified by identifiable and unidentifiable causative antigens (N= 377)

Variables	Identifiable causative antigens (N=225)	Unidentifiable causative antigen (N=152)	*P* value
Baseline demographic data			
Age, years ± SD	64.6 ± 10.9	64.9 ± 11.3	0.76
Male, N (%)	117 (52.0)	63 (41.4)	0.04
Ever smoker, N (%)	101 (45.5)	65 (43.9)	0.77
HRCT pattern, N (%)			0.68
Typical for HP	142 (63.1)	97 (63.8)	
Compatible with HP	44 (19.6)	25 (16.5)	
Indeterminate for HP	39 (17.3)	30 (19.7)	
HRCT findings, N (%)			
Mosaic attenuation	184 (81.8)	122 (80.3)	0.71
Honeycombing cysts	46 (20.4)	25 (16.4)	0.33
UIP or probable UIP pattern	20 (8.9)	7 (4.6)	0.11
Pathological results, N (%)			0.01
Typical HP	86 (38.2)	80 (52.6)	
Probable HP	23 (10.2)	19 (12.5)	
Indeterminate for HP	39 (17.3)	21 (13.8)	
Serum IgG testing	195 (86.7)	132 (88.2)	0.67
Diagnostic confidence, N (%)			<0.001
Definite	100 (44.4)	55 (36.2)	
High confidence	4 (1.8)	36 (23.7)	
Moderate confidence	89 (39.6)	4 (2.6)	
Low confidence	32 (14.2)	57 (37.5)	
Baseline pulmonary function tests			
FVC, %predicted ± SD	66.8 ± 17.3	63.4 ± 15.9	0.08
DLCO, %predicted ± SD	52.5 ± 16.6	47.4 ± 14.2	0.005
Treatment, N (%)			
No medication	36 (16.0)	7 (4.6)	<0.001
Corticosteroids	185 (82.2)	143 (94.1)	0.001
Second immunosuppressive agents	71 (31.6)	75 (49.3)	<0.001
Steroid burst	98 (44.5)	76 (50.7)	0.25
Death or lung transplantation, N (%)	97 (43.1)	69 (45.4)	0.66
Death	83 (36.9)	57 (37.5)	
Lung transplantation	14 (6.2)	12 (7.9)	

Table2: Causative antigens categorized by positive serum IgG, environmental exposure, or its combinations.

	Number (patients)
1. Positive serum specific IgG* (from 329 patients)	161
1.1 Avian antigen, any positive#	110
1.2 Mold antigen, any positive¶	80
- *Aspergillus* spp.	41
- *Microspora faeni*	39
- *Penicillium chrysogenum*	23
- *Trichoderma viride*	16
- *Phoma* spp.	15
- *Aureobasidium pullulans*	10
- *Penicillium notatum*	7
- *Alternaria alternata*	6
- *Cladosporium herbarum*	6
- *Candida albican*	1
- *Helminthosporium halodes*	1
1.3 Bacteria antigen, any positive¶	27
- *Thermoactinomyces vulgaris*	26
- *Thermoactinomyces candidus*	1
2. Identifiable environmental exposure by history	225
2.1 Exposure to avian antigen	129
- Keeping birds	70
- Use of feather or down containing products	47
- Exposure to bird droppings	29
2.2 Exposure to mold or bacterial contamination in the farm environment	53
2.3 Exposure to mold or bacterial contamination in the home or workplace	55
2.4 Expose to hot tub or sauna	10
2.5 Other specific exposures	11
3. Identifiable environmental exposure by history with related serum specific IgG confirmation	86
3.1 Exposure to avian antigen	57
3.2 Exposure to mold or bacterial contamination in the farm environment	10
3.3 Exposure to mold or bacterial contamination in the home or workplace	16
3.4 Other specific exposures	5
4. Multiple potential antigen exposures**	50

* Serum specific IgG testing by either fluorimetric enzyme-linked immunoassay (¶) or immunodiffusion (#)
** Multiple environmental antigen subtypes (farm and avian for example)

Table 3: Univariable and multivariable Cox regression analysis of identifiable causative antigens and antigen avoidance as predictors of all-cause mortality or lung transplantation

	N	Crude HR	95% CI	*P* value	Adjusted HR*	95% CI	*P* value
1. Antigen identification							
1.1. Overall follow-up	377						
- Unidentifiable causative antigen	152	ref	ref	ref	ref	ref	ref
- Identifiable causative antigens	225	0.72	0.52-0.99	0.04	0.69	0.48-0.99	0.04
1.2. Follow-up up to 3 years	377						
- Unidentifiable causative antigen	152	ref	ref	ref	ref	ref	ref
- Identifiable causative antigens	225	0.91	0.58-1.43	0.68	0.97	0.58-1.64	0.92
1.3. Follow-up after 3 years	377						
- Unidentifiable causative antigen	152	ref	ref	ref	ref	ref	ref
- Identifiable causative antigens	225	0.56	0.36-0.88	0.01	0.51	0.31-0.83	0.006
2. Antigen avoidance	356**						
- Antigen non-avoidance or unidentifiable causative antigens	232	ref	ref	ref	ref	ref	ref
- Antigen avoidance	124	0.49	0.34-0.70	<0.001	0.47	0.31-0.71	<0.001
3. Causative antigen identification and antigen avoidance	356**						
- Antigen avoidance	124	ref	ref	ref	ref	ref	ref
- Identifiable causative antigens with antigen non-avoidance	80	2.07	1.32-3.23	0.001	2.22	1.34-3.69	0.002
- Unidentifiable causative antigen	152	2.06	1.39-3.05	<0.001	2.09	1.34-3.26	0.001

*   Adjusted for age, sex, smoking status, baseline FVC, baseline DLCO, and presence of honeycombing cysts in CT findings

**   21 patients with identifiable causative antigens were excluded according to missing data of antigen avoidance

Table 4: Association of causative antigen subtype with all-cause mortality or lung transplantation

	N	Crude HR	95% CI	*P* value	Adjusted HR**	95% CI	*P* value
Positive serum specific IgG	161*						
1.Serum IgG against bird proteins							
- Negative serum IgG against bird proteins or untested patients	267	ref	ref	ref	ref	ref	ref
- Positive serum IgG against bird proteins	110	0.62	0.42-0.90	0.01	0.63	0.43-0.93	0.02
2.Serum IgG against mold							
- Negative serum IgG against mold or untested patients	297	ref	ref	ref	ref	ref	ref
- Positive serum IgG against mold	80	0.75	0.49-1.15	0.18	0.82	0.53-1.27	0.37
3.Serum IgG against bacteria							
- Negative serum IgG against bacteria or untested patients	350	ref	ref	ref	ref	ref	ref
- Positive serum IgG against bacteria	27	0.70	0.36-1.37	0.30	0.91	0.44-1.88	0.80
Environmental exposures by history	225*						
1.Birds, feathers, or bird droppings							
- Expose to other than birds or unidentifiable environmental exposure	248	ref	ref	ref	ref	ref	ref
- Exposure to birds or feathers	129	0.61	0.43-0.87	0.006	0.61	0.43-0.88	0.008
2.Farm environment							
- Expose to other than farm environment or unidentifiable environmental exposure	324	ref	ref	ref	ref	ref	ref
- Exposure to contaminated farm environment	53	1.31	0.87-1.99	0.20	1.11	0.71-1.73	0.65
3.Contaminated houses or workplaces							
- Expose to other than contaminated houses or workplaces or unidentifiable environmental exposure	322	ref	ref	ref	ref	ref	ref
- Exposure to contaminated houses or workplaces	55	1.03	0.66-1.62	0.89	0.97	0.61-1.54	0.90
Environmental exposure confirmed by positive serum IgG test							
- Environmental exposure without serum IgG confirmation or unidentifiable environmental exposure	291	ref	ref	ref	ref	ref	ref
- Identifiable environmental exposures confirmed by positive serum IgG	86	0.62	0.41-0.93	0.02	0.50	0.33-0.78	0.002
Multiple causative antigen exposures							
- Single causative antigen exposure or unidentifiable causative antigen	327	ref	ref	ref	ref	ref	ref
- Multiple potential antigen exposures	50	1.04	0.65-1.67	0.87	1.20	0.74-1.93	0.46

* Some patients had more than 1 exposures
** Adjusted for age, sex, smoking status, presence of honeycombing cysts in CT findings

Table 5: Documented efforts at antigen avoidance (N= 124; higher total due to overlapping or combined approaches)

| Antigen avoidance approaches	Number
1. Removal of pet birds or feather containing products	85
2. Removal of mold damage with abatement or renovation homes	11
3. Removal of suspected objects or items from the environment *(humidifier/air conditioner 4, others 6)*	10
4. Moving out of a home or property with suspected mold or water damage	5
5. Quitting or resigning from a non-farming occupation with suspected antigen exposure	4
6. Abstaining from a hobby or other activity (non-occupational) with suspected antigen exposure	5
7.Abstaining from particular farm-specific exposures (silo, hay storage, barn, silage, etc.)	5
8. Moving or selling a farm environment	9
9. Abstaining from hot tub or sauna use	8

Figure Legends
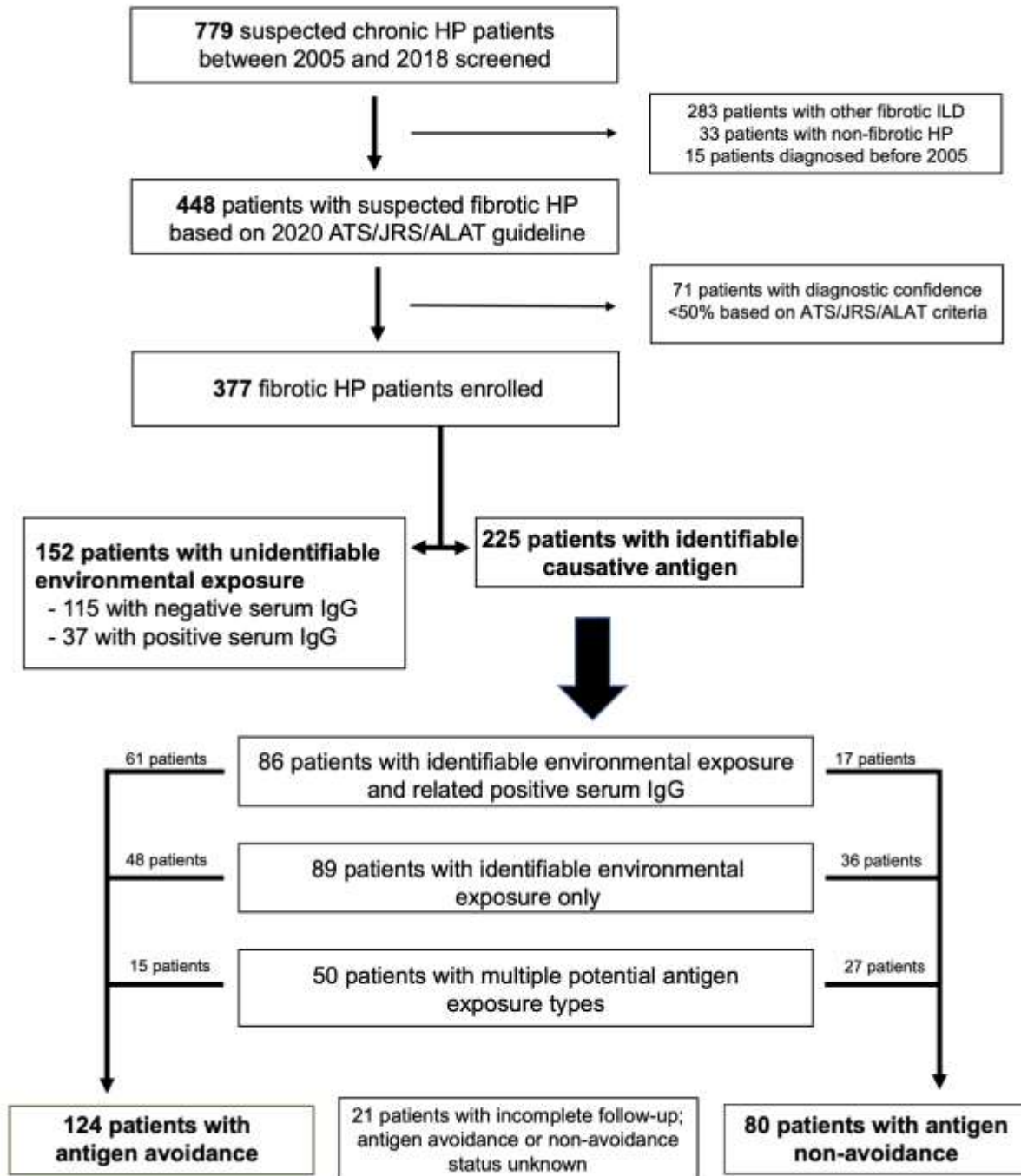
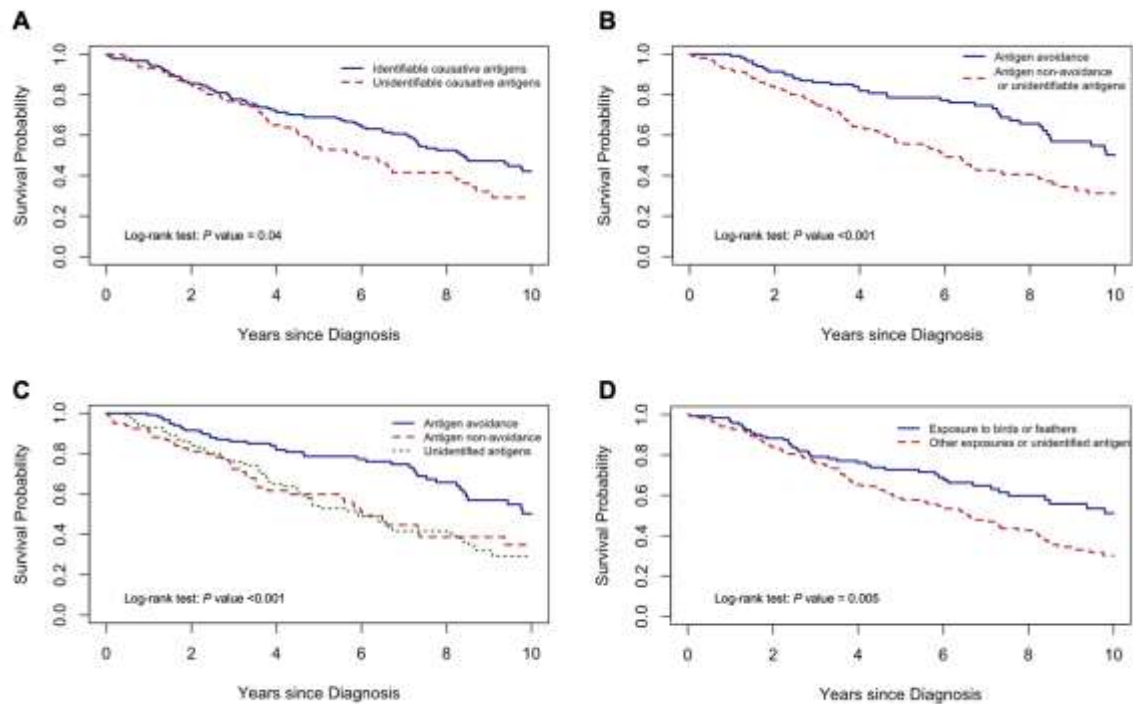**Figure 1:** Flow chart for patient study enrolment

Figure 2: Unadjusted Kaplan-Meier survival curves (Log-rank test) for patients with fibrotic hypersensitivity pneumonitis. A) Comparison of all-cause mortality or lung transplantation stratified by identifiable vs unidentifiable causative antigens. B) Comparison of all-cause mortality or lung transplantation stratified by antigen avoidance vs. non-avoidance or unidentifiable causative antigen C) Comparison of all-cause mortality or lung transplantation comparing antigen avoidance vs. non-avoidance vs. unidentifiable causative antigen. D) Comparison of all-cause mortality or lung transplantation between patients with exposure to avian vs other antigen subtypes

Table E1: Clinical characteristics of excluded patients compared with eligible patients

Variables	Excluded patients (N=71)	Eligible patients (N=377)	P value
Baseline demographic data			
Age, years ± SD	66.4 ± 10.9	64.7 ± 11.0	0.15
Male, N (%)	46 (64.8)	180 (47.7)	0.008
Ever smoker, N (%)	39 (54.9)	262 (44.9)	0.12
Identified causative antigens, N (%)	55 (77.5)	262 (69.5)	0.18
HRCT pattern, N (%)			<0.001
Typical for HP	0 (0.0)	239 (63.6)	
Compatible with HP	12 (16.9)	68 (18.0)	
Indeterminate for HP	59 (83.1)	69 (18.4)	
HRCT findings, N (%)			
Mosaic attenuation	23 (18.3)	306 (81.4)	<0.001
Honeycombing cysts	34 (47.9)	71 (18.9)	<0.001
UIP or probable UIP pattern	21 (29.6)	27 (7.2)	<0.001
Pathological results, N (%)			<0.001
Typical HP	0 (0.0)	166 (44.0)	
Probable HP	0 (0.0)	42 (11.1)	
Indeterminate for HP	25 (29.6)	60 (15.9)	
Baseline pulmonary function tests			
FVC, %predicted ± SD	64.0 ± 18.1	65.4 ± 16.8	0.37
DLCO, %predicted ± SD	47.6 ± 12.4	50.8 ± 16.0	0.19
Death or lung transplantation, N (%)	38 (53.6)	166 (44.0)	0.31
Death	31 (43.7)	140 (37.1)	
Lung transplantation	7 (9.9)	26 (6.9)	

Table E2: **Association of environmental causative antigen subtypes and likelihood of reported antigen avoidance**

Environmental exposure, N (%)	Antigen avoidance (N=124)	Antigen non-avoidance (N=80)	Odds ratio	95% CI	*P* value
1. Patients with single solicited environmental exposure					
- Exposure to birds or feathers	73 (85.9)	12 (14.1)	8.11	3.99-16.50	<0.001
- Exposure to contamination in the farm environment	8 (22.9)	27 (77.1)	0.14	0.06-0.32	<0.001
- Exposure to contamination in the home or workplace	14 (53.8)	12 (46.2)	0.72	0.32-1.65	0.44
- Other specific exposures	14 (87.5)	2 (12.5)	4.96	1.10-22.46	0.02
2. Multiple potential antigen exposures	15 (35.7)	27 (64.3)	0.27	0.13-0.55	<0.001

Table E3: Univariable and multivariable Cox regression analysis of identifiable causative antigens and antigen avoidance as predictors of all-cause mortality or lung transplantation. Thirty-seven patients with positive serum IgG and no identifiable exposure history <u>were reclassified to 'identifiable causative antigen' group</u> (sensitivity analysis model 1).

	N	Crude HR	95% CI	*P* value	Adjusted HR*	95% CI	*P* value
1. Causative antigen identification	377						
1.1. Overall follow-up	377						
- Unidentifiable causative antigen	115	ref	ref	ref	ref	ref	ref
- Identifiable causative antigens	262	0.64	0.46-0.89	0.008	0.67	0.46-0.97	0.03
1.2. Follow-up up to 3 years	377						
- Unidentifiable causative antigen	115	ref	ref	ref	ref	ref	ref
- Identifiable causative antigens	262	0.95	0.59-1.54	0.83	1.03	0.59-1.81	0.91
1.3. Follow-up after 3 years	377						
- Unidentifiable causative antigen	115	ref	ref	ref	ref	ref	ref
- Identifiable causative antigens	262	0.44	0.28-0.69	<0.001	0.45	0.27-0.74	0.002
1.4. Positive serum IgG only							
- Identifiable environmental exposure (with or without positive serum IgG)	225	ref	ref	ref	ref	ref	ref
- Positive serum IgG only	37	0.87	0.45-1.68	0.69	1.08	0.53-2.20	0.84
2. Antigen avoidance	356**						
- Antigen non-avoidance or unidentifiable causative antigen	232	ref	ref	ref	ref	ref	ref
- Antigen avoidance	124	0.49	0.34-0.70	<0.001	0.47	0.31-0.71	<0.001
3.Causative antigen identification and antigen avoidance	356**						
- Antigen avoidance	124	ref	ref	ref	ref	ref	ref
- Identifiable causative antigens with antigen non-avoidance	117	1.85	1.21-2.82	0.004	2.07	1.28-3.34	0.003
- Unidentifiable causative antigen	115	2.28	1.52-3.43	<0.001	2.19	1.39-3.46	<0.001

*   Adjusted for age, sex, smoking status, baseline FVC, baseline DLCO, and presence of honeycombing cysts in CT findings

**   21 patients with identifiable causative antigens were excluded according to missing data of antigen avoidance. 37 patients with positive serum IgG only were defined as antigen non-avoidance.

Figure E1: Kaplan-Meier survival curves demonstrated unadjusted comparison of all-cause mortality or lung transplantation stratified by identifiable (N=262) vs unidentifiable causative antigens (N=115). Thirty-seven patients with positive serum IgG and no identifiable exposure history <u>were reclassified to 'identifiable causative antigen' group</u> (sensitivity analysis model 1).
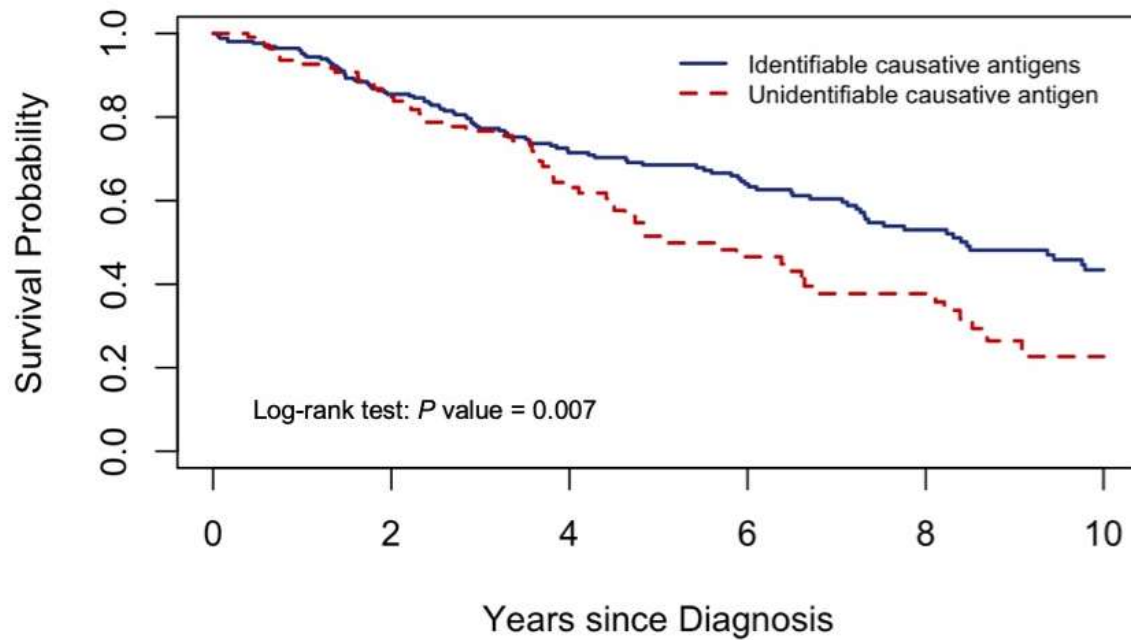
Figure E2: Kaplan-Meier survival curves demonstrated unadjusted comparison of all-cause mortality or lung transplantation comparing antigen avoidance (N=124) vs. non-avoidance (N=117) vs. unidentifiable causative antigen (N=115). Thirty-seven patients with positive serum IgG and no identifiable exposure history were reclassified to 'identifiable causative antigen' group (sensitivity analysis model 1).
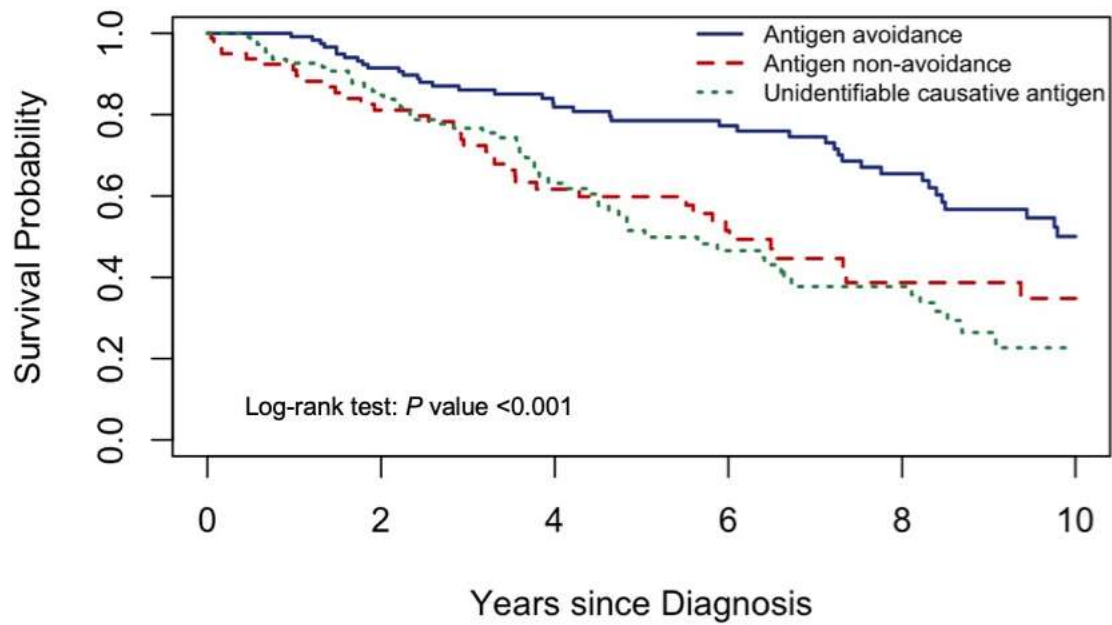
Table E4: Univariable and multivariable Cox regression analysis of identifiable causative antigens and antigen avoidance as predictors of all-cause mortality or lung transplantation. Thirty-seven patients with positive serum IgG and no identifiable exposure history <u>were excluded</u> from the analysis (sensitivity analysis model 2).

	N	Crude HR	95% CI	*P* value	Adjusted HR*	95% CI	*P* value
1. Antigen identification							
1.1. Overall follow-up	340						
- Unidentifiable causative antigen	115	ref	ref	ref	ref	ref	ref
- Identifiable causative antigens	225	0.64	0.46-0.90	0.01	0.66	0.45-0.97	0.03
1.2. Follow-up up to 3 years	340						
- Unidentifiable causative antigen	115	ref	ref	ref	ref	ref	ref
- Identifiable causative antigens	225	0.93	0.57-1.53	0.77	1.06	0.59-1.90	0.85
1.3. Follow-up after 3 years	340						
- Unidentifiable causative antigen	115	ref	ref	ref	ref	ref	ref
- Identifiable causative antigens	225	0.46	0.29-0.73	<0.001	0.45	0.27-0.75	0.002
2. Antigen avoidance	319**						
- Antigen non-avoidance or unidentifiable causative antigens	195	ref	ref	ref	ref	ref	ref
- Antigen avoidance	124	0.46	0.31-0.66	<0.001	0.46	0.30-0.71	<0.001
3. Causative antigen identification and antigen avoidance	319**						
- Antigen avoidance	124	ref	ref	ref	ref	ref	ref
- Identifiable causative antigen with antigen non-avoidance	80	2.07	1.33-3.24	0.001	2.19	1.32-3.63	0.002
- Unidentifiable causative antigen	115	2.29	1.52-3.44	<0.001	2.18	1.38-3.44	<0.001

*   Adjusted for age, sex, smoking status, baseline FVC, baseline DLCO, and presence of honeycombing cysts in CT findings

**   21 patients with identifiable causative antigens were excluded according to missing data of antigen avoidance. 37 patients with positive serum IgG only were defined as antigen non-avoidance.

Figure E3: Kaplan-Meier survival curves demonstrated unadjusted comparison of all-cause mortality or lung transplantation stratified by identifiable vs unidentifiable causative antigens. Thirty-seven patients with positive serum IgG and no identifiable exposure history were excluded from the analysis (sensitivity analysis model 2).
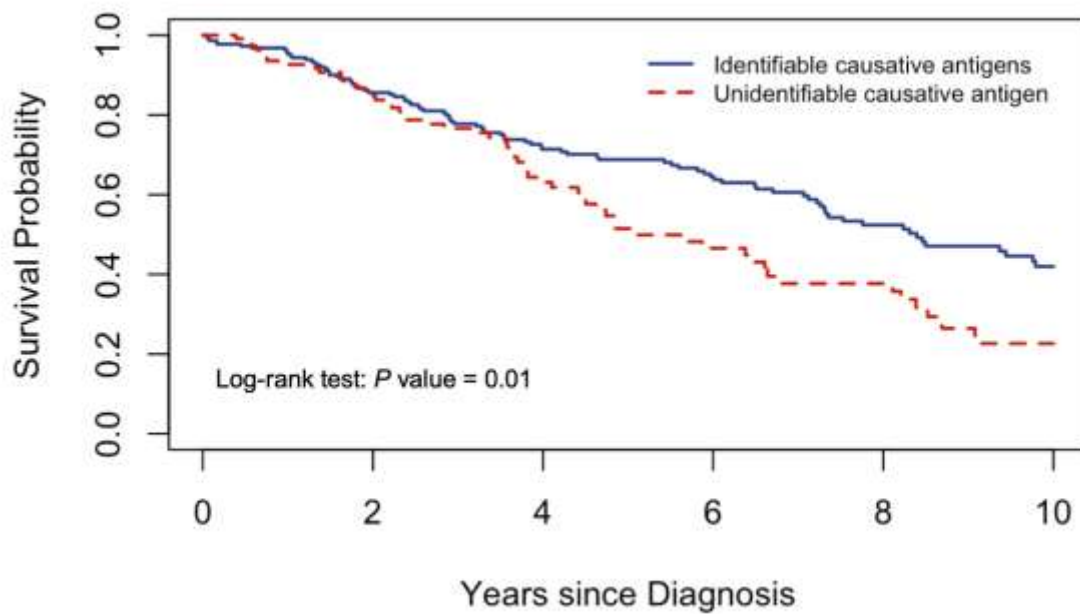
Figure E4: Kaplan-Meier survival curves demonstrated unadjusted comparison of all-cause mortality or lung transplantation comparing antigen avoidance vs. non-avoidance vs. unidentifiable causative antigen. Thirty-seven patients with positive serum IgG and no identifiable exposure history were excluded from the analysis (sensitivity analysis model 2).