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INTERSTITIAL LUNG DISEASE

Occupation *versus* environmental factors in hypersensitivity pneumonitis: population attributable fraction

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ABSTRACT

Background: Despite well-documented case series of hypersensitivity pneumonitis (HP), epidemiological data delineating relative contributions of risk factors are sparse. To address this, we estimated HP risk in a case-referent study of occupational and nonoccupational exposures.

Methods: We recruited cases of HP by ICD-9 codes from an integrated healthcare delivery system (IHCDS) and a tertiary medical care centre. We drew referents, matched for age and sex, from the IHCDS. Participants underwent comprehensive, structured telephone interviews eliciting details of occupational and home environmental exposures. We employed a hierarchical analytic approach for data reduction based on the false discovery rate method within clusters of exposures. We measured lung function and selected biomarkers in a subset of participants. We used multivariate logistic regression to estimate exposure-associated odds ratios (ORs) and population attributable fractions (PAFs) for HP.

Results: We analysed data for 192 HP cases (148 IHCDS; 44 tertiary care) and 229 referents. Occupational exposures combined more than doubled the odds of developing HP (OR 2.67; 95% CI 1.73–4.14) with a PAF of 34% (95% CI 21–46%); nonoccupational bird exposure also doubled the HP odds (OR 2.02; 95% CI 1.13–3.60), with a PAF of 12% (3–21%). Lung function and selected biomarkers did not substantively modify the risk estimates on the basis of questionnaire data alone.

Discussion: In a case-referent approach evaluating HP risk, identifiable exposures accounted, on an epidemiological basis, for approximately two in three cases of disease; conversely, for one in three, the risk factors for disease remained elusive.

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Occupational and environmental factors account for two in three cases of HP. The contributions of risk factors vary markedly depending on case referral source. This could affect clinical ascertainment of cause and the implementation of preventative actions. https://bit.ly/3feAa6P

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Introduction

Hypersensitivity pneumonitis (HP) is a lung disease triggered by an abnormal immune reaction to a variety of inhaled occupational and environmental exposures [1]. The general population incidence of HP is estimated at 1–2 per 100 000 person-years, but this probably is an underestimate due to poor recognition and inaccurate diagnosis [2, 3]. A subset of those with HP develop progressive fibrotic, potentially life-threatening disease, underscoring the need for effective prevention and exposure remediation [4]. Despite a wealth of clinical studies of HP from a myriad of causes, there are sparse population-level data delineating the relative contribution to risk by different exposures, with only one previous case–control investigation addressing this question [5].

Estimating the proportional contributions of occupational *versus* nonoccupational causes to HP is complex because clinical cohorts tend to reflect local or regional exposures. To address the need for a population-based estimate of the burden of HP across a range of potential risk factors, we carried out a study of HP cases and matched referents from the same geographic region (northern California, USA). We performed a systematic exposure assessment that included structured interviews and, in a subset of cases and controls, home visits. We sought to determine the types and sources of exposures (occupational and nonoccupational) associated with HP risk and to assess their contributions to the burden of disease by estimating the population attributable fraction (PAF) for disease.

Methods

Study population

We recruited study participants from two distinct sources in northern California. One was a large, integrated healthcare delivery system (IHCDS) (Kaiser Permanente Health Plan (KPHP)), based on medical diagnostic ICD-9 code 495.0–495.9 (HP, farmer's lung, bagassosis, bird fancier's lung, suberosis, malt worker's lung, mushroom worker's lung, maple bark stripper's lung, ventilation/humidifier lung, other specified allergic alveolitis). The other was the pulmonary subspecialty outpatient practice of a university-based tertiary referral centre (University of California San Francisco (UCSF)) based on a clinic-maintained database of cases diagnosed following multidisciplinary conference review including chest high-resolution computed tomography scans. We identified all referents from the IHCDS membership. This permitted identification of referents who were without the underlying mix of illnesses probably present in the University medical centre case mix and allowed matching to cases by sex and within 5 years of age. If we did not successfully recruit an eligible case or referent, we nonetheless retained their intended match in the study. The UCSF and KPHP committees on human research approved the research protocol.

Study measures

Study measures, their sources, definitions and associated methodologies, are detailed in table 1. Interview-based measures included demographics, smoking, comorbidities and health status and exposure data derived from a telephone-administered questionnaire designed for this study. Participants reporting multiple exposures were assigned a positive response for each. We were careful to differentiate between work-related *versus* nonoccupational exposures to mould and birds in order to appropriately allocate the PAF for these exposures. Home visit-derived variables included visual assessment, lung function (spirometry and exhaled NO) and selected biomarkers [6–11].

Statistical analysis

We tested differences in demographic and smoking characteristics among cases compared to referents using t-tests for continuous variables and the Chi-squared test or Fisher's exact test for categorical variables. We also used Chi-squared to test the association between reported mould exposure (at home or work) and mould precipitins, and between club cell secretory protein 16 (CC16) and soluble suppression of tumorigenicity 2 (sST2).

As the first step in a hierarchical analytic approach, we used Chi-squared to test frequency differences between cases and referents in interview-derived exposure characteristics. Because we considered multiple exposures in these analyses, the p-values were corrected within clusters of exposures (occupational and each of five groups of home environmental exposures) using the false discovery rate (FDR) method of Benjamini and Hochberg [12]. We retained factors for multivariate analysis that achieved an FDR-adjusted p-value <0.20. We used unconditional logistic regression analysis to examine the associations between the retained occupational and environmental risk factors and HP, controlling for age, sex, race and ever-smoking. For the risk factors of interest, we estimated the odds ratio (OR) and the PAF [13]. To assess the potential effect of patient referral source on the pattern of observed risk, given that the UCSF and IHCDS differed in demographics and comorbidities (table A1), we also used a stratified approach, re-estimating these models including only UCSF cases or only IHCDS cases along with the referents (all of

TABLE 1 Study measures: sources, methods and definitions

Source and specific measure	Variable specifics
Structured interview-derived	
Age	Years, continuous
Sex	Male/female, dichotomous
Race/ethnicity	White, non-Hispanic *versus* others, dichotomous
Smoking	Never *versus* ever-smoker, dichotomous
Annual family income	Elicited in US$20 000 increments through US$100 000 and above; dichotomised to ⩽US$40 000 or above
Comorbid conditions	Allergies or hay fever; hypertension; cardiac disease; diabetes mellitus
Short-form general health status	Physical component; mental component
Occupational exposures, longest held job	Epoxies; isocyanates; pesticides; hay/silage; wheat flour; wood dust or natural fibres; animal products (hair, fur, dander, waste); birds (including feathers, down); insect cultivation; sea shells; water humidification systems (including water features, swamp coolers); mouldy/water-damaged workplace; metal cooling fluids; metal dust or fumes; sand/stone/concrete dust
Home-based exposures, last 5 years	Water-damaged or mouldy environment; water humidification systems (including water features, swamp coolers, desert coolers); hot tub or sauna; feather bedding; domestic animals (including birds, mammals, fish tanks, insects)
Hobby exposures or avocations, last 5 years	Hunting; fly fishing; jewellery polishing; working with shells; woodworking; weaving, working with fibres; gardening, composting
Home visit-derived	
Selected visual assessment items	Mould, water damage, humidifiers, hot tubs, swamp coolers, birds, down or feather items
FEV_1% predicted[#]	Spirometry measured by EasyOne Spirometer (ndd Medical Technologies, Chelmsford, MA, USA) [6]. Predicted values based on NHANES III [7]. For collinearity, FEV_1 and FVC < 80% predicted for both were coded as reduced lung volume, defined as a dichotomous variable
FVC% predicted[#]	
Average exhaled NO	Electrochemical quantification (NO Vario; FILT, Berlin, Germany) at three flow rates (50, 100 and 300 $mL \cdot s^{-1}$) yielding the standard measured airway forced expiratory NO (F_{eNO}) and the calculated alveolar NO (AlvNO) [8]
Estimated alveolar NO	
IgG antibody against avian antigens	Serum enzyme immunoassay, Department of Immunology, University of Glasgow, Glasgow, UK [9] Positive response cut-off: >2 $\mu g \cdot mL^{-1}$
Avian precipitins (budgerigar, zebra finch, canary, parrot, nymph parakeet, chicken, pigeon)	Serum double-immunodiffusion-in-gel method of Ouchterlony, Sahlgrenska University Hospital, Gothenburg, Sweden [10, 11]
Mould precipitins (*Aspergillus fumigatus*, *umbrosus*, *niger, oryzae; Alternaria; Botrytis; Cladosporium; Penicillium; Pullularia; Rhizopus; Paecilomyces; Stachybotrys*)	Positive response: 1+ or more in a semi-quantitative scale to any tested avian; 3+ or 4+ in a semi-quantitative scale to any of the tested moulds
High-sensitivity C-reactive protein	$U \cdot mL^{-1}$ (R&D Systems, Abingdon, UK). Results dichotomised using the 90th percentile of values among study referents as the cut-off value for an elevated level, consistent with a one-tailed effect in a non-normal distribution
Krebs von den Lungen-6 factor	$U \cdot mL^{-1}$ (Cusabio Biotech, Stratech, Ely, UK). Results dichotomised as above
Club cell secretory protein	$ng \cdot mL^{-1}$ (Biovendor, Abingdon, UK). Dichotomised as above
Soluble suppression of tumorigenicity 2 receptor	$ng \cdot mL^{-1}$ (Quantikine ELISA, R&D Systems, Abingdon, UK). Dichotomised as above

FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; Ig: immunoglobulin. [#]: differences in age and sex not tested, given referent selection criteria.

whom were IHCDS recruited). Because only a subset of participants agreed to a home visit (53%), analyses combining home visit and interview data were limited to the home visit cohort. To minimise additional loss (table A2), we used multiple data imputation to address missing observations, employing the chained equations method under the assumption that the data were missing at random. All demographic, exposure and clinical variables considered in the analysis of the survey data were included in the imputed models (table A3). Standard errors were calculated using the within and between imputation SE of the estimates applying Rubin's rules [14].

We tested bivariate associations (case *versus* referent) for nine home visit variables, retaining those $p<0.20$ for multiple logistic regression. Final models also included the major risk variables from the previous

survey-based analysis: any occupational exposure, bird (nonoccupational) exposure and mould exposure (nonoccupational). As in the interview-derived variables, we estimated models using the full sample and then in stratified analyses to address the two different sources of case recruitment using UCSF or IHCDS cases only (with all referents in each analysis). We conducted all statistical analyses using either SAS software, version 9.4 or Stata 15.

Results

Figure 1 delineates subject recruitment for the 192 cases and 229 referents ultimately included in this study for interviews and the 118 cases and 106 referents for home visits. There were more who declined among the IHCDS recruited participants than among the UCSF. Per protocol, the primary treating physician granted permission to contact potential participants: this was another cause of exclusions (labelled as "other" among both IHCDS cases and referents).

Table 2 shows demographics, smoking and health status for 421 study participants. There were no statistically significant differences between cases and referents in age or sex, consistent with the matching strategy. Race/ethnicity, income and smoking status also did not differ statistically. Hypertension was more common and SF-12 health status was better among referents. A comparison of UCSF and IHCDS cases for these variables is shown in table A1. A greater proportion of the UCSF compared to the IHCDS HP cases were White, non-Hispanic ($p=0.02$) and, although the proportion of ever-smokers and cumulative pack-years did not differ, there were more current smokers among the IHCDS compared to the UCSF HP cases. Comorbid hypertension and allergies were also more common in the former compared to the latter.

Table 3 shows the frequencies for occupational and environmental exposures in cases compared to referents. Mould or mildew exposure were frequent at home (similar proportions among cases and referents). Mould at work was less frequent overall, but differed statistically among cases *versus* referents ($p<0.0001$). The HP cases were more likely to report any of the interview elicited occupational exposures (56% *versus* 27%, $p<0.0001$). For home exposures, birds (24% *versus* 10%, $p=0.0004$) and fish tanks/reptiles/amphibians (16% *versus* 6%, $p=0.0014$), but not mammalian pets ($p=0.14$) differed statistically among cases compared to referents. Other statistically significant differences included selected hobbies (woodworking or working with fibres; 28% *versus* 13%, $p=0.0002$) and a home desert cooler/humidifier (18% *versus* 10%, $p=0.046$). Water features in the home were paradoxically associated with decreased odds

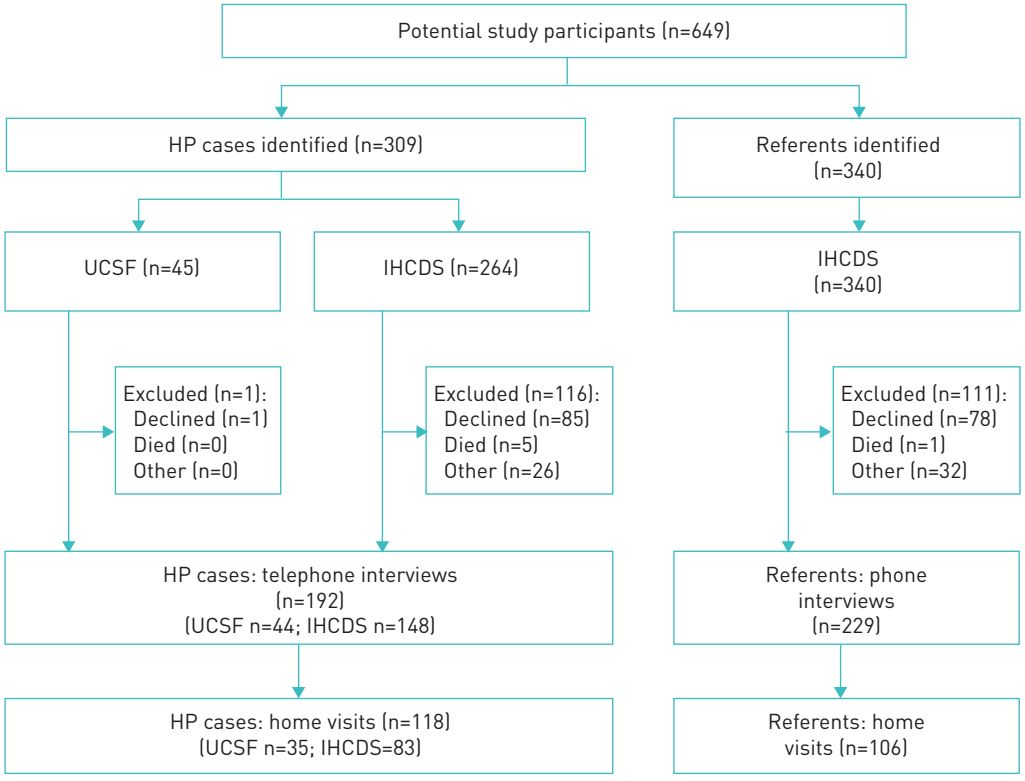

FIGURE 1 Subject recruitment from among potential study participants. HP: hypersensitivity pneumonitis; UCSF: University of California San Francisco; IHCDS: Integrated Health Care Delivery System.

TABLE 2 Demographics, smoking and health status for 421 study participants by disease status

Subject characteristics	HP (n=192)	Referents (n=229)	p-value
Demographics			
Age years[#]	61.7±13.3	63.2±11.8	
Female[#]	121 (63)	139 (61)	
White, non-Hispanic	145 (76)	184 (80)	0.23
Annual family income <US$40 000 (n=363)	57 (31)	45 (25)	0.17
Smoking			
Current smoker	15 (8)	13 (6)	0.38
Ever-smoker	104 (54)	110 (48)	0.21
Packs per day among ever-smokers	0.85±0.68	0.69±0.54	0.11
Pack-years among ever-smokers	21.2±23.9	17.1±19.0	0.18
Comorbidities and health status			
Allergies or hay fever	102 (55%)	116 (51%)	0.61
Hypertension	69 (36%)	125 (55%)	<0.001
Diabetes mellitus	35 (18%)	32 (14%)	0.24
Short-form-12 health status			
Physical component score-12	38.2±11.3	47.8±9.4	<0.001
Mental component score-12	50.9±10.9	53.9±8.7	<0.001

Data are presented as mean±SD or n (%), unless otherwise stated. Income missing for 58 subjects; Short-form-12 for 17 subjects. HP: hypersensitivity pneumonitis. [#]: differences in age and sex not tested, given referent selection criteria.

of HP (OR 0.33, 95% CI 0.09–0.62). All p-values are FDR-corrected within exposure groups (see Methods).

Table 4 presents multivariate modelling in the entire group and also stratified by case source (UCSF *versus* IHCDS). Among all, occupational factors (as listed in table 3) as a group were associated with a more than doubled odds of HP (OR 2.7; 95% CI 1.7–4.1), accounting for a PAF 34%. Pet birds and textile or wood hobbies were associated with OR 2.0, together accounting for a PAF 26%. Thus, these three factors (occupational, birds, textile and wood hobbies) accounted for 60% of the observed disease. Three other risk factor groups were not statistically significant (table 4).

In the stratified analyses, a differing pattern of risk emerged. Limited to IHCDS-derived cases and referents, occupation, bird and hobbies remained significant, and water humidification systems also emerged as a significant risk factor, collectively accounting for a PAF 82%. Among the UCSF case stratum *versus* IHCDS referents, the risks associated with occupation and bird ownership were attenuated and no longer statistically significant, whereas home mould exposure and home fish tank/reptiles/amphibians emerged statistically significant risk factors, together accounting for a combined PAF 55%.

Table 5 presents bivariate analyses of variables from the home visit (n=224; 118 cases, 106 referents). Seven variables reached the *a priori* cut-off of <0.20 for inclusion in further multivariate modelling: combined reduced percent predicted forced expiratory volume in 1 s (FEV_1%) and percent predicted forced vital capacity (FVC%); exhaled NO; alveolar NO; elevated mould precipitins; elevated avian IgG; elevated high-sensitivity C-reactive protein (CRP); elevated CC16; and elevated sST2. Of 193 subjects with both CC16 and sST2 assayed, there was a borderline statistical association between elevation of these biomarkers (p=0.049).

For mould reported at work (longest held job) or home (last 5 years), the proportion with positive precipitins was similar among cases (34%) and referents (33%) (table A4). The proportion of cases reporting home mould with evidence on home visit of mould or water damage (49%) was similar to referents (45%). Of participants reporting a home humidifier, hot tub or swamp cooler, the proportion with mould precipitins ranged from 26% to 60%. Of 95 cases reporting bird exposures, 15% had elevated avian IgG, compared to none of 65 referents reporting such exposure (p<0.001). Of the 14 cases with elevated avian IgG, 12 were reconfirmed positive by precipitin testing.

Table 6 shows the results of multivariable analysis, including the seven lung function or biomarker variables achieving the threshold for inclusion, along with the three major groups of interview-based risks (occupational, bird ownership, home mould). In the entire home visit cohort, only reduced FEV_1% and FVC %, exhaled NO and upper decile CC16 were associated with statistically significant increased odds of HP.

TABLE 3 Occupational and environmental exposures among 192 hypersensitivity pneumonitis (HP) cases and 229 referents

Exposure variables	HP exposure	Referent exposure	p-value
Occupational exposure on longest held job			
Hay	22 (11)	5 (2)	0.0005
Wheat flour	12 (6)	9 (4)	0.2761
Sawdust	34 (18)	12 (5)	0.0003
Plants	37 (19)	17 (7)	0.0007
Compost	24 (13)	7 (3)	0.0007
Animals or animal hairs	31 (16)	14 (6)	0.0015
Birds or feathers	20 (10)	5 (2)	0.0007
Insect cultivation	18 (9)	8 (3)	0.0163
Seashells	3 (2)	0 (0)	0.0681
Humidifier	18 (9)	11 (5)	0.0705
Indoor fountain	38 (20)	24 (10)	0.0105
Swamp cooler	29 (15)	11 (5)	0.0007
Mould	44 (23)	16 (7)	<0.0001
Any work exposure	107 (56)	62 (27)	<0.0001
Home mould or mildew			
Walls	54 (28)	43 (19)	0.0582
Bed	6 (3)	3 (1)	0.3329
Storage areas	15 (8)	6 (3)	0.0582
Air ducts	4 (2)	3 (1)	0.6707
Damp carpet	8 (4)	9 (4)	0.9023
Any of the above (any mould exposure)	61 (32)	57 (25)	0.1175
Pets/animals in last 5 years			
Birds	47 (24)	24 (10)	0.0004
Fish tank/reptiles/amphibians	31(16)	14 (6)	0.0014
Mammalian pets	141 (73)	153 (67)	0.1402
Frequently reported (⩾10%) hobbies/pastimes			
Fine wood working	23 (12)	18 (8)	0.1557
Weaving/working with fibres	35 (18)	15 (7)	0.0004
Either hobby	53 (28)	30 (13)	0.0002
Other home exposures			
Air conditioner	128 (67)	136 (59)	0.2481
Desert cooler/humidifier	35 (18)	23 (10)	0.0457
Hot tub or sauna	42 (22)	43 (19)	0.5164
Water feature	21 (11)	60 (26)	0.0005
Feather bedding	91 (47)	109 (48)	0.9670
Composting	47(24)	44 (19)	0.2867

Data are presented as n (%), unless otherwise stated. Bivariate p-value (Benjamini and Hochberg) by type of exposure group: work exposure; home mould or mildew; pets; hobbies; other home exposures. Combined multiple categories in italics not included in the Hochberg corrections.

This model takes into account occupational risk factors and household bird exposure (both of which retained statistical significance) and household moulds (which was not a statistically significant risk factor).

In the same multivariate model, limited to the IHCDS case stratum, the findings are very similar to the group as a whole. In contrast, limited to the UCSF case stratum only, FEV_1% and FVC% and CC16 among the biomarkers retained statistically significant ORs, although the point estimate of the OR for exhaled NO was similar. Further, sST2 manifested significantly increased odds of HP (OR 3.9; 95% CI 1.1–13.4) not evident in the entire group. Also, in this stratum, household mould exposure was a significant risk factor for HP (OR 3.69; 95% CI 1.25–10.9), whereas mould precipitins were associated with increased but not statistically significant odds of HP (OR 4.0; 0.9–17.8). Re-analysing risk but excluding the questionnaire-based mould item, the precipitin-associated risk estimate increased and was statistically significant (OR 8.0; 95% CI 1.4–46.0) (data not shown in table).

Discussion

This is the first epidemiological study using a case-referent approach to evaluate risk of HP across a range of occupational and nonoccupational exposures, estimating both the odds of disease and the PAF linked to exposure. Because PAF estimates the proportional reduction in disease in the population that theoretically

TABLE 4 Multivariate analyses of hypersensitivity pneumonitis (HP) risk for major categories of exposure

Risk factor	OR (95% CI)	PAF (95% CI)
Model 1. All subjects (192 HP cases and 229 referents)		
Any occupational exposure	2.67 (1.73–4.14)	34% (21–46%)
Desert cooler/humidifier	1.49 (0.80–2.78)	6% (0–14%)
Bird (nonoccupational)	2.02 (1.13–3.60)	12% (3–21%)
Fish tank/reptiles/amphibians	1.69 (0.80–3.59)	7% (0–15%)
Any mould (nonoccupational)	1.20 (0.75–1.93)	5% (0–18%)
Textile or wood hobbies	2.03 (1.16–3.53)	14% (4–23%)
Model 2. IHCDS cases (n=148) and IHCDS referents (n=229)		
Any occupational exposure	3.16 (1.95–5.12)	41% (26–53%)
Desert cooler/humidifier	1.90 (1.05–3.43)	13% (7–18%)
Bird (nonoccupational)	2.34 (1.26–4.31)	15% (4–24%)
Fish tank/reptiles/amphibians	1.16 (0.50–2.67)	2% (0–12%)
Any mould (nonoccupational)	0.96 (0.56–1.64)	0% (0–13%)
Textile or wood hobbies	1.95 (1.08–3.55)	14% (2–23%)
Model 3. UCSF cases (n=44) and IHCDS referents (n=229)		
Any occupational exposure	1.44 (0.69–3.03)	13% (0–37%)
Desert cooler/humidifier	0.72 (0.20–2.62)	0% (0–6%)
Bird (nonoccupational)	1.18 (0.44–3.19)	3% (0–19%)
Fish tank/reptiles/amphibians	3.26 (1.16–9.14)	17% (1–31%)
Any mould (nonoccupational)	2.44 (1.21–4.92)	28% (3–47%)
Textile or wood hobbies	1.74 (0.72–4.25)	12% (0–28%)

All risk factors included in each model, along with age, sex, ever-smoking (100 cigarettes), mammalian pets (nonsignificant in overall model) and water feature (protective factor in overall model; OR=0.33, 95% CI 0.09–0.62). Wald Chi-squared: model 1=59.58; model 2 =59.62; model 3=59.62 (all p<0.0001). PAF: population attributable fractions; IHCDS: Integrated Health Care Delivery System; UCSF: University of California San Francisco.

could be achieved were the exposure in question eliminated, this metric is particularly relevant in assessing the public health impact of risk factors and in developing preventative strategies. We found that the majority (55% to 80%) of HP risk in the population we studied was attributable to discrete occupational (including work-related mould or birds) and home environmental exposures. Conversely, however, 20% to 45% of the risk remained unexplained by our modelling.

Multiple HP series report the proportion of cases clinically attributable to specific exposures. The proportion of cases in which a specific exposure ultimately linked to disease ranges widely, from 40% to 100% [15–27]. It remains to be determined the extent to which antigen-indeterminate HP is due to a limitation in exposure assessment methods, an inability of the participant to recall the exposure, misclassification of HP or a true cryptogenic HP disease process. The distribution of identified exposures

TABLE 5 Lung function and biomarkers associated with hypersensitivity pneumonitis (118 cases and 106 referents)

Variable	Frequency		OR (95% CI)	p-value
	Cases	Referents		
FEV_1% and FVC% both <80% predicted	60.8	25.6	4.48 (3.39–5.09)	<0.0001
Exhaled NO ppb	17.9 (14.1)	15.6 (8.9)	1.03 (1.03–1.04)	<0.0001
Alveolar NO ppb	2.7 (4.3)	1.9 (1.8)	1.08 (1.06–1.09)	<0.0001
Elevated serum avian antibody (>2 μg·mL^{-1})	12.8	8.16	1.65 (1.36–2.01)	0.0016
Elevated serum mould precipitins (3+ to 4+)	15.6	6.1	2.83 (2.29–3.49)	0.044
KL-6>90th percentile (29.3 U·mL^{-1})	20.1	19.4	1.05 (0.90–1.21)	0.56
HSCRP >90th percentile (1.8 μg·mL^{-1})	24.3	21.4	1.18 (1.03–1.36)	0.019
CC16 >90th percentile (16.2 ng·mL^{-1})	36.3	15.0	3.23 (2.79–3.74)	<0.0001
sST2 >90th percentile (20.9 ng·mL^{-1})	29.2	15.8	2.20 (1.90–2.55)	<0.0001

Data are presented as % or median (interquartile range), unless otherwise stated. Bivariate analysis for each variable shown. 90th percentile cut-offs shown in parentheses. Missing data imputed (see Methods). FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; NO: nitric oxide; KL-6: Krebs von den Lungen-6 factor; HSCRP: high-sensitivity C-reactive protein; CC16: club cell secretory protein; sST2: soluble suppression of tumorigenicity 2.

TABLE 6 Risk of hypersensitivity pneumonitis combining home visit and interview data

Risk factor	OR (95% CI)	p-value
Model 1. All home visits participants: cases (n=118) and referents (n=106)		
FEV_1% and FVC% both <80% predicted	2.66 (1.25–5.62)	0.0107
Exhaled NO ppb	1.03 (1.00–1.07)	0.0249
Alveolar NO ppb	1.02 (0.95–1.10)	0.5368
Elevated serum avian antibody	0.90 (0.17–4.69)	0.8959
Elevated serum mould precipitins	2.03 (0.59–6.90)	0.2576
HSCRP >90th percentile	1.69 (0.44–3.13)	0.7539
CC16 >90th percentile	3.07 (1.21–7.83)	0.0184
sST2 >90th percentile	1.67 (0.70–3.95)	0.2465
Any occupational exposure	2.83 (1.44–5.53)	0.0024
Bird (nonoccupational)	2.78 (1.08–7.17)	0.0341
Mould (nonoccupational)	1.43 (0.70–2.93)	0.3214
Model 2. IHCDS cases (n=83) cases and referents (n=106)		
FEV_1% and FVC% both <80% predicted	2.43 (1.08–5.47)	0.0320
Exhaled NO ppb	1.03 (1.00–1.06)	0.0510
Alveolar NO ppb	1.02 (0.95–1.09)	0.6631
Elevated serum mould precipitins	1.54 (0.37–6.36)	0.5499
Elevated serum avian antibody	1.00 (0.18–5.38)	0.9966
HSCRP >90th percentile	1.14 (0.39–3.31)	0.8051
CC16 >90th percentile	2.91 (1.03–8.26)	0.0440
sST2 >90th percentile	1.14 (0.43–3.02)	0.7907
Any occupational exposure	3.53 (1.72–7.23)	0.0006
Bird (nonoccupational)	2.81 (1.09–7.27)	0.0326
Mould (nonoccupational)	0.96 (0.42–2.18)	0.9199
Model 3. UCSF cases (n=35) and IHCDS controls (n=106)		
FEV_1% and FVC% both <80% predicted	3.67 (1.15–11.74)	0.0284
Exhaled NO ppb	1.04 (0.99–1.09)	0.0846
Alveolar NO ppb	1.06 (0.99–1.17)	0.1921
Elevated serum avian antibody	0.65 (0.04–9.59)	0.7518
Elevated serum mould precipitins	3.97 (0.87–17.82)	0.0754
HSCRP >90th percentile	1.07 (0.25–4.58)	0.9923
CC16 >90th percentile	4.26 (1.27–16.11)	0.0327
sST2 >90th percentile	3.86 (1.11–13.43)	0.0343
Any occupational exposure	1.13 (0.37–3.42)	0.8336
Bird (nonoccupational)	1.78 (0.35–9.13)	0.4876
Mould (nonoccupational)	3.69 (1.25–10.93)	0.0184

FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; NO: nitric oxide; HSCRP: high-sensitivity C-reactive protein; CC16: club cell secretory protein; sST2: soluble suppression of tumorigenicity 2; IHCDS: Integrated Health Care Delivery System; UCSF: University of California San Francisco.

in these series also varies widely: some case series are limited to populations of bird fanciers, while farmer's lung or cases due to contaminated metal working fluids dominate other series. Another limitation of the existing literature is that reports often focus on an exposure (*e.g.* mould or birds), without distinguishing by source (occupational *versus* household). A recent series of 206 HP cases from the British Midlands is an exception [28]. It distinguishes, as we did also, mould- and avian-attributed diseases that are from occupational *versus* other sources (mould, 7 of 16 (44%); avian 4 of 37 (11%)). In that series, 49% of the cases overall were considered cryptogenic. A recent European Respiratory Society/ American Thoracic Society estimate of a 19% (95% CI 12–28%) occupational proportion of HP, largely based on case series [29], is lower than ours of 34% (95% CI 21–46%), although the confidence intervals of the two estimates do overlap.

A major aspect of our study is that, by design, it is heterogeneous in respect to HP cases. This includes referral patterns, case definition, recruitment, demographics and comorbidities. For example, IHCDS cases need not have had a specialty subspecialist referral and may reflect less diagnostic precision than the UCSF drawn from a tertiary care centre subspecialty practice; IHCDS cases were defined by ICD-9, while UCSF by expert review; the IHCDS recruitment required primary physician approval prior to outreach, leading to exclusions that did not occur among UCSF cases and there were more who declined (consistent with recruitment from outside the IHCDS); and differences were present in demographics and comorbidities

indicating sources of confounding. Although our findings should be tempered by consideration of this heterogeneity, by drawing cases from the community and an academic tertiary referral setting, our study provides insights into how patterns of risk in HP may vary "in the eye of the beholder." Specifically, our stratified analyses directly address these differences. Cases drawn from the community setting were more likely to be attributable to occupational and household bird-related exposures compared to cases in a referral centre (56% *versus* 16%, respectively). In contrast, the tertiary referral cases were more commonly attributable to nonoccupational mould exposure (28%) and household fish tanks, reptiles or amphibians (17%). This pattern of differences would be consistent with a referral bias, in which cases with less common or more difficult-to-characterise exposures come to tertiary care assessment. Previous literature has demonstrated the geographical heterogeneity of exposure prevalence patterns. Our study, however, further suggests there may be additional differences in apparent risk even within the same broad geographic area, depending on practice setting. This referral effect may widely influence how HP risk is appreciated, representing what we would characterise as a "Rashomon effect" wherein which the tale differs dramatically, depending on the observer.

Overall, occupational exposures and home bird ownership remained significant risk factors for HP. In stratified analyses, these factors retained statistical significance in the IHCDS case subset, whereas home mould exposure was the only significant risk factor for HP among the tertiary referral centre cases. We also demonstrated that mould precipitins were a representative biomarker for mould exposure, providing biological confirmation of the relevance of mould exposures reported on the survey. Because we defined risk based on case and referent interviews assessing exposures and not on medical record extraction of the clinically attributed cause of HP, we cannot correlate clinical assessments with our epidemiological risk estimation. Medical record review also might have confirmed the diagnostic accuracy of the IHCDS cases. Nonetheless, case misclassification does not appear to be major, given that the exposure findings are typical for HP. Biological measurements (*e.g.* spirometry and precipitins) also argue for the construct validity of the questionnaire that we developed for this study, even though this instrument has not yet been validated further through testing in another population. Random misclassification, whether present from misdiagnosis or exposure misassignment, probably would have biased our findings to the null. Prior clinical assessment for HP might have promoted recall bias insofar as cases, when interviewed, may have reported differentially occupational or household exposures compared to referents. If so, then differences in risk in stratified analyses might also reflect underlying differences between the UCSF and IHCDS cases in the clinical attribution of HP cause as understood by the study participants. Referents were entirely drawn from the IHCDS source rather than jointly from UCSF referrals. This obviates confounding from referents with conditions leading to care in a tertiary facility but could lead to other unmeasured confounding.

Referents were selected broadly matched to cases for age and sex, and we retained all identified cases and referents who ultimately participated. Thus, not all cases had a specific match nor all referents their original case. Therefore, we did not use a conditional logistic analysis that would have assumed tight matching allowing a more powerful, paired statistical approach. We undertook a 1:1 matching strategy that limits study power compared to a 2:1 or 3:1 matching. Due to absent medical record extraction, we were unable to evaluate differences in exposure patterns for the various clinical subtypes of HP (*e.g.* acute–subacute–chronic, fibrotic *versus* nonfibrotic). We also lacked data to examine duration or timing of exposure in relation to disease risk or biomarker prevalence. Also, we did not elicit data on home antigen remediation or job change or duty modification due to illness. Finally, this is a cross-sectional analysis that does not allow causal inference from the associations we report.

We analysed the differences in multiple inflammatory or fibrotic biomarkers and observed a statistically higher prevalence of elevated high-sensitivity CRP, CC16 and sST2 levels in cases compared to referents. In multivariate analysis combining interview-derived and home visit variables, only CC16 was consistently associated with increased odds of HP. This observation is consistent with one other study of serum CC16 in HP, idiopathic pulmonary fibrosis and interstitial lung disease with connective tissue disease compared to healthy subjects [30]. More broadly, because serum CC16 may be a marker of increased leakage across the alveolar barrier, this makes plausible an association with HP [31]. The biomarker sST2, although most frequently studied in cardiac injury, may play a role in various disease states, with particular relevance to inflammation and fibrosis [32]. Even though sST2 was not associated with HP in multivariate analysis of the entire group, it was statistically associated with HP in the tertiary referral case stratum. This is the same stratum in which reported household mould exposure remained the dominant risk factor and mould precipitins were associated with elevated risk when interview-reported exposure at home was not in the model. This raises the possibility that certain biomarkers may be more relevant to selected HP aetiologies. Although there was a borderline statistical association between elevation in sST2 and CC16, the latter was also included in the model and thus the association with sST2 in not likely to be as a surrogate marker for CC16.

Despite its association with HP in bivariate analyses, high-sensitivity CRP was not statistically associated with HP in any of the multivariate analyses. High-sensitivity CRP previously has been found to be elevated in HP in other bivariate analyses [33, 34]. Although we did not find as association with KL-6, this has been observed in in other studies of HP [33–35]. Estimated alveolar NO differed statistically in bivariate analysis, but not in multivariate modelling. In contrast, in multivariate analysis we continued to observe a statistical difference in the exhaled NO levels in HP cases compared to referents. Data assessing the potential role of exhaled NO in HP are inconsistent [36, 37]. Although cigarette smoking attenuates exhaled NO, we had too few active smokers to be a confounding factor, which is consistent with other HP series [18, 19].

Conclusion

In conclusion, we found that the population risk for HP is predominantly attributable to environmental exposures, broadly defined and that a large proportion of this risk is attributable to the occupational, not only the household environment. Further, our stratified analyses provide hypothesis-generating observations. Nonetheless, it is important to note that the sample size was small within these strata, even after accounting for missing data. Thus, any conclusions drawn should be considered provisional. In clinical practice, our findings support the need to evaluate thoroughly the exposures in suspected HP not only at home, but also in the workplace. At the public health level, interventions that reduce workplace exposures may have a major impact on HP incidence. Cryptogenic HP, in which the causative factor remains elusive even after epidemiological analysis, remains a clinical and public health challenge.

Author contributions: H. Barnes undertook analyses; A-C. Olin led the exhaled NO and selected biomarkers component; K. Torén led the precipitins component; C. McSharry led the component for selected biomarkers; I. Donelly and M. Lärstad collaborated on selected biomarkers; C. Iribarren led the KPHP collaboration; P. Quinlan led the exhaled NO and selected biomarkers; P.D. Blanc conceived the idea, and directed data collection and analysis; all authors contributed to the analysis, and to drafts and edits of the manuscript.

Data availability: De-identified data may be available on application to the authors.

Conflict of interest: None declared.

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