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Discriminant analysis of symptom pattern and serum antibody titres in humidifier related disease

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Abstract

***Background*—The heterogeneous patterns of symptoms among factory workers exposed to aerosols from contaminated air humidifiers were analysed to assess the association between specific symptoms and the serum IgG antibody response to the humidifier water contaminants, and to test the ability of specific symptoms to predict this antibody response.**

***Methods*—Symptoms from 88 factory workers were surveyed by a doctor administered questionnaire and compared with their serum IgG antibody titres to humidifier water contaminants quantified by enzyme immunoassay.**

***Results*—The strength of association between individual symptoms and antibody showed that fever, shivering or chills, influenza-like symptoms, or headache were individually significantly associated with the presence and higher titres of antibody. This was also true for those subjects whose symptoms were most pronounced during the first day of the working week. Within each subject's full symptom profile there were significant associations between the description of chest tightness, breathlessness, and wheeze; between headache and influenza like symptoms; between fever and shivering or chills; and between intermittent onset and general tiredness. Discriminant analysis of the full symptom profiles showed that there was maximum information content in five independent parameters, namely, the descriptions of fever, headache, and chest tightness, the timing of their onset, and the readiness to describe miscellaneous symptoms in addition to those in the questionnaire. On the basis of these criteria 72% of subjects could be classified according to their antibody state. Cluster analysis with these five independent parameters described four symptom clusters: one associated with high median antibody levels, one with low, and two with zero median levels. These were, respectively: (1) fever with headache and chest tightness; (2) either no or few symptoms; (3) chest tightness and headache with intermittent onset; (4) headache and miscellaneous symptoms with intermittent onset.**

***Conclusions*—The association between serum antibody titres and specific symptom patterns may identify different categories of disease which constitute the spectrum known as humidifier related disease, and strengthens the hypothesis that antibody may be involved in the pathogenesis of some components of the disease.**

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Humidifier related disease can have a wide variety of clinical presentations.[1 2] Humidifier fever has been described as an influenza-like illness with pyrexia and malaise as the main symptoms, but cough, chest tightness, dyspnoea, and weight loss may also be seen.[3] Humidifier related asthma,[4–6] extrinsic allergic alveolitis (humidifier lung in North American literature),[3 4 7 8] some cases with significant pulmonary interstitial fibrosis,[9] and pneumonia like diseases[8] have also been described. In addition, symptoms can develop in air conditioned premises which are unrelated to exposure to contaminated humidifers. These include work related headache, lethargy, mental fatigue, irritation of eye, nose and throat, nausea, dizziness, wheezing, chest tightness, itching, drowsiness, and dry skin, and are described by the terms "sick building syndrome"[10 11] or "building related illnesses".[9] Some of these disorders are likely to appear to a greater or lesser extent in any study of symptoms caused by exposure to aerosolised humidifier contaminants in enclosed environments. This broad range of clinical presentations obscures the question of whether immune hypersensitivity has any role in the pathogenesis of the disease. The correlation between disease and levels of serum antibody to antigens from humidifier water is good in some studies,[12 13] but is absent in others.[1 6 14] In order to identify whether serum antibody levels may contribute to some components of the disease spectrum, and to determine whether the symptom pattern could predict levels of serum antibody, we have investigated whether antibody is associated with any particular symptoms or symptom patterns described by factory workers exposed to contaminated air humidification systems.

Methods

SUBJECTS

Most workers in one area of a microprocessor factory (factory 1, n = 66) and all of the sub-

Table 1 Number of individuals reporting each symptom listed in decreasing frequency

Symptoms	*n*	χ^2	*p*
Any symptom	74	3·1	NS
Work specific	58	3·29	NS
Influenza-like symptoms	52	5·76	0·05
First day onset	49	7·3	0·01
General tiredness	48	0·02	NS
Headache	46	5·4	0·05
Shivering or chills	40	10·74	0·01
Breathlessness	31	1·2	NS
Cough	30	0·78	NS
Fever	29	15·0	0·001
Chest tightness	28	2·0	NS
Miscellaneous	23	6·61	0·05
Wheeze	18	0·76	NS
Poor appetite	15	0·32	NS
Weight loss	5	0·38	NS

NS—not significant.

jects on the factory floor of a printing works (factory 2, n = 22) were investigated where workers had been affected by intermittent symptoms of ill defined periodicity which appeared to be related to work. The air in each factory was humidified intermittently by spray humidifiers. The temporal association between symptoms and humidifier use has been confirmed and published.[15] Each factory was visited several times and the workers were asked if specific symptoms were present (table 1) and a questionnaire was completed. This detailed the nature, frequency, and severity of several respiratory and systemic symptoms, together with age, smoking history, and work history. The clinical details and patient selection criteria have been outlined previously[15]; briefly, the study included 74 subjects who approached their factory medical officer with any symptoms they considered related to work, and 14 asymptomatic volunteers. All donated a blood sample.

SEROLOGY

Serum antibody titres to antigens extracted from the humidifier water were visualised by precipitin formation and quantified by enzyme immunoassay.[14] The antigens from both factory humidifiers were immunologically identical and cross reacted with antigens from other outbreaks in Britain and elsewhere.[14 16] The antibody titre was calculated as a percentage binding index with reference to control negative and strongly precipitin positive sera to give arbitrarily designated values of 0 and 100 respectively. The full method including quality control and estimates of test error have been published.[14] Levels of serum cotinine, a marker of cigarette smoking, were measured by gas liquid chromatography[17]; non-smokers generally have levels below 15 ng/ml.

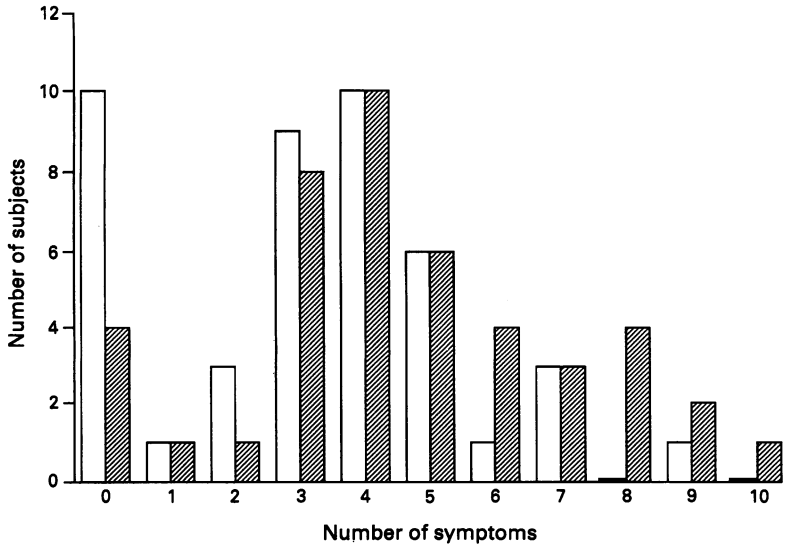

Figure 1 The distribution of the number of symptoms described by precipitin positive (hatched bars) and precipitin negative subjects (clear bars).

STATISTICS

The data were analysed throughout with Minitab and SPSS. The data set was tested for homogeneity of distribution between various demographic parameters by one way analysis of variance and χ^2 analysis. The significance of association between each symptom and antibody was established by χ^2 analysis and the comparison of antibody titres was performed by the Mann-Whitney U test.

In order to clarify which, if any, of the symptoms (categorical variables) were of use in predicting the levels of antibody, a discriminant analysis was performed on the basis of the presence or absence of antibody as classes, with the categorical variables as discriminating variables. All 88 (unweighted) cases were included. In order to verify the discriminant analysis, a hierarchical cluster analysis was carried out. The cosine measure was used as a similarity measurement, reflecting the categorical nature of the symptoms.

Results

Of the 88 subjects interviewed, 74 admitted to having had at least one symptom and 44 were precipitin positive. Twenty five of the 58 symptomatic subjects from factory 1, and 15 of the 16 symptomatic subjects from factory 2, were precipitin positive. The profile of numbers of symptoms in the antibody positive and negative subjects is shown in fig 1. Antibody and symptoms were not significantly associated ($\chi^2 = 3·1$, p = 0·08), but the mean number of symptoms described by the antibody positive subjects was significantly greater (4·59 *v* 3·18, T = 2·8, p = 0·007).

The effects of cigarette smoking and antigen exposure on the antibody response were assessed. Table 2 lists the smoking history, and current smoking status was confirmed by significantly raised cotinine levels. There were no significant differences in the antibody titres between any of the smoking categories (Mann-Whitney U test). Furthermore, there was no significant correlation or association between antibody titre and cotinine levels ($r = -0·09$, $\chi^2 = 2·14$).

There was a significant correlation between antigen exposure (years of employment) and antibody titre ($r = 0·432$) which was also true for the 14 asymptomatic subjects ($r = 0·63$). The ages of the subjects correlated signifi-

Table 2 Median and interquartile range levels of serum cotinine and IgG antibody to humidifier antigens in relation to cigarette smoking habit.

Smoking history	*Cotinine (ng/ml)*	*Antibody (% binding)*
Current (n=23)	243·1 (145·6–359·4)	0 (0–68·2)
Never (n=57)	0·65 (0·4–1·08)	15 (0–68·8)
Ex-smokers (n=8)	0·3 (0·15–1·9)	8 (0–56·5)

cantly with antibody titre ($r = 0·39$) but, since age and exposure were also significantly correlated ($r = 0·574$), this effect was likely to be related to exposure. Antibody positive subjects at any given age were more likely to have had a greater period of exposure (approximately 50% more) than the antibody negative subjects.[14] There was no significant correlation between antigen exposure and cotinine level ($r = 0·13$), nor between age and cotinine level ($r = 0·18$).

The number of times each individual symptom was described is listed in decreasing order in table 1. The association between the occurrence of each symptom and the presence of antibody was significant for fever, shivering or chills, influenza-like symptoms, and headache. In addition, a quantitative estimate of antibody titre measured by enzyme immunoassay in the subjects who did or did not describe each symptom is illustrated in fig 2. Those subjects describing fever ($p < 0·001$), shivering or chills ($p < 0·001$), influenza-like symptoms ($p < 0·01$), and headache ($p < 0·01$) had significantly higher antibody titres than those who did not have these symptoms. In contrast, there was a significantly lower antibody titre in subjects who described extra symptoms in addition to those in the questionnaire ($p < 0·005$), and those whose symptoms occurred other than during the first day at work ($p < 0·01$).

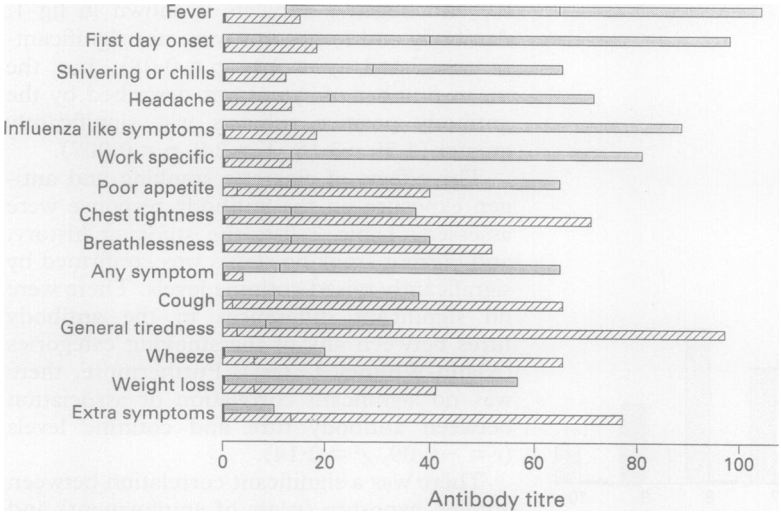

Figure 2 The titre of antibody for each group of subjects with (heavily shaded bars) and without (lightly hatched bars) each symptom. The titres are illustrated as bars in which the median is shown as a vertical line (occasionally zero) and the extremities as the interquartile range.

DISCRIMINANT ANALYSIS

In order to test the association between the presence of antibody and the full symptom profile of each individual, discriminant analysis was performed. The unstandardised canonical discriminant function coefficients for the five main independent criteria which had maximum information content were fever (1·35), headache (0·8), chest tightness (0·58), onset of symptoms (−0·62), and miscellaneous symptoms (−0·7). The other parameters did not have any additional discriminating value. The discriminating power remaining after the group had been separated according to "fever" was poor for "chills" because "fever" and "chills" were themselves significantly correlated. A search for parameters which discriminate best between antibody positive and negative subjects generated a positive association of antibody with fever, headache, and chest tightness, and a negative association with the readiness to describe other symptoms and their variable onset. With the use of these criteria we could correctly classify 71·6% of grouped cases according to antibody state (table 3, $\chi^2 = 17·1$, $p = 4 \times 10^{-5}$).

There was a significant association ($\chi^2 > 11·26$, 1 df; $\chi^2 > 14·28$, 2 df) between chest tightness, breathlessness, and wheeze; between headache and influenza-like symptoms; between fever and chills; and between intermittent onset and general tiredness.

CLUSTER ANALYSIS

The five independent criteria identified by stepwise discriminant analysis above were used for a cluster analysis. The solution with four clusters appeared to give clear groupings (table 4).

Cluster 1 consisted of a group of 21 subjects with no or few symptoms and a low median antibody titre.

Cluster 2 was a group of 25 subjects all describing fever, with headache in 17 and chest tightness in 10; nine had all three. This pattern was associated with high serum antibody titres, particularly in those with three symptoms (median = 79%).

Cluster 3 was a group of 18 subjects with the prominent symptom of chest tightness in all, 17 of these with an intermittent onset and 10 also with headache. This pattern was associated with a zero median antibody titre.

Cluster 4 consisted of a group of 24 subjects in which the symptoms were of an intermittent onset in 23. These consisted primarily of 16 with miscellaneous symptoms, 13 of whom had headache. This pattern of

Table 3 Comparison of actual antibody status of patients with that predicted by discriminant analysis.

	Predicted group membership	
Actual group membership	*Antibody negative*	*Antibody positive*
Antibody negative (n=41)	32	9
Antibody positive (n=47)	16	31

Table 4 Cluster analysis of subjects according to symptom pattern and antibody status

	Cluster			
	1	*2*	*3*	*4*
n	21	25	18	24
Antibody titre:				
Median	5	67	0	0
Interquartile range	0–74·5	14–104	0–20·5	0–0
Number of subjects describing:				
Chest tightness	0	10	18	0
Headache	6	17	10	13
Fever	4	25	1	3
Miscellaneous	0	0	8	16
Intermittent onset	0	8	17	23

symptoms was associated with the absence of serum antibody.

Discussion

The role of serum antibody in the pathogenesis of humidifier related disease is unresolved and, in this study, on first observation, there was no clear association between the presence of antibody and being symptomatic. This is in agreement with other literature reports of humidifier related disease in a variable proportion of the workforces in various workplaces.[1 6 8 13 14 18–20] When the pattern of symptoms was studied, we found that those subjects with antibody described significantly more symptoms, and that there was a significant association of both the presence and higher titres of antibody with a particular symptom profile. This consisted of fever, shivering or chills, influenza-like symptoms, and headache, occurring on the first day back at work. This is entirely in keeping with the accepted symptom profile described for humidifier fever,[3] but in this case the profile was derived from a group with heterogeneous symptoms and was based on their serological response.

It should be noted that this symptom profile is fairly non-specific in that it is also associated with exposure to aerosols from stagnant water which has become contaminated,[21 22] as well as pneumonia and influenza. It is therefore even more important that an objective parameter—for example, serum antibody—is identified in order to probe the aetiology of the disease.

In contrast, symptoms described intermittently or all week long, or the tendency to describe extra symptoms in addition to any of those listed, were associated with significantly lower prevalences of antibody as well as lower titres. This non-specific pattern is perhaps more in keeping with "sick building syndrome" which is not usually attributed to contaminated humidifiers.[11] In this study, however, there was clinical improvement in all subjects irrespective of symptoms and antibody state when the humidifiers were cleaned and modified, and resolution was complete only when humidifiers were replaced. This was powerful evidence that, despite the spectrum of symptoms with and without antibody, these were diseases which were humidifier related.

Discriminant analysis and cluster analysis were used to describe the data. Discriminant analysis with stepwise elimination will exclude those predictor variables (that is, symptoms) which correlate with variables already selected, hence give no added information and also exclude those which give no information—that is, variables whose values are uniformly distributed among the predicted categories. The discriminant function thus contains those predictor variables which are most useful in determining the predicted variable (that is, antibody). To test the validity of this approach, a discriminant function for half the data set could have been used to obtain the predicted variable for the other half. Because of the relatively small size of the data set, however, cluster analysis was used instead as a confirmation of the discriminant analysis in the present study. These methods represent a useful description of the data, and the choice of variables may form the basis for future studies. This approach may also lend itself to the analysis of symptoms and serology where the association is not clear cut, such as in occupational lung disease or extrinsic allergic alveolitis resulting from avian exposure and among farmers.

Other factors which might have had a bearing on the antibody response were considered. Cigarette smoking, which has been shown to have an inhibitory effect on serum IgG antibody production to inhaled antigens in some reports of humidifier related disease[23] and in other systems[24], did not have this effect on the present study group. This agrees with previous findings.[15 25 26] The antibody titres are negligible in non-exposed controls but are increasingly positive with increasing exposure, as shown in previous work.[23] This is therefore of value in confirming exposure and may also be of importance in identifying subjects with subclinical disease.[27] Any reduction in the antibody titre is likely to be of importance in monitoring antigen avoidance. The simplest way to achieve this is by the basic engineering principle of replacing the humidifiers.

The lack of association between the presence of antibody and humidifier related disease in general in this and other reports is perhaps a reflection of the heterogeneity of the symptom profile of the reported disease states, rather than indicating that antibody has no pathogenic role. Recent reports have strongly suggested that exposure to contaminated humidifiers tends to result in a disease spectrum rather than discrete syndromes[2 8 14 28] and that some syndromes may coexist after the same exposure.[25] It is well recognised that the more acute symptoms generally begin towards the end of the working day, usually the first after an absence for weekends or holidays, and a tolerance to exposure develops over the rest of the working week. Even in one affected subject, therefore, the description of symptoms will vary. Some cases of humidifier related disease may

simply be caused by endotoxin exposure and many of the symptoms can be reproduced by exposure to endotoxin,[29] but some outbreaks investigated with sensitive endotoxin assays suggested that this was not the cause.[16 27]

In conclusion we contend that antibody titres can be used to identify subgroups of symptomatic subjects, and that the antibody may play a part in the immunopathogenesis of the disease in some subjects.

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