

Original article

Symptoms after mould exposure including *Stachybotrys chartarum*, and comparison with darkroom disease

Background: Mould-attributed symptoms have included features which overlap with unexplained syndromes such as sick building syndrome.

Objectives: We describe questionnaire and chart review findings in patients following exposure to moulds which include *Stachybotrys* and compare responses with two control groups.

Methods: Thirty-two patients presented with symptoms attributed to mould exposures. Exposure identification for 25 patients had reported *S. stachybotrys chartarum* as well as other mould (*Aspergillus*, *Penicillium*), 88% at work. The remaining seven had professionally visualized or self-reported/photographic exposure evidence only. A chart review was performed and a follow-up with a questionnaire, including questions on current health status, and nonspecific symptoms.

Results: Cough, shortness of breath and chest tightness (at presentation) were reported in 79%, 70% and 64%, respectively, and persisted >6 weeks in 91%. Skin test(s) were positive to fungal extract(s) in 30%. Seventeen returned questionnaires were obtained 3.1 (SD 0.5) years after the initial clinic assessment. Among this subgroup, persisting asthma-like symptoms and symptoms suggestive of sick building syndrome were frequent, and similar to a group previously assessed for darkroom disease among medical radiation technologists. The mould-exposed group more commonly reported they were bothered when walking in a room with carpets, complained of a chemical or metallic taste in their mouth, and had problems in concentration when compared with a control physiotherapist group ($P < 0.005$).

Conclusions: Although only a minority with health concerns from indoor mould exposure had demonstrable mould-allergy, a significant proportion had asthma-like symptoms. Other symptoms were also common and persistent after the initial implicated exposure.

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Key words: darkroom disease; mould; multiple chemical sensitivity; sick building syndrome; *Stachybotrys chartarum*.

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Accepted for publication 19 July 2009

Public concern about potential health effects of indoor mould exposure has increased partly by fears of toxigenic effects from moulds such as *Stachybotrys chartarum*, the 'toxic black mould' (1, 2). There are reports of associations between *Stachybotrys* and other mould exposure and human illness in water-damaged buildings (3–6). Multiple subjective complaints may also include headache, fatigue, chest tightness and mucous membrane irritation, among other nonspecific symptoms (3–6).

The nonspecific symptom complex shares features which overlap with other unexplained syndromes such

as multiple chemical sensitivity (idiopathic environmental intolerance) (7, 8), sick building syndrome (SBS) and darkroom disease (DRD) (9, 10). Symptoms are usually transient although little has been published about the long-term health impact of patients after potential airborne exposure to *Stachybotrys* and other mould in water-damaged buildings (11, 12). Fatigue and nausea have been attributed by some authors to a psychological response to the odor of volatile substances from fungi (13). Situational depression, acute stress, adjustment disorder and post-traumatic stress have also been reported in association with exposures (14).

This study aimed to identify symptoms among patients exposed to *Stachybotrys* and other mould, and compare findings with subjects who have 'darkroom disease' (features of sick building syndrome), to determine overlap and differences.

Abbreviations: DRD, darkroom disease; HDQ, Health and Demographics Questionnaire; MRT/DRD, medical radiation technologists with 'darkroom disease'; *S. chartarum*, *Stachybotrys chartarum*; TMS, Toxic mould syndrome.

Methods

Study design

A chart review and follow-up questionnaire was performed among clinic patients reporting exposure to indoor mould including *S. chartarum*. Ethics approval was received from the Research Ethics Board of the University Health Network. Patients referred to the asthma, allergy or occupational lung disease clinics of one of the authors (S.M.T.) at the Toronto Western Hospital, University Health Network, from 1996 to 2004 for concerns related to potential exposure to *S. chartarum* were identified during clinic visits. They were included in the study if there was documented evidence of *S. chartarum* (among other mould exposure) based on a professional environmental assessment in 25 cases (or solely on the presence of visible mould as assessed professionally in three cases or by self report/photographic documentation in four cases).

Professional environmental assessments were performed by commercial companies independent of the study, at a mean $\pm$ S.D. 14.5 ± 16 months before the initial patient visit. Four of the 32 patients did not have professional exposure assessments but reported and provided photographic evidence of extensive black mould. The professional assessments were performed for 22 of the remaining 28 patients by one company (Company #1) and by other companies for the other six patients. Detailed methods were provided only in the reports by Company #1: visual inspection was made inside and outside the building, identifying and recording water damage and contamination, including inspection of the ventilation systems. Eight air samples were collected using Zefon Air-O-Cell cassettes (Zefon International, Ocala, FL, USA) and a Quicktake pump (SKC incorporated, Eighty Four, PA, USA) at a flow rate of 15 l/min for 10 min. Six of the samples were collected indoors, one was collected from outdoor air and one was used as a field blank. The slides were stained with lactophenol cotton blue and analysed microscopically at $630\times$ magnification. Fungal types were identified to genus/group level and the results were reported as spores or fungal hyphal elements per cubic metre of air (cts/m³). Spores occurring in chains were counted individually. A scale of 0–3+ was used to rate abundance of fungal fragments and non-fungal material, with 3+ indicating the largest amount. Results were reported as spores and as fungal hyphal elements per cubic metre of air (cts/m³). Of the 28 patients with a professional report available, four patients had only the results from microscopy of bulk or swipe samples from indoor surfaces without air sample results and three provided professional visual reports only.

A chart review performed to extract data from clinic visits included mould-attributed symptoms, previous history of asthma and allergic symptoms and the environmental assessment information. In those with a history consistent with upper or lower respiratory or skin allergy, allergy skin tests were performed.

Skin testing was performed using the prick technique. Histamine dihydrochloride 10 mg/ml was used as a positive control, and diluent 0.9% saline solution as a negative control. The skin test panel consisted of 13 common aeroallergens (*Alternaria* 1 : 10 w/v, *Aspergillus* 1 : 10 w/v, *Cladosporium* 1 : 10 w/v, house dust extract (Omega Laboratories, Montreal QC, Canada), cockroach 1 : 20 w/v (Alk Abello, Port Washington, NY, USA), cat pelt 10 000 BAU/ml, dog 1 : 20 w/v, horse 1 : 20 w/v, feathers 1 : 20 w/v, *D. farinae* 10 000 AU/ml, *D. pteronyssinus* 10 000 AU/ml, tree pollen 1 : 20 w/v, grass pollen 1 : 20 w/v (Western Allergy Services, Victoria, BC, Canada), ragweed 125 AgE units/ml (Alk Abello, Port Washington, NY, USA). An additional 13 fungal allergen extracts comprised *Sporobolomyces* 10% w/v, *Pullularia* 10%, *Penicillium* 10%, *Neurospora* (M4) 10%, *Fusarium* 10%, *Rhizopus nigricans* 10%, *Helminthosporium* 1 : 20, *Curvularia* 1 : 20 w/u, *Trichophyton* 1 : 20

w/u, *Mucor* 1 : 20 w/u, *Chaetomium* 1 : 10 w/u, *Rhodotorula* 1 : 20 w/u, (Western Allergy, Victoria, BC, Canada) *S. chartarum* 1 : 20 w/v (Allergy Canada, Thornhill, ON, Canada). Allergen solutions along with a positive and negative control were tested on the forearm. Responses were read at 15 min. A positive skin test was defined as an average wheal diameter at least 3 mm larger than the negative control. Atopy was defined as at least one positive skin prick test to the common allergen panel including *Alternaria*, *Aspergillus* and *Cladosporium* but excluding the other fungal skin test results from the atopy definition.

Pulmonary function tests, methacholine challenges and chest radiographs were performed when lower respiratory symptoms were reported.

Follow-up questionnaires

An information letter was sent to explain the purpose of the follow-up questionnaire. One week later, these patients were contacted by telephone to identify their interest in receiving further study information and to obtain their verbal consent. Those willing to participate were given a verbal explanation of the study and were sent a questionnaire survey for self-completion and mail-back.

The questionnaire package included a Health and Demographics Questionnaire (HDQ) structured to obtain a demographic and health profile and to provide standardized information source for descriptive features (15). An additional section asked for details of recent health symptoms (within the past 2 months) and addressed 18 different symptoms in addition to the lower respiratory symptoms which were previously reported (9). Questions included: 'On how many days in the past 12 months (choices: none, 1–7, 8–14, 15 or more), have you had the following symptoms (listed), and were they worse at work (no/yes)?' The listed symptoms were: (i) fever or night sweats, (ii) nausea, (iii) dizziness or lightheadedness, (iv) skin rash, (v) mouth soreness, (vi) mouth ulcers, (vii) palpitations, (viii) ringing in the ears, (ix) swelling or hives on skin, (x) sore, itchy or runny eyes, (xi) persistent itchy or runny nose or sneezing (not including colds/flu), (xii) sore throat, (xiii) pain on urinating, (xiv) headache, (xv) blurred vision, (xvi) numbness or tightening of face, hands, or feet, (xvii) abdominal pain, (xviii) chemical taste in mouth. The questionnaire also enquired about the following: abnormal tiredness, problems concentrating, short memory, difficulty getting the meaning from reading newspapers or books (and whether worse at work, no/yes).

Results were compared with two historical cohort groups from whom data were available from mailed responses with the same health questionnaire (9): a cohort of 233 medical radiation technologists with 'darkroom disease' (MRT/DRD), based on a definition derived from sick building syndrome; and a control group (unexposed to chemicals) of 1779 physiotherapists (PT) who were not selected on the basis of any symptoms (9). No information on possible mould exposure was available for these two comparison groups.

Statistical analysis

All analyses were performed using SAS version 9 (SAS Institute Inc., Cary, NC, USA). Statistical comparisons across symptomatic and control groups were made using one-way ANOVA for continuous variables and Fisher exact tests for categorical variables. A *P*-value of <0.05 was considered statistically significant for the demographic variables only. For the continuous variable ANOVA analyses, adjustment for multiple comparisons was made using the Tukey–Kramer procedure. For the multiple categorical analyses, a Bonferroni adjustment was used to correct for multiple comparisons.

For the three pairwise comparisons from three groups, an adjusted *P*-value < 0.017 (0.05 ÷ 3) was considered significant.

Results

Characteristics of total group of mould exposed adult patients

A total of 32 adult mould exposed patients (excluding two children) was seen in the tertiary clinic 1996–2004 for symptoms attributed to exposures to mould including *S. chartarum* (Table 1). The mean age for the group was 47 years and 82% were female. The mean $\pm$ SD (range) of time between the onset of exposure-attributed symptoms and initial clinic assessment was 1.9 ± 2.9 years (< 1–10 years). Most (82%) of the patients had mould exposure at work and accordingly, 57% had been relocated to different work areas. Onset of symptoms was reported by 58% of patients to start after > 4 weeks of identified mould exposure, and persisted > 6 weeks in 91% of patients. In 82% the symptoms were present at work, and in 28% the symptoms had cleared by the time

of the first clinic visit. The main initial symptoms reported after exposures were: cough (79%), shortness of breath (70%), chest tightness (64%), wheeze (54%), nasal stuffiness (54%), and headache (51%) (Table 2).

Previous asthma was reported by seven patients (22%), perennial allergic rhinitis by five patients (15%), and seasonal allergic rhinitis by eight patients (24%). Most patients (24 of 31 tested [77%]) had positive skin prick tests to common allergens (including *Alternaria*, *Aspergillus* and *Cladosporium*). Additional fungal allergy extracts were skin tested in 24 patients and nine (37%) of these had positive tests to at least one among all the fungal extracts tested. The most common allergen responses in order of frequency were tree pollen in thirteen patients (42%), ragweed in thirteen (42%), house dust mix and cat each in twelve (39%), grass pollen in eleven (35%), dog and dust mite each in ten (32%), cockroach in five (16%) and horse and feather in three each (10%). The fungal allergen responses were positive to *Aspergillus* in six patients (19%), *Alternaria* in five (16%), and *Cladosporium* in three (10%). Five patients

Table 1. Baseline characteristics of mould-exposed patients seen clinically 1996–2004

	Mould exposed patients	Subjects responding to the questionnaire (including the partner of one patient)	Subset not responding to the questionnaire	<i>P</i> -value*
Number	32	17	16	
Mean age $\pm$ SD	46.8 $\pm$ 10.6	47.2 $\pm$ 11.7	46.4 $\pm$ 10.5	0.98
Female	27 (82%)	15 (88%)	12 (75%)	0.398
<i>Exposure</i>				
Work	27 (82%)	13 (76%)	14 (87%)	1.00
Home	5 (15%)	3 (18%)	2 (12%)	
Unknown	1 (3%)	1 (6%)	0	
<i>Previous:</i>				
Asthma	7 (22%)	6 (35%)	1 (6%)	0.085
Chronic cough	4 (12%)	0	4 (25%)	0.103
Perennial allergic rhinitis	5 (15%)	2 (12%)	3 (19%)	1.00
Seasonal allergic rhinitis	8 (24%)	6 (35%)	2 (12%)	0.398
<i>Onset of symptoms after mould</i>				
< 4 weeks	7 (21%)	3 (18%)	4 (25%)	1.00
> 4 weeks	18 (58%)	10 (59%)	9 (56%)	
Unknown	7 (21%)	4 (23%)	3 (19%)	
<i>Duration of symptoms</i>				
> 6 weeks	30 (91%)	14 (82%)	16 (100%)	0.227
Unknown	3 (9%)	3 (12%)	0	
<i>Symptoms present</i>				
At work	27 (82%)	13 (76%)	14 (87%)	0.656
Off work	24 (73%)	12 (71%)	12 (75%)	1.00
Symptoms cleared by visit	9 (28%)	6 (35%)	3 (19%)	0.438
<i>Mould exposure to S. chartarum confirmed by air sampling or bulk sampling from indoor surfaces</i>	25 (78%)	16/17 (94%)	10/16 (63%)	0.061
<i>Area remediated</i>				
At work	18 (54%)	10 (59%)	8 (50%)	0.732
Relocated to a different area	19 (57%)	10 (59%)	9 (56%)	1.00

**P*-value based on differences between responding and non-responding individuals. *T*-test for continuous variables and Fisher exact test for non-categorical variables.

Table 2. Symptoms among mould-exposed patients as reported at clinic visit

Symptom	Mould exposed patients	Subset responding to the questionnaire	Subset not responding to the questionnaire	P-value (Fisher exact test)*
Number	32	16	16	
Cough	26 (79%)	12 (70%)	14 (87%)	0.398
Shortness of breath	23 (70%)	11 (65%)	12 (75%)	0.708
Chest tightness	21 (64%)	11 (65%)	10 (62%)	1.00
Wheeze	18 (54%)	8 (47%)	10 (62%)	0.491
Nasal stuffiness	18 (54%)	8 (47%)	10 (62%)	0.491
Headache	17 (51%)	9 (53%)	8 (50%)	1.00
Fatigue	17 (51%)	7 (41%)	10 (62%)	0.303
Sputum	15 (45%)	6 (35%)	9 (56%)	0.303
Eye irritation	13 (39%)	7 (41%)	6 (37%)	1.00
Rhinitis	12 (36%)	5 (29%)	7 (44%)	0.481
Throat irritation	8 (24%)	4 (23%)	4 (25%)	1.00
Sinus pains	7 (21%)	5 (29%)	2 (12.5%)	0.398
Disorientation	7 (21%)	3 (18%)	4 (25%)	0.688
Muscle aches	7 (21%)	2 (12%)	5 (31%)	0.225
Difficulty sleeping	5 (15%)	2 (12%)	3 (19%)	0.656
Nausea	6 (18%)	5 (29%)	1 (6%)	0.175
Vomiting	6 (18%)	5 (29%)	1 (6%)	0.175
Palpitations	4 (12%)	2 (12%)	2 (12.5%)	1.00
Loss of appetite	4 (12%)	1 (6%)	3 (19%)	0.335

*Fisher exact test between responding and nonresponding individuals.

had responses to the other fungal extracts, one to *Neurospora*, *Fusarium*, *Rhizopus* and *Trichophyton*, one to *Helminthosporium*, one to *Mucor*, one to *Penicillium*, one to *Neurospora*. Two had borderline (1–2 mm wheal) responses to *Stachybotrys*.

Seven patients were nonatopic (23%) with negative skin test responses to the common allergen extracts including six tested with the additional fungal extracts. These patients had similar symptoms to those with positive skin tests (Table 3). Symptoms also were similar among those with mould exposures at home or at work and in different work environments (Table 3). Measured levels of stachybotrys and other indoor mould are shown in Table 4 and symptoms also did not clearly differ with the different measured exposures.

Pulmonary function tests including methacholine challenge tests were performed in the 22 patients with airway symptoms during the period of initial assessment. Objective evidence of asthma (methacholine $PC_{20} \leq 8$ mg/ml or a postbronchodilator increase in FEV₁ $\geq 12\%$ and 200 ml), was present in five patients (23% of those tested, and 15% of all the patients).

Professional exposure data were available for the environment of 28 patients (11 workplaces and four homes) and presumed exposure by visual self-reports/photographs in the remaining one workplace and three homes (citing 'extensive black mould'). Sixteen of the patients had exposure while at work in contaminated Courthouse buildings (12 at Courthouse #1, 2 at Courthouse #2 and 2 at Courthouse #3). The remaining 12

patients with professional exposure assessments had these performed at their different sites of work or home. Professional reports of bulk or swipe sample results for four patients (patient # 26, 27, 31, 32) and a spouse (#33) and air sample results for 21 patients showed *S. chartarum* for 25/25 patients as well as other mould [*Aspergillus* (24/25 patients), *Penicillium* (25/25), *Cladosporium* (19/25), and *Alternaria* (1/25)]. The quantitative air sample results available are shown in Table 4. Three of the professional assessments only indicated 'visible mould' (patients # 17, 23, 30), and the remaining four patients self-reported visible mould (patients # 21, 25, 28, 29).

Follow-up

A total of 17 follow-up questionnaires was obtained, at a mean time of 3.1 ± 0.5 years (range <1–4 years) after the first clinic visit, and included a response from the partner of one patient who reported symptoms with the same fungal exposure at home. None had ongoing exposure to mould. There was no statistical difference in symptoms initially reported among respondents compared with those not responding to the follow-up questionnaire (Table 2).

In the follow-up, symptoms consistent with asthma in the past 12 months were also common (Table 5): chest tightness on exertion (65%), asthma attack in 63%, wheezes (53%), cough (47%), waking with dyspnoea (41%). Current rash was reported by 24%. Abnormal fatigue in the past 12 months was also common (53%) with other symptoms such as sore throat, nasal and conjunctivitis symptoms in 18–35% (Table 5). Combinations of two or more, three or more and four symptoms of fatigue, sore throat, nose and eye symptoms (consistent with sick building syndrome) within the past 12 months, were reported by 82%, 77% and 41%. Questions which had been included in the questionnaire to identify possible over-reporting (e.g. pain on urination) were answered positively in a higher proportion of mould-exposed patients (12%) than the comparison groups (Table 5).

Comparisons of mould-exposed group who completed follow-up questionnaires ($n = 17$) with darkroom disease individuals and physiotherapists

The 17 mould-exposed subjects who completed the follow-up questionnaire answered a subsection of questions identical to those previously used to identify a group of medical radiation technologists (233) with 'darkroom disease' (defined as a symptom cluster identical to symptoms of sick-building syndrome – MRT/DRD). A control group of 1779 physiotherapists (PT) had also previously completed this questionnaire.

The mould-exposed group was older than the other groups. Females were the majority in all three groups,

Table 3. Exposures, main symptoms and skin prick test to common and fungal allergens

Subject No.	Exposure	Main symptoms	Positive skin tests
1	Courthouse #1, Exposure company #1	Cough, shortness of breath, chest tightness, wheeze, nasal stuffiness, headache, sputum	None (all negative)
2	Courthouse #1, Exposure company #1	Shortness of breath, chest tightness, nasal stuffiness, headache, fatigue, eye irritation, muscle aches, nausea, vomiting	None (all negative)
3	Courthouse #1, Exposure company #1	Cough, shortness of breath, chest tightness, headache, difficulty sleeping	Cat, dog, horse, dust, dust mite, tree, ragweed
4	Courthouse #1, Exposure company #1	Shortness of breath, nasal stuffiness, fatigue, eye irritation	Cat, dog, horse, feathers, dust, dust mite, tree, ragweed
5	Courthouse #1, Exposure company #1	Cough, nasal stuffiness, headache	None (all negative)
6	Courthouse #1, Exposure company #1	Cough, shortness of breath, chest tightness, nasal stuffiness, headache, fatigue, muscle aches, nausea	Dust, dust mite
7	Courthouse #1, Exposure company #1	Nasal stuffiness, eye irritation, rhinitis, throat irritation, disorientation	Cockroach, dust mite, ragweed, neurospora
8	Courthouse #1, Exposure company #1	Cough, shortness of breath, chest tightness, nasal stuffiness, headache, sputum, eye irritation	None (all negative)
9	Courthouse #1, Exposure company #1	Eye irritation, rhinitis	Grass, ragweed, alternaria, aspergillus, cladosporium, helminthosporium
10	Courthouse #1, Exposure company #1	Cough, shortness of breath, chest tightness, eye irritation, rhinitis	Cat, dust, aspergillus (extra fungi not tested)
11	Courthouse #1, Exposure company #1	Cough, chest tightness, headache, sputum, vomiting	Cat
12	Courthouse #1 Exposure company #1	Popping ears	Did not perform skin prick test
13	Courthouse #2, Exposure company #1	Cough, shortness of breath, chest tightness, wheeze, nasal stuffiness, sputum, throat irritation	Ragweed, Mucor
14	Courthouse #2, Exposure company #1	Cough, shortness of breath, chest tightness, nasal stuffiness, headache, fatigue, sputum, rhinitis	Penicillium
15	Courthouse #3, Exposure company #1	Cough, shortness of breath, nasal stuffiness, sputum, sinus pains	Dust, dust mite, tree, alternaria, aspergillus, neurospora, fusarium, rhizopus, trichosporium
16	Courthouse #3, Exposure company #1	Cough, shortness of breath, chest tightness, wheeze, headache, fatigue, sputum, throat irritation, sinus pains, disorientation, palpitation	None (all negative)
17	University building Exposure company #2	Cough, chest tightness, wheeze, headache, fatigue, eye irritation, throat irritation, sinus pains, disorientation, muscle aches, difficulty sleeping, nausea, palpitation	Cat, dog, feathers, dust, tree, grass, ragweed, alternaria, aspergillus, cladosporium (other fungi not tested)
18	Home exposure Exposure company #1	Cough, shortness of breath, chest tightness, nasal stuffiness, headache, eye irritation, rhinitis, throat irritation	Tree
19	Home exposure (spouse of patient #18) Exposure company #1	Shortness of breath, sinus pains	Cat, dog, dust, grass
20	Office exposure Exposure company #1	Cough, shortness of breath, chest tightness, headache, fatigue, eye irritation, disorientation, muscle aches, nausea, loss of appetite	Feather, cockroach, tree, grass, ragweed
21	Office exposure Visible mold	Cough, shortness of breath, chest tightness, sputum, eye irritation, sinus pains	Tree, grass, ragweed
22	Office exposure Exposure company #1	Cough, chest tightness, rhinitis, vomiting	Tree

Table 3. (Continued)

Subject No.	Exposure	Main symptoms	Positive skin tests
23	Home exposure Exposure company #1	Cough, shortness of breath, chest tightness, wheeze, nasal stuffiness, headache, sputum, throat irritation, palpitation	None (all negative)
24	Office exposure Exposure company #3	Cough, shortness of breath, sputum, rhinitis, difficulty sleeping, vomiting	Cat, dog, dust, tree, grass, ragweed (extra fungi not tested)
25	Office exposure Visible mold	Cough, nasal stuffiness, headache	Cat, cockroach, tree, grass, ragweed, alternaria, aspergillus, cladosporium (extra fungi not tested)
26	Home exposure Exposure company #4	Cough, shortness of breath, chest tightness, sputum, rhinitis, vomiting	Dust mite
27	Office exposure Exposure company #1	Cough, shortness of breath, nasal stuffiness, fatigue, sputum, rhinitis, disorientation, muscle aches, nausea	None – all negative (extra fungi not tested)
28	Home, visible mould-roof and basement area	Shortness of breath, wheeze, nasal stuffiness, fatigue, sputum, eye irritation, rhinitis, disorientation, loss of appetite	Cockroach, dust, dust mite, tree
29	Office, visible mold	Cough, sinus pains	Cat, dog, dust mite, tree, grass, ragweed, alternaria, aspergillus, (other fungi not tested)
30	Museum workplace, visible mould Exposure company #5	Cough, shortness of breath, chest tightness, sputum, eye irritation, sinus pains	Cat, dog, dust, dust mite, grass, ragweed
31	Storage Company, Exposure company #6	Cough, chest tightness, nasal stuffiness, fatigue, rhinitis, loss of appetite	Dog, dust, dust mite, tree grass, ragweed
32	School, Exposure company #7	Cough, shortness of breath, chest tightness, nasal stuffiness, headache, fatigue, sputum, eye irritation, throat irritation	Cat, dog, horse, dust
33 (not seen as a clinic patient, performed questionnaire only)	Home exposure (partner of patient #26) Exposure company # 4	Cough, shortness of breath, sputum, rhinitis, difficulty sleeping, vomiting	Did not perform skin prick test

Table 4. Quantitative exposure results for stachybotrys and for total counts of other indoor vs outdoor moulds

Location/patient	Stachybotrys		Other moulds (total)	
	cts/m ³ inside	cts/m ³ outside	cts/m ³ inside	cts/m ³ outside
Courthouse #1 (pt# 1–12)				
Mar 11	25	0	33	63
Mar 12	369	0	196	100
Mar 18	1200	0	235	44
Mar 27	169	0	345	313
July	2134	0	4528	436
Courthouse #2 (pt# 13, 14)	5132	0	16,337	175
Courthouse #3 (pt# 15, 16)	2060	0	8657	375
Home (pt# 18,19)	3457	0	1152	234
Office (pt# 20)	1596	0	9456	23982
Office (pt# 22)	140	0	5167	5341
Office (pt# 24)	13	0	26	2552

71% of the mould-exposed group had a history of ever smoking compared to 33% of MRT/DRD patients, and 53% had a history of diagnosed asthma as compared with

22% of MRT/DRD. A history of (i) recent abnormal fatigue, (ii) sore itchy and runny eyes, (iii) itchy, runny nose and sneezing and (iv) sore throat was statistically less common than the MRT/DRD group but not statistically different from the physiotherapists control group (Table 5).

The mould-exposed group had a similar frequency of recent asthma-like symptoms and urticaria when compared with the MRT/DRD group but were more likely to have smoked (Table 5) compared to the two comparison groups. They were more likely than the PT group to have recent respiratory symptoms; a history of asthma, and recent urticaria, but also had greater frequency of positive responses to the question on pain on urination, which had been included in the survey to assess possible over-reporting (Table 5).

As compared with the PT group (Table 6), a significantly greater percentage (59%) of mould-exposed patients reported symptoms when walking in a room with brand new carpets, complained of a chemical or metallic taste in their mouth, and had problems in

Table 5. Comparisons for symptoms of mould-exposed group who completed follow-up questionnaires, with medical radiation technologists meeting criteria for darkroom disease (MRT/DRD) and a control group of physiotherapists (PT)

Subjects (no.)	Mould (17)	MRT/DRD (233)	PT (1779)	<i>P</i> -value†	
				Mould vs MRT	Mould vs PT
Age ± SD	48 ± 12	42 ± 9	40 ± 10	0.021	0.003
Female	87%	92%	85%	0.370	1.0
Ever smoked	71%	33%	17%	0.003	<0.0001
Current smoker	7%	30%	16%	0.063	0.710
Wheeze*	53%	47%	19%	0.803	0.002
Cough on exertion	47%	37%	11%	0.441	0.0003
Chest tightness on exertion	65%	38%	9%	0.040	<0.0001
Woken by wheeze	24%	16%	5%	0.497	0.011
Woken by dyspnoea	41%	22%	7%	0.081	0.0001
Wheeze with smoke	53%	42%	18%	0.451	0.001
Wheeze with dust	53%	50%	20%	1.0	0.002
Ever had asthma	53%	22%	16%	0.008	0.0005
Asthma attack in the past 12 months	63%	72%	44%	0.681	0.438
Taken asthma medication	75%	72%	53%	1.0	0.290
MD ever diagnosed eczema	36%	22%	17%	0.317	0.082
Hay fever	44%	55%	27%	0.442	0.160
Urticaria	38%	27%	10%	0.393	0.003
Current rash	24%	19%	13%	0.750	0.264
Past year – Abnormal fatigue	53%	84%	29%	0.005	0.055
Sore itchy/runny eyes	35%	74%	13%	0.001	0.018
Itchy, runny nose/sneezing	29%	82%	15%	<0.0001	0.103
Sore throat	18%	40%	5%	0.074	0.042
Pain on urinating	12%	3%	1%	0.136	0.005

*Symptoms in the last 12 months.

†A *P*-value <0.017 is taken as significant, adjusting for three pairwise comparisons from three groups.

Significant *P*-values are shown in bold font.

concentration and short term memory. These findings in the mould-exposed patients were similar to findings in the MRT/DRD group.

Discussion

Symptoms among mould-exposed patients referred to a tertiary clinic were similar to darkroom disease (sick building-type symptoms). Previous asthma/allergic rhinitis were common and the vast majority of patients reported both upper and lower respiratory symptoms on exposure to mould. Previous asthma was common among the mould-exposed group (22% in total and 35% of the subset responding to the questionnaire) and a majority (70%) had asthma-like symptoms at their initial clinic visit. Nevertheless, most of these had no objective evidence of asthma at their clinical visit when symptomatic. Most patients had the onset of symptoms after over

4 weeks of identified mould exposure and for most, symptoms persisted over 6 weeks. Most were still symptomatic by the time of their initial clinic visit despite moving to a different work or residential area. A majority were atopic by skin testing (73%), and although a minority (30%) had positive skin tests to available fungal allergen extracts in this study, it is possible that upper and/or lower respiratory or skin symptoms in at least a subset may have been on the basis of an allergic response to fungi or to other common allergens. Of note, there were only borderline (1–2 mm wheal) skin prick test responses to the extract of *stachybotrys* in this study. We have similarly also observed only one patient with a positive skin test to a *stachybotrys* extract in an earlier survey of allergy patients in Toronto (11) and others have reported lack of specific antibodies found among those with symptoms suggesting it may be a weak antigen (3). A significant proportion had ongoing nonspecific symptoms at follow-up which were not likely to be entirely explained on an allergic basis, although it is possible that this proportion may be over-represented in the results if those with ongoing symptoms may have been more likely to self-select to participate in the follow-up questionnaire. In addition, there may have been some over-reporting in this group as may be suggested by increased positive responses (in 12%) to a question on pain on urination (Table 5).

Four of the patients in this study were included without objective exposure assessments. They described and provided photographs of black mould in their environment but there may not have been *Stachybotrys* present, and even for those in whom the presence was confirmed, we are not able to say that symptoms were caused by *Stachybotrys* rather than other fungal exposures or other concurrent exposures or conditions.

Allergic responses to inhaled fungi can trigger allergic rhinitis, asthma (16, 17) and hypersensitivity pneumonitis in predisposed individuals (11, 12). Irritant effects on mucous membranes and skin has been suggested from volatile fungal compounds such as glucans, as reviewed by Douwes (18). Knowledge of potential human toxic responses from inhaled mould metabolites remains limited although these have been reported in animals (19–21). Two 'chemotypes' of *S. chartarum* exist. One chemotype elaborates macrocyclic trichothecene mycotoxins with significant toxic potential in mice which act as stress kinase activators and translational inhibitors, whereas a second chemotype produces atranones with potential to cause pulmonary inflammation (22). Other products that could contribute to pathogenic effects include hemolysins, proteinases, glucans, spirocyclic drimanes and volatile organic compounds (22). Some authors described measurements of the proteinaceous hemolysin, stachylysin, in serum and environmental samples as a potentially useful indicator in assessing human exposure to *S. chartarum* and in determining the presence of this indoor mould (23). Elevated air and serum measures of

Table 6. Comparisons for specific questions of mould-exposed group who completed follow-up questionnaires with medical radiation technologists meeting criteria for darkroom disease (MRT/DRD) and a control group of physiotherapists (PT)

Subjects (no.)	Mould (17)	MRT/DRD (233)	PT (1779)	<i>P</i> -value†	
				Mould vs MRT	Mould vs PT
Unusually sensitive to everyday Chemicals*	35%	54%	20%	0.1403	0.1221
Chemical sensitivity Special diet	19%	5%	4%	0.060	0.020
Home precautions	38%	36%	16%	1.0	0.028
Wear/avoid clothes	25%	25%	10%	1.0	0.069
Trouble shopping	27%	21%	6%	0.746	0.013
Sensitive to cologne	53%	71%	45%	0.167	0.627
Sensitive to detergents	41%	51%	18%	0.617	0.023
Beauty parlor/barber shop	47%	48%	23%	1.0	0.036
New carpets	59%	46%	24%	0.451	0.003
Newspaper	18%	32%	12%	0.283	0.439
Secondhand smoke	88%	92%	84%	0.636	1.0
Chemical/metallic taste	19%	20%	1%	1.0	0.001
Difficulty concentrating	53%	54%	19%	1.0	0.002
Short memory	53%	62%	23%	0.452	0.008
SF12 physical component score	45.8 ± 15.3	44.5 ± 9.7	46.4 ± 4.6	0.340	0.641
SF12 mental component score	47.3 ± 11.4	42.7 ± 11.4	50.3 ± 10.1	0.074	0.230

*Symptoms in the last 12 months.

†A *P*-value <0.017 is taken as significant, adjusting for three pairwise comparisons from three groups. Significant *P*-values are shown in bold.

ochratoxin A, from *Aspergillus* species, have been reported in workers exposed to contaminated foods such as coffee and spices (24). Although the allergic response to moulds is documented, there has been no association between IgE or IgG antibodies and other adverse health effects of moulds (3, 4).

Other potential adverse effects of mould exposure include reports of carcinogenic effects from some mycotoxins such as aflatoxins from *Aspergillus* species (25). Immunosuppressed individuals may also develop systemic infection from fungi such as *Aspergillus* (26).

An additional potential response when a suggested health risk is identified in a stressful setting is that of anxiety or panic responses, as has been suggested to be a feature of some medically unexplained syndromes such as multiple chemical sensitivity (also known as idiopathic environmental intolerance) (8), and sick building syndrome, and toxic mould syndrome (27, 28). Individuals who have been informed of contamination of their home or workplace building by 'toxigenic' mould such as *S. chartarum*, often after flooding, may understandably undergo emotional stress in relation to concerns about possible health effects, work changes and costs related to clean-up and/or litigation.

Recently, there has been a significant focus on health effects associated with general indoor fungal exposure. Several studies show that the presence of visible mould growth is associated with bronchitis, phlegm production, and chest illness (29–33). Most of these studies relied on self-reported assessment of mould growth. In children, an association between home dampness and lower respiratory symptoms has been well documented

(34–36). However, it is unknown whether this association partly relates to fungi, which thrive in damp conditions.

Stark et al. (37) have explored the association between exposure to fungi and fungal products and lower respiratory illness early in life. They demonstrated a strong association between high household fungal levels and an increased incidence of doctor-diagnosed lower respiratory illness in infants.

'Sick-building' symptoms are commonly nonspecific, including upper respiratory symptoms suggestive of mucous membrane irritation, headaches, fatigue and rash, associated in time with a particular building; this overlaps strongly with so called 'darkroom disease' reported by medical radiation technologists or MRTs (9). In our study the mould-exposed group frequently reported these symptoms at a mean follow-up time of 3 years, although less commonly than reported by the MRT/DRD group when currently working. The frequency of these symptoms was intermediate between the MRT/DRD group and the normal control group of PTs, but the persistence despite remediation of the initial exposure conditions suggests a different mechanism from that causing more transient symptoms among occupants of 'Sick Buildings'.

The follow-up scores of the mould-exposed group for some features associated with multiple chemical sensitivity were similar to those of the MRT/DRD group and showed marked differences from the normal control group of physiotherapists, suggesting a possible degree of overlap for the mould-exposed group with multiple chemical sensitivity/idiopathic environmental intolerance. Questionnaires were included for anxiety, depression and

panic in this study as previously reported for a separate group of subjects with MCS and healthy controls (8) (data not shown) and although scores for panic and anxiety scales were intermediate between the previously reported MCS and normal scores, differences were not significant. However, in this study, the mould-exposed group did not complete the questionnaires at the time of the initial assessment, nor at the time of mould exposures when emotional stress may have been greater. Future studies assessing serial changes in scores for panic, anxiety and agoraphobia in mould-exposed subjects with health concerns may be helpful to further address responses related to exposures.

Limitations of this study include selection bias by our inclusion of patients from a tertiary referral clinic and by the relatively long duration between onset of symptoms and initial clinic assessment: thus this population may have more severe and/or prolonged effects than might be found in all symptomatic exposed individuals. A relatively small number of subjects were assessed, especially in the follow-up component of the study and the follow-up was limited to subjective questionnaire responses. Nevertheless, our findings indicate that at least a subgroup of mould-exposed subjects have long-term respiratory symptoms following mould exposure which cannot be explained on the basis of asthma; the mechanisms for these symptoms deserve further investigation.

Conclusion

We describe the health outcome of a cohort population after exposure to *Stachybotrys* and other moulds, with a comparison to a cohort of patients with darkroom disease (having symptoms of sick-building syndrome). In many cases some or all initial symptoms in the mould-exposed group could be attributed to allergic responses to common allergens or mould. Additional symptoms attributed to mould exposure appear to represent findings similar to sick building syndrome, but persist despite removal from/remediation of mould exposure.

Conflict of interest statement

The authors declare that they have no conflict of interests.

Acknowledgments

The authors thank Yan Zhang for assistance with initial statistical analyses and we thank the patients for their participation. This study was supported in part by a grant from the Centre for Research Excellence in Occupational Disease, Toronto.

Dr Ribeiro was supported in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil.

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