Ann Agric Environ Med 2008, 15, 317–322

RESPIRATORY DISORDERS IN TWO WORKERS OF CUSTOMS DEPOSITORIES OCCUPATIONALLY EXPOSED TO MOULDY TOBACCO

Barbara Mackiewicz[1], Czesława Skórska[2], Ewa Krysińska-Traczyk[2], Lennart Larsson[3], Bogumiła Szponar[4], Janusz Milanowski[1], Elżbieta Czekajska-Chehab[5], Jolanta Sitkowska[2], Grażyna Cholewa[2], Jolanta Szymańska[6], Jacek Dutkiewicz[2]

[1] Department of Pneumonology, Oncology and Allergology, Medical University of Lublin, Lublin, Poland
[2] Department of Occupational Biohazards, Institute of Agricultural Medicine, Lublin, Poland
[3] Department of Laboratory Medicine, Division of Medical Microbiology, Lund University, Lund, Sweden
[4] Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
[5] 1st Department of Radiology, Medical University of Lublin, Lublin, Poland
[6] Department of Paedodontics, Medical University of Lublin, Lublin, Poland

Mackiewicz B, Skórska C, Krysińska-Traczyk E, Larsson L, Szponar B, Milanowski J, Czekajska-Chehab E, Sitkowska J, Cholewa G, Szymańska J, Dutkiewicz J: Respiratory disorders in two workers of customs depositories occupationally exposed to mouldy tobacco. *Ann Agric Environ Med* 2008, **15**, 317–322.

Abstract: Work-related respiratory symptoms, including dyspnoea, cough, fever, tiredness and malaise, were recorded in two customs officers employed in 2 depositories of confiscated cigarettes, of which one showed signs of dampness. Microbiological sampling of the air and the cigarettes stored in a damp depository revealed the presence of potentially pathogenic fungi and bacteria and the biochemical markers of bacterial lipopolysaccharide and fungal biomass. The *Penicillium* species (*P. simplicissimum, P. inflatum, P. commune*) dominated in the damp depository, while in the other one *Aspergillus fumigatus* was prevalent. The patients under study did not show a specific sensitization to microbial allergens in the precipitin test, the test for inhibition of leukocyte migration and the bronchial provocation challenge, except for a weak reaction to fungal allergens in the test for inhibition of leukocyte migration. Moreover, one patient responded with subjective symptoms after exposure to inhalation of increased doses of *Penicillium simplicissimum* antigen. Both cases were diagnosed as a specific form of organic dust toxic syndrome (ODTS). It is hypothesized that the symptoms were evoked most probably by the non-specific action of low molecular fungal metabolites, such as mycotoxins or VOCs (volatile organic compounds), with the possible contribution of bacterial endotoxin. However, as there is no a direct proof to support this presumption, and the effects of nicotine and other tobacco constituents cannot be excluded, further studies are needed to elucidate etiopathogenesis of the disorders associated with the exposure to stored tobacco.

Address for correspondence: Dr Barbara Mackiewicz, Department of Pneumonology, Oncology and Allergology, Medical University of Lublin, Jaczewskiego 8, 20-090 Lublin, Poland. E-mail: b.mack@wp.pl

Key words: customs depository workers, occupational exposure, tobacco, work-related respiratory symptoms, organic dust toxic syndrome, bioaerosol, fungi, bacteria.

INTRODUCTION

The harmful effects of tobacco and tobacco smoke are caused by nicotine and numerous chemical substances [16]. Moreover, people occupationally engaged in processing tobacco leaves and cigarettes could be exposed to various microorganisms growing on tobacco and dispersed into air during the production processes. These microorganisms, comprising mould fungi, bacilli, cocci, and Gram-negative bacteria, may evoke allergic and immunotoxic reactions and mycoses in exposed people [3, 7, 8, 10, 11].

In November 2007, the Department of Occupational Biohazards of the Institute of Agricultural Medicine in Lublin was notified by the Customs Chamber in locality D.,

Received: 10 September 2008
Accepted: 22 October 2008

situated at the eastern border of Poland, about the occurrence of work-related symptoms in the officer employed in the depository of confiscated cigarettes and asked an assessment of the potential microbial hazards in the work environment. Accordingly, during the first half of 2008 we performed the microbiological examination of the work environment in customs depository "D" situated in locality D. and in another depository "K" situated in the locality K., which had been rented for improving storing conditions. Thereafter, 2 customs officers complaining of work-related symptoms, one working in depository "D" (patient M. P.) and another working in depository "K" (patient J. K.), were subjected to clinical examinations at the Clinic of Pneumonology, Oncology and Allergology of the Medical University in Lublin.

MATERIALS AND METHODS

Microbiological examination of work environment. Two customs depositories "D" and "K", situated in the localities D. and K. on the territory of Lublin province (eastern Poland) and covering areas of 420 m^2 and 3,600 m^2 respectively were examined. In the depositories, circa 2.5 and 4 million confiscated packs of cigarettes respectively were stored in plastic bags or cardboard boxes on shelves or in piles. In the depository "D" visible signs of dampness and mouldiness (damp and mouldy patches on floor, moist cigarettes) were recorded. Air samples of the volume 1,500 l each were collected at 3 sites of each depository on polypropylene filters (FIPRO-37, Instytut Włókiennictwa, Łódź, Poland) with an AS-50 aspirator (TWO-MET, Zgierz, Poland). Filters were extracted in saline (0.85% NaCl) and serial 10-fold dilutions were examined for the presence of various types of microorganisms and microbial markers, as described earlier [8, 13, 14, 15]. Moreover, in depository "D", 5 samples of damp cigarettes and 1 sample of mouldy floor scraps were examined by dilution plating.

Briefly, 0.1-ml aliquots of each dilution were spread on duplicate sets of different agar media for cultivation and determination of total mesophilic bacteria (blood agar), Gram-negative bacteria (EMB agar), thermophilic actinomycetes (half-strength tryptic soya agar), and fungi (malt agar and potato-dextrose agar). The bacteria were identified by using the API 20E, API 20NE (bioMérieux, Marcy l'Etoile, France), and BIOLOG (Biolog, Inc., Hayward, CA, USA) systems. The fungi were identified by using microscopy [8, 15].

An integrated method for determining microbial markers in examined extracts by gas chromatography – tandem mass spectrometry (GC-MSMS) was performed at the University of Lund, Sweden. The method includes a protocol for preparation and analyzing samples for: 3-hydroxy fatty acids (3-OH FAs) of 10–18 carbon chain lengths as markers of lipopolysaccharide (LPS, endotoxin), muramic acid (MuAc) as marker of peptidoglycan, and ergosterol (Erg) as marker of fungal biomass [8, 13, 14]. Moreover, the presence of biologically active endotoxin (LPS) was determined by the *Limulus* (LAL) test [15].

Clinical examinations. Included: anamnesis, physical examination, routine laboratory examination of blood, gasometry, radiography, spirometry, computer tomography of

Table 1. Concentration of microorganisms and microbial compounds in the air of customs cigarette depositories.

Organism/compound	Custom depository "D"		Custom depository "K"	
	Concentration[a] (median, range)	Dominant species (percent of total isolates)	Concentration[a] (median, range)	Dominant species (percent of total isolates)
Mesophilic bacteria (cfu/m^3)	60 (30–183,790)	*Providencia rettgeri* (91.4) *Alcaligenes xylosoxidans* (8.2)	4700 (460–45,420)	*Enterococcus* spp. (73.2) *Staphylococcus* spp. (16.6)
Gram-negative bacteria (cfu/m^3)	0 (0–0)	0	0 (0–10)	*Pseudomonas mesophilica* (100)
Thermophilic actinomycetes (cfu/m^3)	0 (0–0)	0	200 (10–360)	*Thermoactinomyces vulgaris* (70.7) *Thermoactinomyces thalpophilus* (19.0)
Fungi (MA)[b] (cfu/m^3)	310 (10–7,090)	*Penicillium simplicissimum* (94.6) *Penicillium inflatum* (5.4)	110 (0–510)	*Aspergillus fumigatus* (41.9) *Trichoderma viride* (32.3)
Fungi (PDA)[c] (cfu/m^3)	220 (20–2,000)	*Penicillium simplicissimum* (50.0) *Penicillium inflatum* (49.5)	110 (0–220)	*Aspergillus fumigatus* (70.0) *Rhizopus nigricans* (21.2)
LPS (GC-MSMS, ng/m^3)	N. d.		138.24 (30.10–1,116.48)	
LPS (*Limulus*, ng/m^3)	0.41 (0.21–4.2)		0.42 (0.42–1,739.3)	
Muramic acid (GC-MSMS, ng/m^3)	N. d.		N. d.	
Ergosterol (GC-MSMS, ng/m^3)	N. d.		0.88 (0.84–8.51)	

[a] Data for 3 sampling points; [b] Malt agar; [c] Potato-dextrose agar; N. d.: not detected.

chest, and bronchofiberoscopy following British Thoracic Society guidelines [2], comprising bronchoalveolar lavage (BAL) and histopathological examination of biopsy specimens.

Both patients were tested for sensitization to 12 extrinsic microbial antigens associated with organic dusts: *Acinetobacter calcoaceticus, Arthrobacter globiformis, Pantoea agglomerans, Saccharopolyspora rectivirgula, Streptomyces albus, Thermoactinomyces vulgaris, Aspergillus candidus, Aspergillus clavatus, Aspergillus fumigatus, Penicillium citrinum, Penicillium commune, Penicillium simplicissimum*. They were selected on the basis of the presence in the work environment and/or known allergenic properties [4, 9]. The antigens were lyophilized saline extracts of microbial cells, prepared at the Institute of Agricultural Medicine, as described earlier [5, 9].

The following allergological tests were applied:

• Agar-gel precipitation test, performed by Ouchterlony double diffusion method in purified 1.5% agar using staining with azocarmine B as earlier described [5] with all 12 antigens.

• Test for inhibition of leukocyte migration (MIF test) in the presence of specific antigen, performed by the whole blood capillary microculture method, as described earlier [5], with the antigens of *Aspergillus fumigatus* (both patients), *Pantoea agglomerans* (both patients), *Penicillium commune* (patient M. P.), *Penicillium simplicissimum* (patient M. P.), *Streptomyces albus* (patient M. P.), and *Thermoactinomyces vulgaris* (patient J. K.). The test was considered as positive at the migration index (MI) equal to 0.790 or lower.

• Bronchial provocation challenge, performed by Lung Test 1000 spirometer provided with ISPA adapter for inhalation challenge (MES, Kraków, Poland) with the antigens suspended in saline (0.85% NaCl), selected on the basis of abundant presence in the work environment: *Penicillium simplicissimum* (patient M. P.), and *Aspergillus fumigatus* (patient J. K.). The breath-steering protocol was applied with increasing concentrations of allergen after initial inhalation of saline. Both patients were tested with increasing concentrations of allergen up to 20 µg/ml (1, 3, 5, 10, 20 µg/ml). Moreover, patient M. P. was tested at another time with increasing concentrations of allergen up to 50 µg/ml (12.5, 25, 30, 35, 40, 50 µg/ml). The results were assessed on the basis of spirometry, blood morphology and occurrence of subjective symptoms.

RESULTS

Microbial pollution of work environment. The air of both depositories was polluted with various bacteria and fungi (Tab. 1). The maximal concentrations of bacteria in the depositories "D" and "K" and the maximal concentration of fungi in the depository "D" exceeded the proposed threshold limit value of 5,000 cfu/m^3 [1, 6]. Among airborne fungi, prevailed potentially pathogenic species: *Penicillium simplicissimum* and *Penicillium inflatum* in the depository "D" and *Aspergillus fumigatus* in the depository "K". Among fungi recovered from damp cigarettes and the mouldy floor in depository "D" distinctly prevailed the species *Penicillium commune*, while among bacteria the most numerous were, respectively, *Enterococcus cecorum*

Table 2. Concentration of microorganisms and microbial compounds in stored cigarettes and scrapings of mouldy floor in customs cigarette depository "D".

Organism/compound	Cigarettes[a]		Scrapings of mouldy floor[b]	
	Concentration[a] (median, range)	Dominant species (percent of total isolates)	Concentration[b]	Dominant species (percent of total isolates)
Mesophilic bacteria (cfu × 10^3/g)	1,050 (70–56,010)	*Enterococcus cecorum* (92.7) *Bacillus subtilis* (2.4)	4,510	*Streptomyces albus* (84.4) *Bacillus* spp. (6.3)
Gram-negative bacteria (cfu × 10^3/g)	0 (0–0)	0	0	0
Thermophilic actinomycetes (cfu × 10^3/g)	0 (0–50)	*Thermoactinomyces vulgaris* (100)	0	0
Fungi (MA)[c] (cfu × 10^3/g)	0 (0–70)	*Penicillium commune* (100)	730	*Penicillium commune* (85.6) *Oidiodendron cerealis* (14.4)
Fungi (PDA)[d] (cfu ×10^3/g)	1 (0–90)	*Penicillium commune* (94.8) *Penicillium ramusculum* (5.2)	595	*Penicillium commune* (75.6) *Penicillium sclerotiorum* (15.1)
LPS (GC-MSMS, µg/g)	104.8 (68.8–109.6)		N. t.	
LPS (*Limulus*, µg/g)	6,250.0 (312.5–31,250.0)		N. t.	
Muramic acid (GC-MSMS, µg/g)	3.6 (3.2–5.3)		N. t.	
Ergosterol (GC-MSMS, µg/g)	21.0 (11.0–144.0)		N. t.	

[a] Data for 5 packs; [b] Data for 1 sample; [c] Malt agar; [d] Potato-dextrose agar; N. t.: not tested.

Table 3. Test for inhibition of leukocyte migration in the presence of environmental antigens.

Patient	Migration index (MI) in the presence of antigens					
	Pantoea agglomerans	*Streptomyces albus*	*Thermoactinomyces vulgaris*	*Aspergillus fumigatus*	*Penicillium commune*	*Penicillium simplicissimum*
M. P.	0.9033	0.9936	N. t.	0.8535	0.8493	0.8493
J. K.	0.8987	N. t.	0.9557	0.8101	N. t.	N. t.

N. t. = not tested

and *Streptomyces albus* (Tab. 2). The abundant presence of moulds in stored cigarettes was confirmed by the detection of considerable amounts of ergosterol which is a biomarker of fungal growth (Tab. 2). The cigarettes also contained bacterial endotoxin (LPS) which is a biomarker of Gram-negative bacteria (Tab. 2). The presence of low concentrations of ergosterol and LPS was also detected in the air of depository "K" (Tab. 1).

Case description

CASE 1. On 11 May 2008, a 33-year old patient: M. P. (183 cm tall, 96 kg weight) was admitted to the clinic; he has been a non-smoker for 2 years, and worked at the Customs Chamber for 4 years. He had suffered from pneumonia in the early childhood and had not reported the complaint ever since. The family history was negative for respiratory system diseases. In 2006–2007, the patient bred pigeons. On admission, he reported symptoms related to work in a cigarette storeroom for 2 years. The symptoms were: dyspnoea, raised body temperature, general discomfort, the sensation of excessive tiredness. The complaints occurred directly during exposure and lasted about 8 hours after finishing work, when they declined spontaneously, without treatment.

Auscultation revealed physiological vesicular murmur, bilaterally over the lung fields. Laboratory blood examination: Hb – 14.l g/dl; Hct – 0.39, RBC – 4.55 T/l, WBC – 6.34 G/l, neutrophils – 59%, eosinophils – 3%, basophils – 0.6%, lymphocytes – 28%, monocytes – 5.4%. Arterial capillary blood gasometry: pO_2 – 76 mm Hg, pCO_2 – 42 mm Hg, pH – 7.44, O_2SAT – 95%. Radiological examination of the chest did not show significant abnormalities. Spirometric test fell within the normal values range: FEV_1 – 4,200 ml (94% of the predicted value), VC – 5,920 ml (105% of the predicted value), FEV_1%VC - 87%, MEF 25 – 1,490 ml/s (57% of the predicted value), MEF 50 – 5,300 ml/s (95% of the predicted value), MEF 75 – 8,850 ml/s (103% of the predicted value), PEF – 10,280 ml/s (103% of the predicted value).

Bronchofiberoscopy included bronchoalveolar lavage (BAL) and taking biopsy specimens for histopathological examination. Bronchofiberoscopy results: larynx, trachea, main carina – normal. Mucosa slightly swollen and hyperemic, a net of individual, submucous vessels visible in the main left bronchus. Two biopsy specimens were taken from the carina between RB4 and RB5a, and one specimen from the area of the middle lobe bronchus opening.

BAL results. RB4b was washed out with 140 ml of 0.85 NaCl, 102 ml of the fluid was recovered. The lavage fluid contained: lymphocytes – 21.17%, granulocytes – 0.95%, macrophages – 72.67%. The cytometric results for the lymphocyte subpopulations were following: CD3+ – 81.72%, CD19+ – 0.22%, CD4+ – 60.61%, CD8+ – 18.27%, CD4+, CD8+ – 2.01%, CD4:CD8 index – 3.32, NK cells – 3.96%, CD3+, HLA DR+ – 69.69%. BAL test revealed an increased proportion of lymphocytes, especially T HLA DR+ activated lymphocytes, and a slightly increased CD4:CD8 index.

Results of histopathological examination of the bronchus specimens. In the serially examined material, fragments of the mucosa were partially covered by ciliated epithelium. Swelling and lymphocyte inflammatory infiltrations were found in the stroma.

Computer tomography of the chest. Normally air-filled lung parenchyma without infiltrative lesions. A single 3 mm nodule in segment 6 of the right lung. Trachea and main bronchi showed no signs of obturation. Bilateral pleural cavities were fluid-free. Lymph nodes in the mid thorax were not enlarged. Expirational HRCT section revealed air traps.

Allergological tests. No precipitins were found in patient's serum against 12 microbial antigens tested. MIF test with 5 antigens was negative. Nevertheless, the MI values with fungal antigens were distinctly lower compared to those with bacterial ones (Tab. 3), indicating low grade sensitivity. Inhalation challenge with increasing concentrations of *Penicillium simplicissimum* allergen up to 20 µg/ml caused neither specific symptoms nor spirometric and blood morphology changes. The challenge with the greater concentrations of this allergen up to 50 µg/ml resulted in the appearance of specific symptoms: at 35 µg/ml hoarseness, at 40 µg/ml cough and nausea, and chest tightness 20 hours post challenge. No changes were recorded in spirometry and blood morphology. Similarly, computer examination of the chest before and after the inhalation challenge did not show significant differences.

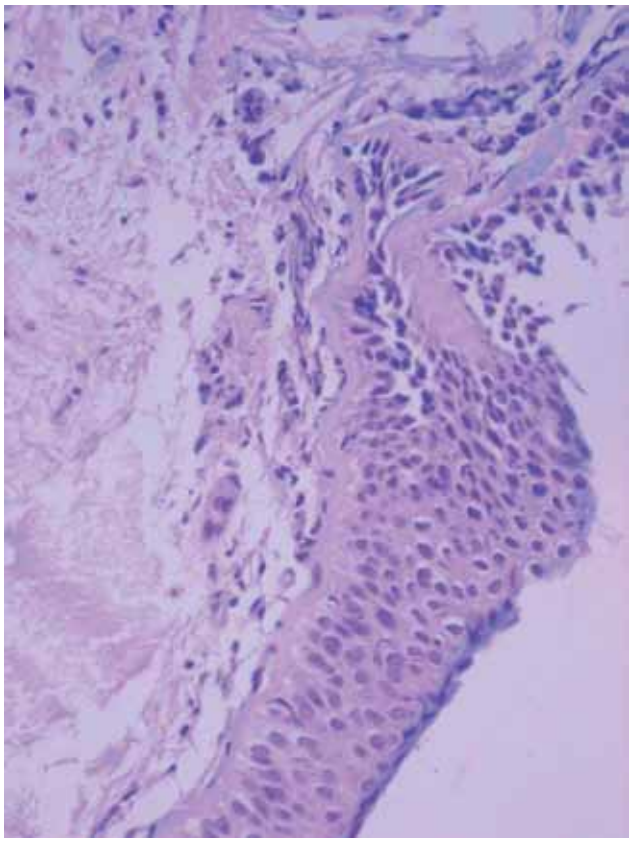

Figure 1. Patient J. K., bronchus biopsy specimen. Inflammatory infiltrations of lymphocytes in stroma. H+E, × 200.

Final diagnosis. On the basis of the obtained results (anamnesis positive for work-related symptoms, the nature of these symptoms, histopathological examination results) the final diagnosis of organic dust toxic syndrome (ODTS) was established with the recommendation of avoidance of exposure to organic dusts.

CASE 2. On 22 June 2008, a 46-year old patient: J. K. (181 cm tall, 93 kg weight) was admitted to the clinic; he has been a non-smoker for 23 years and was employed as a storekeeper at the Customs Chamber for 3 years. A year ago he had bronchitis and did not report any other respiratory system diseases. His father suffered from a chronic obturative lung disease. On admission, the patient reported having symptoms related to work in a tobacco depository since circa 6 months. The symptoms were: headaches, dry cough, dyspnoea, chest tightness, general discomfort, anxiety, nausea, conjunctival itch, lacrimation. The symptoms occurred at work and lasted about 12 hours afterwards, when they declined spontaneously, without treatment.

Auscultation revealed vesicular murmur over the lung fields. Laboratory blood examination: Hb – 14.8 g/dl; Hct – 0.41, RBC – 4.64 T/l, WBC – 7.24 G/l, neutrophils – 48.3%, eosinophils – 5.4%, basophils – 0.4%, lymphocytes – 36.1%, monocytes – 6.8%. Arterial capillary blood gasometry: pO_2 – 84.3 mm Hg, pCO_2 – 32.8 mm Hg, pH – 7.47, O_2SAT – 97%. Radiological examination of the chest did not show significant abnormalities. Spirometric test fell within the normal values range: FEV_1 – 3,680 ml (94% of the predicted value), VC – 5,030 ml (100% of the predicted value), FEV_1%VC – 93%, MEF 25 – 1,170 ml/s (54% of the predicted value), MEF 50 – 4,130 ml/s (82% of the predicted value), MEF 75 – 8,150 ml/s (102% of the predicted value), PEF – 8,450 ml/s (92% of the predicted value).

Bronchofiberoscopy included bronchoalveolar lavage (BAL) and taking biopsy specimens for histopathological examination. Bronchofiberoscopy results: larynx, trachea, main carina – normal. Bronchial tree accessible for examination was patent, showed normal breathing mobility, a net of dilated vessels was visible in the area of left minor carina. One biopsy specimen each was taken from the carina between RB4, RB5a, and RB8b, and one from the left minor carina.

BAL results. RB5 was washed out with 140 ml of 0.85% NaCl, 95 ml of the fluid was recovered. The lavage fluid contained: lymphocytes – 20.0%, granulocytes – 0.93%, macrophages – 75.89%. The cytometric results for the lymphocyte subpopulations were following: CD3+ – 71.32%, CD19+ – 3.97%, CD4+ – 49.68%, CD8+ – 23.58%, CD4+, CD8+ – 0.63%, CD4:CD8 index – 2.1, NK cells – 10.28%, CD3+, HLA DR+ – 31.37%. BAL test revealed a slightly increased proportion of lymphocytes.

Results of histopathological examination of the bronchus specimens. Fragments of the mucosa were covered by ciliated pseudostratified epithelium, numerous infiltrations due to chronic inflammation were found in the stroma (Fig. 1).

Computer tomography of the chest. Normally air-filled lung parenchyma without infiltrative lesions. Pleural cavities were fluid-free. Lymph nodes in the mid-thorax were not enlarged. Fibrotic lesions located in the left subpleural area.

Allergological tests. Similarly to Case 1, the precipitin and MIF tests were negative with all antigens tested. Also, the MI value with the dominant fungal antigen *A. fumigatus* was distinctly lower compared to those with bacterial antigens (Tab. 3). Inhalation challenge with increasing concentrations of *Aspergillus fumigatus* allergen up to 20 µg/ml caused neither specific symptoms nor spirometric and blood morphology changes. Computer examination of the chest before and after the inhalation challenge did not show significant differences.

Final diagnosis. On the basis of the obtained results (anamnesis positive for work-related symptoms, the nature of these symptoms, histopathological examination results), the final diagnosis of organic dust toxic syndrome (ODTS) was established with the recommendation of avoidance of exposure to organic dusts.

DISCUSSION

The described cases provide evidence that occupational exposure to stored tobacco products could be a cause of work-related respiratory symptoms which may occur in customs officers (as presented above), warehousemen, transportation workers, and others. The environmental examinations showed that the symptoms may be associated with the exposure to airborne fungi and bacteria, mostly moulds of the genera *Penicillium* and *Aspergillus*, which may develop on damp floors and in damp cigarettes, and are released to the air of stores. As the patients under study did not show a specific sensitization to fungal allergens (except for a weak reaction in the test for inhibition of leukocyte migration), the symptoms were evoked most probably by non-specific action of low molecular fungal metabolites, such as mycotoxins or VOCs (volatile organic compounds) [12], with the possible contribution of bacterial endotoxin. However, as there is no direct proof to support this presumption, and the effects of nicotine and other tobacco constituents cannot be excluded, further studies are needed to elucidate etiopathogenesis of the disorders associated with the exposure to stored tobacco.

Acknowledgements

The skillful assistance of Ms. Wiesława Lisowska and Ms. Halina Wójtowicz in performing the study is gratefully acknowledged.

REFERENCES

1. Brandys RE, Brandys GM: *Worldwide Exposure Standards for Mold and Bacteria – Historical and Current Perspectives*. Occupational & Environmental Health Consulting Services, Inc., Hinsdale, IL 2003.

2. British Thoracic Society: Guidelines on flexible bronchoscopy. *Thorax* 2001, **56(Suppl. 1)**, i1-i21.

3. Dutkiewicz J: Airborne bacteria as occupational allergens. **In:** Frankland AW, Stix E, Ziegler H (Eds): *The 1st International Conference on Aerobiology, Munich 13–15 August 1978: Proceedings*, 232-242. Umweltbundesamt Berichte 5/79. Erich Schmidt Verlag, Berlin 1980.

4. Dutkiewicz J, Jabłoński L: *Biologiczne Szkodliwości Zawodowe* [*Occupational Biohazards*]. PZWL, Warsaw 1989 (in Polish).

5. Golec M, Skórska C, Mackiewicz B, Dutkiewicz J: Immunologic reactivity to work-related airborne allergens in people occupationally exposed to dust from herbs. *Ann Agric Environ Med* 2004, **11**, 121-127.

6. Górny RL, Dutkiewicz J: Bacterial and fungal aerosols in indoor environment in Central and Eastern European countries. *Ann Agric Environ Med* 2002, **9**, 17-23.

7. Huuskonen MS, Husman K, Järvisalo J, Korhonen O, Kotimaa M, Kuusela T, Nordman H, Litting A, Mäntyjärvi R: Extrinsic allergic alveolitis in the tobacco industry. *Br J Ind Med* 1984, **41**, 77-83.

8. Larsson L, Szponar B, Ridha B, Pehrson C, Dutkiewicz J, Krysińska-Traczyk E, Sitkowska J: Identification of bacterial and fungal components in tobacco and tobacco smoke. *Tobacco Induced Diseases* 2008, Epub 4:4.

9. Milanowski J, Dutkiewicz J, Potoczna H, Kuś L, Urbanowicz B: Allergic alveolitis among agricultural workers in eastern Poland: A study of twenty cases. *Ann Agric Environ Med* 1998, **5**, 31-43.

10. Minárik L, Skalský T, Heyná A, Šteklačova E: Plucne aspergilózy profesionálneho pôvodu [Occupational aspergillosis of the lung]. *Rozhl Tuberk* 1969, **29**, 169-173 (in Slovak).

11. Reiman M, Uitti J: Exposure to microbes, endotoxins and total dust in cigarette and cigar manufacturing. *Ann Occup Hyg* 2000, **44**, 467-473.

12. Samson RA, Flannigan B, Flannigan ME, Verhoeff AP, Adan OCG, Hoekstra ES (Eds): *Health Implications of Fungi in Indoor Environments*. Elsevier, Amsterdam 1994.

13. Sebastian A, Larsson L: Characterisation of the microbial community in indoor environments: a chemical-analytical approach. *Appl Environ Microbiol* 2003, **69**, 3103-3109.

14. Sebastian A, Szponar B, Larsson L: Characterisation of the microbial community in indoor environments by chemical marker analysis: an update and critical evaluation. *Indoor Air* 2005, **15(Suppl 9)**, 20-26.

15. Skórska C, Sitkowska J, Krysińska-Traczyk E, Cholewa G, Dutkiewicz J: Exposure to airborne microorganisms, dust and endotoxin during processing of peppermint and chamomile herbs on farms. *Ann Agric Environ Med* 2005, **12**, 281-288.

16. World Health Organization: *WHO Report on the Global Tobacco Epidemic, 2008: The MPOWER Package*. Geneva, World Health Organization 2008.