

ALLERGIC ALVEOLITIS DUE TO HERB DUST EXPOSURE

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Abstract: We report an episode of allergic alveolitis in a female farmer due to massive exposure to organic dust contaminated with microorganisms during threshing of herbs (thyme). The patient's medical history, the results of exposure test, inhalation challenge, and bronchoalveolar lavage suggested the diagnosis of allergic alveolitis.

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CASE DESCRIPTION

Background. In March 1998, a screening study was performed to assess correlation between exposure to bioaerosols at work and condition of respiratory system of individual farmers from eastern Poland. Among them, a subgroup of 47 persons working at herb threshing were examined. The inquiry examination was carried out using two questionnaires: the Ferris Questionnaire recommended by American Thoracic Society for epidemiological studies concerning diseases of respiratory system, and a questionnaire prepared by the Institute of Agricultural Medicine in Lublin, dealing with exposure to organic dust and work-related symptoms. The physical examination, respiratory function tests, skin-prick test with the use of 4 environmental microbial antigens (EMA) produced in the Institute of Agricultural Medicine in Lublin, agar gel double diffusion test with 12 EMA, and test for inhibition of leukocyte migration by the whole blood capillary microculture method with 4 EMA were performed using the techniques described by Dutkiewicz *et al.*, and Skórska *et al.* [16, 18]. Additionally, some persons (with

work-related symptoms and abnormal spirometry values) had an X-ray examination of the chest.

Exposure of examined farmers to bioaerosols was assessed by the determination of viable microorganisms, total microorganisms, total dust and endotoxin as described by Krysińska *et al.*, and Mackiewicz [9, 12]. Air samples for viable bacteria and fungi were taken on blood agar (for Gram-positive bacteria), EMB agar (for Gram-negative bacteria) on tryptic soya agar (for thermophilic actinomycetes) and on malt agar (for fungi) with a custom-designed particle-sizing slit sampler, enabling estimations of the total and respirable fractions of the microbial aerosol. Concentration of the total microorganisms (dead and living) in the air was determined microscopically by the epifluorescence method, the concentration of dust by gravimetric method and the concentration of endotoxin by *Limulus* test.

Exposure. On 11 March 1998, the female patient J.K. worked for four hours together with six other persons at the threshing of dried thyme (*Thymus vulgaris*). The herbs had been harvested in August 1997 and stored in a barn.

Table 1. Exposure of the patient J.K. to bioaerosols during threshing of thyme.

Total microorganisms (cells/m ³)	Viable microorganisms (cfu/m ³)					Respirable fraction (%)	Dust (mg/m ³)	Endotoxin (µg/m ³)
	Gram-positive bacteria	<i>Pantoea agglomerans</i>	Other Gram-negative bacteria	Therm. actinomycetes	Fungi			
3.7 × 10 ⁷	29.6 × 10 ⁴	1.0 × 10 ⁴	6.4 × 10 ⁷	0.6 × 10 ⁴	9.7 × 10 ⁴	74.2	1319.6	2448.8

Respirable fraction, expressed as a percent of the total count of viable microbes, presents the amount of microorganisms below 3 µm which are potentially able to penetrate down to alveoli. Therm. = Thermophilic

During machine threshing of whole dried plants in the barn, the stems were removed and leaves transferred to another machine for final sorting and cleaning. The bioaerosol examination performed during work revealed an extremely large pollution of the air with dust and endotoxin and large pollution with microorganisms (Tab. 1).

The patient J.K. was chosen from this seven-person group of workers because of abnormal spirometry values and strongly positive work-related symptoms (described below as a positive exposure test). Despite unusually high exposure, the other workers did not report work-related symptoms and did not show a fall of spirometric values except for one male (the husband of the patient) who showed a weak response. The patient J.K. was advised to visit the Clinic of Lung Diseases of Medical Academy in Lublin for detailed further examination.

Medical history. On 12 May 1998, the patient J.K. - a 51-year old female farmer, a non-smoker, 162 cm in height, and weighting 80 kg - was admitted to the Clinic of Lung Diseases in Lublin. She has been treated for 10 years for arterial hypertension. She reported that for the last 3–4 years she has suffered ailments related to exposure to organic dusts: dry cough, dyspnoea, chest tightness, chest pain, headache, tiredness, rhinostegnosis and burning sensation of conjunctivas. These symptoms occurred during the work and persisted for 10–12 hours after it.

Results of medical examinations

Routine clinical examinations. Physical examination revealed numerous bilateral crepitations at the base of lungs. Laboratory data: Hb - 8.86 mmol/l, Hct - 0.43, RBC - 5.20 T/l, WBC - 6.7 G/l, neutrophils - 52%, eosinophils - 2%, lymphocytes - 45%, monocytes - 1%. Blood gases: pO₂ - 71.8 mm Hg, pCO₂ - 44.0 mm Hg, pH - 7.403, O₂ SAT - 91.7%. The X-ray examination of the chest revealed intensified interstitial lung markings and reticular changes in the lower parts of the lung (Fig. 1). The spirometry revealed decreased ventilation reserve of restrictive type: FEV₁ - 1380 ml (55% of pred. value), VC - 1670 ml (55% of pred. value), FEV₁ % VC - 82.7%, MEF 25 - 1430 ml/s (93% of pred. value), MEF 50 - 3150 ml/s (82% of pred. value), MEF 75 - 3540 ml/s (64% of pred. value), PEF - 3650 ml/s (58% of pred. value).

Exposure test. After 4-hours working at thyme threshing and massive exposure to thyme dust, the patient complained of severe dyspnoea and dry cough. Her appearance was changed because of breathing difficulties and redness of skin. The crepitations at the base of lungs were intensified. The spirometric values of the patient dropped by 20-30% (Fig. 2).

Allergological tests. The results of skin prick tests with the antigens of *Saccharopolyspora rectivirgula* (synonyms: *Micropolyspora faeni*, *Faenia rectivirgula*), *Streptomyces albus*, *Pantoea agglomerans* (synonyms: *Erwinia herbicola*, *Enterobacter agglomerans*) and *Aspergillus fumigatus* were negative. Negative results were also obtained from the double diffusion test for serum precipitins with the antigens of *Saccharopolyspora rectivirgula*, *Thermoactinomyces vulgaris*, *Streptomyces albus*, *Bacillus subtilis*, *Arthrobacter globiformis*, *Pantoea agglomerans*, *Acinetobacter calcoaceticus*, *Alcaligenes faecalis*, *Aspergillus fumigatus*, *Aspergillus candidus*, *Penicillium citrinum* and *Alternaria alternata*.

The test for inhibition of leukocyte migration in the presence of specific antigen was performed with the extracts of four environmental microbial antigens. A positive



Figure 1. Chest radiogram of patient J.K. Reticular changes are seen in the lower parts of the lungs.

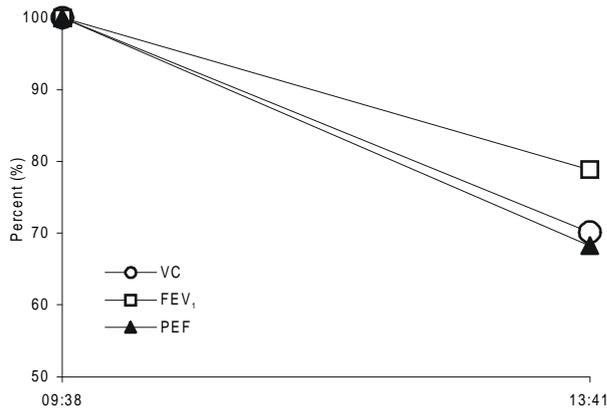


Figure 2. Results of the exposure test to thyme dust in patient J.K. Note 30% drop in VC and PEF and over 20% drop in FEV₁ after 4 hours of work at thyme threshing.

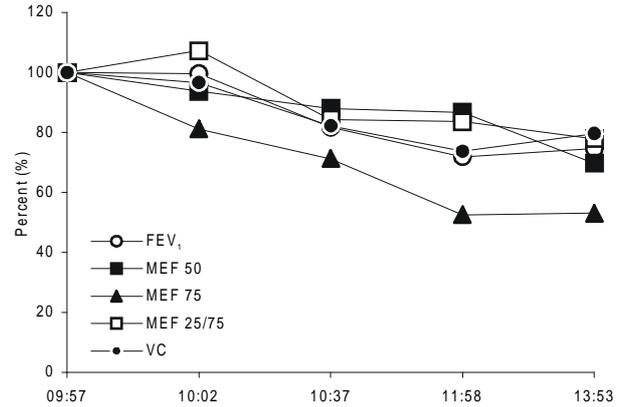


Figure 3. Results of the inhalation challenge with the extract of *Pantoea agglomerans* in patient J.K. Note the significant decrease of spirometric values, in particular a 50% drop of MEF 75 at 2-4 hours post challenge.

result was obtained only with the antigen of *Pantoea agglomerans*. The results of this test with the antigens of *Saccharopolyspora rectivirgula*, *Arthrobacter globiformis* and *Aspergillus fumigatus* were negative.

Cytokine determination. The ELISA tests for the presence of IL-1, IL-6 and TNF α gave negative results.

Inhalation challenge. The bronchial provocation test was performed with the extract of *Pantoea agglomerans* produced in the Institute of Agricultural Medicine. The lyophilised extract was dissolved in 0.85% NaCl in the concentration of 50 μ g/ml. The test was performed as described by Milanowski *et al.* [16], by 20 inhalations of the aerosolised solution. The challenge revealed a strongly positive reaction with significant decrease of FEV₁, MEF 50, MEF 75 and VC values during the second and the third hour after inhalation (Fig. 3). In addition, the body temperature was raised to 37.7°C, dyspnoea increased, tachycardia and general discomfort occurred.

Bronchoalveolar lavage (BAL) was performed as described by Milanowski *et al.* [16], by the instillation of 140 ml 0.9% NaCl into the 5th segment of right lung through a fiberoptic bronchoscope. As much as 105 ml of the instilled fluid was recovered. The composition of the BAL fluid was following: lymphocytes -12.8%, macrophages -86.5%, granulocytes - 0.7%.

Flow cytometry of BAL fluid and peripheral blood. The results are presented in Table 2. The most important finding is the dominance of CD8⁺ over CD4⁺ in BAL fluid amounting to 2.8:1.

Final diagnosis and treatment. On the basis of a complete clinical picture, chronic form of extrinsic allergic alveolitis was diagnosed. During hospitalisation the following treatment was applied: Euphillinum, Hydrocortisone and Enalapril. After treatment, a relief in

dyspnoea was observed. The patient was discharged from the hospital in a satisfactory general condition, and was ordered to avoid any contact with herb dust.

DISCUSSION

Allergic alveolitis is a disease that consists of many entities characterised by a very similar clinical picture, but evoked by different etiological factors [5, 15]. The pathogenesis of this disease is not precisely known. Probably the specific mechanisms are involved: allergic reaction of type IV (delayed), allergic reaction of type III (Arthus), allergic reaction of type I (immediate) [1], and non-specific mechanisms: foreign-body reaction, alternative complement activation and direct inflammatory cells activation, chiefly alveolar macrophages [13, 14]. Allergic alveolitis may occur in acute or chronic form and depends

Table 2. The results of flow cytometry in blood and BAL fluid.

Lymphocytes	Blood	BAL fluid
CD3+	83.5%	90.0%
CD19+	5.3%	1.3%
CD4+	44.2%	24.2%
CD8+	38.6%	67.2%
CD4+8+	1.9%	2.1%
NK cells	8.4%	0.9%
HLA DR+	5.3%	4.2%
HLA DR+, CD3+	8.7%	5.6%
CD25 α +	3.1%	5.1%
CD 122+	3.4%	0.3%
CD 45RO+	2.0%	12.5%
CD 45RO+, CD4+	5.7%	5.7%
CD95+	51.9%	46.3%

on the character of exposure (short or long) and kind of antigens [8, 14, 16, 17]. In Poland, this disease is most often caused by contact with cereals, hay, poultry, pigeons and other birds [5, 15, 16].

In the case described in the present paper, the ailments were evoked by dust released during threshing of herbs. Herbs are commonly cultivated by farmers in eastern Poland and delivered to industrial plants for production of spices, drugs and cosmetics. Earlier studies performed by our group in a herb processing plant revealed very high concentrations of dust, microorganisms and endotoxin [4] and occurrence of work-related respiratory symptoms among workers [11]. Nevertheless, none of examined workers was diagnosed as having typical allergic alveolitis [6]. To the best of our knowledge, there is only scant or no information about the cases of allergic alveolitis caused by exposure to herb dust.

In the present work we have diagnosed allergic alveolitis in a farmer heavily exposed to herb dust. We realise that this diagnosis has some limitations, as the patient did not reveal all the features ascribed to allergic alveolitis. No specific precipitins were found, there was not prevalence of lymphocytes in BAL fluid and we were not able to confirm the diagnosis by diffusion capacity examination. Nevertheless, we believe in the reliability of our diagnosis on the basis of the strong positive exposure test and inhalation challenge, positive test for inhibition of leukocyte migration, significant fall in spirometric values, typical work-related symptoms and distinct prevalence of CD8+ over CD4+ in BAL fluid [2].

The extract of *Pantoea agglomerans* was chosen for inhalation challenge on the basis of the positive result of the test for specific inhibition of leukocyte migration. The strong positive result of the inhalation challenge indicates that most probably *Pantoea agglomerans* was one of the main causative agents of the disease in the examined patient. This Gram-negative epiphytic bacterium occurs in large quantities on grain and has been identified as the most common cause of allergic alveolitis among grain farmers in eastern Poland [10, 16]. *P. agglomerans* was described over 20 years ago as a source of endotoxin possessing high biological activity [3]. The airborne endotoxin produced by this organism may either evoke ODS-like symptoms by itself or contribute to evoking allergic alveolitis as an adjuvant factor. Although the concentration of this organism in the air polluted with thyme dust was not large compared to other microorganisms, the extremely large concentration of endotoxin suggests that the initial concentration of *P. agglomerans* in thyme could be high, and decreased during the more than half year period of storage. Despite the strong endotoxigenic properties of *P. agglomerans*, the positive result of inhalation challenge was not due to non-specific endotoxin activity. The solutions used for test did

contain endotoxin but in small amounts (below 1 ng/ml) which do not cause non-specific decrease in FEV₁, as has been proved in healthy persons and many patients.

The result of this work suggests that exposure to herb dust could be a cause of allergic alveolitis and that *Pantoea agglomerans* is most probably one of the etiologic factors.

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