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ORIGINAL ARTICLE

Acute hypersensitivity pneumonitis in woodworkers caused by inhalation of birch dust contaminated with *Pantoea agglomerans* and *Microbacterium barkeri*

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Abstract

Case description. Five workers (2 males and 3 females) employed in a furniture factory located in eastern Poland developed hypersensitivity pneumonitis (HP) after the pine wood used for furniture production was replaced by birch wood. All of them reported onset of respiratory and general symptoms (cough, shortness of breath, general malaise) after inhalation exposure to birch dust, showed crackles at auscultation, ground-glass attenuations in HRCT examination, and lymphocytosis in the BAL examination. The diagnosis of acute HP was set in 4 persons and the diagnosis of subacute HP in one.

Identification of specific allergen. Samples of birch wood associated with evoking disease symptoms were subjected to microbiological analysis with the conventional and molecular methods. Two bacterial isolates were found to occur in large quantities (of the order 10^8 CFU/g) in examined samples: Gram-negative bacterium of the species *Pantoea agglomerans* and a non-filamentous Gram-positive actinobacterium of the species *Microbacterium barkeri*. In the test for inhibition of leukocyte migration, 4 out of 5 examined patients showed a positive reaction in the presence of *P. agglomerans* and 2 in the presence of *M. barkeri*. Only one person showed the presence of precipitins to *P. agglomerans* and none to *M. barkeri*. In the inhalation challenge, which is the most relevant allergological test in the HP diagnostics, all patients reacted positively to *P. agglomerans* and only one to *M. barkeri*. The results indicate that *P. agglomerans* developing in birch wood was the main agent causing HP in the workers exposed to the inhalation of dust from this wood, while the etiologic role of *M. barkeri* is probably secondary.

Conclusion. The results demonstrate that apart from fungi and filamentous actinobacteria, regarded until recently as causative agents of HP in woodworkers, Gram-negative bacteria and non-filamentous actinobacteria may also elicit disease symptoms in the workers processing wood infected with large amounts of these microorganisms. The results obtained also seem to indicate that cellular-mediated reactions are more significant for causing disease symptoms compared to those that are precipitin-mediated.

Key words

hypersensitivity pneumonitis, wood dust, bacteria, *Pantoea agglomerans*, *Microbacterium barkeri*, inhalation challenge, occupational exposure

INTRODUCTION

Hypersensitivity pneumonitis (HP) is an infrequent, immune-mediated disease of the lower parts of the lungs, initiated by the interaction of immunocompetent cells with large quantities of offending allergens, mostly belonging to fungi, actinobacteria, nontuberculous mycobacteria and bird proteins, which are repeatedly inhaled on exposure to various organic dusts. This interaction leads to lymphocytic and often granulomatous inflammation of the peripheral airways, alveoli and surrounding interstitial tissue and at long-lasting exposure, to fibrosis [1–6]. The immunopathogenic allergens are usually inhaled at work, hence the subunits of the disease are named after occupation at risk, such as farmer's lung or bird breeder's lung [1, 4, 6].

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One of the occupational groups at high risk of HP are workers exposed to wood dust, such as wood cutters, sawmill workers, furniture factory workers, carpenters and joiners [7]. Moreover, people exposed to wood dust at home while using wood chips as a fuel, remodeling wooden houses, or simply while exposed to rotten wood, may also develop HP [8–11]. To the best of the authors' knowledge, until recently at least 21 HP cases or groups of cases caused by the exposure to wood dust have been described. The first description was published in 1932 by Towey at al. [12], who described a pulmonary disease in sawmill workers caused by fungi *Cryptostroma corticale* which developed under the bark of maple trees, and released in large amounts during debarking of logs in sawmills. Thirty-four years later, Emanuel et al. [13] classified this condition as a HP subunit 'maple bark disease'. Subsequent years brought numerous descriptions of various HP-conditions caused by the inhalation of wood dusts, of which the most known are: 'suberosis' a disease evoked by exposure to mouldy cork processed in Portugal, and caused mostly by *Penicillium glabrum* (synonym: *P. frequentans*) [14], and 'wood trimmer's disease' elicited by exposure of sawmill workers in Scandinavia to planks overgrown by mould fungi, mostly *Rhizopus microsporus* after kiln-drying [15–17]. Today, these diseases no longer pose an occupational health problem due to significant improvements in working conditions [17].

Until recently, in at least 21 papers, the main causative agents eliciting HP symptoms after exposure to wood dust have been successfully determined, of which, the most numerous were fungi responsible for 17 single or group disease cases (80.9%), specifically: *Penicillium* spp. (3 cases) [14, 18, 19], *Penicillium glabrum* and *Neurospora* (*Chrysonila*) *sitophila* (1) [20], *Rhizopus microsporus* (3) [15–17], *Aspergillus* spp. (2) [8, 21], *Trichoderma koningii* (2) [22, 23], *Aureobasidium pullulans* and *Graphium* spp. (1) [24], *Alternaria* spp. (1) [25], *Paecilomyces* spp. (1) [26], *Mucor* spp. (1) [27], *Leucogyrophana pinastri* (1) [10], and *Serpula lacrymans* (1) [11]. The remaining organisms identified as HP-causing agents in people exposed to wood dust were filamentous bacteria classified within phylum Actinobacteria (9.5%): *Thermoactinomyces vulgaris* (1) [28] and *Saccharomonospora viridis* (1) [29], and Gram-positive bacterium classified within phylum Firmicutes (4.8%): *Bacillus subtilis* (1) [9]. In one case the allergen of the wood Cabreuva itself (4.8%) has been identified as an adverse allergen [30]. So far, none Gram-negative bacteria were identified as causative agents of HP caused by the inhalation of wood dust.

The presented study describes typical cases of acute HP in the workers of a furniture factory exposed to the inhalation of dust from birch wood. It was found that this wood had been colonized by large amounts of non-filamentous bacteria, mostly not belonging to Actinobacteria, and proved for the first time that Gram-negative bacteria belonging to phylum Proteobacteria could be a cause of HP in people exposed to wood dust.

MATERIALS AND METHODS

Patients

In May 2015, a worker employed in the furniture factory located in a village in the Lublin Region of eastern Poland was admitted to clinic with symptoms corresponding to HP. Next, between May – November 2017, 4 other workers of this factory were also hospitalised with similar symptoms. The initial diagnosis of hypersensitivity pneumonitis (HP) was established and all the patients were subjected in 2017–2019 to detailed clinical and allergological examinations in order to confirm or reject this diagnosis.

Altogether, 5 patients with the diagnosis of HP (further marked as patients No. 1–5) were examined. The group consisted of 3 females and 2 males, aged 40–53 yrs, mean 46.6 ± 6.0 yrs. Four persons never smoked cigarettes, one person was an ex-smoker. The job duration of the patients was 4–16 years, and all of them had been exposed during work to the inhalation of wood dust. During the first 3–10 years of their employment, the sawmill produced furniture made of Scots pine (*Pinus sylvestris*) and the exposure to dust from pine wood had not caused any adverse symptoms. After changing production from pine to white warty birch (*Betula pendula*) wood, the patients began to feel complaints related directly to work on exposure to dust of birch origin. The period of exposure to birch dust ranged from 1–4 yrs (Tab. 1).

Clinical examination

Routine clinical examination comprised:

1) detailed anamnesis of symptoms and time of exposure to wood dust;
2) physical examination;
3) laboratory examination;
4) chest X- ray;
5) high resolution computed tomography (HRCT);
6) bronchoscopy with bronchoalveolar lavage (BAL);
7) pulmonary function tests comprising: spirometric measurements (FVC, FEV_1, FEV_1%FVC, TLC, RV) with the LUNGTEST 1000 (MES, Kraków, Poland), measurements of diffusion lung capacity for carbon monoxide (DLCO), and bodypletysmography;
8) arterial-blood gas test with the RAPIDLAB 348 (Siemens, Germany).

Bronchoalveolar lavage (BAL). Bronchoalveolar lavage was performed in accordance with the ATS guidelines [31]. During flexible bronchoscopy with the Olympus BF-1T180

Table 1. General characteristics of the group of patients diagnosed with hypersensitivity pneumonitis (HP)

Patient No.	Gender	Age	Smoking	Type of wood reported to cause symptoms	Total job duration (years)	Job duration at processing birch wood (years)
1	M	53	No	birch	16	4
2	F	43	Ex	birch	8	3
3	F	53	No	birch	11	4
4	F	44	No	birch	4	1
5	M	40	No	birch	12	2

M – male; F – female; Ex – ex-smoker

videobronchoscope, the device was placed in a wedge position in middle lobe: lateral (RB4) or medial (RB5). 150 ml normal saline was instilled through the endoscope, divided into 7 aliquots. Negative suction pressure was performed. From total retrieved volume, 20 ml was sent to cellular analysis. The lavage was filtered through gaze immediately after the absorption and centrifuged for 5 min at 1,300 g. The precipitate was resuspended in 1 ml of sterile saline, added to cytometer tubes in the amount of 100 µl per tube and incubated with fluorescently-labelled antibodies for 20 min. After incubation, the cells were again suspended in 1 ml of sterile saline, centrifuged and 50 µl of saline was added to the precipitate. The data on particular cell subpopulations were obtained during regular diagnostic BAL analysis. Two-colour (FITC and PE) BD Simultest IMK Plus (BD Biosciences, USA were used to analyze T-cell subpopulations on BD FACSCalibur cytometer, using CellQuest Pro analysis software. For proper analysis of cell populations, lymphocyte purity was kept at >85% and recovery within gate at >90%.

Microbiological examination of birch wood

Culture. Aiming to determine potential microbial agents causing disease symptoms, the samples of birch wood associated with evoking respiratory and general symptoms in patients were examined. Cylindrical blocks of 18 cm long, 6 cm wide and 3 cm thick were cut from the transverse section of birch log. Microbiological samples of wood were taken from various sites of the block with an original, manually operated drilling device [US patent 5,078,553 assigned Jan. 7, 1992] that collects the pulverized wood into a flask attached beneath the bit in a one-step sterile process [32]. 100 mg of each sample was suspended in 10 ml of sterile saline (0.85% NaCl) containing 0.1% (v/v) of Tween 80, and after vigorous shaking, serial 10-fold dilutions in saline were made up to 10^{-8}. The concentration and species composition of microbiota were determined by dilution plating, as reported earlier [33], with some modifications. The 0.1 ml aliquots of each dilution were spread on duplicate sets of the following media:

- Blood agar plates for estimation of the total mesophilic bacteria.
- Eosin methylene blue (EMB) agar (*Difco*) plates for estimation of Gram-negative bacteria.
- Tryptic soya agar (*Difco*) plates for estimation of thermophilic actinobacteria.
- Malt agar (*Difco*) plates for estimation of fungi.

The blood agar and EMB plates were subsequently incubated for one day at 37°C, 3 days at 22°C and 3 days at 4°C. The tryptic soya agar plates were incubated for 5 days at 55°C. The malt agar plates were subsequently incubated for 4 days at 30°C and 4 days at 22°C. The grown colonies were counted and differentiated, and the data were reported as CFU (colony forming units) per gram of the sample. The total concentration of microorganisms per gram of sample was obtained by the addition of the concentrations of mesophilic bacteria, thermophilic actinobacteria and fungi. Bacterial isolates were identified with microscopic and biochemical macro-methods, as recommended by Bergey's Manual [34], and with metabolic micro-tests ENTEROtest 34 N (Erba Lachema, Brno, Czech Republic) and Biolog Microbial Identification Gen III System (BIOLOG Inc., Hayward, CA, USA). Fungi were classified with microscopic methods, according to Samson et al. [35], Watanabe [36], and Larone [37].

The species composition of the total microbiota (mesophilic bacteria, thermophilic actinobacteria, and fungi) was determined and presented in percentage.

Molecular identification of microorganisms. For the identification of prevailing Gram-negative isolate, total DNA was isolated from culture using Qiamp DNA Mini Kit (Qiagen, USA) according to the manufacturer's instructions. *Pantoea agglomerans* DNA was detected by amplification of 16S rRNA gene fragment using the universal oligonucleotide primers 16S start and 16S stop according to Chun and Goodfellow [38]. DNA sequencing of amplicons was performed using ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA, USA), with the use of Abi Prism Big Dye Terminator v. 3.1. Cycle Sequencing Kits and Big Dye XTerminator Purification Kit (Applied Biosystems). Sequences were analyzed and compared with sequences in the GenBank database using the BLAST server at the National Center for Biotechnology Information (Rockville Pike, Bethesda, Maryland, USA).

For identification of the prevailing Gram-positive isolate to the species level, the procedure of determining the 16S rRNA sequence described in Pasciak et al. [39] with the modification that the PCR product was sequenced directly, was used.

Allergological tests

The inhibition of leukocyte migration (MIF), and agar-gel precipitation tests, as well as inhalation challenge were performed for all patients. The tests were based on antigens derived from two bacterial isolates which prevailed in the samples of birch wood associated with disease symptoms (see the Results section below): *Pantoea agglomerans* and *Microbacterium barkeri*.

Moreover, in the agar-gel precipitation test, another 10 microbial antigens were used, derived either from standard strains indicated in the literature as important agents causing HP, or from strains noted in own studies as associated with HP-causing materials: antigens from 4 strains of non-filamentous, mesophilic Gram-negative and Gram-positive bacteria: *Acinetobacter baumannii*, *Pantoea agglomerans*, *Aeromonas* sp., and *Arthrobacter globiformis*; antigens from 4 strains of thermophilic filamentous bacteria (Actinobacteria): *Saccharopolyspora rectivirgula* (syn. *Micropolyspora faeni*), *Thermoactinomyces vulgaris*, 2 strains of *Thermomonospora*-like actinobacteria (X-1, X-2) isolated from compost used for cultivation of mushrooms; antigens from 2 strains of fungi: *Aspergillus fumigatus* and *Penicillium citrinum* [2, 8, 33].

Preparation of antigens. Antigens for all the tests were prepared according to the unified procedure and used in different concentrations, depending on the test. All antigens were prepared in the Institute of Rural Health in Lublin.

In all tests, lyophilized saline extracts of microbial cell mass were used. In the case of *Pantoea agglomerans*, *Microbacterium barkeri* and other mesophilic, non-filamentous bacteria the biomass was harvested from full strength tryptic soya agar cultures which had been incubated in Roux bottles at 34°C for 48 hrs. The mass was then extracted in saline (0.85% NaCl) in the proportion 1:2.5 for 48 hrs at 4°C, with intermittent disruption of cells with the UP 200H Hielscher ultrasonic homogenizer (Hielscher Ultrasonic Technology, Germany). Afterwards, the supernatant was separated by centrifugation,

dialysed against distilled water for 24 hrs, concentrated by evaporation to 0.1–0.15 of previous volume and lyophilized.

The mass of thermophilic actinobacteria was harvested from nutrient broth cultures which had been incubated in Erlenmeyer flasks at 55°C for 4 weeks, homogenized and further extracted and processed as above. The mass (mycelium) of fungi was harvested from sugar broth cultures which had been incubated in Erlenmeyer flasks at 30°C for 4 weeks, homogenized and further extracted and processed as above [33].

Test for inhibition of leukocyte migration in the presence of specific antigen (MIF test). The test was performed by the whole blood capillary microculture method, according to Bowszyc et al. [40], with some modifications. Patient's blood and Parker's culture medium were added in the volumes of 0.5 ml and 0.12 ml, respectively, to 2 silicon test tubes. Then, 0.12 ml of the antigen solution in the concentration of 200 μg/ml was added to one tube, while 0.12 ml of the diluent (PBS) was added to the other as a control. Both suspensions were incubated for 30 min at room temperature and thereafter distributed to heparinised glass capillaries 75 × 1mm. Capillaries were sealed at both ends with a 4:1 mixture of paraffin and vaseline, centrifuged for 10 min at 1,500 rpm and fastened tangentially on microscopic slides with sticky tape at an angle of 10°. The microcultures thus obtained were incubated for 4 hrs at 37°C in a humid chamber. The leukocyte migration distances, visible as distinct white zones, were measured under the binocular microscope. The results were expressed as a migration index (MI), e.g. the ratio of the mean migration distance of leukocytes in microcultures with antigen, to the analogical distance in microcultures without antigen. The test was considered as positive at the MI equal to 0.799 or lower.

Agar-gel precipitation test. The test was performed by the Ouchterlony double-diffusion method in pure 1.5% agar (*Difco*). The patient's serum was placed in the central well and antigens, at the concentration of 30 mg/ml, in peripheral wells. Each serum was tested twice: not concentrated, and 3-fold concentrated for the detection of low level of precipitins. The plates were incubated for 6 days at room temperature, then washed in saline and in 5% sodium citrate solution (to prevent false positive reactions), and stained with azocarmine B [41].

Inhalation challenge. All patients were informed in detail about the aim and possible effects of the test and gave written consent for its performance. The tests were performed with antigens of 2 bacterial strains isolated from the birch wood causing HP symptoms: *Pantoea agglomerans* and *Microbacterium barkeri*. The antigens were dissolved in 0.85% NaCl at a very low concentration of 20 μg/μl, sterilized and checked for sterility and lack of toxicity. The antigenic solutions were administered to patients by the LUNGTEST 1000 (Inhalation Allergological Provocation System, MES, Poland) device during 20 breaths. The absorbed dose of allergen was about 2.16 μg. The tests with bacterial allergens were preceded with the control test with saline (PBS), applied for the measurement of diurnal variability of spirometric values. At each test, all the measurements and observations, including spirometry, blood pressure, pulse rate, body temperature, peripheral blood analyses, auscultation and observations for the appearance of general and respiratory symptoms were performed before the test (at 08:00) and after 2 min, 30 min, 2 hrs, 4 hrs, 8 hrs and 24 hrs post inhalation challenge. The spirometric measurements comprised determination of forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1). The decrease of FVC and/or FEV_1 by 10% of initial value was considered as a positive result of the test. Peripheral blood analyses comprised erythrocyte sedimentation rate, concentration of leukocytes, blood morphology, oxygen saturation, procalcitonin (PCT) level, C Reactive Protein (CRP) level, and interleukin 6 (IL-6) level.

The use of the inhalation challenge as well as all other diagnostic procedures used in the present study were approved by the Bioethics Commission at the Medical University in Lublin (decision no. KE-0254/258/2017).

Statistical analysis. The results of inhalation challenge were analyzed with chi-square test, using STATISTICA v. 6.0 package (Statsoft, Tulsa, Oklahoma, USA). To obtain reliable results, the values measured in the tests for IL-6 and CRP were multiplied by 10 and 100, respectively.

RESULTS

Clinical characteristics of the patients

The results of clinical examinations are shown in Table 2. All patients reported symptoms related with exposure to birch dust. The frequency of symptoms was: dry cough (in all 5 patients), shortness of breath (in all 5), general malaise (in all 5), chest pain (in 4), fever (in 3), fatigue (in 3), hoarseness (in 1) and blockade of the nose (in 1).

Physical examinations by auscultation found inspiratory crackles in each of 5 patients. The morphological and biochemical blood tests did not show significant abnormalities (data not shown).

Chest X-ray revealed interstitial diffuse changes in 4 patients mostly in lower and middle lung fields (Fig. 1). In HRCT ground-glass attenuations were observed in all 5 patients (Fig. 2). Pulmonary fibrosis to a delicate degree was found in 1 patient (Fig. 3).

Pulmonary function tests showed a small degree of obstruction in one patient and a small reduction of diffusion lung capacity for carbon monoxide (DLCO) in the same patient. In 4 other patients, pulmonary function tests were normal (Tab. 3).

All patients demonstrated a typical lymphocytic alveolitis in BAL examination (Tab. 4). In an arterial-blood gas test an abnormality was found in one patient who showed a decrease of pO_2 value (Tab. 5).

According to the clinical observations and diagnostic tests, 4 patients were diagnosed with acute HP and one with subacute HP.

Microbiological composition of birch wood

The samples of white warty birch (*Betula pendula*) wood associated with work-related symptoms in exposed workers showed an extremely large, site-depending diversity in the concentration and species composition of microbiome. The concentration of microorganisms colonizing a brownish ring in the central part of the wooden blocks was about 10,000 times lower compared to those colonizing the peripheral, yellowish

Table 2. Clinical symptoms and examination results of HP patients

Patient No.	Symptoms	Auscultation	Chest X-ray	HRCT	Diagnosis
1	C, SB, F, FA, GM	crackles	ID	GG, FB	subacute HP
2	C, SB, F, CP, FA, GM, H	crackles	ID	GG	acute HP
3	C, SB, CP, FA, GM, BN	crackles	ID	GG	acute HP
4	C, SB, CP, GM	crackles	normal	GG	acute HP
5	C, SB, F, CP, GM	crackles	ID	GG	acute HP

Symptoms: C – cough; SB – shortness of breath; F – fever; CP – chest pain; FA – fatigue; GM – general malaise; H – hoarseness; BN – blockage of the nose.
Chest X-ray: ID – interstitial diffuse changes.
HRCT: GG – ground-glass attenuation; FB – fibrotic changes.

Table 3. Pulmonary function tests

Patient No.	FVC ml (%N)	FEV1 ml (%N)	FEV1%FVC	DLCO ml (%N)	TLC ml (%N)	RV ml (%N)
1	6520 (116)	4780 (110)	73%	11220(117)	8150(105)	1950(81)
2	3940(119)	3440(127)	87,3%	8530(106)	6290(114)	2970(103)
3	2510(89)	2020(84)	80%	7790(101)	4870(105)	1910(110)
4	4280(126)	3100(105)	72%	7700(87)	4760(109)	1830(109)
5	4340(84)	3210(77)[1]	71,4%	7580(68)[2]	7020(98)	2100(104)

[1] small degree of obstruction; [2] small reduction of DLCO

Table 4. BAL fluid analysis of HP patients

Patient No.	Lymphocytes	Macrophages	Neutrophils	CD4+	CD8+	CD4/CD8
1	78.63%	17.37%	0.36%	20.48%	68.14%	0.3
2	69.98%	21.2%	0.64%	38.24%	48.22%	0.79
3	88.34%	4.16%	1.4%	15.54%	58.21%	0.23
4	30.77%	64.57%	0.56%	49.61%	30.74%	1.61
5	56.01%	18.57%	1.04%	27.9%	48.2%	0.58

Table 5. Arterial – blood gas test of HP patients

Patient No.	pO_2 mmHg	pCO_2 mmHg	Saturation (%)	pH
1	84.6	41.7	96.5	7.418
2	81.1	39.8	96.1	7.426
3	67.9[1]	33.8	94.5	7.467
4	79.5	41.9	95.7	7.402
5	76.3	41.3	95.6	7.423

[1] significant decrease of pO_2

ring (Fig. 4) (Tab. 6). In the peripheral ring, characterized by a large concentration of microorganisms, mesophilic bacteria prevailed (99% of the total count), of which the most common were Gram-negative bacteria belonging to phylum Proteobacteria and the species *Pantoea agglomerans* (38.7%) and *Aeromonas* spp. (16.6%), and Gram-positive, coryneform actinobacterium belonging to the phylum Actinobacteria and species *Microbacterium barkeri* (43.7%). In the central ring with a small concentration of microorganisms, the prevalence of mesophilic bacteria was much lower (58.3%), and the rest of microbiome formed fungi (41.7%). Among mesophilic bacteria, the most numerous were *Pantoea agglomerans* (45.8%) and *Microbacterium barkeri* (10.4%), while among fungi *Penicillium* spp. (22.9%) and *Paecilomyces* spp. (14.6%). No thermophilic actinobacteria were found in the examined samples of birch wood (Fig. 5).

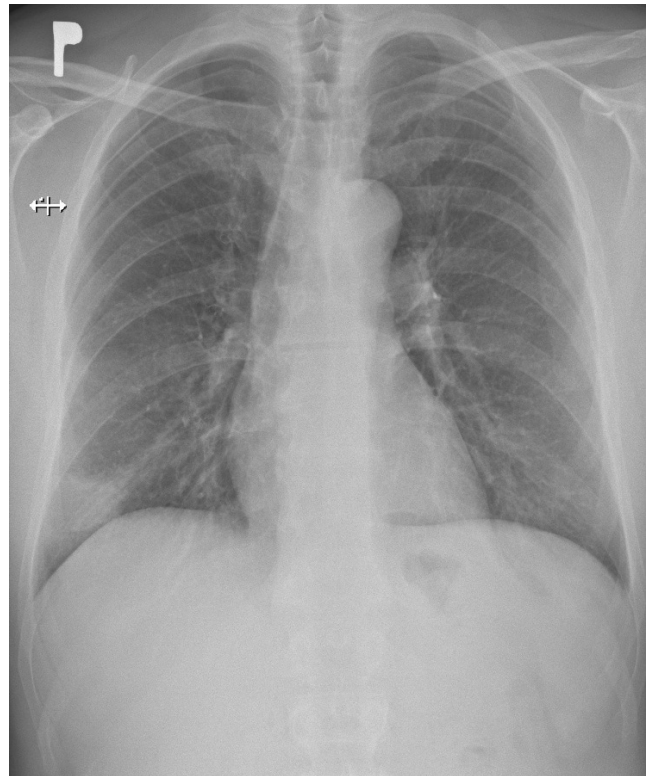

Figure 1. Chest radiogram of patient No. 1. Visible are interstitial diffuse changes with inflammatory infiltrate in the lower part of right lung

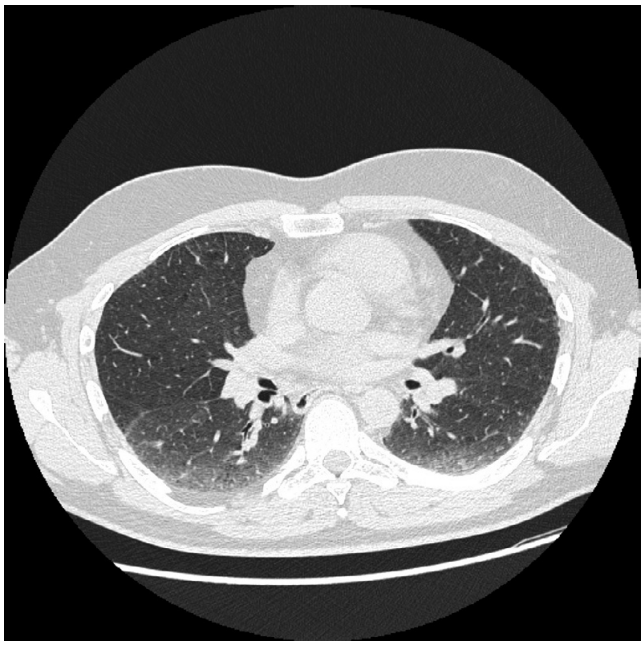

Figure 2. HRCT of patient No. 5. Visible are ground-glass attenuations in the lungs

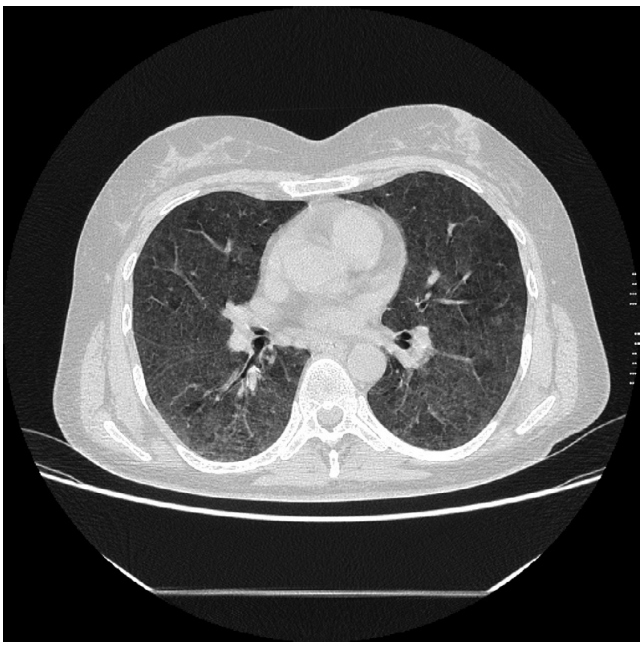

Figure 3. HRCT of patient No. 3. Visible are ground-glass opacities and reticular fibrosis in the lungs

Table 6. Concentration and species composition of microorganisms in blocks of birch wood associated with work-related symptoms

Site A: central, brownish ring[1]			Site B: peripheral, yellowish ring[1]		
No. of microorganisms (× 10^4 CFU/gram)			No. of microorganisms (× 10^4 CFU/gram)		
Microbial species	No.	Percent of total microbiome	Microbial species	No.	Percent of total microbiome
Pantoea agglomerans	2.2	45.8%	*Pantoea agglomerans*	16,050.0	38.7%
Vibrio parahaemolyticus	0.1	2.1%	*Vibrio parahaemolyticus*	0.0	0.0
Aeromonas spp.	0.0	0.0	*Aeromonas* spp.	6,900.0	16.6%
Other Gram-negative bacteria	0.0	0.0	Other Gram-negative bacteria	1.9[2]	<0.001%
Microbacterium barkeri	0.5	10.4%	*Microbacterium barkeri*	18,150.0	43.7%
Total mesophilic bacteria[3]	2.8	58.3%	Total mesophilic bacteria[3]	41,101.9	99.0%
Total thermophilic actinobacteria[4]	0.0	0.0	Total thermophilic actinobacteria[4]	0.0	0.0
Penicillium expansum	0.7	14.6%	*Penicillium expansum*	0.0	0.0
Penicillium glabrum	0.4	8.3%	*Penicillium glabrum*	0.0	0.0
Paecilomyces spp.	0.7	14.6%	*Paecilomyces* spp.	400.0	0.98%
Mucor spp.	0.1	2.1%	*Mucor* spp.	10.0	0.02 %
Trichoderma spp.	0.1	2.1%	*Trichoderma* spp.	0.0	0.0
Total fungi[5]	2.0	41.7%	Total fungi[5]	410.0	1.0%
Total microorganisms	4.8	100%	Total microorganisms	41,511.9	100%

[1] mean of 5 measurements
[2] *Enterobacter amnigenus* biogroup 2, *Enterobacter kobei*
[3] grown on blood agar
[4] grown on tryptic soya agar
[5] grown on malt agar

Identification of the most prevalent bacterial isolates was confirmed by 16S r RNA analysis. The sequence analysis of Gram-negative isolate showed 100% homology to sequences of *Pantoea agglomerans* deposited in GenBank under the accession numbers: MH884044.1, MH883910.1, MH769189.1, CP031649.1, MK026815.1. The 16S rRNA gene sequence of the Gram-positive, coryneform isolate showed the highest similarity (97.50%) to *Microbacterium barkeri* DSM 20145 (GenBank accession no. X77446).

For immunologic tests, including inhalation challenge, 2 species were selected: *Pantoea agglomerans* and

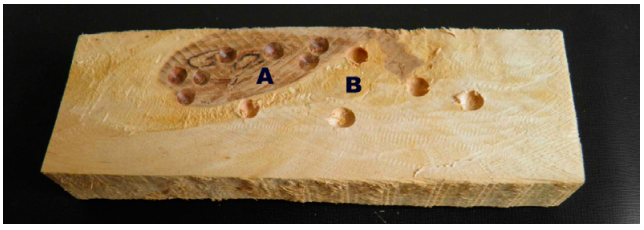

Figure 4. Cylindrical block cut from the transverse section of birch log (*Betula pendula*).
A – central, brownish ring; B – peripheral, yellowish ring. Hollows in the wood indicate sites of sampling with the drilling device

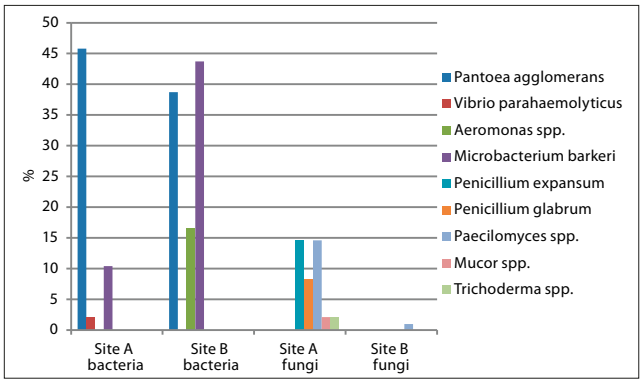

Figure 5. Species composition, expressed as percentage of the total microbiome, in the central site A of wood sample (chacterized by a low concentrations of microbes of the order 10^4 CFU/g), and in peripheral site B (chacterised by a high concentrations of microbes of the order 10^8 CFU/g)

Microbacterium barkeri, which distinctly prevailed in the samples of birch wood associated with causing of work-related symptoms in the exposed workers, and from these strains antigens were prepared.

Allergic reactions in symptomatic workers

Specific inhibition of leukocyte migration. As seen in Table 7, a significant inhibition of leukocyte migration assumed as positive (MI≤0.799) was found in 4 out of 5 workers in the presence of the *Pantoea agglomerans* antigenic extract, and in 2 out of 5 workers in the presence of the *Microbacterium barkeri* extract. The results indicate that antigens derived from these 2 species have a potential to initiate the immunopathologic cellular reaction that is most significant in the pathogenesis of HP. This potential seems to be distinctly greater in the case of *Pantoea agglomerans*.

Agar gel precipitation test. In one serum sample, the precipitin antibodies directed against *Pantoea agglomerans* antigenic extract were present, whereas no precipitins against *Microbacterium barkeri* extract were found (Tab. 7). This seems to indicate that the time of workers' exposure was not sufficiently long enough to produce precipitins that are known markers of long-lasting exposure to adverse antigens, but not necessarily of disease [6]. Of the parallelly tested 10 standard antigens, one worker reacted positively to *P. agglomerans* (the same one who reacted to the birch strain of this organism) and 2 workers to *Arthrobacter globiformis*. None of the workers reacted to the remaining 8 antigens tested (*Acinetobacter baumannii*, *Aeromonas* sp., *Saccharopolyspora rectivirgula*, *Thermoactinomyces vulgaris*, 2 strains of *Thermomonospora*-like actinobacteria isolated from compost, *Aspergillus fumigatus* and *Penicillium citrinum*).

Specific inhalation challenge with *Pantoea agglomerans*. The results of the inhalation challenge with *Pantoea agglomerans* and *Microbacterium barkeri* antigens are summarized in Tables 8 and 9, respectively. As seen in Table 8, all 5 patients showed a positive response to the challenge with allergenic extract of *Pantoea agglomerans*. The decrease of spirometric values was best expressed after 8 hours post challenge when all the patients showed a decrease of forced vital capacity (FVC) below 10% of initial value, assumed as a threshold level (Figs. 6–7). Moreover, all patients showed a highly significant decrease of FVC (P<0.001) when assessed by χ^2 test, indicating a steady lowering during a whole measurement period rather than a drop in one time interval. Three of 5 five patients tested showed also a decrease of forced expiratory volume in one second (FEV_1) below 10% of initial value. However, when assessed by χ^2 test, the drop of FEV_1 appeared to be highly significant in all the patients, with P<0.001 in 4 persons, and P<0.01 in one person.

The results of spirometric measurements were supported by the occurrence of subjective respiratory symptoms after the inhalation challenge with *P. agglomerans*. All the patients responded with cough which was intensive in 2 of them. Additionally, dyspnoea was reported by one patient with intensive cough. The results of the auscultation examination after challenge were abnormal in all patients. Rales were noted in all 5 persons, and wheezing in one of them.

In the results of laboratory blood analysis, the most remarkable were those concerning the interleukin 6 (IL-6) level. All the patients responded after challenge with increase of IL-6 level, which was assessed as positive in 4 persons, and as statistically significant in 2 persons. Although an increase of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) was noted in some workers, others, however, showed decrease of test values; hence, the results of these 2 tests must be assessed as ambiguous. No significant changes after the challenge with *P. agglomerans* were noted

Table 7. Immunologic tests of patient blood with antigens derived from *Pantoea agglomerans* and *Microbacterium barkeri*

Test Patient No.	Test for specific inhibition of leukocyte migration #		Agar gel precipitation test	
	Pantoea agglomerans	*Microbacterium barkeri*	*Pantoea agglomerans*	*Microbacterium barkeri*
1	neg. ↓ MI = 0.802	neg. ↓ MI = 0.803	neg.	neg.
2	**pos. ↓ MI = 0.799**	neg. ↓ MI = 0.842	neg.	neg.
3	**pos. ↓ MI = 0.749**	**pos. ↓ MI = 0.764**	**pos.**	neg.
4	**pos. ↓ MI = 0.793**	neg. ↓ MI = 0.859	neg.	neg.
5	**pos. ↓ MI = 0.786**	**pos. ↓ MI = 0.790**	neg.	neg.
Mean (x±S.D.)	**pos. ↓ MI = 0.786±0.021**	neg. ↓ MI = 0.812±0.039	N. A.	N. A.

pos. = result positive (marked in bold); neg. = result negative; ↓ = decrease of initial value; MI = migration index; N. A. = not applied
# = positive result was assumed at the decrease of migration index (MI) to or below 0.799.

Table 8. Results of inhalation challenge with *Pantoea agglomerans* (8 hrs post challenge)

Test Patient No.	Lung function tests #		Laboratory blood analysis ##				Subjective symptoms	Auscultation
	FVC	FEV_1	IL-6	CRP	ESR	LC		
1	**pos. ** ↓ 13.9%**	neg. ** ↓ 8.5%	**pos. ** ↑ >130%**	neg. * ↓ 42.8%	neg. ↔ 0%	neg. ↑ 5.3%	**Yes Intensive cough**	**Rales**
2	**pos. ** ↓ 10.2%**	**pos. ** ↓ 15.5%**	**pos. ↑ >55%**	neg. ↑ 40.9%	neg. ↑ 33.3%	neg. ↓ 6.9%	**Yes Cough**	**Rales**
3	**pos. ** ↓ 17.3%**	neg. * ↓ 8.4%	**pos. ↑ >60%**	**pos. ** ↑ 272.2%**	neg. ↔ 0%	neg. ↑ 8.1%	**Yes Cough**	**Rales**
4	**pos. ** ↓ 15.0%**	**pos. ** ↓ 23.3%**	neg. ↑ >10%	**pos. ** ↑ 943.8%**	**pos. ↑ 50.0%**	neg. ↑ 29.3%	**Yes Cough**	**Rales**
5	**pos. ** ↓ 40.1%**	**pos. ** ↓ 21.4%**	**pos. ** ↑ >80%**	neg. ↓ 30.2%	**pos. ** ↑ 183.3%**	neg. ↑ 12.9%	**Yes Intensive cough, dyspnoea**	**Wheezing**
Mean* (x±S.D.)	**pos. ↓ 19.3±11.9%**	**pos. ↓ 15.4 ±7.0%**	**pos. ↑ >80%**	n. c.	n. c.	n. c.		

pos. = result positive (marked in bold); neg. = result negative; ↓ = decrease of initial value (in percent); ↑ = increase of initial value (in percent); ↔ = no change in measured value (<1%);
* – ** = changes in the course of test statistically significant (χ^2); * = P<0.01, ** = P<0.001.
IL-6 = interleukin 6; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; LC = concentration of leukocytes in peripheral blood; n. c. = not calculated (when a majority of results was not positive).
= positive result was assumed at the fall equal to or below 10%.
= positive result was assumed at the rise equal to or above 50%.

Table 9. Results of inhalation challenge with *Microbacterium barkeri* (8 hrs post challenge)

Test Patient No.	Lung function tests #		Laboratory blood analysis ##				Subjective symptoms	Auscultation
	FVC	FEV_1	IL-6	CRP	ESR	LC		
1	neg. ↑ 2.5%	neg. ↑ 1.5%	neg. ↔ 0%	neg. ↓ 26.8%	neg. ↔ 0%	neg. ↔ 0%	None	Normal
2	**pos. ** ↓ 10.6%**	**pos. ** ↓ 14.1%**	neg. ↔ 0%	**pos. ** ↑ 240.0%**	neg. ↑ 40.0%	neg. ↔ 0%	None	Normal
3	neg. ** ↓ 9.1%[1]	neg. ** ↓ 3.4%[1]	neg. ↔ 0%	neg. ** ↓ 66.0%	neg. ↔ 0%	neg. ↔ 0%	None	Normal
4	neg. ↑ 3.1%	neg. ↑ 1.4%	neg. ↔ 0%	**pos. ** ↑ 355.0%**	neg. ↔ 0%	neg. ↔ 0%	None	Normal
5	neg. ↓ 2.6%	neg. ↓ 2.3%	neg. ↔ 0%	neg. ↑ 8.1%	**pos. ** ↑ 168.7%**	neg. ↔ 0%	None	Normal
Mean (x±S.D.)	neg. ↓ 3.3±6.4%	neg. ↓ 3.4±6.4%	neg. ↔ 0.0±0.0%	n. c.	n. c.	n. c.		

pos. – result positive (marked in bold); neg. – result negative; ↓ – decrease of initial value (in percent); ↑ – increase of initial value (in percent); ↔ – no change in measured value (<1%)
*- ** – changes in the course of test statistically significant (χ^2); * – P<0.01; ** – P<0.001
[1] result classified as borderline positive
IL-6 – interleukin 6; CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; LC – concentration of leukocytes in peripheral blood; n. c. – not calculated (when a majority of results was not positive)
– positive result was assumed at the fall equal to or below 10%
– positive result was assumed at the rise equal to or above 50%

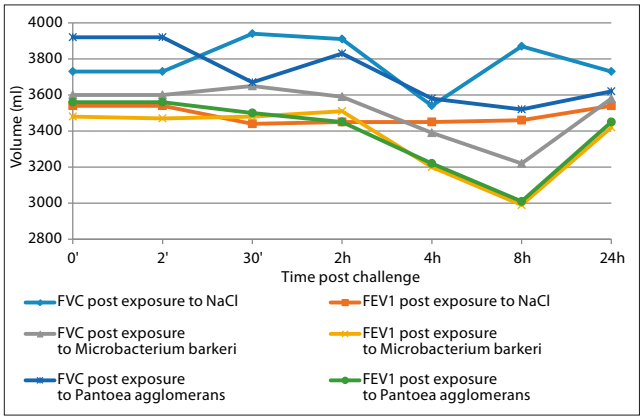

Figure 6. Results of inhalation challenge with the extracts of *Microbacterium barkeri* and *Pantoea agglomerans* in patient No. 2. Note a distinct drop of FEV_1 values 8 hrs post exposure to *M. barkeri* and *P. agglomerans*, drop of FVC value 8 hrs post exposure to *M. barkeri*, and a dual response in FVC post exposure to *P. agglomerans*, manifested by the drop of FVC values after 30 min and then after 4–8 hrs

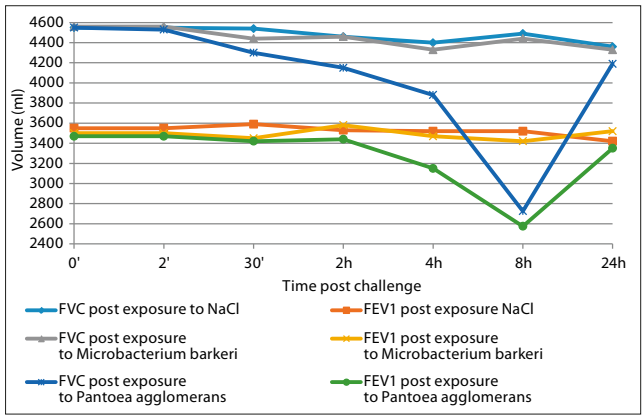

Figure 7. Results of inhalation challenge with the extracts of *Microbacterium barkeri* and *Pantoea agglomerans* in patient No. 5. Note the strong drop of FVC and FEV_1 values 8 hrs post exposure to *P. agglomerans*, and lack of positive response to *M. barkeri*

in: FEV_1%FVC index, the concentration of leukocytes in peripheral blood, blood morphology, procalcitonin blood level, blood pressure, pulse rate, blood oxygen saturation and body temperature.

Summarizing, the distinct decrease of vital capacity and the presence of subjective respiratory symptoms after the inhalation challenge with *Pantoea agglomerans* univocally support the positivity of the test, and indicate the most probable role of this bacterium as a main disease agent causing HP symptoms in exposed woodworkers.

Specific inhalation challenge with *Microbacterium barkeri*. Compared to *Pantoea agglomerans*, the effects of the inhalation challenge with *Microbacterium barkeri* were weaker in all time intervals. One patient (No. 2) responded with a significant drop of spirometric values of FVC and FEV_1 below the threshold level of 10% (Fig. 6), while in another patient (No. 3) the decrease of FVC was slightly above the assumed threshold level (9.1%). As the statistical analysis indicated the drops of FVC and FEV_1 as highly significant ($P<0.001$) in both these patients (Tab. 9), the result of the inhalation challenge was classified as positive in patient No. 2 and borderline positive in the patient No. 3. The 3 remaining patients tested negative in the challenge and 2 of them even showed a slight increase of spirometric values (Tab. 9).

The value of the positive spirometric records after the inhalation challenge was diminished by the lack of subjective respiratory symptoms and auscultation changes in examined patients. Among the results of laboratory blood analysis, a positive and significant increase of C-reactive protein (CRP) level was noted in 2 cases, and a positive increase of erythrocyte sedimentation rate (ESR) in one case. However, because of the great variability of the results noted in both tests and the presence of results that showed decrease of initial value or no change, the final assessment of the noticed effects needs further studies.

No significant changes after the challenge with *M. barkeri* were noted in: FEV_1%FVC index, IL-6 level, the concentration of leukocytes in peripheral blood, blood morphology, procalcitonin blood level, blood pressure, pulse rate, and blood oxygen saturation. In one patient (No. 5) an elevation in body temperature from the initial value of 36.5°C to 37.5°C at 24 hrs was noted after challenge.

DISCUSSION

The diagnosis of HP is usually based on clinical, functional and radiological criteria supplemented by additional tests such as inhalation challenge and/or bronchoalveolar lavage. All patients in this study revealed signs and symptoms complying to these criteria.

For establishing proper diagnosis, a detailed anamnesis aimed at identifying exposure agents suspected to cause the disease, characteristic symptoms and type of relation between the potential etiological agents and particular symptoms, is very important. Five patients who developed disease after occupational exposure to the inhalation of wood dust displayed dyspnea, dry cough, fever and malaise, reported as typical symptoms for the acute stage of HP [1–6]. Physical examination revealed rales over lung fields, regarded as characteristic for HP, in all the patients. For example, Lacasse et al. [43] expressed an opinion that the finding of rales in patients with documented exposure and positive immunologic tests allows for diagnosis of HP in 80% of cases.

In lung function tests, small degrees of obstruction and reduction of DLCO were found in one patient, whereas the others did not show abnormalities. Although restrictive changes are reported as typical for HP, they usually occur in the chronic stage of disease [42, 43]; hence, the lack of such changes does not abolish the presented diagnosis of acute HP.

Radiologic examination of chest revealed pathologic changes in the lungs of 4 out 5 patients, whereas the HRCT examination, regarded as very important in the HP diagnosis [43], displayed abnormal picture in all patients. The HRCT examination enabled differentiation between the acute and subacute stages of disease. In 4 patients with acute HP dominated ground-glass attenuations with features of air trapping, and in one patient with the subacute HP, a fine reticular fibrosis was found in addition to ground-glass changes.

Fibrobronchoscopic (BAL) examination used in the presented group of patients for estimation of the lung cell composition and lymphocyte subpopulations, revealed lymphocyte level ranging from 30.77–88.34%. Such a level is regarded by Lacasse et al. [44] as significant for HP diagnosis, whereas Selman [43] proposed as significant lymphocytosis values exceeding 40% or even 50%. Nevertheless, these levels of lymphocytosis were also exceeded in 4 out 5 of the patients, ranging from 56.01–88.34%. The ratio of CD4/CD8 ranged in these patients from 0.23–1.61. Only in one patient (the same with relatively low lymphocytosis equal to 30.77%) this ratio slightly exceeded (to the value of 1.61) the threshold of 1.0, regarded by Girard et al. [45] as significant for HP diagnosis. In 4 other patients, the ratio ranged from 0.23–0.79, which is below the threshold proposed by these authors. In another paper, Girard et al. [46] expressed an opinion that the ratio CD4/CD8 may depend on the size and type of exposure to pathogenic allergen. They also postulated that the excess of CD8+ cells correlates with acute stage of HP, whereas the excess of CD4+ cells with chronic stage of this disease. In conclusion, the results of bronchoalveolar lavage examination fully confirm the diagnosis of HP in the presented patients.

Until recently, no cases of HP caused by exposure to birch dust had been described. Nevertheless, Määttä et al. [47] demonstrated in the mouse model that repeated airway exposure to birch dust can elicit lung inflammation, accompanied by induction of several proinflammatory cytokines and chemokines. In an epidemiological study in Sweden, Gustafson et al. [48] found that exposure to birch dust may contribute to the risk for idiopathic pulmonary fibrosis in men. As in both studies, microbiological analyses were not performed, and presumable pathogenic agents were not indicated, the problem of specific causative agents responsible for adverse reactions and diseases associated with exposure to wood dust from birch remains open.

Immunopathologic reaction in HP is initiated by the exposure to large amounts of adverse factors, usually microorganisms [33]; hence, the organisms prevailed in the material associated with evoking symptoms are almost surely the disease-causing agents, as demonstrated many times, beginning from classic works by Towey et al. [12] and Pepys et al. [49]. Accordingly, in the presented study, the extracts of the biomass of the bacterial strains belonging to species *Pantoea agglomerans* and *Microbacterium barkeri* were used as test allergens because they distinctly prevailed in the

samples of birch wood associated with evoking of disease symptoms in the workers.

It is known that bacteria and fungi may colonize apparently undecayed timber logs stored in the lumber yards [50, 51], posing a potential hazard for woodworkers who are engaged later in processing these logs. Studies performed on microbiota of 10 species of timber logs in the USA and Poland revealed large concentrations of bacteria and fungi in the sapwood and heartwood of examined logs, ranging in most cases from 10^3–10^7 CFU/g. The numbers of microorganisms were usually greater in the sapwood (containing more nutrients) than in heartwood. Gram-negative rods prevailed among bacteria, while yeasts were most numerous among fungi [51, 52]. The concentrations of bacteria in the logs of white warty birch (*Betula pendula*) amounted to 10^3–10^4 CFU/g, being closer to the lower limit of microbial content [52]. Further studies conducted by Prażmo et al. [53–55] on microbiota of 6 species of timber logs in Poland, confirmed the common occurrence of endotoxin-producing Gram-negative bacteria in the heartwood of examined birch logs (up to 10^5 CFU/g) as well as in other timber species (European beech, common oak, Scots pine, Norway spruce, silver fir). The most common species included *Rahnella aquatilis*, *Pseudomonas maltophilia* and *Pantoea agglomerans*. These species and bacterial endotoxin were recovered from the air of the log-processing sawmills posing a potential hazard of respiratory disorders for the workers [53, 54].

It is noteworthy that in previous studies [51–55], *P. agglomerans* prevailed rather in the peripheral part of the timber log called 'sapwood' (light colour on the transverse section) then in the central part called 'heartwood' (dark colour on the transverse section). This is in line with the findings of the current study, where the concentration of this bacterium (and some other bacterial species) was circa. 10,000 times higher in the yellowish peripheral part of the log transverse section, corresponding to 'sapwood', than in the brownish central part of the section corresponding to 'heartwood', according to the available references [56].

Pantoea agglomerans (synonyms: *Enterobacter agglomerans*, *Erwinia herbicola*), identified as a main cause of HP cases described in the current study, is probably one of the most widespread organisms in the world. Originally a plant bacterium, it is known both as an epiphytic microbe developing on the surface of plants and as an endophytic organism living inside the plants. The bacterium also occurs abundantly in plant and animal products, in the body of arthropods and other animals, in water, soil, dust and air, and occasionally in humans. From the human viewpoint, the role of this organism is ambiguous – both deleterious and beneficial: on the one hand it causes disorders in people exposed to the inhalation of organic dusts and diseases of crops, and on the other, it produces substances effective in the treatment of cancer and other diseases of humans and animals, suppresses the development of various plant pathogens by antibiotic production and/or competition, promotes plant growth by nitrogen fixation and other mechanisms, and appears as a potentially efficient bio-fertilizer and bio-remediator [57, 58].

P. agglomerans has been found in the airborne and settled grain dust [59, 60] and has been identified as a common cause of HP in the Polish agricultural workers exposed to the inhalation of plant dusts, mostly from grain [33, 61, 62], less often from flour [61, 62], clover [33] and herbs [63]. The bacterium also caused a case of HP described in a German farmer [64].

To the best of the authors' knowledge, *P. agglomerans* has never been described previously as the cause of HP in woodworkers. In the present study, however, *P. agglomerans* was identified as a main causative agent of acute HP in the workers of furniture factory exposed to the inhalation of wood dust from white warty birch (*Betula pendula*). This finding was evidenced by the inhalation challenge which was distinctly positive in all the workers showing HP symptoms. As *Pantoea agglomerans* produces a biologically potent endotoxin [65, 66] which may cause respiratory disorders in workers exposed to dust from cotton, grain and other plant products [57, 58, 60], the possibility was considered before testing of an unspecific respiratory response that could be elicited by this substance; therefore, the inhalation tests were carefully calibrated to avoid a diagnostic mistake. Accordingly, the allergenic extract of *P. agglomerans* for inhalation challenge was strongly diluted in saline to the concentration of 20 μg/ml, which did not provoke any reaction in healthy human volunteers.

In the presented work, during the test one patient inhaled a total dose of circa 2.16 μg of the diluted allergenic extract, equal to 0.0216 μg of pure LPS (endotoxin), as determined by the *Limulus* test [58, 60]. Such a dose was circa. 1,400 times smaller compared to the doses mentioned by Rylander et al. [67] and Thorn [68], who assumed that the inhalation of 30–40 μg LPS seems to be a threshold level for inducing clinical symptoms and lung function changes in healthy human subjects. Thus, the decrements of spirometric values and subjective symptoms observed in the current study after the inhalation challenge with *P. agglomerans* extract were not non-specific reactions caused by endotoxin, but specific reactions to *P. agglomerans* antigen(s) occurring only in sensitized individuals. The specificity of the inhalation challenge does not exclude the fact that in real life the airborne endotoxin produced by *P. agglomerans* may augment the allergenic effects of this bacterium and exacerbate the clinical course of HP.

Out of 5 woodworkers with HP symptoms, 4 showed positive response in the test for specific inhibition of leukocyte migration (MIF test), and only one in the test for the presence of specific precipitins. This result needs a comment because until now some authors regard the presence of precipitins as important for establishing the HP diagnosis. This is in line with the original concept of Pepys [41], that immune complexes of precipitating antibody and antigen initiate the immunopathogenic process in HP, which has been shared by some authors until recently. However, most of the recent studies rather support the view that Th1 lymphocytes play a major role in the HP pathogenesis [3, 69], and hence the tests related to cellular reactions, such as MIF, are more reliable than the precipitin test. The latter shows merely a contact of the patient with a suspected antigen, and as such may be very helpful in diagnosis, but does not answer the question whether or not the patient has HP [3, 6, 69]. Therefore, the MIF test, and in particular inhalation challenge, the latter being regarded by some authors [6, 70] as a 'gold standard', have much greater value for establishing the HP diagnosis than the precipitin test, which can be negative in otherwise well-documented cases of HP [6, 33, 69].

Microbacterium barkeri, a coryneform, non-filamentous organism classified within phylum Actinobacteria, is

another bacterial species that occurred in large numbers in birch wood, also in peripheral tissues of log described as "sapwood". In the paper by Milanowski et al. [33], the related bacteria belonging to the species *Arthrobacter globiformis* and *Brevibacterium linens* were found to dominate in samples of barley and barley dust associated with cases of chronic HP in exposed workers, and identified as the causative agents of the disease. In the presented study, *M. barkeri* appeared slightly more numerous in the wood than *P. agglomerans;* however, its allergenic effects seem to be weaker. The inhalation challenge with the allergenic extract of *M. barkeri* resulted in one positive and one borderline positive result in 2 out of 5 workers with the diagnosis of acute HP, and in 2 positive results in the MIF test. Thus, *M. barkeri* should be regarded as a secondary pathogen causing HP in exposed workers. It cannot be excluded that this bacterium can enhance the allergenic (and endotoxic) effects of *P. agglomerans*, but this presumption needs experimental confirmation.

For future prevention of HP cases among woodworkers, the fact is noteworthy that some of the HP patients in this study had worked in the furniture factory for up to 10–12 years without any disorders related to occupation before delivery of the contaminated birch logs that appeared to be a source of allergenic dust. This fact clearly demonstrates that even a single part of logs heavily colonized with adverse microorganisms may cause severe disease in woodworkers.

For the prevention of similar incidents in the future, the managers of wood processing factories should be obliged to stop the processing of such material at the first occurrence of the disease symptoms in workers, and to submit samples of such wood for microbiological examination. At the detection of heavy microbial contamination, production should not be resumed until the workers are equipped with highly efficient respirators.

CONCLUSION

Gram-negative bacterium *Pantoea agglomerans* occurring in large numbers in the sapwood of birch wood was identified as the main causative agent of HP in the group of exposed workers. *Microbacterium barkeri*, a non-filamentous actinobacterial species prevalent also in this wood, should be regarded as a secondary agent probably exacerbating the disease. These results clearly demonstrate that apart from fungi and filamentous actinobacteria, Gram-negative bacteria and non-filamentous actinobacteria may also be a cause of occupational HP in woodworkers.

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