Immunological Aspects and Affections of Rubbish Collectors Caused by Bioaerosols

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Abstract: Immunological Aspects and Affections of Rubbish Collectors Caused by Bioaerosols: Stephan Becher, et al. Rheinischer Gemeindeunfallversicherungsverband—From the point of view of industrial medicine, the maintenance of industrial health and safety standards to minimize health hazards for staff is the most important item in dealing with biological materials at work. With regard to the rubbish industry, it is therefore mandatory to recognize every possible health hazard in order to supplement preventive measures and to increase industrial medical prevention. In a cross-sectional study of industrial medicine, 83 trash collectors were medically examined, their case history having been taken by means of a standardized questionnaire. The immunoblot examination consisted of the measurement of specific IgE and IgG antibodies to Aspergillus fumigatus. An immunoblot was used to supplement the investigation and to illustrate individual epitopes of allergens of Aspergillus fumigatus. In 16 trash collectors (19%) there was a specific sensitization against Aspergillus fumigatus Type I and Type III simultaneously, as proven by the standards of Coombs and Gell. Specific antibodies to Aspergillus fumigatus were discovered in 27 employees. Neither the information given by the dust collectors nor medical examination results allowed the deduction of the presence of an allergy in the sense of typical health disturbances. Based on the results of this study, however, the immunological parameters may indicate a possible sensitization of the employees in the rubbish industry.

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Key words: Rubbish, Trash collectors, Rubbish industry, Sensitisation, Aspergillus fumigatus, AIBlot

Since the introduction of recycling of so-called reusable material, the disposal of rubbish has changed dramatically in view of the new sorting arrangement for refuse. In Germany, the rubbish disposal system is based on the distinction between different types of rubbish which are sorted by individual households. Rubbish is divided into paper waste, packages (yellow bin), glass and compost (biological) waste.

A possible health hazard due to the micro-organisms contained in the waste is a topic of discussion among waste workers and specialists in industrial medicine alike⁶⁻⁹. So far, studies have shown the level and quality of exposure to micro-organisms⁶⁻⁹. The results, however, are inconsistent in quality and quantity. There is also a lack in reference values allowing orientation which makes evaluation of these measurements difficult. For this reason, data can only be improved by further industrial medical studies.

Materials and Methods

A target group of 83 local garbage collectors whose work consisted of emptying and collecting household dustbins on a daily basis was analysed.

The selection of subjects was undertaken by specialists in industrial medicine who looked after the garbage collectors from a medical point of view.

Altogether, 166 men (83 rubbish collectors and a further 83 in a control group) took part in the study.

The rubbish collectors had been employed in their field for a long time - the mean was 125 ± 109 months of employment in the rubbish industry.

For most of the rubbish collectors, several types of rubbish were collected simultaneously or within a temporal connection. An isolated contact with compost waste only was reported by one employee. Two workers dealt solely with bulky refuse. Eleven employees had regular contact with all types of waste (yellow dustbin,
compost, garden waste and remaining waste). 31 subjects were in regular contact with remaining waste, compost and garden waste. Fig. 1 lists the frequency of the individual types of waste.

The control group also consisted of 83 comparable employees from public places of work who were similar in age, sex, social class and work intensity. An explicit exclusion criterium was an increased private or work-linked exposure to micro-organisms.

To evaluate specific health disturbances in the target as well as in the control group, the following parameters were analysed.

1. Evaluation of general subjective health as seen by target and control groups by means of a specially designed questionnaire.
2. Medical history questionnaire focussing on allergies and health disturbances, leaving room for striking medical diagnoses.
3. Examination of specific IgE and IgG antibodies to Aspergillus fumigatus.
4. ALA-Blot (Allergo-Link-Adsorbent) is to illustrate specific epitopes of main and side allergens of Aspergillus fumigatus.

Eight specialists in industrial medicine from different cities took part in the study. All employees were informed about the objective of the examination and gave their written consent.

**Immunological-allergic examinations**

In the context of the immunological investigations the detection of the precipitating antibodies to Aspergillus fumigatus, Type IgE and IgG was successfully undertaken (RAST-Radio-allergosorbent Test).

The Immuno-Blot examinations for epitopes of Aspergillus fumigatus were performed by an experienced laboratory familiar with the procedure. The method of Western blot of the DPC Biemann Ltd., Bad Nauheim, Germany was used. This method was installed in monitoring disease activity in patients with Aspergillus-induced lung diseases.

Immunoblotting is the method of choice for analysis of a spectrum of allergens, especially of a complex extract.

In this particular method, proteins and glycoproteins of a purified complex allergen extract were split into bands according to their molecular weight by means of SDS-PAGE (Polyacrylamide-gel-electrophoresis) and transferred to a nitrocellulose membrane.

The procedure of ALA-Blot-Systems is based on a ligand-coated tube and uses ligand-combined liquid allergens. During the incubation phase, the patient’s specific IgE binds to the ligand-combined allergen. An anti- ligand is then added to the liquid mixture. The complex formed during the first phase of the reaction binds to the ligand-coated walls of the incubation tubes.

After marking with an according conjugate (anti-human-IgE) and the subsequent substrate reaction, bound patient antibodies become visible as bands after several rinses. By means of comparison with defined bands of molecular weight markers from the reference system of the manufacturer as well as from available literature, the IgE antibodies present in the samples are characterized as regards epitopes according to their specificity.

Fungi are regarded as highly complex types of allergens. The airborne spores of fungi carry relevant...
amounts of inhalable allergens of the microorganism. With regard to their incidence in the rubbish industry and sensitizing potency, especially species of Aspergillus and Penicillium must be noted.

The description of the examination was undertaken according to the methods of descriptive statistics.

**Results**

In most cases, there was mixed exposure to the various types of rubbish. The focus on biological waste (compost) and remaining waste is obvious. Personal protective devices were available to all employees and were used by 65 (79%) on a daily basis. The frequency of sick-leave is an important criterion as regards susceptibility and work satisfaction. The frequency of illness among the rubbish workers is low. Six workers had already been at least one time unfit to work for more than three months. Typical health disturbances caused by biological causes leading to prolonged inability to work were not listed. "Lung disease" was listed by two workers. In one case this turned out to be allergic asthma due to house-dust mite sensitivity. In the second case, the reason was chronic bronchitis (chronic obstructive airway disease) in a smoker.

The Epitope of the main allergic compounds of A. fumigatus was found in all workers with a positive ALA-Blot result (in 18.9 kDa) at high intensity. In the specific characterization of proteins the tape was seen in 27.6 kDa six times in the control group and in all workers with positive ALA-Blots. The tape of protein of A. fumigatus Asp f2 was found in 36.9 kDa in the workers.

Regarding the immunological examinations, the ALA-Blot examination verified the house-dust mite induced allergy (epitope at 27.6 kDa). Furthermore, three protein bands typical of A. fumigatus were proven (Table 1). Specific lGgE-antibodies were not found. A sensitization of type lGg to A. fumigatus is immunologically illusratable. In the rubbish collector with chronic bronchitis, lGg antibodies were found. lGgE antibodies were not isolated, and the ALA-Blot was negative.

The regular occurrence of skin defects was negated by 59 individuals (72%). 24 rubbish collectors claimed to have skin changes such as skin inflammation, pruritus, vesiculation (all were mentioned on the questionnaire) and had the following immunological details: 46 % (11 subjects) were lGg positive, 12 % (3 subjects) were ALA-Blot positive, and 3 subjects (12%) were ALA-Blot and lGg positive (Fig. 2).

Among the subjects without skin changes, the following immunological details were found: 32 subjects were lGg positive, 13 subjects tested positive for ALA-Blot and 13 subjects tested positive for lGg and ALA-Blot (Table 2). On comparing the relative frequency of the immunological data, it becomes apparent that positive results (evidence of antibodies) occur more commonly in the group of rubbish collectors without skin changes. In view of the results of the study, a correlation between skin changes in dustmen, exposure to bioaerosols and a specific sensitisation to Aspergillus fumigatus cannot be found. Eighteen of the subjects (22%) were on regular medication.

10 subjects claimed to have an allergy. According to the subjects themselves, the type of allergy was drug-related in 3 cases, 2 subjects were allergic to preservatives, one subject was allergic to house-dust mite and one was allergic to pollen. None of the subjects claimed to be sensitized towards fungi or allergic to fungi in a clinically relevant manner.

Nevertheless, 6 workers with a history of allergies showed a positive sensitization against A. fumigatus type lGg. Of them also had evidence of the main allergen of A. fumigatus (18.9 kDa) in the ALA-BLOT investigation.

In comparing the subjects with a history of allergies with the remaining subjects, it may be stated that in these no further more frequent specific sensitization of type lGg of A. fumigatus or more frequently positive ALA-BLOT findings were found.

Therefore, this study does not prove that existing allergies in rubbish collectors lead to an increased sensitization to A. fumigatus or to clinical allergic symptoms.

Specific antibodies of TpTy lGgE to A. fumigatus were

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found in 4 subjects in the rubbish workers' group. In the control group a specific sensitization of type IgE to fungi could not be found in any subject.

The proof of specific A. fumigatus antibodies, type IgE was linked with a specific sensitivity test of IgG specific antibodies in 4 cases, and the ALA-BLOT investigation (A. fumigatus) showed between three and ten epitopes in the range 18.9 to 12.6 kDa, in this group of subjects. The main allergen of A. fumigatus is always included.

In the rubbish workers' group one can find specific IgG (A. fumigatus) as well as a specific ALA-BLOT (A. fumigatus) along with specific IgE (A. fumigatus), so that a specific sensitization of Type I and III to A. fumigatus in these rubbish collectors (N=4) is obvious. These dust collectors have only medium to short employment periods and accordingly a professionally linked exposure to bioaerosols (120, 108, 80 and 12 months).

An allergy is presumed existent when the immunologically proven sensitization co-exists with typical clinical symptoms. This is not the case with the specifically sensitized rubbish collectors. Typical allergic complaints as an expression of an allergy are not mentioned in the questionnaires. Accordingly no examination findings were described by the doctors involved in the study. Specific type IgG antibodies were found in 43 subjects in the rubbish collectors' group and in 31 in the control group. The mean antibody titre was 1.58 ± 4.5 U/l in the dustmen's group and 0.56 ± 0.86 U/l in the control group.

The IgE-specific ALA-BLOT to Aspergillus fumigatus showed three to eight protein bands (epitopes) of the fungal allergen in 16 cases (19.3%) in the dustmen's group. The epitope of the main allergen of Aspergillus fumigatus (Asp11) was proven to be present in all positive ALA-BLOTs of the examined group at 18.9 kDa. In only four rubbish collectors with protein bands in the ALA-BLOT investigation were there additional specific IgE antibodies.

It is especially common to find a high rate of false positive or false negative IgE results (RAST or ELISA) when investigating for fungi. Due to the high specificity and the demonstration of singular epitopes the ALA-BLOT is superior to the proof of specific IgE (RAST or ELISA). Therefore, one can conclude that there is specific sensitization in the rubbish collectors with positive ALA-BLOT results even if the proof of IgE does not function with RAST or ELISA.

Due to the ubiquitous occurrence of A. fumigatus, the proof of antibodies/the positive ALA-BLOT against Aspergillus allergens cannot provide complete evidence of professional exposure.

The suspicion of a professionally acquired disease with Aspergillus fumigatus can only be reinforced by
evaluating clinical, cultural and serological results (with regard to allergies, for example by clinical improvement on freedom from contact with the particular allergen).

The medical examination revealed 5 eczema subjects in the dust collectors’ group, and 3 showed evidence of flexural eczema. 16 subjects had rhinitis. In the control group, only two subjects showed evidence of eczema, and flexural eczema was found in three subjects (3.6%).

The frequency of infection was higher in the dust collectors’ group than in the control group (8 in the dust collectors’ group, and 2 in the control group). Generalized itch or conjunctivitis was found in 9.7% and 8.4%, and in the control group the percentages were 2.4 and 1.2% respectively (Fig. 3).

Discussion

The value of monitoring Aspergillus-infected patients by measuring antibody responses is still under discussion.

Within the group of dust collectors an increased sensitization of Type I and III with regard to the fungus Aspergillus fumigatus was found. At the time of examination, objective medical disturbances were more common in the group of rubbish collectors than in the control group. The medical findings were in accordance with the health problems mentioned in the history questionnaire. The reasons were infectious, allergic or irritative in origin. The high incidence of rhinitis in the group of dustmen as opposed to the control group is indicative of an allergic origin.

In view of the higher incidence of infection in the group of dustmen, a differential diagnosis of organic dust toxic syndrome (ODTS) is possible. This is a syndrome related to the exposure to biological toxins. The symptoms vary and include fever, headache, muscular and joint pain, dyspnoea and chronic cough. Possible sources of toxins seem to be gram negative bacteria and some species of Aspergillus and Legionella.

With regard to the immunological parameters, the antibodies to Aspergillus fumigatus of types IgE and IgG as well as Aspergillus fumigatus specific epitopes were demonstrated to occur more frequently, but establishment of a correlation between sensitization and subjective or objective complaints regarding the underlying study data was not possible.

The IgE-specific ALA-BLOT of Aspergillus fumigatus showed 3 to 8 protein bands (epitopes) of the fungal allergen in 16 cases within the group of dustmen. The epitope of the main allergen of Aspergillus fumigatus (Asp f1) was proven at 18.9 kDa in all positive ALA-BLOTs. In the specific protein characterization by means of an ALASTAT-BLOT, the protein band is reproducible in 6 cases as the only protein band in the control group at 27.6 kDa; in the dustmen group it is found in all ALA-BLOTS. The weak degree found especially in the control group leads us to believe that the protein pattern shows a crossed over allergic reaction.

It is obvious that the epitope at 27.6 kDa is in accordance with that of the house-dust mite (Dermatophagoides pteronyssinus) which has a molecular weight of 28 kDa.

The protein bands of the main allergen of Aspergillus fumigatus (Asp f1) is demonstrable at a significant
intensity in all ALA-BLOTs of the dustmen group.

Protein bands of Aspergillus fumigatus allergens (Asp f2) are also found at 39.6 kDa along with other incompletely characterized protein bands which are probably Aspergillus fumigatus allergens.

The high IgG titre as well as the high standard deviation are striking in the dust collectors' group. This is in turn indicative of the correlation between the level of exposure and the IgG antibody titre in employees in the rubbish industry as described in the literature.\(^\text{12, 13}\).

**Conclusion**

It was demonstrated that in the area of rubbish disposal (in dealing with microorganisms), a higher rate of sensitization against fungi is possible. In examining such employees from the point of view of industrial medicine, it is important to bear this in mind initially and in follow-up examinations.

The determination of IgE is not suitable to establish useful information on the sensitization to fungi. Moreover, specific antibodies of type IgG are more ideal. A supplementary ALA-BLOT can also be helpful in combination with a good anamnesis. Bearing in mind these results of this study of industrial medicine will help you to minimize health problems in trash collectors.

**References**


