

Diagnosis and Immunotherapy of Mould Allergy

IV. Relation between Asthma Symptoms, Spore Counts and Diagnostic Tests

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Twenty-four adult asthmatics with autumnal asthma and positive bronchial provocation test to the mould species *Cladosporium* were evaluated by daily symptom scores during 11 weeks in the peak mould spore season. A significant association with fluctuation in *Cladosporium* spore count was found regarding the relative weekly symptom score (mean of subjective asthma and peak flow scores), relative use of antiasthmatic medication and the combined (total) score (mean of symptom and medication scores). The median weekly symptom, medication, and total scores were positively correlated to *Cladosporium* spore count, but only significantly so in the medication score. Eighteen patients were allergic to both *Cladosporium* and *Alternaria*, but asthma symptoms were not associated to *Alternaria* spore counts; on the contrary, a negative correlation indicated that *Alternaria* only played a minor role in eliciting asthma. Neither mugwort nor house dust mites seemed to be of importance. The results of diagnostic tests (bronchial provocation test, quantitative skin prick test, RAST and histamine release) were correlated to the mean absolute symptom score of the spore season. The highest correlation to asthma score, peak flow score, medication score, and the combined score was found with the bronchial provocation test. The data indicate that autumnal asthma, to a high degree, is elicited by *Cladosporium* spores, and further, that the specific allergic diagnosis can only be established by a combination of positive diagnostic tests and careful recording of symptoms elicited by the causative allergen.

Key words: asthma; diagnostic tests; mould allergy; spore counts; symptom score.

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Recently, the importance of relating the outcome of diagnostic tests to clinical symptoms in order to obtain a specific diagnosis of mould-induced asthma has been emphasized (15). A good correlation between the patients' symptoms and pollen counts has been found in a number of studies (8, 16, 22, 26). However, most studies are concerned with the relation between pollen and rhinoconjunctivitis symptoms and only very few concern asthma (5, 6, 19). In the study by Bruce et al. (6) on the relation between ragweed pollen count and asthma score, a higher incidence of correlation for mould spores than for ragweed was found.

The importance of relating asthma symptoms to airborne pollen or spores is considerable as asthma is a multifactorial diseases. Most patients are allergic to various inhalant allergens, and symptoms may be caused not only by the relevant allergen, but in many patients to a higher degree by unspecific factors as infections, exercise, air pollution. etc. (2). Another factor invalidating the high correlation obtained in hay fever patients is the non-specific hyperreactivity causing delayed asthma symptoms for more than 1 week after exposure to the causative allergen (2).

In the evaluation of allergic patients establishment of a test to predict the clinical sever-

ity of the disease might be valuable. A significant correlation has been found between total seasonal symptoms and clinical sensitivity estimated by RAST and nasal challenge (23) and quantitative skin test and histamine release (17). In the case of autumnal asthma the severity of disease was not predictable by bronchial provocation test (BPT), skin test, histamine release (HIST) or radioallergosorbent test (RAST) (6). No extensive studies concerning the relation in mould-allergic patients have been published.

To investigate the clinical relevance of mould allergens, and especially *Cladosporium herbarum*, on asthma symptoms in patients selected for specific immunotherapy with *Cladosporium*, the relation between asthma symptoms and spore counts was evaluated. Further, the severity of asthma disease was correlated to the clinical sensitivity assessed by BPT, quantitative skin prick test (SPT), RAST and HIST.

MATERIAL AND METHODS

Patients

From a population of 33 asthmatics evaluated for *Cladosporium* allergy (15), 24 patients with a positive allergy to *Cladosporium* were investigated (8 women and 16 men, median age 25 years (range 16–54)). Positive allergy was defined as positive BPT ($> 20\%$ decrease in FEV₁ using a concentration $< 10,000$ BU/ml) and clinical symptoms in the mould season. All patients had exogenous allergy with no signs of chronic airway obstruction. In periods without asthma all had normal lung function. Seventy-five per cent of the patients showed IgE-reactivity to the mould genus *Alternaria* and 83% to other inhalant allergens estimated by SPT. Twenty-five per cent had positive SPT reactions to grass pollen, 38% to *Artemisia vulgaris*, 71% to animal dander (horse, dog, cat) and 50% to house dust mite (*Dermatophagoides pteronyssinus*). Moulds, however, were considered the major allergens as patients were either symptom-free (the majority) or only had minimal asthma outside the *Cladosporium* season (late summer and autumn). No patients had been

treated with mould immunotherapy within the last 2 years. Eight patients (4 women and 4 men, median age 31 years, range 16–58) with negative BPT but positive SPT to *Cladosporium* in three cases and with clinically suspected mould-induced asthma served as controls.

Clinical assessment

The patients completed daily symptom scores during an 11-week period (weeks 31 to 41) in 1981. Asthma symptoms were recorded twice a day according to a grading system: 0: no symptoms, 1: mild symptoms, 2: moderate symptoms and 3: severe symptoms. Peak flow (Mini Wright Peak Flow Meter) was measured in the morning and in the evening at least 4 h after inhalation of nebulized bronchodilator, and the highest of three consecutive readings was recorded. The daily antiasthmatic medication was kept at a minimum to avoid complete absence of symptoms.

The clinical severity of the asthma disease was estimated both relatively and absolutely.

The mean weekly value of asthma symptoms, peak flow and drug consumption for each individual patient was used to estimate the relative severity of the asthma disease. Asthma symptoms were scored continuously in per cent from no symptoms (score 0) to maximum (score 100) observed in the registration period. Likewise, peak flow reduction was scored in per cent in relation to the highest (score 0) and lowest (score 100) value observed in the mould season. In the same way medication was evaluated in per cent from no intake (score 0) to maximum (score 100). Symptom score was defined arbitrarily as the mean of weekly asthma and peak flow scores, and the total disease score as the mean of symptom score and medication score and related to the mean weekly mould spore count.

The absolute severity of the asthma disease in the entire registration period was assessed for each patient. The mean of the daily subjective recording of asthma symptoms graded 0–3 was used. Peak flow reduction in the *Cladosporium* season was calculated in per cent of the maximum value observed for each patient outside

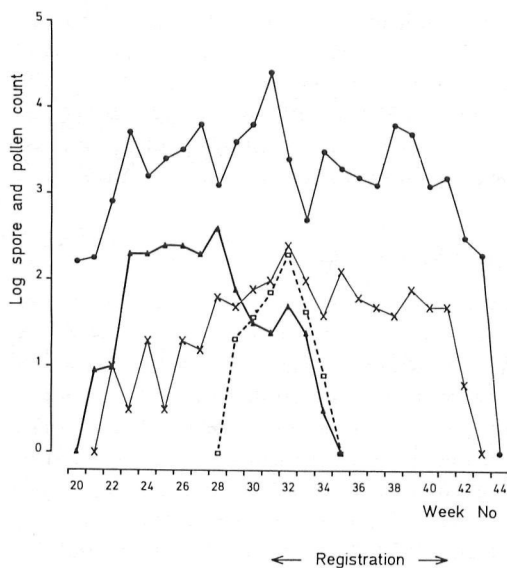


Fig. 1. Weekly spore and pollen counts (log value) during the 1981 peak season. *Cladosporium* ●, *Alternaria* ×, grass pollen ▲, and mugwort (*Artemisia vulgaris*) □.

the season and scored one point for each 10 % reduction. Medication was scored in the following way: 1 point for every 2 puffs of β_2 -agonist aerosol, 2.5 mg of oral terbutaline, 300 mg of sustained release theophylline (Theo-Dur®), 4 puffs of beclomethasone and 2.5 mg of oral prednisolone. The sum of mean asthma score, mean peak flow score and medication score represents the total severity of asthma disease and was used for calculating the correlation of diagnostic test sensitivity and severity of disease.

Spore counts

Daily spore counts were performed from a BIAP spore trap (12) on the roof of a two-storey building located in the eastern part of Denmark. Spores were recorded during a 10-min period each day at maximum sporulation (11). Identification of various mould species and publication of spore data were performed by the Danish Aerobiological Group (27). The registration period (weeks 31 to 41) was chosen to avoid the interference of grass pollen and partly

of *Artemisia* (Fig. 1). Eighty-three per cent of the patients lived within 50 km of the spore trap.

Allergen extract

Lyophilised, partly purified and biologically standardised preparations of *Cladosporium herbarum* and *Alternaria tenuis* (Pharmalgen®, Pharmacia, Uppsala, Sweden) were reconstituted and diluted in human serum albumin-containing saline. For SPT, BPT and HIST extracts were reconstituted on the day of testing. An intermediate product of the same raw extract was used for coupling to the RAST discs (15).

Bronchial provocation test

Inhalation challenge was performed simultaneously with SPT and blood sampling outside the pollen and spore seasons (February 1982). Drugs interfering with the immediate response were avoided (18). Using the tidal volume, 1 ml of aerosolized extract in a 10-fold dilution was inhaled. Forced expiratory volume in first second (FEV_1) was recorded (Vitalograph Spirometer®, Buckingham, England) and the allergen concentration causing a 20 % decrease in FEV_1 calculated (bronchial sensitivity = PC_{20}). The reproducibility of repeated BPT was within 1/2 log step of allergen dilution (15).

Skin prick test

Quantitative SPT was performed in quadruplicate using at least three 10-fold allergen dilutions as previously described (14). Linear regression analysis was performed on the wheal area as a function of allergen concentration (log system), and only tests with a coefficient of correlation > 0.9 were accepted. The skin sensitivity was calculated as the allergen concentration eliciting a wheal equal to the histamine reference (histamine dihydrochloride 1 mg/ml). The reproducibility was of the same magnitude as that of BPT (14).

Blood sampling

Serum was obtained prior to BPT and SPT and stored in small aliquots at -20°C until ana-

lysed. Samples were only frozen and thawed once.

Radioallergosorbent test

Circulating specific IgE was measured by RAST using paper discs as solid phase (15). Patient serum was diluted to obtain binding of ^{125}I -anti-IgE at the steepest part of the dose response curve. The assay was calibrated by use of a human reference and expressed in RAST classes according to the Pharmacia RAST system. All samples were run in one set-up and the coefficient of variation of double determinations was less than 10%.

Histamine release

Basophil histamine release was performed with incubated Ficoll-Hypaque isolated mononuclear cells with 10-fold allergen dilutions as previously described (15). The release was calculated in per cent of the total histamine contents. The cell sensitivity was expressed as the allergen concentration resulting in a 20% histamine release after correction for spontaneous release (15).

Statistics

Concordance and discordance between spore counts and clinical symptoms were evaluated using a Chi-square test. Correlation of scores and spore counts and symptom score versus diagnostic test sensitivity was analysed by Spearman's rank correlation coefficient (ρ).

RESULTS

The association between fluctuation in spore counts (*Cladosporium* and *Alternaria*) in the registration period and median symptom, medication and total scores in patients with positive allergy to *Cladosporium* is shown in Fig. 2. Concordant increases and decreases of symptom score, medication score and the combined (total) score for all patients were significantly ($P < 0.05$) associated with changes in *Cladosporium*

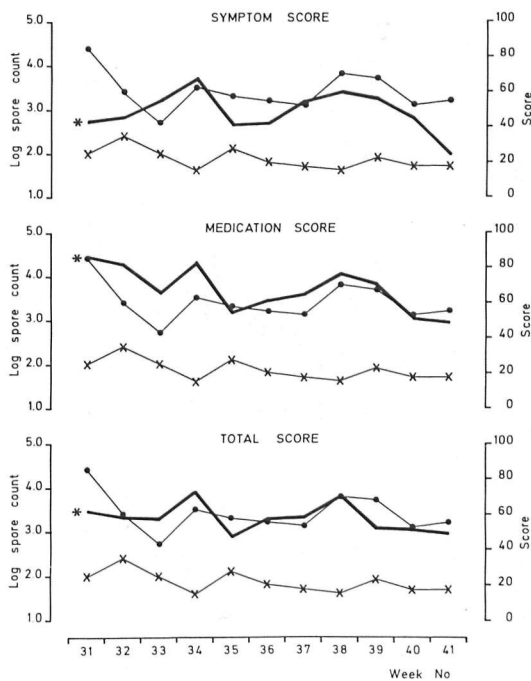


Fig. 2. Relation of weekly spore count and median relative symptom, medication and total scores (for further details of definition of scores see text). *Cladosporium* ● *Alternaria* ×. * Represents data based on only eight patients.

spore count. The score in week 31 is based on eight observations only, and thus not as representative as the other data.

No direct correlation between median scores and weekly spore counts was found, although some degree of positive correlation exists between medication score and *Cladosporium* spore count ($\rho = 0.62$, $P = 0.05$) (Fig. 3). The correlation obtained with total score was lower ($\rho = 0.50$, NS), and for the symptom score, as low as 0.20 (NS). To evaluate the possibility of tachyphylaxis, i.e. a reduction of asthma score during the observation period, or an increase in scores elicited by an equal number of spores, an index of weekly score/log spore was calculated. As is seen in Table 1, this phenomenon does not occur as the index of the symptom, medication and total scores was rather constant during the 11-week registration period.

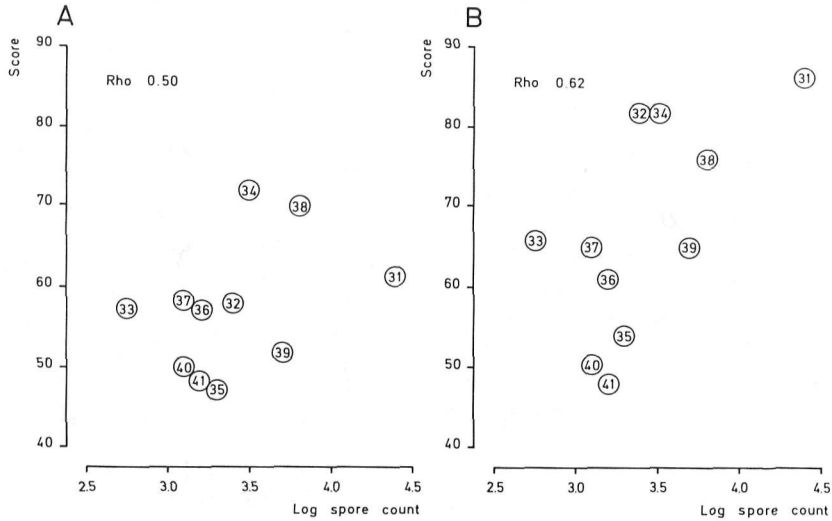


Fig. 3. Correlation between weekly *Cladosporium* sporre count and median relative total score (A) and medication score (B). The week numbers are indicated.

As evident from Fig. 2, the influence of the *Alternaria* spore count on the asthma score of the examined patients appears minimal, although 75% of the patients were allergic to this species as estimated by both SPT and BPT. To clarify this problem the median weekly scores of 18 patients allergic to both *Cladosporium* and *Alternaria* were related to *Alternaria* spore counts. As is shown in Fig. 4, which only states the results of total scores of these 18 bi-mould allergic patients, the score increases and decreases are significantly ($P < 0.05$) related to *Cladosporium* spore counts, but an almost inverse relation to the *Alternaria* spore counts was found. This inverse relation of

asthma score and *Alternaria* spore counts is reinforced by the data presented in Fig. 5 showing a negative, although statistically not significant, correlation ($Rho = -0.12$).

No significant correlation of individual absolute mean score (calculated as stated in Material and Methods) and the results of the diagnostic tests was demonstrated. The highest value of correlation was found for total score/BPT sensitivity ($Rho = -0.39$). In all calculations BPT obtained a higher correlation than SPT, RAST and HIST. The value of medication score and BPT was -0.33 , peak flow score and BPT -0.30 and asthma score and BPT -0.10 .

Table 1
Weekly score/log spore count index

Week No.	31	32	33	34	35	36	37	38	39	40	41
Symptom score	9.5	13.4	20.1	19.2	12.4	13.1	17.5	15.9	15.0	14.3	7.5
Medication score	19.4	24.3	24.1	23.5	16.3	19.1	20.8	20.2	17.7	16.4	15.1
Total score	13.9	17.2	20.8	20.6	14.2	17.8	18.6	18.6	14.0	16.2	15.1

Data of week 31 based on only eight patients and of the remaining weeks on 24 patients.

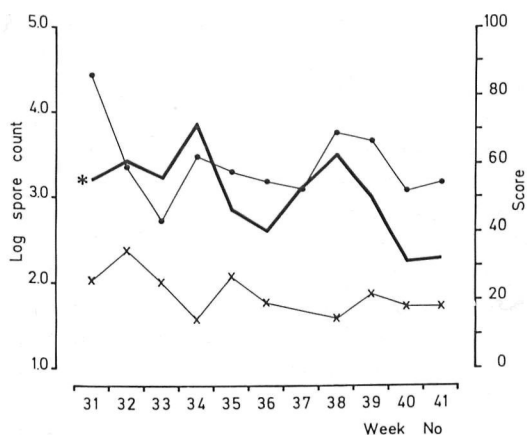


Fig. 4. Relation of weekly spore count and median relative total symptom score in 18 patients allergic to both *Cladosporium* ●, *Alternaria* ×. * Represents data based on six patients.

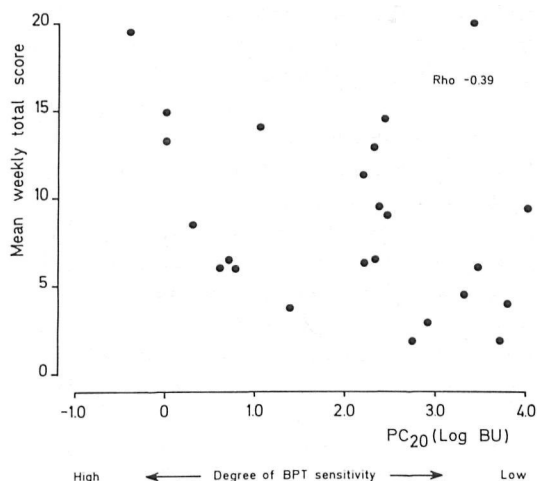


Fig. 6. Correlation between degree of BPT sensitivity (PC_{20}) and mean absolute total score of the 11-week observation period.

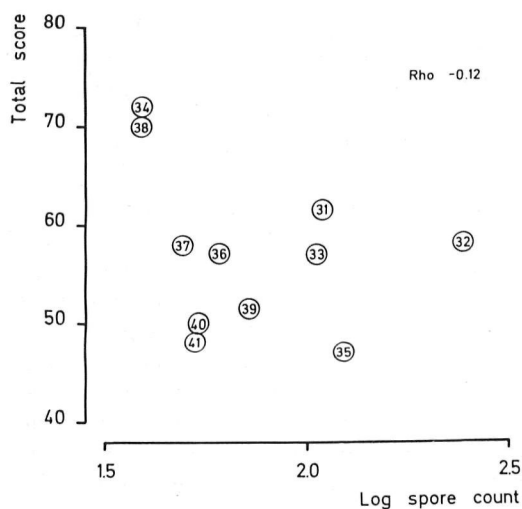


Fig. 5. Correlation between weekly *Alternaria* spore count and median relative total score. The week numbers are indicated.

DISCUSSION

The role of mould spores in eliciting clinical allergy has been recognized for more than 60 years (9, 21). Most reports, however, are based

on observations in single patients (7). Demonstration of a cause-and-effect relationship of moulds to clinical allergy in larger groups of patients has virtually not been published, and surveys of mould allergy are primarily based on demonstration of IgE reactivity (skin test, RAST and challenge test) (20). The mould genera *Cladosporium* and *Alternaria* (1, 13) have been recognized as those most often producing hypersensitivity skin reactions in allergic individuals, they also have the highest spore counts (12, 20) and, in northern Europe, a rather well-defined season (12). The only report revealing a relation between mould spores and summer-autumn asthma in a larger group of patients was published by Bruce et al. (6), who found significant correlation between mould spores and clinical symptoms in 83% of patients participating in a ragweed allergy immunotherapy study. The authors furthermore question the role of pollen as a causative agent in asthma disease as only 45% had asthma symptoms correlating with ragweed counts. The same weak correlation between asthma and grass pollen counts has been published in another immunotherapy study. (26).

The patients investigated in the present study

all have clear evidence of immediate type (IgE) hypersensitivity to the mould species *Cladosporium* as estimated by SPT, RAST, HIST and BPT. The significant association between increases and decreases of *Cladosporium* spore count and clinical symptoms in a 2 1/2-month observation period, during which other airborne allergens were only present in the first 4 weeks and in decreasing amounts, is highly suggestive of a causal relationship. To avoid interference of persistent/delayed asthma (2), no day-to-day relation between clinical symptoms and spore counts was calculated. However, the mean weekly relative score of symptoms (asthma + peak flow), medication and the combined (total) score fluctuates significantly with the *Cladosporium* spore counts (Fig. 2). By the use of relative scores (changes in scores) in contrast to absolute scores (stated by a fixed score system for all patients) in the evaluation between spore counts and symptoms, the problem of interindividual scoring of asthma symptoms and the use of antiallergic medication was avoided. The direct cause- and-effect relationship found in the present study is as clear-cut as that found in many studies on seasonal hay fever symptoms (8, 16, 22, 26).

The relative role of pollen and mould spores in producing seasonal asthma has been discussed by several authors (3). During the 4-week period when airborne pollens were present, we found no evidence of interaction in the relation between symptoms and *Cladosporium* spore counts of either grass or mugwort pollen, whereas they seemed to be responsible for hay fever, as these symptoms disappeared (50 % of the patients) or declined significantly after week 35. The house dust mite is a well-known cause of asthma, and half of the patients had a positive SPT to *Dermatophagoides pteronyssinus*. Thus an expected increase in asthma symptoms by the termination of the registration period (due to increase in mite counts in autumn) was observed in only six patients, whereas another six patients recorded a decreasing asthma score. These observations may lead to the conclusion that neither pollen nor mite allergy constitutes a major role in these selected mould-allergic

patients. Allergy to animal dander was found in about 70 % of the patients, but a possible influence could not be elucidated from the weekly data.

The problems involved in performing direct correlations between symptom score and pollen counts have been pointed out by Wilkinson et al. (24). The use of rank correlation partly overcomes these problems. In the present study we only found a significant correlation regarding medication score, although both symptom and total scores were positively correlated to *Cladosporium* count. The low correlation found for symptom score ($Rho = 0.20$) might, to a certain degree, be caused by unspecific hyper-reactivity (2), although this was not indicated by the score/spore index as no tendency of elevated values in the week following a high-spore-count-week was observed (Table 1). A possible interpretation could be that for a large group of patients the relative use of antiasthmatic drugs is superior to subjective scoring as an expression of the severity of the disease.

Contrary to expectation, *Alternaria* does not seem to play a major role in eliciting asthma symptoms in patients allergic to both *Cladosporium* and *Alternaria* (1, 12, 13, 20). This might be a consequence of the lower spore counts, but might also be due to the larger dimension of the *Alternaria* spore compared with the *Cladosporium* spore (10), with the possibility that a larger proportion of spores is retained in the upper airways and is thus not inducing asthma. The data do not exclude that *Alternaria* is responsible for some of the symptoms. The important fluctuations, however, are caused by *Cladosporium* spores. Based on quantitative immunoelectrophoresis, no cross-reactivity of the involved *Cladosporium* and *Alternaria* allergens exists (unpublished results).

Contrary to hay fever (23), no significant correlation exists between the degree of asthma symptoms and the objective measurements of allergic sensitivity as estimated by BPT, SPT RAST and HIST. In spite of the problems involved in BPT (18) this objective measurement of allergy to *Cladosporium* appears to be the most preferable for predicting the severity of the disease. This indicates the complex nature of

asthma and emphasizes that the diagnostic tests are useful only in revealing IgE-reactivity, and are of limited value in establishing a specific diagnosis. The specific diagnosis or the specific allergic etiology of symptoms can only be confirmed by careful registration of the symptom patterns elicited by allergens producing positive diagnostic tests. The nine patients with negative BPT to *Cladosporium* showed no fluctuations related to *Cladosporium* spore counts. Thus a negative BPT may exclude important clinical allergy, but a positive test does not predict anything about the clinical relevance. This point is extremely important in the evaluation of the relative value of diagnostic tests in studies establishing a specific diagnosis by BPT (15, 25).

Contrary to Bruce et al. (6), we do not consider autumnal asthma a multifactorial disease, but, to a high degree, elicited by mould spores, especially *Cladosporium*, and thus likely to respond to immunotherapy with a single allergen. Data from a placebo controlled, double-blind immunotherapy study with *Cladosporium*, including the present patients, are being studied.

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