

The incidence of allergic aspergillosis in chronic asthma

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Hinson and colleagues¹ described bronchopulmonary infections with Aspergillus fumigatus associated with chronic asthma, expectoration of brown plugs containing mycelia, fever, transient pulmonary infiltrates, and sputum and blood eosinophilia. Several series of cases of allergic aspergillosis accompanied by positive skin tests and precipitating antibody have been reported from England. In Baltimore from December, 1964, to June, 1966, 53 patients with chronic asthma were studied during exacerbations to determine the incidence and significance of Aspergillus infection. An additional patient was referred with the clinical diagnosis of aspergillosis. Sputum from only 2 patients grew A. fumigatus on more than one occasion. In the referred patient, Aspergillus undoubtedly represented a complication of bronchiectasis, but the accompanying wheezing, fever, sputum eosinophilia, and positive skin test indicated an allergic response. The other patient presented the characteristic clinical features of the syndrome of allergic bronchopulmonary aspergillosis except mycelia in the sputum. Allergic pulmonary aspergillosis in the course of chronic or recurrent asthma appears to be infrequent in the Maryland area.

In 1952, Hinson, Moon, and Plummer¹ reported 3 cases of "allergic bronchopulmonary aspergillosis." The essential features of the syndrome were episodes of wheezing with low-grade fever, expectoration, transient pulmonary infiltrates, and eosinophilia of the blood and sputum. The sputum grossly contained small "brown plugs," which on microscopic examination proved to be clumps of mycelia. Eosinophils were prominent, and Charcot-Leyden crystals were sometimes present. Cultures were positive for *Aspergillus fumigatus*. The syndrome was differentiated from secondary aspergillosis complicating cav-

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ities due to tuberculosis, bronchiectasis, lung abscess, or tumor, and from severe *Aspergillus* bronchial infection occurring in patients whose resistance was compromised by severe underlying disease.¹⁻³

Since Hinson's report, Pepys and colleagues⁴ and Campbell and Clayton⁵ have described many more patients with allergic aspergillosis in whom immediate positive wheal and erythema skin reactions and precipitins to *Aspergillus* extracts were frequently demonstrated. Many such patients had had asthma for years, and during an exacerbation, the characteristic clinical manifestations described were present and *Aspergillus* was cultured from the sputum.⁶ Recrudescence or recurrence of the infection accompanied by asthmatic symptoms over months or years was described by Hinson.¹ Frankland and Hamilton⁶ also emphasized that many of these patients had had asthma due to other causes before developing aspergillosis.

Reports of this condition have come principally from England. The syndrome of asthma with pulmonary infiltrates, eosinophilia of blood and sputum, and sometimes fever has often been described in the United States, but never to our knowledge related to *Aspergillus* infection,* although secondary aspergillosis is well known. How frequently *Aspergillus* pulmonary infection causes chronic or recurrent asthma or acute exacerbations thereof in the United States is not known, and this question prompted the present study. If *Aspergillus fumigatus* infection could be demonstrated to be a significant etiologic factor, it would confirm the reports from England and provide a fruitful field for future studies on the etiology of asthma.

In the present study, an initial attempt was made to select patients who presented the clinical features of the syndrome described above. Preliminary surveys, however, revealed no patients satisfying these criteria; hence, 35 ambulatory patients with chronic asthma seen in the Allergy Clinic and 18 suffering from severe exacerbations and admitted to the Hospital were eventually selected for studies, including culture of sputum for bacteria and fungi, skin testing with *Aspergillus* antigens, and examination of sputum and blood for eosinophils.

MATERIALS AND METHODS

Selection of patients

Fifty-three patients were selected because they had suffered from chronic asthma for years, and many were studied during a period of exacerbation which, in 18 patients, was severe enough to require hospitalization. All were following the usual therapeutic regimen. Twenty-six patients were receiving

*Loeffler's syndrome, as originally described, consisted of transient pulmonary infiltrates, and blood eosinophilia with minimal or no systemic symptoms; observations since Loeffler's report indicate a similar syndrome occurs in, and may be a characteristic feature of, other disease entities; in this broader concept, the syndrome of allergic aspergillosis may be considered a variant of Loeffler's syndrome of a more chronic nature. In the United States in 1935, Craven⁹ described 3 cases with long-standing asthma which presented with cough, sputum, fever, mycelia in the sputum, heavy growth of *Aspergillus fumigatus* from the sputum, and positive immediate or delayed skin reactions to *Aspergillus* extract. Although Craven did not examine the sputum of blood for eosinophils and did not postulate the presence of an allergic reaction to *Aspergillus*, these cases appear to be good examples of the syndrome under discussion.

Table I. *Ranges of age and asthma duration*

<i>Age distribution</i>		<i>Duration of asthma</i>	
<i>Age (years)</i>	<i>No. of patients</i>	<i>Years</i>	<i>No. of patients</i>
1-10	4	½- 5	15
11-20	9	6-10	18
21-30	5	11-15	5
31-40	7	16-25	6
41-50	13	26-35	9
51-60	13	35-45	0
Over 60	3	36-45	1
Total	54	Total	54

Table II. *Past history and concomitant conditions of patients studied*

<i>History and/or concomitant conditions</i>	<i>No. of patients</i>
Hayfever or allergic rhinitis	18
Acute infections, including bronchitis, pneumonia, pneumonitis	14
Urticaria and rash	13
Sinusitis	7
Bronchiectasis, emphysema, cor pulmonale	6
Tonsillitis and sore throat	5
Nasal polyps	4
Atopic dermatitis or eczema	2

maintenance daily steroids (prednisone), 30 had received antibiotics from time to time, and 16 were being desensitized to common pollens and household inhalants. As noted below, 1 patient without prior asthma was referred specifically for consideration of aspergillosis. There were 23 Caucasians and 31 Negroes. Twenty were men and 34 were women. The ages ranged from 7 to 71 years. The duration of asthma varied from less than 1 year to 52 years (Table I).

Twenty-nine patients had a family history of asthma, hay fever, urticaria, and/or atopic dermatitis. Twenty-three had no atopic family history. The relevant past histories and concomitant conditions of the patients studied are shown in Table II.

Sputum examination

Sputum was collected in a sterile test tube or Petri dish and examined the same day. Specimens were obtained at bronchoscopy from the 19 inpatients. Sputum was examined grossly for color, consistency, and for the presence of brown plugs and/or flecks. Septate hyphae were searched for by direct microscopy. An equal amount of 2 per cent pancreatin solution was added to the collected sputum for one hour. After a wire loop had been used to pick out flecks or plugs, the homogenized material was spread lightly on Sabouraud maltose agar slants. Two sets of three types of culture media were inoculated:

plain Sabouraud medium, Sabouraud medium with 0.04 Gm. chloramphenicol,* and Sabouraud medium with cyclohexamide. One set was incubated at 27° C. and the other at 37° C. Both sets were read after 1 week. If no growth was observed, the culture tubes were incubated for 2 more weeks.⁷ The presence of eosinophils on smear was graded as follows: 1+, few to occasional eosinophils; 2+, many eosinophils but other cells predominant; 3+, predominant eosinophils but other cells noted; and 4+, cells almost exclusively eosinophils in large numbers. Bacterial cultures were also made with blood agar, chocolate agar, and desoxycholate agar plates. The bacterial culture plates were incubated at 37° C. for 24 hours, and then examined for growth.

Skin tests

The patients were skin-tested intradermally to extracts of three species of *Aspergillus*: *A. fumigatus*, *A. terreus*, and *A. niger*. Extracts were prepared as follows: cultures obtained from the bacteriology laboratory of The Johns Hopkins Hospital were grown in Sabouraud's broth for 4 weeks at 27° C. The mycelial mat was harvested and washed with normal saline solution and then dried with 95 per cent alcohol and ground. Ten cubic centimeters of Coca's solution was added to 1 Gm. dry weight of mat, and the mixture was passed through a Seitz filter.

Precipitating antibodies against *A. fumigatus* were determined in the 2 patients suspected of allergic aspergillosis by the agar gel diffusion test with Ouchterlony's method. The plates were incubated at 27° C. for 3 to 7 days, and the precipitation lines were observed.

RESULTS

A variety of fungi were grown from the sputa (Table III); yeast, particularly *Candida albicans*, was the most common. In 2 patients, *A. fumigatus* was grown in more than a single culture, and these 2 patients will now be described in detail:

Case 5

A 34-year-old television technician was admitted to this hospital on January 11, 1965, with cough and fever which had persisted for 3 weeks. A severe pneumonia in infancy had resulted in chest deformity. At age 32, hemoptysis was followed by surgical removal of emphysematous bullae from the right lung. In April, 1964, he developed a spontaneous right pneumothorax followed by an empyema which was drained and a thoracoplasty was carried out. Following this procedure, chronic cough and expectoration developed and he was admitted. Chest x-ray revealed scarring of the right lung and bronchogram showed bronchiectasis of the right lung. Gross inspection of the sputum showed a few brownish flecks, and a stained smear showed many eosinophils and a few septate hyphae. This patient was referred to the authors for study. *A. fumigatus* was grown on a number of occasions. The patient was at times wheezy, but not severely so. A positive immediate skin reaction to 1:10 dilution of *A. fumigatus* antigen was demonstrated. The peripheral blood smear showed 14 per cent eosinophilia on one occasion but otherwise the counts varied from 0 per cent to 3 per cent eosinophils. The patient's fever subsided on penicillin therapy and he was discharged on January 31, 1965, only to return on February 3

*Suggested by Dr. A. W. Frankland, St. Mary's Hospital, London, as being less inhibitory to *Aspergilli* than cyclohexamide but still inhibitory to bacterial growth.

Table III. *Fungi grown from the sputum of 54 patients*

<i>Fungus</i>	<i>No. of patients</i>
No growth after 3 weeks	27
Yeast (<i>C. albicans</i> mostly and <i>Saccharomyces</i> sp.)	16
<i>Pullularia pullulans</i>	4
<i>Penicillium</i> sp.	3
<i>Mucor</i> sp.	2
<i>Aspergillus fumigatus</i>	4

because of recurrence of fever. *A. fumigatus* was again grown from the sputum and an empyema spontaneously drained through the chest wall. At operation the empyema was explored; a bronchopleural fistula was demonstrated and closed by further thoracoplasty. A pure culture of *A. fumigatus* was obtained from the cavity. No further febrile episodes have occurred and cultures for *A. fumigatus* have been sterile. Serum precipitins against *A. fumigatus* were not demonstrated.

This patient presented with *A. fumigatus* infection, complicating bronchiectasis, bronchopleural fistula, and empyema. The presence of sputum eosinophilia, a positive skin test, and wheezing suggests that the patient had an allergic reaction to the fungus.

Case 10

An 11-year-old boy from a coastal city in Florida was admitted to this hospital on February 26, 1965. Severe asthma had been present episodically for 2 years. Expiratory wheezing and hyperaeration of the lungs were present. Eosinophilia of sputum was 4+ and of the nasal secretions, 1+; the blood eosinophils were not increased, although the referring physician had reported 23 per cent on January 31, 1965. Positive immediate skin reactions to dust, live oak, and elm and several mold spores, including *A. fumigatus*, were demonstrated. On March 10 an exacerbation of asthma developed which was accompanied by a low-grade fever for 2 days and blood eosinophilia of 21 per cent. A sputum referred to the general bacteriology laboratory did not grow fungi. The acute episode subsided after 2 days. Chest x-ray was not obtained until the next flare-up on March 31, 1965; small infiltrates in the right upper and left lower lobes were present. The sputum was not examined for *Aspergillus* until April 3, and a second specimen was obtained on April 10. Smears on both specimens were negative for septate hyphae but showed 1+ eosinophilia. Eventually a few colonies of *A. fumigatus* were grown from both specimens. Improvement was observed on 10 to 20 mg. prednisone daily, but occasional wheezing still occurred during the following year. He returned for re-examination in June, 1966, when having only occasional mild asthmatic episodes. The bronchoscopic examination was normal. An expectorated specimen of sputum was negative for *Aspergillus*, other fungi, and eosinophils. The skin test with *A. fumigatus* antigen was now negative, and eosinophilia of the blood was not present.

Although this patient lacked the brown plugs in the sputum and also a heavy growth of *Aspergillus* (characteristic of the typical syndrome described by Hinson and co-workers¹), he represents a possible case of allergic aspergillosis on the basis of wheezing, fever, eosinophilia, transient pulmonary infiltrates, two isolations of *A. fumigatus*, and a positive immediate skin test to *A. fumigatus* antigen. Serum precipitins for *A. fumigatus* were never demonstrated. If this patient had an *Aspergillus* infection which caused an exacerbation of his asthma, it was an intercurrent event in the course of chronic asthma originally due to other causes, such as inhalants, pollens, and infections with common respiratory pathogens.

In 2 other patients one or a few colonies of *A. fumigatus* were isolated from a single sputum specimen. Other specimens from the same patients were

negative, and one could conclude they represented examples of *Aspergillus* spores transiently present in sputum but without relationship to the patient's disease.

Skin tests

All of the 54 patients in this study had been skin-tested and gave significant skin reactions to one or another of either household inhalants (dust, feathers, kapok, cottonseed, dog, and cat hair) or the common pollens (rag-weeds, grasses, and trees) or various spores. There was only 1 patient—Case 13—who did not show significant reactions to these allergens. Thirty-five patients were tested intradermally with 1:10 dilution of specially prepared antigens of *A. fumigatus*, *A. terreus*, and *A. niger*. The data reveal that the three antigens were equally active and usually elicited skin reactions of similar size. Seventeen were considered positive and 18 were negative. The negative reactors gave no evidence of primary irritative reactions to the antigenic preparations. One patient—Case 2—with a history of bronchial asthma and perennial rhinitis for 15 years, gave a 3+ reaction, but there was no evidence to indicate that the current condition was related to *Aspergillus* infection. Of the 2 patients just described in detail from whom *A. fumigatus* was isolated, one had a 1+ and the other a 2+ skin reaction to *A. fumigatus* antigens.

Of the 54 patients, 38 had sputa positive for eosinophils, and in 15 patients they were predominant (3 to 4+). No attempt was made to compare blood and sputum eosinophilia since these determinations were not always done simultaneously.

Febrile episodes with a temperature between 99° to 102° F. occurred in only 4 patients, including the 2 patients presented in detail. There were only 2 patients whose sputum contained brown flecks suggestive of fungus as described by Ilinson and colleagues.¹ They were Cases 5 and 52. Cultures on Case 5 grew *Aspergillus*, as mentioned previously; Case 52 did not. Thirty-five patients had chest x-rays and their interpretation is shown in Table IV. Nothing unusual was revealed except peribronchial infiltrates in 4 patients, including the 2 patients described in detail above. The bacterial cultures and gram stain smears of sputum revealed essentially normal bacterial flora, including alpha streptococci, diphtheroids, *Neisseria* sp., *Klebsiella* sp., and *Diplococcus pneumoniae*.

Table IV. *Chest x-ray findings in 34 patients*

<i>Interpretation</i>	<i>No. of patients</i>
Essentially normal	12
Hyperaeration	5
Peribronchial infiltrates	4
Emphysema	5
Prominent bronchovascular markings	2
Pneumonia	2
Mild atelectasis	1
Others (calcific changes, cardiomegaly, pleuritis)	4

DISCUSSIONS

Aspergillus fumigatus is a ubiquitous fungus often contaminating sputum cultures and present at times in the respiratory tract without harmful effects. The mere growth of *A. fumigatus* in sputum is not *prima facie* evidence of aspergillosis; septate hyphae should be demonstrated in the sputum, and the organism should be grown from several specimens. Frequently the demonstration of septate hyphae is missed. In the present study, only 2 patients, 5 and 10, out of the 54 patients grew *A. fumigatus* from more than one sputum specimen. Only in Case 5 were septate hyphae demonstrated. Case 10, despite the inability to demonstrate hyphae, is the only case otherwise satisfying the criteria for the syndrome of allergic bronchopulmonary aspergillosis described by Hinson.

The difficulty of making the diagnosis of allergic aspergillosis on clinical grounds is illustrated by the several patients who had one or another feature of the syndrome without receiving laboratory confirmation of the presence of *Aspergillus*. As already noted, in this series of 54 patients, 2 had brown flecks in the sputum, 2 others had febrile episodes with their asthma, and another 2 had peribronchial infiltrates. None of these individuals, however, had any of the other distinguishing features of the syndrome.

Pepys and coauthors⁴ surveyed 2,080 patients with various chest disorders and found that 145 had positive cultures for *A. fumigatus*, 42 of which had asthma. Campbell and Clayton⁵ reported that of 269 patients suspected of having bronchopulmonary aspergillosis, 244 had positive cultures for *A. fumigatus*. Two hundred and ten of the suspects were asthmatic, and 87 had the Hinson syndrome of allergic bronchopulmonary aspergillosis. Only one of this group did not grow *A. fumigatus*.

The incidence of allergic type of bronchopulmonary aspergillosis noted in these studies and our own cannot be compared. The earlier studies included patients preselected to conform to one of the syndromes in which *Aspergillus* occurs, whereas our cases represent an otherwise unselected series of patients with chronic asthma. The association of bronchial aspergillosis and asthma may be more frequent in England, as most recognized cases have come from that country. The infrequency of aspergillosis in our series of asthmatic patients suggests that it is a rare cause of asthma in this area. It may be, however, that more frequent examination of asthmatic sputum by smear and culture might uncover more cases. The methods of examination used in this study can be accomplished by most hospital bacteriology laboratories.

Aspergillosis as a secondary infection is well recognized in this hospital because the autopsy files of this hospital from 1951 to 1963 contain records of 26 patients who had aspergillosis histologically. The majority of these patients died in the latter part of this period. The diagnoses were made post mortem and represent the opportunistic or secondary type of aspergillosis.⁸

Of interest is the demonstration by Pepys and colleagues⁴ and Campbell and Clayton⁵ of precipitating antibodies to *A. fumigatus*. These antibodies are more frequently demonstrated in the secondary type than in primary allergic bronchopulmonary aspergillosis; and the number of precipitating

bands is more numerous in the former than in the latter. In our 2 patients with allergic manifestations who grew *A. fumigatus*, no precipitins were demonstrated.

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