Invasive Pulmonary Aspergillosis
Difficulties in Establishing the Diagnosis and Distinguishing Primary from Secondary Infection

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Reports of aspergillosis are appearing with increasing frequency since the widespread use of broad-spectrum antibiotics, corticosteroids, radiation and anticancer chemotherapy. This may also be partly due to increased awareness of fungus diseases in general.

In our review of well over 100 publications on pulmonary aspergillosis, we found the evidence for establishing the diagnosis to be rather dubious in many of them. Hison, Moon and Plummer and more recently, Finegold and associates published excellent reviews of the subject with extensive bibliographies. We have seen no subsequent publication that would justify any significant modification of their comprehensive discussions and we will avoid a repetitious review at this time.

The present case report deals with the difficulties of: (a) establishing an indisputable diagnosis of invasive pulmonary aspergillosis without a specimen of diseased tissue available for complete studies, (b) distinguishing primary from secondary infection, and (c) excluding all possibilities of any pre-existing pulmonary disease.

Case Report

This 65-year-old white man was admitted on January 5, 1960. In April, 1930, a routine x-ray film of the chest revealed minimal infiltration in the left apex. He was treated for minimal pulmonary tuberculosis for five months in 1930 and again for 14 months in 1934. In 1950, he was hospitalized for psychosis; serial x-ray films revealed progression of his pulmonary lesion by early 1952. He was then transferred to Sunmount where he received 1 gm. of streptomycin twice weekly and the equivalent of 12 gm. of para-aminosalicylic acid from February, 1952 to February, 1953. Tubercle bacilli were never recovered and serial x-ray films revealed essentially no change despite a prolonged course of "original" chemotherapy. In 1956, he was admitted elsewhere for psychiatric treatment and a routine x-ray of his chest revealed progression of his disease. However, he received no further antituberculosis drug-therapy.

In the late summer of 1959, he noticed loss of weight, poor appetite, increasing cough productive of a moderate amount of purulent sputum which was intermittently streaked with blood, and an occasional hemoptysis of an ounce or two. By the fall of 1959, he was febrile and very ill. He consulted a local physician in late December, 1959, received penicillin for a few days, and was then referred for admission.

On admission to Sunmount on January 5, 1960, he was seriously ill, cachetic, and complained of a severe cough, productive of several ounces of purulent, blood-tinted sputum daily. His temperature was 39°C., weight 107 lb., and height 5 ft., 7 inches. Physical examination revealed a bleeding rectal polyp (excised on January 12, 1960, because of profuse bleeding). Examination of the chest revealed dullness, markedly diminished breath sounds and scattered rales over the left hemithorax.

The admission white blood count was 15,000 per cmm., neutrophils 75 per cent, lymphocytes 16 per cent, monocytes 7 per cent, eosinophils 1 per cent, hemoglobin 9.9 gm. per cent, hematocrit 36 per cent and sedimentation rate (Cutler method) 24 mm. per hour. On January 24, 1960, the white blood count was 19,600 per cmm. with a similar differential. Several blood counts before operation revealed 0 to 1 per cent eosinophiles. Repeated urinalyses were within normal limits. The blood urea nitrogen was 17.4 mg. per cent and the fasting blood sugar 112 mg. per cent. The glucose tolerance test was normal. Serum sodium was 129 mEq., chloride 89 mEq., and potassium 3.75 mEq. per liter. The VDRL was nonreactive. Serologic tests for histoplasmosis, coccidioidomycosis, and blastomycosis were

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negative. Skin tests for tuberculosis (PPD no. 2 repeated), histoplasmosis, coccidiodomycosis and blastomycosis were nonreactive.

Serial roentgenograms revealed minimal infiltration in the left apex in April, 1930 (Fig. 1) that progressed to moderately advanced stage by February, 1953 (Fig. 2) and far advanced extensively involving the entire left lung on preoperative film of February, 1960 (Fig. 3). Planigrams in January, 1960 revealed multiple cavities in left upper lobe and superior segment of lower lobe, but no so-called “fungus-ball" cavity.

Many adequate purulent, blood-streaked sputum specimens were examined by smears, cultures and inoculations of guinea pigs and mice without successful recovery of tubercle bacilli or fungi—with one exception. Aspergillus niger was cultured from a sputum specimen of February 6, 1960 (reported three days before pneumonectomy). Repeated cytologic studies of the sputum revealed no neoplastic cell.

In view of his past history of tuberculosis, the extent of his disease and serious condition, daily streptomycin and para-aminosalicylic acid therapy were started as soon as the first two sputum specimens were obtained for bacteriologic and mycologic studies. His clinical course before resection was variable, but in general unfavorable. His temperature subsided to 37.3° C. during the second week of therapy, only to recur and range from 38.3 to 39° C. (despite addition of penicillin and tetracycline), until the left lung was excised on February 12, 1960.

The gross specimen revealed extensive necrotizing pneumonia throughout the left lung with more fibrotic scarring in the upper than in the lower lobe. The upper lobe contained one large and several smaller cavities; the superior segment of the lower lobe also contained a cavity. The large cavity was completely filled with a fungus-ball. Numerous small abscesses were scattered throughout the lung.

The cellular reaction was composed of plasma cells, lymphocytes, epithelioid cells, atypical giant cells, vascular granulation tissue, and scattered small abscesses containing segmented neutrophils. No evidence of tuberculosis or other specific underlying pulmonary disease was evident. The usual characteristic findings of primary bronchiectasis were absent. Special stains revealed no acid-fast bacilli, but did demonstrate widespread invasion of the pulmonary parenchyma by branching septate hyphae. Hyphae were also seen within the lumina of pulmonary vessels. Lesions of chronic endarteritis obliterans were noted in numerous large, medium and small arteries. Mycelia, heads, stalks and spores characteristic of Aspergillus were prominent within open cavities and lumina of bronchi. In several areas, bronchi appeared to be distended by an expanding mycetoma representing a mass of well stained branching septate hyphae. Several cultures from the ex-
cised specimen revealed luxuriant growth of a fungus which was identified as *Aspergillus niger* by its typical cultural and morphologic characteristics.

After detailed study of many slides from all lobes, our pathologist (M.M.) was of the opinion that the lesions of longest duration (perhaps years) were in the apical-posterior segment of the upper lobe, that the disease gradually extended downward, and that the most recent lesions were in the lower lobe. He is submitting a detailed report of the pathologic and pathogenetic features of this case for publication.

The patient's productive cough ceased abruptly after surgery and his temperature became normal by the third postoperative day. Meanwhile, his hemoglobin, hematocrit, blood count and differential, and his sedimentation rate returned to normal.

During the third postoperative week, his temperature rose to 37.8° C. for six days and a chest roentgenogram revealed moderate new infiltration in his remaining lung. His white blood cell count again rose to 16,250 per cmm., neutrophils 65 per cent, lymphocytes 27 per cent and monocytes 8 per cent (eosinophiles 0 per cent). Several blood cultures were sterile. Smears and cultures of several sputum specimens for predominating organisms revealed a common flora except for one report of a penicillin-resistant, coagulase-positive micrococcii. Several cultures for fungi failed to grow aspergilli or any other fungus. Penicillin therapy was started in hopes of retarding further encroachment upon the remaining lung by any progressive pulmonary infection in such a debilitated patient, and ristocetin was added when recovery of the penicillin-resistant Micrococcus was reported.

Potassium iodide therapy was started on March 6, 1960, and stopped six days later because of an iodide rash. A skin test with autogenous vaccine was nonreactive and desensitization was not pursued. The roentgenographic worsening noted during the third postoperative week was almost completely cleared two weeks later. Whether this postoperative worsening was due to retained secretions (due to ineffective cough), or pneumonia, or both, or possibly an allergic reaction to residual infection with aspergillus, was never proved. Subsequently his clinical condition improved and he regained ten pounds.

During the 14 months since operation, several episodes of transient infiltration have appeared and cleared in varying areas of the remaining right lung (suggestive of Loeffler's pneumonia). However, the roentgenographic appearance of the right lung in April, 1961 (Fig. 4) was, except for technical factors, quite similar to that of the preoperative film of February, 1960 (Fig. 3).

Whereas his clinical condition improved and he gained ten pounds within two months after operation, this case did not represent a therapeutic triumph. A year later he was still severely underweight, his general condition poor and prog-
nosis grave. No specific evidence of systemic aspergillosis has been found. Intensive studies to explain the lack of further improvement have been unrewarding. Recently, he received another course of potassium iodide therapy (February 22 to April 7, 1961), without re-appearance of a rash, but also without noticeable benefit.

**DISCUSSION**

A complete review of all previous hospital records over the past 30 years revealed no evidence that tubercle bacilli were ever recovered from any of the many sputum cultures reported; not even a "positive" smear for acid-fast bacilli. No tuberculin test was reported prior to his current admission. Thus, it seems fair to say only that this patient has had pulmonary disease of undetermined etiology for 30 years.

During his current hospitalization, the tuberculin test (PPD No. 2 repeated) was nonreactive. Tubercle bacilli were persistently absent (by concentrated smears and cultures) from the many adequate purulent, blood-stained sputum specimens of a patient with very extensive destructive pulmonary disease. Thus, a diagnosis of advanced active pulmonary tuberculosis seems remote. Skin tests, serologic tests and sputum cultures, as well as animal inoculations, revealed no definite evidence to support a diagnosis of histoplasmosis, coccidioidomycosis, or blastomycosis.

Since our patient's clinical condition was deteriorating and his prognosis appeared hopeless without surgery, we recommended pneumonectomy. Our reasons for recommending operation were three: (1) to remove a septic lung destroyed by a necrotizing process of unknown etiology, (2) to obtain a resected specimen from which we might identify a specific etiologic agent, test drug-susceptibilities, and revise further therapy accordingly, and (3) to exclude the possibility of an underlying malignant lesion. The resected left lung was extensively invaded by a fungus identified as *Aspergillus niger* by its cultural and morphologic characteristics.

Attempts to trace the source of his exposure to *A. niger* were unsuccessful. He worked irregularly as a laborer at odd jobs since World War I, and also as a janitor at a local theatre for the past ten years. He denied ever working as a farmer, thresher, fur cleaner, hair sorter, or in the production of cereals. He was never a squab or pigeon feeder, bird fancier, nor does he recall exposure to dead birds or penguins. It is safe to assume that he and all living in this area are exposed to soil and periodically to decaying organic matter.

Most of the common predisposing factors were either absent or unidentified in our case. There was no history of treat-
ment with broad-spectrum antibiotics, adrenocorticosteroids, radiation or antineoplastic chemotherapy prior to his present admission. We found no evidence of diabetes, leukemia, lymphomas or other neoplastic disease. He was certainly debilitated on admission, was admitted with extensive pulmonary disease of undetermined etiology, and he did receive tetracycline for two weeks prior to pneumonectomy.

*Aspergillus niger* was recovered from only one of many sputum specimens cultured before surgery. This sole laboratory report was inconclusive. Recovery of aspergilli from cultures of sputum may indicate: (a) a laboratory contaminant, (b) saprophytic "opportunist" in the oropharyngeal or tracheobronchial flora, (c) secondary infection superimposed upon another underlying broncho-pulmonary disease of other etiology such as tuberculosis, bronchiectasis, cyst, carcinoma etc., or (d) it may occasionally be the primary etiologic agent. As a secondary infection, aspergilli may grow as superficial saprophytes within lumina of cavities or bronchi and contribute little or nothing to the patient's illness; or may locally invade pulmonary tissue devitalized by another underlying primary disease and contribute substantially to his illness; or may invade extensively and disseminate, thus becoming the major cause of illness or even death. In general, aspergillosis has been commonly reported as a secondary super-infection and occasionally as a primary infection of the lung.

The difficulties of establishing a conclusive diagnosis of pulmonary aspergillosis and distinguishing primary from secondary invasive disease is self-evident. In the absence of a definitive diagnosis when the primary cause of pulmonary disease eludes us, we may too readily accept the isolation of aspergilli as a resolution of a diagnostic dilemma. Exclusion of all other primary diseases of the lung is not always infallible. Thus, arriving at a diagnosis by this negative approach is indicative of the inadequacies of our present methods and is hardly as convincing as a proven ("positive") diagnosis.

We believe a diagnosis of invasive pulmonary aspergillosis may be questionable unless a specimen of the involved tissue (obtained by biopsy, resection or at necropsy) is available for complete studies. To establish indisputable evidence for such a diagnosis requires demonstration of invasion of the lung parenchyma by the fungus, and isolation and identification of the fungus by its cultural and morphologic characteristics.

Distinguishing primary from secondary invasive pulmonary aspergillosis may be extremely difficult, or impossible in some cases. Can one be sure that all other primary diseases were excluded rather than merely missed? In longstanding cases of pulmonary disease (30 years in our case), aspergillosis might be secondarily superimposed upon a pre-existing specific pulmonary disease wherein specific histologic features were obliterated or obscured with the passage of time. Likewise, we cannot exclude the possibility that our case was one of primary aspergillosis *ab initio* which, by periodic exacerbations, eventually spread downward from apex to the lower lobe. Thus, we may not categorically state that our case is one or the other (primary or secondary aspergillosis); and from our review of the literature we believe the same pertains to many cases reported in the past.

Exceptions to the requirement of pathologic tissue for study before making a diagnosis of invasive aspergillosis may include repeated recovery of the organism from uncontaminated cultures of blood, cerebrospinal fluid, "closed" pleural effusion etc. Such case reports are few. Persistent recovery of aspergilli from the sputum in a patient with recurrent hemoptysis whose chest roentgenogram reveals a typical "fungus-ball" cavity with a crescent of air (or a case of migrating pneumonitis with peripheral eosinophilia and asthmatic-like attacks) may be highly suggestive and justify a tentative diagnosis. However, demonstration of invasion of the lung parenchyma and identifying the invading fungus is by far more satisfying.
In general, invasive and disseminated aspergillosis have been resistant to available medical therapy. Smith and his associates advocate desensitization with autogenous vaccine for patients who are hypersensitive to the vaccine, and then to follow with potassium iodide therapy. Desensitization has been useful in cases presenting as "asthmatic bronchitis" or migrating pneumonia (Loeffler's syndrome). Whereas high dosages of potassium iodide were effective in eliminating aspergilli from the sputum in non-invasive aspergillosis, toxicity posed a problem in the dosages used by Utz et al. Nystatin may be effective in surface infections including secondary infection of the pleural space. Although aspergilli are notably resistant to amphotericin B, this drug may be tried in cases failing to respond to other treatment. Eulicin does inhibit growth of *A. niger* in vitro. However, no specific drug now available is known to be reliably effective in invasive and disseminated aspergillosis. Amphotericin B, nystatin, eulicin, 2-hydroxyzystilbamidine, (and we hope new and better drugs) will require further evaluation. Localized disease is an indication for resection, and case reports of successful excision are appearing with increasing frequency in recent years.

Few physicians see and indisputably diagnose premortem a sufficient number of cases of invasive pulmonary aspergillosis to investigate the efficacy of various therapeutic agents in a carefully controlled concurrent study on man. *A priori*, with present serologic and immunologic limitations, the diagnosis is uncertain unless a resected specimen is available for complete studies. However, in addition to *in vitro* studies of the susceptibility of the different species of aspergilli to various drugs or antibiotics, controlled studies of the therapeutic efficacy of these medications *in vivo* could be tried in an adequate series of different animal species. Such studies might produce some leads toward more effective medical treatment of this disease.

**References**


**ACCELERATED THROMBOPLASTIN GENERATION**

By means of a "retarded" thromboplastin-generation test, acceleration of thromboplastin generation has been demonstrated in 70 per cent of 64 patients with acute arterial occlusion complicating arteriosclerosis obliterans. This hypercoagulable state is produced by excess clotting activity which appears to be a previously unrecognized accelerator of thromboplastin generation. The excessive activity of this accelerator may be a temporary phenomenon or may persist for as long as three years. Coumarin anticoagulant drugs do not appear to exert any direct effect on the activity of this accelerator.

**References**