Original Research Article

Significance of sputum cultures in diagnosis of invasive pulmonary aspergillosis in acute leukemia – A meta analysis

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Abstract

The diagnosis of Invasive Pulmonary Aspergillosis (IPA) and therapy with Amphotericin B appear to improve survival, but the early diagnosis is difficult. There are no pathognomonic clinical findings for IPA. The commonest presentation is compatible with any of bacterial, fungal, viral or protozoan infections. In addition, IPA may coexist with or arise during the therapy of other infections in immune compromised patients. It was thought that ante mortem isolation of Aspergillus species from respiratory secretions in pathologically confirmed cases occur infrequently. Demonstration of tissue invasion by fungal hyphae remained the accepted standard for diagnosis of IPA. Sputum culture is regarded as not useful for the diagnosis of IPA. Colonization of aspergillus may occur without invasion, hence, culture of respiratory secretions were not reliable. Aggressive diagnostic methods to establish the diagnosis of IPA are warranted. However there may exist important subsets of patients where sputum culture of Aspergillus may still be diagnostically useful. The underlying disease, i.e. immune suppression, neutropenia/ leukemia is a critical factor in selecting patients where sputum cultures may be useful. The degree of immune suppression increases, the diagnostic specificity of isolation of aspergillus from sputum. In this article, a meta analysis of 21 studies, spanning 27 years, the methods adopted for diagnosing IPA by various authors is analysed and discussed. The detailed pathogenesis of IPA is also discussed.

Key words

Invasive Pulmonary Aspergillosis (IPA), Sputum cultures, Immunosuppression, Leukemia, Neutropenia.

Introduction

Aspergillus probably is the most common fungal opportunistic invader of the lung. Invasive Pulmonary Aspergillosis (IPA) is of major concern in neutropenic patients who received antibiotics during chemotherapy for Acute Leukemia but also occurs in patients who receive long term corticosteroids. Patients with acute leukemia have an incidence of IPA 20 fold higher than lymphoma patients or organ transplant recipients.

Sputum cultures yield aspergillus in only 8 to 34% of patients with IPA. Investigators have found that positive anterior nares cultures predict IPA in neutropenic patients with acute leukemia and radiologic lung infiltrates [1]. Serological Tests for detection of antibody to aspergillus for diagnosis of IPA have been disappointing [2].

Pathogenesis

Experimental studies

In experimental Studies with IPA, Sidransky, et al., found that normal mice that inhaled viable spores of A. flavus are resistant to lethal infection, while mice pretreated with corticosteroids and antibiotics exposed to spores develop a high incidence of fatal IPA [3]. Studies conducted to gain insight into the pathogenesis of experimental aspergillosis indicated that mice pretreated with cortisone acetate and then injected intraperitoneally with non-germinating spores of A. flavus developed a high incidence of lethal visceral hyphal aspergillosis [4]. This means, the experimental models implicate corticosteroids, granulocytopenia and impaired monocyte chemotaxis as predisposing factors.

To gain further information regarding the pathogenesis of experimental IPA in steroid-treated mice, an ultra structural study was performed on alveolar macrophages obtained by tracheobronchial washings of control and cortisone treated animals 1/4, 1/2, 2, 4, 8 and 66 hours after inhalation exposure to viable spores of A. flavus. Spores phagocytized by alveolar macrophages of control or steroid treated mice were encircled by phagocytic membranes within $1/4^{th}$ hour after inhalation. The lysosomes of alveolar macrophages of control mice appeared to interact promptly with the phagocytic membranes and cytoplasmic degranulation was marked within 2 hours.

In contrast, the lysosomes of alveolar macrophages of cotisone treated mice appeared to show no significant reaction between lysosomes and phagocytic membranes at any of the intervals studied. Whereas spores with in alveolar macrophages of control mice either remained dormant or became fragmented, spores within macrophages of steroid treated mice showed evidence of pre germination and germination after 2-4 hours. These observations suggest that the cortisone induced alterations within alveolar macrophages may be of importance in the subsequent events leading to lethal IPA in steroid treated mice [5].

Defence mechanisms

New non-phagocytic defense mechanism

Evidence suggests that neutrophils are important in the host defenses against IPA and mucormycosis although hyphae in these lesions are too large to be phagocytized. Interactions of neutrophils with hyphae of A.fumigatus and Rhizopus oryzae were studied in vitro. Light and electron microscopic observations indicated that neutrophils attached to and spread over the surfaces of hyphae even in the absence of serum. This was followed by dramatic morphologic changes which suggested severe damage and probably death of hyphae. An assay of neutriphil induced reduction of uptake of radioisotopes was used to quantitate damage to the fungi by neutrophils from normal subjects. Damage to

hyphae was inhibited by a variety of compounds which are known to affect neutrophil surface functions, mobility and metabolism. Use of inhibitors of oxidative microbicidal mechanisms of netrophils indicated the central importance of these mechanisms in damage to hyphae. Inhibitors of neutrophil cationic proteins altered damage only to Rhizophus. Damage to hyphae by lysozyme suggested that it may play a secondary role in the neutrophill, primarily against aspergillus. The new non-phagocytic mechanism may play an important role in host defenses against these and other hyphal forms of fungi [6].

Selective protection against conidia by macrophages and against Mycelia by polymorphonuclear phagocytes by comparing natural immunity to A. fumigatus in vivo with action of human or mouse phagocytes against A.fumigatus in vitro. Andrias Schaffner, et al. [7] delineated two sequential lines of defence against A.fumigatus. The first line of defense was formed by macrophages and directed against spores. Macrophages prevented germination and killed spores in vitro and rapidly eradicated conidia in vivo even in neutropenic and athymic mice. The second was the neutrophilic granulocyte which protected against the hyphal form of A. fumigatus. Human and mouse neutrophills killed mycelia in vitro. Normal, but not neutropenic mice, stopped hyphal growth, and eradicated mycelia. Either line of defense acting alone protected mice from high challenge doses. Natural immunity collapsed only when both the reticuloendothelial system and polymorphonuclear neutrophils were impaired. These findings are in keeping with the clinical observations that high doses of cortisone and neutropenia are the main risk factors for IPA. Cortisone inhibited the conidicidal activity of mouse macrophages in vivo and of human or mouse mononuclear phagocytes in vitro. Cortisone damaged this first line of defense directly and not through the influence of T. lymphocytes or other systems modifying macrophage function as shown in athymic mice and in vitro. In addition, daily high doses of cortisone in mice reduced the mobilization of polymorphonuclear neutrophil so that the second line of defense was also impaired. Thus cortisone can breakdown natural residence on its own. Myelosuppression rendered mice susceptible only when the first line of defense was overpowered by high challenge doses, by activated spores that cannot be killed by macrophages, or by cortisone suppression of the conidicidal activity of macrophages.

The host, thus, can call upon two independent phagocytic cell lines that form graded defense system against aspergillus. These lines of defense, function in the absence of a specific immune response, which seems superfluous in the control and elimination of the fungus [7].

Qualitative disorders of granulocyte function that occur in acute leukaemia may further increase the likelihood of IPA, much as they do in patients with chronic myeloid leukaemia [8]. Pulmonary toxicity from chemotherapeutic agents also raises the possibility of drug induced lung injury may predispose patients to IPA [9]. Colonization by ubiquitous, ambient aspergillus spores may be increased by antibiotics, prolonged use of corticosteroids and cytotoxic chemotherapy [10].

Diagnosis

Although earlier diagnosis of IPA and therapy with Amphotericin B appear to improve survival, early diagnosis is difficult. There are no pathognomonic clinical findings for IPA. The commonest presentation of course, the compatible with any number of bacterial, fungal, viral or protozoan infections. In addition, IPA may coexist with or arise during the therapy of other infections in immune compromised patients, especially those due to candida species and pseudomonas aeruginosa.

Antemortem isolation of Aspergillus species from respiratory secretions in pathologically confirmed cases occur infrequently, with sensitivity rates ranging from 13 to 34 percent.

Because of the low sensitivity the uncertain specificity of culture results, in the diagnosis of IPA, demonstration of tissue invasion by fungal hyphae remains the accepted standard for diagnosis.

Sputum culture is regarded as not useful for the diagnosis of IPA. For example, in the most patients with IPA, sputum samples failed to yield the organism. Likewise the specificity of the sputum culture result is equally unsatisfactory; most patients with aspergillus on sputum culture will not have IPA. Colonization of aspergillus may occur without invasion. Hence, culture of respiratory secretions was not reliable because they often reflect only colonization. Whereas repeated positive cultures or demonstration on wet mounts are desirable, inadequate sputum production may prevent this. Aggressive diagnostic methods like Nasal cultures, Trans bronchial aspiration cultures, bronchoscopic aspiration lavage cultures, Tran bronchial biopsy – histopathology, Open lung biopsy – histopathology, Pleural fluid cultures, Post mortem cultures of lung tissue, and Post mortem histopathology of lung tissue are warranted, to establish the diagnosis of IPA, so that antifungal therapy can be started early. Thrombocytopenia may also limit transbronchial aspiraton or lung biopsy for ante mortem diagnosis of IPA.

Meta analysis

In this meta analysis 21 studies, were reviewed spread over a period of 27 years, from 1959 to 1986. It consisted of 14 studies in acute leukemias [11-24], 2 in post cardiac transplants [25, 26], and 5 in post renal transplant patients [27-31]. The parameters used to diagnose IPA is detailed in **Table - 1**.They used Sputum cultures, Nasal cultures, Trans bronchial aspiration cultures, Bronchoscopic aspiration lavage cultures, Tran bronchial biopsy – histopathology, Open lung biopsy – histopathology, Pleural fluid cultures, Post mortem cultures of lung tissue, and Post mortem histopathology of lung in diagnosing IPA.

Criteria taken for diagnosis of IPA, in 21 studies are depicted in **Table - 2**. Sputum cultures taken as a criteria in 17 studies, 5 studies advocated multiple sputum cultures , No comment in 4 studies, Sputum cultures – not diagnostic in 2 studies, one study advocates invasive procedure (BAL, bronchoscopic biopsy, Open Lung biopsy, Trans thoracic biopsy).

In my study [32], sputum culture was taken as criteria for diagnosing fungal infectins. All of them had mixed bacterial and fungal infections as shown in **Table - 3** and **Table - 4**.

However there may exist important subsets of patients for which ante mortem isolation of Aspergillus may still be diagnostically useful. The underlying disease is a critical factor in selecting patients in whom ante mortem sputum cultures may be useful as the degree of immunosuppression increases, the diagnostic specificity and clinical applicability of isolation of aspergillus from respiratory tract samples increases. Victor L. Yu, et al. [33], in their study found, that none of the patients with positive respiratory tract culture results among the non-immunosuppressed patients or among the patients with solid tumors or lymphoma group, were shown to have IPA. However, for neutropenic/ leukemia patients with positive respiratory tract culture undergoing tissue examination were ultimately shown to have IPA. It is also consistent with the thesis that those leukemic/ neutropenic patients without tissue diagnosis may well have had IPA.

Summary

- The isolation of Aspergillus from respiratory secretions in non-immunosuppressed patients and patients with solid tumors without neutropenia is rarely indicative of IPA.
- In Immunosuppressed but non-neutropenic patients with a transplant, lymphoma, or steroid therapy, isolation of Aspergillus from respiratory secretion is a cause for concern.

- For patients with neutropenia or leukemia (chronic lymphocytic leukemia excluded), isolation of Aspergillus from respiratory secretions were virtually diagnostic of IPA. Positive results of respiratory tract culture are sufficient indication for immediate initiation of Amphotericin B therapy. Invasive diagnostic procedures to obtain lung tissue need not be performed [33].

Table – 1: The parameters used to diagnose IPA.

Parameter studied	**Consisting of 21 Studies ,with total of 474 patients**		
Sputum cultures [11-18, 20-22, 26-31]	315 Pts	Positive	Negative
		135	180
Nasal cultures [19, 22]	8 Pts	positive	negative
		8	0
Why sputum cultures not done? Reasons	12 studies	No expectoration	Reasons not given
		3 studies [12, 17, 18]	9 studies [11, 15, 19, 20, 22-26]
Trans bronchial aspiration cultures [11, 19, 23, 24]	9 pts	positive	Negative
		9	0
Bronchoscopic aspiration lavage cultures [22, 24, 26]	9 Pts	positive	Negative
		4	5
Tran bronchial biopsy – histopathology [11, 18, 24, 25, 31]	11 Pts	positive	Negative
		11	0
Tran cutaneous biopsy – histopathology [15, 17, 18, 25, 27, 31]	19 Pts	positive	Negative
		19	0
Open lung biopsy – histopathology [18, 24, 31]	3 Pts	positive	Negative
		3	0
Pleural fluid cultures [22, 31]	3 Pts	positive	Negative
		3	0
Post mortem cultures of lung tissue [12, 14-21, 24, 25, 28-31]	225 Pts	positive	Negative
		149	76
Post mortem histopathology of lung tissue [12, 14-21, 24, 25, 28-31]	233 Pts	positive	Negative
		233	0

(2 – controlled studies – 95 patients showed negative sputum cultures)

Table – 2: Criteria taken to diagnosis of IPA, in 21 studies.

Criteria taken to diagnose IPA	**21 studies**
Sputum cultures	17
Advocated multiple sputum cultures	5
No comment	4
Sputum cultures – not diagnostic	2
Advocates invasive procedure (BAL, bronchoscopic biopsy, Open Lung biopsy, Trans thorasic biopsy)	1

Table – 3: Fungal species isolated [32].

Species	No. of Episodes (N=18)	ALL (4)		ANLL (14)	
		Adults (3)	Children (1)	Adults (14)	Children (0)
Candida albicans	14 (77.8%)	3	1	10	0
Aspergillus flavus	04 (22.2%)	0	0	4	0

Table – 4: Outcome of Acute Leukemia Patients with Pulmonary Infections [32].

Type of Infection	No. of Episodes (56)	Outcome	
		Recovered	Died
Pure Bacterial Infection	18	16	2
Fungal (Mixed With Bacteria)	18	2	16
No Organisms Isolated Sputum less Pts)	20	20	0

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