The Clinical relevance of *Aspergillus* isolation from respiratory tract samples & detection of Aspergillus galactomannan antigen in serum of patients with acute exacerbation of COPD

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Abstract: Acute invasive aspergillosis is a devastating opportunistic infection in the severely immunocompromised. Patients at risk for invasive aspergillosis include those with prolonged neutropenia (e.g., following cytotoxic regimens for acute leukemia), hematopoietic stem cell transplant (HSCT) recipients, solid organ transplant recipients (particularly lung transplant recipients), and advanced AIDS. There is also a growing appreciation of invasive aspergillosis in persons with less severe levels of immunocompromise. For example, chronic necrotizing pulmonary aspergillosis (CNPA) generally occurs in with patients pre-existing structural lung diseases such as prior tuberculosis or lung abscess or modest immune impairment, as occurs with diabetes, poor nutrition, chronic obstructive pulmonary disease, or low-dose corticosteroids. This study aimed at finding an approximate incidence of IPA in patients with acute exacerbation of COPD and whether a combination of two tests, serological detection and mycological culture yielded a more specific diagnosis of IPA.

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1. Introduction:

Aspergillus is an ancient genus of fungi, with a large number of species, many of which are of medical importance. It is a ubiquitous, thermophilic, filamentous fungus. Aspergillus spp. can also assimilate nutritive material & live outside an animal host, thus they are widely present in the environment. Acquisition of infection by human hosts occurs mainly by inhalation. It is estimated that humans normally inhale about 200 conidia per day. The resultant clinical condition relies on the balance between the infectious load (i.e. the number of conidia inhaled) versus the immune status of the host (Warnock, 2007). The immune system thus requires the ability to recognize inhaled conidia and to control their growth, but also to avoid excessive inflammation (Sherif and Segal, 2010).

Aspergillus spp. exploits many mechanisms that allow their existence as saprophytes & convey virulence factors against human host as true human pathogens. Aspergillus fumigatus possess the capability of invading the lung parenchyma, thus it is more likely to be the causative agent of the serious condition known as invasive Aspergillosis (Sharma and Chwogule, 1998).

Acute invasive aspergillosis is a devastating opportunistic infection. Patients at risk for invasive aspergillosis include those with prolonged neutropenia (e.g., following cytotoxic regimens for acute leukemia), hematopoietic stem cell transplant (HSCT) recipients, solid organ transplant recipients (particularly lung transplant recipients), and advanced AIDS. Mortality from invasive aspergillosis has increased by several-fold in the 1980s and 1990s, a reflection of more patients undergoing treatment for hematologic malignancies and allogeneic HSCT (Sherif and Segal, 2010).

Patients with moderate levels of immune suppression may exhibit different forms of invasive Aspergillosis as in those having a “pre- existing structural lung disease” e.g tuberculosis, lung abscess or chronic obstructive lung disease (COPD). The clinical condition of such patients was described as chronic necrotizing pulmonary Aspergillosis (CNPA) (Ascioglu et al., 2002).

Adequate & proper diagnosis of invasive Aspergillois is a fundamental step in the pathway of management & setting the strategy of treatment. Patients may present with some non- specific symptoms as fever (which is refractory especially in neutropenic patients), anorexia, malaise, cough & dyspnea. In some cases hemoptysis might occur following vascular invasion by Aspergillus filaments which are known to be angioinvasive. Meanwhile other cases may present by pulmonary infarction with chest pain & heaviness (Ascioglu et al., 2002).

The European Organization for the Research and Treatment of Cancer/ Mycoses study Group (EORTC/MSG) had established the criteria for diagnosis of IPA and emphasized the importance of isolation of Aspergillus spp. from sterile body sites as a critical prerequisite in diagnosis of invasive pulmonary aspergillosis (Stevens, 2002).

Further confirmation of diagnosis of Invasive aspergillosis is done by direct demonstration of the hyphae in direct preparations of bronchial secretions or bronchoalveolar lavage (BAL), plus histopathological examination of lung tissue which represents the golden standard giving the diagnosis the description of "proven invasive pulmonary Aspergillosis", the next category is "Propable" IA in which there must be two elements present, one of the predisposing host factors plus laboratory mycological findings as viewing the hyphae in lung tissue (Stevens, 2002).

2. Subjects & Methods:

This study included 30 COPD patients with the criteria of acute exacerbation according to the GOLD guidelines who were admitted at the Chest Department, Ain Shams University Hospital.

All patients were subjected to the following: Full history taking, thorough clinical examination, chest-X-ray, arterial blood gases, ECG, routine laboratory investigations, fibrooptic bronchoscopy to obtain samples of bronchial lavage, blood samples to obtain serum samples.

'Probable' Aspergillus infection of the lower respiratory tract was diagnosed in the COPD patient, who had severe disease (stage III or IV) according to GOLD criteria, with recent exacerbation of dyspnea resistant to appropriate treatment (including antibiotics), accompanied by one of the following: i) Positive culture for Aspergillus from LRT, ii) positive serum antibody test for Aspergillus fumigatus, iii) positive serum Galactomannan (GM) antigen test *(Gao et al., 2010)*. Colonization with Aspergillus was diagnosed if culture was positive for Aspergillus with no other evidence of fungal infection. Culture was performed on Sabouraud Dextrose agar, incubated for 7 days at both 25°C and 37°C. Colonies were identified according to *McClenny, 2005*.

Galactomannan (GM) antigen of Aspergillus was detected in serum specimens by Platelia Aspergillus Ag Enzyme immunoassay (EIA) kit supplied by Bio-Rad, Marnes la Coquette, France.

3. Results:

The descriptive data regarding age and smoking index of the 30 patients is shown in table (1):

Table (1): Age and smoking index data of patients included in the study.

	No.	Min.	Max.	Mean	SD
Age	30	45	71	62.20	6.386
Smoking Index	30	20	40	26.67	5.467

Of the 30 specimens, 7 (24%) yielded a positive culture of Aspergillus fumigatus. 23 (76%) serum specimens were positive for Aspergillus antigen by EIA testing. The 7 positive culture specimens were also positive for Aspergillus antigen, giving a diagnosis of 'Probable' Aspergillus infection of the lower respiratory tract with 2 criteria in 24% of patients, whereas the remaining 16 patients (54%) were diagnosed as 'Probable' Aspergillus infection with only 1 criterion.

Table (2): Correlation between results of Aspergillus EIA and severity.

			Result		Total
			neg	pos	
Severity	Moderate	Count	6	0	6
		% within Result	85.7%	.0%	20.0%
	Severe	Count	1	23	24
		% within Result	14.3%	100.0%	80.0%
	Total	Count	7	23	30
		% within Result	100.0%	100.0%	100.0%

	Value	p
Pearson Chi-Square	24.643[a]	.000

Table (2) shows a highly significant statistical correlation between Aspergillus EIA test results and severity of COPD.

We found no significant statistical correlations between severity and age, or severity and smoking index.

We measured the Diagnostic Validity of the Aspergillus EIA Test, which is the diagnostic ability of the test to detect severe cases from moderate cases. Results are shown in Table (3).

Table (3): Diagnostic validity test- TN=True negative, FN=False negative, FP=False positive, TP=True positive, NPV=Negative predictive value, PPV=Positive predictive value.

TN	FN	FP	TP	Efficiency
6	1	0	23	
Specificity	**Sensitivity**	**NPV**	**PPV**	
100%	95%	85%	100%	96%

Statistical methods:

IBM SPSS statistics (V. 20.0, IBM Corp., USA, 2011) was used for data analysis. Data was expressed as Mean±SD for quantitative parametric measures in addition to both number and percentage for categorized data. The following tests were done: 1. Chi-square test to study the association between each 2 variables or comparison between 2 independent groups as regards the categorized data. The probability of error at 0.05 was considered significant, while at 0.01 and 0.001 are highly significant. 2. Diagnostic validity test.

4. Discussion:

In a study by *Graf et al., 2011*, Probable IPA was diagnosed in 12% of the patients, using either culture or antigen detection as a microbiological indicator. They included 704 patients in their study, which probably makes their results more representative than results of the current study. However, they included patients with severe underlying diseases and some transplant patients, thus their study encompasses a wider range of risk factors.

In another study, *Garnacho-Montero et al., 2005*, found COPD to be a significant risk factor for fungal infection in critically ill patients prior to ICU admission.

Furthermore, a study conducted by *Nguyen et al., 2007*, tested for the presence of Aspergillus GM antigen in non-immunocompromised patients, including those with COPD, but they detected the antigen in BAL. They found that BAL GM testing was no more sensitive than the combination of BAL microscopy and culture and exhibited a lower PPV than these tests, and it only increased the likelihood of obtaining false-positive results. We thus recommend that GM detection be done in serum in combination with BAL culture for Aspergillus.

In the same context, *Gao et al., 2010* reported that the benefit of using 2 markers (β-Glucan and GM) in serum of COPD patients was evaluated. They concluded that such combination is better than using a sole marker for diagnosis of IPA. However, the serum testing of GM & β-Glucan is precluded by the high probability of false positive yield in some cases. Several causes of false positive results of β-Glucan in serum were reported; presence of other fungal infection, treatment with albumin, hemodialysis & the presence of some bacterial infection. They also linked the false positive results of GM to some other factors such as the administration of tazobactam-piperacillin, amoxicillin, clavulanic acid & antifungal drugs.

In the present study, we found a highly significant statistical correlation between the severity of COPD and the presence of Aspergillus galactomannan antigen in serum. We propose that IPA should be thought of in all patients with severe exacerbations of COPD, especially those refractory to antibiotic therapy. The sensitivity and specificity of the GM antigen test obtained in this study, warrants its use, in combination with culture from BAL, as an early indicator of probable IPA. The only drawback of the antigen detection test is its cost, which could be a limitation to its use in facilities with limited resources.

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