

Influenza Associated Pulmonary Aspergillosis: A Retrospective Study from a Tertiary Care Center in Lebanon

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DOI: <http://doi.org/10.26738/poem.v2i1.28>

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Received: March 21, 2024

Revised: June 19, 2024

Accepted: July 4, 2024

ABSTRACT

Influenza-associated pulmonary aspergillosis (IAPA) has been reported in multiple cohort studies, typically in the immunocompromised host. Limited data is available from the Middle Eastern region.

Methods

This is a retrospective study conducted at the American University of Beirut Medical Center (AUBMC) between January 1 2014 and March 1 2019, to describe the clinical characteristics, risk factors and outcomes of patients with influenza and invasive pulmonary aspergillosis (IPA). Six hundred fifty six patients were admitted with influenza pneumonia. Data about positive cultures for *Aspergillus* sp. isolated from sputum, deep tracheal aspirates (DTA) or bronchoalveolar lavage (BAL) or a positive aspergillus galactomannan test (GM) in serum or BAL was collected. Cases were identified based on the definitions and criteria for influenza and invasive aspergillosis suggested by the European Organization for Research and Treatment of Cancer/ Mycoses study Group (EORTC/MSG), EORTC/MSG intensive care unit (EORTC/MSGERC ICU) Working Group, and IAPA definition.

Results

Nine patients had a positive result for *Aspergillus* sp. in culture or GM. Based on the EORTC/MSG criteria, 7 patients were classified as probable cases of IPA, and one as a possible case. On the other hand, only 2 patients were classified as probable cases of invasive fungal infection (IFI) based on the IAPA criteria. Only 1 out of 9 patients died. There was a preponderance of aspergillosis among immunocompromised patients.

Conclusion

Our study showed that the incidence of IPA in influenza patients was low compared to the data reported from European countries. This calls for national and regional surveillance to better understand the epidemiological variation in the regions.

Keywords: Influenza, Aspergillosis, IPA, IAPA

Introduction

The association between influenza and superimposed pulmonary aspergillosis has been described as early as 1979.[1] Influenza-associated pulmonary aspergillosis (IAPA) has been reported in multiple cohort studies.[2, 3] The associated mortality is high ranging between 22.2% to 100%, especially in patients

admitted to the intensive care unit (ICU).[4] While invasive pulmonary aspergillosis (IPA) typically occurs in immunocompromised hosts and isolation of *Aspergillus* species in immunocompetent patients is most often considered a colonization,[5] IAPA has

been described in many patients without the traditional risk factors for IPA, including immunocompetent individuals.[6] Since the associated mortality of IAPA is 51% compared to 28% in patients with influenza admitted to the ICU without IAPA, criteria have been proposed for the diagnosis and early identification of IAPA.[7-11] Limited data on IAPA is available from the Arab countries in the Middle East region.

In Lebanon, a country with low vaccination rates for influenza, seasonal influenza is a significant burden on the population, with a hospital admission rate in one center estimated at 26.6%.[12] The purpose of this study is to describe the clinical characteristics, risk factors and outcomes of patients with influenza who had *Aspergillus* sp. isolated from cultures of respiratory specimens or positive aspergillus galactomannan (GM) test.

Methods

Study design

This is a retrospective chart review conducted at the American University of Beirut Medical Center (AUBMC), a teaching hospital with 364 beds and 50 adult ICU beds. We reviewed the electronic medical records of patients hospitalized between January 1, 2014, and March 1, 2019, before the severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) pandemic started. We included those with a primary diagnosis of influenza (confirmed either by a reverse transcriptase polymerase-chain-reaction (RT-PCR) or by a rapid antigen tests). We then checked which patients had positive cultures for *Aspergillus* sp. isolated from sputum, deep tracheal aspirates (DTA) or bronchoalveolar lavage (BAL) or a positive aspergillus GM in serum or BAL in patients who had worsening respiratory symptoms.

Until 2020, our center had not been speciating the *Aspergillus* sp. nor had access to 1,3- β -D-Glucan (BDG). We collected data on patients characteristics including age, gender, Charlson's score, body mass index (BMI), presence of neutropenia with neutrophils count at the time of influenza diagnosis (<500 neutrophils/mm³), bacterial co-infection at the time of influenza diagnosis, presence of type II diabetes mellitus (DM II), chronic kidney disease (CKD), hemodialysis use, chronic lung disease (chronic obstructive pulmonary disease COPD, asthma and other lung diseases like bronchiectasis), active hematological or solid organ malignancy, and

active chemotherapy intake.

Reviewed hospitalization course included length of hospital stay in days, intake of neuraminidase-inhibitor and/ or corticosteroids, radiographic characteristics of the lung findings, the need for oxygen supplementation, and the need for invasive or non-invasive mechanical ventilation. In-hospital mortality was defined as mortality anytime during the hospital stay.

The study protocol was approved by the AUBMC Institutional Review Board under number BIO-2019-0166 and informed consents were waived due to the retrospective nature of the study.

Case definitions and analysis

Cases were identified by clinical, microbiological and radiological criteria based on the definitions and criteria for influenza and invasive aspergillosis suggested by the European Organization for Research and Treatment of Cancer/Mycoses study Group (EORTC/MSG), [7] European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) ICU Working Group [9] and IAPA definition (supplementary material 1). [8] According to EORTC/MSG criteria, patients were classified into three types of IAPA (proven IPA, probable IPA, and possible IPA). [7] The EORTC/MSERG ICU working group updated definitions specifically for invasive candidiasis and invasive aspergillosis that are relevant for ICU patients, focusing on the host factors. [9]

As for IAPA, although patients were classified as either proven or probable, authors suggested that clinicians should not distinguish between proven and probable disease and might consider these differences only for clinical trials [8]. Two categories were included in this definition: Invasive *Aspergillus* tracheobronchitis (ATB) and IAPA without ATB, based on expert consensus. [8]

Since our study population included all hospitalized patients and not only those admitted to the ICU, we opted to include all the aforementioned definitions to assess our patients.

Results

During the study period, we identified a total of 656 cases of severe influenza pneumonia requiring hospital admission. Of these, nine patients had a

positive result for *Aspergillus* sp. in culture or positive GM. The clinical and laboratory characteristics of the 9 patients are presented in Table 1.

Based on the EORTC/MSG criteria, 7 patients were classified as probable cases of IPA, and one as a possible case. On the other hand, only 3/9 patients were admitted to the ICU and thus met the entry criteria for the IAPA definition and 2 were classified as probable cases of IAPA. (Table 2).

Seven patients were infected by influenza virus A and 2 patients by influenza virus B. Five patients had influenza confirmed by PCR and 4 by a positive rapid respiratory antigen test and had a typical clinical presentation. The mean time from influenza diagnosis to positive aspergillus test in respiratory samples was 4.66 days (interquartile range (IQR) 0–9 days). The mean age of patients was 52.5 years (standard deviation [SD] 16.9). Four out of 9 patients were males.

Six patients had underlying medical conditions classically considered risk factors for IPA, including malignancy under treatment. Three patients had no immune compromising conditions, but two of them had chronic lung disease. None of the patients had DM and one had CKD. Three patients were treated in ICUs, one of which required mechanical ventilation, with a length of ICU stay of 5, 5 and 23 days respectively.

There were 5 patients who underwent bronchoscopy, all of which had a positive GM in BAL, 4 of which had a GM index ≥ 1.0 . Two of those patients grew *Aspergillus* sp. from BAL culture in addition to sputum culture, however they had a negative GM in serum. A total of 5 *Aspergillus* isolates were cultured from 5 different patients. As mentioned previously, during the study period speciation of *Aspergillus* sp. was not available at our center. Among those who did not undergo bronchoscopy, 3 patients had an elevated serum GM >0.5 while 1 had a positive sputum culture for *Aspergillus* sp. None of the patients had evidence of ATB by bronchoscopy.

All patients demonstrated radiologic abnormalities. One patient had a cavitary lesion on chest computed tomography (CT), 6 patients had dense circumscribed lesions, and none showed a halo sign (Table 2).

Prednisone at a dose of 40 mg per day had been administered to 4/9 patients for an average duration of 3 days prior to the positive fungal results. All patients were treated with voriconazole at the recommended dose. Only one of the 9 patients died from respiratory failure during the hospital stay.

Seven patients were treated with antibiotics for superimposed bacterial infections, mostly consisting of quinolones alone or in combination with extended spectrum beta-lactam antibiotics.

Table 1: Clinical characteristics of patients with positive aspergillus cultures or galactomannan

Characteristic		n (n/N) or Mean±SD
Age in years (Mean ± SD)		52.5± 16.9
Male (n/N)		4 (4/9)
Time to aspergillosis diagnosis from admission (Mean ± SD)		4.66 days ±5.57
Underlying risk factors		
Charlson score (Mean ± SD)		3.11 ± 1.69

BMI (Mean \pm SD)		22.9 \pm 5.65
Neutropenia at influenza diagnosis (n/N)		2/9
Respiratory superinfection (bacterial or viral) (n/N)		3/9
Hemodialysis (n/N)		0/9
Active chemotherapy use (n/N)		5/9
Diabetes mellitus II (n/N)		0/9
Chronic kidney disease (n/N)		1/9
	COPD	2/9
Chronic lung disease (n/N)	Other (asthma, bronchiectasis, NSLC ³ status post lobectomy)	1/9
	>1 disease concurrent	2/9
Hematopoietic stem cell transplant (n/N)		5/9
Active hematological/solid malignancy (n/N)		6/9
Clinical Course		
Non-invasive supplemental oxygen therapy (n/N)	BIPAP	1/9
	Face mask	1/9
	High flow Nasal cannula	4/9

Oseltamivir use (n/N)		8/9
Vasopressor use/septic shock (n/N)		1/9
Bacteremia (n/N)		1/9
Treatment with antibiotics (n/N)		7/9
Treatment with corticosteroids (n/N)		4/9
Length of stay in the hospital in days (mean \pm SD)		13.2 \pm 6.65

BMI: body Mass Index; BIPAP: bi-level positive airway pressure; COPD: chronic obstructive pulmonary disease; Neutropenia: neutrophils count at the time of influenza diagnosis <500 neutrophils/mm³; NSCLC: non-small cell lung cancer.

Table 2: Summary of cases per EORTC/MSG and IAPA criteria

Patient	Host factors	ICU admission	Radiologic features	Mycological evidence	EORTC/MSG criteria	IAPA criteria	Outcome
1	Yes (neutropenia, active malignancy)	No	Yes, lobar consolidation	<i>Aspergillus</i> sp. culture from sputum	Probable	Entry criteria not met	Alive
2	Yes (transplant, active malignancy)	No	Yes, lobar consolidation	Elevated galactomannan antigen in serum (2.7)	Probable	Entry criteria not met	Alive
3	Yes (transplant, active malignancy)	Yes	Yes, dense, well-circumscribed lesion	Galactomannan antigen in BAL 0.92	Possible	No IAPA	Alive
4	None	No	Yes, dense, well-circumscribed lesion and lobar consolidation	Elevated galactomannan antigen in BAL (1.78)	No IFI	Entry criteria not met	Alive

5	Chronic respiratory airway abnormality	Yes	Yes, dense, well-circumscribed lesion and lobar consolidation	<i>Aspergillus</i> sp. culture from BAL and sputum	Probable (ICU definition)	Probable	Alive
6	Yes (active malignancy)	No	Yes, dense, well-circumscribed lesion and lobar consolidation and cavitory lesion	<i>Aspergillus</i> sp. culture from BAL and sputum	Probable	Entry criteria not met	Alive
7	Yes (neutropenia, active malignancy)	No	Yes, lobar consolidation	Elevated galactomannan antigen in BAL (5.6)	Probable	Entry criteria not met	Alive
8	Yes (transplant, active malignancy)	No	Yes, dense, well-circumscribed lesion and lobar consolidation	Elevated galactomannan antigen in serum (1.2)	Probable	Entry criteria not met	Alive
9	Chronic respiratory airway abnormality	Yes	Yes, dense, well-circumscribed lesion and lobar consolidation	Elevated galactomannan antigen in serum (1.56)	Probable (ICU definition)	Probable	Dead

EORTC/MSG: European Organization for Research and Treatment of Cancer/Mycoses study Group; IAPA: influenza associated pulmonary aspergillosis; BAL: bronchoalveolar lavage; IFI: invasive fungal infection; ICU: intensive care unit

Discussion

This is the first case series from the Arab countries of the Middle East region describing the association between influenza pneumonia and pulmonary aspergillosis and providing added insight to the regional epidemiology of this disease. Our results demonstrated a low incidence of concurrent influenza infection and pulmonary aspergillosis (1.4%) in our hospitalized patients with influenza and a subsequent low mortality in these patients (1/9). Eight out of the 9 patients met the criteria for definitions set by the EORTC/MSG and/or the IAPA criteria, resulting in a cumulative incidence rate of only 1.2%.

Two very recent systematic review/meta-analyses have been published; one summarizing the clinical characteristics of IAPA in critically ill patients, and the second in all hospitalized patients.[4, 13] Studies on hospitalized patients with a sample size >50 were included and all were observational except for one randomized control trial (RCT).[4] These studies were mostly conducted in Europe and China.[4] Therefore, more high-quality evidence needs to be generated globally, particularly in regions such as the Middle East and Africa in which there is a paucity of data.

Our results demonstrated a very low incidence rate of IAPA in comparison to other published studies. This could partly be explained by the fact that we explored the incidence in all hospitalized patients, and not only those admitted to the ICU. Most studies reporting on the incidence of IAPA have focused on critically ill patients. The proportion of IAPA among this population varies across studies, with as high as 32% in immunocompromised individuals. Even in the non-immunocompromised, the reported incidence was 14% in the same study, including 7 ICUs from the Netherlands and Belgium.[6] However, other studies have also expanded the analysis to include all patients hospitalized for influenza (including those requiring ICU admission). One large scale study from the United States which analyzed 477,556 hospitalizations identified with the principal diagnosis of influenza, found that IAPA was diagnosed in 823 (0.17%) of total admissions. [14] Another study on the total number of influenza admissions in a Chinese institution showed an incidence rate of 5.4%.[15] One cohort study from Canada also found an incidence of 7.2% in ICU patients.[16] Thus, the rates of IAPA are variable according to the countries. Low rates could be partly due to underdiagnosis of aspergillosis probably from the lack of physicians' awareness of the relationship between influenza and aspergillosis.

This has been observed globally, with Thevissen et. al reporting that outside Europe, only a minority of physicians have heard of or diagnosed IAPA in the past 5 years (17% in the United States and 39% in other countries) and lower respiratory sampling were performed less often. Additionally, while 39% of respondents globally did take lower respiratory samples for microbiologic analysis, the majority of respondents (79%) rarely requested GM from BAL samples.[17] The unavailability and the cost of GM particularly in low and middle income countries might play a role in underdiagnosing IAPA. In the absence of a high degree of suspicion physicians may have opted not to pursue the diagnosis of IAPA especially given the low mortality rate in our patient population. Another possible explanation to the low incidence in our study is that our population of admitted influenza patients might be less sick than in other studies as AUBMC is a private hospital and not unfrequently patients are admitted for IV hydration and antipyretics, whereas conditions for admitting influenza patients might be stricter in other settings.

The mean or median ages of hospitalized patients in pooled studies in a systematic review ranged from 52 to 65 years, and the proportions of males ranged from 50.6% to 69.3%.[4] Although we had a low number of patients, this was similar to our findings, where the average age was 52.5. Additionally, the systematic review done by Shi et al found that development of IAPA was significantly associated with a history of DM, COPD, and presence of EORTC/MSGERC host factors.[4] Our study also demonstrated a preponderance of IPA among immunocompromised patients. Only one case occurred in a patient with no risk factors. In our study, four patients were receiving steroids. In the meta-analysis on critically ill patients in particular, pooled studies comparing those with IAPA and no IAPA, showed that significantly more patients with IAPA were on chronic corticosteroids whereas there was no significant difference in pre-existing chronic lung diseases, DM and solid/hematological cancer. Thus, the concerning factors with IAPA despite the variations in prevalence, is the fact that it can manifest in patients without traditional risk factors for aspergillosis.

Our population included mostly hospitalized non-critically ill patients and in general, this group of patients represents a challenge in classification, diagnosis as well as management. The EORTC/MSGERC consensus definitions in combination with the ICU working groups' definition captured the most patients (7 out of 9, 77.7%) in this case series.

Compared to the EORTC/MSGERC definitions, the IAPA definition is more useful in an immunocompetent patient population. However, restricting the inclusion criteria to critically ill patients limits its use in the larger demographic of non-critically ill patients who may still incur significant morbidity such as a prolonged hospital stay. Indeed, studies have shown that in both total hospitalized patients and those only admitted to the ICU, the mortality rate, use of mechanical ventilation, and length of hospital stay are significantly increased in patients with IAPA versus patients without IAPA.[4, 13] Though these results mostly stem from observational data, the available evidence does seem to suggest that infection with aspergillosis in patients with influenza is independently associated with mortality, and not just a marker of clinical severity. [18]

Our results showed that the interval from the diagnosis of influenza to *Aspergillus* growth was on average 4.6 days and is consistent with other studies frequently demonstrating a median time to diagnosis of up to 5 days.[19-22] This relatively short interval suggests that the patient might have been colonized by *Aspergillus* sp. preceding hospital admission through inhalation of spores. Therefore, the environmental surroundings will incur a differential risk to each patient and can also be center specific given differences in ventilation systems.

A recent systematic review summarized the available evidence on the diagnostic performance of the various definitions for the diagnosis of IPA in non-hematological, non-solid organ transplant, critically ill patients. There was significant heterogeneity across studies in terms of population, prevalence, used reference definitions for IPA, (non-standardized) ad hoc variations of reference definitions, limiting comparisons across studies.[23] A proven diagnosis of IAPA in both the EORTC/MSGERC and the IAPA definition requires invasive procedures, though experts generally agree that the distinction between proven and probable IAPA is more important for clinical trials rather than clinical practice.[8] A presumptive diagnosis of IAPA can therefore be established in a relatively easier and quicker manner. This is important to consider, since early initiation of antifungal treatment has been shown to improve outcomes in critically ill patients,[24] however more evidence should be generated to assess early treatment benefit in non-critically ill patients. In our study, all patients received voriconazole as the first line agent, which is the appropriate initial antifungal therapy for

IPA as per guidelines.[24]

There is uncertainty as to whether patients with influenza admitted to the ICU would benefit from prophylactic therapy against infection with *Aspergillus* sp.. One recent randomized control trial assessed posaconazole as a prophylactic agent. The majority of patients were diagnosed within 48 hours of ICU admission, excluding them from the modified intention-to-treat population, while in the remaining patients the incidence of IAPA was not significantly reduced compared to those receiving standard of care. [25]

This study was limited by its retrospective nature, small sample size and single center experience. Additionally, some pulmonary aspergillosis cases could have been missed since diagnostic testing may not have been ordered if a physician did not suspect the diagnosis. We did not compare patients with influenza who developed pulmonary aspergillosis versus those who didn't, which limits the ability to form associations of the different patient factors with outcomes.

Conclusion

Although influenza associated aspergillosis was less common in our study than reported elsewhere, the heterogeneity of incidence rates across studies published in the literature highlights the importance of conducting national and regional surveillance studies to better understand the epidemiological variations. Moreover, there are important differences between hospitalized patients on regular wards and those in the ICU, and the lack of data in the former group warrants more studies in order to optimize diagnosis and management in different patient populations.

List of abbreviations:

IAPA, Influenza-associated aspergillosis; **ICU**, Intensive care unit; **IPA**, Invasive pulmonary aspergillosis; **GM**, galactomannan; **AUBMC**, American University of Beirut Medical Center; **RT-PCR**, Reverse transcriptase polymerase-chain-reaction; **DTA**, Deep tracheal aspirate; **BAL**, Bronchioalveolar lavage; **BDG**, 1,3- β -D-Glucan; **BMI**, Body mass index; **DM II**, Type II diabetes mellitus; **CKD**, Chronic kidney disease; **COPD**, Chronic obstructive pulmonary disease; **EORTC/MSG**, European Organization for Research and

Treatment of Cancer/Mycoses study Group; **EORTC/MSGERC**, European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium; **ATB**, *Aspergillus tracheobronchitis*; **SARS-COV-2**, Severe acute respiratory syndrome coronavirus 2; **IQR**, Interquartile range; **SD**, Standard deviation; **CT**, Computed tomography; **NSLC**, Non-small cell lung cancer ; **BIPAP**, Bi-level positive airway pressure; **IFI**, Invasive fungal infection; **RCT**, Randomized controlled trial.

Funding Statement:

This study did not receive any funding or grant.

Institutional Review Board Statement:

The study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The study protocol was approved by the AUBMC institutional Review Board under number BIO-2019-0166.

Informed Consent Statement:

Patient consent was waived due to the retrospective nature of the study.

Conflict of interests

The authors declare no conflicts of interest.

Data Availability Statement:

The data presented in this study are available on request from the corresponding authors. The data are not publicly available as per our IRB regulations.

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Supplementary material 1:

I- European Organization for Research and Treatment of Cancer/Mycoses study Group (EORTC/MSG) [7]

-Proven IPA required a histopathologic or direct microscopic examination of a needle aspiration or biopsy specimen that showed hyphae and evidence of lung tissue damage. Probable IPA required the following three criteria to be met:

1) One of the following host factors: Recent history of neutropenia ($<0.5 \times 10^9$ neutrophils/L [<500 neutrophils/mm³] for >10 days) temporally related to the onset of invasive fungal disease, hematologic malignancy, receipt of an allogeneic stem cell transplant, receipt of a solid organ transplant, prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a therapeutic dose of ≥ 0.3 mg/kg corticosteroids for ≥ 3 weeks in the past 60 days, treatment with other recognized T-cell immunosuppressants, such as calcineurin inhibitors, tumor necrosis factor- α blockers, lymphocyte-specific monoclonal antibodies, immunosuppressive nucleoside analogues during the past 90 days, treatment with recognized B-cell immunosuppressants, such as Bruton's tyrosine kinase inhibitors, like ibrutinib, inherited severe immunodeficiency (such as chronic granulomatous disease, STAT 3 deficiency, or severe combined immunodeficiency), acute graft-versus-host disease grade III or IV involving the gut, lungs, or liver that is refractory to first-line treatment with steroids such as neutropenia, hematologic malignancy, allogeneic stem cell transplant, solid organ transplant, or prolonged corticosteroids use.

2) Radiological evidence for pulmonary aspergillosis or evidence of tracheobronchitis on bronchoscopic analysis, consistent with an otherwise unexplained pulmonary infectious-disease process. Radiologic findings detected by computed tomography (CT) should include one of the following: dense, well-circumscribed lesions with or without a halo sign, air crescent sign, cavity, wedge-shaped and segmental or lobar consolidation.

3) Mycological evidence including any one of the following: *Aspergillus* sp. recovered by culture from sputum, BAL, bronchial brush, or aspirate; microscopical detection of fungal elements in sputum, BAL, bronchial brush, or aspirate indicating a mold; single serum or plasma galactomannan level ≥ 1.0 , BAL fluid: ≥ 1.0 or single serum or plasma: ≥ 0.7 and BAL fluid ≥ 0.8 ; plasma, serum, or whole blood 2 or

more consecutive PCR tests positive; BAL fluid 2 or more duplicate PCR tests positive; at least 1 PCR test positive in plasma, serum, or whole blood and 1 PCR test positive in BAL fluid; *aspergillus* species recovered by culture from sputum, BAL, bronchial brush, or aspirate.

Cases that meet the criteria for a host factor and a clinical feature but for which mycological evidence has not been found are considered possible invasive fungal disease (IFD).

II- The EORTC/MSGERC ICU Working Group updated definitions specifically for invasive candidiasis (IC) and (2) invasive aspergillosis (IA) that are relevant for ICU patients [9]. Among the relevant modifications to the definition of probable IA proposed by the (EORTC/MSG), was a change in the host factors. One of the following factors must be present to meet the definition: [9]

1. Glucocorticoid treatment with prednisone equivalent of 20 mg or more per day
2. Qualitative or quantitative neutrophil abnormality (inherited neutrophil deficiency, absolute neutrophil count of ≤ 500 cells/mm³)
3. Chronic respiratory airway abnormality (chronic obstructive lung disease, bronchiectasis)
4. Decompensated cirrhosis
5. Treatment with recognized immunosuppressants (eg, calcineurin or mammalian target of rapamycin [mTOR] inhibitors, blockers of tumor necrosis factor [TNF] and similar antifungal immunity pathways, alemtuzumab, ibrutinib, nucleoside analogues) during the past 90 days
6. Hematological malignancies/hematopoietic stem cell transplantation (HSCT) recipient
7. Solid organ transplant
8. Human immunodeficiency virus infection
9. Severe influenza (or other severe viral pneumonia, such as coronavirus disease 2019 [COVID-19])

III- Influenza-Associated pulmonary aspergillosis definition [8]

IAPA definition was developed for clinical research study purposes with the inclusion criteria of patients requiring ICU admission for respiratory distress with a positive influenza test temporally related to ICU admission.

Proven IAPA diagnosis requires a positive lung biopsy that shows evidence of invasive septate hyphae and a positive *Aspergillus* sp. culture or positive *Aspergillus* PCR in tissue.

Proven *Aspergillus* tracheobronchitis (tracheal and/or

bronchial ulcerations or nodules, pseudomembranes or plaques visualized at bronchoscopy), requires hyphal elements suggestive of *Aspergillus* seen on sloughed-off pseudomembrane, and *Aspergillus* identified in tissues through culture or PCR [8].

Probable diagnosis of *Aspergillus* tracheobronchitis requires the presence of airway plaque, pseudomembrane or ulcer and at least one of the following: serum GM index >0.5 or BAL GM index ≥ 1.0 or positive culture or detection of hyphae consistent with *Aspergillus* from BAL culture, tracheal aspirate culture or sputum culture [8]. In this group of patients, a radiographic infiltrate is not required.

In patients with probable IAPA, (and without documented *Aspergillus* tracheobronchitis), it is sufficient to have the following:

- 1- Evidence of a pulmonary cavitating infiltrate (not attributed to another cause) and at least one of the following: positive sputum culture or positive tracheal aspirate culture
- 2- OR evidence of pulmonary infiltrate and at least one of the following: serum GM index >0.5 or BAL GM index ≥ 1.0 or positive BAL culture

In patients with positive sputum culture but negative BAL GM, the probability of IAPA would be considered as low unless there is a pulmonary cavity or evidence of tracheobronchitis.