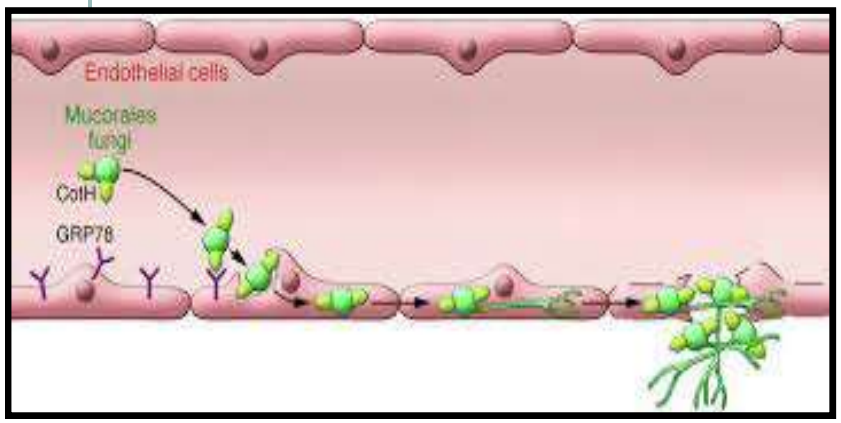
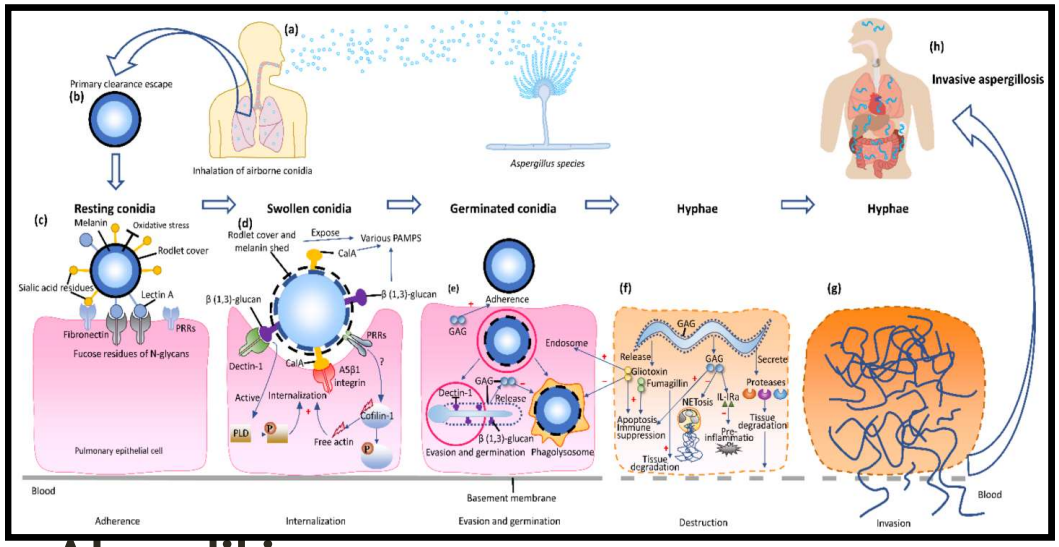
NEW ERA IN ASPERGILLOSIS AND MUCORMYCOSIS DIAGNOSIS

Tehran University of Medical Sciences
School of Public Health





Kazem Ahmadikia

Assistant Professor of Medical Mycology
Department of Medical Parasitology and Mycology
Tehran University of Medical Sciences

Tehran, Iran

Email:Kazem_Ahmadikia@yahoo.com

SYSTEMIC MYCOSES (DEEP MYCOSES)

Pathogenic Mycoses

No need to predisposing factors and occur in healthy hosts (True pathogen)

However, they have particular geographical distribution (endemic in certain areas).

The casual agents of these groups of infection are dimorphic fungi.

Confer specific immunity against the second exposure

Primarily cause asymptomatic or a mild illness

Opportunistic Mycoses

Need a predisposing factor to develop (the condition that lead to weakness of body). (opportunistic pathogen)

Globally distributed.

not dimorphic.

No immunity will be evolved

Cause severe disease

PATHOGENIC SYSTEMIC MYCOSES

Histoplasmsis

Coccidioidomycosis

Paracoccidioidomycosis

Blastomycosis

OPPORTUNISTIC MYCOSES

Yeast associated opportunistic mycoses:

Candidiasis

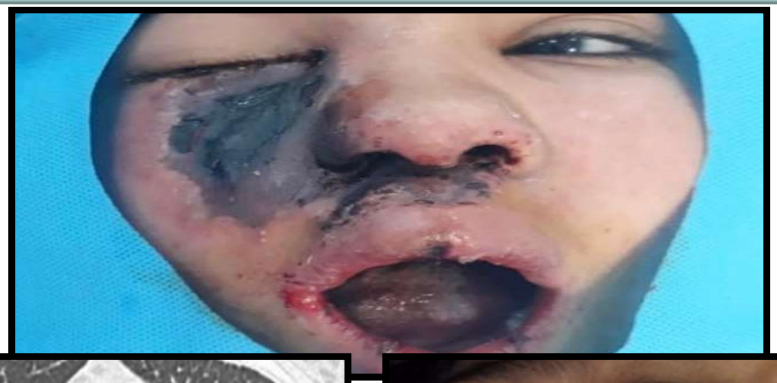
Cryptococcosis

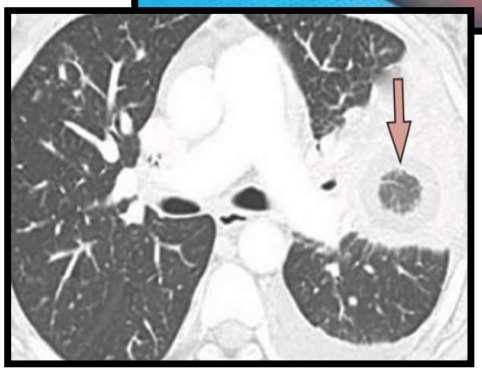
Invasive mold infections

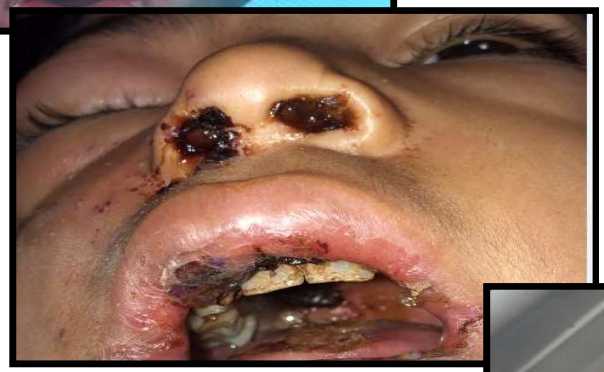
Aspergillosis

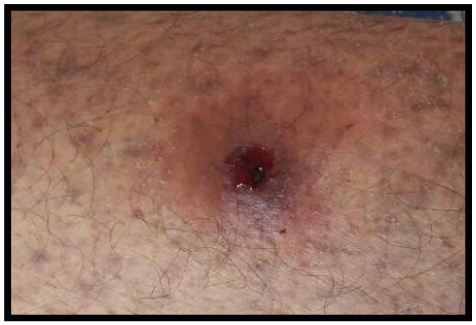
Mucormycosis

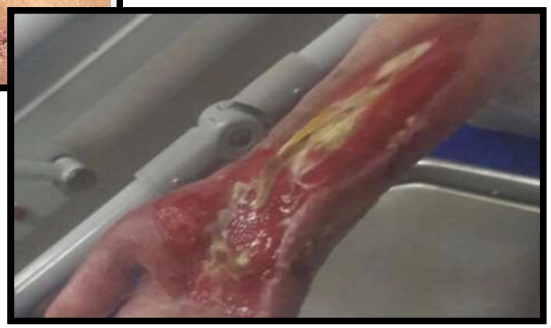
MUCORMYCOSIS



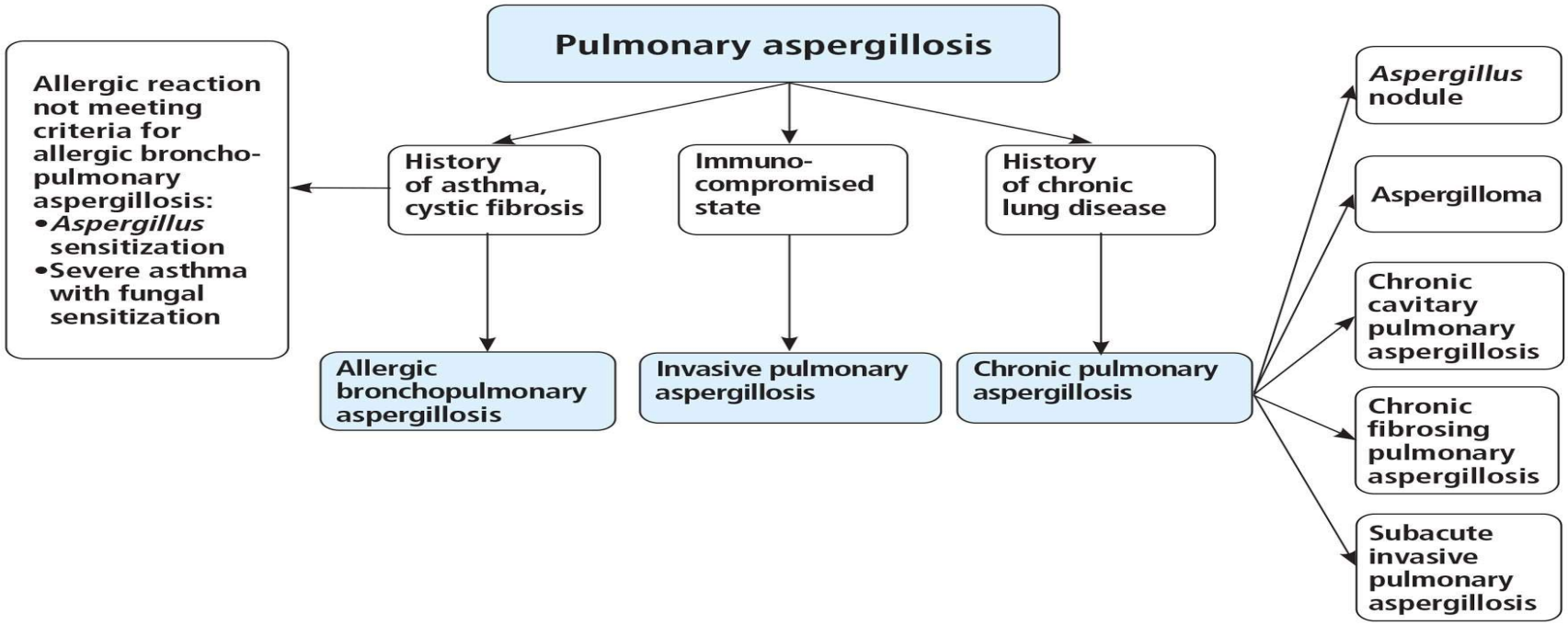








ASPERGILLOSIS



DIFFERENCES AND SIMILARITIES

Aspergillosis

- Mostly involve sinus and lung
- Mortality: 30-60%
- Risk factors: Hematological malignancies, neutropenia, chemotherapy, corticosteroid therapy, SOT and BMT, Severe COVID-19 and Flu
- Treatment: Voriconazole, Isavuconazole
- Dissemination: infrequent
- Outcome: Good

Mucormycosis

- Mostly involves rhino-orbito-cerebral
- Mortality: 60-95%
- Risk factors: Uncontrolled diabetes, COVID-19 with long term corticosteroid therapy, Hematological malignancies, neutropenia, chemotherapy, corticosteroid therapy, SOT and BMT,
- Treatment: Ampho B, Posaconazole+ surgical debridement
- Dissemination: to orbit and brain
- Outcome: Poor

Importance Of Early Diagnosis And Prompt Management

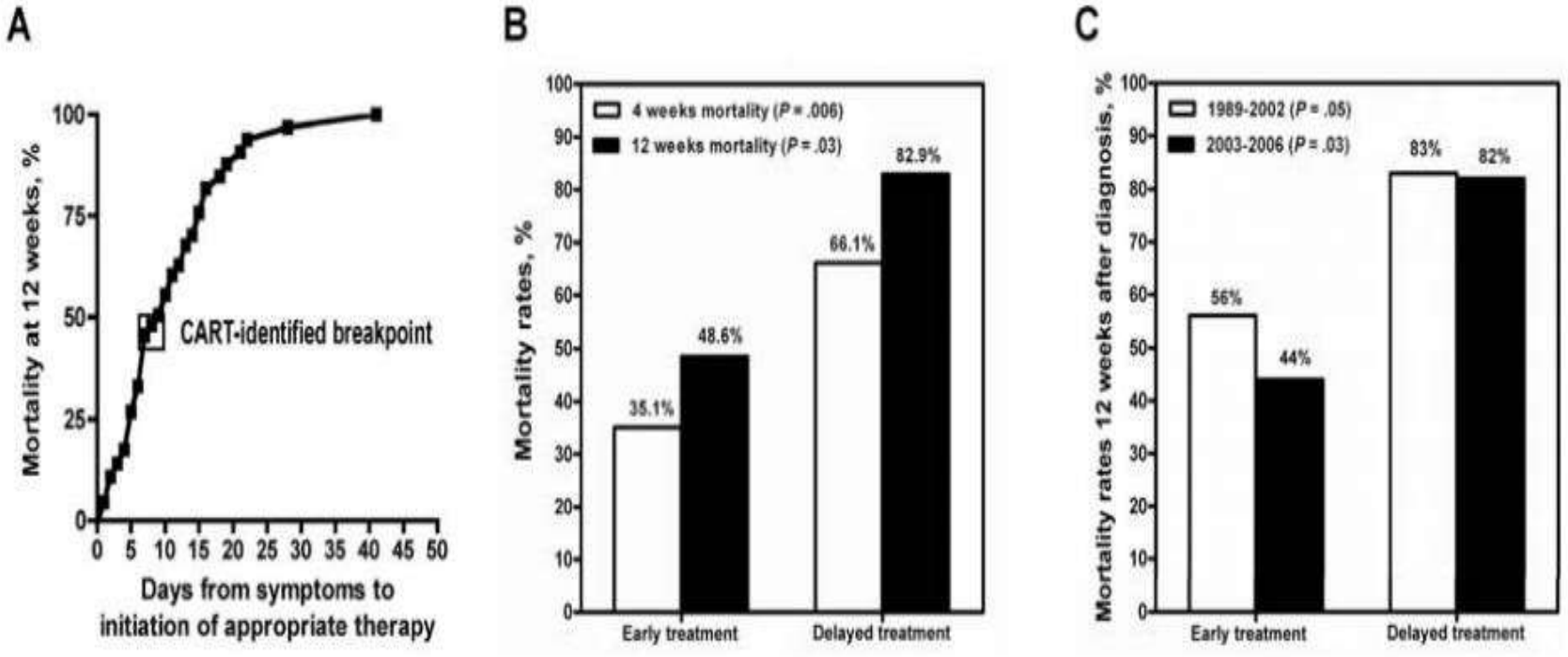
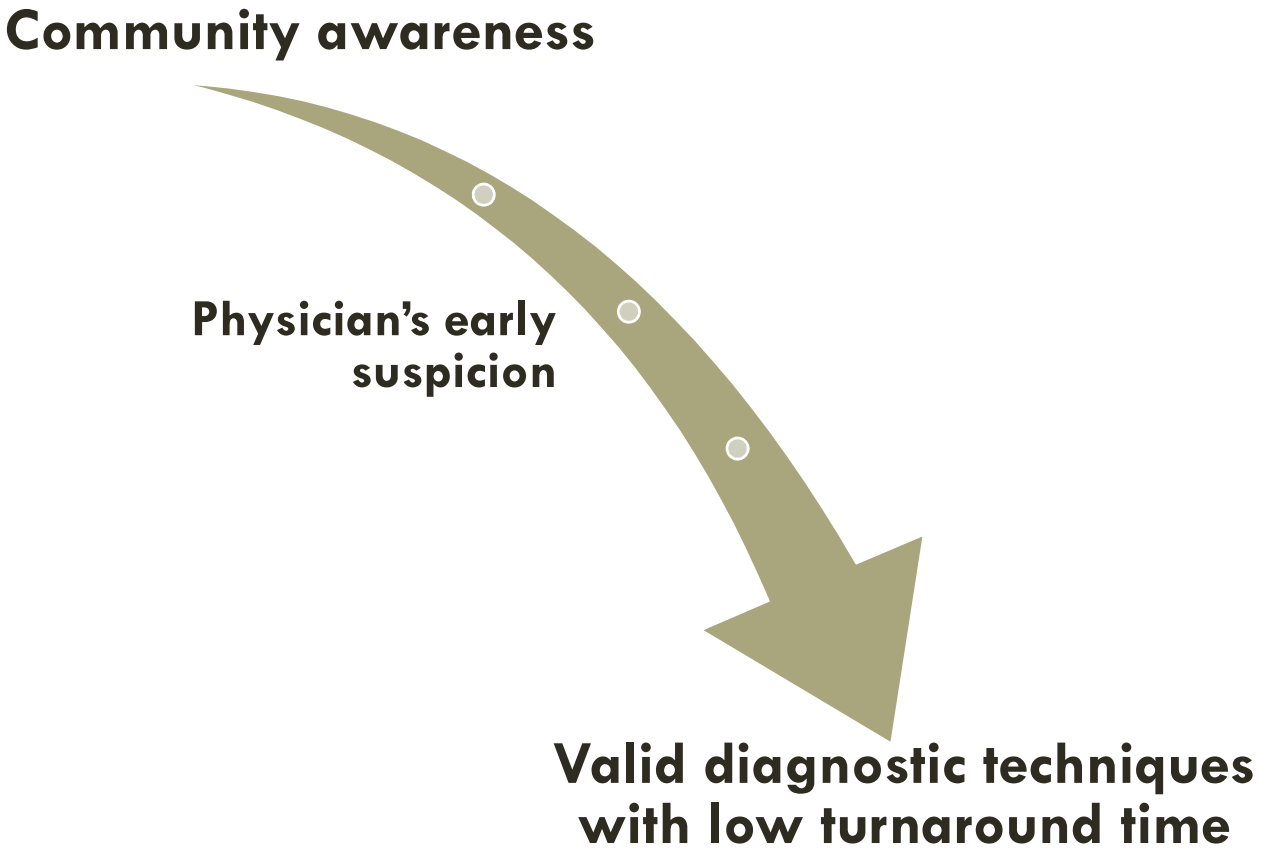


Figure 3. Mortality rates among patients stratified by the classification and regression tree (CART)–derived mortality breakpoint for delayed amphotericin B–based treatment. *A*, CART analysis of the relationship between the interval (in days) from the onset of symptoms of zygomycosis to the administration of effective amphotericin B–based treatment and mortality rate 12 weeks after diagnosis. *B*, Mean 4- and 12-week mortality rates among patients who received early (<6 days after diagnosis) and delayed (≥6 days after diagnosis) amphotericin B–based treatment. *C*, Mean 12-

Pivotal Factors Influencing Early Diagnosis



QUESTIONS

1- Which of the following form are the most frequent clinical presentations of invasive aspergillosis?

CNS aspergillosis

Cutaneous aspergillosis

Sinus and lung involvement

Gastro-intestinal aspergillosis

2- Which of the following form are the most frequent clinical presentation of Mucormycosis?

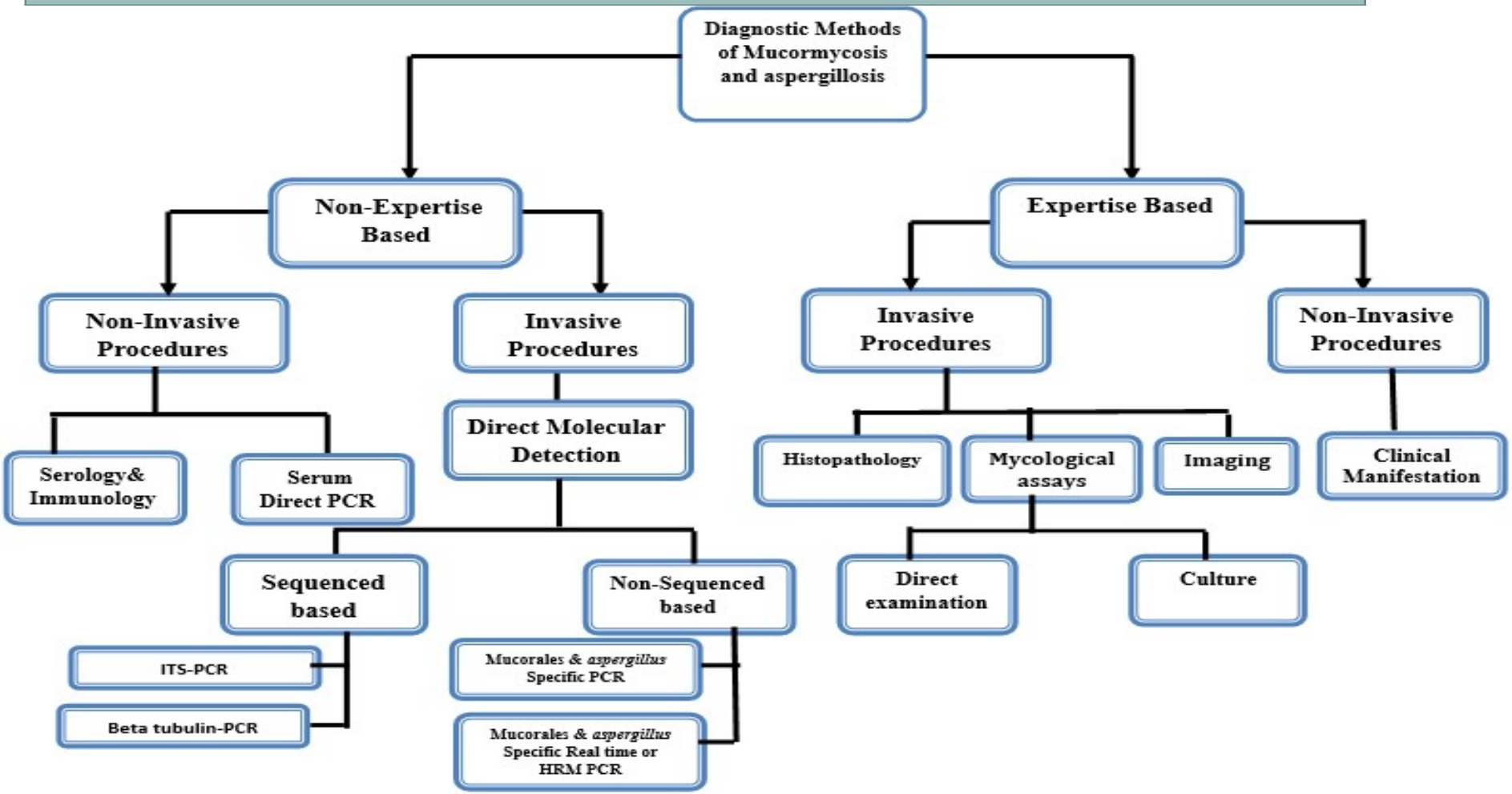
Rhino-orbito-cerebral mucormycosis

Cutaneous mucormycosis

Pulmonary mucormycosis

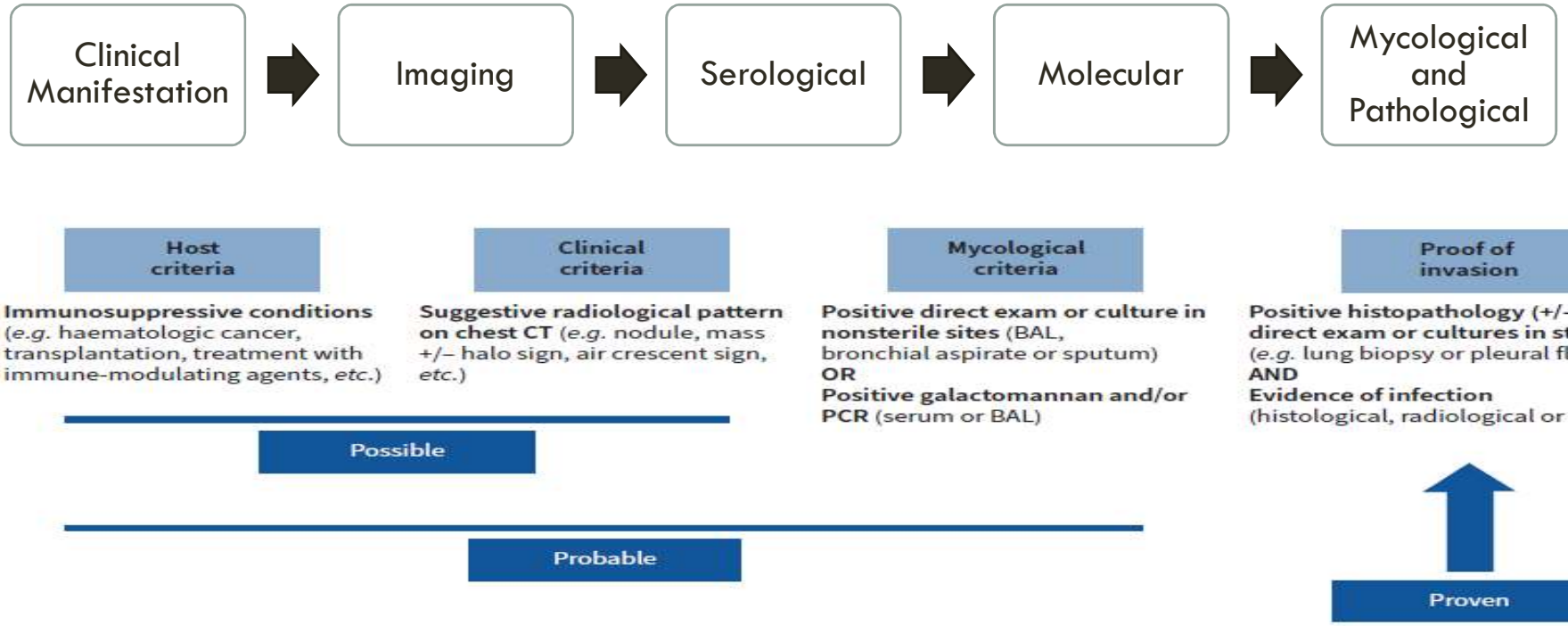
Gastro-intestinal mucormycosis

Diagnostic Approaches



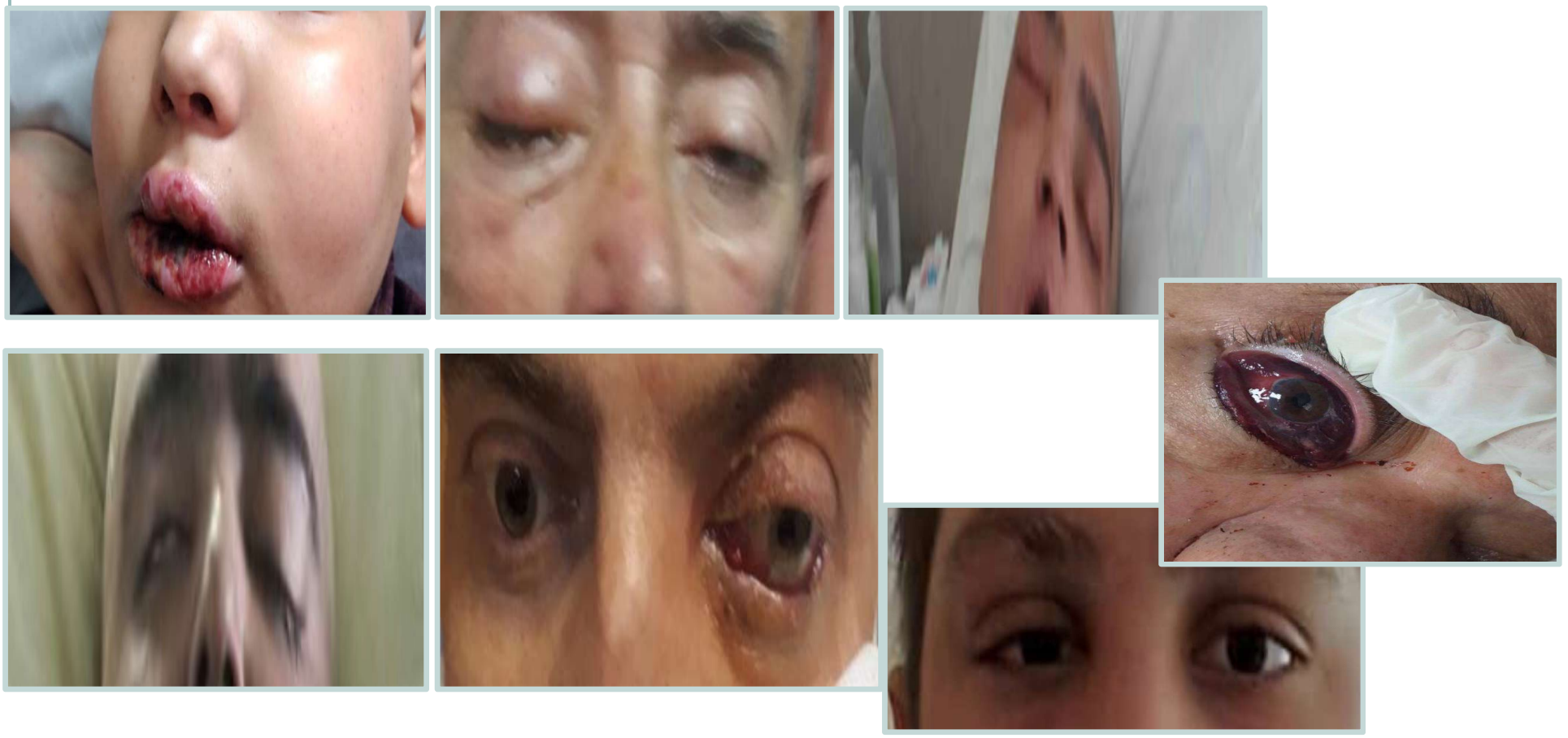
DIAGNOSTIC PATHWAY FOR ASPERGILLOSIS AND MUCORMYCOSIS

SCALE OF PROBABILITY (POSSIBLE, PROBABLE OR PROVEN)



Diagnostic Challenges

Clinical manifestations of different invasive fungal infections may not be distinguishable



RADIOGRAPHIC PATTERNS

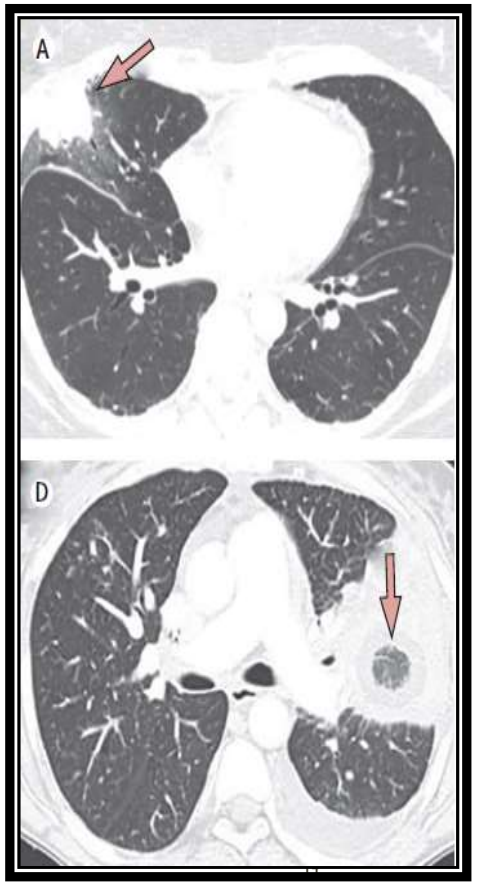
NEITHER SENSITIVE NOR SPECIFIC
EARLY STAGE OF THE INFECTION VS LATE STAGE
HEMATOLOGICAL MALIGNANCIES VS. SOT, FLU, COVID-19 (TYPICAL AND NON TYPICAL PATTERNS)

Aspergillosis

- Early phases: macronodule(s) >1 cm, surrounded by a halo of ground-glass attenuation (halo sign)
- consolidation
- alveolar consolidations
- masses (especially in solid organ transplant (SOT) recipients)
- cavity or air-crescent sign (delayed
- finding)
- Paranasal CT: Mucosal thickening

Mucormycosis

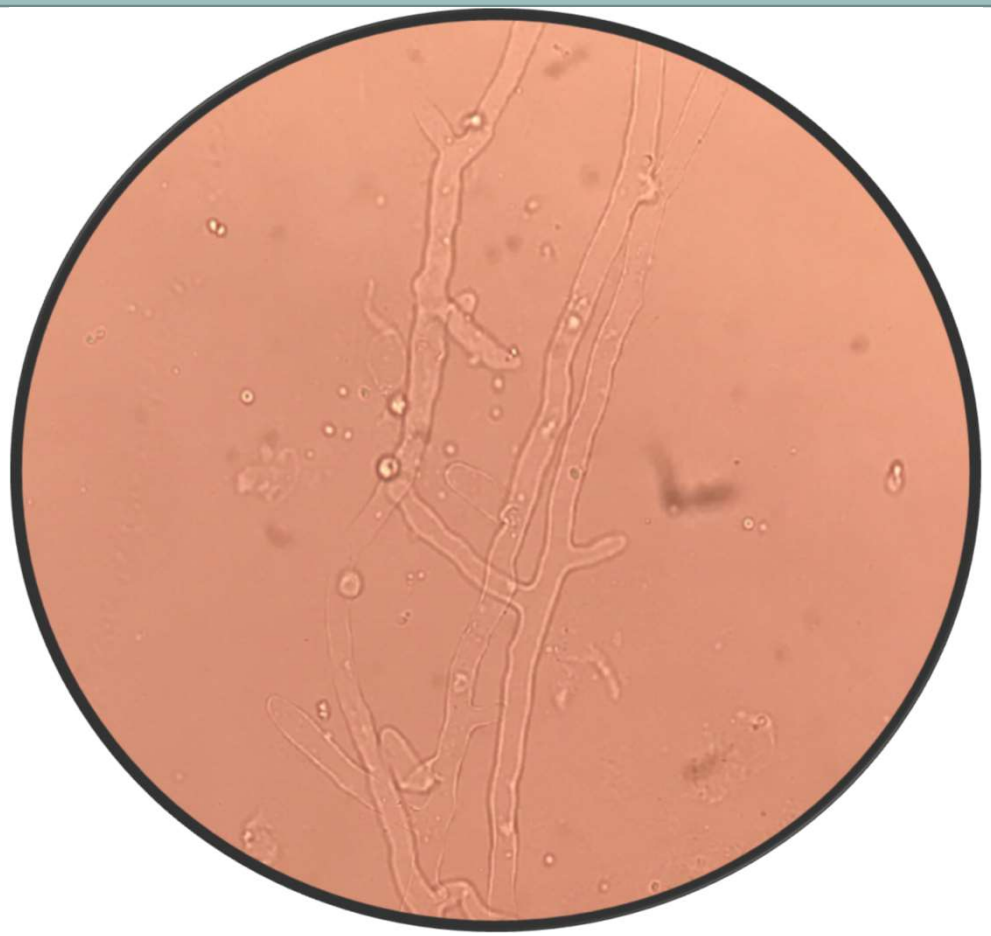
- Typical findings:
- Reverse Halo sign
- >10 nodules
- pleural effusion
- Paranasal CT: Massive Mucosal thickening+Bone destruction
- **Cranial MRI**
- Typical finding: orbit,
- brain involvement

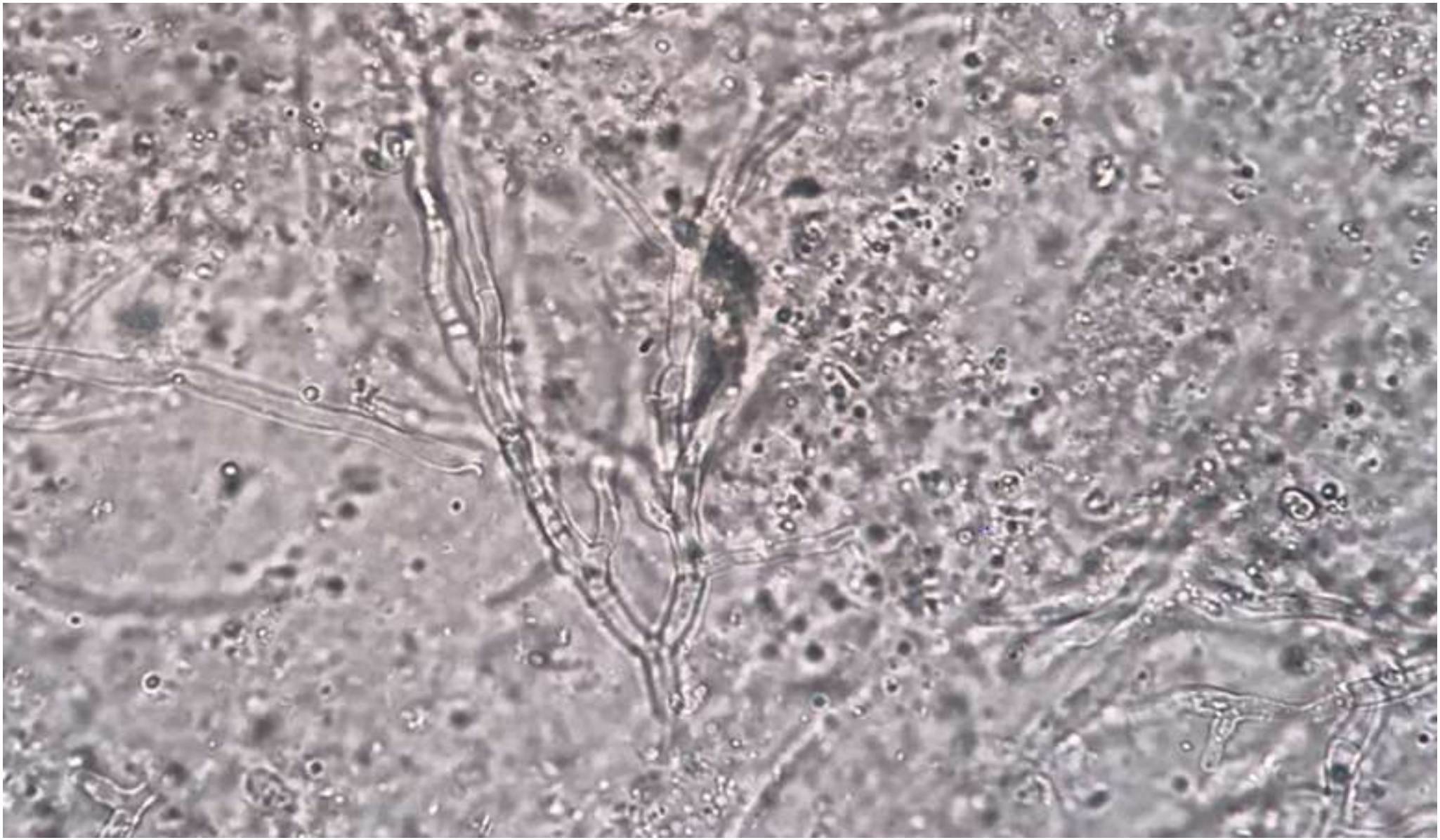


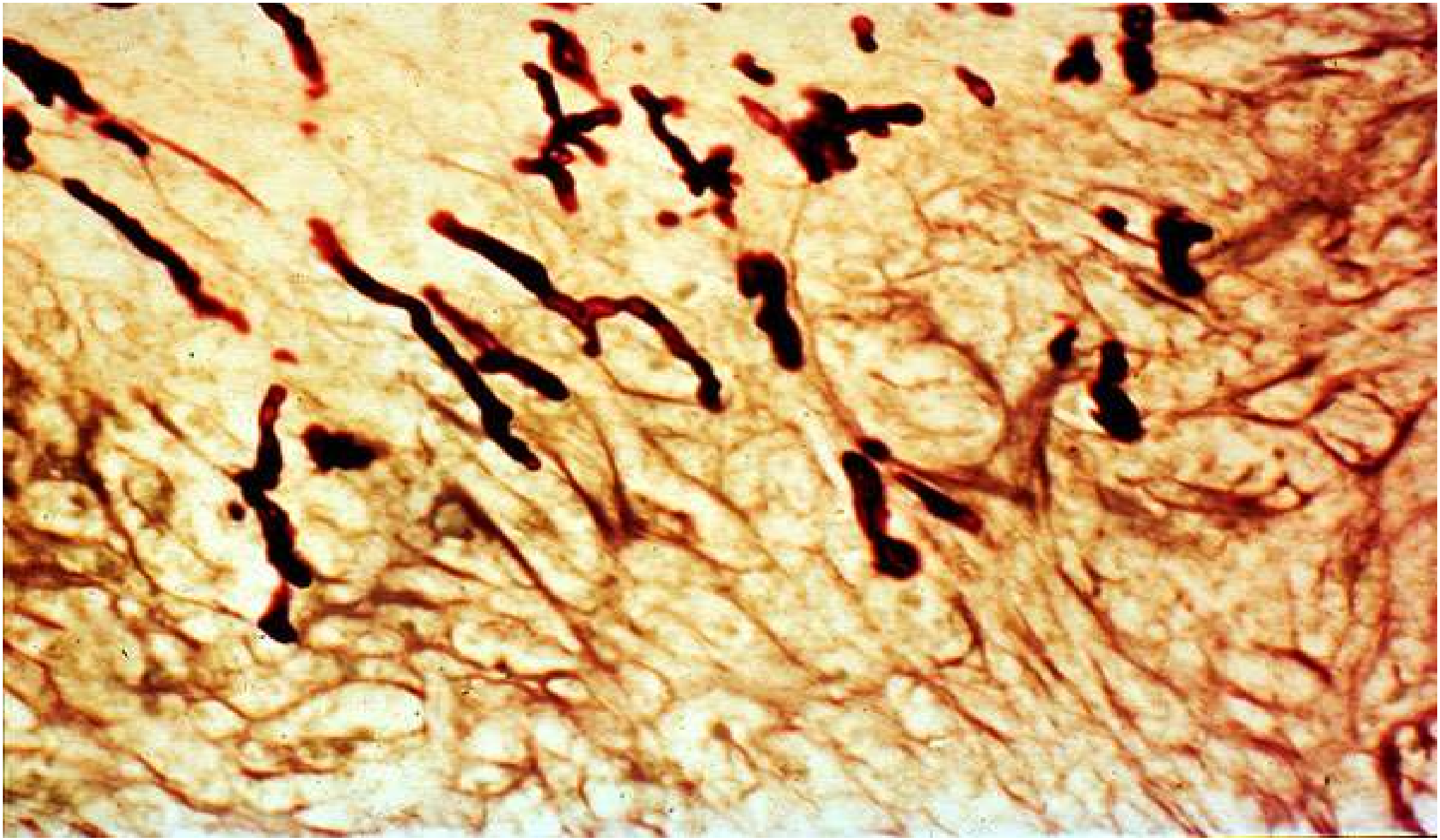
CLINICAL SPECIMENS

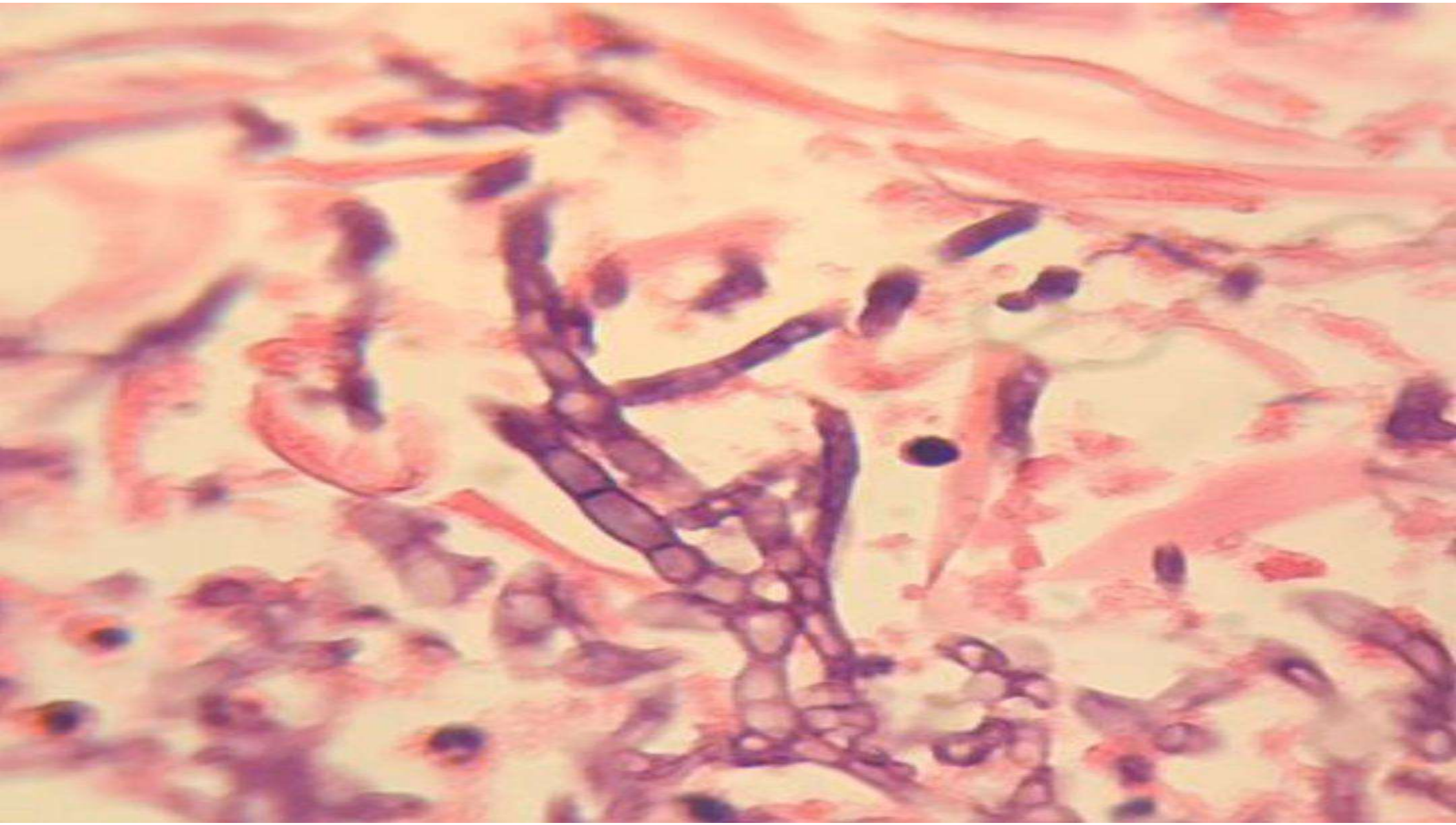
Type of specimen	The diagnostic Methods	Aspergillosis or mucormycosis
Sinus biopsy	Pathology, direct examination, culture, molecular detections	Both
BAL	Pathology, direct examination, culture, molecular detections, serology	Both
Bone biopsy	Pathology, direct examination, culture, molecular detections	Mucormycosis > aspergillosis
Orbit and brain	Pathology, direct examination, culture, molecular detections	Mucormycosis > aspergillosis
Skin and palate biopsy	Pathology, direct examination, culture, molecular detections	Mucormycosis > aspergillosis
Formalin fixed biopsy	Pathology, direct examination, molecular detections	Both
FFPE	Pathology, molecular detections	Both
Whole blood and CSF	molecular detections, serology	Both but serology is more applied for aspergillosis
Serum	molecular detections, serology	Both

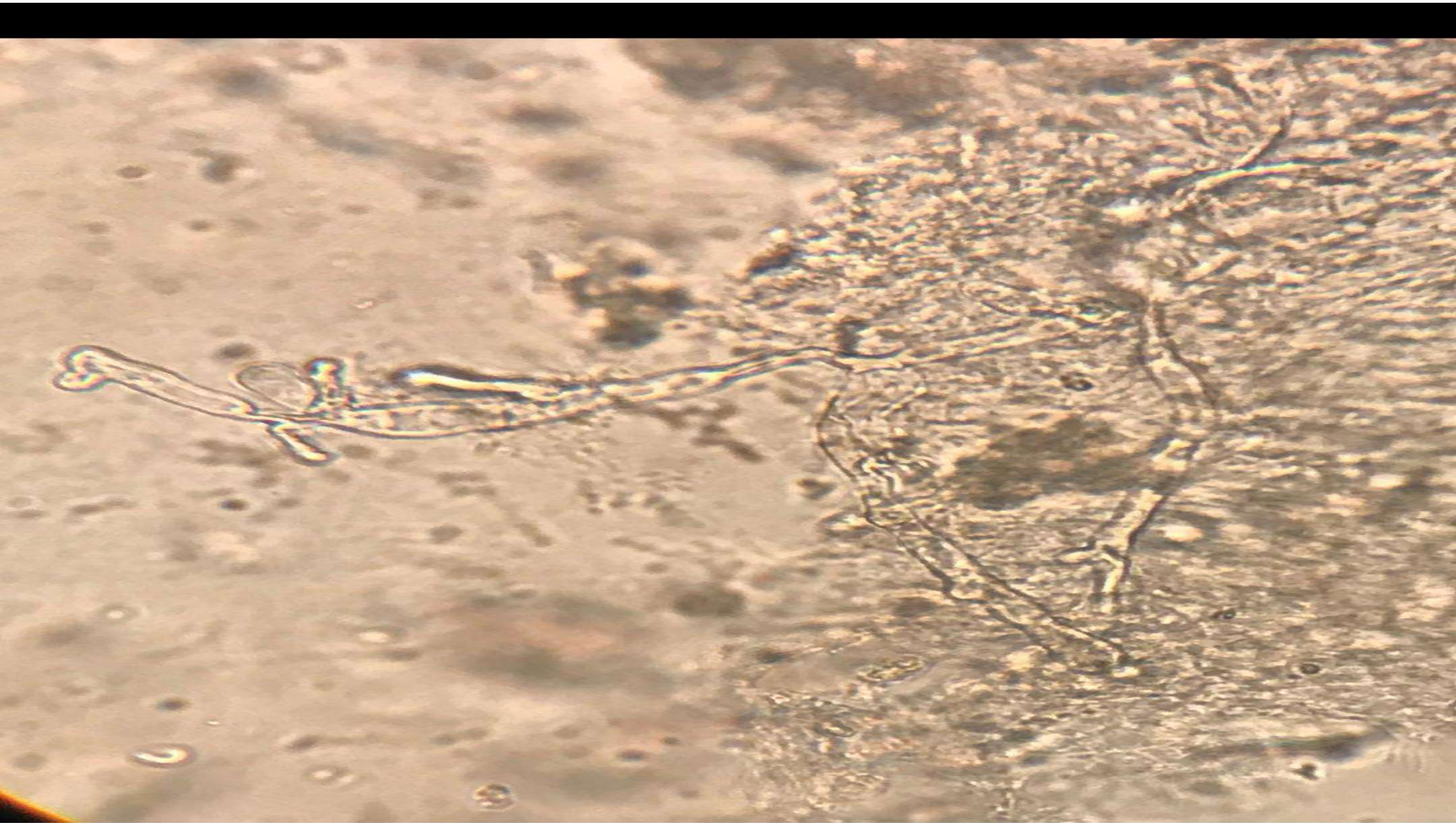
DIRECT EXAMINATIONS: KOH OR FLUORESCENT DYES SUCH AS CALCOFLUOR WHITE™ OR BLANCOPHOR

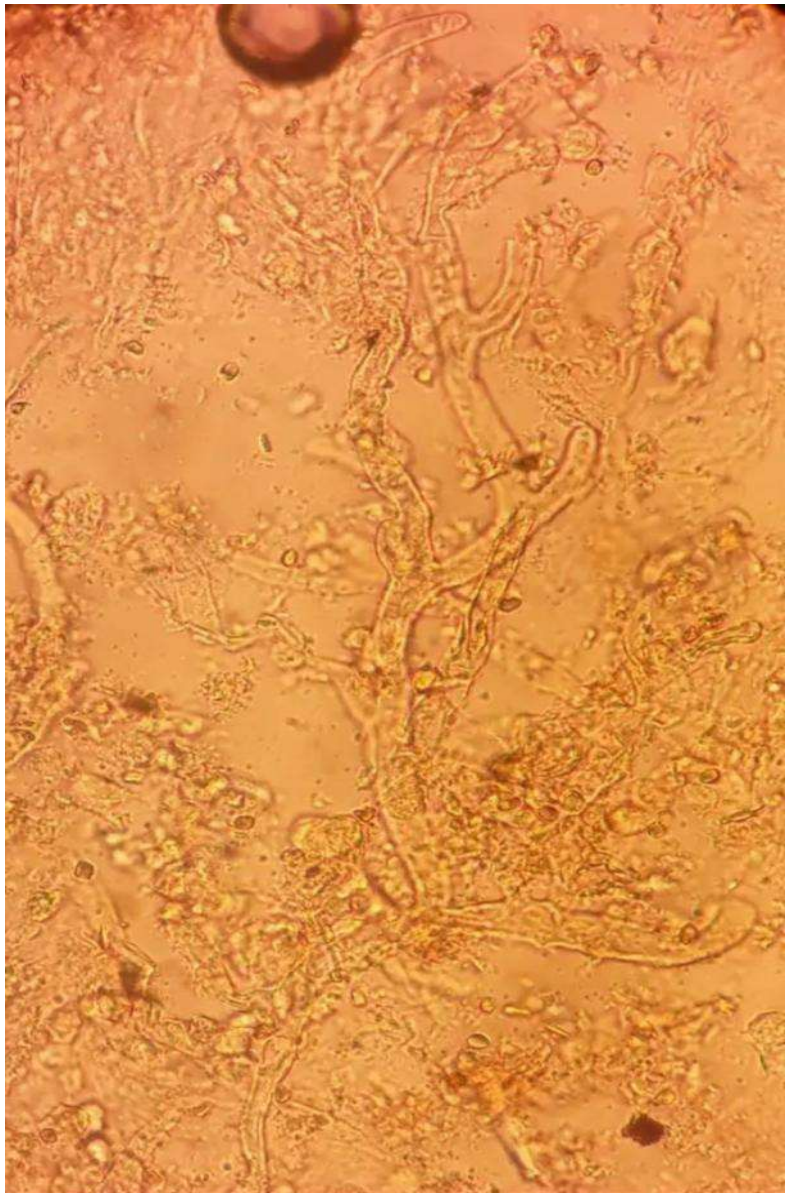


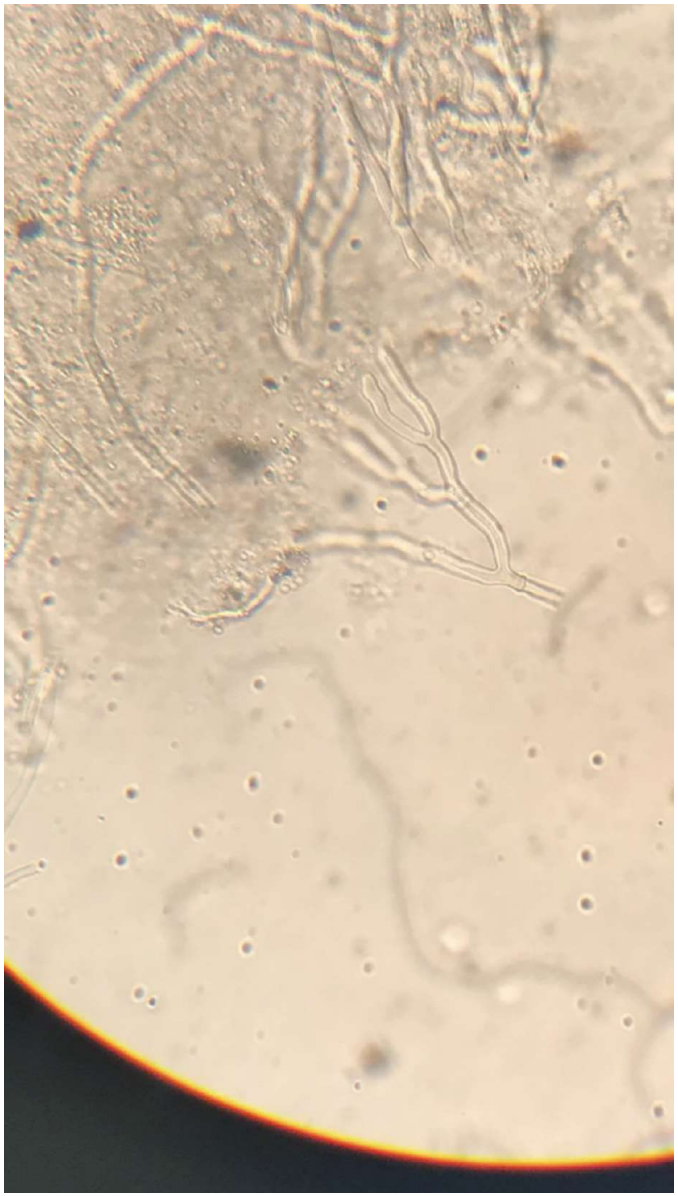












QUESTIONS

6- What is expected to be observed in direct examination of a biopsy or BAL specimen suspected to have invasive aspergillosis?

Ribbon like broad aseptate hyphae with right angle branching

Dichotomously branched septate hyphae

Both septate and aseptate hyphae

Branching hyphae alongside Arthroconidia

7- What is expected to be observed in direct examination of a biopsy specimen suspected to have mucormycosis?

Ribbon like broad aseptate hyphae with right angle branching

Dichotomously branched septate hyphae

Both septate and aseptate hyphae

Branching hyphae alongside Arthroconidia

8- The microscopic hyphal pattern that is observed in invasive aspergillosis is specific and seen only in this disease. Is it true?

False

True

9- The microscopic hyphal pattern that is observed in mucormycosis is specific for the disease among invasive mold diseases. Is it true?

False

True

QUESTIONS

6- What is expected to be observed in direct examination of a biopsy or BAL specimen suspected to have invasive aspergillosis?

Ribbon like broad aseptate hyphae with right angle branching

Dichotomously branched septate hyphae

Both septate and aseptate hyphae

Branching hyphae alongside Arthroconidia

7- What is expected to be observed in direct examination of a biopsy specimen suspected to have mucormycosis?

Ribbon like broad aseptate hyphae with right angle branching

Dichotomously branched septate hyphae

Both septate and aseptate hyphae

Branching hyphae alongside Arthroconidia

8- The microscopic hyphal pattern that is observed in invasive aspergillosis is specific and seen only in this disease. Is it true?

False

True

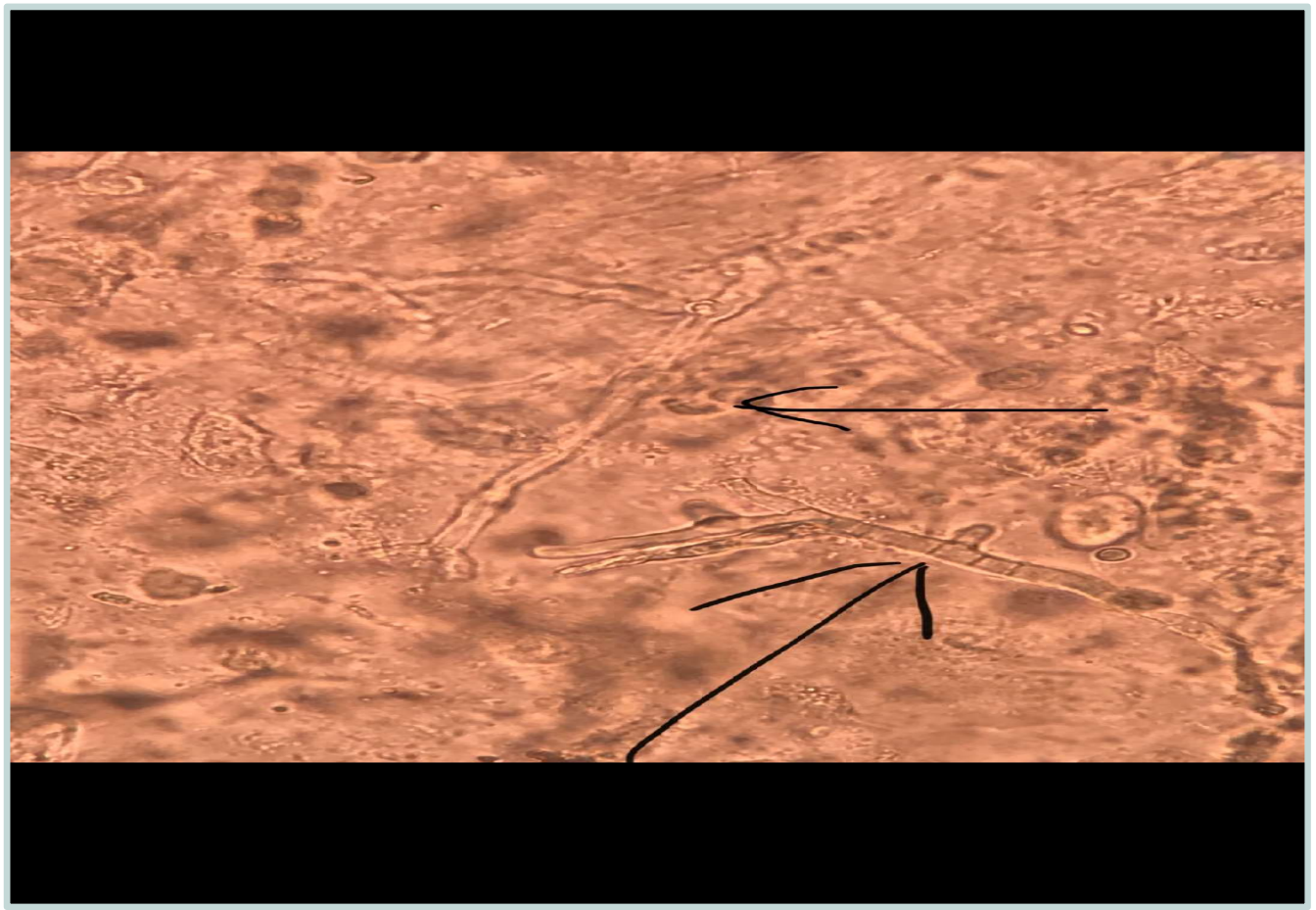
9- The microscopic hyphal pattern that is observed in mucormycosis is specific for the disease among invasive mold diseases. Is it true?

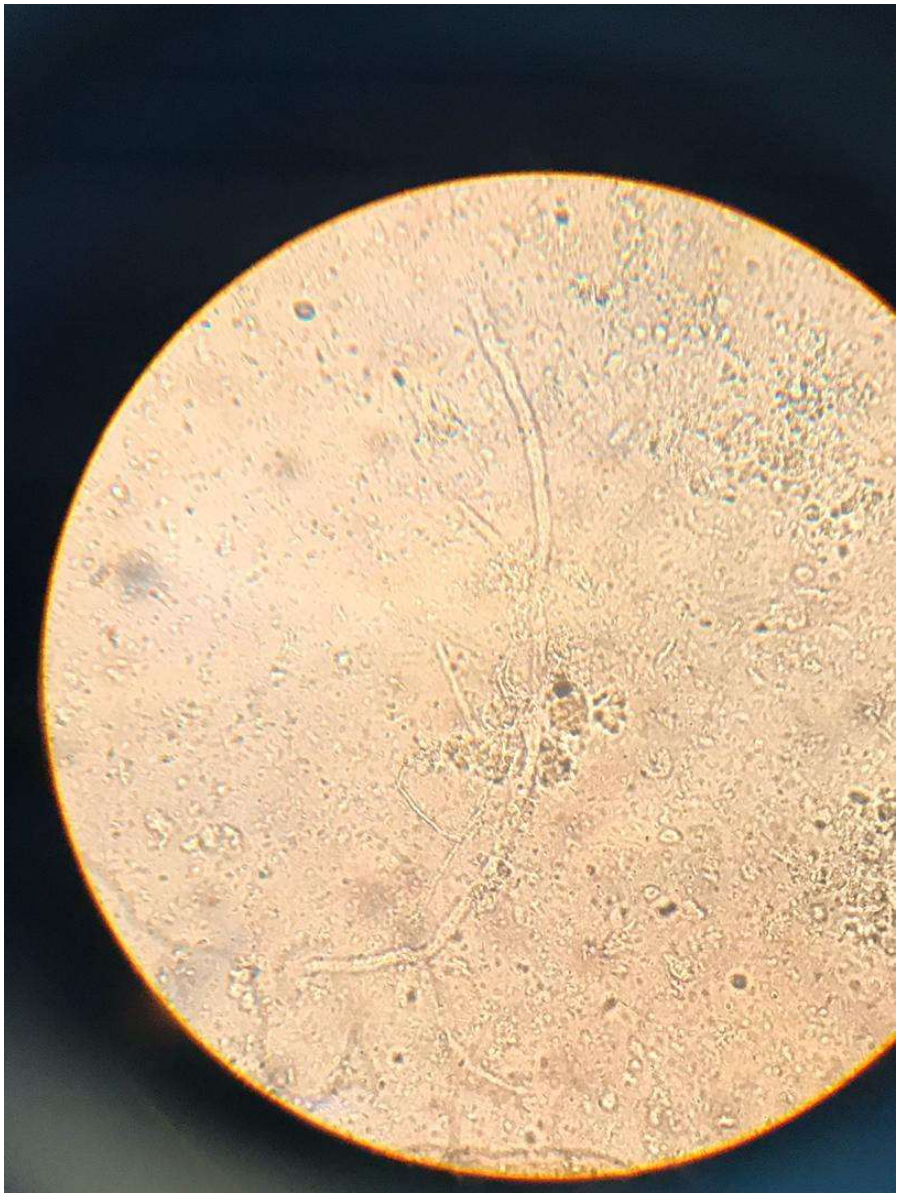
False

True

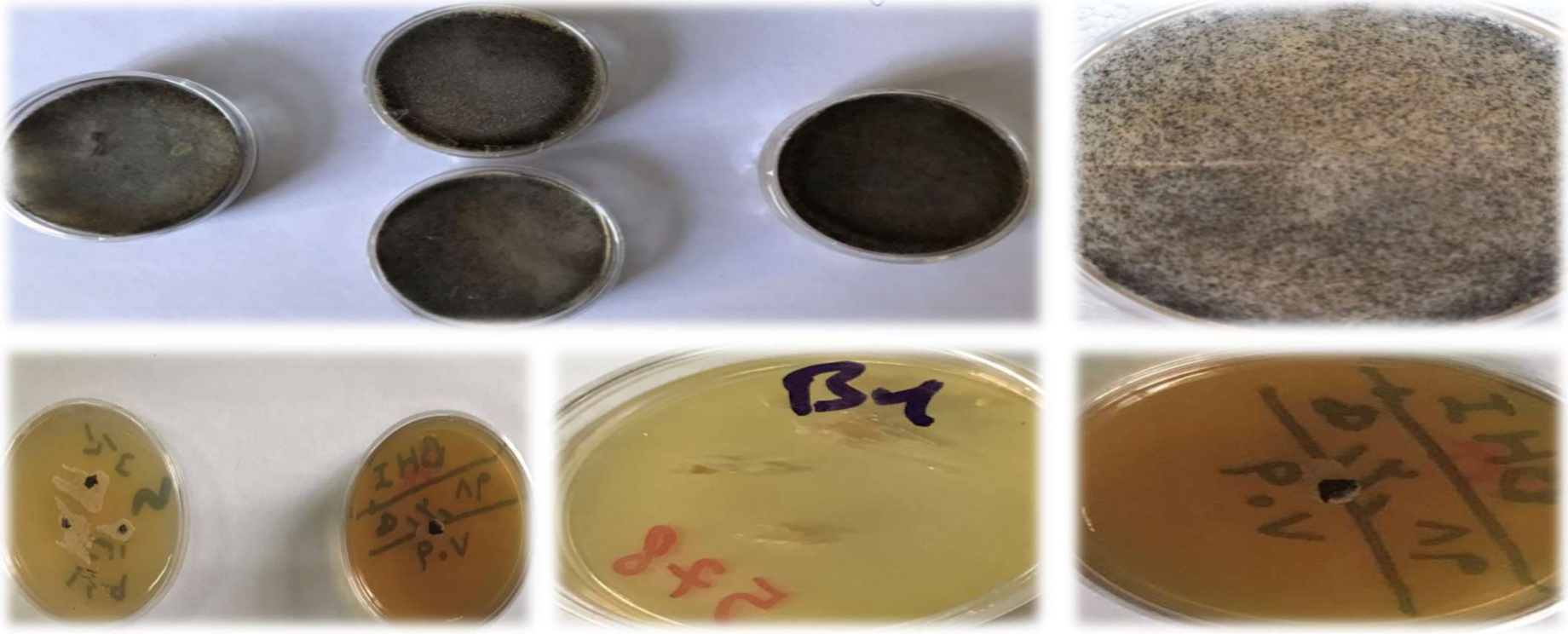
DIAGNOSTIC CHALLENGES OF DIRECT EXAMINATION

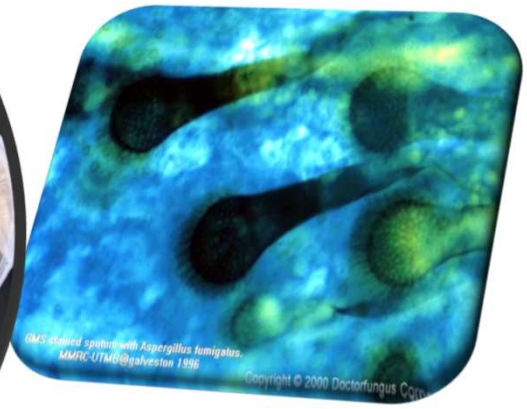
In the absence of a typical ribbon like aseptate hyphae or when septate and non-septate hyphae suggestive of double infections is visible, the method can be confusing

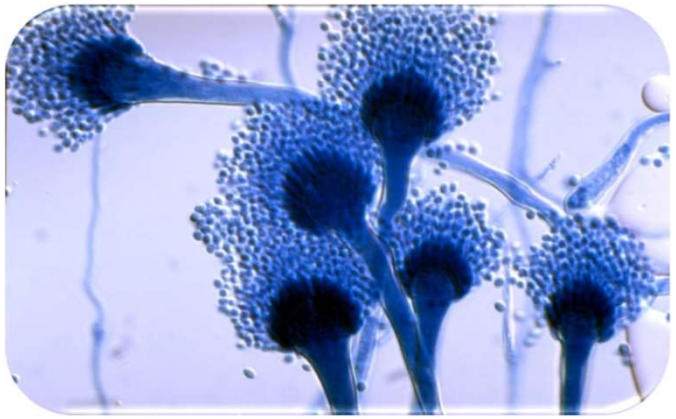


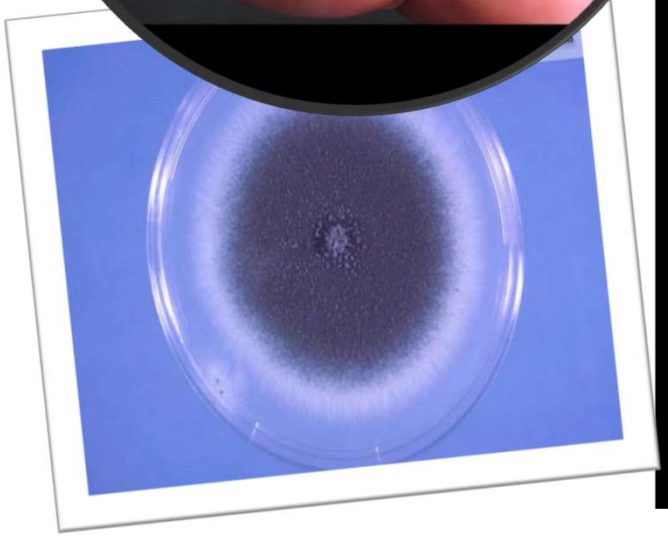


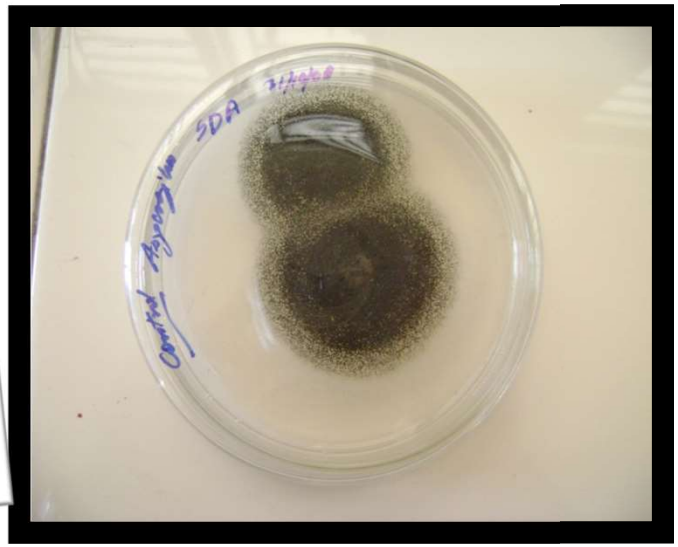
CULTURE AND IMPORTANCE OF IDENTIFICATION




GMS stained sputum with Aspergillus fumigatus.
MMRC-UTMB@galveston 1996
Copyright © 2000 Doctorfungus






SDA

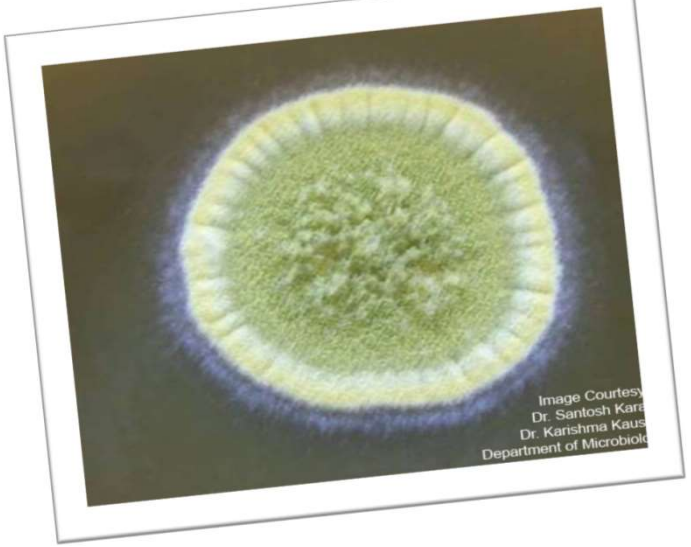


Image Courtesy
Dr. Santosh Kara
Dr. Karishma Kaus
Department of Microbiol

DIAGNOSTIC CHALLENGES OF CULTURE

- The causal agents are ubiquitously distributed.

- It is important in cases with undifferentiated hyphae, for initial identification and for AFST performance

- Blood, CSF and Sputum culture

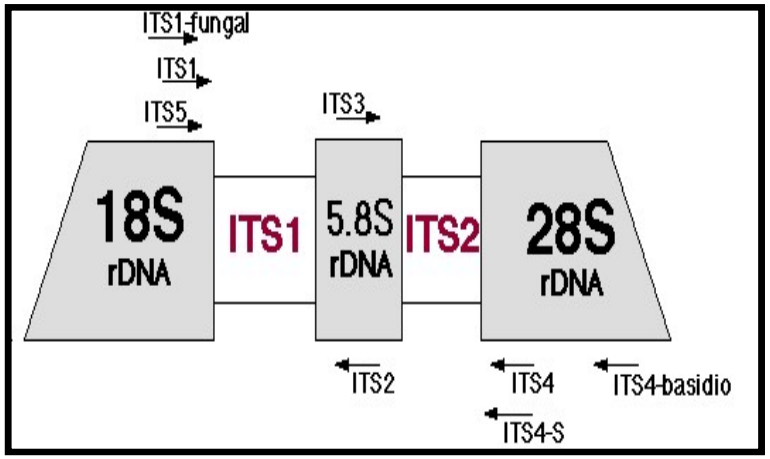
- Overally less sensitive

- On sterile or non sterile specimen

Result of Direct examination	Results of Culture	interpretation
+	+	The diagnosis is confirmed
+	-	The diagnosis is confirmed but for cases with septate hyphae differential diagnosis through molecular methods should be done
-	+	The diagnosis should not be hastily reported unless further assays would be followed
-	-	The diagnosis is confirmed

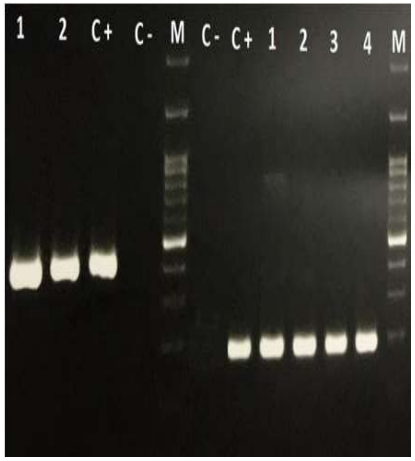
NON-EXPERTISE BASED DETECTION METHODS, INVASIVE PROCEDURE, DIRECT MOLECULAR BASED DETECTION

Sequence based

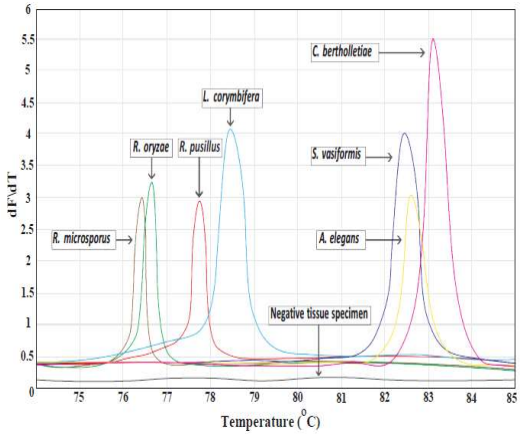


Non-sequence based

Mucorales Specific PCR



Mucorales Specific HRM



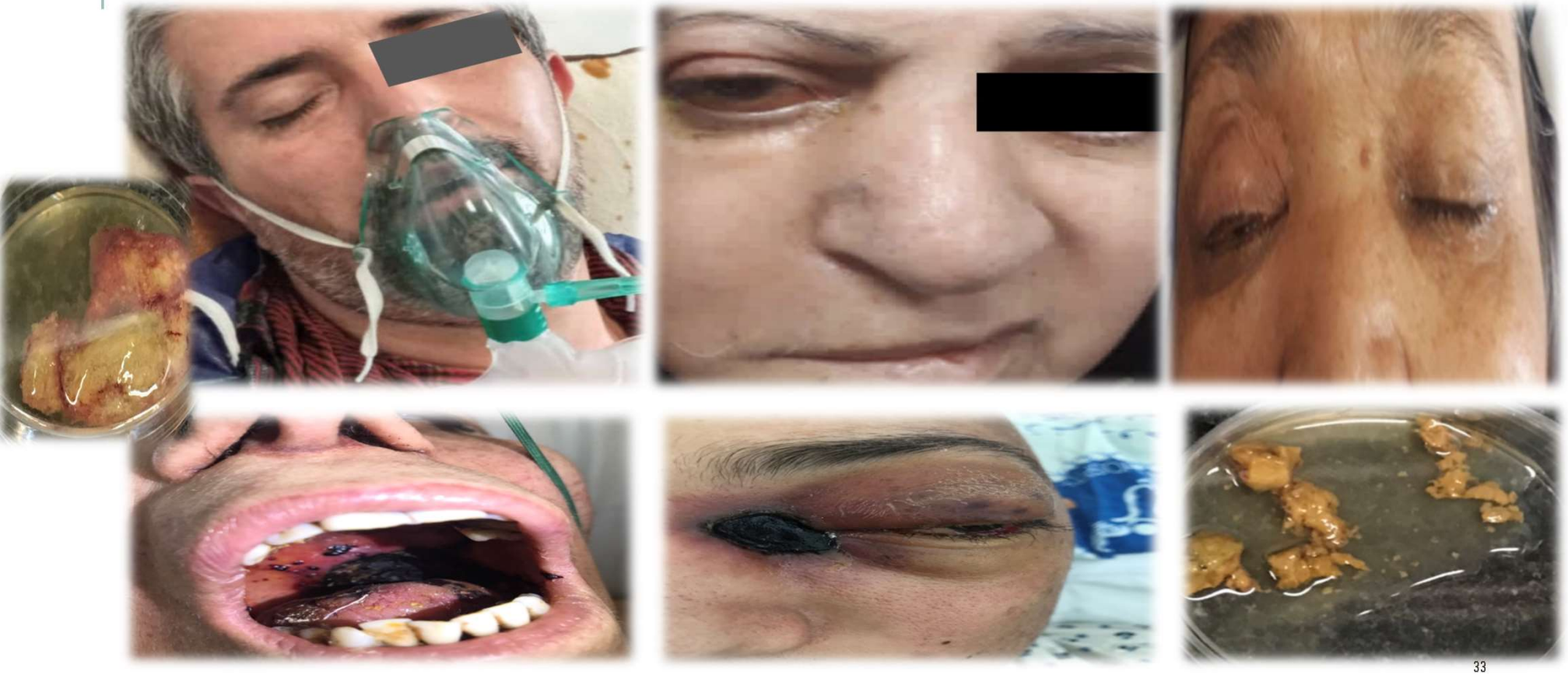
NON-EXPERTISE BASED DETECTION METHODS, INVASIVE PROCEDURE, DIRECT MOLECULAR BASED DETECTION

- In cases with both dual patterns of hyphae
- Case highly suspected to carry mucormycosis or aspergillosis
- When direct examination despite of presence of hyphae is misleading (the type of the hyphae could not be ascertained)
- In cases with septate hyphae but negative culture
- In negative culture cases with proof of fungal hyphae in direct or pathological examination but identification of the causal agent to the species level is important

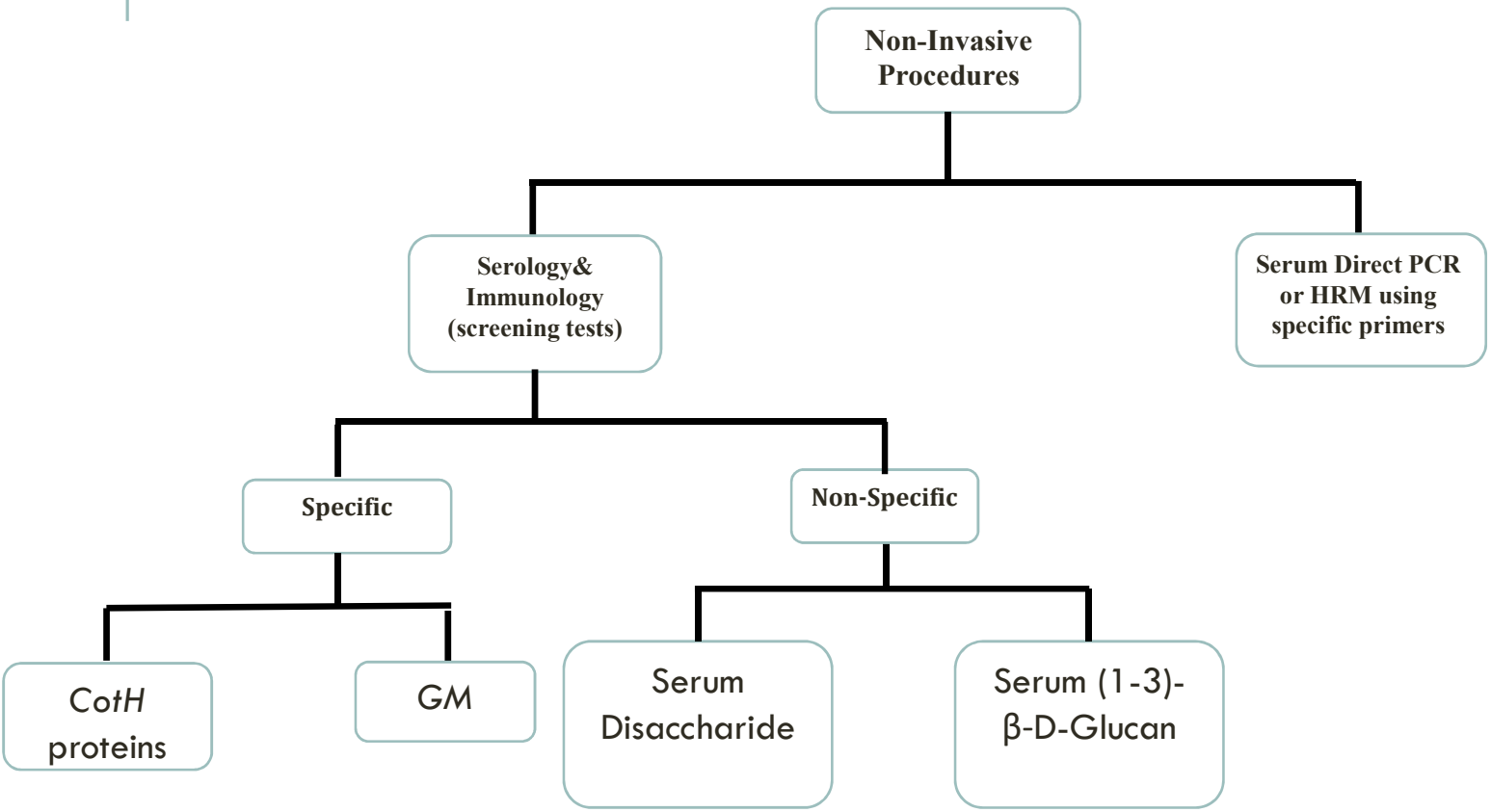
THE DIAGNOSTIC APPROACHES THAT ARE RECOMMENDED FOR THE CASES WITH CO-INFECTION OF ASPERGILLOSIS AND MUCORMYCOSIS DETECTED THROUGH DIRECT EXAMINATION

- **Direct Molecular based detection using specific primers**
- **Culture**
- **GM and BDG**
- **Histopathology**

NON-EXPERTISE BASED DETECTION METHODS, NON-INVASIVE PROCEDURE FOR MUCORMYCOSIS AND ASPERGILLOSIS DETECTION



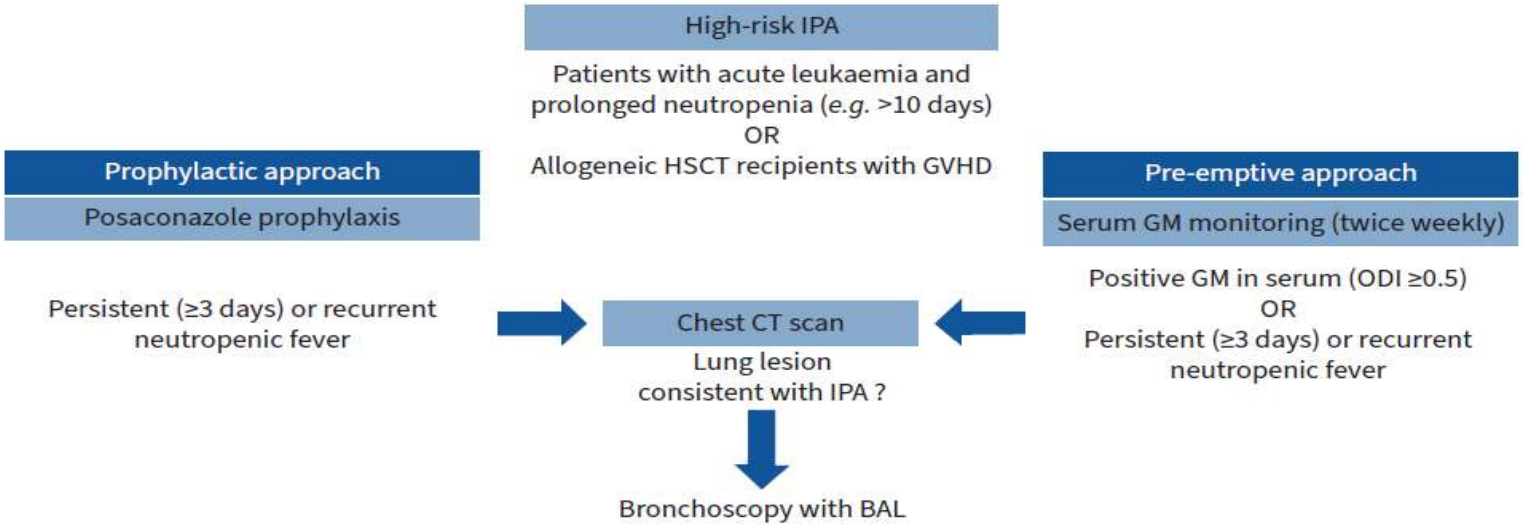
NON-EXPERTISE BASED DETECTION METHODS, NON-INVASIVE PROCEDURE FOR MUCORMYCOSIS AND ASPERGILLOSIS DETECTION



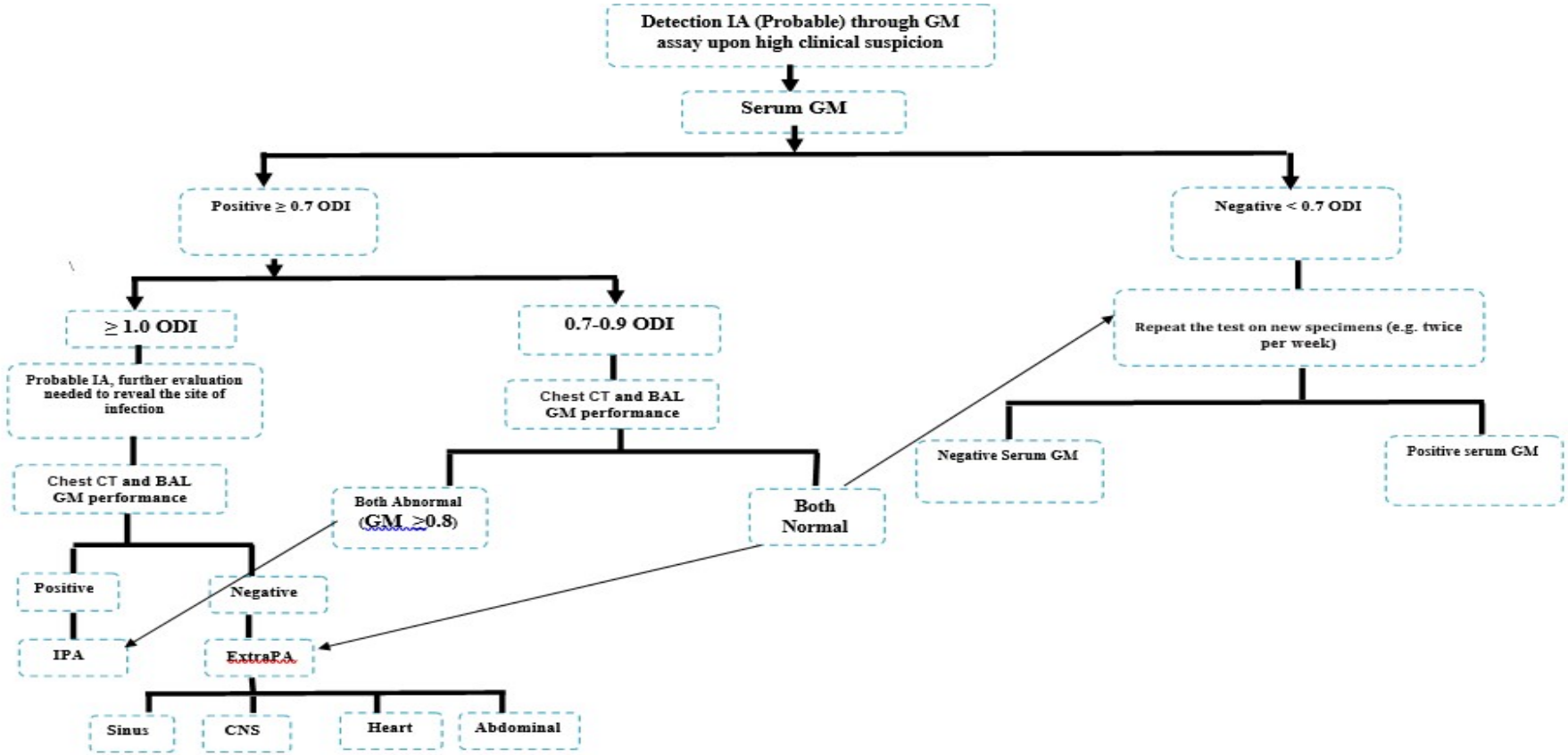
GM ASSAY
(SERUM VS. BAL)
(NEUTROPENIC VS. NON-NEUTROPENIC)
(HEMATOLOGICAL MALIGNANT VS. OTHER COMMUNITY)

- The cornerstone of IPA diagnosis in haematologic cancer patients

- a pre-emptive approach with serial serum GM screening (e.g. twice per week)



GM ASSAY
(IPA VS EXTRAPULMONARY ASPERGILLOSIS)



EXPECTED SEROLOGICAL RESULTS IN DIFFERENT FUNGAL INFECTIONS

Serological assay	Expected result in different fungal infections			
	Aspergillosis	Mucormycosis	Candidiasis	PCP
Serum Disaccharide	+	+	+	Insufficient data
B-D-G	+	-	+	+
GM	+	-	-	-
COTH protein	-	+	-	-

NON-EXPERTISE BASED DETECTION METHODS VS. EXPERTISE BASED DETECTION METHODS

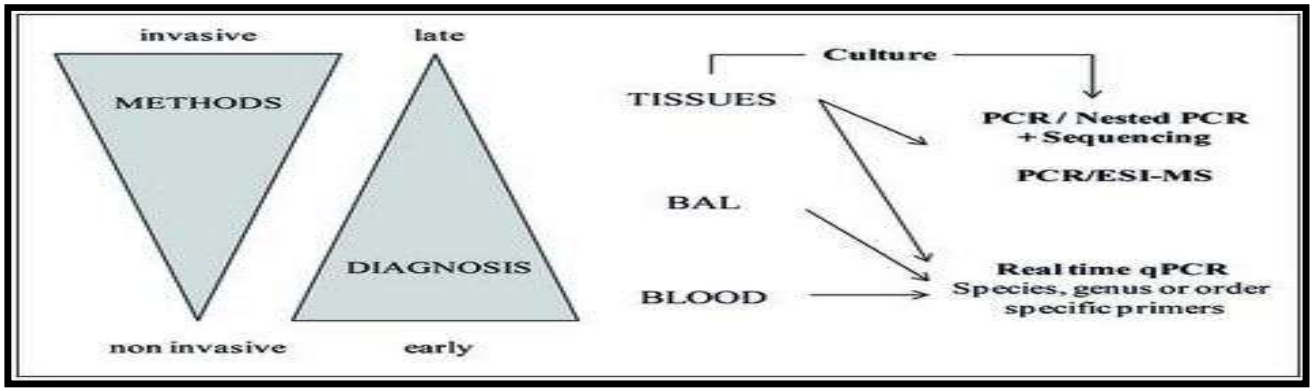
NON-INVASIVE PROCEDURE VS. INVASIVE PROCEDURE

EXPERTISE BASED DETECTION METHODS, invasive PROCEDURE (e.g. histopathology & direct examination)

- (e.g. histopathology & direct examination)
- Make a robust diagnosis (evidence based); categorized the cases as "Proven"
- Detect at an advanced stage of the disease
- Need high level of expertise
- Need tissue biopsy or aspiration

NON-EXPERTISE BASED DETECTION METHODS

- Make a probability or a possibility diagnosis
- Can be detected in early stage of the diseases
- Protocol based
- Don't need invasive specimens



DIAGNOSTIC CHALLENGES OF MUCORMYCOSIS: WHY DIAGNOSIS IS OFTEN DELAYED?

Diagnostic assays		Advantage	Disadvantage
Clinical manifestations		Make an initial suspicion	Non-specific, indistinguishable from aspergillosis, fusariosis or even penicilliosis
Radiology		Make an initial suspicion	Non-specific
Serology	B-D-Glucan	Pan-fungal marker, can be used for differential diagnosis of mucormycosis from aspergillosis	Not applicable in detection of mucormycosis
	GM	Specific for detection of aspergillosis, can be used for differential diagnosis of mucormycosis from aspergillosis or fusariosis, or even in case of aspergillosis/ mucormycosis co-infection can make some clues	Not applicable in detection of mucormycosis
Histopathology& Direct examination		Gold standard	Needs tissue biopsy or aspiration which may cannot be timely achieved in patients with thrombocytopenia, needs great personal expertise, take long time to establish the diagnosis, not be able to identify the fungal aetiology, it will be confusing in case of atypical hyphae or co-infection
Culture		Make an initial identification, important for AFST	Insensitive and in majority of cases, culture remains sterile, take long time to establish the diagnosis, requires a high level of expertise
Molecular techniques		Sensitive, low turnaround time to establish the diagnosis, specific	Not standardized, needs sequencing, should be interpreted alongside other methods

COMPARISON THE DIAGNOSTIC VALUES OF DIFFERENT METHODS EMPLOYED IN OUR STUDY IN TERMS OF TIME CONSUMPTION

Diagnostic test	The turnaround time to establish	
	detection	Identification
Direct examination	1 h	Not be able to identify the causative agent
Histopathological examination	10-14 days	Not be able to identify the causative agent
Culture	48-72 h	48-72 h
Semi-nested PCR	9 h	10-14 days
PCR-HRM	5 h	5 h
Serology	**3 h**	**Not be able**

COMPARISON THE DIAGNOSTIC VALUES OF DIFFERENT METHODS

Provide same day diagnosis

- Direct examination
- Histopathological examination
- Specific PCR
- HRM PCR
- Serological assay

Provide conclusive evidence of invasive disease

- Direct examination
- Histopathological examination
- Positive culture of sterile specimens

FACTORS FAVORING MUCORMYCOSIS OVER ASPERGILLOSIS

Clues	
Epidemiologic and host clues	Clinical, radiologic, and laboratory clues
Institution with high background rates of mucormycosis	Pansinusitis or ethmoid involvement
Iron overload	Orbital and brain involvement
Hyperglycemia with or without DM	Oral necrotic lesions in hard palate or nasal turbinates
Prior voriconazole or echinocandin use	Tooth pain
	Chest wall cellulitis
	Multiple (n >10) nodules in CT and pleural effusion
	Reverse halo sign in CXR or CT

QUESTIONS

12- Which of the following methods has the lowest turnaround time for detection of aspergillosis and mucormycosis once the specimen is collected?

Histopathological assays

HRM PCR

Culture

PCR & sequencing

13- Which of the following methods has the lowest turnaround time for detection of aspergillosis and mucormycosis once the specimen is collected?

Direct examination

HRM PCR

Culture

Serology (e.g. GM and BDG)

14- Which of the following methods of detection is considered as the gold standard for invasive aspergillosis and mucormycosis detection?

Direct examination & Histopathological assays

HRM PCR

Culture

PCR & sequencing

QUESTIONS

15- Which of the following methods is considered as the gold standard of causal agent identification of invasive aspergillosis and mucormycosis?

Direct examination & Histopathological assays

HRM PCR

Culture

PCR & sequencing

16- Through which of the following methods both detection and identification can be simultaneously achieved in a low turnaround time?

Direct examination & Histopathological assays

HRM PCR

Culture

PCR & sequencing

QUESTIONS

17- Which of the following detection methods can be applied as screening test for detection of aspergillosis and mucormycosis in setting with high risk individuals?

Direct examination

Molecular methods (HRM PCR)

Culture of sterile specimens

Serology (e.g. GM and BDG)

18- The serological patterns that is predicted to be yielded in mucormycosis and aspergillosis, respectively is as follow ……?

GM (+) and BDG (+) -- GM (+) and BDG (+)

GM (+) and BDG (+) -- GM (-) and BDG (-)

GM (-) and BDG (-) -- GM (-) and BDG (+)

GM (-) and BDG (-) -- GM (+) and BDG (+)

QUESTIONS

19- When direct molecular based methods for detection of aspergillosis and mucormycosis are inevitably recommended?

In cases with both dual patterns of hyphae

Case highly suspected to carry mucormycosis or aspergillosis

When direct examination despite of presence of hyphae is misleading (the type of the hyphae could not be ascertained)

In cases with septate hyphae but negative culture

All above choices

20- Which of the diagnostic approaches are recommended for the cases with co-infection of aspergillosis and mucormycosis detected through direct examination?

Direct Molecular based detection using specific primers

Culture

GM and BDG

Histopathology

a, b, c