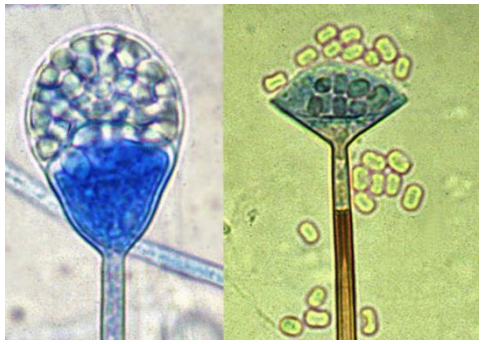
Updates in invasive mould infections



Arunaloke Chakrabarti

Director, Doodhdhari Burfani Hospital & Research Institute, Haridwar, India
Immediate Past-President, International Society for Human & Animal Mycology

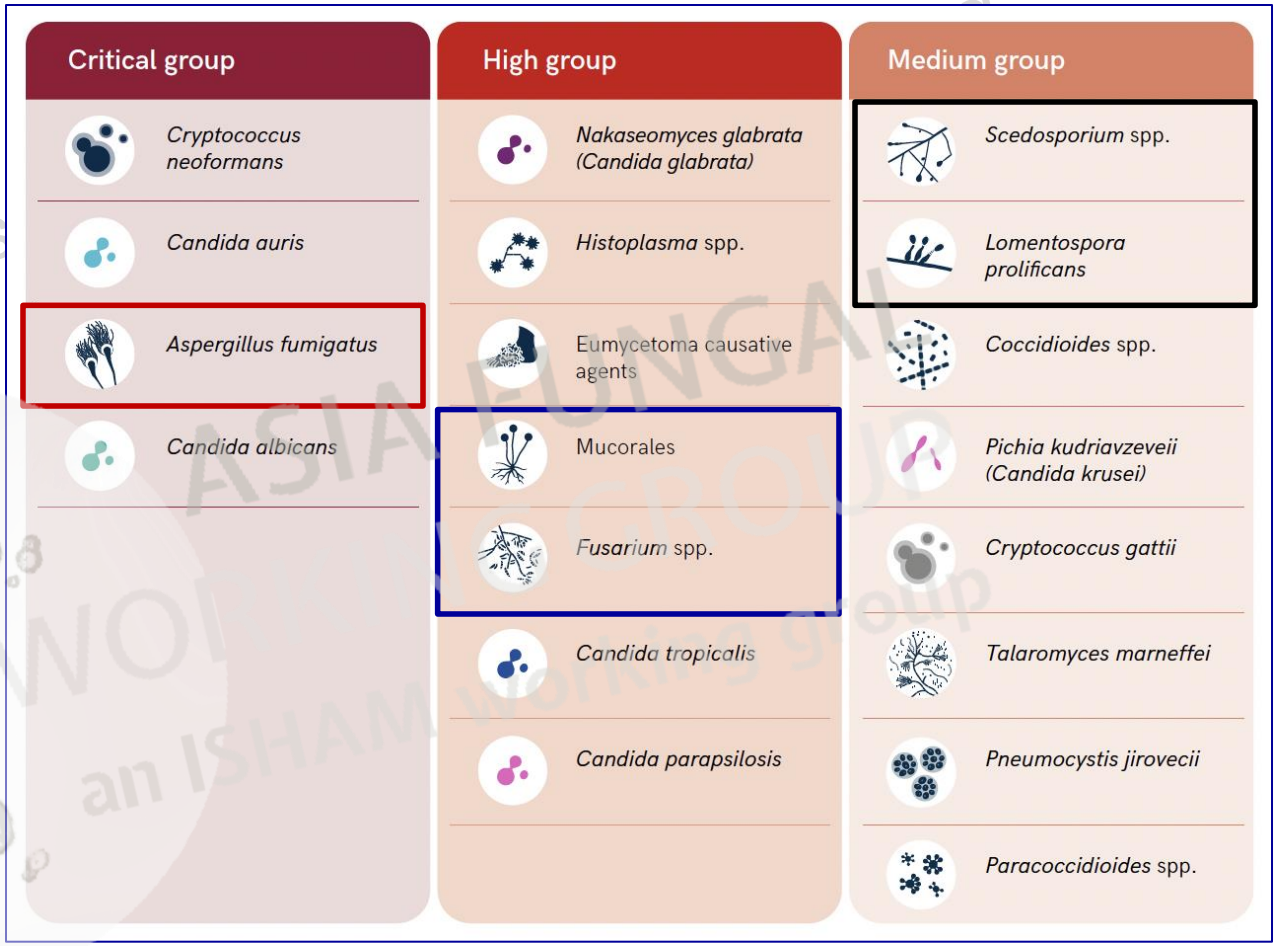
PP-CRB-VNM-0176

Updates

- **Prevalence**
- **Risk factors**
- **Etiological agents**
- **Diagnosis**
- **Management**

- **WHO released first Fungal Priority Pathogen List** (FPPL) in 2022 of 19 pathogen posing greatest threat to public health

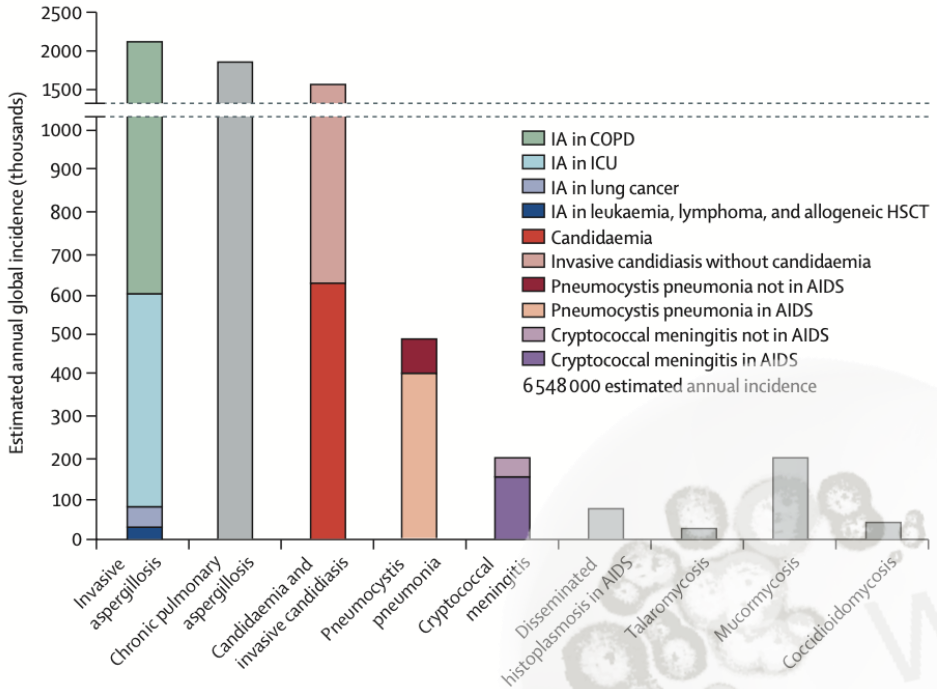
WHO- fungal priority pathogens



Missing moulds which are prevalent in Asian countries

- *Aspergillus favus*
- *Cladophialophora bantiana*

Epidemiology of invasive fungal disease



Annual incidence of 6·5 million invasive fungal infections & 3·8 million deaths, of which about 2·5 million were directly attributable

Denning DW. Lancet Infect Dis. 2024 Jan 12:S1473-3099(23)00692-8;

Since 1980s, Invasive mould infections rapidly evolved

- Increased prevalence
- New species emerged causing human infection
- COVID-19 impact - CAPA, CAM
- Antifungal resistance – one health issue
- Public health importance

Major challenges – challenge of 50%

- Difficult to diagnose – 50% diagnosed post-mortem
- Difficult to treat – 50% mortality despite therapy
- Stewardship issue – 50% inappropriate therapy

Specific challenges in developing countries in Asia

- **Tropical environment** where fungi easily thrive – mycelial infection from high spore count in air
- **Large number of susceptible popul**ation – diabetics, COPD, cirrhosis etc

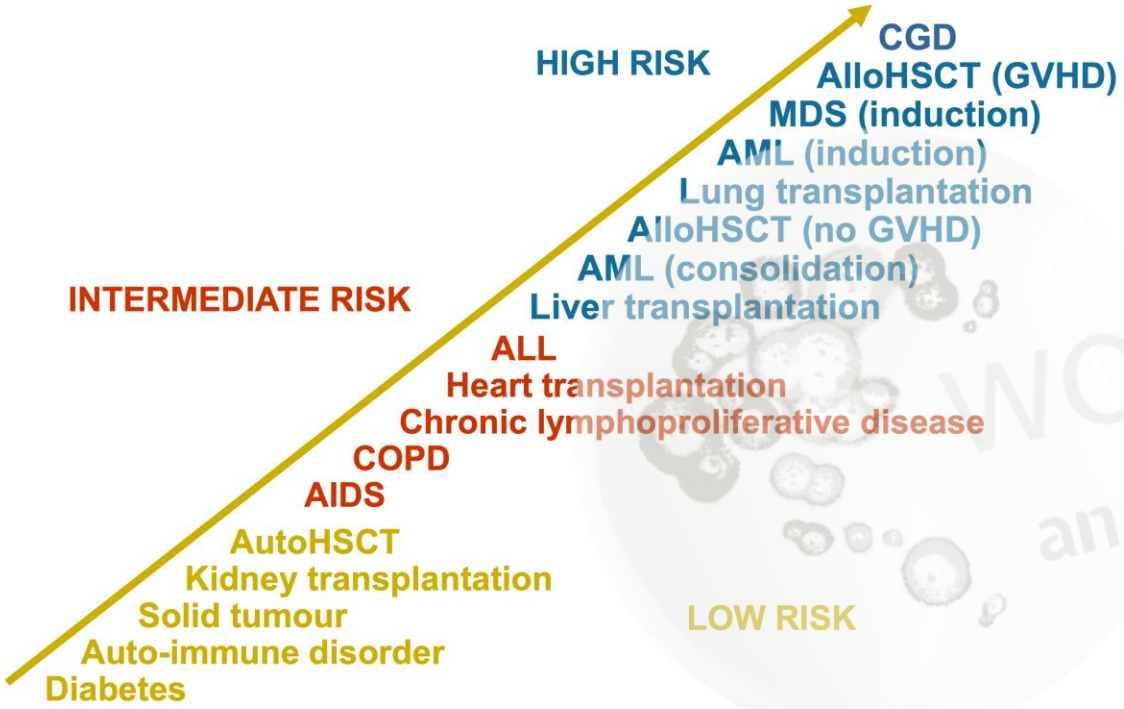
Very high rate of IMI in Asian countries including Indonesia & India

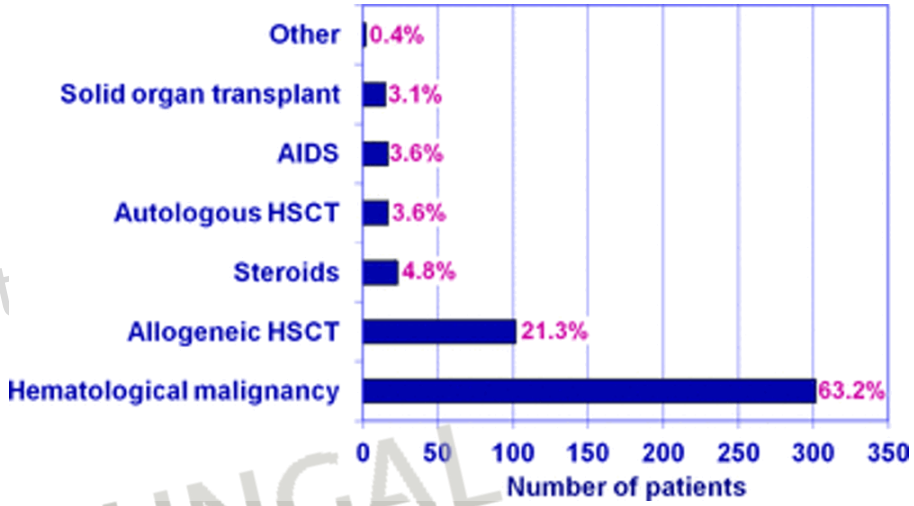
- **2.9 - 4.1%** population suffer from serious fungal infection
  - **Invasive aspergillosis –18.0-18.6/100,000**
  - **Chronic pulmonary aspergillosis – 125-142/100,000**
  - **Chronic fungal rhinosinusitis – 109-110/100,000**
  - **Mucormycosis – 0.2-14.0/100,000**

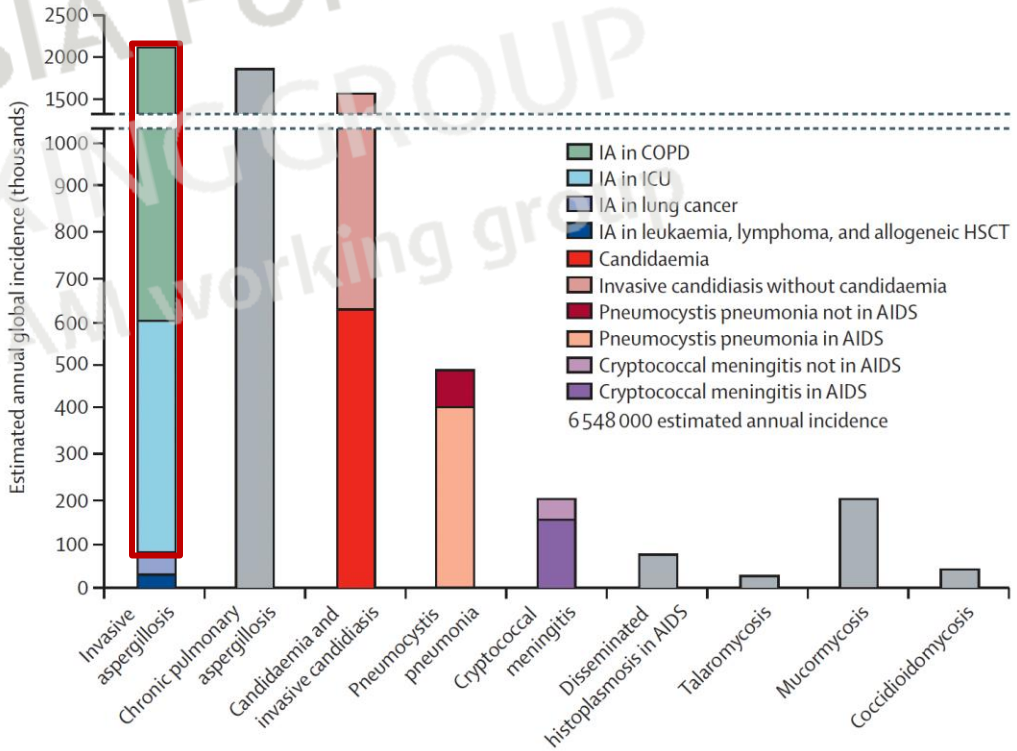
Ray A, *et al*. Open Forum Infect Dis 2022; 9: ofac603; Wahyuningsih R, *et al*. Mycoses 2021; 64: 1203-1212

Vulnerable population

Risk stratification of IA in immunosuppressed







Herbrecht et al Ann NY Acad Sci 2012

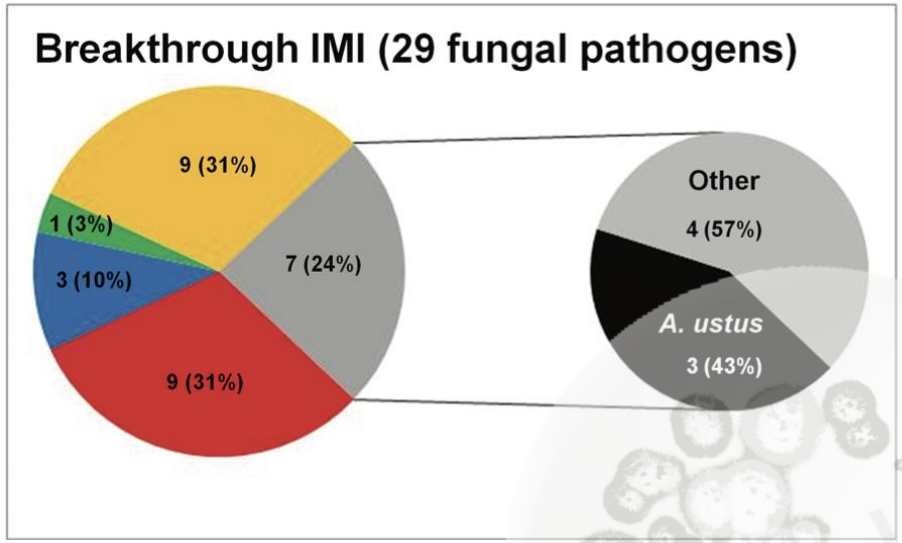
Denning DW. Lancet Infect Dis. 2024 Jan 12:S1473-3099(23)00692-8; Verma N *et al*. Mycoses 2022; 65: 266-284

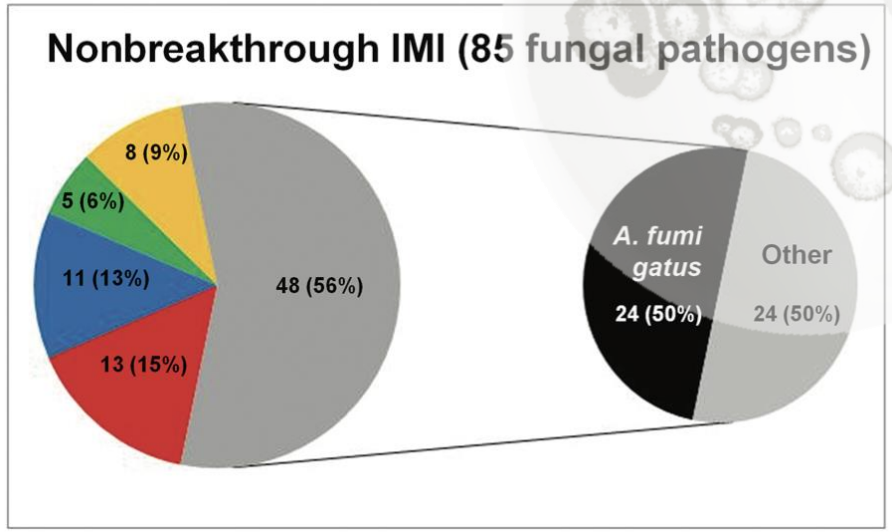
Invasive mould infections

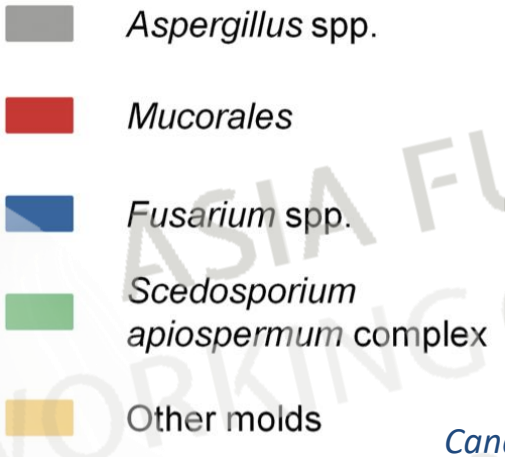
- **New risk factors**
  - **prolonged ICU stay, COPD, structural lung defect, chronic liver & kidney disease, ARDS**
  - **Influenza & COVID-19** (tocilizumab, advanced age, steroid)
  - **Immunobiologicals** [TNFα blockers, Tyrosine kinase inhibitor (ibrutinib), anti-B cell inhibitors, anti-rejection, check-point inhibitors]
- **Selection/breakthrough after prophylactic antifungal**
  - **After introduction of fluconazole** prophylaxis
    - **Increase in invasive mould disease** (aspergillosis, mucormycosis, fusariosis & others) (Pagano *et al*, 2006; Nucci *et al*, 2013)
  - **After introduction of mould active prophylaxis** (voriconazole & posa)
    - **Breakthrough mould infection, mucormycosis** (Auberger *et al*, 2012)
    - **Emergence of triazole resistant aspergillosis** (van der Linden *et al*, 2013)

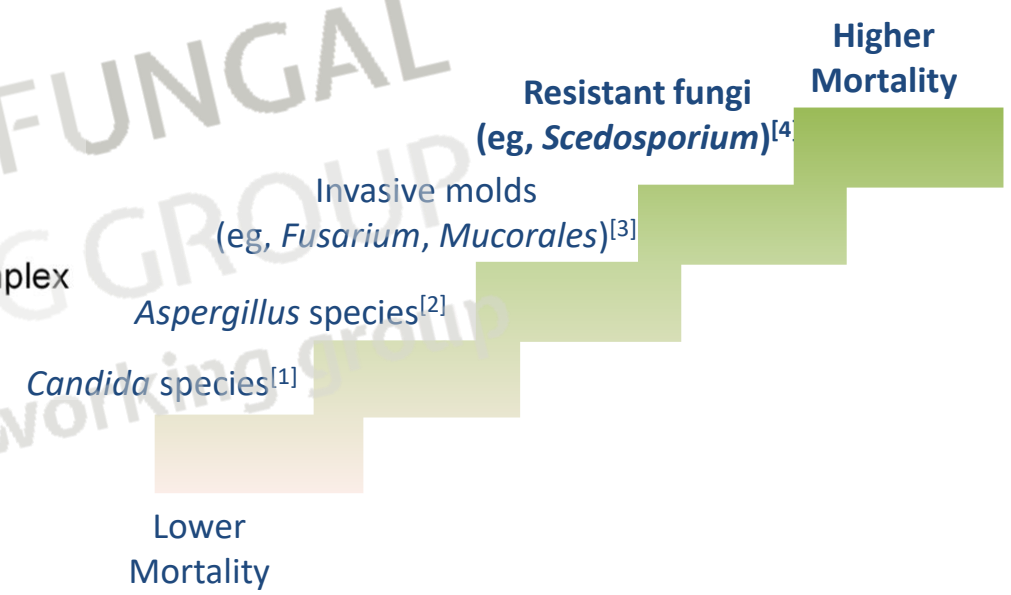
Cadena J, *et al*. Infect Dis Clin N Am 2021; 35: 415-434; Bernardes & Hohl. Curr Clin Microbiol Rep 2020; 7: 142-149; Chamiols G, *et al.* Clin Infect Dis 2018; 66: 140-148

Comparison of breakthrough vs. non-breakthrough mould infection









Lamoth F, *et al*. Clin Infect Dis 2017; 64: 1619-21; Andes. Clin Infect Dis. 2012;54:1110. Nivoix. Clin Infect Dis. 2008;47:1176. Park. Emerg Infect Dis. 2011;17:1855. Cortez. Clin Microbiol Rev. 2008;21:157.

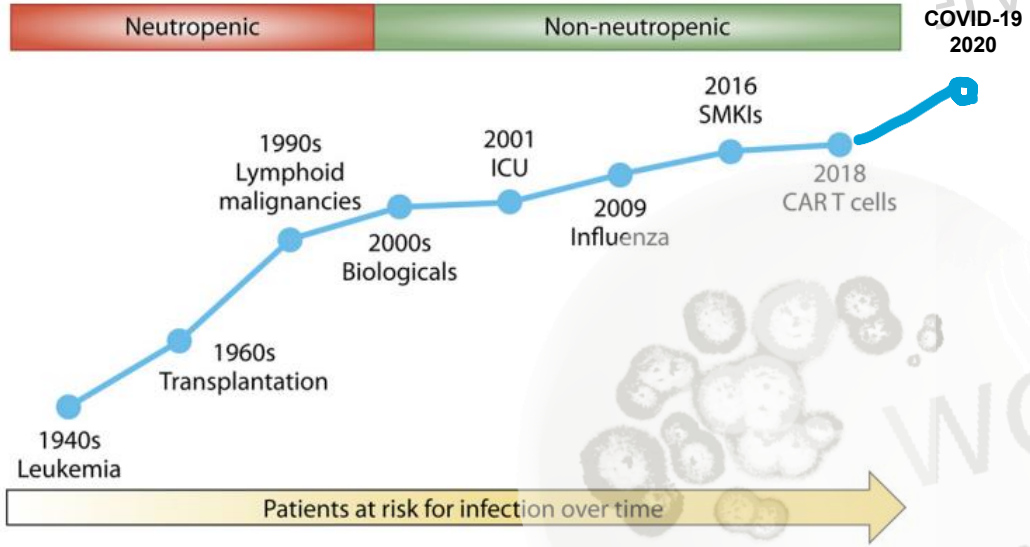
Uncommon mould in clinical practice

Mucormycetes	Hyalohyphomycetes	Phaeohyphomycetes	Parafungi
Rhizopus homothallicus	*Scedosporium* spp.	***Cladophialophora bantiana***	***Pythium insidosum***
***Apophysomyces* spp.**	*Lomentospora prolificans*	*Verruconis gallopava*	
***Saksenaea* spp.**	*Fusarium* spp.	*Alternaria* spp.	
Rhizopus microsporus	*Rasmsonia* spp.	*Bipolaris* spp.	
Thamnostylum lucknowense	*Paecilomyces* spp.	*Exophiala* spp.	
Syncephalastrum racemosum	*Acremonium* spp.		
	***Schizophyllum* spp**		

Hoenigl M, *et al.* Lancet Infect Dis 2021; 21: E 246-E257; Douglas AP, *et al.* Clin Microbiol Infect 2016; 22: 670-680; Rathi A, Chakrabarti A, *et al*. Cornea 2018; 37: 518-522; Chakrabarti A, *et al*. Med Mycol. 2016; 54: 111-11; Prakash & Chakrabarti. Microorganism 2021; 9: 523

New risk groups for aspergillosis

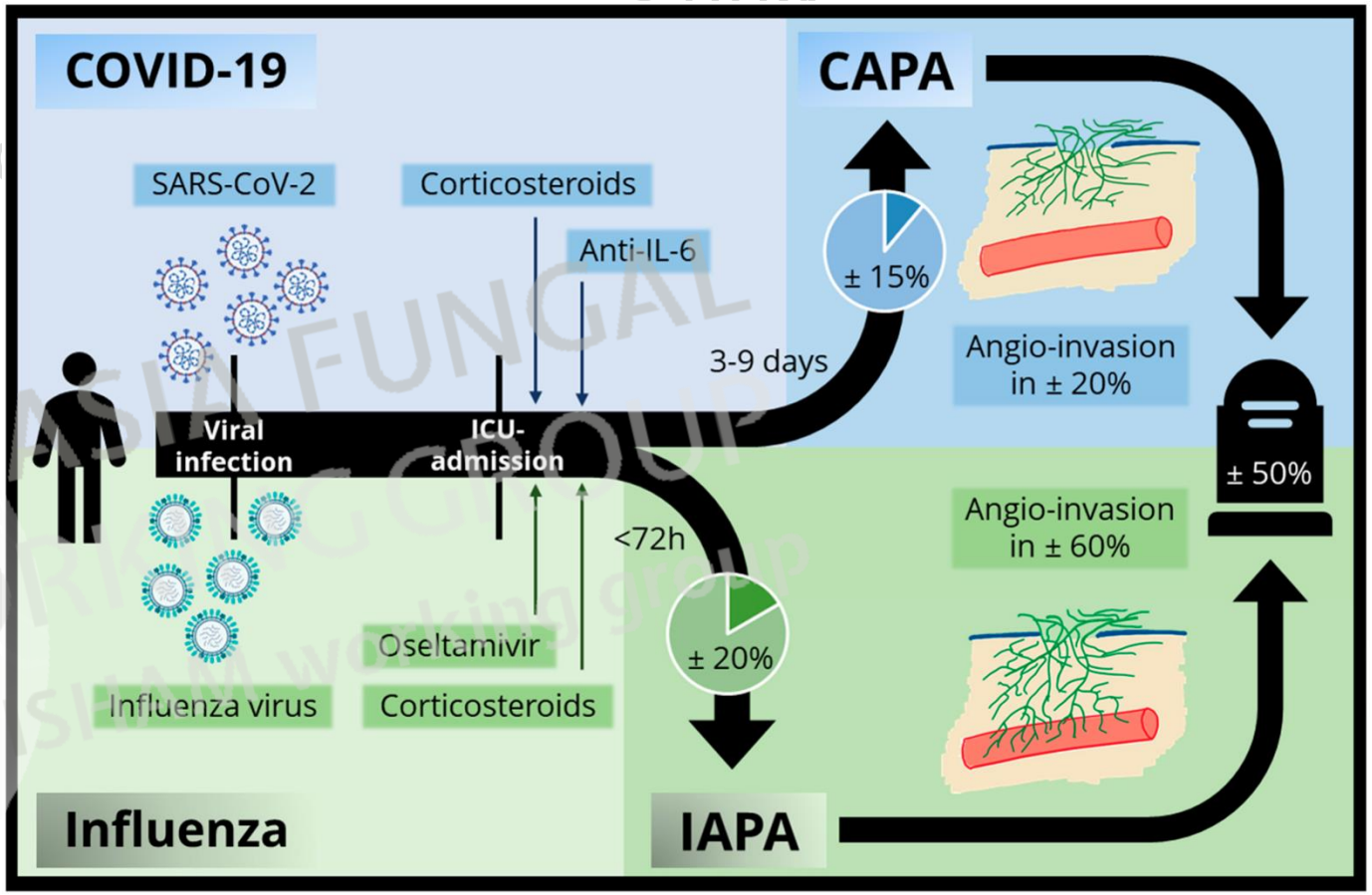
Epidemiological trends of IA



SMKI, small-molecule kinase inhibitor; CAR T cells, chimeric antigen receptor T cells.

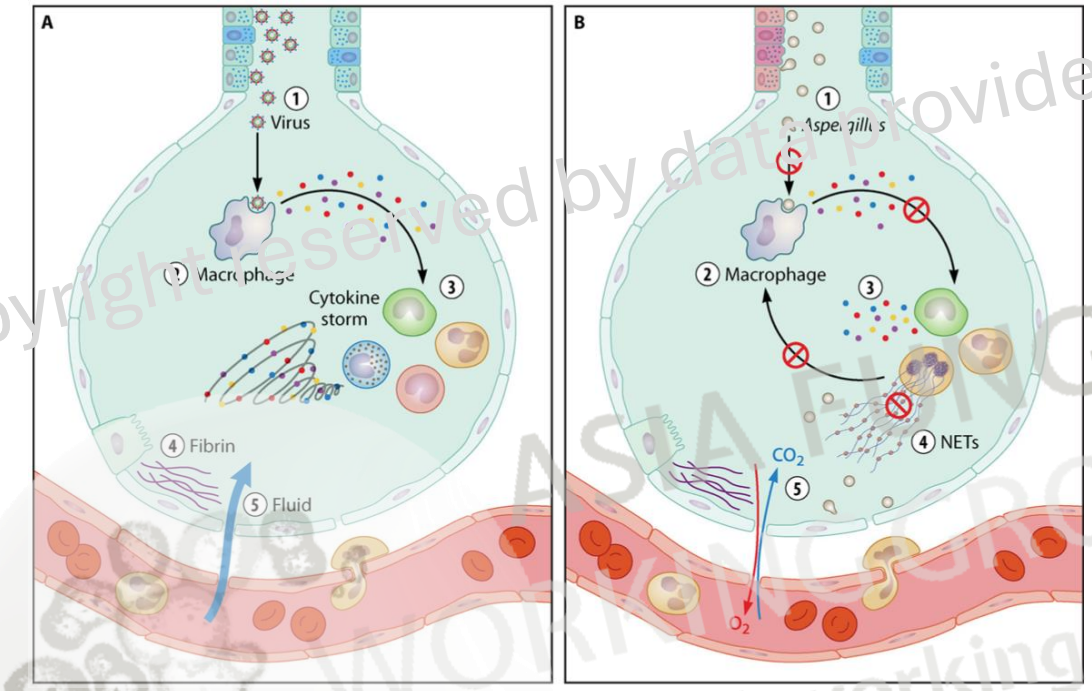
Bassetti M, *et al.* Intensive Care Med 2017; 43: 1225; Latge & Chamilos Clin Microbiol Rev 2020; 33: e00140-18; Meersseman W, *et al*. Clin Infect Dis 2007; 45: 205-16; Rudramurthy SM, *et al*. Indian J Med Microbiol 2016; 34: 529-32

Virus associated pulmonary aspergillosis



Feys S, *et al*. J Fungi 2021; 7: 1067

Respiratory viral-pathogen co-infections

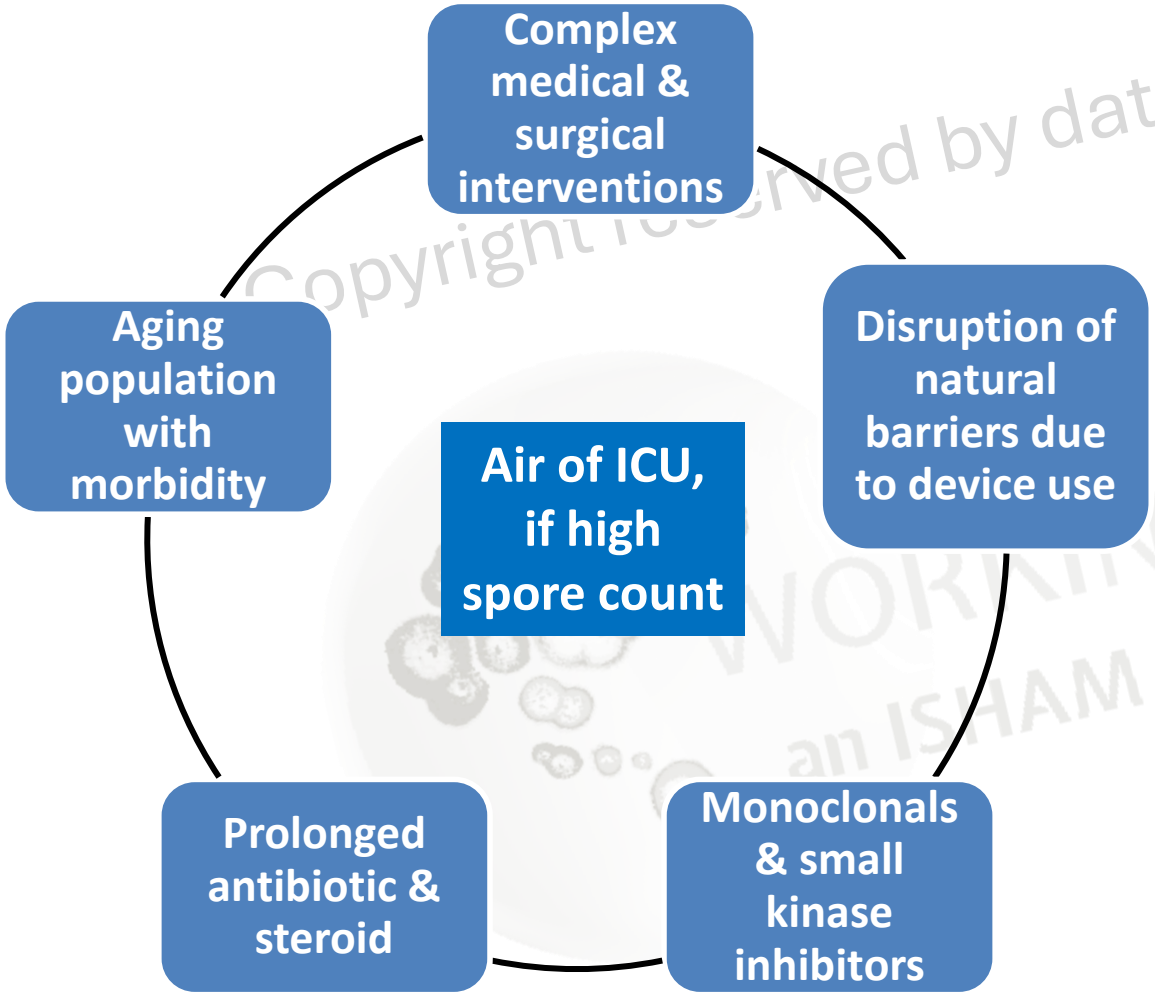


(A) Entry of virus into alveolar space. (1) Virus infects airway epithelium. (2) Alveolar macrophages recognize the virus & produce cytokines. (3) Cytokines attract more immune cells (neutrophils & monocytes). (4) Damage further through the formation of fibrin & scar tissue. (5) Weakened blood vessels allow fluid to seep in & fill the lung cavities, leading to respiratory failure.

(B) Entry of fungi into the alveolar space. (1) damaged epithelium facilitates adhesion of fungal conidia & subsequent invasion. (2) Phagocytosis, fungal killing, & cytokine production by alveolar macrophages impaired. (3) Recruitment of neutrophils also affected. (4) Loss of neutrophils compromises their cytokine production & neutrophil mediated fungal killing. (5) Fibrinous material can cause the obstruction of small airways, decreasing oxygen & carbon dioxide diffusion, leading to hypoxia

Salazar F, *et al.* Clin Microbiol Rev 2022; 35: e00094-21

Why the patients become susceptible for mould infection in ICU?



Which mould infections in ICU?

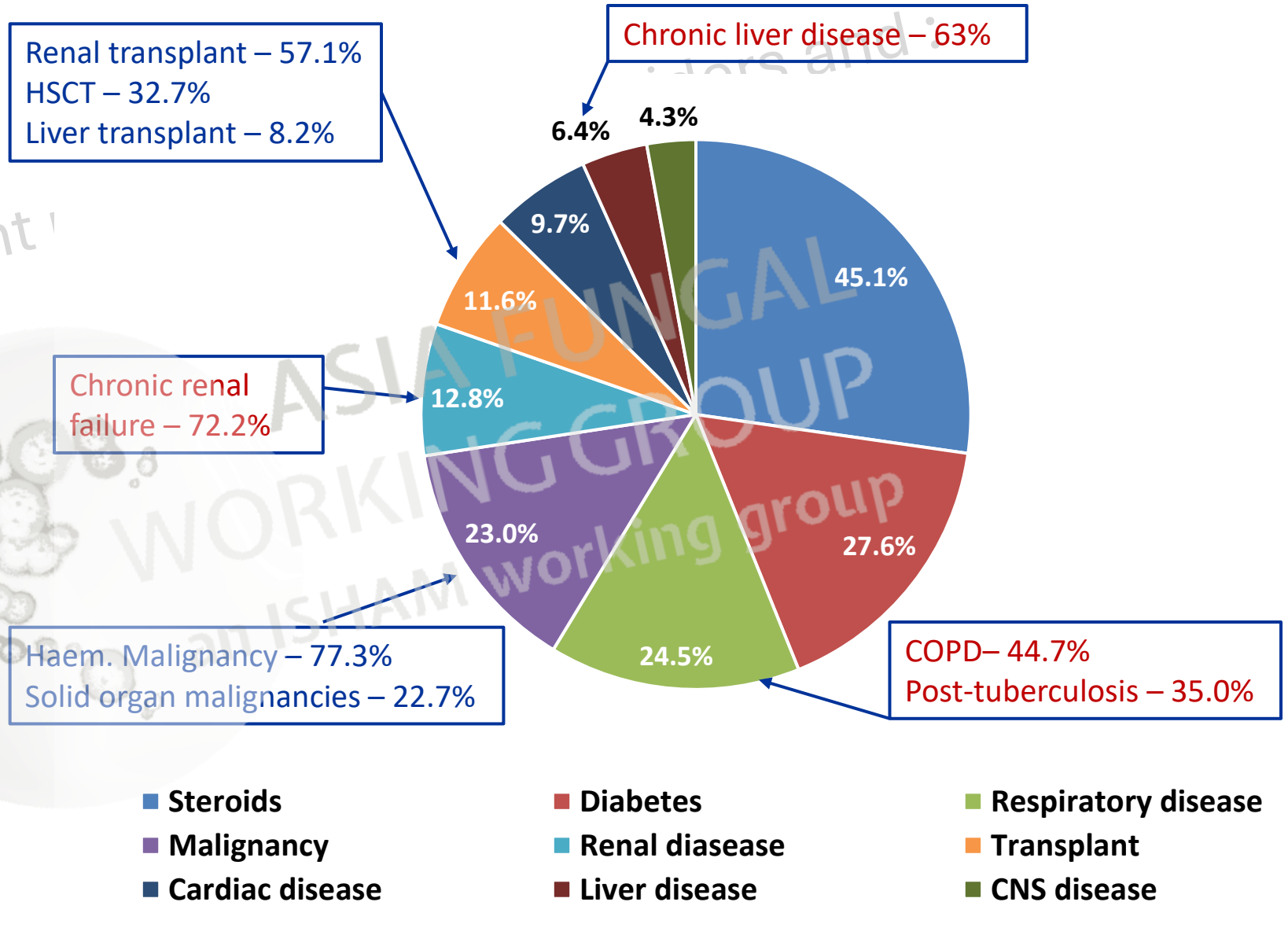
- ***Aspergillus***
  - Invasive aspergillosis
  - Chronic pulmonary aspergillosis
- ***Mucorales***
- Rare moulds
  - *Fusarium*
  - *Scedosporium*
  - Dematiaceous moulds
  - Many others….

Bassetti M, *et al.* Intensive Care Med 2017; 43: 1225

Mould infections in ICU – 11 centers

Indian data

- 10.1/1000 ICU admission
  - **Aspergillosis – 74.8%**
  - **Mucormycosis -23.9%**
- Site of infections
  - **Pulmonary – 80.7%**
  - Rhino-orbito-cerebral – 5.8%
  - CNS– 1.3%
  - Gastrointestinal – 0.8%
  - Subcutaneous tissue – 2%
  - Heart – 0.3%
- ***A. flavus* & *A. fumigatus* equal frequency**
- **Overall mortality – 67.3%**



Chakrabarti A, *et al*. J Crit Care 2019; 51: 64-70;

Impact of invasive aspergillosis in ICUs globally

Prevalence – 0.2% to 17% with mortality >80%

Reference	Country	Positivity	percentage
Valles J, *et al*. 2003	Spain	Pneumonia	19
Meersseman W, *et al*. 2004	Belgium	128/1850	6.9
Montagna MT, *et al*. 2013	Italy	12/5561	0.2
Baddley JW, *et al*. 2013	USA >600 hospitals (4y)	412/6424	6.4
Taccone FS, *et al*. 2015	8 countries, 30 ICUs	94/563	17% proven 36% putative

Chakrabarti & Sharma Curr Fungal Infect Report 2019; 13; 1-10; Bassetti & Bouza. J Antimicrob Chemother 2017; 72 (Suppl 1): i39-i47

New therapies associated with invasive fungal disease

Chimeric antigen receptor T (CAR-T) cells therapy targeting the CD19 antigen also increases the risk of fungal infections

Traditional Inherited or Acquired Host Factors: Immunodeficiency syndromes, Underlying disease, prior therapies, allogeneic HCT, neutropenia, corticosteroids

Novel Therapies Associated with Invasive Fungal Disease

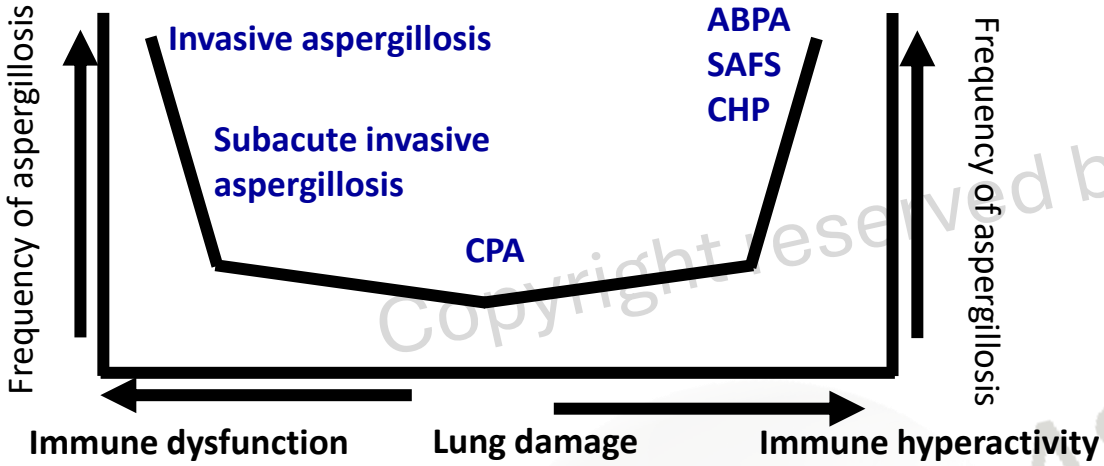
Target	Agents	IFD Association
	Monoclonal Antibodies	
TNF alpha inhibitors	Infliximab, Adalimumab, Etanercept, Golimumab, Certrolizumab	Dimorphic fungal infection
CD52 inhibitors	Alemtuzumab	Pneumocystosis, cryptococcosis
IL17 inhibitors	Brodalumab, Ixekizumab, Secukinumab	Candidiasis (mucosal)
	Small Molecules	
BTK inhibitors	Ibrutinib, Acalabrutinib, Zanubrutinib	Invasive mold infection (CNS), cryptococcosis, blastomycosis
PI3K inhibitors	Duvelisib, Idelalisib	Pneumocystosis
JAK 1/2/3 inhibitors	Baricitinib, Ruxolitinib, Tofacitinib	Dimorphic fungal infection, pneumocystosis, Aspergillosis

Genetic Pathways Associated with Invasive Fungal Disease

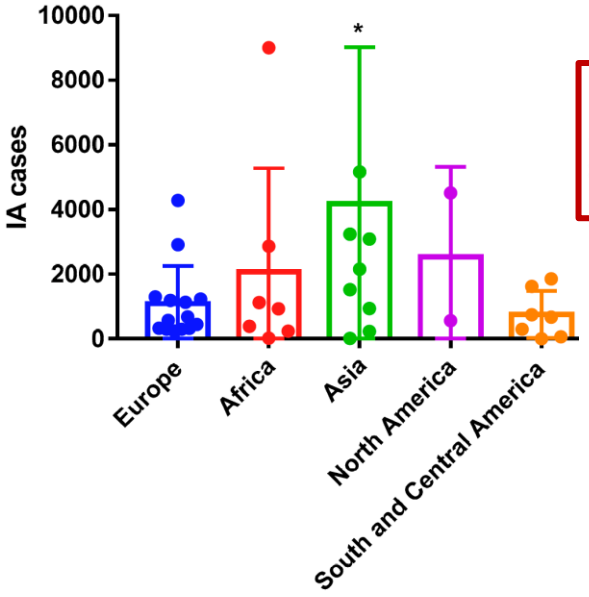
Inherited Defects in Immunogenetic Pathways	IFD Association
IL-12 and interferon-γ	Dimorphic fungal infection
NADPH oxidase, GATA2	Invasive mold infection
CARD9, STAT1, STAT3, AIRE, IL-17, IL-12	Candidiasis

Little J, *et al.* OFID 2024; 11: ofae 363

Aspergillus clinical syndrome

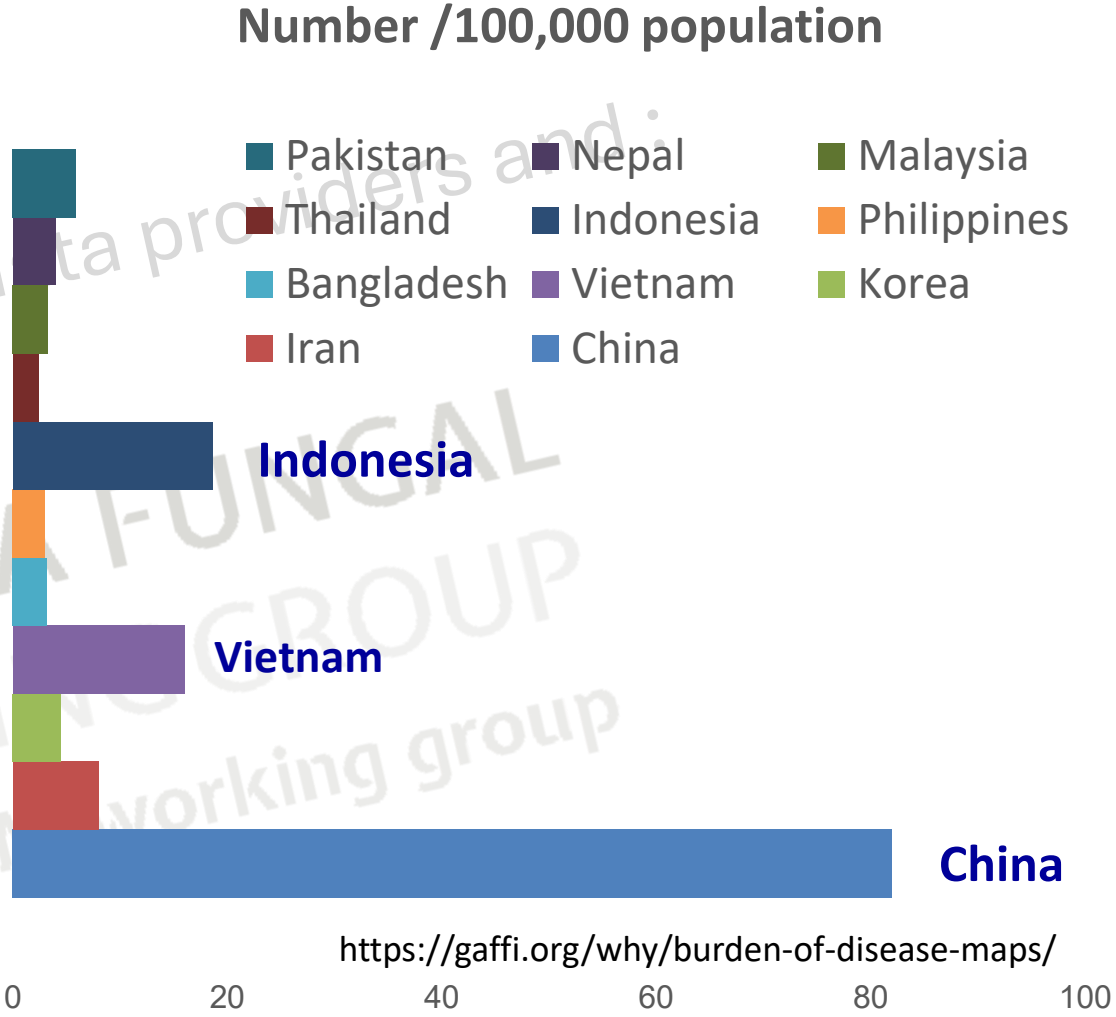


Kosmidis C, et al. Thorax 2015;70:270-277



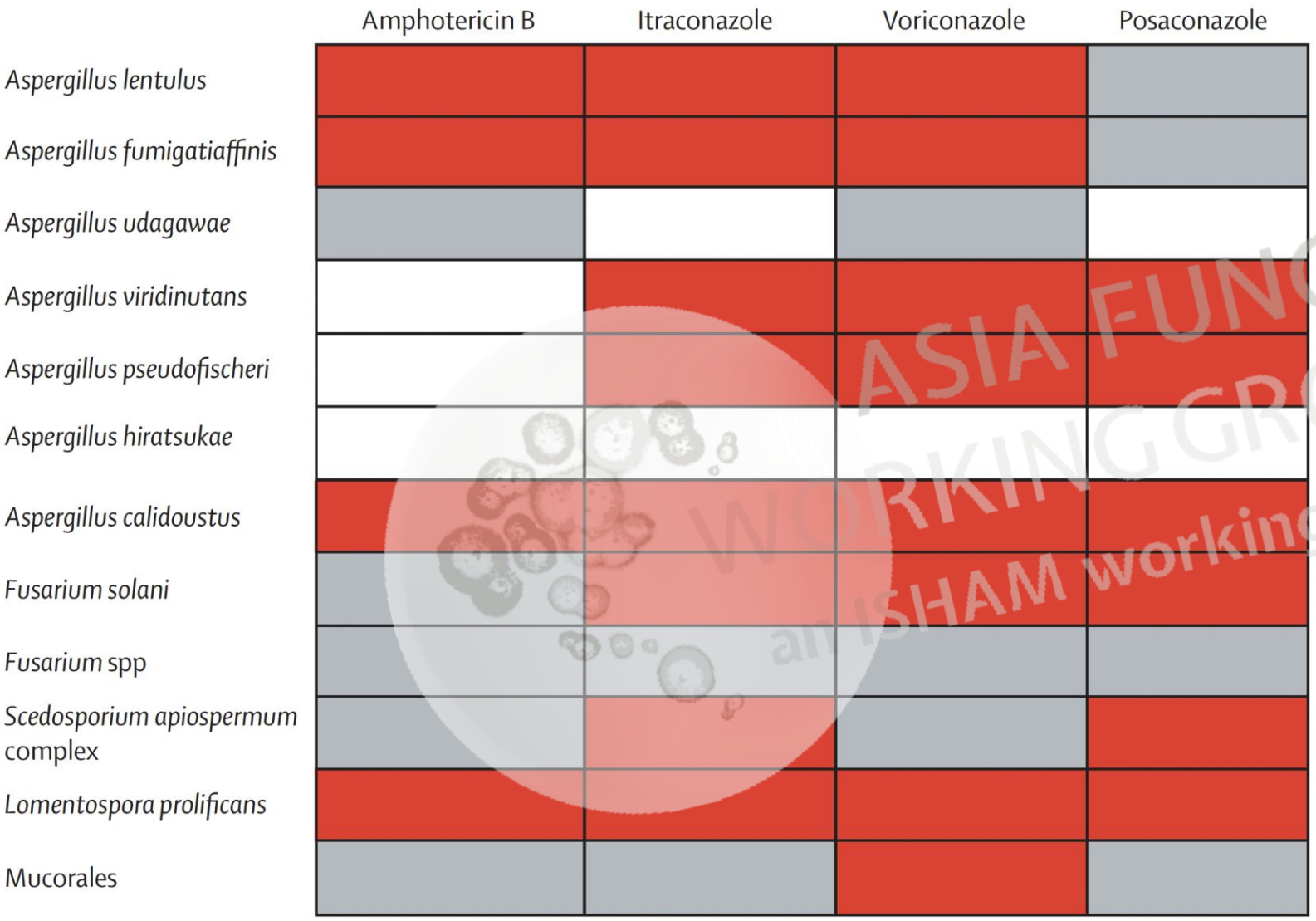
Bongomin F, *et al.* J Fungi 2017; 3: 57

Invasive aspergillosis - magnitude



- ***A. fumigatus*** more common in temperate climate; ***A. flavus*** in tropical climate
- ***A. flavus* in Vietnam: 85% resistant to azole, 50% to itraconazole** (Tran-Dinh N, *et al.* Mycopathologia 2009; 168: 257-68; Dwong TMN, *et al.* J Fungi 2020; 6: 296)

Cryptic species of *Aspergillus*



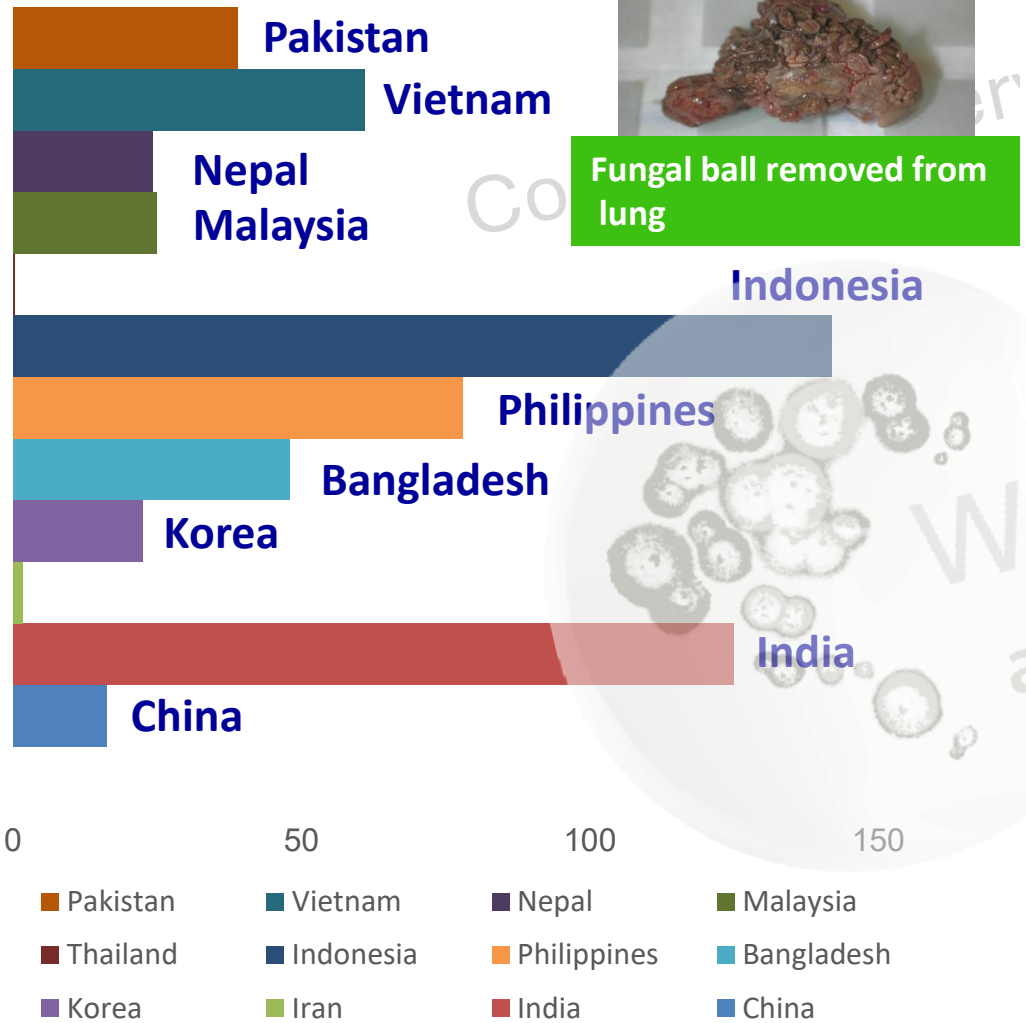
- **Change due to expanding use of *Aspergillus*-active antifungal in prophylaxis, empirical & targeted therapy**
- **Emergence of resistant cryptic *Aspergillus* spp (*A. lentulus, A. ellipticus, A. alliaceus, A. nominus, A. tubingensis, A. montevidensis*) in ~10%**
- Majority had immunosuppression & ICU admission
- Mortality – 40%

Perlin DS, *et al.* Lancet Infect Dis 2017; 17: e383-92

Lass-Florl & Cuenca-Estrella. J Antimicrob Chemother 2017; 72 (Suppl 1): i5-i11; Fernandez-Pittol M, *et al*. Rev Iberoam Micol 2022; 39: 44-49

Chronic pulmonary aspergillosis

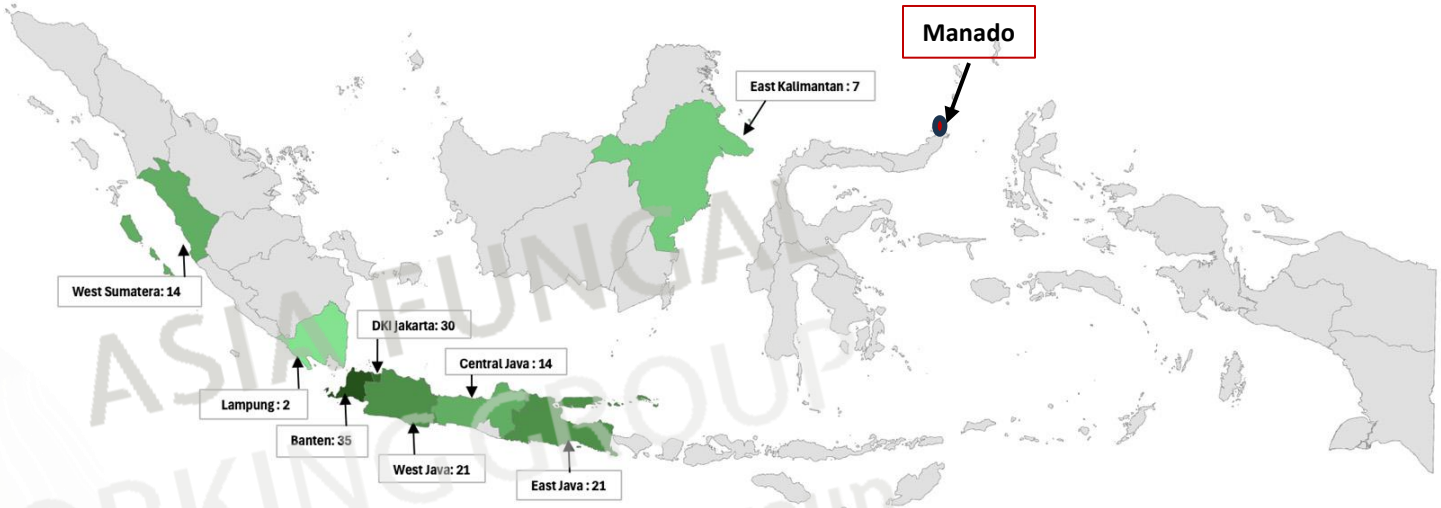
Number/100,000 population



Fungal ball removed from lung

https://gaffi.org/why/burden-of-disease-maps/

Geographic distribution of CPA in Indonesia



Rozaliyani A, *et al*. J Fungi 2025; 11: 329

MDR-TB in Indonesia

- 28% *Histoplasma* & 32% *Aspergillus* serology; 12% dual antibody reactivity
- *Histoplasma* – damp housing, *Aspergillus*- pet

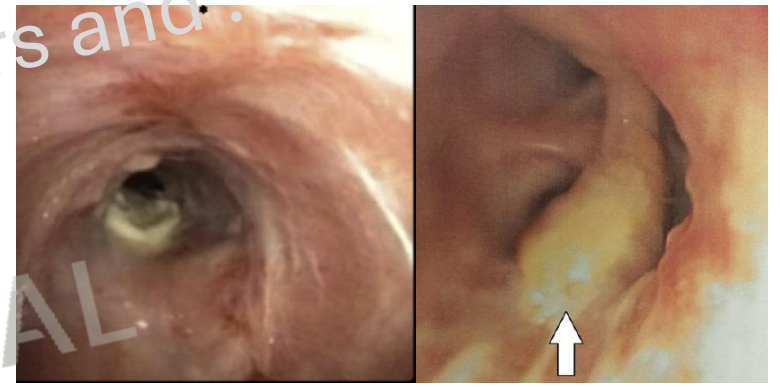
Soeroso NN, *et al*. J Fungi 2024; 10: 529

Barac A, *et al*. Med Mycol 2019; 57: S104

Clin Infect Dis. 2020 Jan 15; 70(2): 349–350.
Published online 2019 May 11. doi: 10.1093/cid/ciz391
PMCID: PMC6938972
PMID: 31077266

Influenza Coinfection: Be(a)ware of Invasive Aspergillosis

Paul E Verweij,[1,2] Roger J M Brüggemann,[2,3] Joost Wauters,[4] Bart J A Rijnders,[5] Tom Chiller,[6] and Frank L van de Veerdonk[2,7]

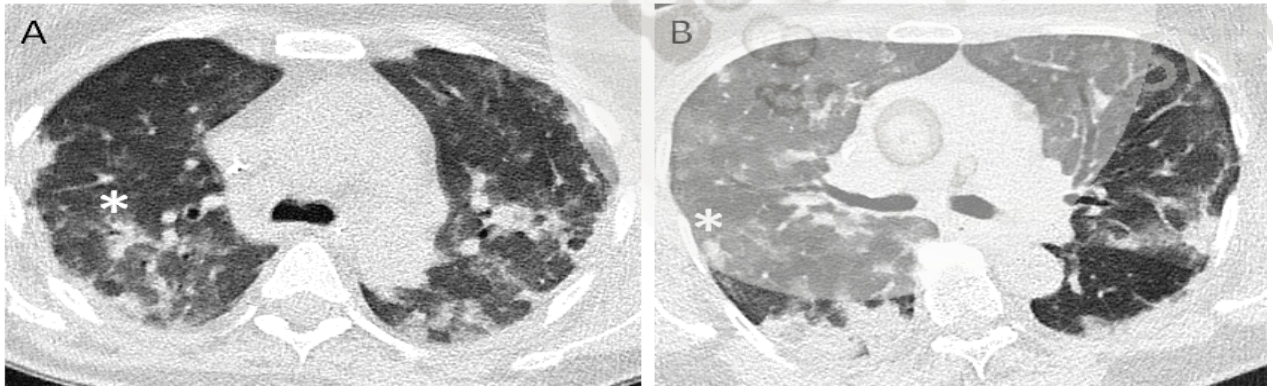


- 3 cohort studies performed in Belgium & the Netherlands - influenza-associated aspergillosis (IAA) in **16%–23% of influenza** patients in the intensive care unit (ICU)
- IAA cases have been reported in **at least 16 countries**
- **Mortality of influenza patients with IAA was 51%** compared with 28% in those without IAA
- **Influenza virus causes ulceration of the tracheobronchial epithelium**, thus providing an opportunity for *Aspergillus* to cause invasive infection; steroid plays additional role
- Clinical presentation includes **tracheobronchitis** & other forms of pulmonary aspergillosis

Verweij PE, *et al*. Intensive Care Med 2020; 46: 1524-1535

Influenza/COVID-19 associated pulmonary aspergillosis

Factor	IAPA	CAPA
Incidence	**10% of ICU patients**	**6.9% of ICU, 10.3% mechanically ventilated**
Tracheobronchitis	**Up to 55% patients**	**Very few cases**
Aspergillus diagnostic	**BAL GM positive in > 88%**	**BAL GM commonly positive**
	Serum GM positive in 65%	**Serum GM positive in 21%**
Diagnostic algorithm	Consensus algorithm	4 algorithm with controversies



Confirmed aspergillosis **Without fungal infections**

28 experts group recommends

- **Bronchoscopy & BAL GM**
- CT, serum GM/BDG, sputum & tracheal aspirate do not help in diagnosis

Verweij PE, *et al*. Intensive Care Med 2021; 47: 819-834

Fungal rhinosinusitis

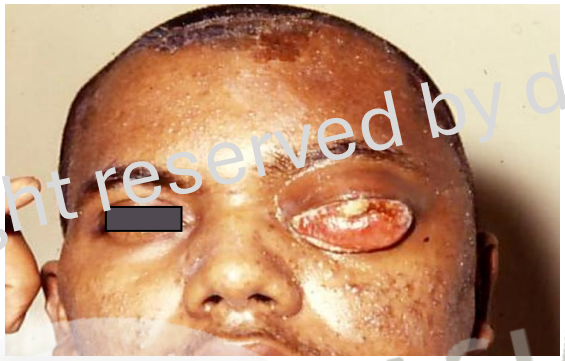
India

The Laryngoscope
© 2009 The American Laryngological, Rhinological and Otological Society, Inc.

Contemporary Review

Fungal Rhinosinusitis: A Categorization and Definitional Schema Addressing Current Controversies

Arunaloke Chakrabarti, MD; David W. Denning, FRCP, FRCPath; B. J. Ferguson, MD; Jens Ponikau, MD; Walter Buzina, MD; Hirohito Kita, MD; Bradley Marple, MD; Naresh Panda, MS; Stephan Vlaminck, MD; Catherine Kauffmann-Lacroix; Ashim Das, MD; Paramjeet Singh, MD; Saad J. Taj-Aldeen, PhD; A. Serda Kantarcioglu, PhD; K. K. Handa, MS; Ashok Gupta, MS; M. Thungapatra, PhD; M. R. Shivaprakash, MD; Amanjit Kaur, MD; Annette Fothergill; B. D. Radotra, MD



- Study in north India – 1.4% adult suffer from CRS,
- 8.1% of them are FRS (0.11% of population)
- *A. flavus* in 97.6% cases

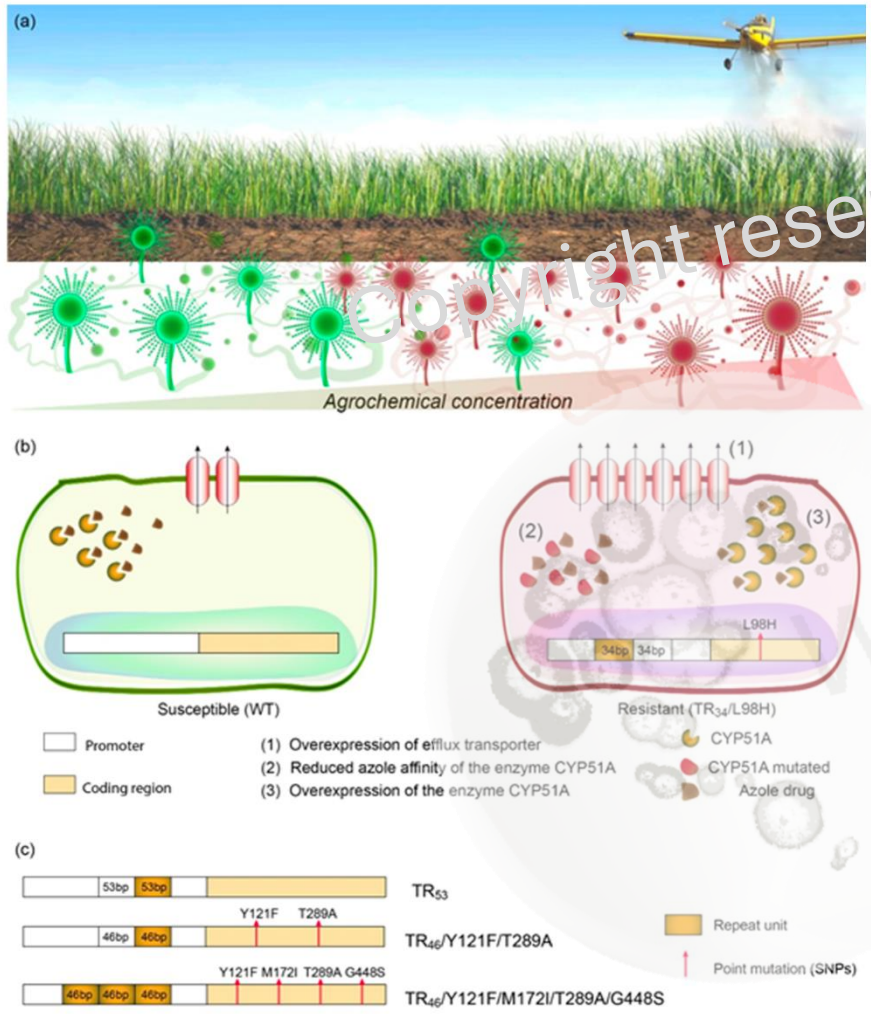
Chakrabarti A, *et al.* Mycoses 2015; 58: 294

Indonesia

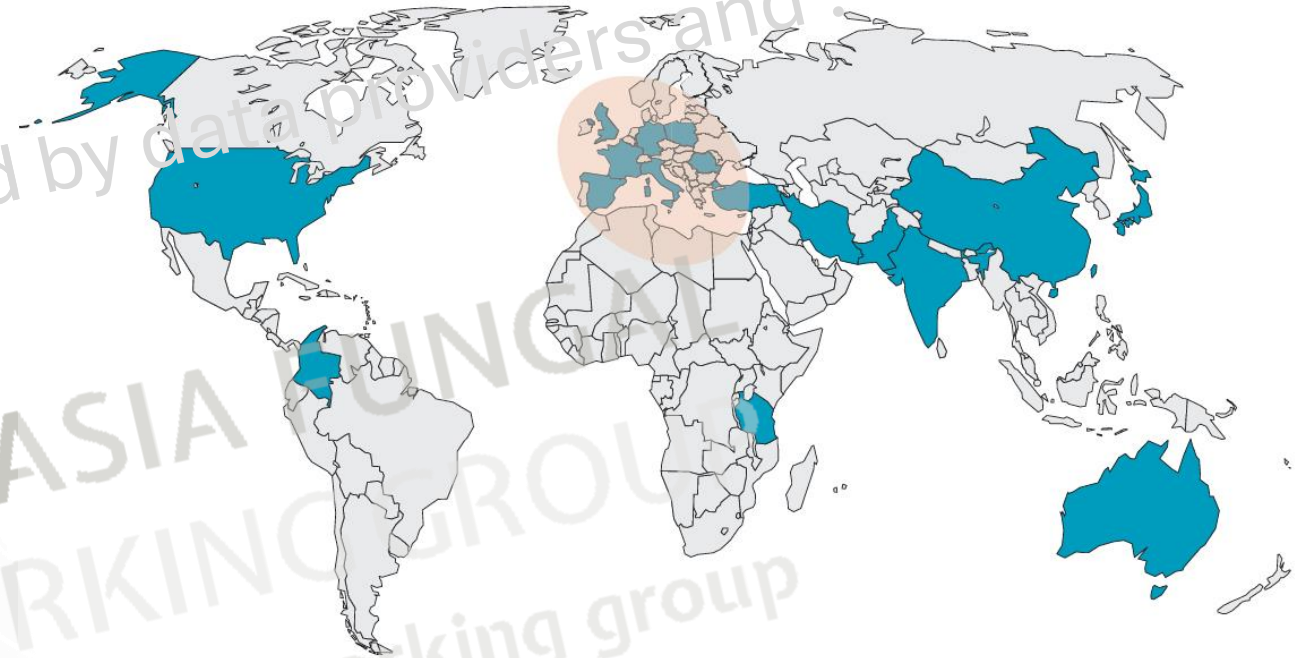
Infection	Incidene or prevalence	None	Rate/100K	Total burden
Oesophageal candidiasis	I	-	10	28,560
Cryptococcal meningitis	I	340	8.7	8,670
Pneumocystis pneumonia	I	-	11.5	30,800
Histoplasmosis	I	?	0.4	1,060
Talaromyces marneffei infection	I	-	0.4	210
Invasive aspergillosis	I	-	18.6	49,500
Mucormycosis	I	-	0.20	530
Chronic pulmonary aspergillosis	P	-	142	378,700
Allergic bronchopulmonary aspergillosis (ABPA)	P	-	126	336,200
Severe asthma with fungal sensitisation (SAFS)	P	-	166	443,800
Chronic fungal rhinosinutis	P	294,000	110	294,000

Wahyuningsih R, *et al*. Mycoses 2021; 64: 1203-1212

Aspergillus azole resistance



Countries reporting resistance



- **Increasingly recognized** : clinical, environmental isolates
- **In European countries - 0.6% to 30%, having reached the highest rate (>20%) in the Netherlands, UK, & Germany**
- **China (5.5%), India (1.7%), Iran (3.5%), Japan (12.7%), Thailand (3.2%), Australia (2.6%), and the United States (0.6% to 11.8%)**

Perlin DS, *et al.* Lancet Infect Dis 2017; 17: e383-92

Bastos RW, *et al.* PLoS Pathogen 2021; 17: e1010073; Friedman & Schwartz. J Fungi 2019; 5: 67; Verweij PE, *et al.* Clin Infect Dis 2016; 62: 362-368

J. Fungi **2020**, *6*, 296; doi:10.3390/jof6040296

Drug-Resistant *Aspergillus flavus* Is Highly Prevalent in the Environment of Vietnam: A New Challenge for the Management of Aspergillosis?

Tra My N. Duong [1,2], Phuong Tuyen Nguyen [2], Thanh Van Le [2], Huong Lan P. Nguyen [3], Bich Ngoc T. Nguyen [4,5], Bich Phuong T. Nguyen [6], Thu Anh Nguyen [1,6], Sharon C.-A. Chen [1,7], Vanessa R. Barrs [1,8], Catriona L. Halliday [1,7], Tania C. Sorrell [1,9], Jeremy N. Day [2,10] and Justin Beardsley [1,2,3,*]

Resistant/Non-WT Pattern	ITC	POS	VRC	AmB
Resistant/non-WT (n/N)	17/35	27/35	6/35	9/35
Resistant/non-WT % (95% CI)	48.6% (31.4–66%)	77.1% (59.9–89.6%)	17.1% (6.6–33.7%)	25.7% (12.5–43.3%)

WT = wild-type; ITC = itraconazole; POS = posaconazole; VRC = voriconazole; AmB = amphotericin B; CI = confidence interval.

Country	ITC		POS		VRC		AmB	
	MIC Range (mg/L)	MIC GM (mg/L)	MIC Range (mg/L)	MIC GM (mg/L)	MIC Range (mg/L)	MIC GM (mg/L)	MIC Range (mg/L)	MIC GM (mg/L)
Vietnam	**1–8**	**1.52**	**0.5–2**	**0.91**	**1–4**	**2.16**	**2– >16**	**4**
Brazil	0.5–8	1.41	0.03–0.25	0.188	0.5–2	1.017	-	-
Iran	0.031–2	0.25	0.03–0.5	0.13	0.063–2	0.55	1–16	3.4
India	0.03–0.125	0.06	0.015–0.06	0.022	0.15–1	0.5	-	-
Europe *	0.03–0.25	-	0.06–0.125	-	0.125–0.25	-	-	-

A systemic review 3633 *A. flavus* isolates- 538 (14.3%) resistance to amphotericin B

Fakhim H, *et al.* J Med Mycol 2022; 32: 101310

We tested 208 *A. flavus* isolates

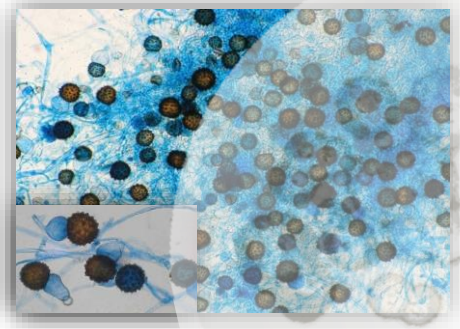
Antifungals		Mean (µg/mL)	Range (µg/mL)
Amphotericin B	EUCAST	4.45	1–16
	CLSI	0.64	0.25–2
Itraconazole	EUCAST	0.40	0.25–1
	CLSI	0.19	0.062–0.5
Voriconazole	EUCAST	1.45	0.25–4
	CLSI	1.26	0.5–4
Posaconazole	EUCAST	0.27	0.125–0.50
	CLSI	0.13	0.062–0.25
Isavuconazole	EUCAST	1.36	0.25–4
	CLSI	0.75	0.125–2
Caspofungin	EUCAST	0.49	0.25–1
	CLSI	0.51	0.25–1
Anidulafungin	EUCAST	0.01	0.008–0.016
	CLSI	0.01	0.008–0.016
Micafungin	EUCAST	0.08	0.008–0.25
	CLSI	0.03	0.008–0.125

Rudramurthy SM, et al Diagn Microbiol Infect Dis 2011; 71: 370-377

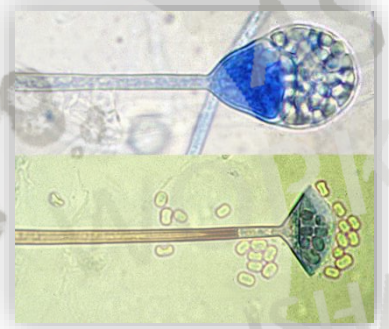
Mucormycosis in Asia - challenges

- **Incidence & mortality – very high** in China, India, Iran, and Pakistan; **70 times higher rate in India** compared to western countries; **Mortality ~50%, high even in rhino-cerebral type**
- **Uncontrolled diabetes** overshadows all other risk factors; **>60% world diabetics reside here**
- **Mucormycosis in immunocompetent hosts** –**cutaneous & renal mucormycosis** prevalent in China & India
- **Many new Mucorales** have emerged causing infections

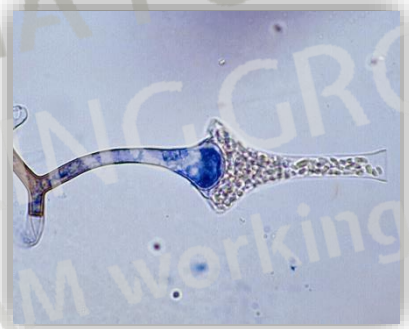
Mucor irregularis



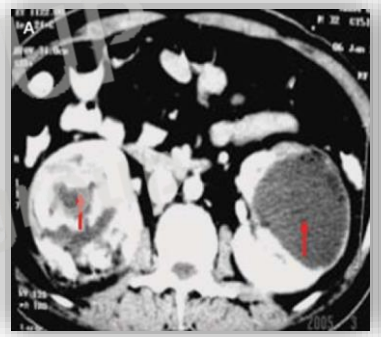
Rhizopus homothallicus



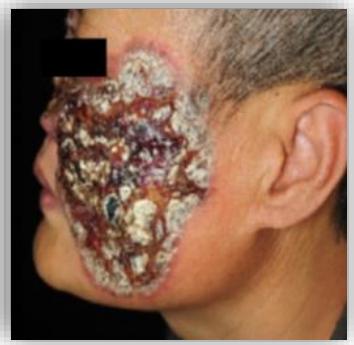
Apophysomyces variabilis



Saksenaea vasiformis



Renal mucor



Chronic cutaneous

- **Outbreak - CAM was declared as notifiable disease in India - 47,508 reported cases within 45 days**
- **'likely that the actual figures are considerably higher than this'**

https://governmentstats.com/mucormycosis/index.html

Prakash & Chakrabarti. J Fungi 2019; 5: 26; Prakash & Chakrabarti. Microorganisms. 2021;9:523; Chakrabarti A. Clinical Practice of Med Mycol in Asia 2020; 279-92;

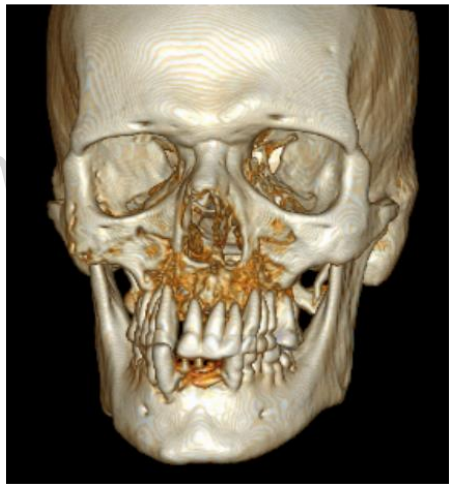
COVID-19 associated mucormycosis outbreak

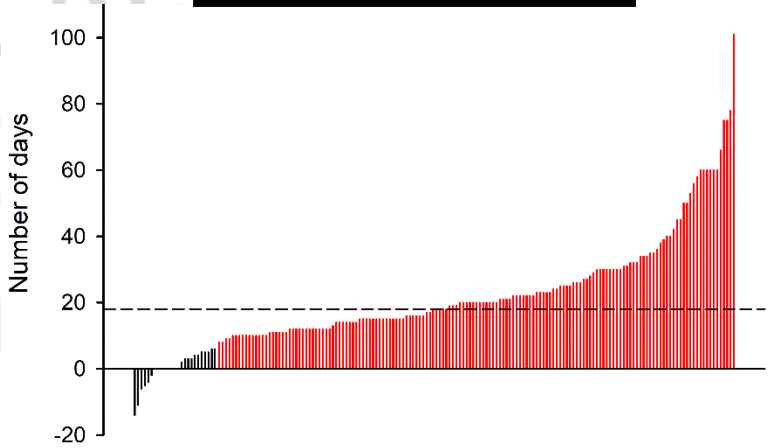
- COVID19 associated mucormycosis outbreak – ravaged Asia; >100,000 cases in India
- Presentation
  - Nasal blockade or congestion, nasal discharge, facial pain
  - Toothache, loosening of maxillary teeth, jaw involvement
- CAM prevalence – 0.27% in COVID 19; 1.6% treated in ICU
- Cases were diagnosed median 18 days after COVID diagnosis
- 86.1% naso-orbital mucormycosis, 23.5% brain involvement
- Diabetes – 62.7%; Steroid – 78.1%
- Mortality – 6 weeks (38.3%), 12 weeks (45.7%)

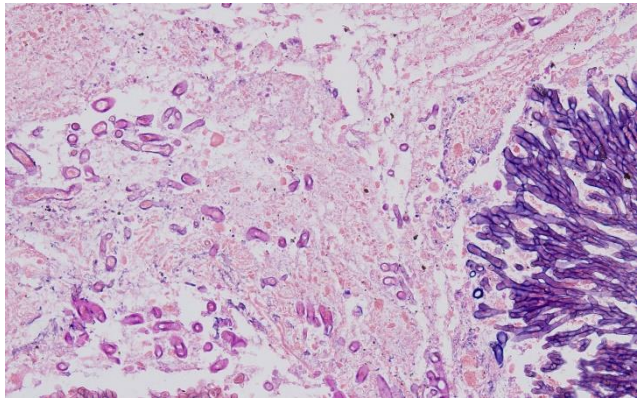
Patel A,…Chakrabarti A. Emerg Infect Dis 2021; 27: 2349-2359

- The prevalence of mixed mould infection was 20%

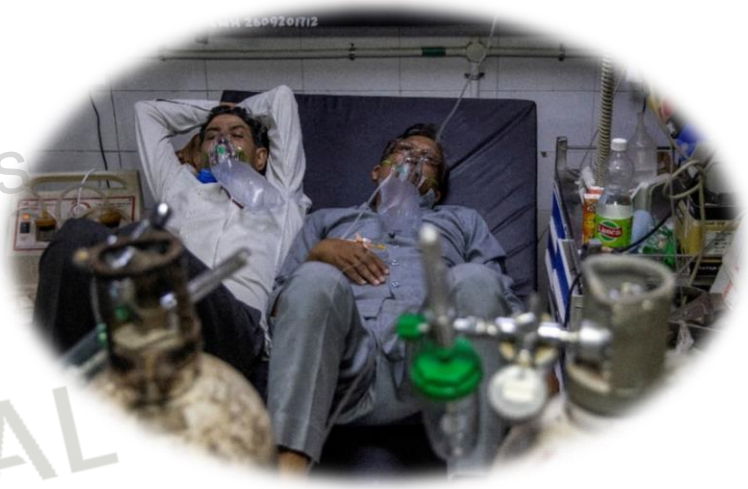
Muthu et al. Mycoses. 2024; 67: e13745







Second wave



Steroid

Patient factor (Diabetes)

Mucormycosis

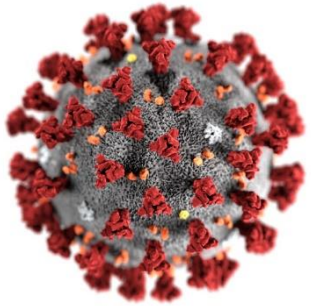
Covid causing problem

Environment

Possible reasons for CAM outbreak

Host

- Uncontrolled & poorly controlled diabetes
- Indiscriminate steroid use



COVID-19

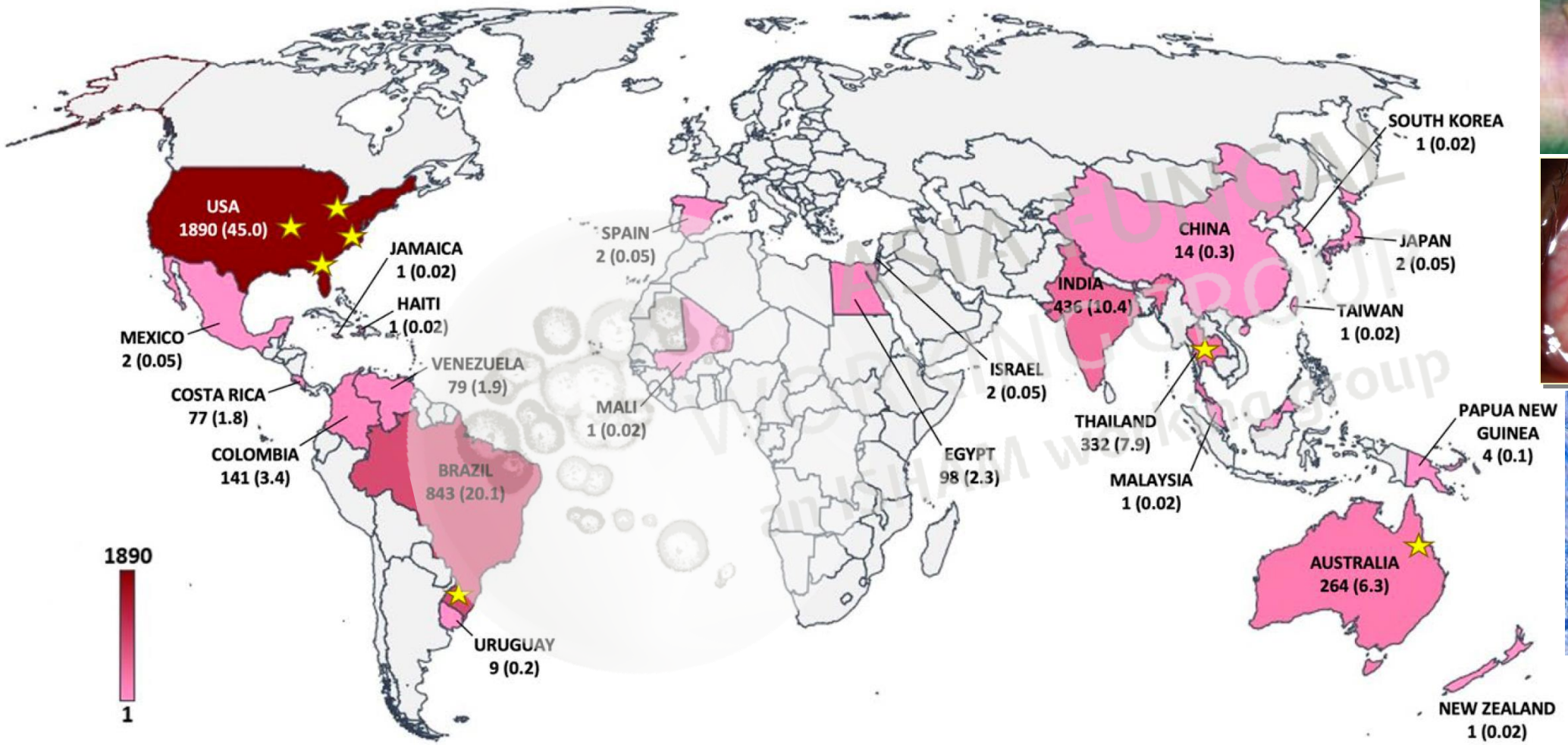
Fungus

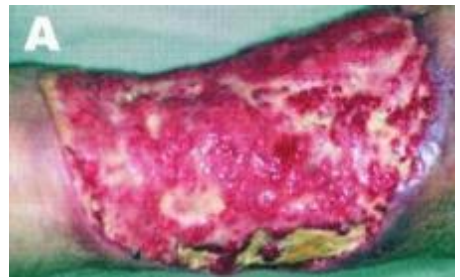
Environment

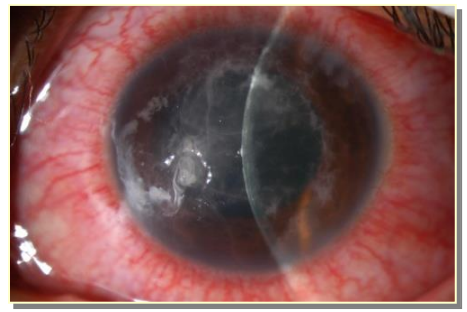
- Virus damages beta-cell pancreas
- Causes enrichment scores for complement & coagulation pathway

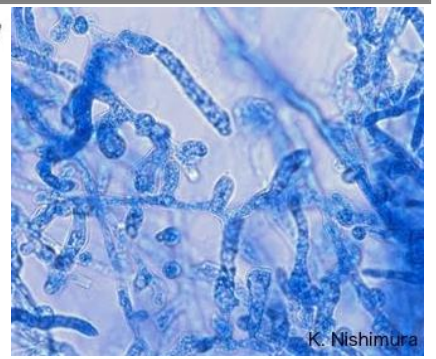
- High Mucorales spore in hospital and home environment

Distribution of pythiosis cases (4203 cases during 1980-2021)



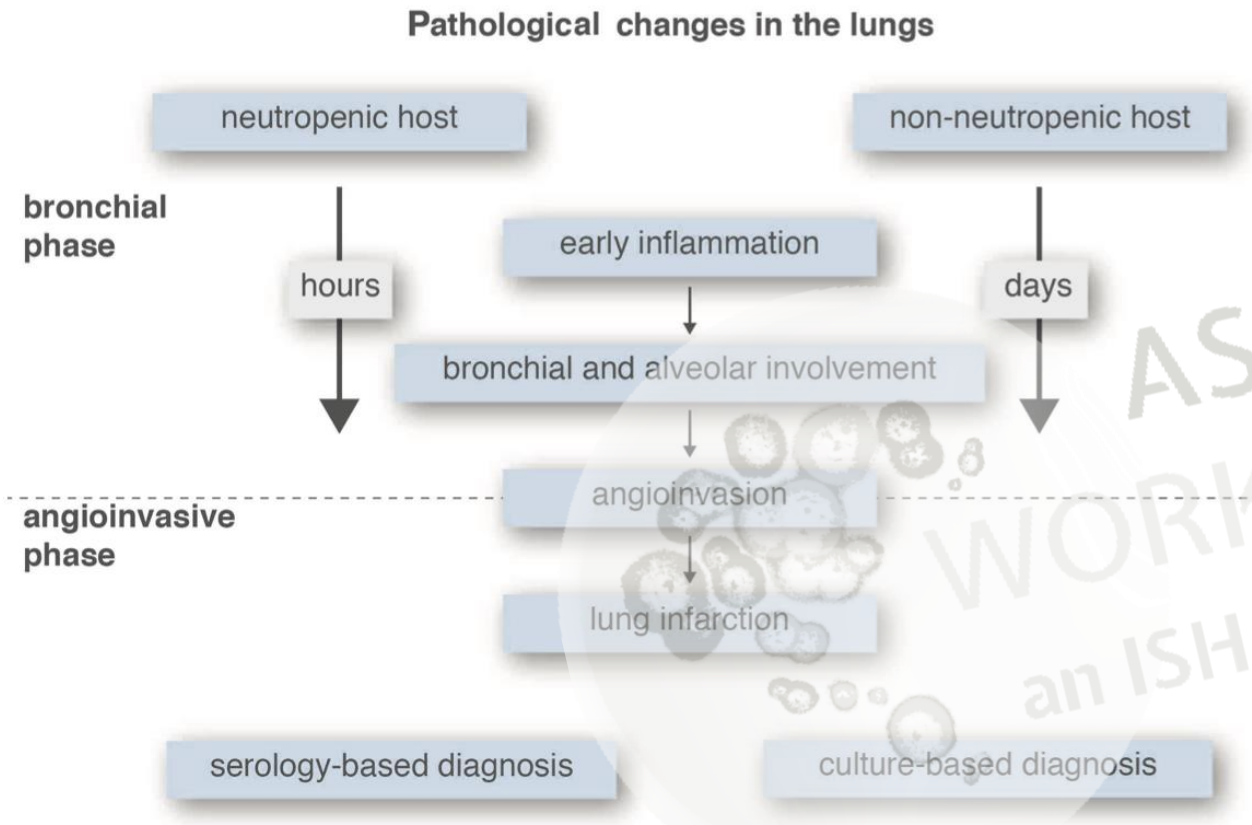




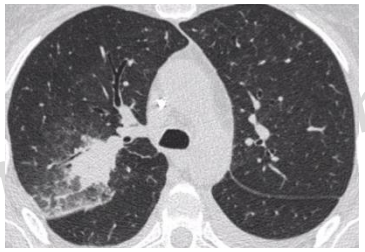


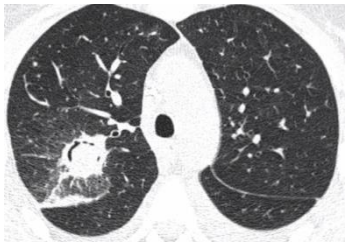
Yolanda & Krajaejun. J Fungi 2022; 8: 182

Can Imaging help?



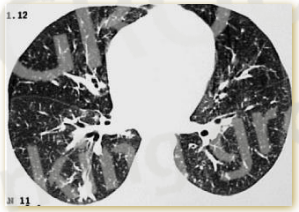
Neutropenic host: primarily angio-invasive

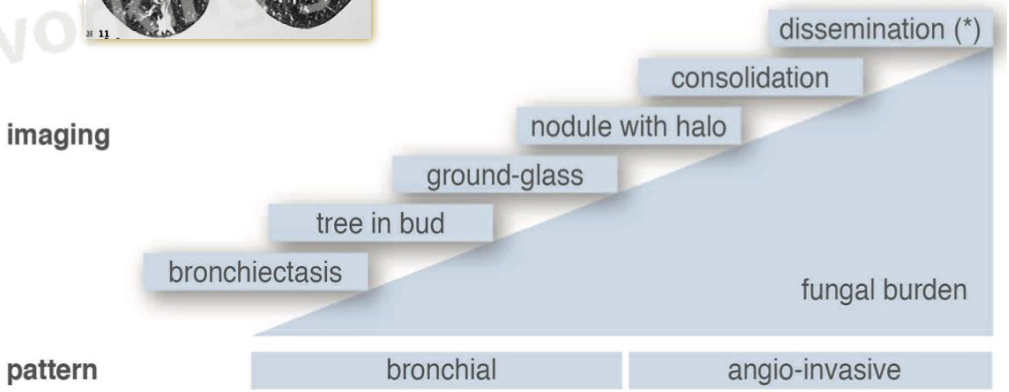




Non-neutropenic host: primarily airway invasive

- Radiological findings are often non-specific

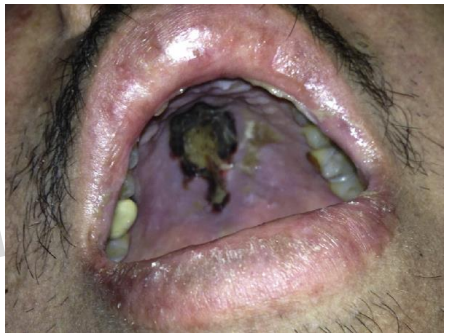




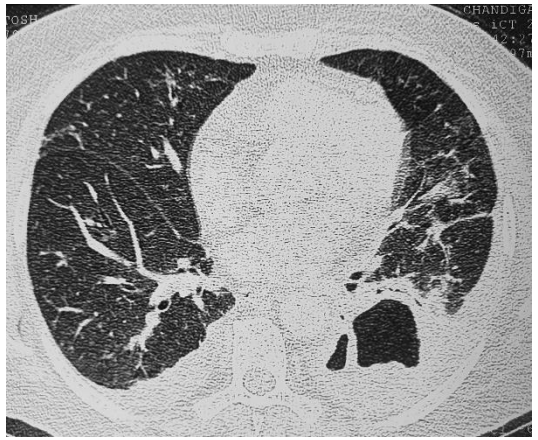
Panackal AA, *et al.* Curr Treat Options Infect Dis 2014; 6: 309-325; Nucci m, *et al.* Haematologica 2013; 98: 1657-1660

You may think of mucormycosis

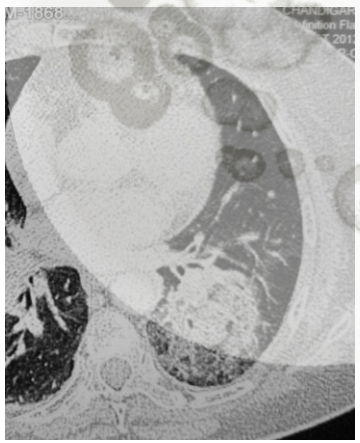
Farmakiotis & Kontoyiannis. Infect Dis Clin N Am 2016; 30: 143



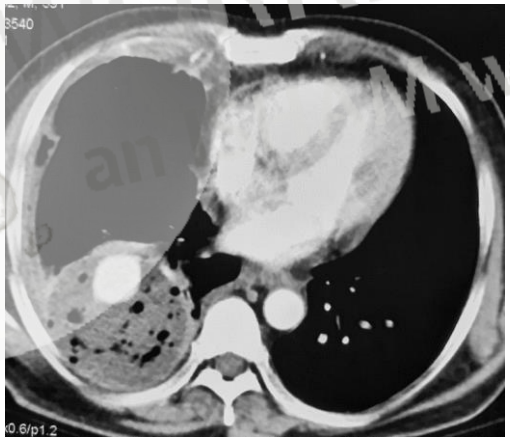
- **Presence of severe sinusitis, eschar**
- **Fever, worsening of cough, brown/black sputum, chest pain, hemoptysis**
- **Patient is on voriconazole or echinocandins**
- **Acute & aggressive vascular event**
- **Repeated absence of galactomannan & beta - glucan**



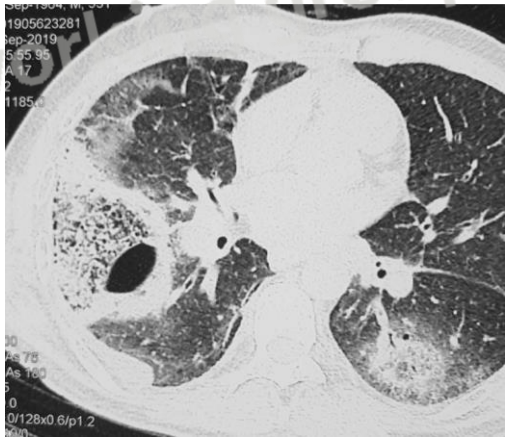
Cavity



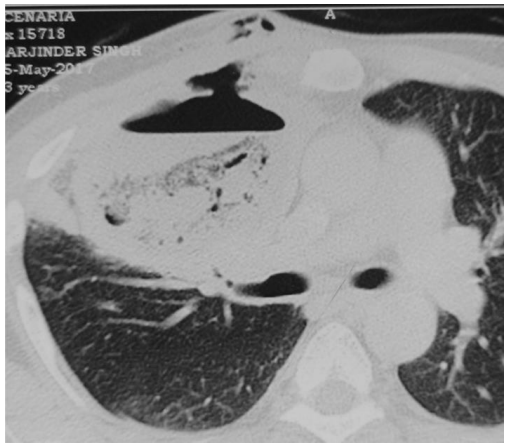
Consolidation



Mycotic aneurysm



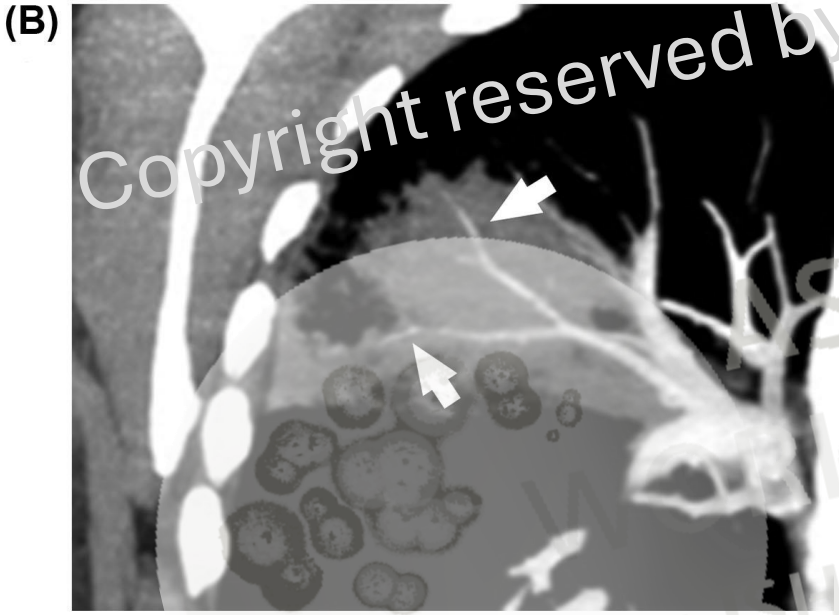
Bird's nest sign



Cavitating mass

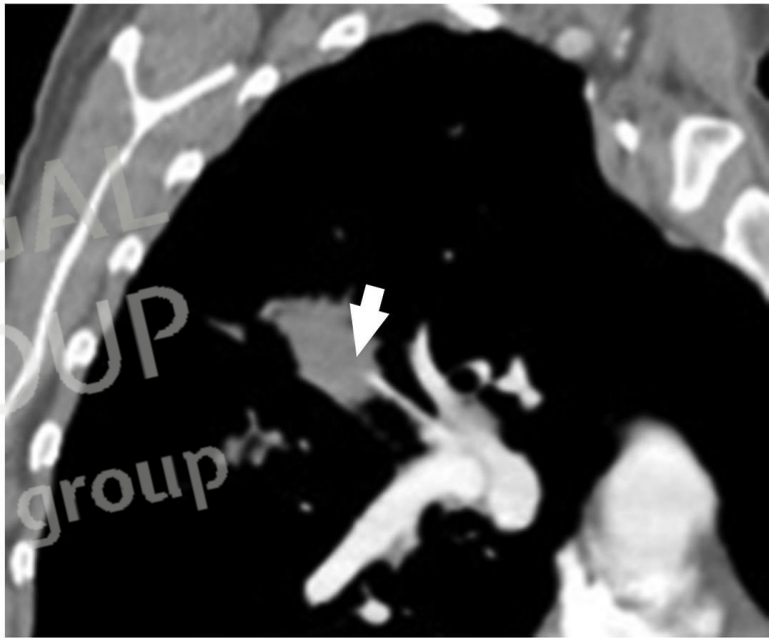
Vessel occlusion sign in CT pulmonary angiography

Intact arms of the Venus di Medici, Uffizi Museum, Florence, Italy



A negative vessel occlusion sign in a neutropenic patient with *P. aeruginosa* pneumonia

Broken arms of the Venus de Milo, Louvre Museum, Paris;



A positive vessel occlusion sign in a neutropenic patient with invasive aspergillosis

Stanzani M, *et al*. Med Mycol 2019; 57: S274-S286

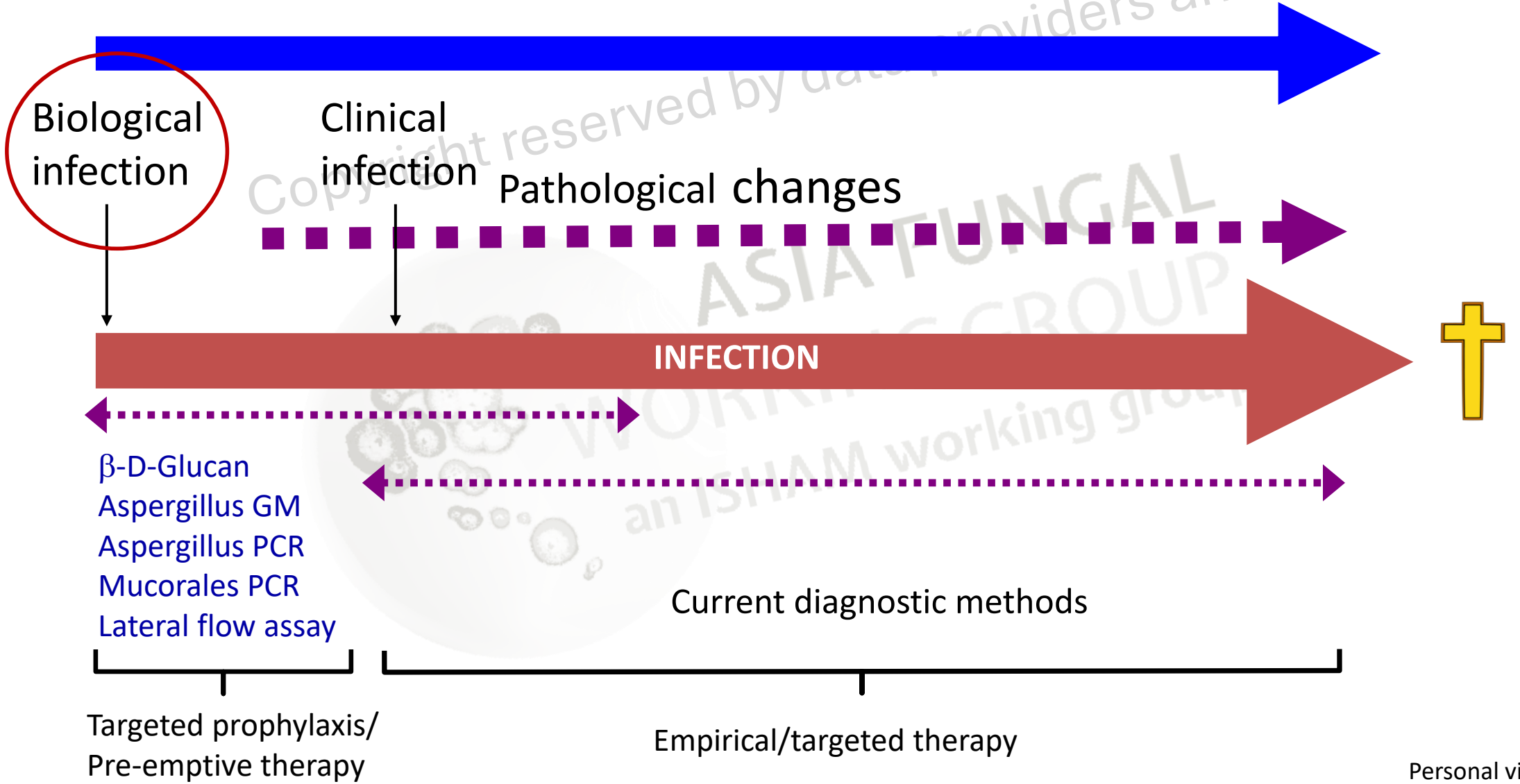
Flexible bronchoscopy may improve diagnosis

In a study from India, FB was diagnostic in 71% cases of suspected invasive mould disease (n=107)

- Bronchoscopic abnormalities more frequent in those with confirmed pulmonary mucormycosis (67%) than invasive pulmonary aspergillosis (27%)
- Endobronchial nodule or growth in 22% of pulmonary mucormycosis & 5% of pulmonary aspergillosis
- Adherent mucus in 33% of the 27 confirmed pulmonary mucormycosis

Muthu et al. Mycoses. 2021; 64: 668-677

What is desirable for diagnosis of Invasive mould infection?



Personal view

Advanced diagnostic techniques improve sensitivity and TAT[1,2]

Next-generation sequencing
- Metagenomics
- Cell-free DNA

Molecular
- PCR – specific, panfungal
- *Aspergillus, Mucor*, others
- PCR + galactomannan

Antigen detection
- Galactomannan
- BDG
- LFA/LFD

	Specific PCR[3,4]	Panfungal PCR[3,4]
Advantages	• High sensitivity • Rapid turnaround • Helps in early diagnosis • qPCR helps in burden quantification	• Can detect most pathogens • No need of clinical suspicion • Medium sensitivity
Disadvantages	• Difficult to distinguish colonisation versus infection • Need of clinical suspicion	• Less helpful for samples from non-sterile sites (commensals) • Require second step for species

Lateral flow assay for aspergillosis[5]

- Simple, rapid (30 min) digital read-out could improve data
- Mixed population: BAL sensitivity (77%) and specificity (81%), blood sensitivity (70%) and specificity (96%)
- Culture-positive samples: BAL LFA sensitivity (92%) and specificity (91%)

BAL, bronchoalveolar lavage; BDG, β-d-Glucan; LFA, lateral flow assay; LFD, lateral flow device; min, minute; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; TAT, turnaround time.

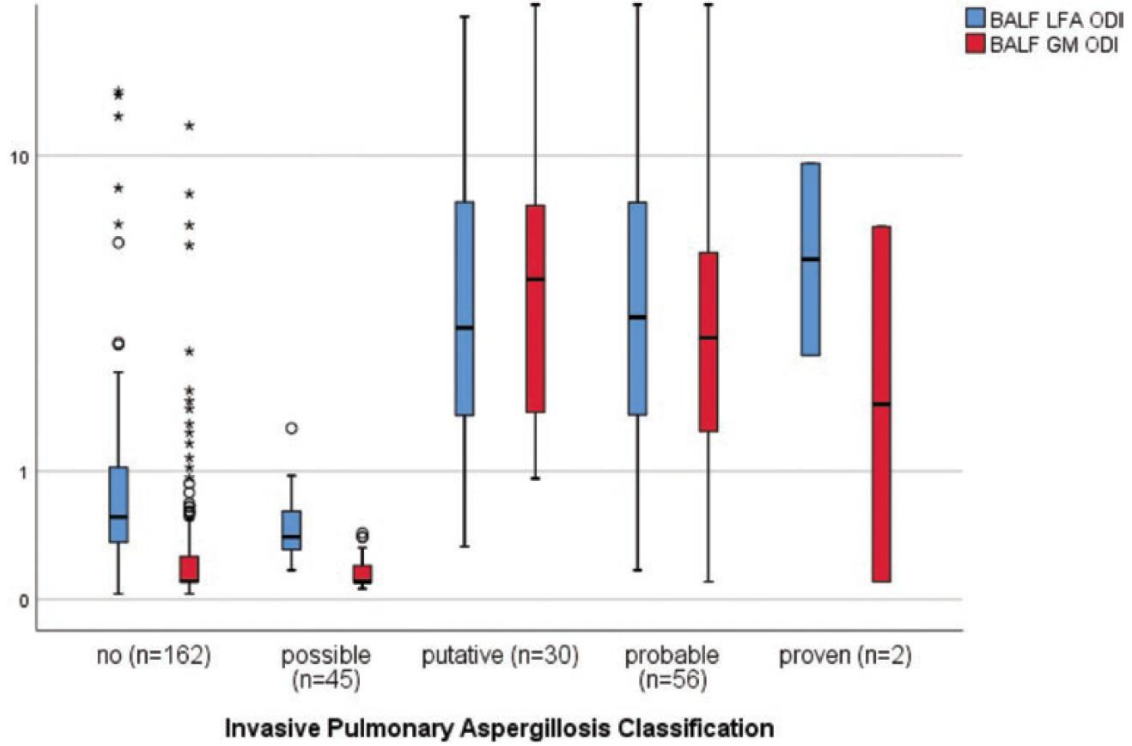
1. Yan G, et al. *Mycopathologia*. 2021;186(5):575–82; 2. Chakrabarti A, personal opinion, 2025; 3. Valero C, et al. *J Fungi (Basel)*. 2022;9(1):59; 4. Friedman DZP and Schwartz IS. *Infect Dis Clin North Am*. 2023;37(3):593–616;
5. Lass-Florl C, et al. *Clin Microbiol Infect*. 2021;27(9):1230–41.

Aspergillus lateral flow assay

- LFD, CE marked in 2017
- **Detects Mab J5 extracellular protein secreted by *Aspergillus* hyphal tip**
- Small volume of sample (75-150 µl)
- <10 min sample handling – heat & centrifuge serum & BAL
- Visual reader to avoid subjectivity
- **Result of test – after 15 minutes**
- Provides quantitative result
- **Cross reacts with *Penicillium* spp**.

- LFA, CE marked, FDA approval pending
- **Detects ME-A5 mAb against GM epitope & another mAb against undisclosed Ag**
- Small volume of sample (300 µl)
- <15 sample handling – heat & centrifuge all sample types
- Visual reader to avoid subjectivity
- **Result of test - after 30 minutes**
- Provides quantitative result
- **Cross reacts *Fusarium, Scedosporium***

BAL GM vs. BAL LFA



- **Multi-centre study**
- **Majority (65%) were ICU patients**

Jenks JD, *et al.* Clin Infect Dis 2021; 73: e1737-44

Comparison LFA & LFD

LFD for protein **LFA for GM**

Sensitivities	ICU
LFD (OLM) in BALf	+/-80%
LFA (IMMY) in BALf	60%–94%
LFA (IMMY) in serum	20%–56%

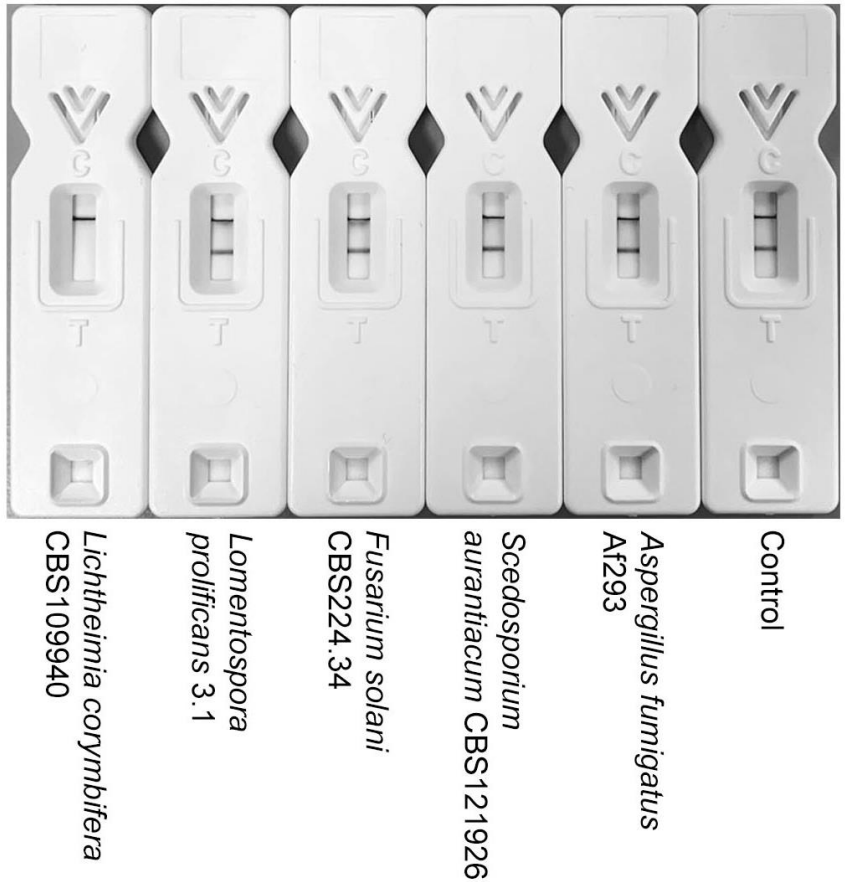
Guo J et al.. Eur J Clin Microbiol Infect Dis 2023; Mercier T, *et al.* Clin Infect Dis 2021; 72: 1577-84

PoC testing for mucormycosis

Development of a monoclonal antibody and a lateral-flow device for the rapid detection of a Mucorales-specific biomarker

Christopher R. Thornton[1,2*†], Genna E. Davies[2†] and Laura Dougherty[1]

[1]Biosciences, Faculty of Health and Life Sciences, University of Exeter, Exeter, United Kingdom, [2]ISCA Diagnostics Ltd., Hatherly Laboratories, Exeter, United Kingdom



- IgG2b murine mAb **TG11** (binding to 20-250 kDa EPS secreted during hyphal growth)
- Pan-Mucorales
- No cross-reactivity with other moulds or *Candida*
- **Competitive assay:** T-line must be negative for a positive result

Currently under evaluation in human serum and BALf samples

Thornton CR, Davies GE, Dougherty L. Front Cell Infect Microbiol 2023; 13:1305662.

PCR and molecular diagnosis – where do we stand?

Parameter	Aspergillosis	Mucormycosis	Hyalohyphomycosis	Phaeohyphomycosis	Panfungal
Commercial option	Yes	Yes	No	No	Yes
Standardisation	Extensive	In progress	No	No	Limited
Clinical validation	Blood: 79% (sen), 80% (spec) BAL: 80% (sen), 95% (spec)	Blood: >75% (sen), >85% (spec) BAL: >90% (sen), >94% (spec)	*Fusarium* Blood: 93% (sen), 100% (spec)	Little or none	Blood: 75% (sen), 70% (spec) BAL: 90% (sen), 75% (spec)
Species/genus differentiation	Variable	Variable	Target specific species	Target specific species	Assay dependent, sequencing desired
Genetic resistance marker	Limited to environmental strains	No	No	No	No

BAL, bronchoalveolar lavage; PCR, polymerase chain reaction; sen, sensitivity; spec, specificity.
White PL. *Curr Opin Infect Dis.* 2024;37(6):451–63.

Recent emphasis in diagnosis

- **Point of care test – LFA**
- **Whole genome sequencing**
- **Cell free fungal DNA in blood**

Clinical Infectious Diseases

MAJOR ARTICLE

IDSA Infectious Diseases Society of America | hivma hiv medicine association | OXFORD

Clin Infect Dis 2020; 71: 1367-1376

Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium

J. Peter Donnelly,[1] Sharon C. Chen,[2] Carol A. Kauffman,[3] William J. Steinbach,[4] John W. Baddley,[5] Paul E. Verweij,[6] Cornelius J. Clancy,[7] John R. Wingard,[8]

Proven mould infection

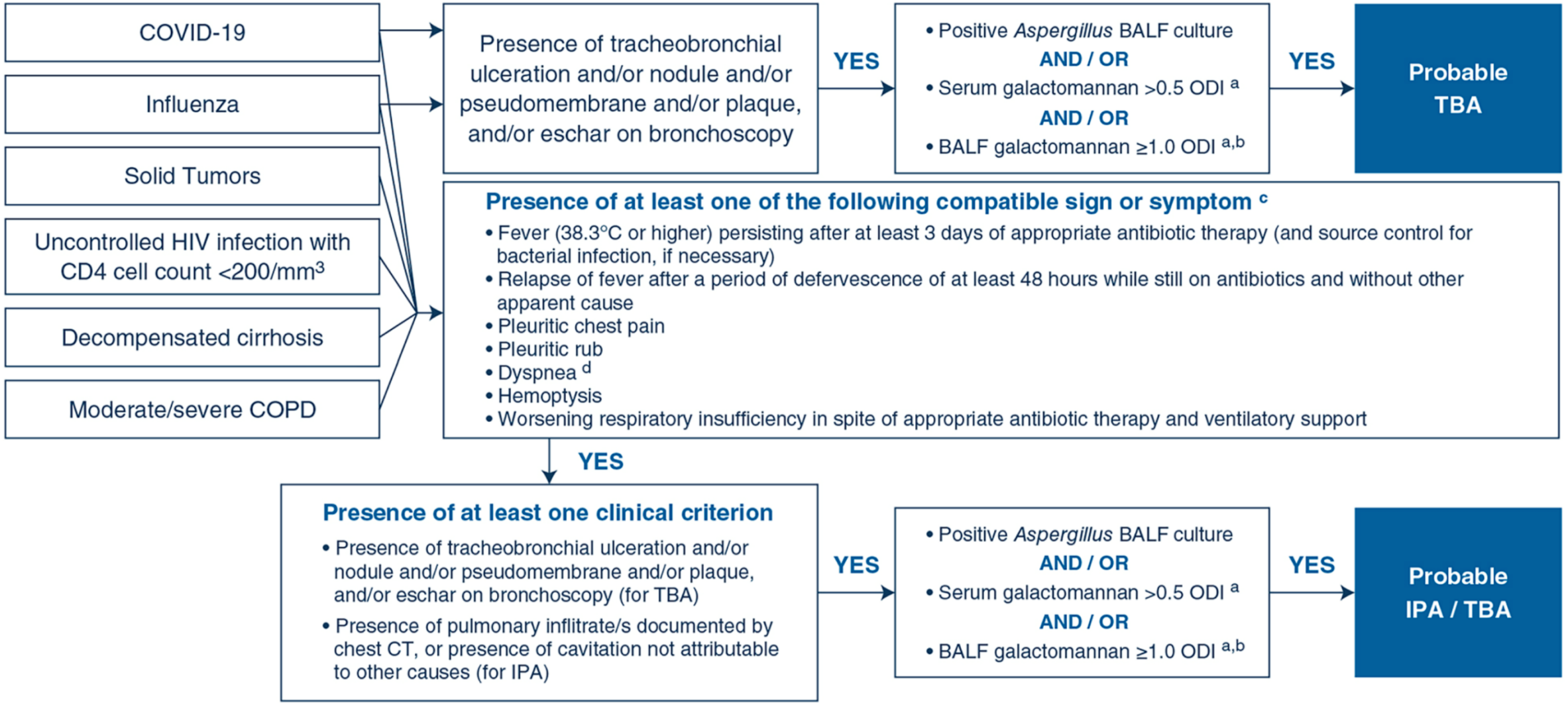
Fungus	Microscopic Analysis: Sterile Material	Culture: Sterile Material	Blood	Serology	Tissue Nucleic Acid Diagnosis
Molds[a]	Histopathologic, cytopathologic, or direct microscopic examination[b] of a specimen obtained by needle aspiration or biopsy in which hyphae or melanized yeast-like forms are seen accompanied by evidence of associated tissue damage	Recovery of a hyaline or pigmented mold by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BAL fluid, a paranasal or mastoid sinus cavity specimen, and urine	Blood culture that yields a mold[c] (eg, *Fusarium* species) in the context of a compatible infectious disease process	Not applicable	Amplification of fungal DNA by PCR combined with DNA sequencing when molds are seen in formalin-fixed paraffin-embedded tissue

Probable invasive pulmonary aspergillosis

	2008 EORTC/MSG	2020 EORTC/MSGERC
Host factors	>10 days neutropaenia Allogenic SOT >3 weeks corticosteroids T-cell immunosuppressants Inherited severe immunodeficiency (CGD, SCID)	>10 days neutropaenia Allogenic SCT, haematological malignancy (active/remission), Acute GVHD grade III/IV >3 weeks corticosteroids T-cell (including calcineurin inhibitors) or B-Cell (Bruton's tyrosine kinase inhibitors) immunosuppressants Inherited severe immunodeficiency (CGD, SCID, STAT 3)
Clinical features	*At least 1* CT sign: • Dense, well-circumscribed lesion(s) with or without a halo sign • Air-crescent sign • Cavity	*At least 1* CT sign: • Dense, well-circumscribed lesion(s) with or without a halo sign • Air-crescent sign • Cavity • Wedge-shaped and segmental or lobar consolidation

	2008 EORTC/MSG	2020 EORTC/MSGERC
Mycological evidence	Positive cytology, direct microscopy, or culture in: sputum, BAL, bronchial brush or aspirate GM antigen ≥0.5 (plasma, serum, BAL, or CSF) B-D-glucan (serum)*	Direct microscopy, or culture in: sputum, BAL, bronchial brush or aspirate GM antigen (*at least 1*): • Single serum or plasma ≥1.0 • Single BAL fluid ≥1.0 • Single serum or plasma ≥0.7 and BAL fluid ≥0.8 • Single CSF ≥1.0 Aspergillus PCR (*at least 1*): • ≥2 consecutively plasma, serum, or whole blood PCR+ • ≥2 duplicate BAL fluid PCR+ • ≥1 PCR+ in plasma, serum, or whole blood **and** PCR+ in BAL fluid

Consensus definition for IA from ESGCIP, EFISG, ESICM, ECMM, MSGERC, ISAC, ISHAM



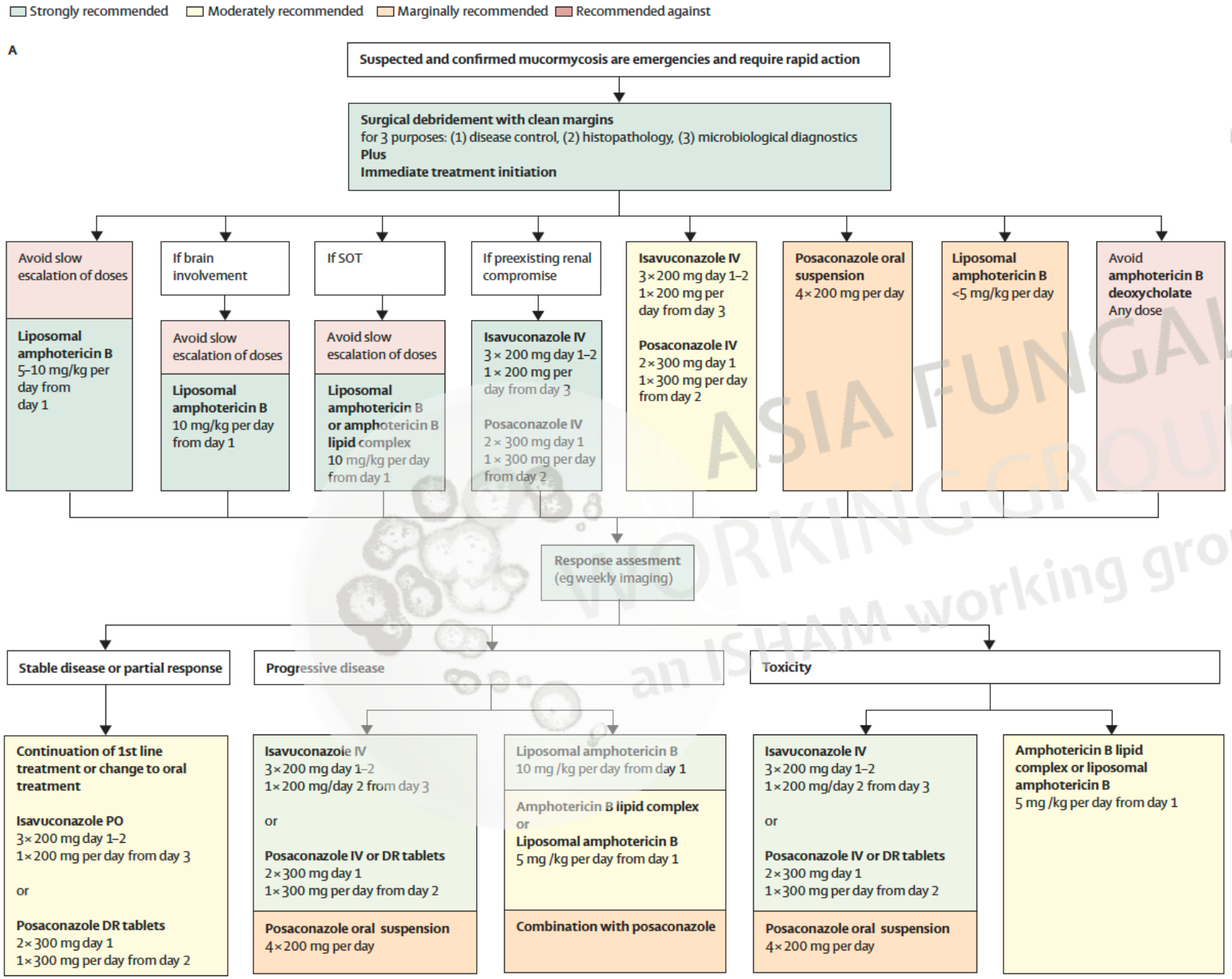
Bassetti M, *et al*. Intensive Care Med 2024; 50: 502-515

Treatment of invasive aspergillosis – requires a multi-disciplinary approach

- **Prophylaxis -** Posaconazole, voriconazole, echinocandins
- **Empiric/pre-emptive therapy -** LAMB, echinocandins or voriconazole (when mucormycosis excluded); **Isavuconazole or LAMB when mucormycosis not excluded**
- **Targeted therapy - Voriconazole or isavuconazole or posaconazole first choice;** Liposomal amphotericin B or other lipid preparations, echinocandins, itraconazole
- **Surgery –** massive haemoptysis, endocarditis, sinus disease, pericardium or great vessel involvement
- **Ulcerative trancheo-bronchitis** – neublized LAmB
- **Response monitoring** – HRCT every 7-10d
- **Failure** (↑symptoms, new lesion, no decrease, intolerance) – alternate therapy (combination)
- **Reduce immunosuppression, especially steroid during voriconazole therapy to avoid steroid myopathy** (methyl prednisolone)

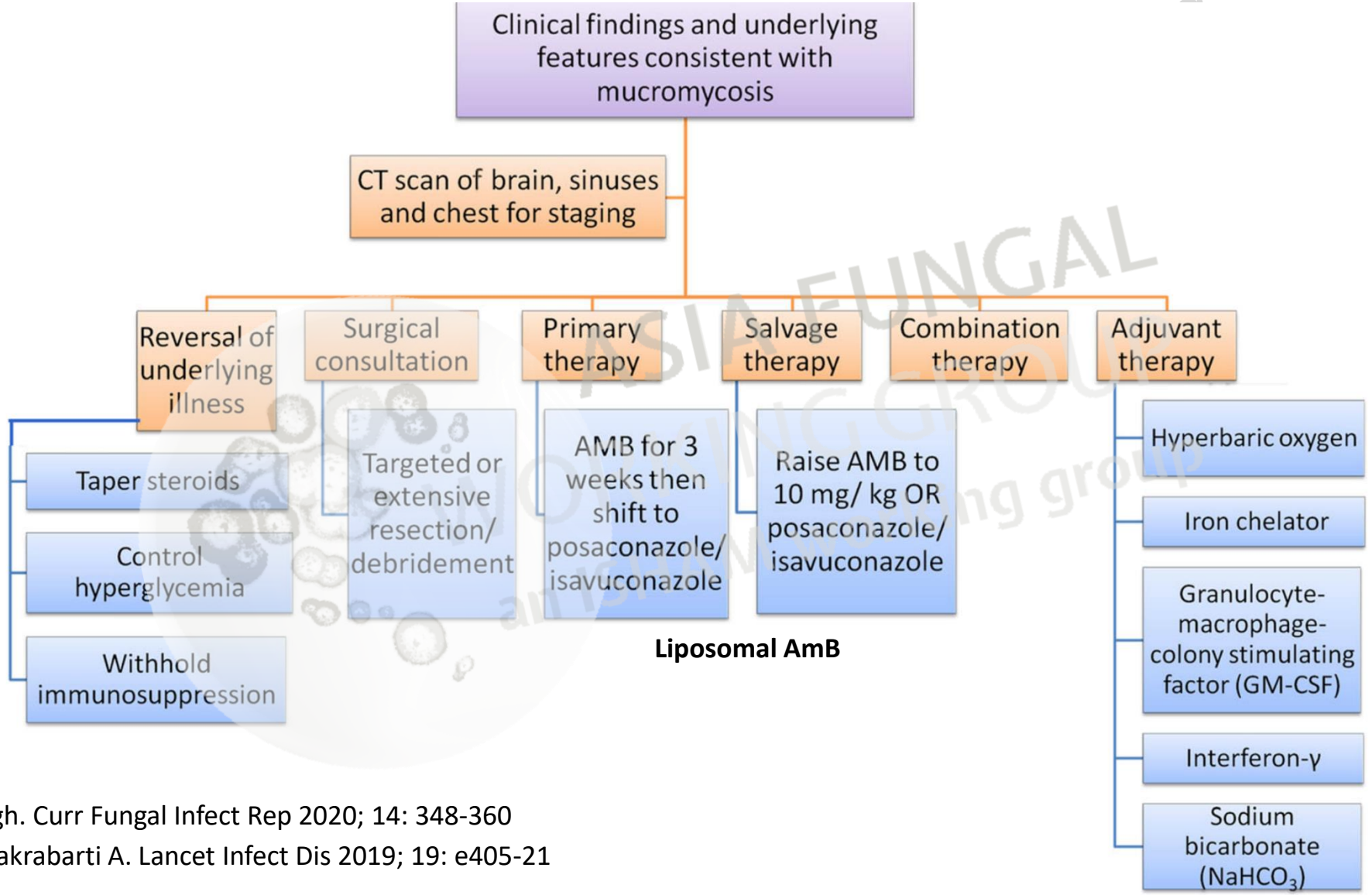
Bhattacharya PK, *et al.* Indian J Crit Care Med 2024; 28 (Suppl2): S20-S41; Patterson *et al*. Clin Infect Dis 2016; 63: 433

Global guideline -treatment of mucormycosis



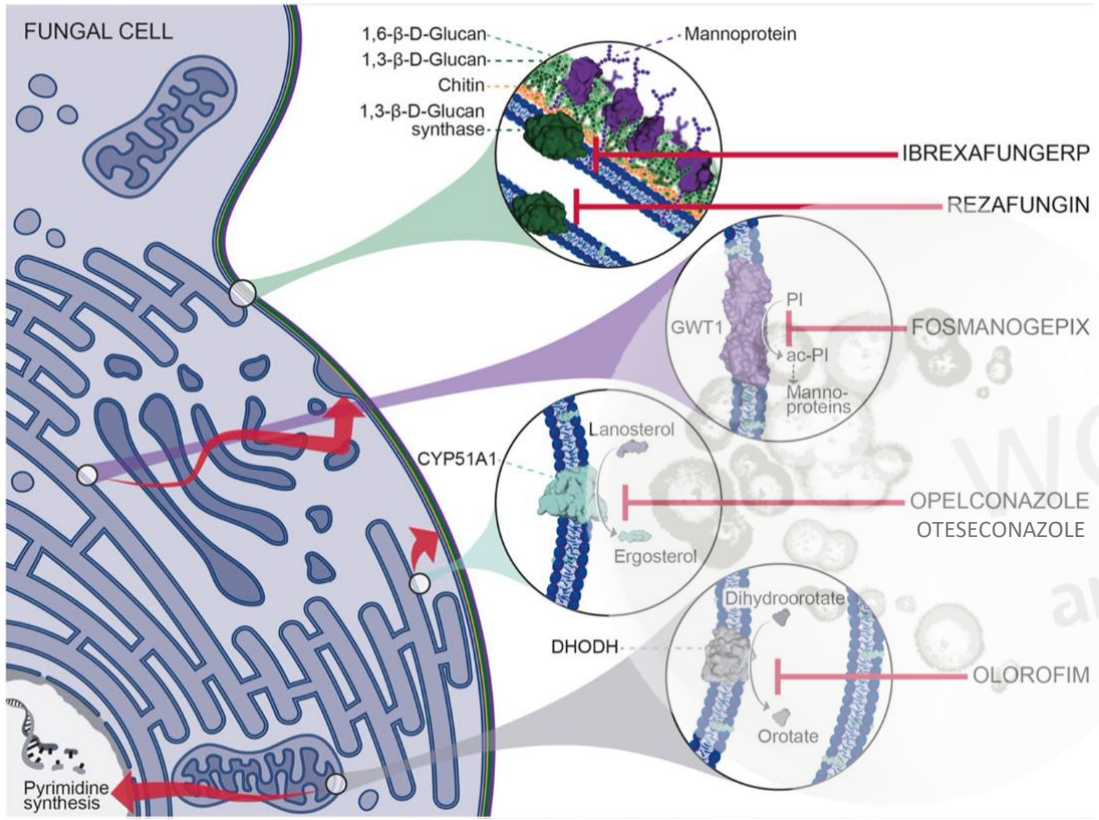
Cornely OA…….Chakrabarti A.
Lancet Infect Dis 2019; 19: e405-21

Treatment of mucormycosis – multimodal approach



Chakrabarti & Singh. Curr Fungal Infect Rep 2020; 14: 348-360
Cornely OA…….Chakrabarti A. Lancet Infect Dis 2019; 19: e405-21

Novel antifungals



Drug	Family	Target of action
Ibrexafungerp	Triterpenoid	1-3-β-D-glucan synthase inhibition
Rezafungin	Echinocandin	1-3-β-D-glucan synthase inhibition
Fosmanogepix	Gepix mannoprotein synthase	Inhibits GPI-anchored cell wall transfer protein 1 (Gwt1) [affects anchoring of mannoprotein in cell wall]
Olorofim	Orotomide	Inhibits pyrimidine biosynthesis enzyme dihydroorotate dehydrogenase (DHODH)
Opelconazole	Triazole	14-α-demethylase inhibition
Oteseconazole	Tetrazole	14-α-demethylase inhibition

Hoenigl M, *et al*. Drugs 2021; 81: 1703-1729

Hoenigl M, *et al*. Clin Microbiol Rev 2024; April 11: e0007423

Activity of newer antifungal

Antifungal drug	*Aspergillus*		*Mucorales*	*Fusarium*	*Scedosporium*
	Aspergillus spp.	Azole-resistant			
Isavuconazole		Variable activity	Variable activity		
Posaconazole		High-dose		Salvage therapy	
Rezafungin					Variable activity
Ibrexafungerp					Variable activity
Olorofim				Variable activity	
Fosmanogepix			Variable activity		

Kourti & Roilides. Mycopathologia 2024; 189: 88

Summary

- Prevalence of invasive mould infection is on rise globally; more in Asian countries
- The spectrum of vulnerable host range has increased
- New species & cryptic species cause challenging infection
- *Aspergillus flavus* is equally isolated as *A. fumigatus*
- COVID19 had impact – CAPA & outbreak of CAM
- Drug resistance in *Aspergillus fumigatus* & *A. flavus* has emerged
- Diagnosis & management of invasive aspergillosis & mucormycosis are serious challenge due to low sensitivity in diagnosis & high mortality
- POCT in aspergillosis; molecular diagnosis in IMD have raised hope for early diagnosis
- Posaconazole & isavuconazole are recommended for first line therapy in aspergillosis
- Newer antifungal – Fosmanogepix & Olorofim are new hopes for managing IMD

THANK YOU