Laboratory diagnosis of Invasive Aspergillosis for outcome prediction

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Aspergillosis

ETIOLOGY

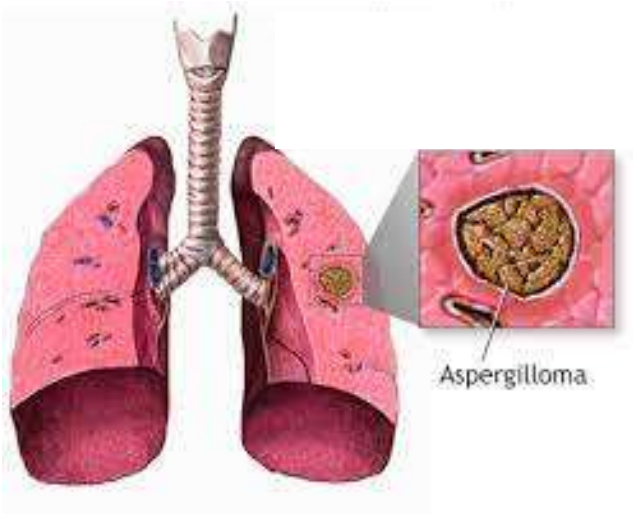
- Aspergillus fumigatus
- Aspergillus flavus
- Aspergillus niger
- Aspergillus lentulus
- Aspergillus Tereus

- infection or disease caused by the **genus Aspergillus**
- wide range of disease from allergic reactions to disseminated invasive disease

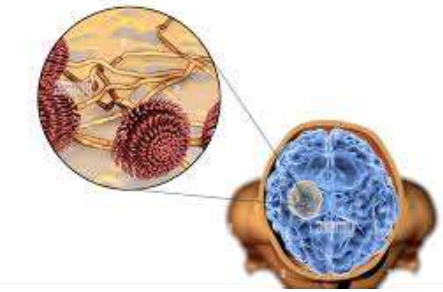
1. Richard C. Barton Hindawi Publishing Corporation Scientica Vol 2013, Article ID 459405

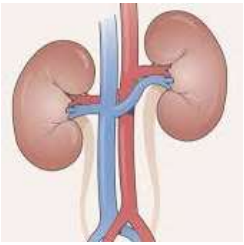
Invasive Aspergillosis

- Most cases involving the lung " parenchymal Invasive pulmonary aspergillosis (IPA)" [2]
- 10%–25% of cases, disseminated particularly to the brain, liver, kidney, gut, and sinuses [2]



INVASIVE ASPERGILLOSIS
is a severe and aggressive fungal disease that occurs in profoundly
IMMUNOCOMPROMISED HOSTS[1]

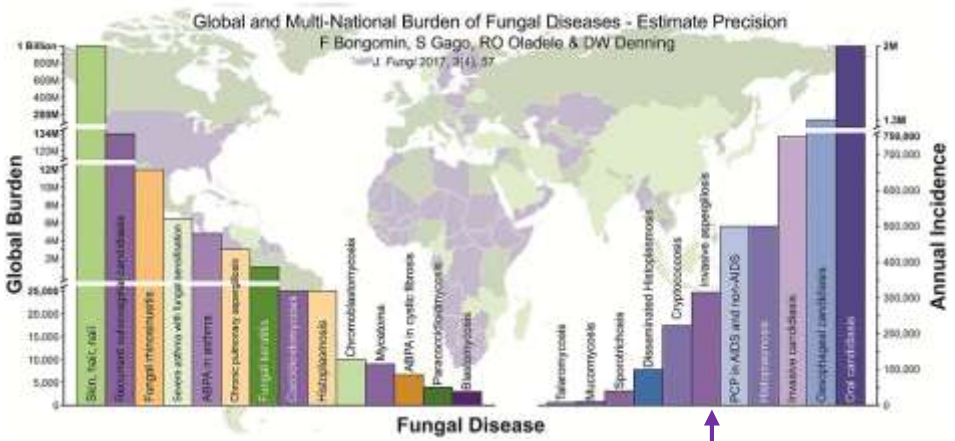




1. Bongomin F et al. *J Fungi (Basel).* 2017;3(4):57 ; 2. Richard C. Barton Hindawi Publishing Corporation Scientica Vol 2013, Article ID 459405

Incidence of Invasive Aspergillosis

Incidence



~200,000 cases/year[1,2]

recently updated to >300,000 cases/year

ICU: 0.3 – 5.8%[3]

Mortality in Hematological patients:

- 29% despite treatment
- ICU: 46–80%[2]

USA

~15,000 aspergillosis-associated hospitalizations occurred in the United States in 2014 [3]

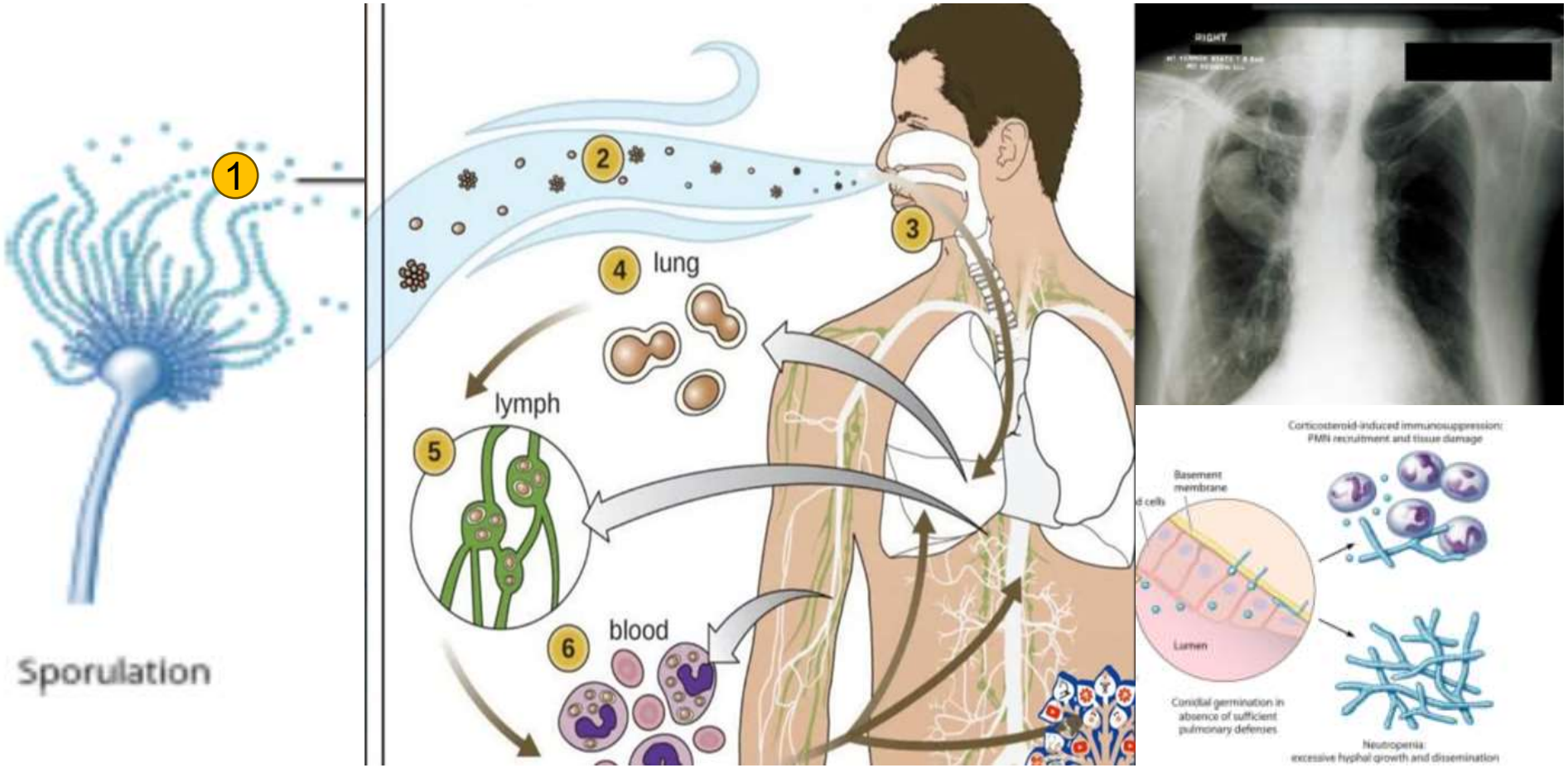
INDONESIA

The annual incidence of invasive aspergillosis:
~ 49,500 [3]
Allergic bronchopulmonary aspergillosis prevalence in adults :
~ 336,200 [3]

BSI, blood stream infection; IA, invasive aspergillosis; ICU, intensive care unit

1. Felix Bongomin et all , J. Fungi 2017, 3, 57; doi:10.3390/jof3040057; 2. Lass-Florl C et al. *J Antimicrob Chemother*. 2017;72(Suppl. 1):i5–i11; 3. Bassetti M et al. *J Antimicrob Chemother*. 2017;72(Suppl. 1):i39–i47; 3 US-CDC; 4. ; 5. Retnowahyuningsih et all, jurnal UI

Pathogenesis of Invasive Aspergillosis



Taylor R.T.Dagenais et all, Clinical Microbiology review, July 2009, p447-465

Risk Factors - Invasive Aspergillosis

CLASSIC FACTORS

- Acquired neutrophil defect
- Neoplastic disease with persistent neutropenia
- High dose of corticosteroids
- Immunosuppressants
- Bone marrow and solid organ transplants
- Aplastic anemia/myelodysplastic syndrome/myelofibrosis
- AIDS –CD4 <50 cells
- Primary defect of neutrophils
- Chronic granulomatous disease
- Hyper-IgE syndrome (Job's)

NON-NEUTROPENIC FACTORS

- Chronic obstructive pulmonary disease
- Chronic and acute liver disease
- Intracranial surgery
- Reactive airway disease
- Rheumatoid arthritis
- ICU stay

NEWLY RECOGNIZED FACTORS

- Intravenous infusion/drug abuse
- Diabetes mellitus
- Burn wound
- Application of tapes/ECG leads
- Allergic broncho pulmonary aspergillosis/asthma
- Influenza (Independent risk factor)
- Ibrutinib (Independent risk factor)

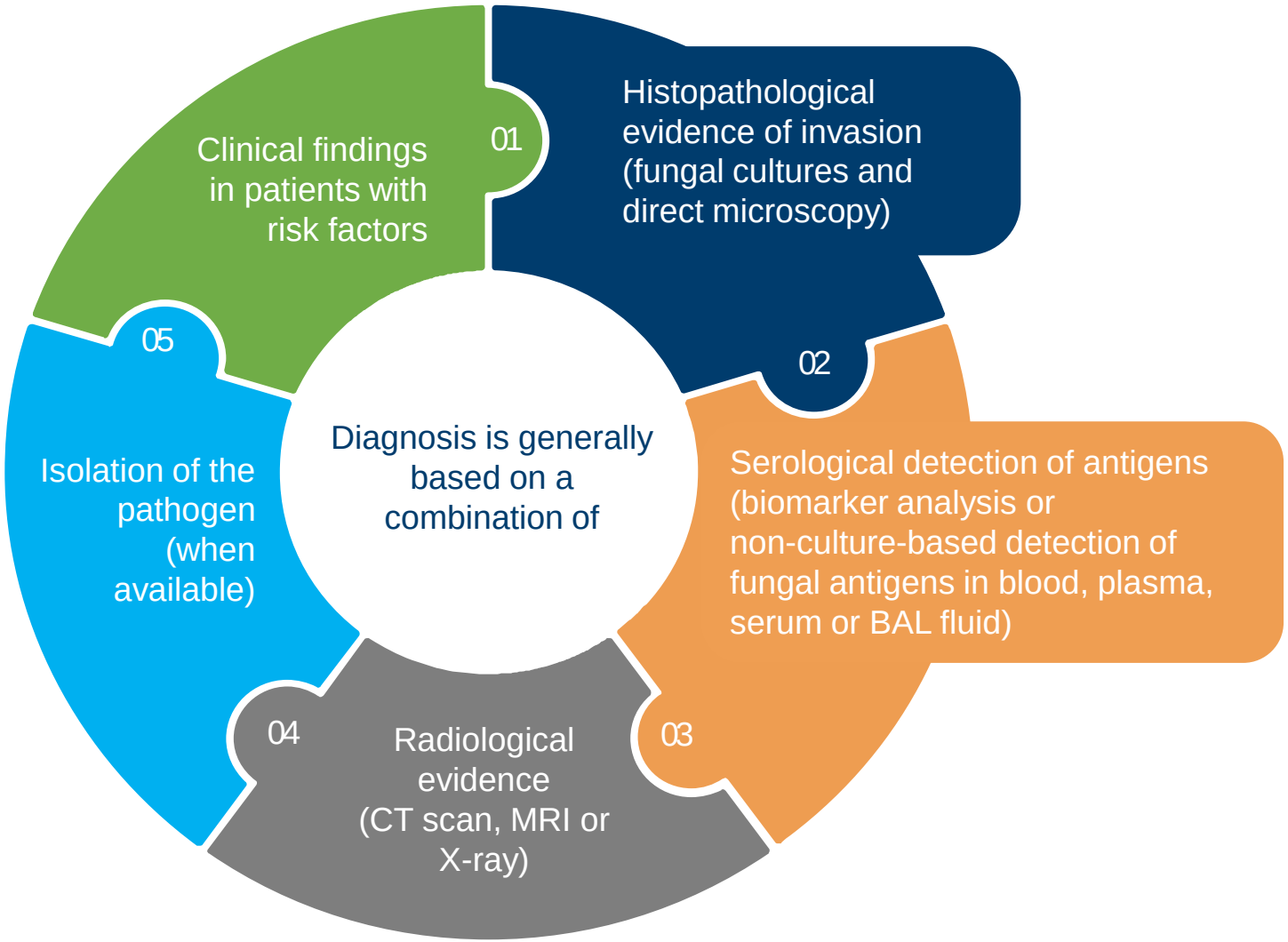
ICU, intensive care unit; AIDS, Acquired immunodeficiency syndrome; CD4, Cluster of differentiation 4; ECG, Electrocardiography

1. Bassetti M et al. *J Antimicrob Chemother*. 2017;72(Suppl. 1):i39–i47; 2. Chakrabarti A et al *Med Mycol*. 2011;49 Suppl 1:S35-S47; 3. Schauwvlieghe et al *Lancet Respir Med* 2018; 6: 782–92; 4. Varughese et al. *Clin Infect Dis* 2018 Aug 16;67(5):687-692

Diagnosis of Aspergillus Infections

European Organization for Research and Treatment of Cancer/Mycoses Study Group Education and Researches Consortium (EORTC/MSGERC)

- Histopathological examination and culture remain the ‘gold standard’ for diagnosing IA [3]
- Appropriate clinical specimens.[3]
- Non-culture-based diagnosis of IA [3]



BAL, bronchoalveolar lavage; CT, Computed tomography; IA : Invasive Aspergillosis; MRI, Magnetic resonance imaging

1. Lamoth F et al. *J Antimicrob Chemother*. 2017;72(Suppl. 1):i19–i28; 2. Johnson G, et al. *Biomark Med*. 2014;8(3):429–51; 3. Abby P. Douglas et all Internal Medicine Journal (2021) 51(Suppl. 7) 143–176.

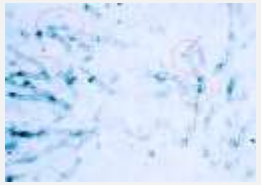
Diagnosis of Invasive Aspergillosis

Category	Host factor	Clinical presentation	Mycological evidence
Proven IA [1]	Not required	Not required	• Pathology evaluation showing compatible hyphae and associated tissue damage **and** • Culture showing *Aspergillus* in specimen obtained **by a sterile procedure from a normally sterile site** • Amplification of fungal DNA by PCR combined with DNA sequencing when molds are seen in formalin-fixed paraffin-embedded tissue
Probable IA	Risk factors for IA[1] Addition of • Use of B cell immunosuppressants (e.g. ibrutinib) • Explicit addition of solid organ transplants[2]	The presence of 1 of the following **4 patterns on CT:** Dense, well-circumscribed lesions(s) with or without a halo sign, Air crescent sign, Cavity, Wedge-shaped and segmental or lobar consolidation[1,2]	At least one of the following non-definitive tests: • Cytology, direct microscopy and/or culture showing *Aspergillus* species in a lower respiratory tract specimen • Serum GM in 2 consecutive samples ODI ≥0.5 BAL GM in single sampel ODI ≥1.0 or BAL GM ODI ≥0.8 AND serum/plasma GM ODI ≥0.7 • Aspergillus PCR positive from 2 blood or BAL specimen or positive from 1 blood and 1 BAL [2]

BAL, bronchoalveolar lavage; GM, galactomannan;, IA, invasive aspergillosis ; ODI : Optical density Index

1.Bassetti M et al. *J Antimicrob Chemother.* 2017;72(Suppl. 1):i39–i47; 2. Abby P. Douglas et all Internal Medicine Journal (2021) 51(Suppl. 7) 143–176

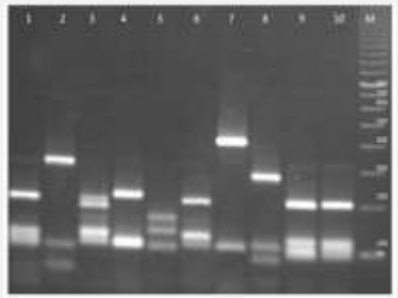
Diagnosis of Invasive Aspergillosis– Direct microscopic And Culture

Method	PROS	CONS	Sensitivity and specificity
Direct Microscopic [1] 	• Easy	• Sensitivity depend on various methods (Fluorescent dyes (e.g. calcofluor white) • Lacking in specificity • Aspergillus sp. rarely sporulate in vivo and hyphae seen may represent any number of filamentous fungi	Sensitivity 0-90%
Culture 	• Morphological identification based on microscopic and macroscopic examination is the method of choice for identifying filamentous fungi [2] • Thermotolerance test for *A. fumigatus* [1] • Low cost[3]	• Primary isolation from : deep sites and sterile samples (e.g. biopsies, CSF) [3] • Yield of BAL culture is notoriously low[2] • Slow speed : up to 3 weeks (2–5 days minimum) [3] • Potential for contamination[3] • Positive BAL culture may reflect colonization and no infection[2]	BAL[2] Sensitivity: 20–50%
	• Morphological identification based on MALDITOF-MS or sequencing ITS region [1]	• MALDITOF Need In-house databases all species to improve species identification[1] • Sequencing ITS region not necessary if organism has typical growth characteristics.[1] • High cost[1]	

BAL, bronchoalveolar lavage; MALDI TOF MS, matrix-assisted laser desorption/ionization time of-flight mass spectrometry

1. Abby P. Douglas et all Internal Medicine Journal (2021) 51(Suppl. 7) 143–176; 2. Lamoth F, et al. J *Antimicrob Chemother*. 2017;72(Suppl. 1):i19–i28; 3. Johnson G, et al. *Biomark Med*. 2014;8(3):429–51; 3..

Diagnosis of Invasive Aspergillosis - Molecular Test

Method	PROS	CONS	Sensitivity and specificity
PCR	• PCR testing can be done using whole blood, serum, plasma or BAL[1] • A single negative test is sufficient to exclude IA[1] • Early detection[2] • Species-specific[2] • Quantitative (qPCR) [2] • High-throughput (qPCR) [2]	• Important variations in results were observed across studies, which may in part be due to differences in PCR methods, procedures for DNA extraction, choice of target sequences and primers, and spectrum of detection[2] • BAL : a positive result cannot distinguish colonization from IA[3] • Not easily used at POC[2]	Serum[1] Sensitivity: 88% Specificity: 75% • Two consecutive tests increased specificity to 87% BAL[1] Sensitivity: 91% Specificity: 92%
Board range Pan-Aspergillosis PCR [3,4] 	• Helpful in the identification of Aspergillus and other fungal pathogens	• Strongly recommended for biopsy specimens which demonstrate fungal hyphae • High cost	

BAL, bronchoalveolar lavage; PCR, polymerase chain reaction; PE : Paraffin embedded; POC, point of care; qPCR, quantitative polymerase chain reaction;

1. Lamoth F, et al. J *Antimicrob Chemother*. 2017;72(Suppl. 1):i19–i28; 2. Johnson G, et al. *Biomark Med*. 2014;8(3):429–51; 3. Abby P. Douglas et all Internal Medicine Journal (2021) 51(Suppl. 7) 143–176; 4. P. Lewis White, cid 2021:72 (Suppl 2)

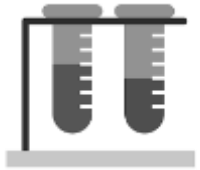
Diagnosis of Invasive Aspergillosis - Biological Markers

Method	PROS	CONS	Sensitivity and specificity
LFD	• Rapid POC diagnosis of IA using serum or BAL[2] • The combination of LFD with GM or PCR results in high diagnostic accuracy[1] • Low cost[2] • User-friendly[2]	• Despite promising results, few centres currently use the *Aspergillus* LFD, which has not yet been approved by the FDA or the EORTC/MSG committee[1]	Serum[1] Sensitivity: 68% Specificity: 87% BAL[1] Sensitivity: 86% Specificity: 93%
Galactomannan[1] Polysaccharides consisting of a mannose with galactose, component aspergillus cell wall released during growth	• The test is validated for use in serum and BAL samples and has demonstrated some utility for the diagnosis of cerebral aspergillosis in CSF samples	• The requirement for two consecutive tests with ODI ≥0.5 in serum was shown to provide the best diagnostic accuracy • Exposure to mould-active antifungals reduces the sensitivity of GM assay[3] • ELISA Platelia Bio-RAD	Serum Sensitivity: 60–80% Specificity: 80–95% BAL Sensitivity: 85–90% Specificity: 90–95%
BDG	• Rapid[2] • Compared with the GM assay, BDG tests have a broader spectrum of detection, including *Aspergillus spp*. and most other pathogenic fungi (except for *Mucorales* and *Cryptococcus spp*.)[1]	• The role of BDG testing in BAL seems to be limited by a poor specificity[1] • Low PPV[2]	• Serum[1] • Sensitivity: 60–80% • Specificity: 80–90%

BAL, bronchoalveolar lavage; BDG, (1–3)-β-D-glucan; CSF, cerebrospinal fluid; GM, galactomannan; IA, invasive aspergillosis; ODI, optical density index; PPV, positive predictive value; LFD, lateral flow devices; PCR, Polymerase Chain Reaction; BGD, blood group degradation

1. Lamoth F et al. *J Antimicrob Chemother*. 2017;72(Suppl. 1):i19–i28; 2. Johnson G, et al. *Biomark Med*. 2014;8(3):429–51; 3. Donnelly JP, et al. *Clin Infect Dis.* 2019 [Epub ahead of print].

Challenges Of Current Diagnostic Tools In Hematology Patients



LIMITATION OF CONVENTIONAL TECHNIQUES
(histopathology and culture)

- Relatively low sensitivity
- Slow turnaround
- Invasive nature of specimen collection required for testing[1]
  - Serious practical problem as requiring the use of lung biopsies and BAL[3]
  - At-risk patients are usually characterized by thrombocytopenia and neutropenia, which precludes the use of invasive procedures[3]

LIMITATION OF BLOOD CULTURE

- Fungal pathogens are rarely present in the blood or other bodily fluids[3]
- Complete identification need sophisticated methods (MALDITOF, PCR-Sequencing)

- **Empiric therapy**

1. Salcedo DT, et al. *J Clin Microbiol*. 2020 [Epub ahead of print];2. Donnelly JP, et al. *Clin Infect Dis*. 2019 [Epub ahead of print]; 3. Johnson G, et al. *Biomark Med*. 2014;8(3):429–51.

Empiric Therapy

- **Treatment provided only when:**
  - Fever is persistent or recurrent in spite of broad-spectrum antibiotic therapy or other clinical or radiological features suggestive of IA in high-risk patients with prolonged neutropenia.[1]
  - High incidence of IFD and the low diagnostic yield of conventional microbiological methods.[1]

DISADVANTAGES: of Empirical Antifungal Approach

- It may be associated with the risk of overtreatment with potentially toxic and/or expensive drugs, knowing that only a minority of patients are probably affected by an IFD.[2]
- It allows a less targeted antifungal treatment compared with diagnostic driven strategies, although broad-spectrum antifungal drugs are used.[2]

With evolution of diagnosis approach to IA, empiric strategy is not recommended.[1]

IFD: Invasive Fungal Disease; IA: Invasive Aspergillosis

1. Garcia-Vidal C, et al. Update by the GEMICOMED-SEIMC/REIPI Documento de consenso del GEMICOMED perteneciente a la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC) sobre el tratamiento de las infecciones invasoras producidas por Aspergillus. Available from: https://www.seimc.org/contenidos/documentoscientificos/borrador/seimc-GuiaClinica-BR-2018-Management_of_aspergillosis.pdf . Accessed on Nov 2, 2020

Guidelines Recommendation For DDA

ESCMID-ECMM-ERS guideline

Early diagnosis of IA is a challenge and should be based on the integration of clinical, radiological and microbiological data.[1]

Pre-emptive treatment is a diagnosis-driven strategy. In most cases, it is defined by positive GM testing. However, chest CT with pulmonary infiltrates could apply as well. The use of BDG and PCR testing as alternative biomarkers for GM have considerable merit though BDG is not specific for *Aspergillus* disease.[1]

GEMICOMED-SEIMC/REIPI Guideline

Diagnostic-driven antifungal therapy may be based on the screening (at least on a twice-a-week basis) for serum GM antigen or *Aspergillus* DNA detection at regular intervals throughout the entire at-risk period (AII).[2]

IA, invasive aspergillosis; CT, computerized tomography; BDG, (1→3)-β-d-glucan; PCR, Polymerase chain reaction; DNA, Deoxyribonucleic acid; SEIMC, Spanish Society of Infectious Diseases and Clinical Microbiology; ESCMID, European Society of Clinical Microbiology and Infectious Diseases; ECMM, Excellence Centre of Medical Mycology; ERS, European Respiratory Society

1. Ullmann A.J. et al. *Clinical Microbiology and Infection* 24 (2018) e1-e38. 2. Garcia-Vidal C. et al. *Enferm Infecc Microbiol Clin.* 2019;37(8):535-541.

Diagnostic Driven Approach (DDA)

ADVANTAGES

A diagnostic approach to treating invasive fungal infections can

- Aid in both early treatment of patients as well as improving the chances of survival.[1]
- It allows the early detection of asymptomatic infections thanks to the use of screening markers.[2]
- Targeted antifungal treatments may be used.[2]
- It allows reduction in the use of antifungal drugs compared with an empirical approach.[2]
- It allows effective antifungal control.[2]

IFD: Invasive Fungal Disease

1. Greene RE, et al. *Clin Infect Dis.* 2007; 44(3): 373–9. 2. Girmenia C, et al. *J Clin Oncol*. 2010 Feb 1;28(4):667-74.

Use Of Aspergillus Galactomannan And PCR: Reduces Use Of Antifungal Treatment

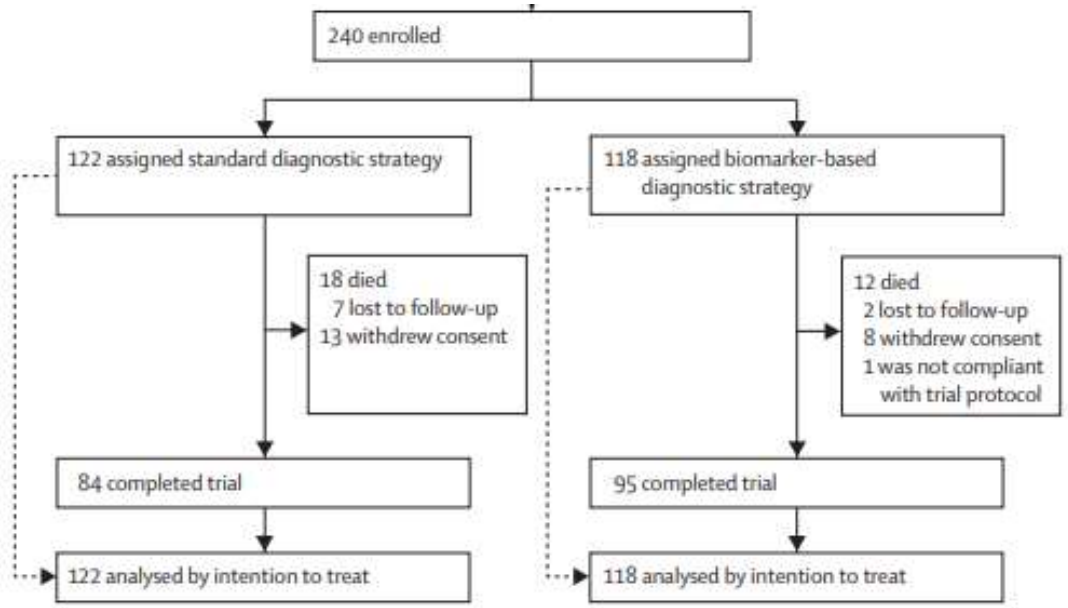
BACKGROUND

Empirical treatment with antifungal drugs is often used in haematology patients at high risk of invasive aspergillosis

OBJECTIVE

Randomised, controlled, open-label, parallel group trial to compare the efficacy and safety of biomarker-based diagnostic strategy with the standard diagnostic strategy of culture and histology in high-risk haematology patients

STUDY DESIGN



PCR; polymerase chain reaction

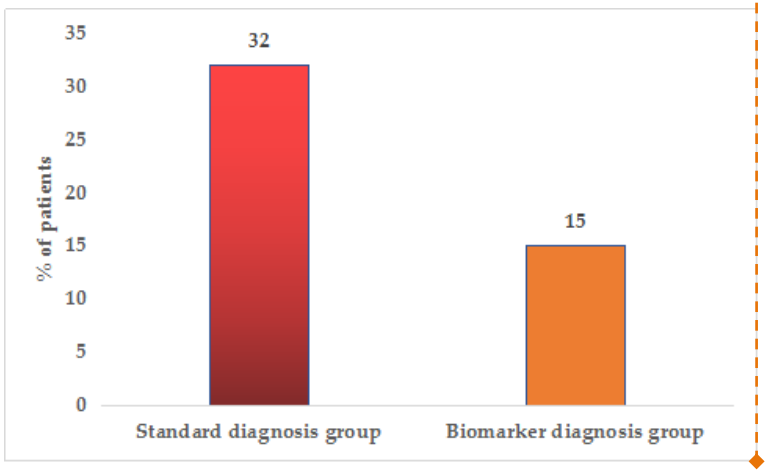
Morrissey CO, et al. *Lancet Infect Dis.* 2013;13(6):519-528.

Use Of Aspergillus Galactomannan And PCR: Reduces Use Of Antifungal Treatment

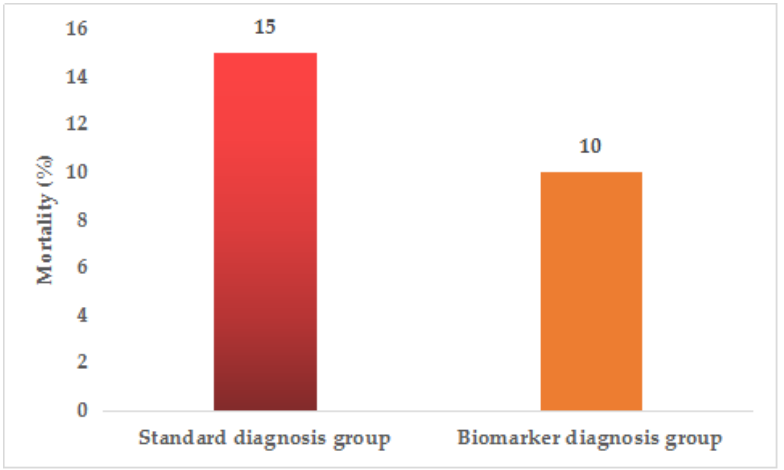
RESULTS

Biomarker-based diagnostic strategy (galactomannan and PCR)

Reduces use of empirical antifungal treatment



Reduces all cause mortality



CONCLUSION

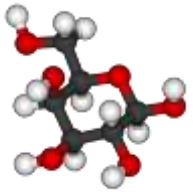
Biomarker-based diagnostic strategy significantly reduced the use of empirical antifungal treatment and increased the sensitivity of invasive aspergillosis diagnosis, which led to better directed antifungal treatment with no increase in mortality

PCR; polymerase chain reaction

Morrissey CO, et al. *Lancet Infect Dis.* 2013;13(6):519-528.

Role of Biomarker Galactomannan for outcome predictor

- Miceli et al. CID 2008:46
- Seung Beom Hanet et all. Han et al. BMC Infectious Diseases (2015) 15:271
- Toine Mercier et all. Frontiers of Microbiology, vol 9 04 April 2018
- Abby P. Douglas et all. Internal Medicine Journal (2021) 51(Suppl. 7) 143–176



Galactomannan

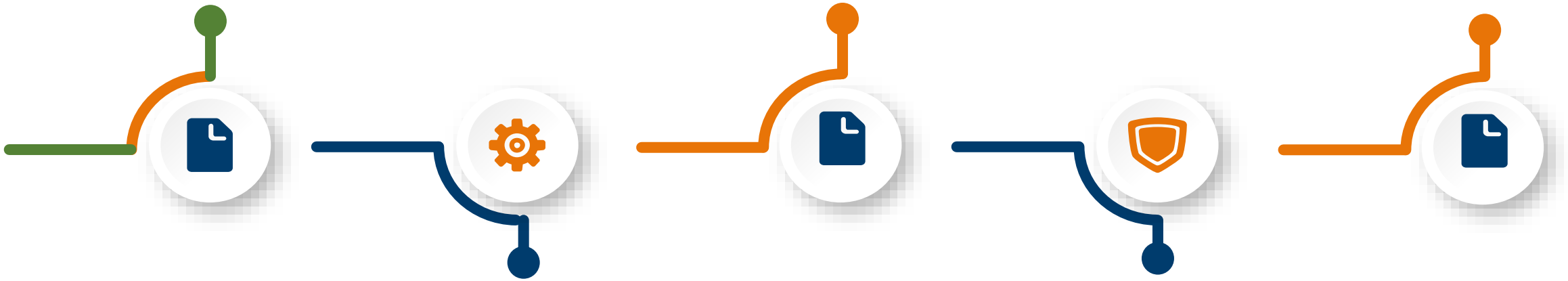
- Serum GM in baseline good predictor of of therapy response and survival in short-term and long-term
- A relatively consistent relationship observed between rate of serum GM decline and treatment response
- A suggestion that early clearance of serum GM may be an early surrogate marker of response
- The outcome of IPA was significantly associated with the early trend of serum serum GM during antifungal therapy.

Summary

Increasing prevalence and mortality of IA Patient with risk factor .[1]

Early diagnosis and treatment of IA: Crucial to decreasing the clinical and economic burden along with mortality.[2,3]

Diagnostic driven approach recommended by several guidelines such as GEMICOMED-SEIMC/REIPI, ESCMID-ECMM-ERS.[4]



Risk factors: Hematological malignancies, various transplantations, chronic granulomatous disease, advanced AIDS, ICU stay.[5]

Clinicians face many challenges in early diagnosing/treatment of IA.[2]

DIAGNOSTIC DRIVEN APPROACH[6]

- Reduces the use of unnecessary antifungals with a superior to equivalent outcome.
- Allows for a more rational utilization of available antifungals
- Reduces costs and adverse events associated with the widespread use of empiric therapy
- Improves quality of care in the high-risk patient population

- **Galactomannan as predictor of outcome**

1. Lass-Florl C, et al. *J Antimicrob Chemother.* 2017;72(Suppl. 1):i5–i11; 2. Chandrasekar P. *Eur J Haematol.* 2010;84(4):281-290. 3. Warris A, et al. *Clin Microbiol Infect*. 2019 ;25(9):1096-1113; **4.** Garcia-Vidal C, et al. *Enferm Infecc Microbiol Clin.* 2019;37(8):535-541; 5. Bassetti M, et al J *Antimicrob Chemother*. 2017;72(Suppl. 1):i39–i47; 6. Dib RW, et al. *BMC Infect Dis*. 2018;18(1):656.


Dziękuję
Спасибо
Dankie
D'Akujem
хвала
Gracias
Merci
Sagolun
Arigatô
Köszönöm
Kiitos
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Tak
Thank You
Merci
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Xièxie
Matondo
Obrigado
Mahalo
Grazie
Chokrane
Efharisto
Dank Je
Toda
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Hvala
Faleminderit
Terima Kasih