Advances in Diagnosis of Invasive fungal infection
Invasive aspergillosis

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Management of haematological patients at high risk of IA

Profound and prolonged neutropenia or active graft-versus-host disease

Primary prophylaxis

No screening with biomarkers

Start antifungal

Diagnostic work-up

Empiric treatment

No screening with biomarkers

Persistent fever or new fever after resolution

Trigger to start antifungal

Diagnostic work-up

Pre-emptive strategy

Screening with biomarker(s)

Positive biomarker

New pulmonary infiltrate (X-ray/CT)

Trigger to start antifungal

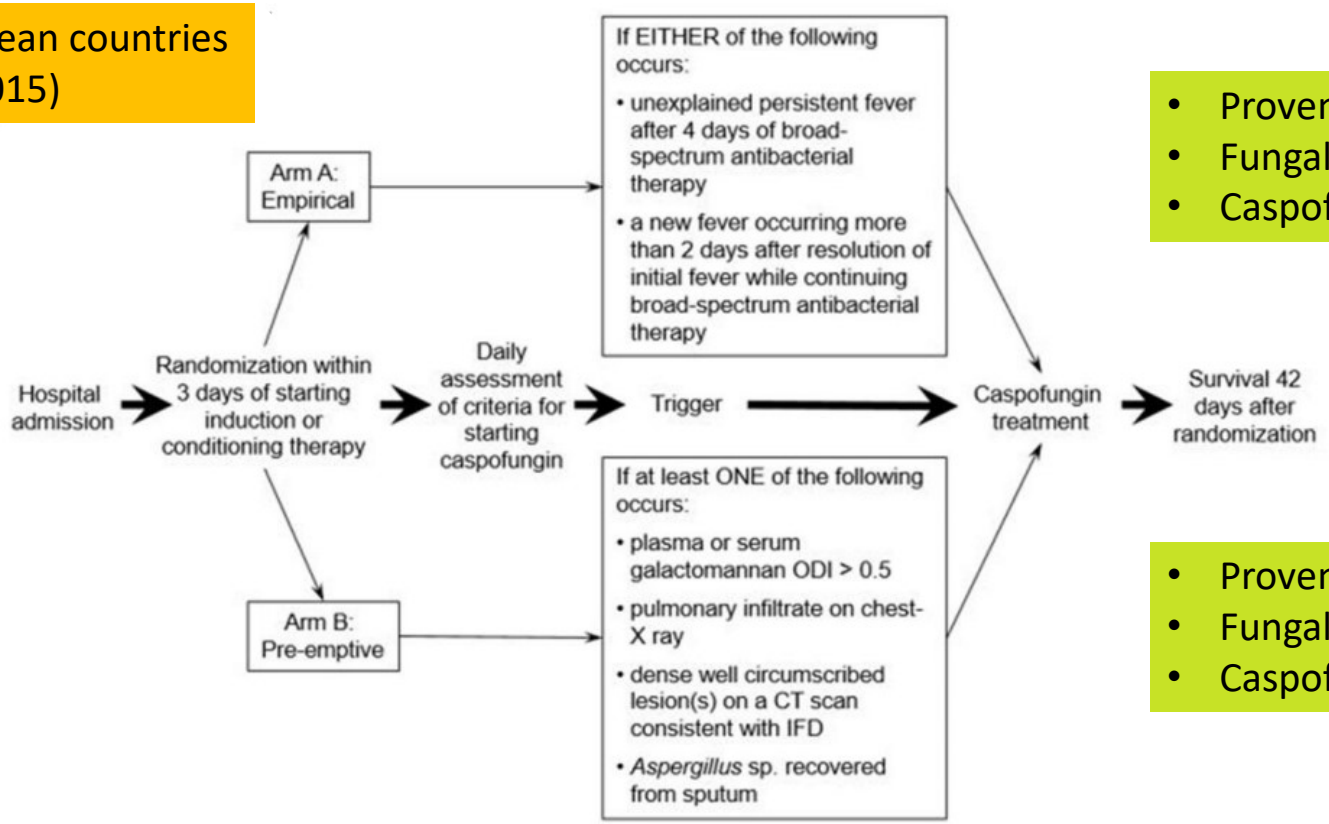
Diagnostic work-up

JOURNAL ARTICLE ACCEPTED MANUSCRIPT

Empiric versus pre-emptive antifungal strategy in high-risk neutropenic patients on fluconazole prophylaxis: a randomized trial of the European organization for Research and Treatment of cancer (EORTC 65091)

mITT population = 549

15 sites in 6 European countries (2012-2015)



- Proven/prob IFD: 6.6%
- Fungal free survival: 88.3%
- Caspofungin R/: 63%

- Proven/prob IFD: 7.7%
- Fungal free survival: 90.6%
- Caspofungin R/: 27% (p< 0.001)

J. Maertens et al. CID 2022

Empric versus pre-emptive antifungal strategy

Conclusions randomized EORTC trial

Pre-emptive antifungal strategy that includes twice weekly galactomannan screening and CT-scan on demand does not impact overall survival of adults with prolonged neutropenia who are at high-risk for IDF while receiving fluconazole prophylaxis

Pre-emptive strategy is not associated with an increase risk of proven or probable IFD

Pre-emptive strategy reduces the use of antifungals by half

J. Maertens et al. CID 2022

General reflections on *Aspergillus* PCR

Low fungal load regularly encountered when testing blood samples, become negative promptly after starting treatment

In BALf fungal often higher than in blood in patients with invasive aspergillosis (however often still a low load)

Clinical significance of weak positive PCR tests: due to testing specimens not directly associated with the infected site or contaminants?

Optimal use of *Aspergillus* PCR is in combination with an antigen detection test:

- Both test are negative, sensitivity is sufficient to exclude invasive aspergillosis
- Both assays are positive: high specificity, strongly supports the diagnosis of invasive aspergillosis
- Discordant results are frequently encountered in clinical practice and remain difficult to interpret

Lewis PL et al. CID 2021, 72: S95S101

Evolving risk factors for invasive mould infections

Risk factors for invasive aspergillosis

Haematological malignancy
Neutropenia
Allogeneic haematopoietic stem-cell transplantation
Solid organ (lung) transplantation

Invasive aspergillosis in the ICU:
Prolonged treatment with corticosteroids before admission to the ICU
Chronic obstructive pulmonary disease
Liver cirrhosis

Critically ill with viral pneumonitis
Influenza
COVID-19

Meersseman W, et al. Clin Infect Dis. 2007;45:205–16; Wauters J, et al. Intensive Care Med. 2012;38:1761–8; Dewi IMW, et al. Curr Opin Microbiol. 2021;62:21–7.

Management of ICU patients at high risk of IA

Patients with severe influenza or COVID-19

Primary prophylaxis

Not standard of care

Studies ongoing but majority of influenza associated aspergillosis already diagnosed upon admission

Infection probably later in COVID-19 patients

Empiric treatment

Not standard of care

To be evaluated in patients with severe influenza while waiting results of diagnostic work-up?

Pre-emptive strategy

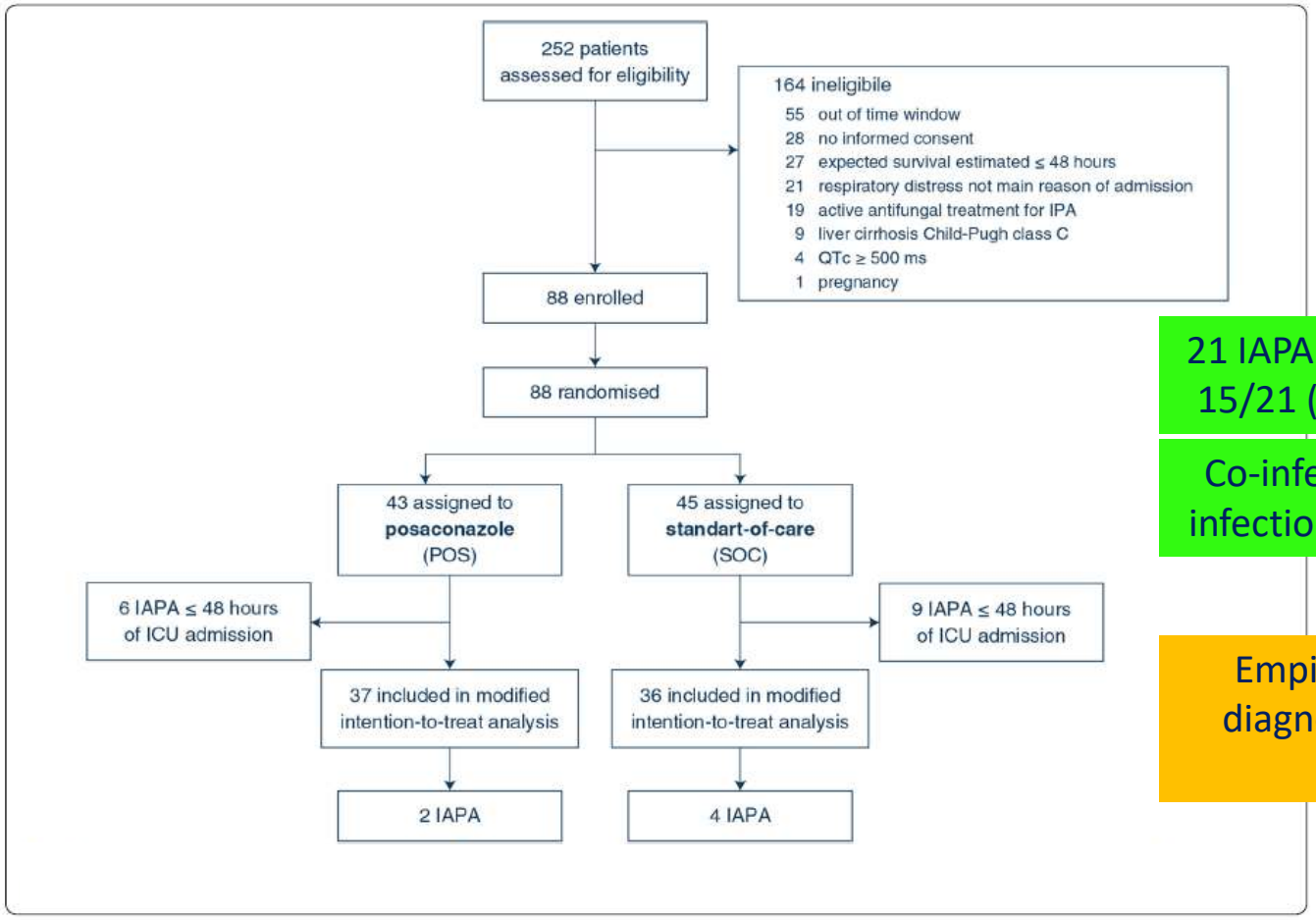
Not standard of care

Screening of blood for GM and BDG is not advised

Screening of tracheal aspirates for *Aspergillus* in COVID-19 patients?

Bartoletti M, et al. CID 2021;73:e3606-3614
Verweij P, *et* al. Intensive Care Med. 2021;47:819–34
Vanderbeke L, et al. Intensive Care Med. 2021;47:674–86.
Van Grootveld et al. Mycoses 2021, 64: 641-650

The majority of invasive aspergillosis is diagnosed within 48h of ICU admission in influenza patients



Early IAPA

Late IAPA

21 IAPA cases (21/88 patients, 24%)
15/21 (71%) diagnosed within 48h

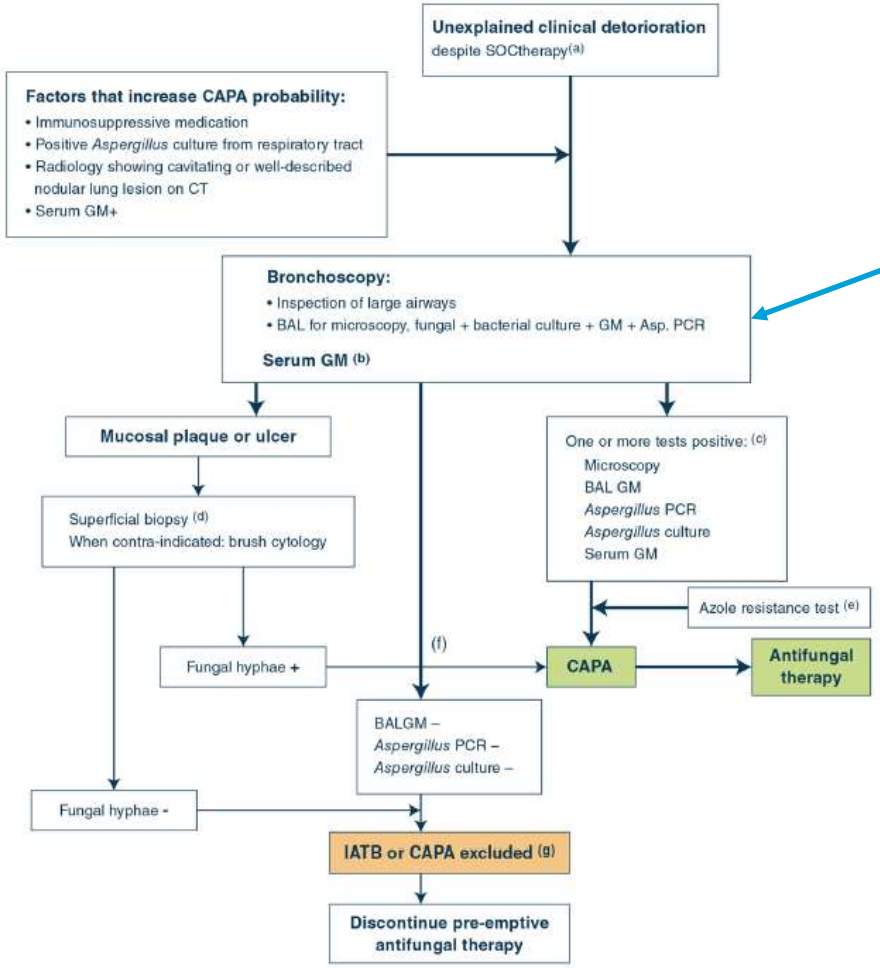
Co-infection instead of secondary infection in majority of the patients

Empirical antifungal therapy + diagnostic mycological work-up within 24–48h?

Vanderbeke L, et al. Intensive Care Med. 2021;47:674–86.

Proposed clinical guidance for the management of CAPA

Consider empirical antifungal treatment if visible plaques in trachea/bronchi or while awaiting results of diagnostic BAL tests in patients with rapidly deteriorating clinical condition



Cornerstone of CAPA diagnosis

Verweij P, *et* al. Intensive Care Med. 2021;47:819–34

Aspergillosis antigen detection assays

Choices are expanding rapidly, based on detection of

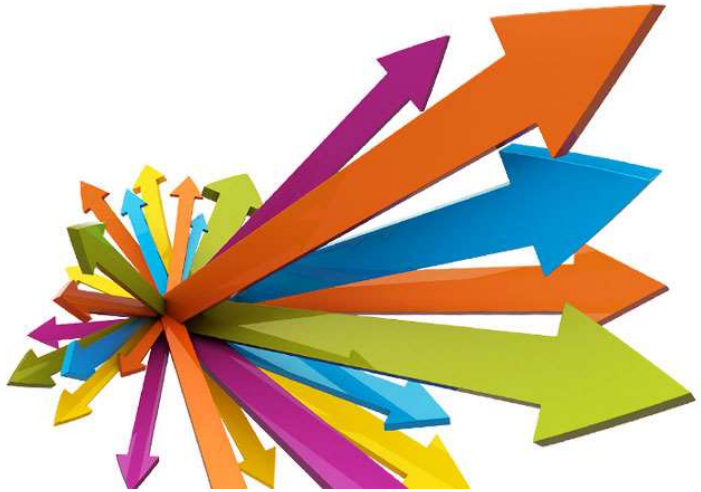
- Galactomannan
- Mannoprotein

Lateral flow assays/devices:

- Initial naming LFD for OLM test, LFA for IMMY test
- But now also other assays available

Other single test format assays

Validation data of most assays still limited



RESEARCH Open Access

Point of care aspergillus testing in intensive care patients

Toine Mercier[1,2*†], Albert Dunbar[3†], Vincent Veldhuizen[3], Michelle Holtappels[1], Alexander Schauwvlieghe[2,3,4], Johan Maertens[1,2], Bart Rijnders[3†] and Joost Wauters[5†]

Table 1 Patient characteristics

	Case	Control	p
n	55	123	
Center = Rotterdam (%)	22 (40.0)	54 (43.9)	0.747
Age, years (median [IQR])	63 [56, 68]	57 [46, 66]	0.073
Male gender (%)	34 (61.8)	66 (53.7)	0.395
Weight, kg (median [IQR])	70 [60, 84]	70 [62, 85]	0.910
Underlying disease (%)			0.355
Pulmonary disease	22 (40.0)	59 (52.2)	
Hematologic malignancy	9 (16.4)	10 (8.8)	
Heart disease	4 (7.3)	10 (8.8)	
Liver disease	3 (5.5)	5 (4.4)	
Gastrointestinal disease	3 (5.5)	2 (1.8)	
Other malignancy	2 (3.6)	9 (8.0)	
Other	12 (21.8)	18 (15.9)	
Neutropenia (%)	8 (17.0)	7 (5.7)	0.094
Influenza (%)	17 (30.9)	47 (38.2)	0.442
COPD (%)	6 (10.9)	15 (12.2)	1.000
Positive culture (%)	28 (50.9)		
Positive microscopy (%)	4 (7.3)		
BALf GM (median [IQR])	4.80 [2.73, 5.68]		

IQR interquartile range, *BALf GM* bronchoalveolar lavage fluid galactomannan, *COPD* chronic obstructive pulmonary disease

IMMY LFA
BAL fluid of 178 patients, including 55 cases (proven and probable IPA)

Depending on the definitions used:

- sensitivity 0.88–0.94
- specificity was 0.81
- area under the ROC curve 0.90–0.94

Conclusions:

- good overall test performance in ICU patients
- the LFA on BAL fluid and can be used as a rapid screening test while waiting for other microbiological results

Crit Care 2020: 24

Evaluation of IMMY LFA for diagnosis of CAPA

- Multicenter retrospective study
- ICU admission between March 2020 and April 2021
- CAPA patients classified according to the ECMM/ISHAM criteria with the exclusion of *Aspergillus* LFA
- No CAPA patients randomly selected

	0.5 ODI cutoff		1.0 ODI cutoff	
	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Respiratory samples				
Tracheal aspirate (TA) (N_{CAPA}=16; $N_{ØCAPA}$=18)	100% (79–100)	44% (22–69)	81% (54–96)	67% (41–87)
Nondirected bronchial lavage (NBL) (N_{CAPA}=20; $N_{ØCAPA}$=52)	90% (68–99)	83% (70–92)	80% (56–94)	88% (77–96)
Bronchoalveolar lavage fluid (BALF) (N_{CAPA}=29; $N_{ØCAPA}$=61)	72% (53–87)	79% (66–88)	52% (33–71)	98% (91–100)
BALF and NBL combined[b] (N_{CAPA}=49; $N_{ØCAPA}$=113)	80% (66–90)	81% (72–87)	63% (48–77)	94% (88–97)
All combined[b] (N_{CAPA}=58; $N_{ØCAPA}$=127)	83% (71–91)	76% (67–83)	66% (52–78)	90% (83–94)
Serum samples (N_{CAPA}=46; $N_{ØCAPA}$=102)	20% (9–34)	93% (86–97)	9% (2–21)	99% (95–100)

- Aspergillus GM LFA shows good performance especially on respiratory samples with the 1.0 ODI cutoff
- Can be implemented as screening test on tracheal aspirates, triggering BAL analysis if positive
- Isolated ODI slightly above the 0.5 ODI should lead to further mycological investigations

B. Autier et al. JCM 2022

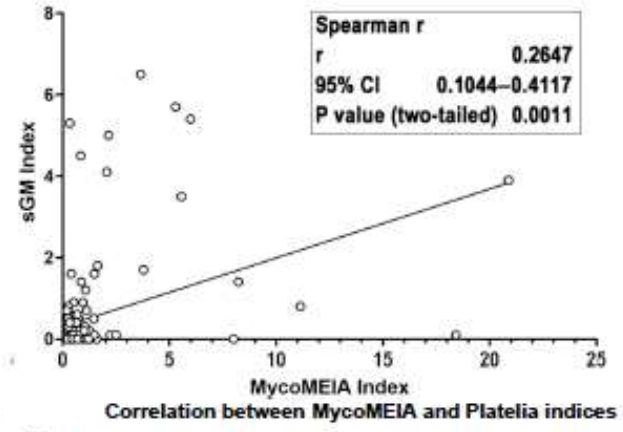
Some future perspectives

Antigen detection in urine

Next Generation Sequencing

MycoMEIA *Aspergillus* Assay for urine testing

- Platelia assay is insensitive to detect galactomannan in human urine samples
- MycoMEIA assay is optimized to detected specific β-galactofuranose in urine
- Antigen detected by mAb476 is abundantly present in urine on fungus-derived extracellular vesicles and also as a free glycan
- ELISA kit CE marked 4/2022; 510k planned 4Q2022
- Dipstick test is being developed and validated
- Promising results from small cohorts, significant but low correlation with Platelia galactomannan assay



Correlation between MycoMEIA and Platelia indices

Pearl DIAGNOSTICS

Aerts et al., TIMM 2021

NGS for detection and identification of fungi directly in clinical specimens

Likely will be the future in clinical mycology

Potential to identify to a species level, detect antifungal resistance and genotype during outbreak

But several limitations need to be overcome

- Identification of optimal genes to provide a sufficient degree of species differentiation while maintaining the required analytical sensitivity
- Optimization of the entire process
- Overcoming the lack of required NGS bioinformatic tools and pipelines
- Detection of a pathogen in the context of an overwhelming presence of single commensal/colonizing species with limited clinical importance (*Candida* species in respiratory mycobiome)

Lewis PL et al. CID 2021, 72: S95S101