Best-of :
New tools in laboratory diagnosis of invasive fungal infections

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Conflicts of interest

- **Lecture for Pfizer symposium**

How to diagnose an invasive fungal disease ?

Male 38 yo

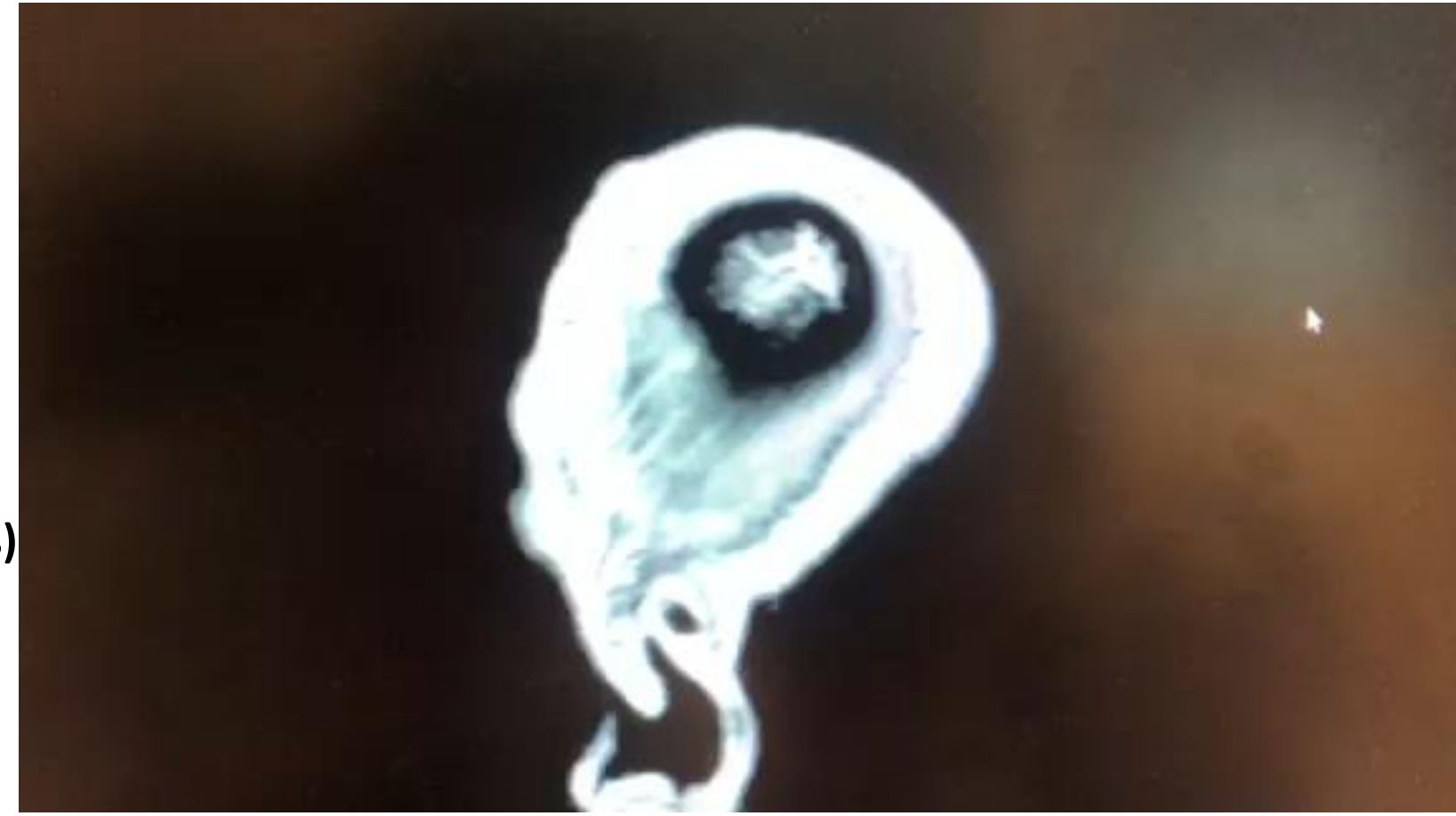
Severe combined immunodeficiency

Large lymphocyte leukemia

multiple brain lesions

(confusional syndrome and gait disorders)

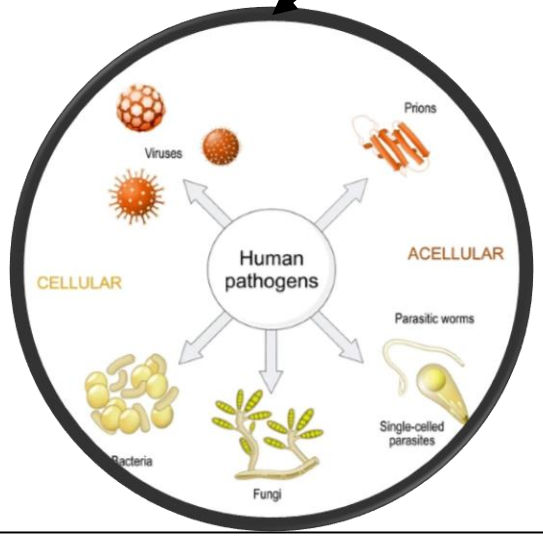
following recent *Nocardia* pneumonia



How to diagnose an invasive fungal disease ?

?

Lymphoprolifération ?

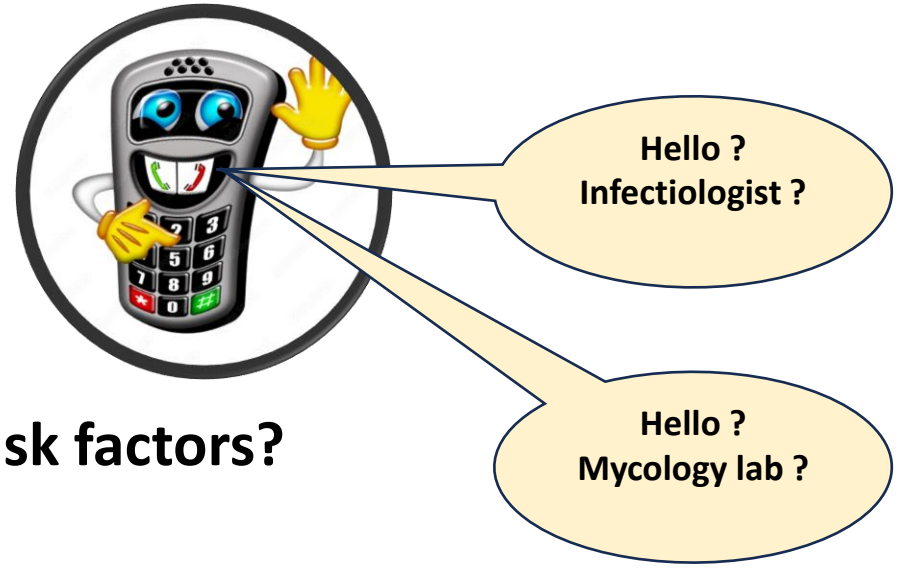


Neuromeningeal tuberculosis or nocardiosis ?

And why not a fungi ?

Granulomatosis
Vasculitis ?

Learn to think together



Immune status of the patient ? Risk factors and NEW risk factors?

Possible “exotic” pathology ?

Other organ affected ?

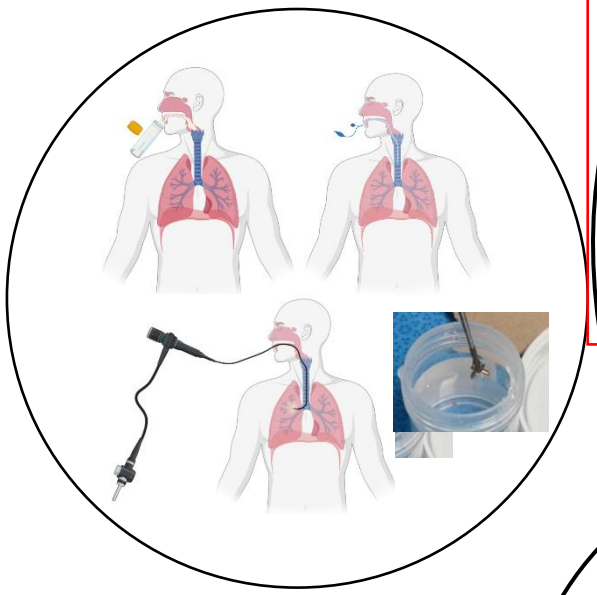
In order of frequency ?

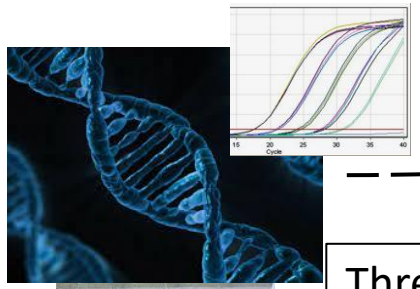
Can we sample ?

=> Or Should we settle for an indirect diagnosis ?

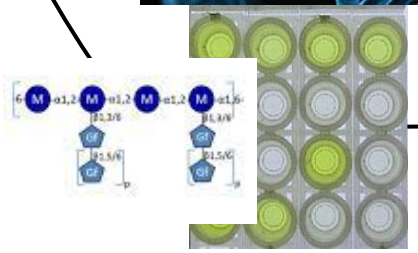
(Antigen, circulating DNA)

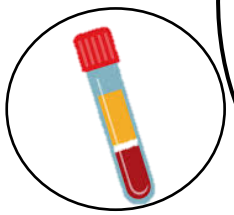
Tools	Invasive Candidiasis	Cryptococcosis	Invasive aspergillosis	Mucormycosis	Fusariosis	Histoplasmosis	Other	Pneumocystosis
Direct examination/ Histology	Evocative morphological aspects Poor sensitivity							
Specificities		Capsule Indian ink Other type of staining						Specific aspects Direct IF
Culture	Poor sensitivity							
Specificities						Class 3 Pathogen (L3) !	Class 3 Pathogen (L3) ? Specific media	
Antigen	Mannan Spécific Transient Poor sensitivity Serum, (CSF) Diagnosis Deep-seated localisation	Glucuronoxylomannan Sensitivity +++ Spscificity +++ Serum, CSF (BAL, urine) Diagnosis Prognosis	Galactomannan Sensitivity host- and clinical entity dependant Specificity Cross reaction Other fungi Serum, BAL, CSF (bronchial aspiration) Screening, Diagnosis, Prognosis		Some species cross react with GM	Specific Ag Not available in F Cross react with GM	Ag Coccidioides Not available in F	
BDG	Species dependant		Disappointing		Sensivity 50%		Basidiomycota	NPV Long-time persistance
PCR	T2MR (5 species) Blood (CSF/AH) Necker/Marseille (few species)	St-Louis	*A. fumigatus*/spp All matrices Resistance	All matrices (4 species in Lille)	St-Louis	St-Louis	Rare test catalog ANOFEL	Interpretation threshold
Panfungal sequencing	Moderate sensitivity if direct examination is positive !							
NGS/WGS	Sensitivity, quantification, interpretation… ?							





Threshold ?



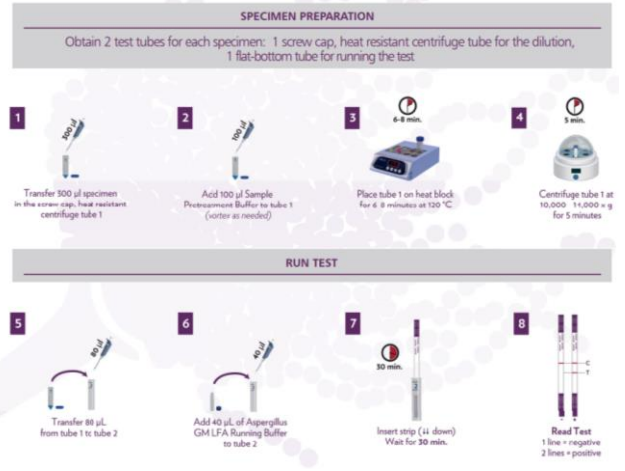


LFA; IMMY

LFD; OLM

Diagnostic tools for *Aspergillus*

GM Ag alternative
PoC LFA/LFD



LFD OLM 15 min

LFA IMMY 30 min

Serum and BAL
Interest if few samples or in emergency

Performance varies depending on classification
LFA > LFD

	Samples with IPA n (%)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Total[a] n
Blot putative IPA						
LFD	27 (16)	88.9 (70.8-97.7)	55.1 (46.7-63.3)	26.7 (22.5-31.3)	96.4 (90.2-98.8)	174
LFA cut-off 1	30 (16)	93.3 (77.9-99.2)	46.1 (38.1-54.3)	25.2 (22.1-28.7)	97.3 (90.2-99.3)	184
Schauwvlieghe modified definition of IPA						
LFD	111 (63)	68.5 (59.0-77.0)	67.2 (54.3-78.4)	78.4 (71.4-84.0)	55.1 (47.1-62.9)	175
LFA cut-off 1	116 (62)	85.3 (77.6-91.2)	72.9 (60.9-82.8)	83.9 (77.9-88.5)	75.0 (65.4-82.6)	186
EORTC/MSG probable IPA						
LFD	30 (30)	73.3 (54.1-87.7)	49.3 (37.2-61.4)	37.9 (30.8-45.6)	81.4 (69.8-89.2)	101
LFA cut-off 1	31 (29)	87.1 (70.2-96.4)	50.6 (39.0-62.2)	41.5 (35.3-48.1)	90.7 (79.2-96.2)	108
Blot putative IPA + entry criterion GM ≥ 1						
LFD	56 (32)	60.7 (46.8-73.5)	47.1 (38.0-56.4)	34.7 (28.9-41.0)	72.2 (64.0-79.1)	177
LFA cut-off 1	59 (31)	79.7 (67.2-89.0)	45.0 (36.2-54.0)	39.8 (35.1-44.8)	82.9 (73.8-89.2)	188

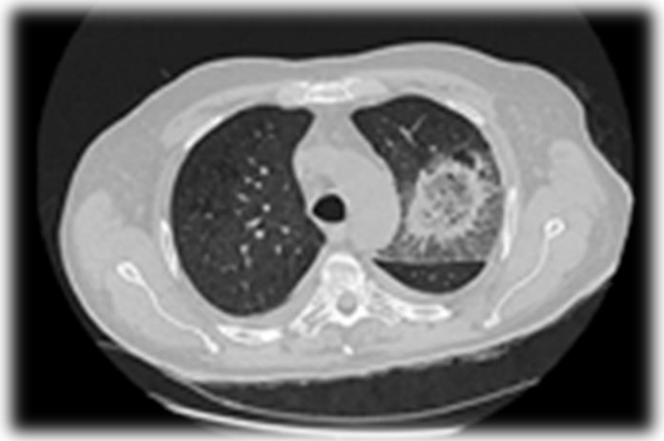
Scharmann *et al*. Mycoses. 2020; 200 LBA => LFA : correlation Platelia

Better specificity for GM index 1 in BAL

	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)

Autier *et al*. J Clin Microbiol. 2022; LFA seul, CAPA

Mucormycosis Something new ?



Method of Millon *et al.* :

R*hizomucor*, *Lichtheimia* et *Mucor*/Rhizopus

robustness of the method (CIL)

Se 85,2%/ Sp 89,8% (MODIMUCOR trial)

Commercialized methods :

Good performance

Internal PCR control

More Genus

Order specific

MycoGENIE with Aspergillus

	MucorGenius® Real-Time PCR	MycoGENIE® Aspergillus Species—Mucorales Species	Fungiplex® Mucorales RUO PCR Kit
Diagnostic specimens	Bronchoalveolar lavage Biopsy samples, paraffin embedded Serum	Serum Biopsies Lower respiratory tract samples	Not specified
Species detected	*Rhizopus* spp. *Mucor* spp. *Lichtheimia* spp. *Cunninghamella* spp. *Rhizomucor* spp.	*Rh. Pusillus* *M. indicus* *M.circinelloides* *M.plombeus* *R. arrhizus* *R. stolonifera* *L. corymbifera* *L. glauca* *C. bertholletiae* *Mycotypha* sp.	*Rhizopus* spp. *Lichtheimia* spp. *Cunninghamella* spp. *Rhizomucor* spp. *Mucor* spp. *Actinomucor* spp. *Apophysomyces* spp. *Saksenaea* spp. *Syncephalastrum* spp.
Manufacturer	PathoNostics	Ademtech	Bruker
Reference	[23,24,25]	[26]	[27]

Dannaoui. J. Fungi. 2022

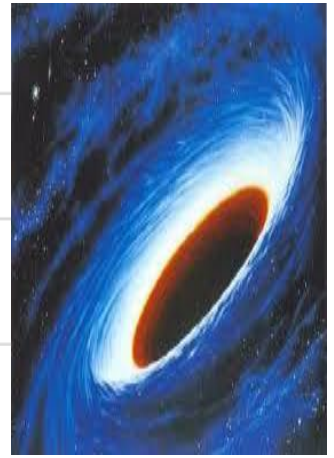
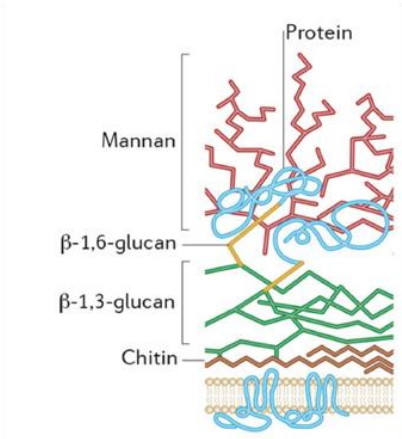
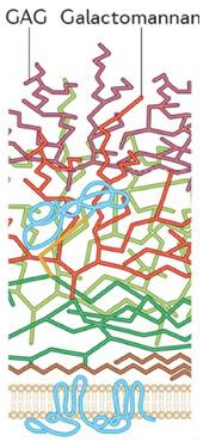
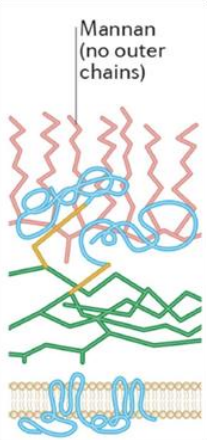
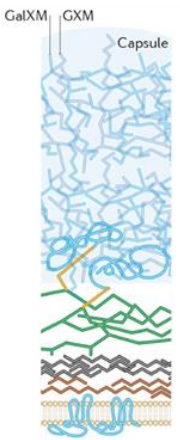
Interpretation Ct > 35

Se PCR >> Se Culture

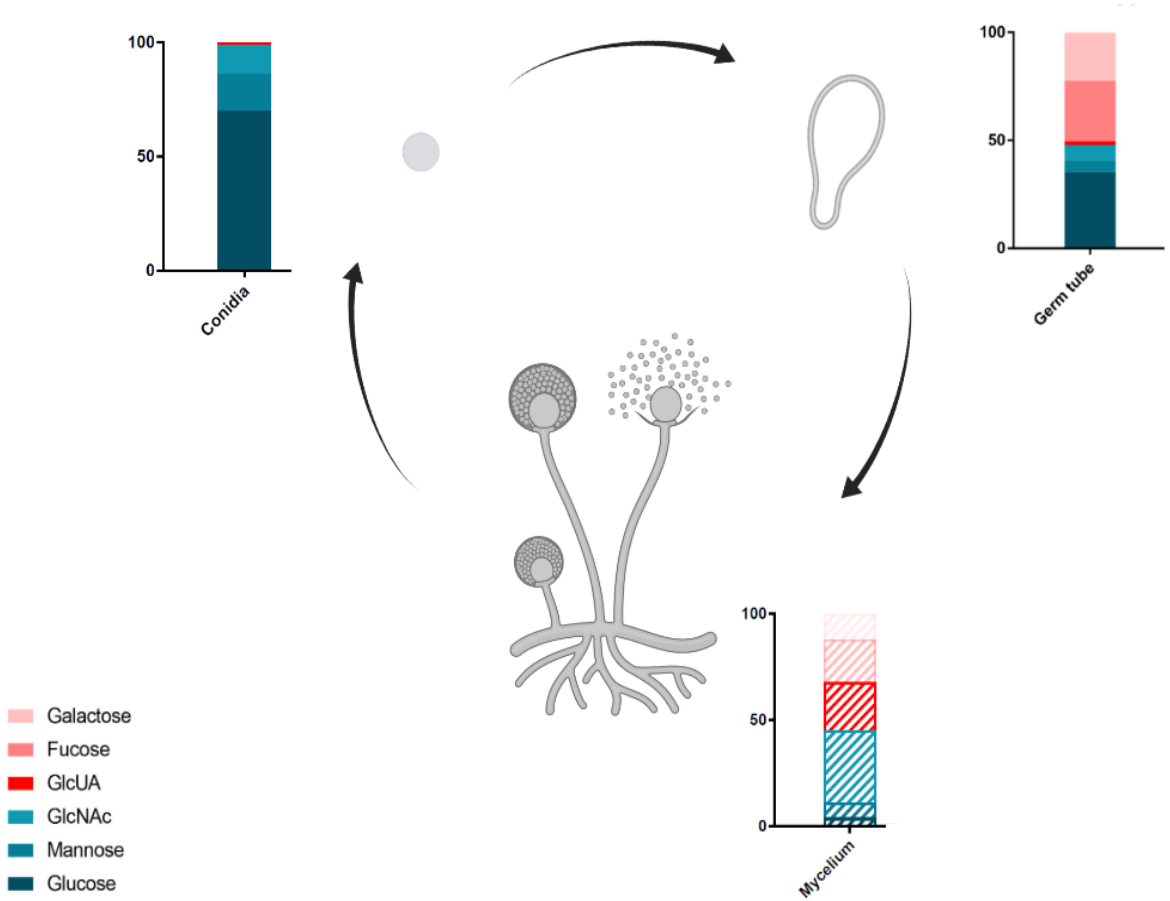
PCR not include in diagnostic criteria of IFI

Early diagnosis, prognosis, non invasive

Biomarkers in mucormycosis : BIM study

Biomarqueurs	Candidose invasive	Aspergillose invasive	Pneumocystose	Cryptococcose	Mucormycose
Galactomannane		✓			
Mannane	✓				
Glucurono-xylomannane				✓	
(1,3)-β-D-glucane	✓	✓	✓		
Organisation structurelle de la paroi fongique (Gow *et al.* 2017)					?

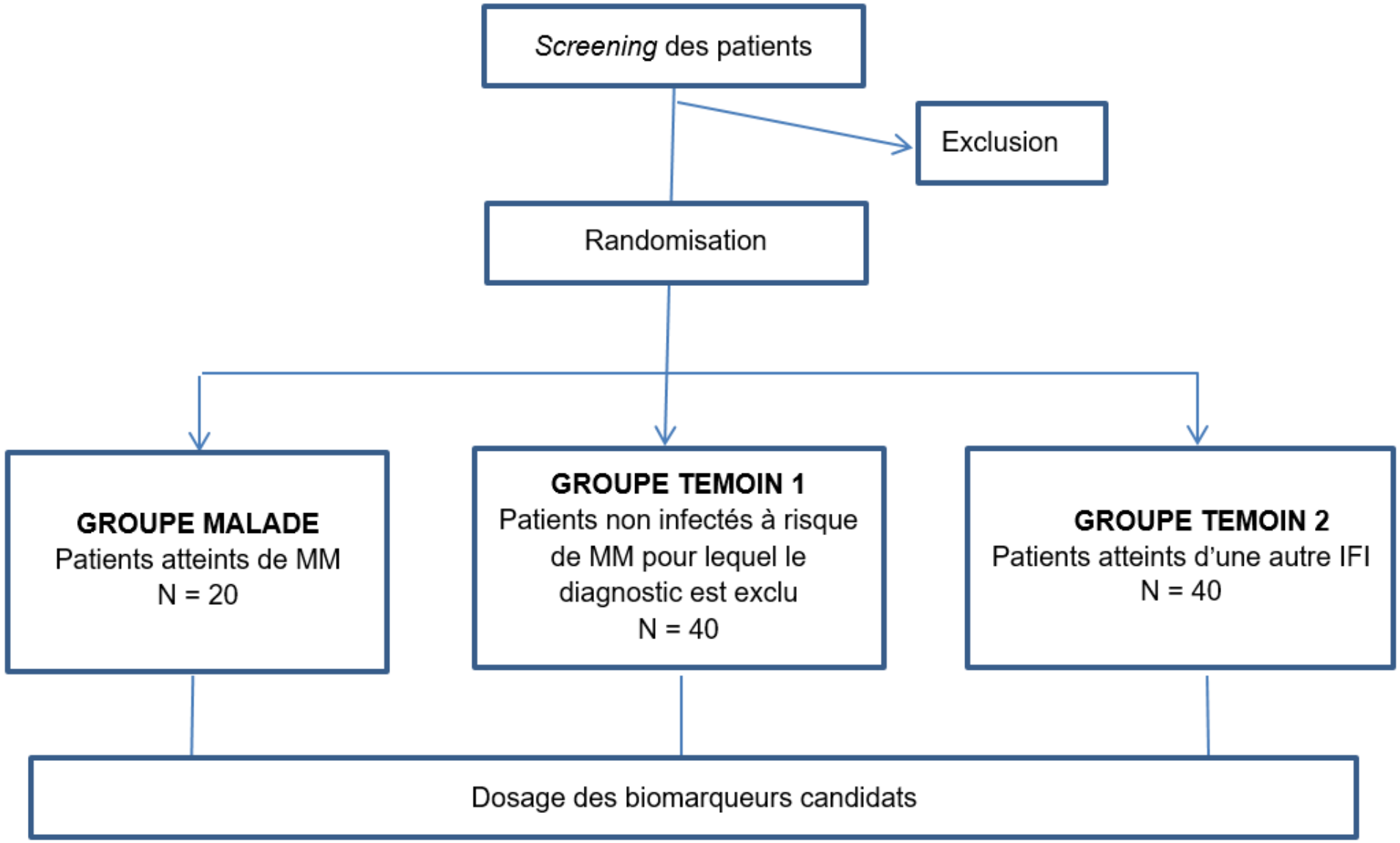
Biomarkers in mucormycosis : BIM study



Production of a monoclonal antibody directed against extracellular polysaccharides from *R. arrhizus*
ELISA test

Evaluation on patients sera from Lille and Amiens
Prospective inclusion of cases and controls (infected and non infected)
Samples at different time point

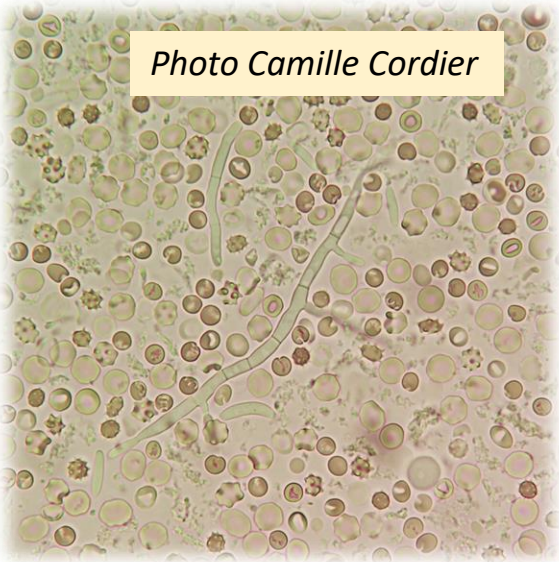
Biomarkers in mucormycosis : BIM study



Fusarium PCR
Not so bad !

Photo adelaide.edu.au

Photo Camille Cordier



In-house PCR

APHP St-Louis

Retrospective study : 15 patients (sera and biopsies)

Sensitivity 93% in sera, 100% in biopsies

Biomarkers : Sensitivity GM 7,1% (but aspergillosis co-infection), BDG 54,5%

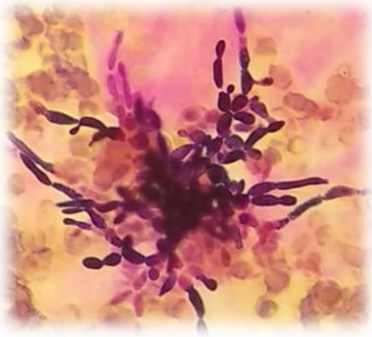
May have prognosis value ? (High fungal load, persistance)

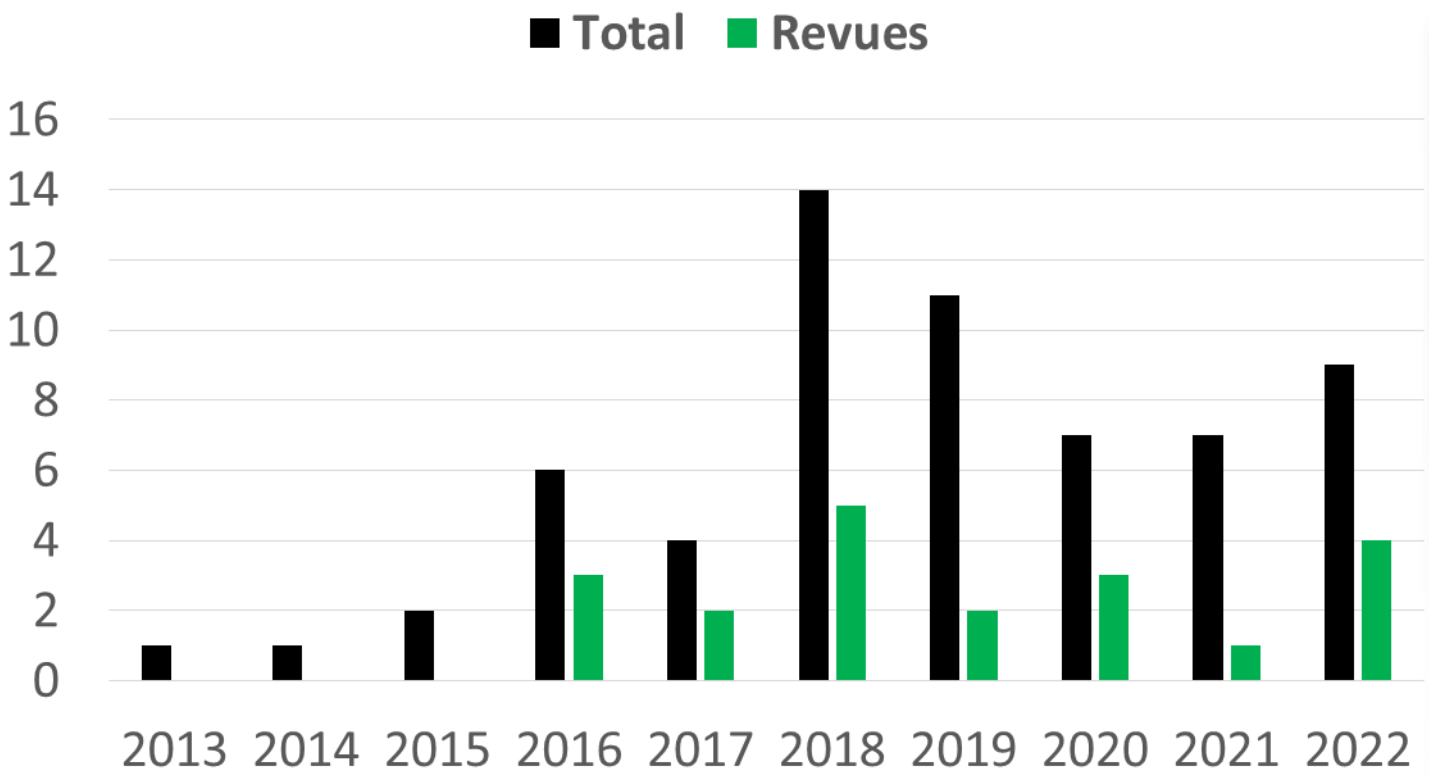
Non invasive (circulating DNA)

Prospective study ?

Dellière et al. Med Mycol. 2022

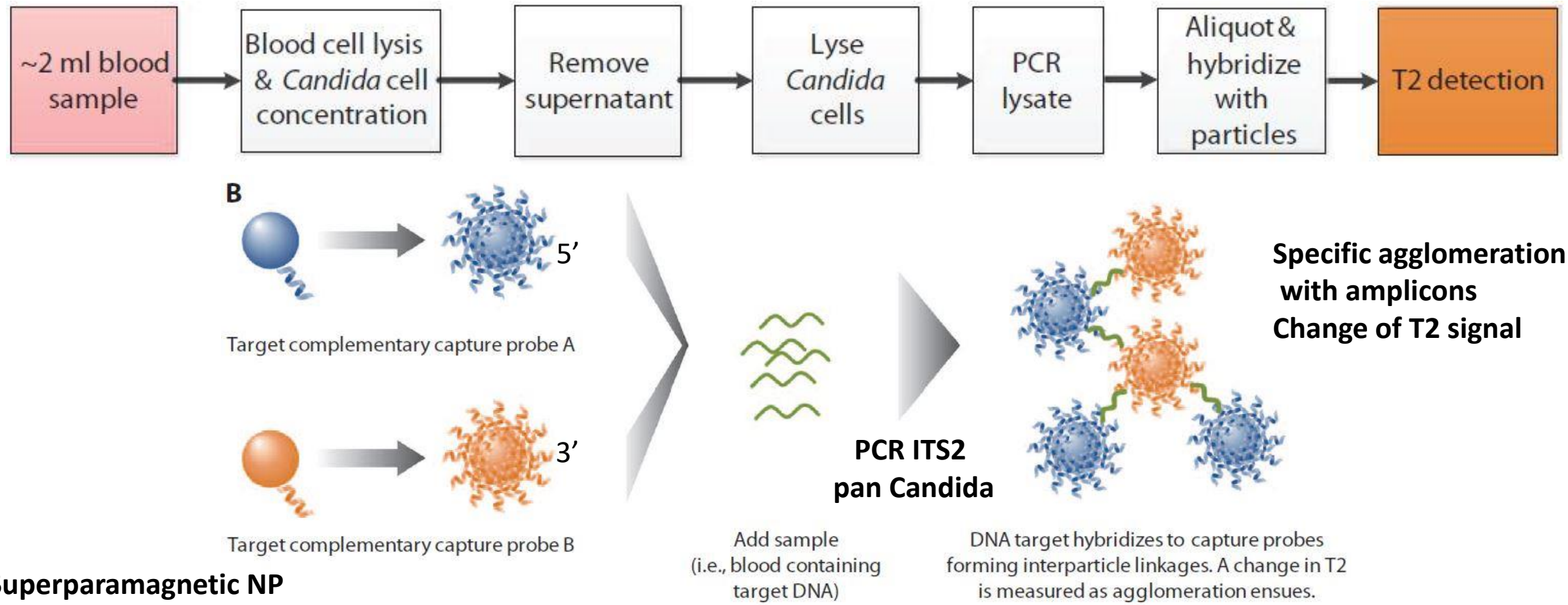
How to diagnose invasive candidiasis in 2023





Pubmed 58 articles

T2Candida :
The principle : Nanoparticles coupled to PCR



Superparamagnetic NP
Coupled with specific species probes

Neely *et al.*. Sci Transl Med. 2013

T2*Candida* : How does it work ? It works alone !

Repetability : CV 0,23%
Intermediate precision : CV 0,45%
Reproducibility inter-instrument: CV 2,57%

Save time x 10 ***vs.*** **Hémoculture**
LOD: 1 à 3 CFU/mL ***vs.*** **1 à 100 CFU/mL**
vs. **100 à 1000 PCR**

? CA/CT CG/CK CP

<3h

Sample holding

Lysis buffer

Reagents

Connection

Introduction in the drawer

Neely *et al.*. Sci Transl Med. 2013; Zervou *et al.*. Methods Mol Biol. 2017

T2*Candida* : our experience in ICU

- Prospective observational study
- 62 patients / 4 months => 38 suspicions of IC
- (Median sofa score =10, SAPSII = 54.5, Candida score =2)
- BC *vs*. T2*Candida*, Mannans
- 69 samples :
  - 7 BC + => T2MR +
  - 12 T2MR + => 5 false positive ? from 3 patients dont 2/3 Mannan +

- Prevalence: 10,1%

Se=100%	**PPV= 58,3%**
Sp=91,9%	**NPV= 100%**

Pr S. Nseir, Dr A. Rouzé,
Dr O. Pouly, Pr J.Poissy
Presented at ESICM

T2*Candida* in summary

- **Benefits**
  - T2MR *vs*. BC :
    - Sensitivity, time to result
  - T2MR *vs.* PCR
    - Technical time, time to result, LOD, PPV
  - Invasive candidiasis diagnosis
    - Candidemia, abdominal candidiasis
  - Follow-up, prognosis value
    - Persistence = complications
  - Aqueous humor, CSF, other
  - Medico-economic aspects

- **Limits**
  - Does not replace BC !

    => 5 species only and absence of AFST
  - What about discordants results ?

    Gold standard= BC?
  - % invalid results (Pediatrics ++)
  - Instrument failure +++, Delay in delivery of reagents
  - Only 7 drawers
  - Cost

    Selection of patients with the highest pre-test probability

    Each center/service must know the epidemiology and prevalence of invasive candidiasis

Take home messages

- No perfect diagnostic solution
- Diagnostic performance varies depending on context and tests
- Early diagnosis, follow-up, prognosis
- Some revolutions (circulating fungal DNA detection)
- Target prescriptions, be smart with interpretation

- WGS/NGS: the future ???