DES de Néphrologie : Enseignement l'Option SOINS INTENSIFS en NÉPHROLOGIE

Outils diagnostiques des agents infectieux – focus sur le diagnostic fongique

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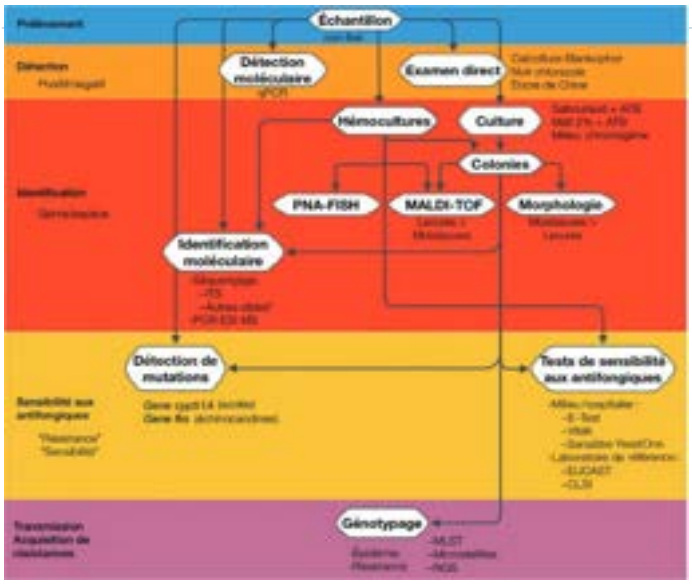
31/01/23

OBJECTIFS du COURS

A. Connaître les différents outils disponibles pour le diagnostic des infections fongiques
B. Appréhender les limites et les avantages des différents outils

Démarche diagnostique pour les infections fongiques



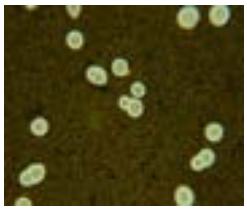
Alanio, A. & Bretagne, S. Nouvelles stratégies pour le diagnostic microbiologique des mycoses viscérales: Anticiper le diagnostic et adapter au plus vite les traitements. Rev Prat 65, 1325–1326 (2015).

Identification

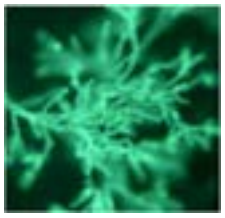
- **Examen direct**
  - **Levures** +/-filament
  - Champignons filamenteux (*Aspergillus* spp. vs. mucorales)
- **Culture**
  - **Identification** des colonies
    - Phénotypes (morphologie, assimilation des sucres, MALDIToF)
    - Moléculaire
  - **CMI** (détection de mutations par biologie moléculaire)
  - **Genotypage**
- **Anatomo-pathologie**

Ask the laboratory for Mycology Direct Examination
<15 minutes

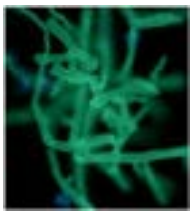
Yeast= filamentous yeast (*C. albicans)*



Capsule = *Cryptococcus*



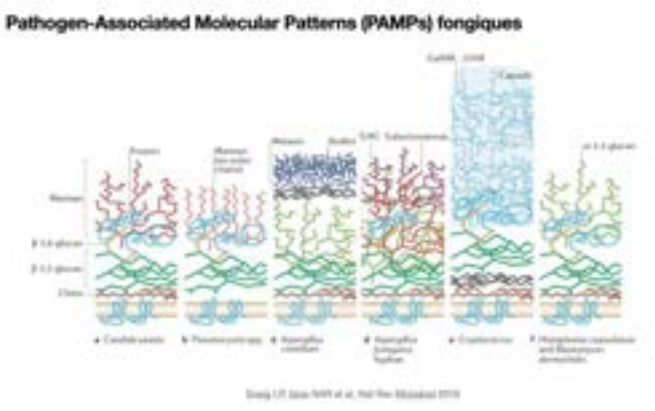
Thin regular hyphae = *Aspergillus* like



Thick irregular hyphae = Mucorales like

Current non cultural biomarkers

- Ag
  - GM
  - BDG
  - Mn *Candida* spp.
  - GMX *Cryptococcus*
  - *Histoplasma* sp.
- DNA
  - *Aspergillus* spp.
  - Mucorales
  - Histoplasma
  - Fusarium
  - *Candida* spp.



QCM auto-évaluation 1 (exemple)

Enoncé 1 : Vous souhaitez chercher une aspergillose invasive sinusienne

Q1 – Quel examen biologique souhaitez vous prescrire en priorité?

A. PCR Aspergillus fumigatus
B. Prélèvement sinusien orienté par examen ORL
C. Ecouvillons naso pharyngé
D. Antigène galactomanne sérique

Mycological evidence
Any mold, for example, *Aspergillus*, *Fusarium*, *Scedosporium* species or Mucorales recovered by culture from sputum, BAL, bronchial brush, or aspirate
Microscopical detection of fungal elements in sputum, BAL, bronchial brush, or aspirate indicating a mold
Tracheobronchitis
Aspergillus recovered by culture of BAL or bronchial brush
Microscopic detection of fungal elements in BAL or bronchial brush indicating a mold
Sino-nasal diseases
Mold recovered by culture of sinus aspirate samples
Microscopic detection of fungal elements in sinus aspirate samples indicating a mold
Aspergillosis only
Galactomannan antigen
Antigen detected in plasma, serum, BAL, or CSF
Any 1 of the following:
Single serum or plasma: ≥1.0
BAL fluid: ≥1.0
Single serum or plasma: ≥0.7 and BAL fluid ≥0.8
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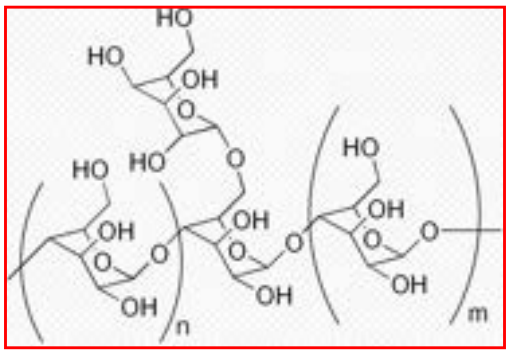
Aspergillus PCR
Any 1 of the following:
Plasma, serum, or whole blood 2 or more consecutive PCR tests positive
BAL fluid 2 or more duplicate PCR tests positive
At least 1 PCR test positive in plasma, serum, or whole blood and 1 PCR test positive in BAL fluid
Aspergillus species recovered by culture from sputum, BAL, bronchial brush, or aspirate

Donnely P et al. CID 2020

Galactomannan



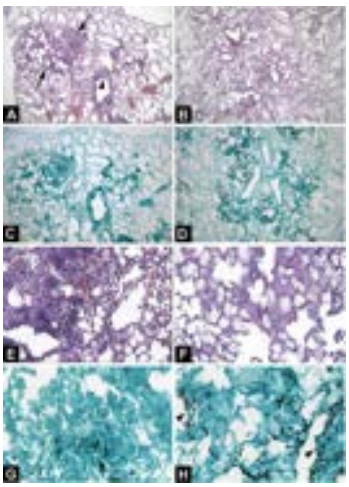
Polysaccharides consisting of a mannose backbone with galactose side groups

Meta-analysis (Pfeiffer et al, CID, 2006)

	Cases of proven IA			
Studies	TP/(TP + FP)	Pooled sensitivity (95% CI)	TN/(TN + FP)	Pooled specificity (95% CI)
All	163/229	0.71 (0.68–0.74)	3601/4055	0.89 (0.88–0.90)
Studies limited to patients with hematological malignancy	106/152	0.70 (0.62–0.77)	2570/2808	0.92 (0.90–0.93)
Studies limited to patients undergoing BMT	49/60	0.82 (0.70–0.90)	722/843	0.86 (0.83–0.88)
Studies limited to solid-organ transplant recipients	2/9	0.22 (0.03–0.60)	180/215	0.84 (0.78–0.88)

Differences in Patterns of Infection and Inflammation for Corticosteroid Treatment and Chemotherapy in Experimental Invasive Pulmonary Aspergillosis

- Corticosteroid-treated mice :
  - A : large foci of pneumonia and exudative bronchiolitis with the destruction of bronchi and alveolae.
  - E : Hemorrhagic necrosis with neutrophil infiltration was also observed.
  - G : *A. fumigatus* was observed in small numbers
- Chemotherapy-treated mice
  - B : only a few bronchiolitis lesions and diffuse pneumonia with edema and congestion within alveolae
  - F : with no inflammatory exudate involving PMN or other cells
  - D and H : alveolae and parenchyma invaded by numerous hyphae of *A. fumigatus*



Balloy et al Infect Immunity 2005; Stergiopoulou et al Am J Clin Pathol 2007

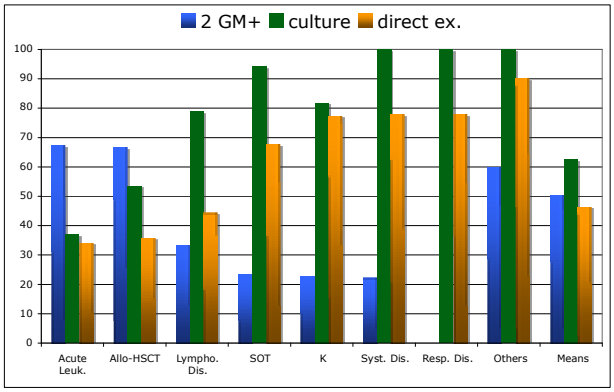
Comparison of galactomannan indices according to neutropenia and steroids use

GM index	Group 1 (n = 18) <100 PMN	Groups 2 + 3 (n = 81) ≥100 PMN	p
GM index ≥1	8 (44.4)	8 (9.9)	0.001[a]
GM index ≥0.7	8 (44.4)	12 (14.8)	0.009[a]
GM index ≥0.5	11 (61.1)	15 (18.52)	0.001[a]
GM index, median (range)	0.61 (0.08–5.84)	0.19 (0.04–5.31)	0.01[b]
Steroid administration			
Yes	4.63 (4.37–5.84)	0.2 (0.04–1.93)	0.001[b]
No	0.44 (0.08–2.48)	0.18 (0.04–5.31)	0.19[b]
Potentially GM-contaminated antibiotic(s) use			
Yes	4.36 (0.11–5.84)	0.24 (0.04–5.31)	0.76[b]
No	0.57 (0.08–4.96)	0.18 (0.04–2.01)	0.003[b]
Antifungal therapy			
Yes	0.54 (0.08–2.48)	0.17 (0.1–2.29)	0.694[b]
No	1.21 (0.1–5.84)	0.20 (0.04–5.31)	0.006[b]

[a]p-value of Fischer's exact test.
[b]p-value of the non-parametric Kruskall-Walls test.

C. Cordonnier et al, CMI 2009

Lower yield of GM in SOT recipients



Decrease of GM yield in Lymphoprolierative Diseases and other categories [1]
- poor performances in SOT [2]
- neutropenia/steroid ratio [3]

Lortholary et al CMI 2011

What do we call false positives?

- Unreproducible positive results
  - False+ves due to the GM kit
  - Each positive serum must be tested twice! (Guigue et al Plos One 2015)
- Wrong false+ves in non diagnosed invasive aspergillosis
  - Non pulmonary aspergillosis (Pautas *et al*, J Infection 2001)
- False+ves related to other sources of GM than infection
  - Digestive absorption (children)?
  - Antibiotics (Ansorg R, Mycoses, 1997)
  - Massive and variable contamination of piperacilline-tazobactam batches (Adam, O, *et al* CID 2004; Viscoli C, *et al* CID 2004; Walsh TJ, *et al* JCM 2004)

Factors that influence GM performance

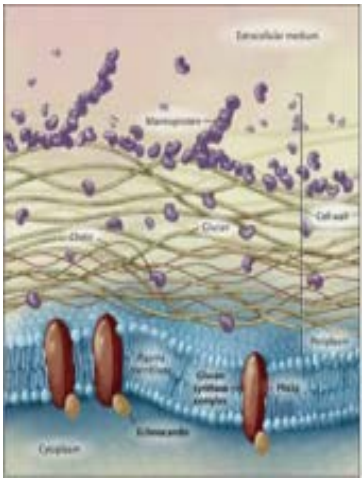
Table 2 Biological and epidemiological factors that influence the performance of GM detection in invasive aspergillosis[3]

Biological factors	*Epidemiological factors*
Site of infection	Patient population
Aspergillus species causing infection	Sampling strategy
Microenvironment at the site of infection: nutrients, oxygen level, pH	Definition of a positive result
Exposure to antifungal agents	Definition of an IFD
Molecular structure of released galactomannan	Prevalence of IFD
Underlying condition/neutropenia/ level of immunosuppression	Cutoff for positivity
Renal clearance, hepatic metabolism	Laboratory experience
Circulating galactomannan antibodies	Nutritional factors (galactomannan-containing food)
Storage of clinical sample	Treatment with semi-synthetic β-lactam antibiotics
Pre-analytical treatment procedure	

Marchetti et al BMT 2011

(1,3)beta-D-Glucan

- Therapeutic target of echinocandins
- Ag common to most of the fungal species (excepted cryptococcus et Mucorales)
  - *Candida, Saccharomyces, Aspergillus, Fusarium, Acremonium, …*
  - *Pneumocystis jirovecii*



Bennet, NEJM, 2006

β-D-Glucan Assay for the Diagnosis of Invasive Fungal Infections: A Meta-analysis

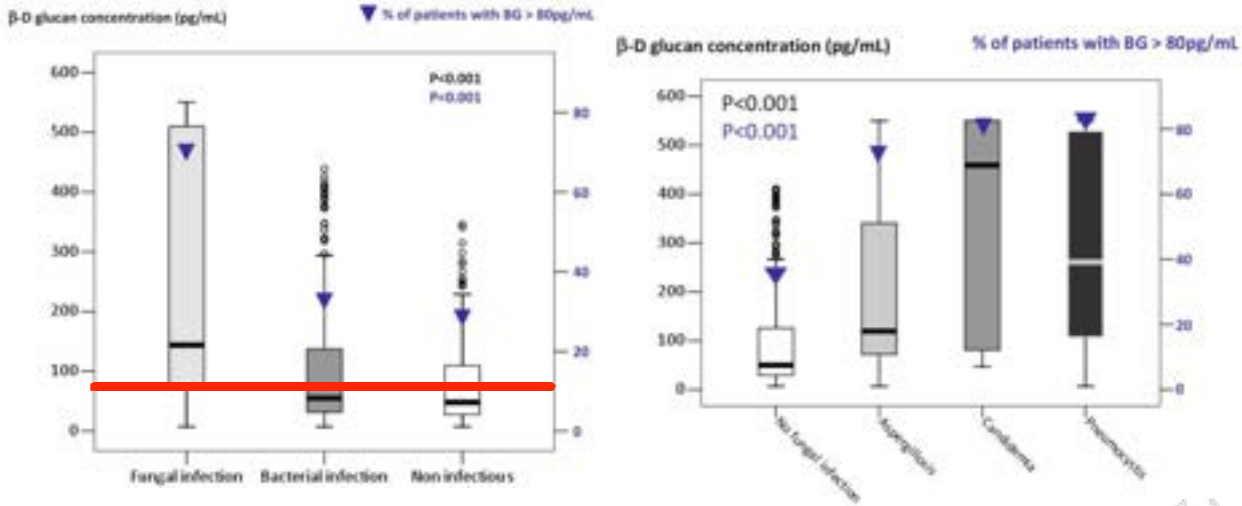
Drosos E. Karageorgopoulos,[1,2] Evridiki K. Vouloumanou,[1] Fotinie Ntziora,[1,2] Argyris Michalopoulos,[1,2] Petros I. Rafailidis,[3,4] and Matthew E. Falagas[1,4,5]

[1]Alfa Institute of Biomedical Sciences; [2]Department of Medicine, Laikon General Hospital, and [3]Intensive Care Unit and [4]Department of Medicine, Henry Dunant Hospital, Athens, Greece; and [5]Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts

- 2979 patients, 594 proven or probable IFI, 16 studies
- Sensitivity: 76.8% [CI: 67.1-84.3%]
- Specificity: 85.3% [CI: 79.6-89.7%]
- Conclusion: « It can be useful in clinical practice, if implemented in the proper setting and interpreted after consideration of its limitations »
- Highly heterogeneous studies

Karageorgopoulos et al CID 2011

ß-D-glucans in ICU



Azoulay et al., Oncotarget 2016

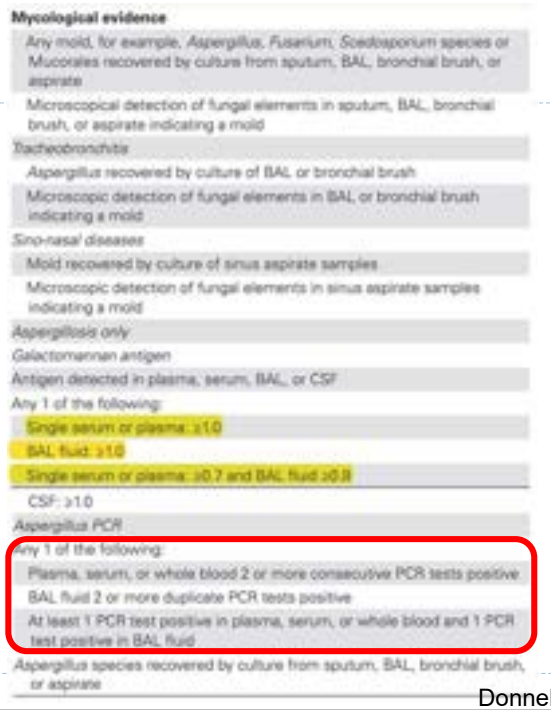
BDG - in 2/13 proven PC
84% sensitivity

False positives

Treatments	Immunoglobulines Albumin Coagulation factors Antibiotics (piperacillin – tazobactam) Others? (chemotherapies …)
Patient cares	Hemodialysis with cellulose membranes Gauze or other materials that contain glucans Tubes handling
Bacterial infections	Gram negative bacteria Some streptococci
Patient linked	Mucosal damages (yeast colonization) Hemolytic or lipemic samples …

Pickering JW et al, JCM 2005, 43 : 5957-62

DNA detection



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Donnely P et al. CID 2020

Perspectives PCR

- Questions techniques “résolues”
  - qPCR (validation MIQE)
  - Prévention enzymatique (UNG)
  - Emploi impératif d’un contrôle interne (pas d’ADN humain)
  - Extraction d’ADN: dépend de l’échantillon et donc de votre stratégie diagnostique
- Questions techniques en suspens
  - ADN cible (pan-fongique versus espèce-spécifique)
- Questions non résolues
  - Origine de l’ADN détecté
  - Et donc meilleur échantillon clinique
  - Et donc la meilleure technique d’extraction
- Kits commerciaux (en cours mais pas forcément mieux que des PCR maison validées)
- Intégration des qPCR fongiques dans la prise en charge des patients

Biomarker performances in IFD

Biomarkers and Aspergillosis

Table 3. Performance of Fungal Biomarker Assays for the Detection of Invasive Aspergillosis as Determined by the Relevant Published Meta-analyses

	Assay												
	GM-EIA				β-Glucan				PCR				
	Leeflang [77]	Pfeiffer [80]	Zou [5]	Guo [60]	Lamoth [9]	Karageorgopoulos [10]	He [11]	Onishi [12]	Tuon [15]	Sun [16]	Avni [18]	Mengoli [17]	Arvanitis [19]
Parameter	Serum		BAL		Plasma/Serum				BAL			Blood	
Sensitivity, %	79.3	79.3	83.6	85.7	56.8	77.1	77.0	77.3	78.4	79.6	76.8	88.0	84.0
Specificity, %	80.5	86.3	89.4	89.0	97.0	85.3	81.3	83.4	93.7	94.1	94.5	75.0	76.0
PPV, %	30.3	46.9	64.4	66.7	55.6	30.2	28.7	33.4	74.1	79.2	79.9	36.7	41.3
NPV, %	97.3	98.5	95.9	96.0	97.1	97.8	97.3	97.2	94.9	94.2	93.4	97.4	95.9
Positive LR	4.06	5.81	7.89	7.81	18.90	5.24	4.12	4.65	12.43	13.38	13.87	3.52	3.50
Negative LR	0.26	0.24	0.18	0.16	0.44	0.27	0.28	0.27	0.23	0.22	0.25	0.16	0.21
DOR	15.8	24.2	43.0	48.6	43.0	19.4	14.7	17.2	53.8	61.7	56.52	22.1	16.6

Biomarkers and candidemia

Table 3. Sensitivity of (1→3)-β-D-Glucan (BDG) Testing to Detect Proven or Probable Systemic *Candida* Infection in Comparison with Invasive Aspergillosis As Reported in Different Studies

Study	Cutoff(pg/mL)	Systemic Candida infections, proportion (%)	Invasive aspergillosis, proportion (%)
Hachem et al 2009 [27]	80 (2 consecutive values)	13/21 (62)	14/21 (67)
Koo et al 2009 [28]	80	26/41 (63)	24/32 (75)
Obayashi et al 2008 [19]	30	3/3 (100)	28/28 (100)
Persat et al 2008 [31]	80	22/26 (85)	48/70 (69)
Senn et al 2008 [32]	7 (2 consecutive values)	10/17 (59)	9/15 (60)
Akamatsu et al 2007 [33]	40	7/14 (50)	5/5 (100)
Ostrosky-Zeichner et al 2005 [36]	80	83/107 (78)	8/10 (80)
Odabashi et al 2004 [20]	60	9/11 (82)	4/4 (100)
Mori et al 1997 [43]	1000	11/12 (92)	4/4 (100)
Mitsutake et al 1996 [44]	60	27/32 (84)	5/5 (100)
Miyazaki et al 1995 [45]	10	11/11 (100)	3/3 (100)
Total from all studies[a]	...	222/295 (75)	152/197 (77)

[a] Total represents cumulative data

No difference between *Candida* and *Aspergillus* [1]

[1] *Karageorgopoulos et al CID 2011*

Prospective Evaluation of Serum β-Glucan Testing in Patients With Probable or Proven Fungal Diseases

Table 2. Comparison of Demographic and Clinical Characteristics of the Patients With Candidemia Who Tested Positive or Negative for BG at the Time of Diagnosis

	Total (N = 41)	Patients With Negative BG at TOD (n = 15) (%)	Patients With Positive BG at TOD (n = 26) (%)	P Value[a]	Multivariate OR[b]	P[b]
Sociodemographic data						
Gender						
Female	18	8 (40.0)	10 (38.5)	1.00		
Male	25	9 (60.0)	16 (61.5)			
Average age (years) (range)	40.8 (0, 77)	34.5 (1–75)	44.2 (0–77)	.28		
Medical data						
Category of patient						
ICU pediatric patients	13	7 (46.7)	6 (23.1)	.17	1.0	0.09
Hematology or Renal transplant adult patients	28	8 (53.3)	20 (76.9)		4.7 (0.9–26.4)	
Candida species responsible for candidemia						
Non-albicans Candida species	25	14 (93.3)	11 (42.3)	<.01	1.0	0.0
Candida albicans	16	1 (6.7)	15 (57.7)		25.4 (3.6–680.3)	
Median time interval between blood culture sampling and yeast growth (hours) (range)	31 (10–67)	29 (15–60)	34 (10–67)	.37		
Median duration of candidemia (days) (range)	2 (1–11)	2 (1–5)	1.5 (1–11)	.84		
Use of catheter at time of 1st positive blood culture	39	14 (93.3)	25 (96.2)	1.00		
Positive culture of catheter	11	4 (26.7)	7 (26.9)	.75		
Systemic antifungal drugs before candidemia	17	6 (40.0)	11 (42.3)	1.00		
Outcome						
Positive	25	13 (86.7)	12 (46.2)	.02	—	
Worsening invasive candidiasis and/or death within 30 d	16	2 (13.3)	14 (53.8)			
Median time interval between candidemia and 1st BG sampling (day) (range)	2 (−3,7)	3 (−1,7)	2 (−3,7)	.21	—	
Neutropenia at time of candidemia	22	6 (40.0)	16 (61.5)	.21	—	
Albumin during 1 mo before candidemia	3	1 (6.7)	2 (8.0)	1.00		
Surgery within 15 d before candidemia	8	2 (13.3)	6 (23.1)	.69		
Mucitis or digestive GVH disease within 15 d before candidemia	15	5 (33.3)	10 (38.5)	1.00		
Hemodialysis within 15 d before candidemia	8	1 (6.7)	7 (26.9)	.22	—	

Biomarkers and candidemia

Prospective Evaluation of Serum β-Glucan Testing in Patients With Probable or Proven Fungal Diseases

Cécile Angebault,[1,2] Fanny Lanternier,[1,3,4] Frédéric Dalle,[5] Cécile Schrimpf,[6] Anne-Laure Roupie,[7] Aurélie Dupuis,[1] Aurélie Agathine,[1] Anne Scemla,[1,2,3] Etienne Poulalle,[1,2] Denis Caillot,[8] Bénédicte Neven,[9] Pierre Frange,[3,4] Felipe Suarez,[1,2] Christophe d'Enfert,[6,10] Olivier Lortholary,[1,3,4] and Marie-Elisabeth Bougnoux[1,2,3,10]

Results. (1-3)-β-D-glucan was undetectable at TOD in 36% and 48% of patients with candidemia and IA, respectively; there was no correlation between negative BG results at TOD and patients' characteristics, localization of infection, or prior antifungal use. Nevertheless, patients with candidemia due to *Candida albicans* were more likely to test positive for BG at TOD (odds ratio = 25.4, $P = .01$) than patients infected with other *Candida* species. In 70% of the patients with a follow-up, BG negativation occurred in >1 month for candidemia and >3 months for IA. A slower BG decrease in patients with candidemia was associated with deep-seated localizations ($P = .04$). Thirty-nine percent of patients with rare IFD had undetectable BG at TOD; nonetheless, all patients with chronic subcutaneous IFD tested positive at TOD.

Angebault et al, OFID 2017

Biomarkers and PCP

Parameter	Study		
	Summah[a]	Fan	Lu
Cases/Total (n/N)	506/2330	606/1793	416/2505
Sensitivity (%)	97	98	99
Specificity (%)	94	91	90
PPV (%)	82	85	66
NPV (%)	99	99	>99
LR +tive	16.2	10.9	9.9
LR -tive	0.03	0.02	0.01
DOR	540	545	990

Upper respiratory tract specimens - Induced Sputum * Se: 99% Sp: 96%

Performance similar irrespective of HIV status – FPCRI meta-analysis (in preparation)

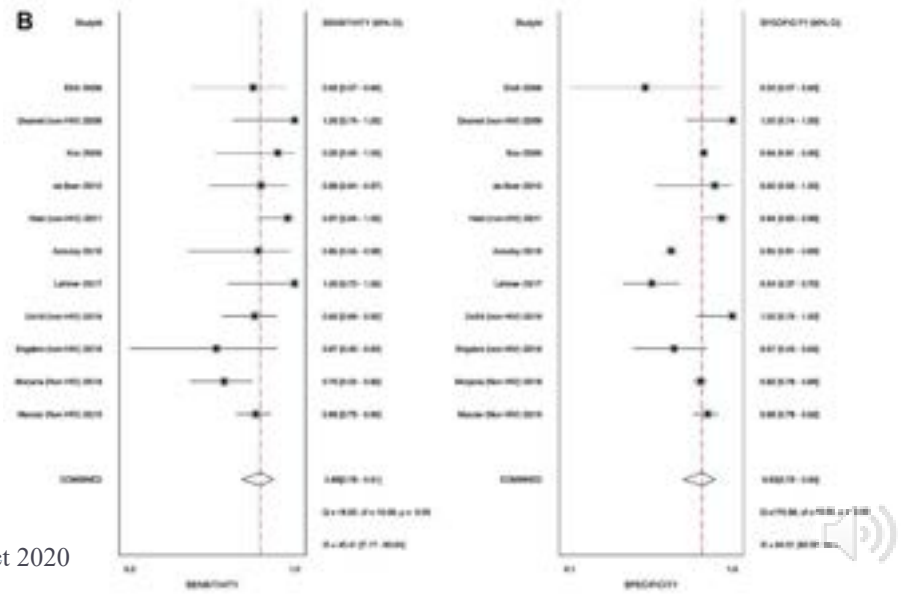
Summah *et al* Chinese Medical Journal: May 2013 126:10 -1965-1973; Fan *et al* PLoS One.2013;8(9):e73099., Lu *et al* J Clin Microbiol. 2011 Dec; 49(12): 4361–4363; *Senecal et al CMI 2022

Biomarkers and PCP

Publications (<sept 2019)

86% sensitivity

83% specificity



Corpo et al., Clin Microb Infect 2020

Biomarkers and PCP

BDG for diagnosis of *Pneumocystis* pneumonia (PcP):

- Some novelties in cancer patients…

Study	Gold standard	Sensitivity[1]	Specificity[1]	PPV[1]	NPV[1]
Morjaria et al. (CID 2019)	PCR+	**70%**	81%	35%	95%
Szvalb et al. (J Infect 2020)	PCR 1000 cp/ml	**75%**	78%	11%	99%
Mercier et al. (J Fungi	PCR+ and radiology	**77%**	82%	NA	NA
Corpo et al. (CMI 2020)	Meta-analysis	**86%**	83%	85%[2]	95%[2]

[1] For BDG cut-off recommmended by manufacturer (Fungitell: 80 pg/ml)
[2] For a pre-test probability of 50%

High pre-test probability required to achieve acceptable PPV

Low pre-test probability required to achieve 95-100% NPV

⇒ **A negative BDG cannot reliably rule out PcP if pre-test probability is intermediate/high**

QCM auto-évaluation 2

Q2 - Parmi ces tests, lequel aura les meilleurs performances diagnostiques chez le patient transplanté?

A. - PCR pneumocystis dans un LBA
B. - PCR pneumocystis dans un prélèvement naso pharyngé
C. - Antigène galactomannane sérique
D. - BD-glucan
E. - Hémoculture

Conclusion

- Do not neglect **classical mycology** (direct examination, culture)
  - Identification of new species
  - MIC, resistance
- Biomarkers
  - Acknowledge the limits of **glucan and galactomannan** detection
  - Specially in SOT recipients (prevalence of the IFD, risk factors (steroids>neutropenia)
  - Think twice before implementing routine screening tests
  - **Prevalence of the disease (>5%)**
  - *Aspergillus* PCR are included in the EORTC criteria
  - Added value of **combined tests: diagnosis yes**; screening ??
- General considerations
  - Epidemiological trends, new treatments, new other markers …
  - Dialogue biologists-clinicians

References

- https://ecil-leukaemia.com/images/resources/2022part2/ECIL9_Update_on_fungal_diagnostics_Revised_Guidelines.pdf

- Donnelly JP, et al. 2019. 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. Clin Infect Dis 71:1367–1376.

Réponse aux QCM de l'auto-évaluation initiale

- Reprenez ici les 2 QCM des diapos 3 et 4 et indiquer les réponses

QCM auto-évaluation 1 (exemple)

Enoncé 1 : Vous souhaitez echercher une aspergillose invasive sinusienne

Q1 – Quel examen biologique souhaitez vous prescrire en priorité?

A. PCR Aspergillus fumigatus
B. **Prélèvement sinusien orienté par examen ORL**
C. Ecouvillons naso pharyngé
D. Antigène galactomanne sérique

QCM auto-évaluation 2

Q2 - Parmi ces tests, lequel aura les meilleurs performances diagnostiques chez le patient transplanté?

A. **- PCR pneumocystis dans un LBA**
B. - PCR pneumocystis dans un prélèvement naso pharyngé
C. - Antigène galactomannane sérique
D. - BD-glucan
E. - Hémoculture