Solid Organ Nakli Sonrası Gelişen İnvazif Fungal Enfeksiyonlar Toplantısı
24 Şubat 2018, İstanbul

Solid Organ Nakil Hastalarında İnvazif Fungal Enfeksiyonların Mikolojik Tanısı

Prof. Dr. Sevtap Arıkan Akdağlı
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Tıbbi Mikrobiyoloji AD

Sunum Planı

- Hangi mantarlar?

- Mikrobiyolojik Yöntemler
  - Direk mikroskopik inceleme
  - Kültür
  - Serolojik Yöntemler: Galaktomannan, Beta-D-glukan, Mannan-Anti Mannan
  - Moleküler Yöntemler

- Sonuç

SOT:
Kılavuz Önerileri
Uzman Görüşleri
Araştırma Sonuçları

İnvazif fungal enfeksiyonlar, çeşitli nedenlerden ötürü önem taşır

Olumsuz konak koşulları

Dirençli cins ve suşlar

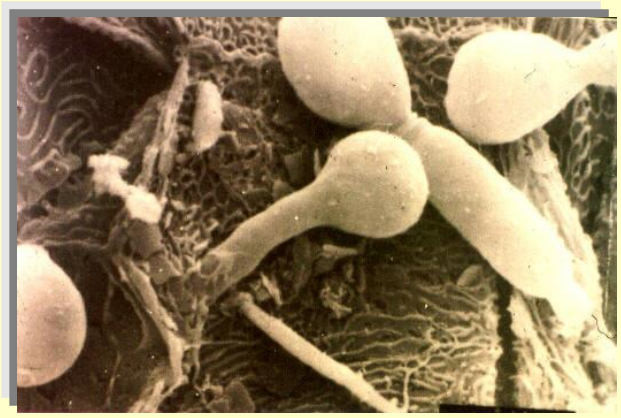
- Düşük klinik yanıt oranları
- Yüksek mortalite

Erken tanıda zorluklar

Antifungal tedaviye suboptimal yanıt

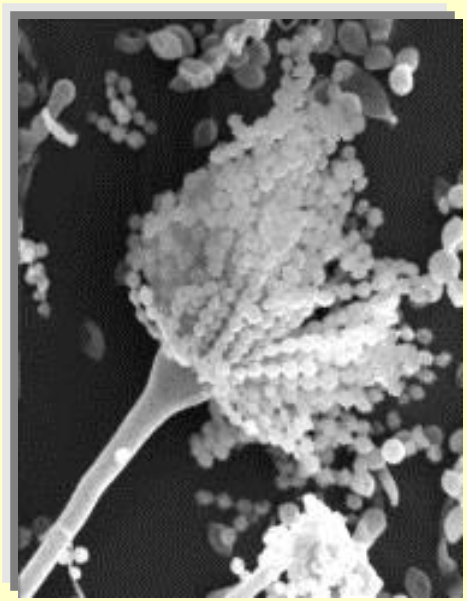
Richardson. JAC 2005; 56(Suppl. S1): i5; Bille et al. Curr Opin Infect Dis 2005; 18: 314

Fırsatçı İnvazif Mikozlar-Etkenler



- CANDIDA
- ASPERGILLUS
- DİĞER

C. neoformans
Mucorales
Fusarium
Scedosporium
Dematisiyöz küfler.....



Invasive fungal infections in solid organ transplant recipients

J. Gavaldà[1], Y. Meije[1], J. Fortún[2], E. Roilides[3], F. Saliba[4], O. Lortholary[5], P. Muñoz[6,7,8,9], P. Grossi[10], M. Cuenca-Estrella[11] on behalf of the ESCMID Study Group for Infections in Compromised Hosts (ESGICH)

Clin Microbiol Infect 2014; **20** (Suppl. 7): 27–48

İNVAZİF KANDİDOZ

TABLE 1. Risk factors for invasive candidiasis

Transplant type	Target population
Liver	**High-risk liver transplant recipients:** **Major:** MELD score >30 Re-transplantation, fulminant hepatic failure, Renal failure requiring replacement therapy, **Minor:** MELD score 20–30, split, living-donor >40 transfusion blood products, choledochojejunostomy (Roux-en-Y) Renal failure not requiring replacement therapy (CrCl <50 mL/min) Early re-intervention, multifocal colonization/infection by *Candida* spp.
Pancreas	Post-perfusion pancreatitis, acute rejection and poor initial allograft function Vascular thrombosis, enteric drainage, anastomotic problems, haemodialysis Laparotomy after transplantation
Intestinal	Acute rejection and poor initial allograft function, haemodialysis, laparotomy after transplantation, anastomotic problems, over-immunosuppression
Heart	Acute rejection, haemodialysis, re-exploration after transplantation

Cr CL, creatinine clearance; MELD, model for end-stage liver disease; over-immunosuppression (high immunosuppression drug levels, under corticoid bolus).

Invasive fungal infections in solid organ transplant recipients

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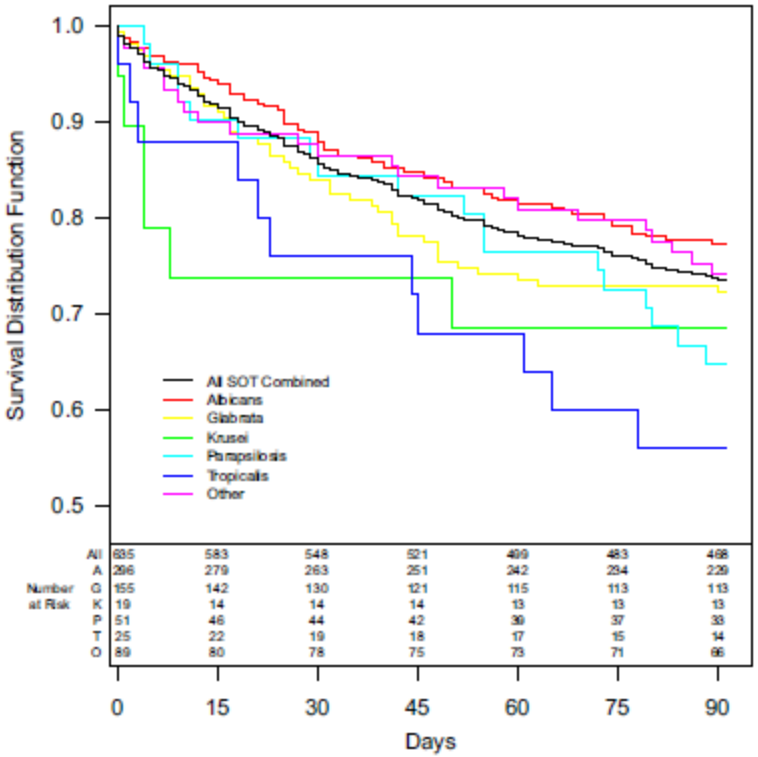
Clin Microbiol Infect 2014; **20** (Suppl. 7): 27–48

İNVAZİF ASPERGİLLOZ

TABLE 2. Risk factors for invasive aspergillosis

	Early IA	Late IA (>3 months post-transplant)
Liver transplant	Re-transplantation Kidney failure, especially post-transplant Haemodialysis Fulminant hepatic failure Complicated surgery or reoperation	More than 6 g of accumulative prednisone in the third month after transplantation Post-transplant renal failure Post-transplant haemodialysis Leukopenia (<500/mm^3) Chronic graft dysfunction
Lung transplant	Bronchial anastomotic ischaemia or bronchial stent placement Acute rejection Single-lung transplant *Aspergillus* spp. colonization before or during first year post-transplant	Chronic graft dysfunction
Heart transplant	*Aspergillus* spp. colonization of the respiratory tract Re-operation Post-transplant haemodialysis Hypogammaglobulinaemia (IgG < 400 mg/dl)	ICU readmission Kidney transplantation >2 acute rejection episodes
Kidney transplant	Graft lost and haemodialysis Post-transplant haemodialysis Prolonged high corticosteroid doses	
	CMV infection Over-immunosuppression	

The epidemiology and outcomes of invasive *Candida* infections among organ transplant recipients in the United States: results of the Transplant-Associated Infection Surveillance Network (TRANSNET)



David R. Andes[1] | Nasia Safdar[1] | John W. Baddley[2] | Barbara Alexander[3] | Lisa Brumble[4] | Allison Freifeld[5] | Susan Hadley[6] | Loreen Herwaldt[7] | Carol Kauffman[8] | G. Marshall Lyon[9] | Vicki Morrison[10] | Thomas Patterson[11] | Trish Perl[12] | Randall Walker[4] | Tim Hess[1] | Tom Chiller[13] | Peter G. Pappas[2] | The TRANSNET Investigators

Results: A total of 639 cases of IC were identified. The most common species was *Candida albicans* (46.3%), followed by *Candida glabrata* (24.4%) and *Candida parapsilosis* (8.1%). In 68 cases >1 species was identified. The most common infection site was bloodstream (44%), followed by intra-abdominal (14%). The most frequently affected allograft groups were liver (41.1%) and kidney (35.3%). All-cause mortality at 90 days was 26.5% for all species and was highest for *Candida tropicalis* (44%) and *C. parapsilosis* (35.2%). Non-white race and female gender were more commonly associated with

Transplant Infectious Disease 2016; 18: 921–931

Epidemiological features of invasive mold infections among solid organ transplant recipients: PATH Alliance® registry analysis

25 Medical Centers in US & Canada

Shahid Husain[1,*], Fernanda P. Silveira[2], Nkechi Azie[3], Billy Franks[3] and David Horn[4]

Table A1. Infections by type of solid organ transplant.

Type of organ transplant, *n* (%)	All	*Aspergillus*	*Mucorales*	Other mould	Unidentified mould	Multiple
Any	333 (100)	246 (73.9)	13 (3.9)	33 (9.9)	3 (0.9)	38(11.4)
Kidney	45 (100)	28 (62.2)	4 (8.9)	7 (15.6)	0	6 (13.3)
Liver	33 (100)	23 (69.7)	7 (21.2)	1 (3.0)	1 (3.0)	1 (3.0)
Lung	209 (100)	156 (74.6)	0	23 (11.0)	2 (1.0)	28 (13.4)
Heart	22 (100)	18 (81.8)	1 (4.5)	1 (4.5)	0	2 (9.1)
Small bowel	4 (100)	4 (100)	0	0	0	0
Multiple	20 (100)	17 (85.0)	1 (5.0)	1 (5.0)	0	1 (5.0)

"Prospective Antifungal Therapy Alliance"

Medical Mycology, 2017, 55, 269–277

IC

Balkan Med J 2013; 30: 282-6 • DOI: 10.5152/balkanmedj.2013.9256

Bloodstream Infections among Solid Organ Transplant Recipients: Eight Years' Experience from a Turkish University Hospital

Ayşegül Yeşilkaya[1], Özlem Kurt Azap[1], Melike Hamiyet Demirkaya[1], Mehtap Akçıl Ok[2], Hande Arslan[1], Aydıncan Akdur[3]

[1]*Department of Infectious Diseases and Clinical Microbiology, Başkent University Faculty of Medicine, Ankara, Turkey*
[2]*Department of Statistics and Computer Science, Başkent University Faculty of Science and Letters, Ankara, Turkey*
[3]*Department of General Surgery, Başkent University Faculty of Medicine, Ankara, Turkey*

Table 1. Characteristics of the recipients of a solid organ transplantation with bloodstream infection, according to the type of transplantation

	Kidney	Liver	Heart	Total
Transplants performed	556	307	64	927
Living donor	441	246	0	687
Number of BSI episodes	70	228	19	317
Number of BSI episodes >1	10	55	4	69
Number of patients with BSI	58 (10%) (CI_{95} 8.7-11.3)	121 (39%) (CI_{95} 36-42)	12 (19%) (CI_{95} 14-24)	191 (21%) (CI_{95} 19.7-22.3)
Ratio BSI episodes/patients	1.2	1.9	1.6	1.7
Incidence by episodes	12.5%	74.2%	29.6%	34.1%
Incidence by patients	10.4%	39.4%	18.8%	20.6%
Microbiology of BSI				
Gram negative	42	115	11	168 (61.1%)
Gram positive	20	75	4	99 (36%)
Ratio of gram positive to gram negative BSI	0.47	0.65	0.36	0.58
Candidaemia*	1	7	0	8 (2.9%)
Polymicrobial	7	31	4	42 (13.2%)

**Candida albicans* (4), *Candida glabrata* (1), *Candida spp.* (3)
BSI: bloodstream infection

IA

Experimental and Clinical Transplantation (2017) Suppl 1: 214-218

Culture-Positive Pulmonary Aspergillosis Infection: Clinical and Laboratory Features of Solid-Organ Transplant Recipients

Balam Er Dedekarginoglu,[1] Serife Savas Bozbas,[1] Gaye Ulubay,[1] Fusun Oner Eyuboglu,[1] Mehmet Haberal[2]

From the [1]Department of Pulmonary Diseases and the [2]Department of General Surgery, Baskent University, Ankara, Turkey

Results: **Of the 15 study patients, 7 were heart transplant, 6 were kidney transplant, and 2 were liver transplant recipients. Three patients had positive aspergillosis cultures from extrapulmonary specimens (1 brain biopsy and 2 wound swap cultures). Other patients with positive cultures were from bronchoalveolar lavage (6 patients), sputum (4 patients), both bronchoalveolar lavage and sputum (1 patient), and deep tracheal aspiration specimen (1 patient).** ***Aspergillus fumigatus*** **was the most common species.**

Table 3. Timeline of Patients With Positive *Aspergillus* Cultures at Time of Hospitalization for Transplant

Patient Description	Time of Positive Culture After Transplant, days
Heart transplant (patient 1)	76
Heart transplant (patient 2)	44
Heart transplant (patient 3)	32
Heart transplant (patient 4)	30
Kidney transplant patient	18
Liver transplant patient	20

IA

Transplantation Proceedings, 36, 2703–2707 (2004)

Aspergillus Pneumonia in Renal Transplant Recipients at a Medical Center in Turkey

M. Usta, S. Kahvecioglu, I. Akdag, M. Gullulu, B. Ozdemir, B. Ener, A. Ersoy, Y. Cirak, K. Dilek, and M. Yavuz

From the Ulu Dağ University School of Medicine, Ulu Dağ, Turkey.

We present five patients of ages ranging between 34 and 43 years who displayed aspergillus pneumonia between 1991 and 2000. All patients received cyclosporine, azathioprine, and prednisone for maintenance immunosuppressive therapy. Their ages ranged from 34 to 43 years with the onset of infection between 1 to 25 months posttransplant. In all cases, the infection was localized to the lungs. Standard methods of fungal culture and identification were used. No coinfections with tuberculosis or other fungi or bacteria were identified.

Pneumocystis Pneumonia in Solid Organ Transplantation

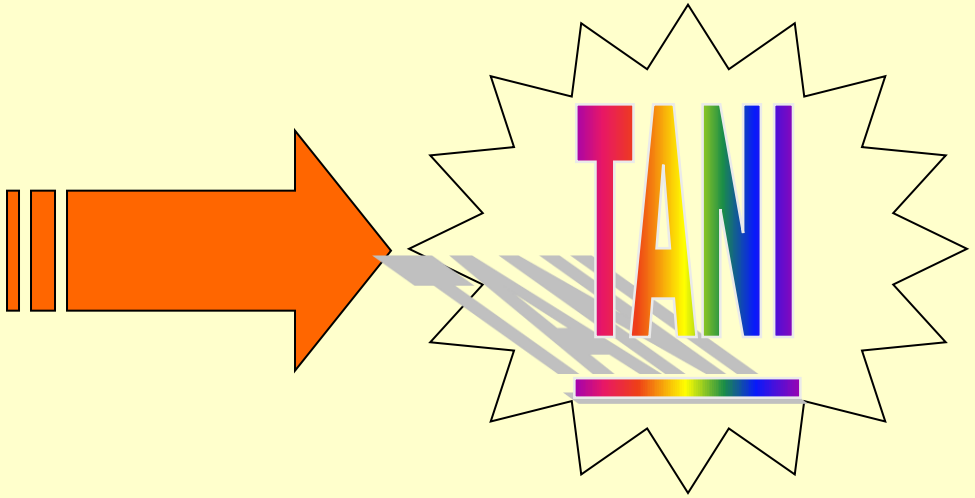
S. I. Martin[a,*], J. A. Fishman[b] and the AST Infectious Diseases Community of Practice

Table 1: Risk factors for the development of *Pneumocystis* pneumonia expected or observed in solid organ transplant recipients

Risk factors	Comments
Immunosuppressive therapies	
Corticosteroids	• Retrospective case series in non-HIV patients identified corticosteroids in up to 90% • Median dose and duration of therapy in one series of non-HIV patients with PCP was 30 mg/day of prednisone for 12 weeks (13)
Antilymphocyte therapy	• Antilymphocyte antibodies are linked to the highest risk for PCP in the 1–6 month posttransplant period (14) • Alemtuzumab, a monoclonal antibody with activity against B-, T-, and NK cells may confer the highest risk (15)
Mycophenolate mofetil	• The anti-*Pneumocystis* effects of mycophenolate mofetil *in vitro* and in animal models have not been confirmed in prospective clinical trials (16)
Calcineurin inhibitors	• At a single institution where cyclosporine A replaced azathioprine in renal transplantation, the incidence of PCP increased from 3% to 9% (17) • One retrospective study suggested a higher incidence of PCP among renal transplant recipients on tacrolimus-based regimens compared to cyclosporine A (18)
Other clinical factors	
CMV disease	• CMV may be an independent risk factor for PCP (19) • Coinfection with CMV and PCP may be observed in solid organ transplantation (20–22)
Allograft rejection	• PCP has been related to the intensity of immunosuppression in transplant recipients (18) • PCP has been linked to treatment and number of episodes of acute rejection (21)
Low CD4+ T cell counts	• In HIV infection, the risk for PCP is linked to CD4+T cell counts <200 cells/mL, or <20% of the total circulating lymphocytes (23) • PCP has been linked to decreased CD4+T cell counts in HSCT recipients (24), solid tumor patients receiving chemotherapy (25), autoimmune disease and hematological malignancy patients (26) • Transplant patients with CD4+T cell lymphopenia are expected to be at risk for PCP, though clinical data to support this are lacking (19)
Neutropenia	• Prolonged neutropenia is a potential risk factor for PCP in transplant recipients (19)
Exposure	• In solid organ transplant recipients not taking effective prophylaxis, being in close proximity to other transplant recipients with PCP may increase the risk for developing infection (6–11)

CMV = cytomegalovirus; GVHD = graft vs. host disease; HIV = human immunodeficiency virus; HSCT = hematopoietic stem cell transplant; PCP = *Pneumocystis* pneumonia.

- Klinik
- Mikrobiyoloji
- Histopatoloji
- Radyoloji



İFE -Tanı: Uluslararası Kılavuzlar

ECIL	Konvansiyonel Tanı
ECIL	Biyolojik Belirteçler
ECIL	Mukormikoz
ESCMID	ESCMID – *Candida* Tanı
IDSA-ASM	Enf. Hastalıklarının Tanısında Mikrobiyoloji Lab.nın Kullanımı
ESCMID-ECMM	Feohifomikoz
ESCMID-ECMM	Hyalohifomikoz
ESCMID-ECMM	Mukormikoz
ESCMID-ECMM	Nadir Görülen İnvazif Maya Enfeksiyonları
IDSA	Aspergilloz
ESCMID-ECMM	Aspergilloz

British Society for Medical Mycology best practice recommendations for the diagnosis of serious fungal diseases

Review

Lancet Infect Dis 2015; 15: 461–74

Published Online March 12, 2015

Silke Schelenz, Rosemary A Barnes, Richard C Barton, Joanne R Cleverley, Sebastian B Lucas, Christopher C Kibbler, David W Denning, on behalf of the British Society for Medical Mycology

Panel 1: Microbiology best practice recommendations

Microscopy and stains

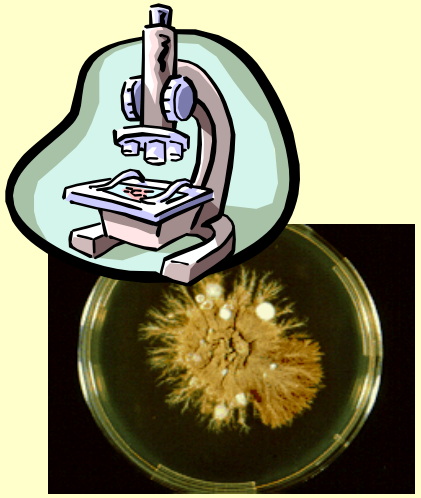
- Fluids from usually sterile sites and bronchoalveolar lavage (BAL) from patients with suspected infection should be examined by direct microscopy with suitable methods for fungal detection*
- Adequate tissue for histology and culture should be ensured before direct microscopy is done on the rest of the sample
- Optical brighteners are recommended for microscopy on all samples from immunocompromised patients
- Direct fluorescent-antibody staining, PCR, or both is recommended for patients with suspected pneumocystis infection
- India ink staining of cerebrospinal fluid samples from immunocompromised patients is recommended in addition to Gram staining if cryptococcus capsule antigen (CRAG) testing is not available on site

Fungal serological and molecular testing

- Serum samples from immunocompromised patients with presentations consistent with cryptococcal meningitis for whom a CSF specimen is not available (eg, cases in which lumbar puncture is contraindicated) should be tested for *Cryptococcus* spp antigen (CRAG)
- Galactomannan screening of serum (two times per week) from patients with haematological malignancies at high risk of invasive aspergillosis should be considered in those not receiving mould-active prophylaxis; optical density (OD) index threshold of 0·5 has a high negative predictive value, enabling invasive aspergillosis to be excluded
- Galactomannan testing of BAL from patients at high risk of invasive aspergillosis should be considered, although the current OD index cutoff of 0·5 might change
- β-D-glucan screening of serum from patients at high risk of invasive fungal disease should be considered; a negative result has a high negative predictive value, enabling invasive fungal disease to be excluded
- PCR screening of serum for aspergillus from patients at high risk of invasive fungal disease should be considered; a negative result has a high negative predictive value, enabling invasive fungal disease to be excluded
- Combination testing with aspergillus PCR plus another antigen test improves the positive predictive value and diagnosis of invasive fungal disease
- Patients with pulmonary cavities of uncertain cause (with or without an aspergilloma) should have serum samples tested for antibodies to aspergillus
- Patients with suspected allergic bronchopulmonary aspergillosis should have serum samples tested for total IgE and aspergillus-specific IgE

Mikrobiyolojik Tanı Yöntemleri

- **KONVANSİYONEL YÖNTEMLER**
  - **Direk mikroskopik inceleme**
  - **Kültür**



- DİĞER YÖNTEMLER
  - Serolojik yöntemler
  - Moleküler yöntemler

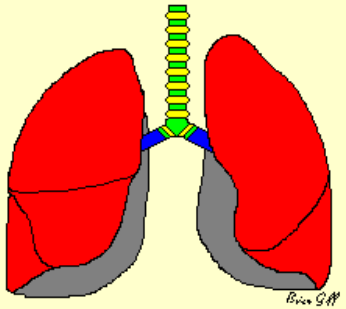
Uygun Örnek Seçimi

Bir küf mantarına bağlı alt solunum yolu enfeksiyonu şüphesinde uygun örnek? - -IA

<u>İzolasyon şansı</u>

Biyopsi > BAL > DTA > Balgam

≥2 örnekte aynı küf



Kalp – Balgam: A.fumigatus

Vol. 75, 326–329, No. 3, February 15, 2003

THE ISOLATION OF *ASPERGILLUS FUMIGATUS* FROM RESPIRATORY TRACT SPECIMENS IN HEART TRANSPLANT RECIPIENTS IS HIGHLY PREDICTIVE OF INVASIVE ASPERGILLOSIS[1]

PATRICIA MUÑOZ,[2,4] LUIS ALCALÁ,[2] MATILDE SÁNCHEZ CONDE,[2] JESÚS PALOMO,[3] JUAN YÁÑEZ,[3] TERESA PELAEZ,[2] AND EMILIO BOUZA[2]

Results. **During the 10-year study period, *Aspergillus* spp. was recovered from 30 episodes from 27 heart transplant recipients (incidence: 10.5%). Three episodes were classified as indeterminate and were included in the analysis in a double way, first considering them as true positives and afterward as true negatives, so ranges were obtained. After applying diagnostic criteria, 18 of 30 episodes were proven or probable IPA, and 9 episodes were colonizations. Accordingly, 7 to 8% of heart transplant recipients suffered an IPA, and the overall positive predictive value (PPV) was 60% to 70%. When analyzed by species, the PPV of recovering *Aspergillus fumigatus* was 78% to 91%, whereas it was 0% for other species. The PPV increased to 88% to 100% when *A. fumigatus* was recovered from a respiratory specimen other than sputum and decreased to 50% to 67% when it was recovered from sputum. The sensitivities of fungal and conventional media for the recovery of *Aspergillus* spp. were 95% to 100% and 33% to 38%, respectively.**

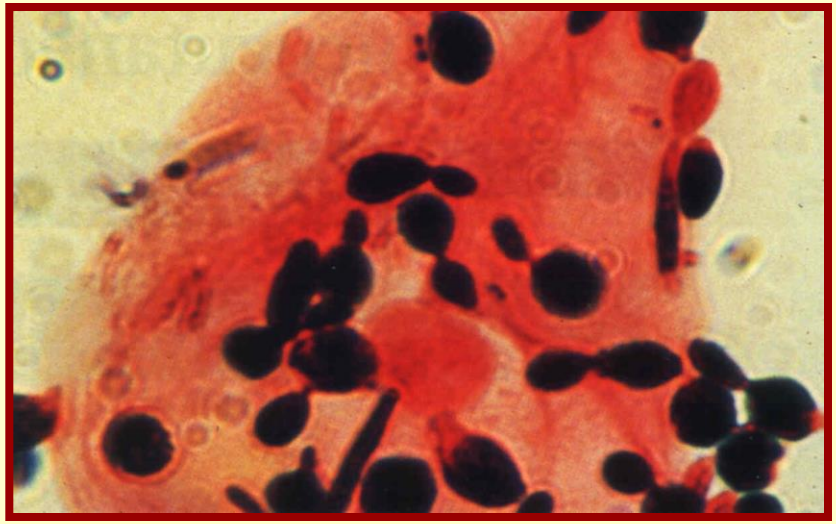
Conclusion. **The isolation of *A. fumigatus* from the respiratory tract of a heart transplant recipient is highly predictive of invasive aspergillosis.**

Mikroskopik inceleme: Yöntemler ve Gözlenen Yapılar

YÖNTEM	UYGULAMA AMACI	SIK GÖZLENEN YAPILAR
Gram boyası	Bakteriyal etkenlerin ve maya mantarlarının saptanmasında kullanılır	Bakteriler, Gram pozitif, tomurcuklanan maya hücreleri (+ psödohif, gerçek hif)
Potasyum hidroksit (KOH) ile ıslak preparat hazırlama	Örnekteki organik bileşikleri eriterek örnekte bulunan olası bir mantarın görülmesini kolaylaştırır	Hif, artrokonidyum
"Kalkoflor" beyazı ile floresan boyama	Mantara ait yapıların görülme olasılığını artırır. KOH'den önemli ölçüde daha duyarlıdır.	Hif, artrokonidyum, maya hücreleri
Çini mürekkebi	Kapsül incelenmesinde yardımcıdır.	Kapsül boşluğu, maya hücreleri

Larone DH. Medically Important Fungi-A Guide to Identification; MCM 9th ed. 2007

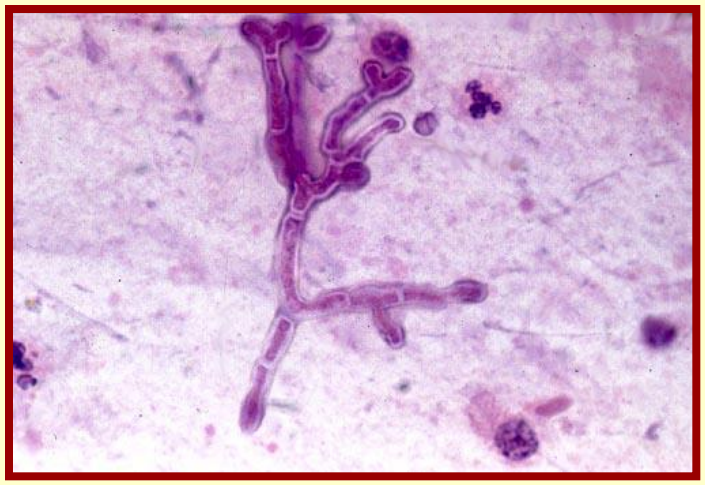
Mikroskopik inceleme



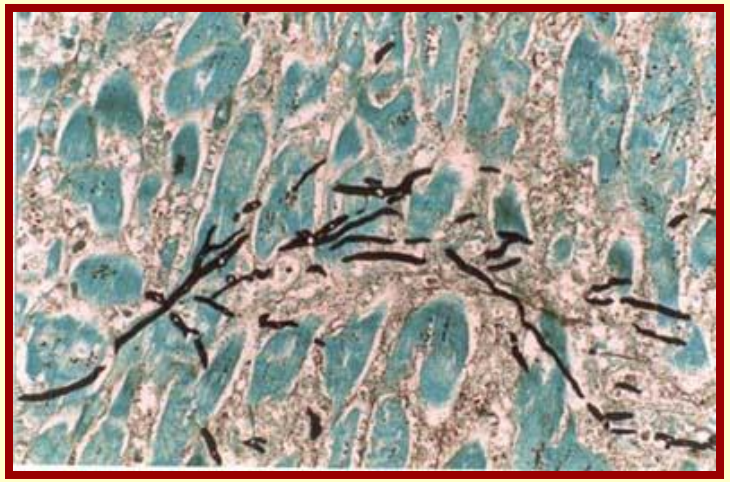
Tomurcuklanan maya hücreleri ve psödohif (Gram)

Kapsüllü maya hücreleri (çini mürekkebi)

Mikroskopik / Histopatolojik inceleme

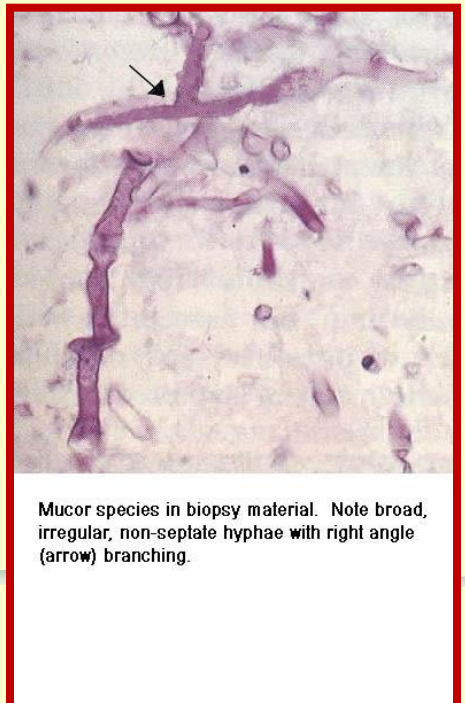


Septalı hif (Gram)



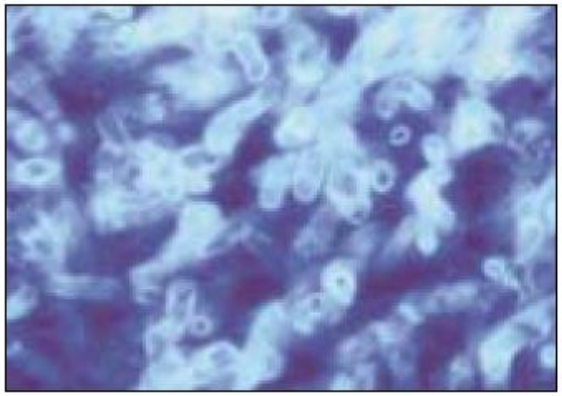
Septalı hif (GMS)

Hif (KOH)

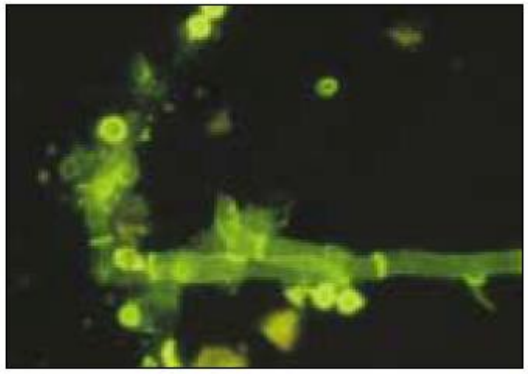


Mucor species in biopsy material. Note broad, irregular, non-septate hyphae with right angle (arrow) branching.

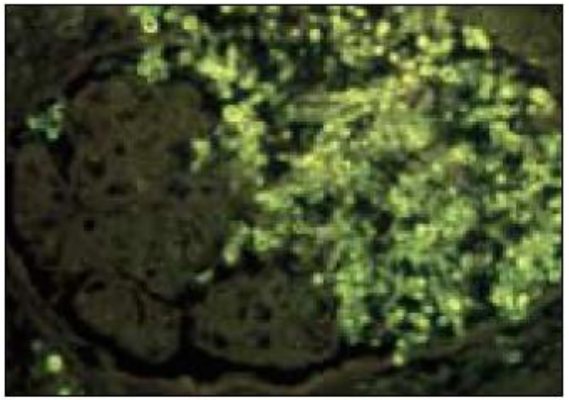
Septasız hif



[I1] Lung section; *Aspergillus* sp.; CFW; UV illumination, no barrier filter. The CFW-stained hypahe are seen as bright blue-colored.



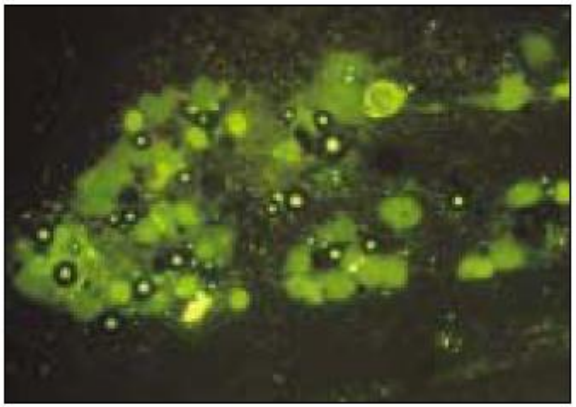
[I21] Ear discharge smear. CFW, UV excitation. Septate branching hyphal fragments and spores. Culture grew *Aspergillus niger*.



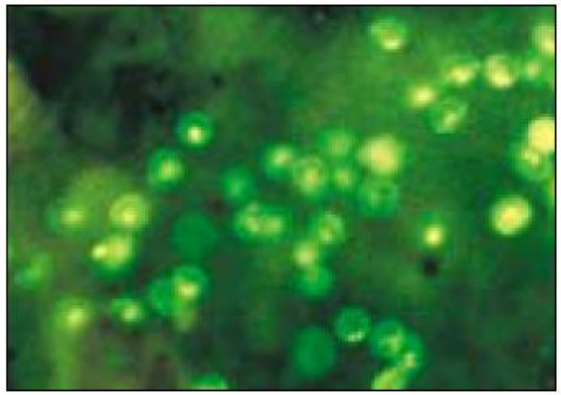
[I8] *Candida albicans* in section of kidney, CFW, UV excitation.

AII (Duyarlılık↑)

ESCMID-ECMM-ERS Asp guideline



[I16] Smear of bone marrow. *Cryptococcus neoformans*. CFW, UV excitation. The dark (unstained) halo around the yeast cells is the polysaccharide capsule, which does not fluoresce with CFW.



[I17] Smear of BAL. *Pneumocystis carinii* cysts. CFW, UV excitation. The "double parenthesis" bodies are clearly seen in most of the cysts; note that the positions vary depending on the orientation of the cyst in the specimen smear.

Kalkoflor beyazı

Laboratory Medicine 2003; 34: 361-7

Direk mikroskopik inceleme: Sonuç Raporu

***Olası* hızlı tanı;**

ancak:

- Tanımlama, kesin tanı Ø
- Olası etken ile uyum
- Duyarlılığı değişken

Direk mikr.: Ön/Ara Raporlar ve Kritik Değerler

- Steril örnek – Direk mikr. – Fungal Yapı
- İmmünkompr. –Direk mikr. – Hif

...Kültür:

Kesin tanı için altın standart

"SoR" : Temel Tanı Yöntemi "A"

Kültür - -
Hangi besiyerleri, hangi ekim koşulları?

- (Rutin bakteriyolojik besiyerleri)
- Sabouraud dekstroz agar (birden fazla besiyerine)

  (Küfler için kontaminasyonu ekarte etmeye yardımcı ekim yöntemleri)
- (Gerektiğinde) antibiyotik eklenmiş besiyeri
- 30°C, 35°C

Kültürün Artıları ve Eksileri

AVANTAJLAR	DEZAVANTAJLAR
"ALTIN STANDART"	Duyarlılık ve özgüllüğü değişken
Tanımlama	Eşik koloni değerleri mevcut değil
Antifungal duyarlılık profilinin tahmini	Kontaminasyon riski nedeniyle (özellikle küf mantarları için) yalancı pozitiflik olasılığı
Antifungal duyarlılık testlerinin uygulanması	Üreme süresi uzun (özellikle küf); erken tanıya yardımı sınırlı

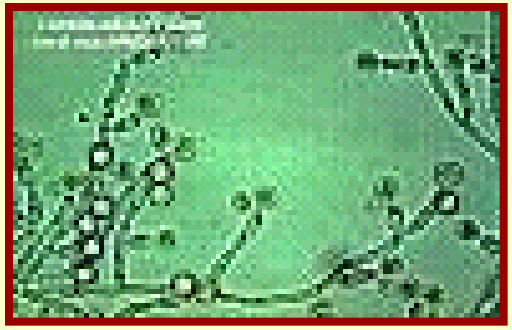
Kültür: Ön/Ara Raporlar ve Kritik Değerler

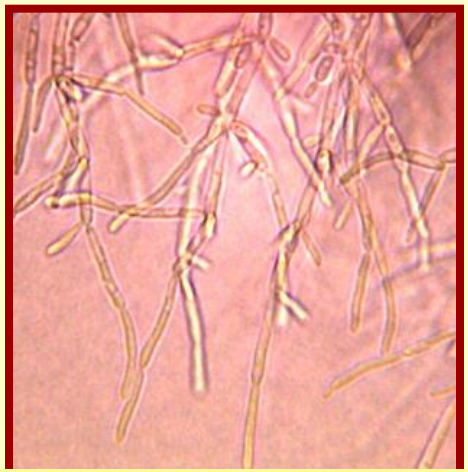
- Steril Örnek – Kültür - Mantar
- İmmünkompr. -Kültür – Küf (Ön Rapor)

Kültürlerin Değerlendirilmesi,Tanımlama, Sonuç Raporu

MAYALAR

- Koloni morfolojisi
- Germ tüp testi
- Mısır unlu tween 80 besiyerinde morfolojik görünüm
- Kromojenik besiyerleri (+ Primer izolasyon)
- Asimilasyon reaksiyonları
- Diğer (kapsül varlığı, üreaz aktivitesi...)
- MALDI-TOF MS



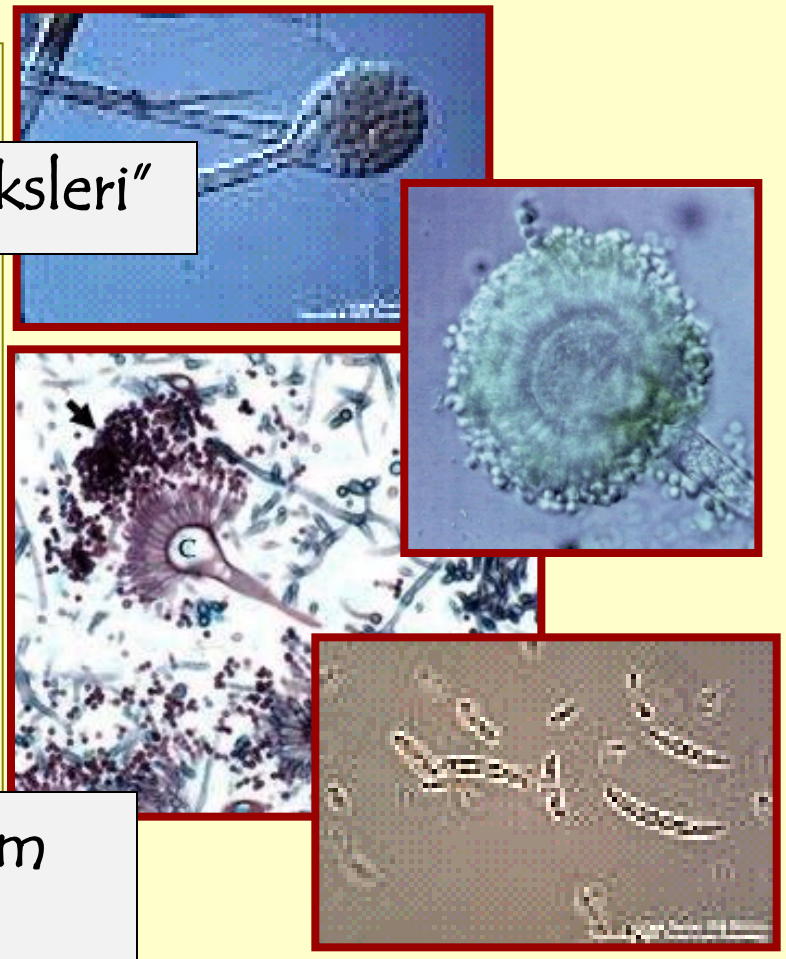


- Epidemiyolojik dağılım
  - Antifungal ilaçlara duyarlılık profili

Kültürlerin Değerlendirilmesi, Tanımlama, Sonuç Raporu

KÜFLER

- Koloni morfolojisi "Tür kompleksleri"
- Mikroskopik görünüm
- Diğer testler
  - Moleküler yöntemler ile doğrulama
- MALDI-TOF MS



- Epidemiyolojik dağılım
  - Antifungal ilaçlara duyarlılık profili

Konvansiyonel tanı: zorluklar...

Uygun, steril klinik örnek → X

	DİREK MİKROSKOPİ	KÜLTÜR
ERKEN TANI	✓ (Olası tanı)	X (küfler)
DUYARLILIK	X	X
ÖZGÜLLÜK	✓	✓ / X (küfler)
KESİN TANI (ALTIN STANDART)	X	✓

KAN KÜLTÜRÜ

Candida ve diğer mayalar: ✓

Fusarium: ✓

Scedosporium: ✓

Aspergillus: X

Kandidemide kan kültürünün duyarlılığı: (ortalama) ~%50 ; optimal koşullarda bile %70'in üzerinde değil

Ostrosky-Zeichner et al. Crit Care Med 2006; 34: 857

Sims et al. Arch Med Res 2005; 36: 660

(Otopsi Çalışmaları)

Berenguer et al. DMID 1993; 17: 103

Kan Kültürü (KANDİDEMİ) – Diğer Sorunlar

JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2011, p. 325–334

National Surveillance of Fungemia in Denmark (2004 to 2009)▽

Maiken Cavling Arendrup,[1]* Brita Bruun,[2] Jens Jørgen Christensen,[3] Kurt Fuursted,[4] Helle Krogh Johansen,[5] Poul Kjældgaard,[6] Jenny Dahl Knudsen,[7] Lise Kristensen,[8] Jens Møller,[9] Lene Nielsen,[10] Flemming Schønning Rosenvinge,[11] Bent Røder,[12] Henrik Carl Schønheyder,[13] Marianne K. Thomsen,[14] and Kjeld Truberg[15]

$P = 0.003$). The variation in distribution of *C. glabrata* between centers using Bactec (17.9%, 231/1,289) and those using BacT/Alert (23.6%, 381/1,612) was statistically significant ($P = 0.0002$). This was also the case if the analysis was performed separately for the group of 60 to 79 years of age (Bactec, 19.2% [131/684]; BacT/Alert, 24.9% [219/880], $P = 0.0071$). *C. krusei*,

Recent reports have suggested that the choice of blood culture system may influence the recovery of *C. glabrata* and that the Bactec system may be inferior to the BacT/Alert system, in this respect (6, 44). The present data support this observation, as the recovery rate of *C. glabrata* was significantly lower at centers using the Bactec system than at centers using the BacT/Alert system. A theoretical bias for this observation could be that the age distribution varied between centers using the two blood culture systems, and therefore, the analysis was repeated for the 60- to 79-year-old age group with the same result. It is consequently suggested that the mycosis medium be included in blood cultures at centers using the Bactec system when a patient is at risk for candidemia.

Delaying the Empiric Treatment of *Candida* Bloodstream Infection until Positive Blood Culture Results Are Obtained: a Potential Risk Factor for Hospital Mortality

Matthew Morrell,[1] Victoria J. Fraser,[2] and Marin H. Kollef[1]*

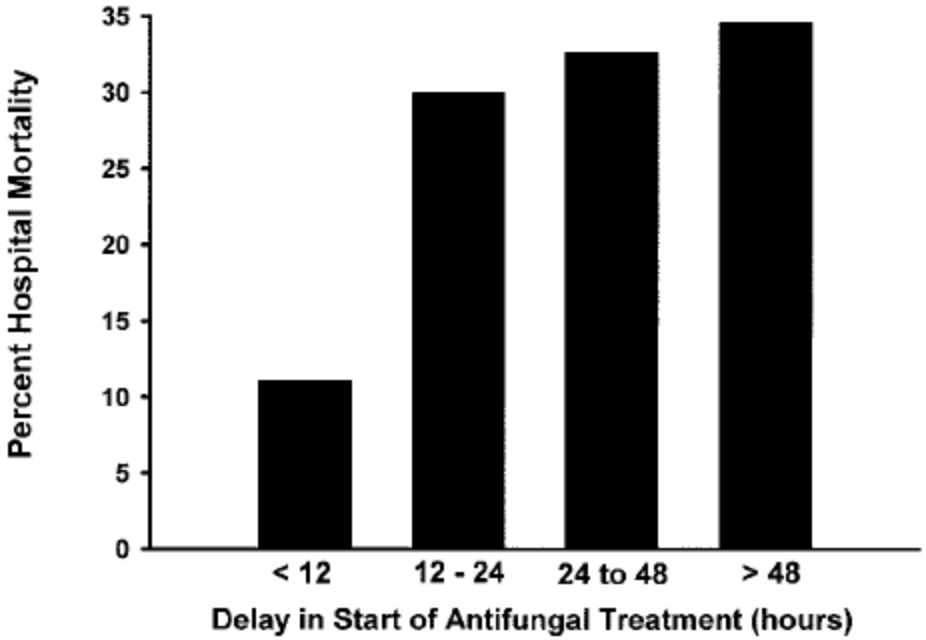


FIG. 1. Relationship between hospital mortality and the timing of antifungal treatment. The timing of antifungal therapy was determined to be from the time when the first blood sample for culture positive for fungi was drawn to the time when antifungal treatment was first administered to the patient.

Nosocomial Candidiasis: Antifungal Stewardship and the Importance of Rapid Diagnosis

Michael A. Pfaller[1,2,3,4,*] and Mariana Castanheira[2]

Table 6. Commercially available culture-independent diagnostic test for candidiasis.

Test	Vendor/manufacturer	Specimen type	Limit of detection	% Sens	% Spec	Comments
β-D-glucan (Fungitell)[a]	Assoc. Cape Cod, Inc.	Serum or plasma	80 pg/ml	75.3	85.0	FDA cleared; not Specific for *Candida*
Mannan/Antimannan[b]	Platelia	Serum	≥0.5 ng/ml (mannan)	83.0	86.0	Not available in US; not FDA cleared
Real-time PCR[c]	Quest Diagnostics	Serum	1–350 CFU/ml	NA	NA	Reference laboratory; not FDA cleared
Real-time PCR[d]	Viracor.IBT	Serum or plasma	≤1 CFU/ml	80.0	70.0	Reference laboratory; not FDA cleared
SeptiFast Real-Time PCR[e]	Roche Diagnostics	Whole blood	30–100 CFU/ml	61.0	99.0	Not available in US; not FDA cleared; Whole blood tested after extraction
T2Candida[f]	T2Biosystems	Whole blood	1-3 CFU/ml	91.1	99.4	FDA cleared; whole blood tested without extraction

Medical Mycology, 2016, 54, 1–22

Nosocomial Candidiasis: Antifungal Stewardship and the Importance of Rapid Diagnosis

Michael A. Pfaller[1,2,3,4,*] and Mariana Castanheira[2]

Table 7. Risk stratification and the predictive value of rapid diagnostic test for candidiasis.

Test	Vendor/Manufacturer	Sens/Spec (%)	Prevalence of Disease (%)	Predictive value (%)[a]	
				PPV	NPV
Fungitell (BDG)	Assoc. Cape Cod, Inc.	75.3/85.0	2	9.3	99.4
			5	21.1	98.5
			10	35.7	96.8
			30	68.2	88.8
Mannan/antimannan	Platelia	83.0/86.0	2	11.0	99.6
			5	24.0	99.0
			10	39.7	97.9
			30	71.8	92.2
SeptiFast Real-time PCR	Roche Diagnostics	61.0/99.0	2	54.5	99.2
			5	77.5	98.0
			10	87.1	95.8
			30	96.3	85.6
T2Candida	T2Biosystems	91.1/99.4	2	75.0	99.8
			5	88.5	99.6
			10	94.8	99.0
			30	98.6	96.3

[a]PPV, positive predictive value; NPV, negative predictive value.

Medical Mycology, 2016, 54, 1–22

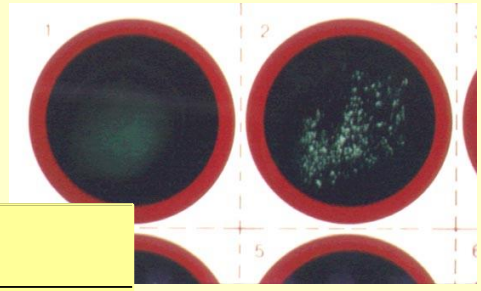
Fırsatçı İFE -Serolojik Testler

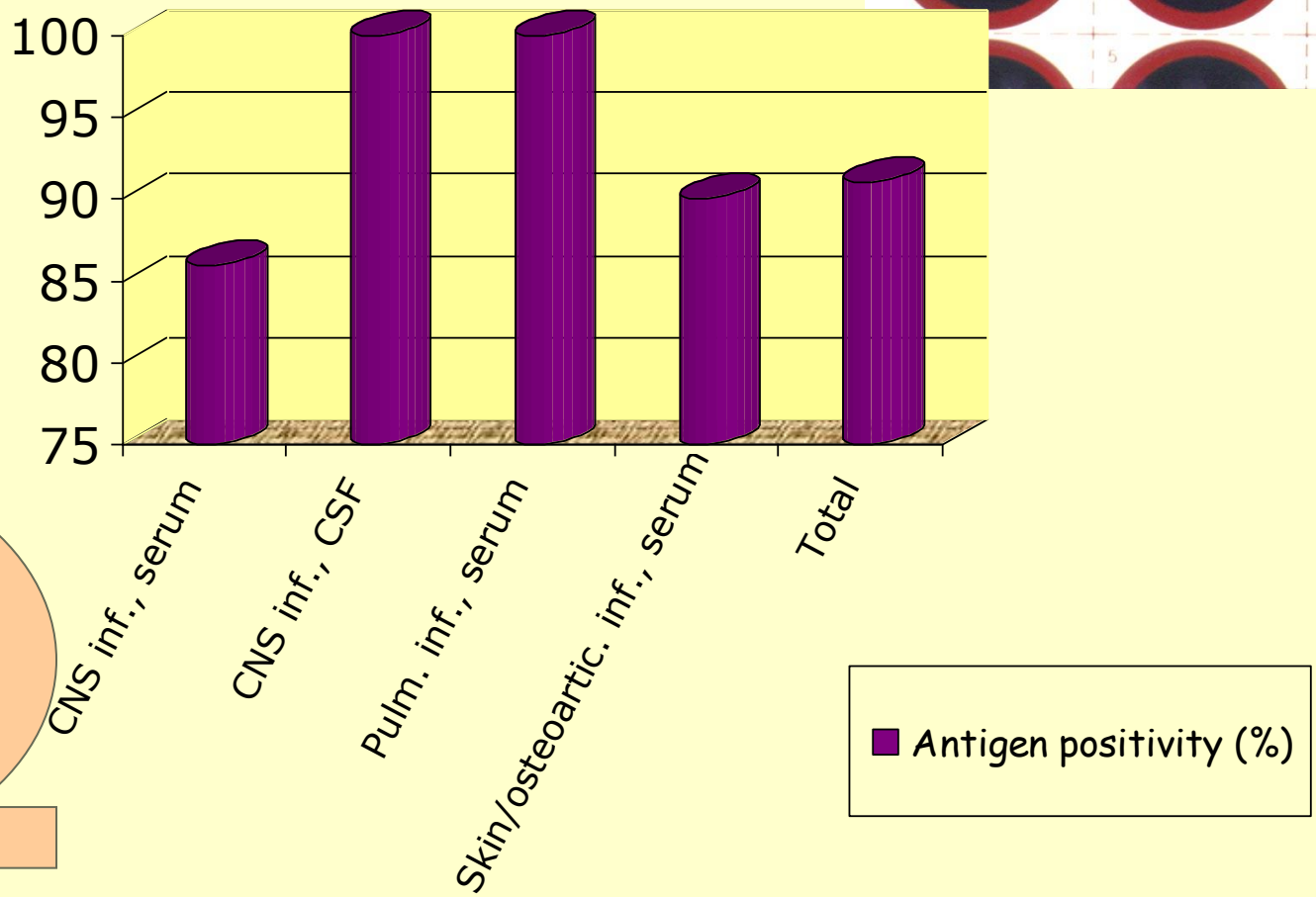
Etken	Tesbit edilen antijen / antikor / enzim / metabolit	Klinik Örnek
C. neoformans	Kapsül polisakkarit	BOS, serum
Aspergillus	Galaktomannan (GM)	Serum, BAL
Aspergillus	Ekstraselüler glikoprotein Ag	Serum, BAL
Mucorales (ve *C. neoformans)* hariç diğer mantarlar	(1,3)-ß-D-glukan (BDG) (G testi)	Plazma/serum
Candida	Mannan, anti-mannan	Serum, (BOS)

	Galactomannan	(1,3)-β-D-glucan
Diagnostic spectrum	Aspergillus[a]	Panfungal[b]
Method	'Sandwich' ELISA	EIA-based
Commercial kit	Bio-rad (France) Pastorex Aspergillus Dynamiker Biotechn (China)	Fungitell (USA) Fungitec-G MK (Japan) Wako (Japan Maruha (Japan) Dynamiker Biotech (China)
Cut-off (serum) **BAL**	0.5 1	Fungitell 80 pg/mL Fungitec, Wako 11-30 pg/mL Dynamiker 95 pg/ml)
Early Diagnosis	5-8 days	3-10 days
Serum, plasma **Sensitivity** **Specificity**	29-100% 20-100%	47-98% 86-98 %
BAL (Sensitivity, Specificity)	56-100%, 76-100%	50-93%, 55-73%
Use in monitoring treatment	+	Ø
Cost	+	++

Arikan-Akdagli et al. Turk J Haematol 2014; 5:31

Cryptococcus antijen testi (LA)





178 transplant rec.
145 renal
20 liver
10 heart
3 lung

Husain et al. Emerg Infect Dis 2001; 7: 375

Wu et al. Transpl Infect Dis 2002; 4: 183

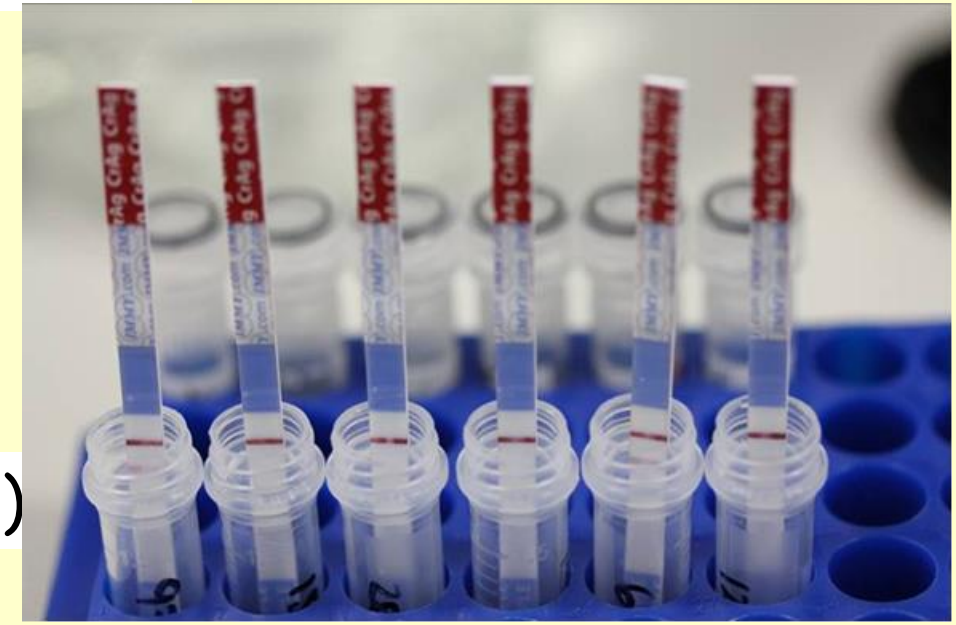
Cryptococcus Ag "Lateral Flow Assay" ("POC" test)

Immy Inc. (Norman, OK USA) 2009

Hızlı sonuç (10 dk.)

40 µL örnek (serum/plazma/idrar/BOS)

Duy(%96-100) Özg(%97-99.5)



life-worldwide.org/en/media-centre/article/what-are-the-point-of-care-tests-for-fungal-infections

Tdv. takibinde kullanılmaz (Ag-yavaş klerens)

Prattes et. al. Curr Fungal Inf Rep 2016; 10: 43

...GM testi:Meta-analiz

Serum

27 çalışma
~4000 olgu

EORTC-MSG veya benzeri tanı kriterleri ile

KANITLANMIŞ ASPERGİLLOZ
DUYARLILIK: %71
ÖZGÜLLÜK: %89

~

KANITLANMIŞ+YÜKSEK OLASILIKLI ASPERGİLLOZ
DUYARLILIK: %61
ÖZGÜLLÜK: %93

"Cut-off"
5 çalışma: 0.5
13 çalışma: 1.0
11 çalışma: 1.5

Testin doğruluk oranı:
Orta düzeyde

Test, hematolojik malignansi veya HSCT olgularında, solid organ transplant olgularına göre daha yararlı

Yüksek "cut-off" → doğruluk oranı artıyor

Pfeiffer et al. CID 2006; 42: 1417; Rex et al. CID 2006; 42: 1428

Table 2. Characteristics of studies included in the meta-analysis of diagnosis of invasive aspergillosis using a galactomannan assay.

Study	Year	Patient population	Age group	Study design	Purpose of study	Reference standard	Blinded	Threshold for positive test result[a]	No. of samples required for positivity	Antifungal therapy reported
Becker et al. [19]	2003	Patients with hematological malignancy	Adult	Prospective	Surveillance	EORTC/MSG	Yes	1	2	No
Platelia package insert [20]	2003	Patients with hematological malignancy	NR	Pospective	Surveillance	EORTC/MSG	Yes	0.5	2	No
Bretagne et al. [21]	1997	Patients with hematological malignancy	All	Prospective	Surveillance	Other	No	1	2	No
Bretagne et al. [22]	1998	Bone marrow transplant recipients	All	Retrospective	Surveillance	Other	No	1	2	No
Buchheidt et al. [23]	2004	Patients with hematological malignancy	Adult	Prospective	Surveillance	EORTC/MSG	No	1.5	2	No
Challier et al. [24]	2004	Immunocompromised	Adult	Prospective	Surveillance	EORTC/MSG	NR	1	1	No
Challier et al. [24]	2004	Immunocompromised	Pediatric	Prospective	Surveillance	EORTC/MSG	NR	1	1	No
Costa et al. [25]	2002	Patients with hematological malignancy	Adult	Retrospective	Surveillance	EORTC/MSG	No	1.5	1	No
Fortun et al. [26]	2001	Solid-organ transplant recipients	Adult	Retrospective	Surveillance	Other	Yes	1	1	No
Herbrecht et al. [27]	2002	Patients with hematological malignancy	All	Prospective	Surveillance	EORTC/MSG	No	1.5	1	No
Husain et al. [28]	2004	Solid-organ transplant recipients	Adult	Prospective	Surveillance	EORTC/MSG	No	0.5	1	No
Jarque et al. [29]	2003	Patients with hematological malignancy	Adult	Prospective	Surveillance	EORTC/MSG	NR	1.5	2	No
Kami et al. [30]	2001	Patients with hematological malignancy	Adult	Prospective	Surveillance	Other	Yes	1.5	2	No
Kwak et al. [31]	2004	Solid-organ transplant recipients	Adult	Prospective	Surveillance	EORTC/MSG	no	0.5	2	Yes
Machetti et al. [32]	1998	Bone marrow transplant recipients	NR	Prospective	Surveillance	Other	No	1.5	2	No
Maertens et al. [33]	FP, no. of cases with false-positive result; TN, no. of cases with true-negative result; TP, no. of cases with true-positive result.								2	No
Maertens et al. [6]	2002	Bone marrow transplant recipients	Adult	Prospective	Surveillance	EORTC/MSG	No	1	2	No
Maertens et al. [34]	2004	Patients with hematological malignancy	Adult	Prospective	Surveillance	EORTC/MSG	Yes	0.5	2	No
Marr et al. [16]	2004	Patients with hematological malignancy	All	Prospective	Surveillance	EORTC/MSG	No	1	1	Yes
Moragues et al. [35]	2003	Patients with hematological malignancy	Adult	Retrospective	NR	EORTC/MSG	No	1.5	2	No
Pazos et al. [36]	2003	Patients with hematological malignancy	NR	Prospective	Surveillance	EORTC/MSG	NR	1.5	2	No
Pinel et al. [37]	2003	Patients with hematological malignancy	All	Pospective	Surveillance	EORTC/MSG	No	1.0	2	No
Rovira et al. [38]	2004	Bone marrow transplant recipients	Adult	Prospectivw	Surveillance	EORTC/MSG	NR	1.5	1	No
Suhalian et al. [39]	2001	Bone marrow transplant recipients	Adult	Prospective	Surveillance	Other	No	1.5	1	No
Sulahian et al. [40]	1996	Bone marrow transplant recipients	NR	Prospective	Surveillance	Other	Yes	1	1	No
Sulahian et al. [39]	2001	Patients with hematological malignancy	Pediatric	Prospective	Surveillance	Other	No	1.5	2	Yes
Ulusukarya et al. [41]	2000	Patients with hematological malignancy	All	Retrospective	Surveillance	Other	No	1	1	No
Verweij et al. [42]	1995	Patients with hematological malignancy	Adult	Retrospective	Surveillance	Other	No	1	2	No
Yoo et al. [43]	2005	Patients with hematological malignancy and bone marrow transplant recipients	Adult	Prospective	Surveillance	EORTC/MSG	NR	0.5	2	No

KC → Fortun et al. [26]

AC → Husain et al. [28]

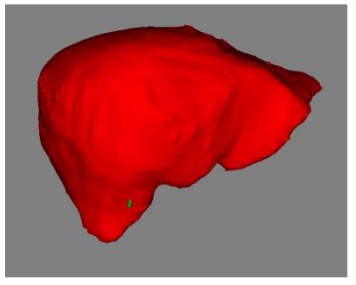
KC → Kwak et al. [31]

	Cases of proven IA				Cases of proven or probable IA			
Studies	TP/(TP+FP)	Pooled sensitivity (95% CI)	TN/(TN+FP)	Pooled specificity (95% CI)	TP/(TP+FN)	Pooled sensitivity (95% CI)	TN/(TN+FP)	Pooled specificity (95% CI)
Studies limited to solid-organ transplant recipients	2/9	0.22 (0.03–0.60)	180/215	0.84 (0.78–0.88)	9/22	0.41 (0.21–0.64)	210/247	0.85 (0.80–0.89)

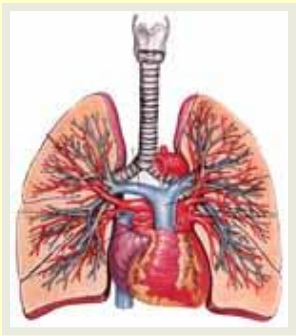
FP, no. of cases with false-positive result; TN, no. of cases with true-negative result; TP, no. of cases with true-positive result.

GM test-Yalancı pozitiflikler

Transplantation



13%



20%

Hematopoietic stem cell transplantation

5-14%

Singh & Paterson. CMR 2005; 18: 44

GM testi – Yalancı pozitiflik nedenleri

Cross reaction *(Penicillium chrysogenum, P. digitatum, Paecilomyces variotii, Saprochaete capitata, Histoplasma, Fusarium); (Bifidobacterium-premature babies), Listeria monocytogenes*

False positive *from various foo food...)*

Daha sonraki veriler önemli bir pozitiflik oranı düşündürmüyor.

Mikulska et al. JAC 2012

Vergidis et al. JCM 2014

•Kasap ve ark. 3. Ulusal Kl. Mikr. Kongresi 2015, SS45 (Türkiye; ilaç piyasasındaki 3 farklı pip/tazo - - pozitiflik yok)

Drugs *(Piperacillin, Piperacillin-tazobactam, Amoxicillin-clavulanic acid, Uricase, Cytotoxic chemotherapeutics, "Plasmalyte")*

Letscher-Bru et al. J Mycol Med 1998, 8: 112; Verweij et al.43rd ICAAC 2003, abst. no. M-1027;Sulahian et al.43rd ICAAC 2003, abst no. 2033; Viscoli et a CID 2004; 38: 913; Singh & Paterson. CMR 2005; 18:44; Weisser et al. CID 2005; 15;41:1143; Mennink-Kersten et al. JCM 2005; 43: 3925, Giacchino et a JCM 2006; 44: 3432; Machetti et al. JAC 2006; 58: 806; Hage et al. JCM 2007; 45: 676; Narreddy et al. J Infect 2007; Nov 3, Penack et al. DMID 2007; Sept 20; Petti et al. DMID 2013 March 18

Plasmalyte: No Longer a Culprit in Causing False-Positive Galactomannan Test Results

Isabel Spriet,[a] Katrien Lagrou,[b] Johan Maertens,[c] Ludo Willems,[a] Alexander Wilmer,[d] Joost Wauters[d]

False-positive galactomannan (GM) results have been reported in patients treated with gluconate-containing solutions, such as Plasmalyte. The GM optical density index was tested on 33 distinct batches of Plasmalyte and was found to be negative in all of the batches, confirming that Plasmalyte is no longer a cause of false-positive GM results.

March 2016 Volume 54 Number 3 795-797 Journal of Clinical Microbiology

BAL'da GM

Comparison of two different cut-offs for BAL: 0.5 vs. 0.85

89 BAL GM tests

%

CUT-OFF for BAL	Sensitivity	Specificity	PPV	NPV
0.5	73	89	73	89
0.85	67	95	83	87

ECIL recommended "cut-off": 1 (BIII)

- BAL GM more sensitive than cytology (0%), BAL culture (27%), transbronchial biopsy (40%), or serum GM (67%) for diagnosing IPA.

- BAL GM was ≥0.85 and ≥0.5 in 86 and 100% of patients with proven or probable IPA who received a mould-active agent for ≤3 days. BAL GM not impacted by short courses of mould-active agents.

Nguyen et al. Biol Blood Marrow Transplant 2011 Jul;17:1043

Antifungal treatment affects the laboratory diagnosis of invasive aspergillosis

Elaine McCulloch,[1] Gordon Ramage,[2] Ranjith Rajendran,[2] David F Lappin,[2] Brian Jones,[3] Peter Warn,[4] Raghdaa Shrief,[4] William R Kirkpatrick,[5] Thomas F Patterson,[5] Craig Williams[1]

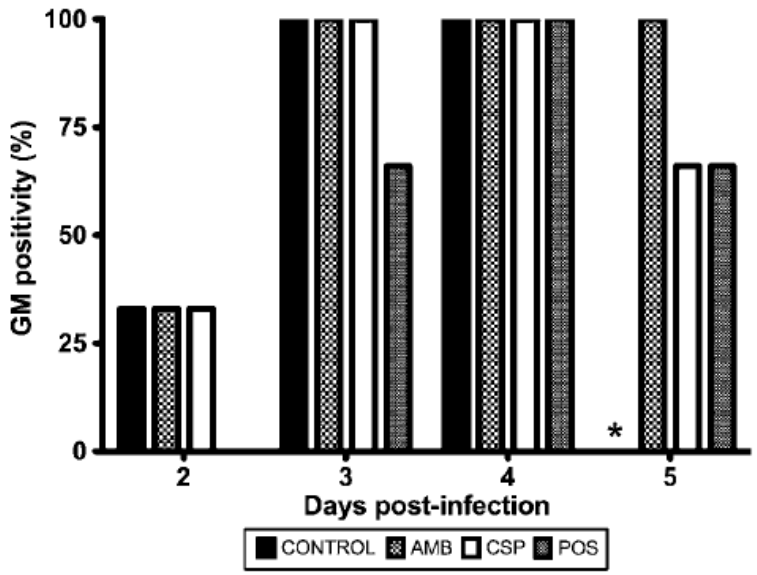


Figure 1 Effect of antifungal treatment on galactomannan (GM) release in serum. Three groups of rats (n=12 per group) were treated with caspofungin (CSP; 5 mg/kg), posaconazole (POS; 2.5 mg/kg) or amphotericin B (AMB; 1 mg/kg) daily following infection, and compared with a vehicle-treated infected control group (n=12). Terminal blood samples were taken at days 2, 3, 4 and 5, post-infection, and serum samples were used in a galactomannan enzyme immunoassay. A galactomannan index of ≥0.5 was defined as positive for invasive aspergillosis. Note the delayed galactomannan positivity of the posaconazole-treated animals, and the biphasic galactomannan positivity of both caspofungin and posaconazole. *Sample not performed.

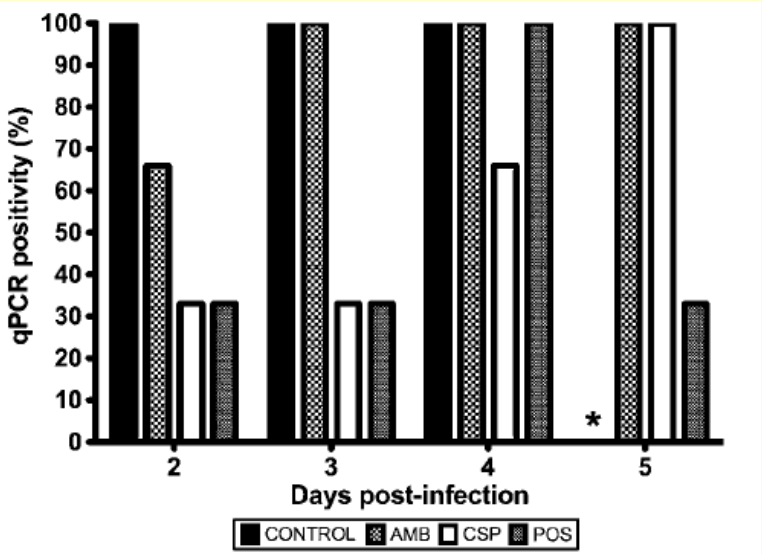


Figure 2 Effect of antifungal treatment on DNA detection in clotted blood. Three groups of rats (n=12 per group) were treated with caspofungin (CSP; 5 mg/kg), posaconazole (POS; 2.5 mg/kg) or amphotericin B (AMB; 1 mg/kg) daily following infection, and compared with an untreated infected control group (n=12). Terminal blood samples were taken at days 2, 3, 4 and 5, post-infection, and clot samples used for qPCR analysis. A Ct value of less than 40 was defined as positive for invasive aspergillosis. Note how qPCR detection was sensitive in vehicle-treated controls, and that amphotericin B-treated rats had similar sensitivity. The kinetics of detection was delayed for caspofungin and posaconazole and shows a short window of maximum sensitivity following posaconazole therapy. *Sample not performed.

GM-Serum/BAL ve Küflere Etkili Antifungal İlaç Kullanımı

IDSA-Asp 2016

GM-Serum: Küflere etkili tdv. / proflaksi + ise tarama amaçlı kullanımı önerilmez.

GM-BAL: Küflere etkili tdv. / proflaksi + olgularda bakılabilir. Güçlü Ö, KD Yüksek

ESCMID-ECMM-ERS-Asp

GM: Küflere etkili proflaksi + ise tarama amaçlı kullanımı önerilmez.

ESCMID-ECMM-ERS Aspergillus Guideline

Blood galactomannan

Population	Intention	Intervention	SoR	QoE	Comment
Solid organ recipients	To diagnose invasive aspergillosis	Galactomannan in blood	C	II	Low sensitivity, good specificity; most data for lung Tx (few other SOT patients with IA included)

Meersseman 2008, Guinea 2008, Tabarski 2012, Husain 2004, Pfeiffer 2006, Guique 2013, Martin-Rabadan 2013, Petraitiene 2011, Mikulska 2013, Vergedis 2012, Nucci 2014; Huang 2007

ESCMID-ECMM-ERS Aspergillus Guideline

Population/Test: GM-BAL

Intention	Intervention	SoR	QoE	Comment
Any	To diagnose pulmonary aspergillosis	A	II	GM in the BAL is a good tool to diagnose Optimal cut-off is between 0.5 to 1.0 (<0.5 rules out IA)

D'Haese 2012, Heng 2013, Luong 2012, Zou 2012, Fisher 2013, Reinwald et al. 2012

GM-CSF

Population	Intention	Intervention	SoR	QoE	Comment
Any	To diagnose cerebral aspergillosis	Galactomannan in CSF	B	II	No validated cut-off

Viscoli 2002, Verweij 1999

GM - Biopsies

Population	Intention	Intervention	SoR	QoE	Comment
Any	To detect galactomannan in tissue	To apply GM-test on lung-biopsies	B	II	Cut-off 0.5; high sensitivity (90 %) and specificity (95%); specimens need to be sliced; precondition for doing so is that sufficient material is available; dilution in isotonic saline

Klont 2004, Lass-Flörl 2007

GM, BAL

Diagnosis of Invasive Aspergillosis in Lung Transplant Recipients by Detection of Galactomannan in the Bronchoalveolar Lavage Fluid

(*Transplantation* 2010;90: 306–311)

Alessandro C. Pasqualotto,[1,2,3,6] Melissa O. Xavier,[1] Letícia B. Sánchez,[4] Clarice D. A. de Oliveira Costa,[4] Sadi M. Schio,[4] Spencer M. Camargo,[4] Jose J. Camargo,[4] Teresa C. T. Sukiennik,[2] and Luiz Carlos Severo[1,3,5]

Methods. Herein, we prospectively studied BAL fluid samples from 60 lung transplant patients to determine the optimal cutoff for BAL GM testing. Only one sample per patient was studied. BAL samples were vortexed and processed according to the manufacturer's instructions for serum samples. Sensitivity, specificity, and likelihood ratios were calculated in reference to proven or probable IA cases using receiver operating characteristic analysis.

Results. Eight patients had IA during the study (incidence 13.3%), including four patients with proven IA. Aspergillosis increased 5-fold the risk of death in lung transplant recipients. The positive predictive value of a positive BAL GM test at the 0.5 cutoff was low (24.2%). Raising the cutoff improved test specificity without compromising sensitivity. The best cutoff was defined at 1.5 (sensitivity 100% and specificity 90.4%).

Conclusions. This study reinforces the importance of BAL GM testing in lung transplant recipients, particularly to exclude the diagnosis of IA. To minimize the frequency of false-positive results, a higher test cutoff should be applied to BAL samples, in comparison with serum samples.

TABLE 4. Performance of Platelia *Aspergillus* EIA in BAL samples of lung transplant recipients using distinct cutoff values

Cutoff value	Sensitivity (%)	Specificity (%)	LR (+)	LR (−)	PPV[a] (%)	NPV[a] (%)
0.5	100	40.4	1.68	—	20.5	100
0.7	100	61.5	2.60	—	28.6	100
1.0	100	80.8	5.20	—	44.4	100
1.4	100	88.5	8.67	—	57.1	100
1.5	100	90.4	10.40	—	61.5	100
2.0	87.5	94.2	15.17	0.13	70.0	98.0
2.2	75.0	96.2	19.50	0.26	75.0	96.2

[a] Based on a pretest probability of 13.3% (prevalence of invasive aspergillosis observed in this study).

BAL, bronchoalveolar lavage fluid; EIA, enzyme-linked immunoassay; LR, likelihood ratio; +, positive; −, negative; PPV, positive predictive value; NPV, negative predictive value.

GM, BAL

Aspergillus Galactomannan Antigen in the Bronchoalveolar Lavage Fluid for the Diagnosis of Invasive Aspergillosis in Lung Transplant Recipients

Shahid Husain,[1] David L. Paterson,[1] Sean M. Studer,[2] Maria Crespo,[2] Joseph Pilewski,[2] Michelle Durkin,[3] Joseph L. Wheat,[3] Bruce Johnson,[2] Lisa McLaughlin,[4] Christopher Bentsen,[4] Kenneth R. McCurry,[5] and Nina Singh[1,6]

Results. A total of 333 BAL samples from 116 patients were tested. Invasive aspergillosis was documented in 5.2% (6/116) of the patients. Samples analyzed included 9 BALs from two patients with proven IA, 19 BALs from four patients with probable IA, and 305 BALs from 110 patients without IA. At the index cutoff value of ≥0.5, the sensitivity was 60%; specificity was 95%, with positive and negative likelihood ratios of 14 and 0.41, respectively. Increasing the index cutoff value to ≥1.0 yielded a sensitivity of 60%, a specificity of 98%, and the positive and negative likelihood ratios of 28 and 0.40, respectively. Two of six patients with IA receiving antifungal prophylaxis had false-negative results.

Conclusions. A Platelia EIA index cut-off ≥1.0 in the BAL fluid in a lung transplant recipient with a compatible clinical illness may be considered as suggestive of IA.

(*Transplantation* 2007;83: 1330–1336)

Aspergilloz; LFD

A "non-GM" antigen as a surrogate marker for IA

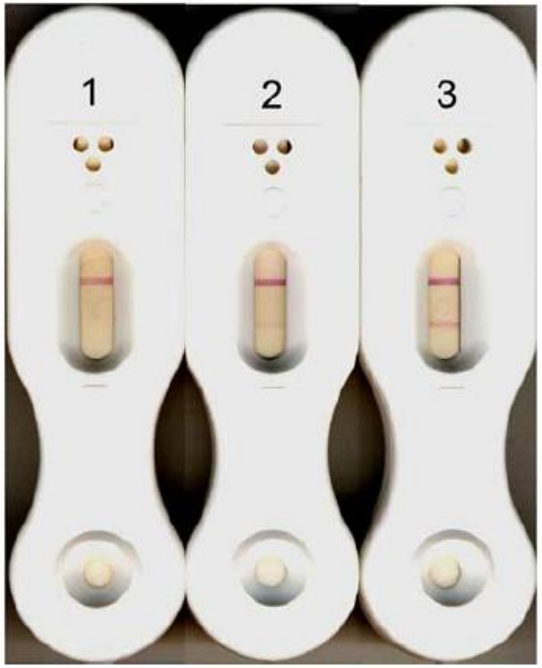
CLINICAL AND VACCINE IMMUNOLOGY, July 2008, p. 1095–1105

Development of an Immunochromatographic Lateral-Flow Device for Rapid Serodiagnosis of Invasive Aspergillosis[▽]

Christopher R. Thornton*

Hybridoma Laboratory, School of Biosciences, Geoffrey Pope Building, University of Exeter, Stocker Road, Exeter, Devon EX4 4QD, United

- ✓Mouse MAb JF5
- ✓Binds to a protein epitope present on an extracellular glycoprotein Ag secreted by Aspergillus spp.
- ✓Used to develop an immunochromatographic LFD
- ✓Detection of Asp Ag in serum in 15 min.
- ✓Highly specific, no cross Rx with other clinically sign.fungi



Negative Weak Strong

Curr Fungal Infect Rep (2016) 10:43–50

Point of Care Testing for the Diagnosis of Fungal Infections: Are We There Yet?

Juergen Prattes[1,2] · Sven Heldt[3] · Susanne Eigl[3] · Martin Hoenigl[1,2,3,4]

Table 1 Aspergillus LFD performance in BALF and serum in various patient cohorts

	Risk group	Sample size (*n* of patients)	Specimen	Sensitivity	Specificity	Reference
Hoenigl 2012	HM	29	BALF	100	81.8	[17]
Miceli 2015	HM	7	BALF	100	83	[18]
Prattes 2015	HM	72	BALF	71	76	[19]
Johnson 2015	HM and non-HM	32	BALF	100	80	[22]
Hoenigl 2012	SOT	10	BALF	100	80	[17]
Willinger 2014	SOT	47	BALF	91	83	[27]
Eigl 2015	ICU	133	BALF	80	81	[28]
Prattes 2014	Respiratory Disease	221	BALF	77	92	[6]
Held 2013	HSCT	101	Serum	40[a] 20[b]	86.8[a] 97.8[b]	[25]
White 2013	HM	103	Serum	81.8[a] 59.1[b]	84.8[a] 98[b]	[26]

HM hematological patients, *SOT* solid organ transplant recipients, *ICU* intensive care unit, *HSCT* hematological stem cell transplantation recipients, *BALF* bronchoalveolar lavage fluid

[a] Single testing = a minimum of one positive LFD results is required for diagnosis

[b] Multiple testing = a minimum of two or more positive LFD results are required for diagnosis

Biomarker/LFD

ESCMID-ECMM-ERS Aspergillus Guideline

Population	Intention	Intervention	SoR	QoE	Comment
Hematological malignancy and solid organ transplant	Diagnose IA	Evaluation of LFD using BAL samples (retrospective study)	B	II	Retrospective study. Sensitivity and specificity of BAL LFD tests for probable IPA were 100% and 81% (PPV 71%, NPV 100%),5 pts with possible IPA had positive LFD, no proven IA

Hönigl et al. 2012, Held et al. 2013, Hönigl et al. 2014

(1-3)-β-D-glukan testi

- Mucorales (ve *C. neoformans*) dışı mantarlar

2003: Serumda beta-glukan tayini: IFI tanısında kullanımı (FDA)

Obayashi et al. Lancet 1995, 345: 17; Kami et al. Nippon Ishinkin Gakkai Zasshi 2001, 42: 181; Miyazaki et al. JCM 1995; 33: 3115; Odabasi et al. CID 2004; 39: 199; Kiuchi et al. 2nd Int. Conf. of Tr. Infect. Dis., 2000; Ostrosky-Zeichner et al. CID 2005; 41: 654; Kedzierska A. Pol J Microbiol 2007; 56: 3; Kelaher A. Clin Lab Sci 2006; 19: 222; Metan et al. SJID 2012; 44: 330

Beta-glukan testi: Duyarlılık ve özgüllük

Duyarlılık	%50-100
Özgüllük	%77-100
NPV	%98-100
PPV	%44-67
Zaman	Klinik tanıdan *4-10 gün* önce (+)

Obayashi et al. Lancet 1995, 345: 17; Kami et al. Nippon Ishinkin Gakkai Zasshi 2001, 42: 181; Miyazaki et al. JCM 1995; 33: 3115; Odabasi et al. CID 2004; 39: 199; Pickering JCM 2005; 43: 5957

Beta-glukan testi: Çok merkezli değerlendirme

Ostrosky-Zeichner et al. CID 2005; 41: 654

Tek serum örneği

ENFEKSİYON (n) (KANITLANMIŞ) Toplam n=135	POZİTİFLİK ORANI Cut-off: 80
Kandidoz (107)	% 77.6
Aspergilloz (10)	% 80
Fuzaryoz (3)	0/3
Mukormikoz (3)	0
Kriptokokkoz (12)	2/12

163 IFI olgusu
170 sağlıklı kontrol

Altta yatan hastalık /risk faktörü: Çeşitli
IFI tanısı: EORTC/MSG kriterleri

%	Duyarlılık	Özgüllük	PPV	NPV
Cut-off: 80	64.4	92.4	89	73

Lower sensitivity of serum (1,3)-β-D-glucan for the diagnosis of candidemia due to *Candida parapsilosis*

Clin Microbiol Infect 2016; 22: Pages 646.e5–646.e8

Abstract

The aim of this study was to evaluate the sensitivity and the levels of 1,3-β-D-glucan (BDG) among patients with candidaemia due to different *Candida* species. Retrospective study of all patients who had a single-species candidaemia and BDG testing performed within 48 h from the onset of candidaemia during 2009–2015 was performed. Factors influencing the sensitivity of BDG, including the presence of a central venous catheter, antifungal therapy and *Candida* species, were analysed in univariate and multivariate models. In all, 107 patients with the following *Candida* distribution were included: 46 (43%) *Candida albicans*, 37 (35%) *Candida parapsilosis*, and 24 (22%) other species. BDG sensitivity and levels were the highest in *C. albicans* candidaemia and lowest for *C. parapsilosis* (respectively, 72% and 410 pg/mL for *C. albicans*, 41% and 39 pg/mL for *C. parapsilosis*, and 63% and 149 pg/mL for other species; p 0.015 and p 0.003). In multivariate analysis, *Candida* species (*parapsilosis* versus others) was the only factor influencing the sensitivity of BDG (OR 0.3, 95% CI 0.1–0.7, p 0.006). The sensitivity of BDG in candidaemia seems highly dependent on the fungal species, with the lowest being for *C. parapsilosis*.

Beta-glukan testinin IFI tanısındaki yeri: Meta-analiz

2979 olgu
16 çalışma

Duyarlılık: % 77

Özgüllük: % 85

Kullanılan istatistiksel analizler önemli ölçüde değişken

Testin tanısal doğruluğu: iyi /(orta) düzeyde

Karageorgopoulos et al. CID 2011; Mar;52:750
(Onishi et al. JCM 2012: 50: 7)

Detection of (1, 3)-β-D-glucan in bronchoalveolar lavage and serum samples collected from immunocompromised hosts

Theel et al. Mycopathologia 2013; 175: 33

109 patients (Fungitell)

For IFI in general: Low PPV
(BAL & serum; 20 vs. 27%).

High NPV
(BAL & serum; 83 vs. 85%)

B-D-glukan, serum

IFE

The (1,3)β-D-Glucan Test as an Aid to Early Diagnosis of Invasive Fungal Infections following Lung Transplantation▽

Barbara D. Alexander,[1,2]* P. Brian Smith,[3] R. Duane Davis,[4] John R. Perfect,[1] and L. Barth Reller[1,2]

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2010, p. 4083–4088

TABLE 3. Per-test sensitivity, specificity, and positive and negative predictive values based on different (1,3)β-D-glucan test positive cutoff values

Glucan positive cutoff (pg/ml)	% (95% confidence interval)			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
60	70.7 (54.5, 83.9)	58.7 (55.1, 62.3)	8.5 (5.8, 12.0)	97.4 (95.4, 98.6)
70	63.4 (46.9, 77.9)	62.6 (59.0, 66.0)	8.4 (5.6, 12.0)	96.9 (95.0, 98.3)
80	63.4 (46.9, 77.9)	65.9 (62.4, 69.3)	9.2 (6.1, 13.1)	97.1 (95.2, 98.4)
100	58.5 (42.1, 73.7)	71.3 (67.9, 74.5)	9.9 (6.5, 14.5)	96.9 (95.1, 98.2)

area under the ROC curve was 0.69. Based on a 60-pg/ml positive cutoff, per-patient sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 64%, 9%, 14%, and 50%, respectively; per-test estimates were 71%, 59%, 9%, and 97%, respectively. The majority (92%) of patients not diagnosed with an IFI had at least one BG level of ≥60 pg/ml, and 90% had at least one BG level of ≥80 pg/ml. Respiratory colonization with mold and hemodialysis significantly affected mean BG levels. In conclusion, the accuracy of the BG test is marginal and its utility as a tool for the early diagnosis of IFI is questionable in the lung transplant population. Although the NPV of the BG test is high, the low PPV limits its utility as a screening tool for early diagnosis of IFI.

B-D-glukan, BAL

IFE

Bronchoalveolar Lavage Fluid (1,3)β-D-Glucan for the Diagnosis of Invasive Fungal Infections in Solid Organ Transplantation: A Prospective Multicenter Study

Wolfgang Mutschlechner,[1] Brigitte Risslegger,[1] Birgit Willinger,[2] Martin Hoenigl,[3] Brigitte Bucher,[4] Stephan Eschertzhuber,[5] and Cornelia Lass-Flörl[1]

Background. Prompt diagnosis of invasive fungal infections (IFI) remains a challenge. (1,3)β-D-glucan detection in bronchoalveolar lavage (BAL) fluid by Fungitell assay aims to further improve upon the test's utility by directly applying it to specimens from the target organ. **Methods.** A prospective multicenter analysis of the Fungitell assay was performed on BAL and serum samples obtained from nonselected solid-organ transplantation patients suffering from probable, proven or no IFI according to the revised criteria of the European Organisation for Research and Treatment of Cancer / Mycosis Study Group. **Results.** Two hundred thirty-three BAL and 109 serum specimens from 135 patients with proven, probable, or no IFI were tested. Based on a 100 pg/mL: cutoff per test sensitivity, specificity, positive and negative predictive values were 79.2%, 38.5%, 27.6%, and 86.3% in BALs and 79.2%, 81.8%, 69.2%, and 83.1% in sera investigated. **Conclusions.** The accuracy of the (1,3)β-D-glucan test is marginal so that its utility as a clinical test for early diagnosis of IFI is questionable in the lung transplant population. Although the high negative predictive value of the Fungitell assay in both, BALs and sera, may support exclusion of pulmonary IFI in solid-organ transplantation patients, the low positive predictive value limits its utility as a screening tool for early diagnosis of IFI.

(*Transplantation* 2015;99: e140–e144)

NPV yüksek; enf. unun ekarte edilmesinde yardımcı olabilir. PPV düşük; tanıdaki yeri sınırlı

Diagnostic Tools - B-D-glucan Assay

Population	Intention	Intervention	SoR	QoE	Comment
Mixed population: Adult ICU, Hematological disorders, SOT	To diagnose IFD (not specific for aspergillosis)	Diagnostic assay	C	II	4 different assays; Fungitell FDA approved and available in US and Europe; others only available in Japan; overall sensitivity of 77% and specificity of 85% Specificity limits its value in this setting Two or more consecutive samples: sensitivity = 65%, specificity = 93%; studies included once to thrice weekly
		Screening assays	C	II	Varies with assay and cut-off: Wako assay sensitivity = 40-97%, specificity = 51-99%

ESCMID-ECMM-ERS Aspergillus Guideline

Karageorgopoulos 2011, Lu 2011

Glukan testi:Yalancı pozitiflik

- Selüloz hemodiyaliz membranları
- Ig preparatları
- Antitümör polisakkarit
- Bakteriyal infeksiyonlar
  (Gram (–) basillerle > gram (+) koklarla)
- Siroz
- Abdominal cerrahi
- Mukozit, enterokolit
- (Glukan pozitif olan) kan / kan ürününün Transfüzyonu
- Antimikrobiyal ilaçlar (antifungaller dahil)

Ikemura et al. JCM 1989, 27: 1965; Kato et al. Nephron 2001, 89: 15; Digby et al. Clin Diagn Lab Immunol 2003; 10: 882; Singh & Paterson CMR 2005; 18: 44; Fungitell Assay Guide; Albert et al. EJCMID 2011 Apr; 10; Liss et al. JAC Dec 13 2015

1,3-β-D-Glucan contamination of common antimicrobials

B. Liss[1,2*], O. A. Cornely[1–5], D. Hoffmann[6], V. Dimitriou[5] and H. Wisplinghoff[6,7]

Journal of Antimicrobial Chemotherapy Advance Access published December 13, 2015

Results: Twenty-five antimicrobials (20 antibiotics and all the tested antifungals) contained enough BDG to trigger a positive test. Depending on the substance, BDG varied between 9 and 2818 pg/mL.

Conclusions: A majority of the available antimicrobial substances contained BDG, potentially limiting the utility of BDG testing in the context of prior exposure to these drugs. As the cumulative effects of repeated BDG exposure are unknown, efforts to reduce contamination should be considered.

Antimicrobial substance	Manufacturer	Batch	BDG (pg/mL) modified limulus amoebocyte lysate assay	SD	immunological assay	SD
Amikacin	B. Braun	16FM0040	41	4.2	36	3.3
Amoxicillin/clavulanic acid	Hikma	J001	**429**	31.1	**420**	28.2
Ampicillin	ratioPharm	N49967	**120**	8.5	**114**	7.8
Ampicillin/sulbactam	Teva	2859	**347**	24.4	**340**	22.8
Cefazolin	Hikma	147087.2	**1569**	71.6	**1672**	61.5
	Fresenius Kabi	147074,1	**982**	67.8	**982**	65.5
Cefepime	Bristol-Myers Squibb	4D02695	**1134**	77.1	**1150**	77.7
Cefotaxime	Fresenius Kabi	B14002	**445**	32.0	**445**	30.4
Ceftazidime	GlaxoSmithKline		**871**	60.8	**864**	58.1
Ceftriaxone	Stragen Nordic	18F3760	**313**	23.0	**305**	20.9
Cefuroxime	Fresenius Kabi	136177,1	**877**	59.9	**872**	58.3
Ciprofloxacin	Fresenius Kabi	137245.2	17	2.3	16	1.4
Clarithromycin	Martindale Pharma	J4	21	2.5	17	1.5
Clindamycin	Fresenius Kabi	141067,2	31	3.0	29	2.3
Colistin	Sobi	7916	**1006**	69.4	**987**	65.9
Trimethoprim/sulfamethoxazole	ratioPharm	N37410	43	3.0	36	2.9
Daptomycin	Novartis	CDF092H	**553**	37.5	**546**	37.2
Doxycycline	ratioPharm	D13761	**2808**	88.9	**2818**	78.7
Erythromycin	Stragen Nordic	318020	**498**	33.7	**482**	33.1
Flucloxacillin	Stragen Nordic	4P66HH	**211**	16.9	**195**	13.9
Gentamicin	B. Braun	147154	19	1.7	9	0.7
Imipenem	Fresenius Kabi	IDEA1294	**432**	31.2	**424**	28.7
Linezolid	Pfizer	14K23U84	**526**	37.6	**521**	35.4
Meropenem	Hospira	600E006A	**213**	15.4	**208**	14.6
Metronidazole	B. Braun	B8953	16	3.3	10	1.4
Moxifloxacin	Fresenius Kabi	42806	23	1.7	14	1.8
Penicillin G	INFECTOPHARM	B011401,1	33	4.3	23	2.3
Piperacillin/tazobactam	IBIGEN	1FL5001DE	**254**	17.8	**226**	15.2
Rifampicin	RIEMSER	4124	**89**	7.3	**83**	6.4
Tobramycin	B. Braun	1965140	41	2.8	40	3.3
Vancomycin	Fresenius Kabi	234/14	32	3.3	23	2.5
Amphotericin B deoxycholate	Bristol-Myers Squibb	46204TB23	**1315**	88.6	**1308**	87.3
Liposomal amphotericin B	Gilead	042471AD	**712**	50.4	**710**	47.4
Caspofungin	MSD	216260	**119**	8.1	**109**	7.5
Fluconazole	B. Braun	13284403	**168**	13.0	**139**	10.1
Voriconazole	Pfizer	Z316902	**229**	15.6	**224**	15.6

Values shown in bold are above the positivity cut-off.

Moleküler Yöntemler

SORUNLAR

- Standardizasyon
- Kontaminasyon
- Kolonizasyon-infeksiyon ayırımı
- Aralıklı pozitiflikler
- Proflaksi/tedavinin DNA salınımına etkisi
- Tedaviye yanıt olmaksızın negatifleşme

AVRUPA GRUBU Aspergillus-PCR standardizasyon çalışmaları

("European Aspergillus PCR Initiative")

Loeffler J et al. JCM 1999, 37: 1200; Lass-Florl et al. Brit J Haematol 2001, 113: 180; Yeo et al. CMR 2002, 15: 465

PCR-serum

IC

Performance of *Candida* Real-time Polymerase Chain Reaction, β-D-Glucan Assay, and Blood Cultures in the Diagnosis of Invasive Candidiasis

Results. PCR using plasma or sera was more sensitive than whole blood for diagnosing IC ($P = .008$). Plasma or sera PCR was more sensitive than BDG in diagnosing IC (80% vs 56%; $P = .03$), with comparable specificity (70% vs 73%; $P = .31$). The tests were similar in diagnosing candidemia (59% vs 68%; $P = .77$), but PCR was more sensitive for deep-seated candidiasis (89% vs 53%; $P = .004$). PCR and BDG were more sensitive than blood cultures among patients with deep-seated candidiasis (88% and 62% vs 17%; $P = .0005$ and .003, respectively). PCR and culture identified the same *Candida* species in 82% of patients. The sensitivity of blood cultures combined with PCR or BDG among patients with IC was 98% and 79%, respectively.

Conclusions. *Candida* PCR and, to a lesser extent, BDG testing significantly enhanced the ability of blood cultures to diagnose IC.

PCR Using Different Blood Components

15 controls. Plasma and serum samples did not differ in sensitivity (81% [13 of 16] vs 75% [12 of 16], respectively; $P = 1.0$) or specificity (67% [10 of 15] vs 73% [11 of 15], respectively; $P = 1.0$). For the remainder of the study, PCR was performed on plasma and/or serum samples.

n=55

Nguyen et al. CID 2012; 54: 1240

PCR-serum

IA

Real-time PCR on the first galactomannan-positive serum sample for diagnosing invasive aspergillosis in liver transplant recipients

F. Botterel, C. Farrugia, P. Ichai, J.-M. Costa, F. Saliba, S. Bretagne.

Transpl Infect Dis 2008: 10: 333–338

2 out of the 4 replicates. Among the 13 probable or possible IA, 8 patients were PCR positive. The other 12 patients who had no IA were all PCR negative. Our data suggest that a concomitant real-time PCR performed on the first GM-positive sample improves the specificity of the first GM-positive assay result.

PCR-BAL

IA

Comparison of an *Aspergillus* Real-time Polymerase Chain Reaction Assay With Galactomannan Testing of Bronchoalvelolar Lavage Fluid for the Diagnosis of Invasive Pulmonary Aspergillosis in Lung Transplant Recipients

Me-Linh Luong,[1] Cornelius J. Clancy,[1] Aniket Vadnerkar,[1] Eun Jeong Kwak,[1] Fernanda P. Silveira,[1] Mark C. Wissel,[2] Kevin J. Grantham,[2] Ryan K. Shields,[1] Maria Crespo,[1] Joseph Pilewski,[1] Yoshiya Toyoda,[3] Steven B. Kleiboeker,[2] Diana Pakstis,[1] Sushruth K. Reddy,[2] Thomas J. Walsh,[4] and M. Hong Nguyen[1]

Clinical Infectious Diseases 2011;52(10):1218–1226

Conclusions. A recently developed pan-*Aspergillus* PCR assay and GM testing of BAL fluid may facilitate the diagnosis of IPA after lung transplantation. *A. fumigatus*– and *A. terreus*–specific real-time PCR assays may be useful in rapidly identifying the most common cause of IPA and a species that is intrinsically resistant to amphotericin B, respectively.

PCR, kan/serum

IA

Table 11. Aspergillus PCR on whole blood, serum or plasma

Population	Intention	Intervention	SoR	QoE
Patients with haematological malignancies	To diagnose IA	PCR on blood samples	B	II
	To diagnose IA	PCR on serum samples		
	To diagnose IA	PCR on whole blood samples		
Haematopoietic stem cell transplantation	To diagnose IA	Prospective screening PCR on whole blood samples	B	II
	To diagnose IA	Prospective screening PCR on blood samples	B	II
	To diagnose IA	PCR and GM in BAL	A	II

ESCMID-ECMM-ERS Aspergillus Guideline

PCR, BAL/CSF

IA

Table 10. PCR on bronchoalveolar lavage or cerebrospinal fluid

Population	Intention	Intervention	SoR	QoE
Patients undergoing allogeneic stem cell transplantation recipients not on mould-active prophylaxis	To diagnose IA	BAL PCR	B	II
Patients with pulmonary infiltrates and haematological malignancies and prolonged neutropenia	To diagnose IA	BAL PCR	B	II
ICU patients,mixed populations	To diagnose IA	BAL PCR	B	II
Patients with haematological malignancies	To diagnose CNS aspergillosis or meningitis	CSF PCR	B	II

ESCMID-ECMM-ERS Aspergillus Guideline

ISHLT GUIDELINES

The 2015 International Society for Heart and Lung Transplantation Guidelines for the management of fungal infections in mechanical circulatory support and cardiothoracic organ transplant recipients: Executive summary

Table 4 Summary of Recommendations for Diagnosis of Aspergillosis in Adult Cardiothoracic Transplant Recipients

Recommendation	Class of recommendation	Level of evidence	Applies to heart Tx	Applies to lung Tx
Routine use of BAL-PCR is not recommended.	II	C	✓	✓
BAL-PCR should only be used in combination with other fungal diagnostics (e.g., chest CT scan, BAL-GM, culture) for IA diagnosis.	II	C	✓	✓

"Conflicting Evidence"
"Expert Opinion"

The Journal of Heart and Lung Transplantation, Vol 35, No 3, March 2016

The 2015 International Society for Heart and Lung Transplantation Guidelines for the management of fungal infections in mechanical circulatory support and cardiothoracic organ transplant recipients: Executive summary

Table 2 International Society for Heart and Lung Transplantation Standards and Guidelines Committee Grading Criteria

Class I	Evidence and/or general agreement that a given treatment or procedure is beneficial, useful, and effective
Class II	Conflicting evidence and/or divergence of opinion about the usefulness/efficacy of the treatment or procedure
Class IIa	Weight of evidence/opinion is in favor of usefulness/efficacy
Class IIb	Usefulness/efficacy is less well established by evidence/opinion
Class III	Evidence or general agreement that the treatment or procedure is not useful or effective and in some cases may be harmful
Level of evidence A	Data derived from multiple randomized clinical trials or meta-analyses
Level of evidence B	Data derived from a single randomized clinical trial or large non-randomized studies
Level of evidence C	Consensus of opinion of the experts and/or small studies, retrospective studies, registries

J Antimicrob Chemother 2016; **71**: 2386–2396

ECIL guidelines for the diagnosis of *Pneumocystis jirovecii* pneumonia in patients with haematological malignancies and stem cell transplant recipients

Alexandre Alanio[1], Philippe M. Hauser[2], Katrien Lagrou[3], Willem J. G. Melchers[4], Jannik Helweg-Larsen[5], Olga Matos[6], Simone Cesaro[7], Georg Maschmeyer[8], Hermann Einsele[9], J. Peter Donnelly[10], Catherine Cordonnier[11]*, Johan Maertens[12] and Stéphane Bretagne[1] on behalf of the 5th European Conference on Infections in Leukemia (ECIL-5†), a joint venture of The European Group for Blood and Marrow Transplantation (EBMT), The European Organization for Research and Treatment of Cancer (EORTC), the Immunocompromised Host Society (ICHS) and The European LeukemiaNet (ELN)

Immunofluorescence assays are recommended as the most sensitive microscopic method (recommendation **A-II**). Real-time PCR is recommended for the routine diagnosis of PCP (**A-II**). Bronchoalveolar lavage (BAL) fluid is recommended as the best specimen as it yields good negative predictive value (**A-II**). Non-invasive specimens can be suitable alternatives (**B-II**), acknowledging that PCP cannot be ruled out in case of a negative PCR result (**A-II**). Detecting β-D-glucan in serum can contribute to the diagnosis but not the follow-up of PCP (**A-II**). A negative serum β-D-glucan result can exclude PCP in a patient at risk (**A-II**), whereas a positive test result may indicate other fungal infections. Genotyping using multilocus sequence markers can be used to investigate suspected outbreaks (**A-II**). The routine detection of dihydropteroate synthase mutations in cases of treatment failure is not recommended (**B-II**) since these mutations do not affect response to high-dose co-trimoxazole. The clinical utility of these diagnostic tests for the early management of PCP should be further assessed in prospective, randomized interventional studies.

Table 3: Diagnostic approaches to *Pneumocystis* pneumonia in transplantation

Test	Estimated yield	Comments
Routine sputum smears	Generally poor	• Organ transplant patients with PCP may have smaller burden of infecting organisms than AIDS patients (39) • Use of fluorescent monoclonal antibody staining may increase the sensitivity of finding the organism over other stains
Induced sputum smears	Improved over routine sputum exam when coupled with antibody staining; yield ≥50% (29)	• Yield from induced sputum in transplant patients may not reflect that found in HIV-infected patients • Sensitivity and specificity in transplant patients unknown • Repeat testing may improve yield (30)
Bronchoalveolar	Generally ≥70% in non-AIDS immunocompromised hosts when coupled with antibody staining	• Older data involving immunosuppressed patients with PCP suggested a yield close to 80% (40)
Transbronchial biopsy	Increases yield of routine BAL (1)	• Multiple biopsies preferred to increase sensitivity with some increased procedural risk
Open lung biopsy	Often considered to be a gold standard, but early patchy disease may decrease yield	• Case reports highlight PCP infections missed on BAL that were subsequently identified from open lung biopsies (41,42) • Cases of missed infection in open lung biopsy also reported (30)
PCR testing of samples	Sensitivity and specificity vary depending on manner of sampling (sputum vs. BAL) and assay employed	• Multiple assays are not standardized. Generally target genes for conserved surface glycoproteins or rRNAs • Specificity unknown
Plasma (1→3) β-D-glucan	Some reports in transplant and HIV patients (43–46). Meta-analysis suggests a sensitivity of almost 95%, but with a specificity in the mid-80% (47)	• (1→3) β-D-glucan is produced in the cyst cell wall and detection in the serum has been associated with underlying infection (also positive in other invasive fungal infections) (48) • Clinical trials data lacking

AIDS = acquired immunodeficiency syndrome; BAL = bronchoalveolar lavage; HIV = human immunodeficiency virus; PCP = *Pneumocystis* pneumonia; PCR = polymerase chain reaction; rRNA = ribosomal ribonucleic acid.

Diagnostic Modalities for Invasive Mould Infections among Hematopoietic Stem Cell Transplant and Solid Organ Recipients: Performance Characteristics and Practical Roles in the Clinic

Ghady Haidar [1], Bonnie A. Falcione [1,2,3] and M. Hong Nguyen [1,3,*]

Table 1. Diagnostic performance of GM, BDG and *Aspergillus* PCR and recommendations for their roles in diagnosis and screening. Unless indicated, performance data are derived from meta-analysis. ¥ Limited data, data shown based on individual reports.

	Sensitivity	Specificity	Recommendations	Caveats
Serum GM	22%	84%	Diagnosis of IA: poor sensitivity. Can be used as adjunct to other diagnosis modalities.	
BAL GM				
Organ transplant [49,50]				
Cut-off ODI of 0.5	82%–100%	84%–96%	Diagnosis of IA: good. Negative BALF GM essentially rules out IPA if the patient is not on anti-mould antifungals; GM should not be routinely tested in surveillance BALF in lung transplant patients due to low specificity.	Optimal cut-off for positivity not clear (probably 1.0 or 1.5) GM in BAL cannot differentiate IPA from *Aspergillus* colonization.
Cut-off ODI of 1.0	82%–100%	91%–97%		
Cut-off ODI of 1.5	100%	92%		
Lung transplant [51,52]				
Cut-off ODI of 0.5	60–100%	40%–95%		
Cut-off ODI of 1.0	60–100%	81%–98%		
Cut-off ODI of 1.5	100%	90%		

SOT

J. Fungi **2015**, *1*, 252-276; doi:10.3390/jof1020252

Diagnostic Modalities for Invasive Mould Infections among Hematopoietic Stem Cell Transplant and Solid Organ Recipients: Performance Characteristics and Practical Roles in the Clinic

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S O T

	Sensitivity	Specificity	Recommendations
Beta-D-glucan	66%	44%	Very limited data. Not useful in lung transplant patients because of very low PPV.
Serum/Blood PCR	No data	No data	No data
BAL Asp PCR	100%	88%	Diagnosis of IPA: good to very good. Cannot differentiate between IPA and fungal colonization.

J. Fungi **2015**, *1*, 252-276; doi:10.3390/jof1020252

Invasive fungal infections in solid organ transplant recipients

J. Gavaldà[1], Y. Meije[1], J. Fortún[2], E. Roilides[3], F. Saliba[4], O. Lortholary[5], P. Muñoz[6,7,8,9], P. Grossi[10], M. Cuenca-Estrella[11] on behalf of the ESCMID Study Group for Infections in Compromised Hosts (ESGICH)

Clin Microbiol Infect 2014; **20** (Suppl. 7): 27–48

Recommendations for the diagnosis of IFD in SOT

- Positive blood cultures that yield yeasts or, in some cases, filamentous fungi (*Scedosporium* spp. and *Fusarium* spp.) are considered diagnostic of IFD (AIII).
- A proven diagnosis of IFD can also be based on the observation of tissues with invasive fungal structures or through isolation from sterile tissue or fluid samples (not obtained through drains) (AIII).
- BDG quantification is recommended to rule out *Candida* infection in adult patients with risk factors and/or symptoms (CIII)
- Detection of GM antigen in plasma or serum should not be used for the routine diagnosis or treatment monitoring of IA in SOT recipients (DIII).
- Detection of GM antigen in BAL (AII) or CSF (BIII) is useful for the diagnosis of IA (AII) and should be performed whenever possible.

- Special considerations for lung transplantation:
  - In the case of a positive sputum culture for *Aspergillus* spp., a bronchoscopy and high-resolution chest CT scan should be performed to rule out tracheobronchial and/or invasive disease (BIII).
  - In the case of a positive GM in BAL, a high-resolution chest CT scan should be performed to rule out invasive disease (AIII).

Invasive fungal infections in solid organ transplant recipients

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Recommendations for the diagnosis of IFD in SOT

[57]. The specificity of GM is reduced by the potential false-positives, which are usually associated with the use of β-lactams [58]. A high frequency of false-positives for GM during the first week after liver transplantation was observed, a finding that was associated with β-lactam prophylaxis [59,60]. Therefore, GM should not be used for routine diagnosis or treatment monitoring.

One potential advance in the diagnosis of IA is the use of GM detection in bronchoalveolar lavage (BAL). A study performed at Pittsburgh assessed the role of GM quantification in BAL of 116 lung recipients [61]. Based on a cut-off of 0.5, the authors found a sensitivity of 60% and a specificity of 95%; when the cut-off was raised to 1.0, the sensitivity was the same and the specificity was 98% [61]. Another study with lung recipients in Florida reported sensitivity and specificity of GM in BAL of 100% and almost 91%, respectively, using an index >1.0 as cut-off [62].

Invasive fungal infections in solid organ transplant recipients

J. Gavaldà[1], Y. Meije[1], J. Fortún[2], E. Roilides[3], F. Saliba[4], O. Lortholary[5], P. Muñoz[6,7,8,9], P. Grossi[10], M. Cuenca-Estrella[11] on behalf of the ESCMID Study Group for Infections in Compromised Hosts (ESGICH)

Clin Microbiol Infect 2014; **20** (Suppl. 7): 27–48

Recommendations for the diagnosis of IFD in SOT

- If invasive pulmonary aspergillosis is suspected, a high-resolution chest CT is recommended, because its sensitivity is higher than the chest radiograph (AII).
- Therapeutic response should be monitored by clinical follow-up, and periodical high-resolution CT should be considered every 7–10 days during the first weeks of therapy in adults (AIII).
- PCR should not be used for routine diagnosis or treatment monitoring of IA in SOT recipients (DIII).

- The detection of mould nucleic acid by PCR in BAL or sputum of a transplant patient should be considered, particularly in lung/heart transplant patients due to the subsequent risk of invasive infection (BII). In any case, these procedures should be considered experimental, and the results need to be validated.
- The detection of β-D-glucan in serum may be helpful in the diagnosis of IFD (other than cryptococcosis and mucormycosis), together with the clinical-radiological criteria and the immunosuppressive-host criteria, although false-positive results have been reported (B-II). The detection of cryptococcal antigen in serum or CSF and the detection of positive blood cultures, skin cultures (in the case of compatible lesions) and urine cultures are the main diagnostic techniques for patients with suspected cryptococcosis (AII).

SOT - IFE ESCMID-ESGICH

YÖNTEM	ÖNERİ DÜZEYİ
Kan kültürü pozitifliği	AIII (Diagnostik)
Dokuda mikr. (+)/doku veya steril örnek kültür (+)	AIII
GM-Serum (IA)	DIII
GM-BAL (IA)	AII
GM-CSF	BIII
BDG, IFE tanısı	BII
BDG, Candida enf. ekarte etmek için	CIII
PCR-Serum (IA)	DIII
PCR-BAL/Balgam (IA)	BII (doğrulanması gerekir)
Cryptococcus Ag-Serum/CSF	AII
Cryptococcus: Kan kültürü/İdrar Kültürü/Deri lezyonundan kültür (+)	AII

Clin Microbiol Infect 2014; **20** (Suppl. 7): 27–48

SONUÇ

- Kültür, invazif fungal enfeksiyonlarının tanısında altın standart olma özelliğini korumaktadır. Ancak, erken tanıya katkısı sınırlıdır, duyarlılık ve özgüllüğü istenilen düzeylerde değildir.

- Bazı serolojik testler erken tanıya yardımcı olabilirse de solid organ nakil olgularında duyarlılıkları sınırlı olabilir. Bu yöntemlerin, tek başlarına değil, konvansiyonel yöntemlerle birlikte kullanılmaları önerilir.

PUBLISHED: 25 JULY 2017 | VOLUME: 2 | ARTICLE NUMBER: 17120

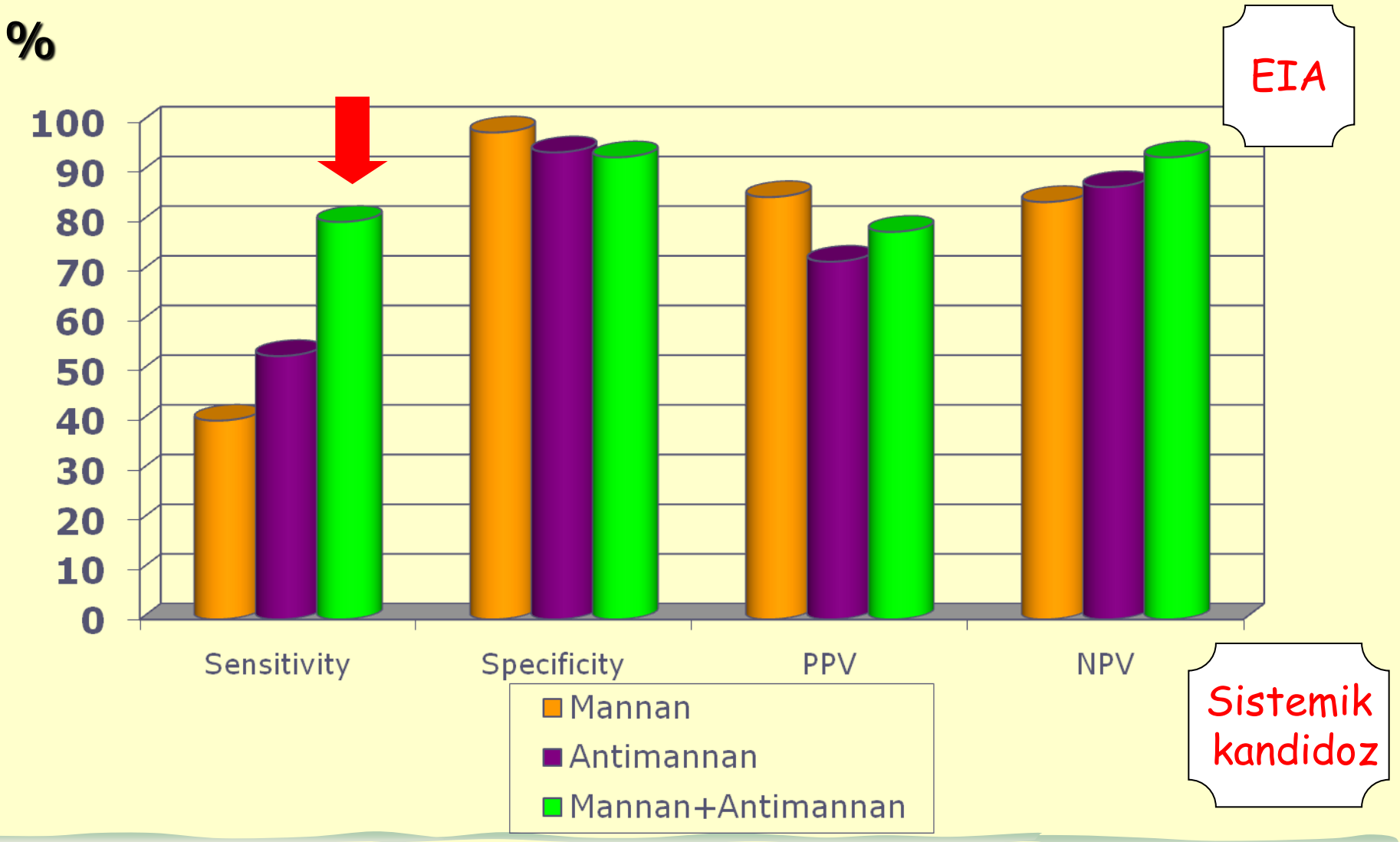
editorial

Stop neglecting fungi

Fungal pathogens are virtually ignored by the press, the public and funding bodies, despite posing a significant threat to public health, food biosecurity and biodiversity.

Experts agree that fungal pathogens are a serious threat to human health, food biosecurity and ecosystem resilience, yet lack of funding translates into inadequate surveillance systems to monitor fungal disease incidence and antifungal drug resistance, which often rely on not-for-profit initiatives, such as the Global Action Fund for Fungal Infections (GAFFI; http://www.gaffi.org/). As highlighted in the World Health Organization (WHO) Global Report on Antimicrobial Resistance Surveillance[8], which devotes fewer than 10% of its pages to fungi, resources allocated for monitoring and reducing antifungal drug resistance are limited. Indeed, the WHO has no funded programmes specifically targeting fungal diseases, fewer than 10 countries have national surveillance programs for fungal infections, and fewer than 20 have fungal reference diagnostic laboratories.

Mannan & anti-mannan



Sendid et al. JCM 1999; 37: 1510

Prospective evaluation of mannan and anti-mannan antibodies for diagnosis of invasive Candida infections in patients with neutropenic fever

Ellis et al. J Med Microbiol 2009; 58: 606

The diagnostic performance and usefulness of the Platelia antigen and antibody test (Bio-Rad) was investigated in a prospective study of haematological patients at risk for invasive Candida infections.

A strategy was developed to determine diagnostic cut-offs from receiver operating characteristic curves with maximal sensitivity and, given this sensitivity, maximal specificity, both being greater than 0. In this patient population, these values were 0.25 ng ml(-1) for mannan (M) and 2.6 arbitrary units ml(-1) for anti-mannan (AM), which are lower than those recommended by the manufacturer. All patients developed at least one positive diagnostic M or AM result during the 10 days of persistent febrile neutropenia (PFN). The optimal overall performance was found when two consecutive positive tests for both M and AM were used [sensitivity, specificity, positive predictive value and negative predictive value (NPV) of 0.73, 0.80, 0.36 and 0.95, respectively]. There was a positive correlation of M with beta-D-glucan (r=0.28, P=0.01). The first positive M test was found up to a mean+/-sd of 8.8+/-8.5 (range 2-23) days prior to a clinical/mycological diagnosis of IC. The low specificities of the test performance may have been due to some of the comparator patients having subclinical Candida infections as evidenced by the high incidence of colonization among them (60% had a colonization index of >or=0.5).

The high NPVs suggest that the tests may be particularly useful in excluding IC. It is feasible to explore the use of serial measurements of M and AM as part of a broader diagnostic strategy for selecting PFN patients to receive antifungal drug therapy.

Specificity of mannan antigen and anti-mannan antibody screening in patients with haematological malignancies at risk for fungal infection

Wiebke Duettmann,[1,2] Christoph Koidl,[3] Robert Krause,[1] Gertrude Lackner,[3] Albert Woelfler[2] and Martin Hoenigl[1,4,5]

Mycoses. 2016 Jun; 59: 374

Summary

Combination of mannan antigen and anti-mannan antibody (Mn/A-Mn) testing has been reported a useful and specific strategy for diagnosis of invasive Candida infections (ICIs). We evaluated Mn/A-Mn as a screening tool in patients with haematological malignancies. This clinical prospective study was performed at the Division of Hematology, Medical University Graz, Austria between July and December 2012. Patients at risk for fungal infection were included into the study and twice weekly screened by Mn/A-Mn testing, yielding 650 samples. Of overall 67 patients 66 had no evidence for ICI. From those, 153/640 serum samples (23.9%) were positive for mannan Ab, and nine (1.4%) for Ag. Most false positive Ab results were observed among 375 samples from patients without haematopoietic stem cell transplantation (34.9% resulted positive). Combined specificity of Mn/A-Mn was 74.8%. Of 10 samples obtained in the single patient with candidemia, five were positive for mannan Ag (from the day of diagnosis up to 40 days after detection of candidemia) and none for Ab. In conclusion, mannan Ab screening yielded a high number of false positive results. While mannan Ag was found to be highly specific and may have potential for diagnostic driven testing, mannan Ab testing cannot be recommended based on our study results.

Uludağ Üniv. Deneyimi

Hicran Akın ve ark. 2013

- 72 kandidemi
  - 63 non-nötropenik
  - 9 nötropenik
- 30 bakteriyemi
- 26 sağlıklı kontrol

n= 128

Prof. Dr. Beyza Ener'in izniyle; Yayınlanmamış veri

Uludağ Üniv. Deneyimi

Hicran Akın ve ark. 2013

	Duyarlılık	Özgüllük	PPV	NPV
Mannan Ag	31,9	76,8	63,9	46,7
Anti-mannan Ab	81,9	46,4	66,3	66,7
Her ikisi (+)	20,8	**89,3**	48,4	46,7
Herhangi biri (+)	**93**	33,9	64,4	**88,1**

Tüm Hastalar (n=128)

Prof. Dr. Beyza Ener'in izniyle; Yayınlanmamış veri

Uludağ Üniv. Deneyimi

Hicran Akın ve ark. 2013

	Mannan Ag (+)	Mannan Ab (+)
Nötropenik hasta (9)	6 (%66,7)	2 (%22,2)
Non-nötropenik hasta (63)	17 (%27)	57 (%90,5)
Bakteriyemili ve sağlıklı kontrol (56)	13 (%23,2)	30 (%53,6)

Prof. Dr. Beyza Ener'in izniyle; Yayınlanmamış veri

ESCMID-ECMM-ERS Aspergillus Guideline

Population/Test: Storage of original samples

Population	Intention	Intervention	SoR	QoE	Comment
Any	To prevent degradation of biomarkers, eg. Galactomannan (GM) in serum or BAL/bronchial washes	Complete assay soon after delivery to laboratory. Avoid short or long-term storage of serum at 4° C.	A	I	Has been established that galactomannan in serum degrades with short-term and long-term storage at 4C. BAL fluid GM index values remain stable. Testing of pos/neg serum and BAL fluid pools showed no decline in galactomannan index over 11 months at –20° C

ASM Manual of Clinical Microbiology, 10th Edition, Johnson et al. 2013, Oren et al. 2012, Furfano et al. 2012, Wheat et al. 2014

IDSA-ASM Kılavuzu; Transport ve saklama koşulları:
Serum: : + 4°C'de 5 güne kadar, > 5 gün: -70°C'de saklama
BAL: Oda sıcaklığı, 2 saate kadar; >2-24 sa. : + 4°C

Impact of pre-analytical variables in the determination of serum galactomannan

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pre-analytical variables interfering on the test. Here we studied the influence of temperature and sample storage duration in GM results, using samples known to be negative and positive (spiked) for GM. We also evaluated the effect of hemolysis and hyperbilirubinemia on GM optical indexes. We found no influence of storage time (up to 96 h) and temperatures (refrigerated vs. RT) on GM results. However, bilirubin ($P = .022$) and haemoglobin ($P = .003$) content influenced GM readings in samples known for being GM positive and negative at baseline, respectively. We conclude that the Platelia GM test does not suffer major influence of pre-analytical variables such as storage conditions, and low levels of hemolysis and hyperbilirubinemia. Nonetheless, massive haemolysis seems to interfere with GM readings in GM-negative samples, and high levels of bilirubin can affect GM readings in samples that are positive for GM at baseline. These findings

testing. In particular, we now know that GM readings are not affected by storage of samples for up to 5 days at RT. Moreover, jaundice may have an influence on GM readings in individuals who are already positive for GM, reducing GM-ODI by up to 44%. Still, Hb may have an influence on GM readings in individuals who are GM-negative, by increasing GM-ODI by up to 42%. These data may be clinically useful when interpreting GM results in patients at risk for IA.

Medical Mycology Advance Access published November 12, 2016

Medical Mycology, 2016, 0, 1–7

Post-diagnostic kinetics of the (1 → 3)-β-D-glucan assay in invasive aspergillosis, invasive candidiasis and *Pneumocystis jirovecii* pneumonia

Koo et al. CMI 2012; 18: E122

IA: 69
olgu
IC: 40
PJP: 18

overall 1-week decline in BG was 0 pg/mL (IQR 0–53) in IA, 0 (IQR – 65 to 12) in IC and 17 (IQR 0–82) in PCP. Most patients with BG values through 6 and 12 weeks had persistent levels >80 pg/mL. Initial BG and the early trajectory of BG were not predictive of 6-week or 12-week clinical failure or mortality. Whereas BG eventually declines in patients with IA, IC and PCP, it lacks prognostic value within a clinically meaningful time frame.