



Highlights in Micologia Medica Diagnostica

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Argomenti

- Ricerca antigeni;
- Ricerca acidi nucleici;
- Identificazione mediante MALDI-TOF MS;
- Marker d'infezione?





Ricerca Antigeni

- Aspergillosi (galattomanano in pazienti pediatrici, su BAL di pazienti oncoematologici, a confronto con β -1,3 glucano);
- IFI (β -1,3 glucano su liquido cefalo-rachidiano, esperienza italiana in ICU);
- Criptococcosi (GXM serico come screening di prevenzione in pazienti HIV positivi).





Threshold of galactomannan antigenemia positivity for early diagnosis of invasive aspergillosis in neutropenic children

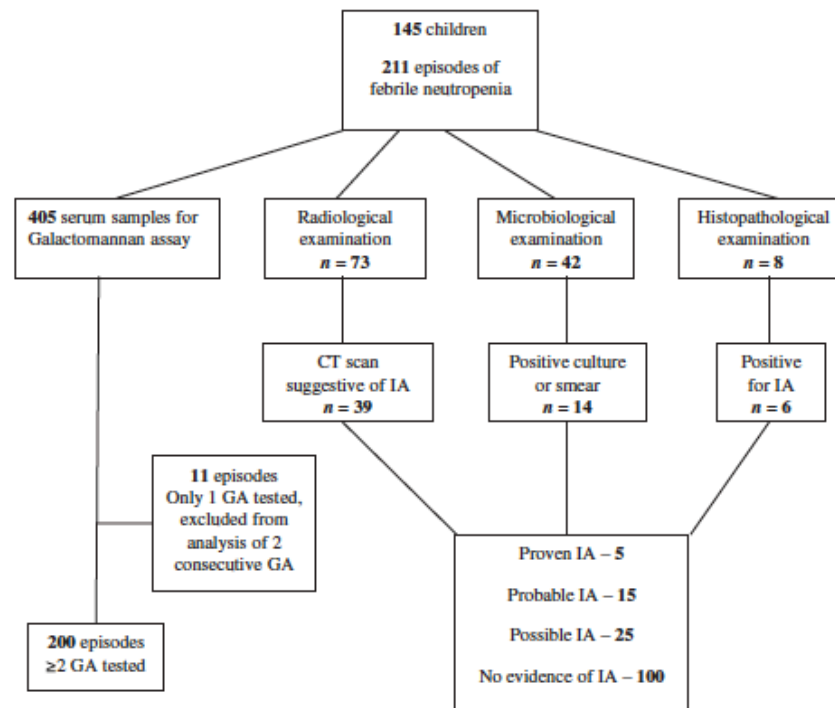


Figure 1. Flow diagram of 145 patients included in the study and investigations to diagnose invasive aspergillosis (IA). GA = galactomannan assay.

Due determinazioni > una;
Decremento falsi negativi;
Negativizzazione del titolo associata a esito favorevole;
Titoli elevati $\geq 1,9$ associati a elevata mortalità.



Galactomannan in bronchoalveolar lavage for diagnosing invasive fungal disease

Scarsa accuratezza diagnostica;

Scarsa corrispondenza con il sospetto radiologico e la positività serica al galattomannano;

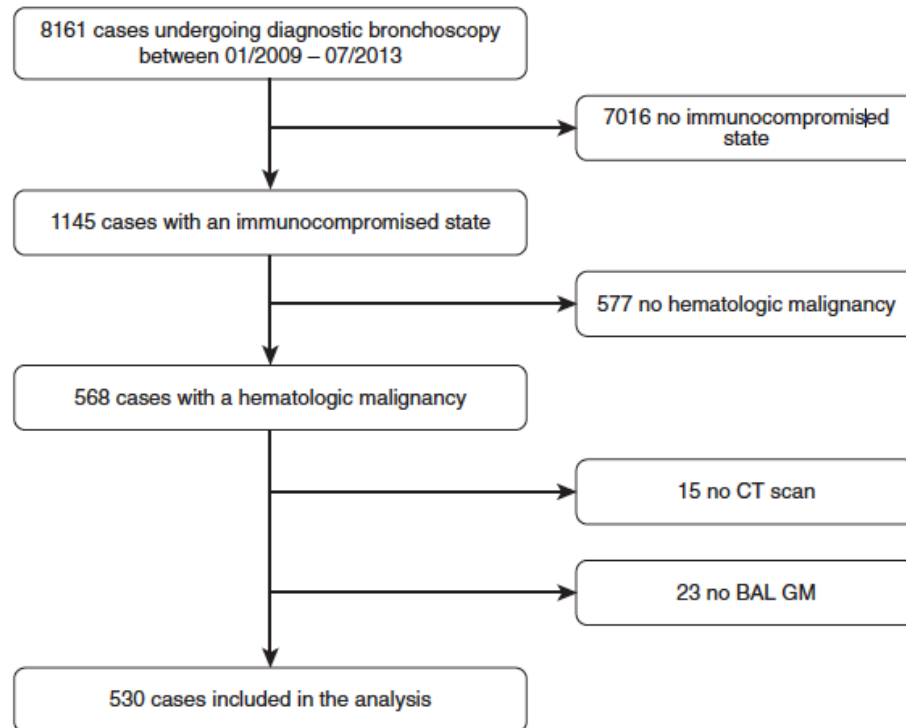


Figure 1. Study design according to the CONSORT guidelines. BAL = bronchoalveolar lavage; CT = computed tomography; GM = galactomannan.

	Sen	Spe	PPV	NPV
Tratt empirico	0,42 0,34-0,5	0,75 0,,71-0,8	0,39 0,31-0,47	0,78 0,73-0,82
Sospetto radiolog	0,34 0,27-0,42	0,73 0,68-0,78	0,37 0,3-0,46	0,7 0,65-0,75





Use and limits of (1-3)- β -d-glucan assay (Fungitell), compared to galactomannan determination (Platelia Aspergillus), for diagnosis of invasive aspergillosis

TABLE 2 Performance of galactomannan and (1-3)- β -D-glucan tests for diagnosis of invasive aspergillosis

Variable	No. (%)		LR (95% CI)
	IA ^a (n = 69)	Control (n = 147)	
Galactomannan test^b			
Negative	35 (51)	142 (97)	0.53 (0.42–0.66)
Positive	34 (49)	5 (3)	14.5 (5.92–35.4)
(1-3)-β-D-Glucan test^c			
Negative	13 (19)	120 (82)	0.23 (0.14–0.38)
Positive	56 (81)	27 (18)	4.42 (3.09–6.33)
GM test/BG test			
Negative/negative	11 (16)	116 (79)	
Positive			0.20 (0.12–0.35)
Negative			3.99 (2.87–5.54)
Negative/positive	24 (35)	26 (18)	
Positive			1.97 (1.22–3.16)
Negative			0.79 (0.66–0.96)
Positive/negative	2 (3)	4 (3)	
Positive			1.07 (0.20–5.68)
Negative			1.00 (0.95–1.05)
Positive/positive	32 (46)	1 (1)	
Positive			68.2 (9.51–488.7)
Negative			0.54 (0.43–0.67)

^a Proven and probable diagnoses according to EORTC/MSG criteria.

^b Specificity, 97% (95% CI, 92 to 99%); sensitivity, 49% (95% CI, 37 to 62%).

^c Specificity, 82% (95% CI, 74 to 88%); sensitivity, 81% (95% CI, 70 to 90%).

Maggiore sensibilità, ma minore specificità di BG vs GM;
Più BG falsi positivi, soprattutto in caso di batteremia;
BG negatività utile per confermare falsi positivi in GM;
Combinazione dei due metodi proposta per migliore diagnosi

J Clin Microbiol. 2014 Jul;52(7):2328-33





The utility of bronchoalveolar lavage beta-D-glucan testing for the diagnosis of invasive fungal infections

Table 2 BAL and serum BDG and GM levels and clinical response in the patients with proven/probable pulmonary IFI (n = 34).

^a IFI	^b Risk factor(s)	BAL BDG (pg/ml)	BAL GM (index)	Serum BDG (pg/ml)	Serum GM (index)	Response at 12 wks
^a IA	CGD	>500	1.02	97	<0.5	Success
^a IA	ID	129	<0.5	NP	NP	Success
IA	AA, HSCT, GVHD, S	>500	2.3	<31	<0.5	Success
IA	Heme, N	>500	5.5	296	1.8	Unknown
IA	Heme, HSCT, S	>500	0.6	<31	<0.5	Death
IA	AA, HSCT, GVHD	>500	7.33	NP	NP	Success
IA	CGD, HSCT, S	450	4.9	>500	5.6	Failure
IA	Heme, N, S	117	0.7	<31	<0.5	Failure
IA	Heme, HSCT, GVHD	86	0.92	370	<0.5	Failure
IA	AA, N	85	4.76	183	3.21	Failure
IA	ID, HSCT, N	<31	11.4	92	1.2	Failure
IA	CGD	<31	<0.5	204	NP	Failure
IA	AA, N	<31	<0.5	39	<0.5	Failure
IA	Heme, HSCT, S	<31	<0.5	<31	<0.5	Failure
^a IFI	CGD	<31	1.02	<31	NP	Failure
^a IFI	ID	<31	<0.5	NP	NP	Success
IFI	Heme, HSCT, N	215	<0.5	137	<0.5	Success
IFI	Heme, HSCT, S	149	<0.5	93	<0.5	Failure
IFI	AA, N	140	<0.5	210	<0.5	Success
IFI	Heme, HSCT, N, S	135	<0.5	109	<0.5	Death
IFI	AA, HSCT, GVHD, N, S	129	<0.5	189	<0.5	Failure
IFI	Heme, HSCT, GVHD	<31	<0.5	>500	<0.5	Death
IFI	Heme, HIV, N, S	<31	<0.5	>500	<0.5	Death
IFI	CGD	<31	NP	139	<0.5	Success
^a <i>Paecilomyces</i>	ID, HSCT, GVHD, N	>500	8.4	370	3.5	Death
^a Mucormycosis	Heme, HSCT, GVHD, S	168	0.56	NP	<0.5	Death
^a Mucormycosis	Heme, HSCT, GVHD	86	0.92	370	<0.5	Failure
^a Mucormycosis	AA, N	<31	<0.5	<31	<0.5	Failure
^a Fusariosis	AA, N	<31	<0.5	74	<0.5	Failure
^a Scopulariopsis	AA, N, S	<31	<0.5	<31	<0.5	Success
Fusariosis	Heme, HSCT, GVHD, N, S	73	<0.5	<31	<0.5	Death
Mucormycosis	Heme, HSCT, N	54	<0.5	NP	NP	Success
<i>Paecilomyces</i>	Heme, HSCT, GVHD, N, S	35	<0.5	<31	<0.5	Death
Mucormycosis	AA, N	<31	<0.5	<31	<0.5	Death

NP = not performed.

^a Denotes proven cases; otherwise all other cases are probable IFI per 2008 EORTC/MSG definitions (IA = invasive aspergillosis; IFI = invasive fungal infection not otherwise specified; causative organism listed if known).

^b Risk factors annotated as follows: AA = aplastic anemia; CGD = chronic granulomatous disease; GVHD = graft versus host disease; Heme = hematologic malignancy; HIV = human immunodeficiency virus; HSCT = hematopoietic stem cell transplantation; ID = other immunodeficiency (e.g. GATA-2, DOCK-8); N = neutropenia; S = steroids (≥ 0.3 mg/kg/d prednisone equivalent).

BG in BAL molto sensibile per casi di pneumocistosi;
Sensibilità comparabile tra BG in BAL e GM in BAL per aspergillosi invasiva e IFI;
Bassa specificità in BAL;
Scarsa riproducibilità del saggio su BAL.

J Infect. 2014 Sep;69(3):278-83





Utility of (1-3)- β -D-glucan testing for diagnostics and monitoring response to treatment during the multistate outbreak of fungal meningitis and other infections

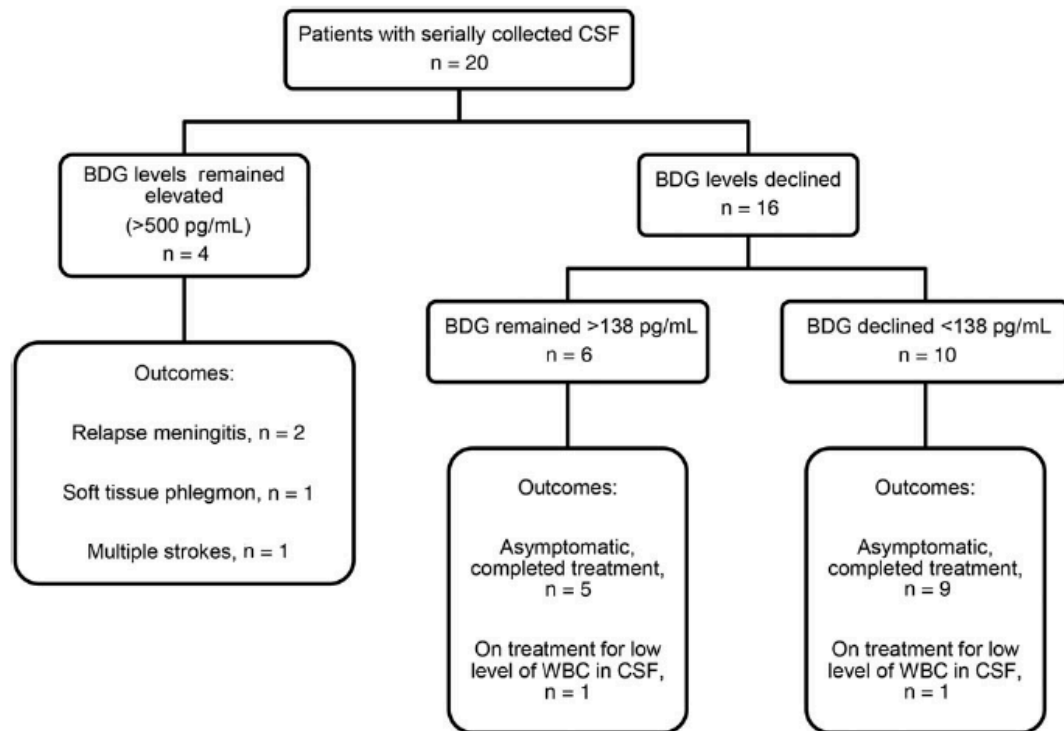


Figure 2. Flowchart showing (1–3)- β -D-glucan levels and outcomes of patients with serially collected cerebrospinal fluid (for individual patient numbers see Table 3). Abbreviations: BDG, (1–3)- β -D-glucan; CSF, cerebrospinal fluid; WBC, white blood cell.

BG maggiore sensibilità rispetto all'amplificazione; Il dato potrebbe essere correlato a una maggiore persistenza del Bg vs DNA per assenza di β -glucanasi; BG come marker utile nel monitoraggio terapeutico

Clin Infect Dis. 2014 Mar;58(5):622-30





Interference of confounding factors on the use of (1,3)-beta-D-glucan in the diagnosis of invasive candidiasis in the intensive care unit

Table 4 Comparison of BG values between the control group and patients receiving different doses of albumin

Albumin infusion	10 g (20 % albumin 50 mL)	20 g (20 % albumin 100 mL)	30 g (20 % albumin 150 mL)	Over 30 g (20 % albumin >150 mL)
BG levels after albumin infusion (median)	87.5 (IQR 0.0–523)	100.0 (IQR 0.0–261.3)	0.0 (IQR 0.0–400)	75.0 (IQR 0.0–341.5)
BG levels in the control group (median)	0.0 (IQR 0.0–140.0)	0.0 (IQR 0.0–140.0)	0.0 (IQR 0.0–140.0)	0.0 (IQR 0.0–140.0)
<i>p</i> -Value	0.2469 (>0.05)	0.0511 (>0.05)	0.6007 (>0.05)	0.0176 (<0.05)
Odds ratio	1.0020	1.0018	1.0018	1.0021
95 % CI	0.9993–1.0048	0.9998–1.0018	0.9994–1.0043	1.0006–1.0037
<i>p</i> -Value	0.1573	0.0833	0.1584	0.0059 (<0.05)

BG (1,3)- β -D-glucan, CI confidence interval, IQR interquartile range

BG can be considered a useful marker for invasive candidiasis in ICU patients;

L'albumina, somministrata due giorni prima della ricerca del BG, aumenta significativamente i livelli serici di BG



Prevalence and correlates of cryptococcal antigen positivity among AIDS patients--United States 1986-2012

Studio retrospettivo per Ag criptococcico nel siero di pazienti con numero di CD4 inferiore a 100 cell/ml;

La positività dell'antigene nel siero di solito precede di 3 settimana la comparsa dei segni e sintomi di meningite criptococcica;

La ricerca del GXM nel siero di pazienti dovrebbe essere utilizzata per soggetti "late presenters";

La positività e la successiva terapia antifungina prevengono non solo la meningite, ma abbassano il tasso di mortalità per tale patologia.





Ricerca Acidi Nucleici

- IFI (PCR in biopsie e BAL);
- DematOMICOSI (identificazione di specie).

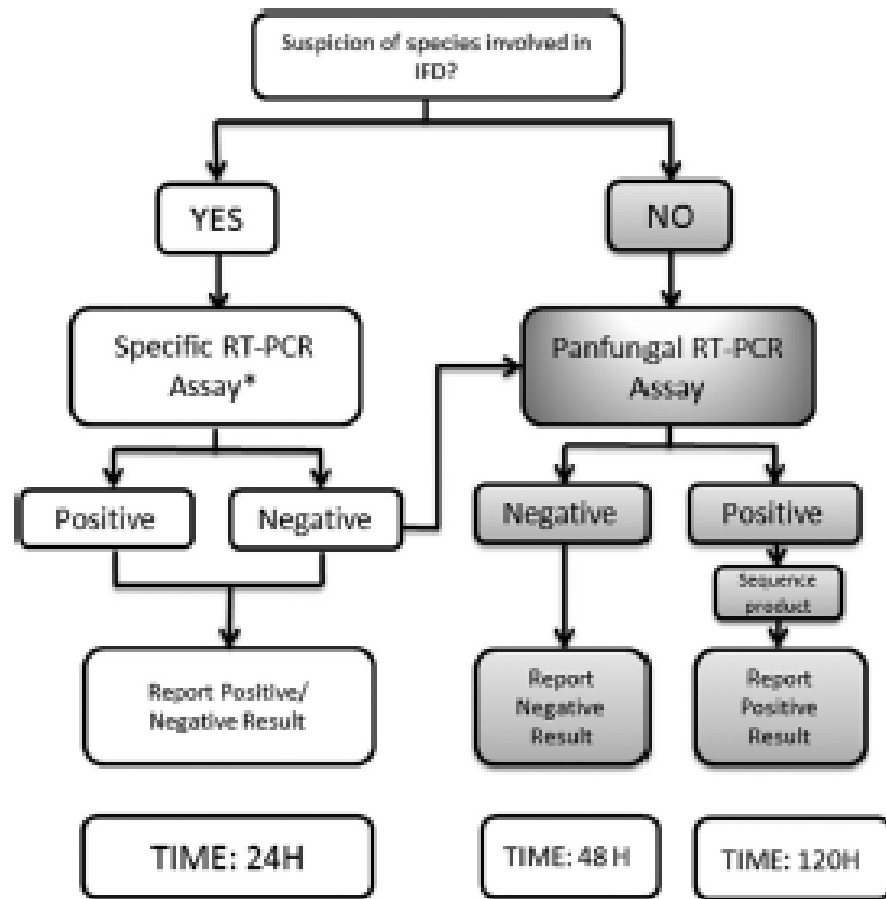
Identificazione mediante MALDI-TOF MS

- Lieviti (*Candida* spp, *Cryptococcus* spp);

Marker d'infezione

- Aspergillosi (proteine seriche in Western Blot e MS)





Performance of panfungal--and specific-PCR-based procedures for etiological diagnosis of invasive fungal diseases on tissue biopsy specimens with proven infection: a 7-year retrospective analysis from a reference laboratory

J Clin Microbiol. 2014 May;52(5):1737-40

FIG 1 Schematic diagram of procedures performed with biopsy samples, depending on clinical suspicion. When specific assay results were negative, a panfungal assay was performed. *, specific assays detected the following species: *A. fumigatus*, *A. terreus*, *A. flavus*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Rhizopus oryzae*, *Rhizopus microsporus*, *Mucor* spp., *Fusarium* spp., *Scedosporium prolificans*, and *Scedosporium apiospermum*.





Difficulties with molecular diagnostic tests for mould and yeast infections: where do we stand?

There are still critical unresolved issues on the origin of the DNA detected in clinical samples, leaving room for studies providing a rationale for modifying the choice of the best clinical specimen and the best DNA extraction procedure. Primer choice is still controversial, that is, species-specific vs. pan-fungal, with pros and cons for each choice. Clinical validation should take into consideration the complex time function of risk factors of IFDs and their management. It is also necessary to determine whether next-generation sequencing strategies will change the way we develop and perform molecular tests on patients.

Clin Microbiol Infect. 2014 Jun;20 Suppl 6:36-41





Clinical evaluation of a *T. rubrum* specific PCR and pan-dermatophyte PCR in the diagnosis of suspected onychomycosis in 183 subjects from Serbia

Br J Dermatol. 2014 Jun 7

Clinical evaluation of a novel commercial multiplex-based PCR diagnostic test for differential diagnosis of dermatomycoses

Mycoses. 2014 Jan;57(1):27-34

The use of a one-step PCR method for the identification of *Microsporum canis* and *Trichophyton mentagrophytes* infection of pets

Acta Biochim Pol. 2014;61(2):375-8





Development and validation of an in-house database for matrix-assisted laser desorption ionization-time of flight mass spectrometry-based yeast identification using a fast protein extraction procedure.

J Clin Microbiol. 2014 May;52(5):1453-8

MALDI-TOF MS for the identification of veterinary non-*C. neoformans*-*C. gattii* *Cryptococcus* spp. isolates from Italy

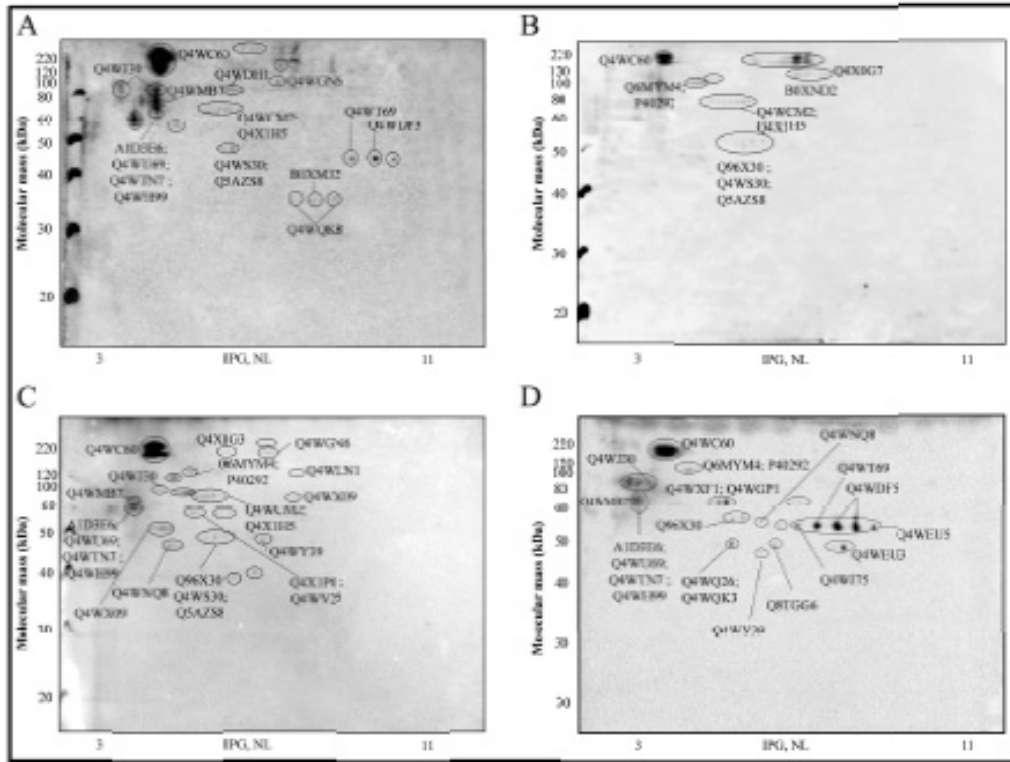
Med Mycol. 2014 Aug;52(6):659-66





Immunoproteome of *Aspergillus fumigatus* using sera of patients with invasive aspergillosis

Figure 2. 2-D Western immunoblot of proteins extracts of *A. fumigatus* germlings from the pool of patient's sera classified as (A) proven/hospital 1; (B) probable; (C) proven/hospital 2; or (D) other-mycosis. The identified antigenic proteins are indicated with the accession number (UniProtKB).



Due proteine come potenziali candidati per la diagnosi di aspergillosi;
 Le proteine individuate derivano da conidi germinanti di *A. fumigatus* e non presentano omologie con proteine umane o di altri funghi.

Int J Mol Sci. 2014 Aug 20;15(8):14505-30

