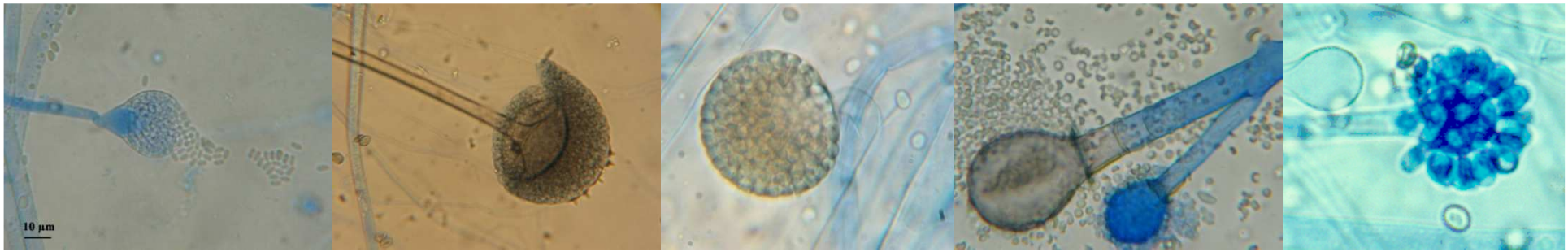

Pulmonary mucormycosis

Olivier Lortholary, M.D.; Ph.D.

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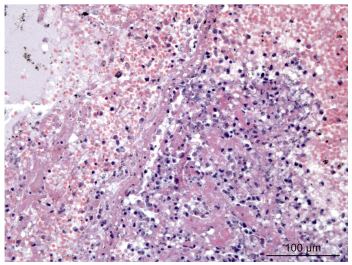
Service de Maladies Infectieuses et Tropicales

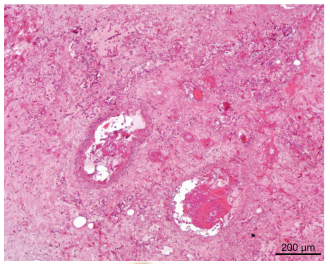
Centre d'Infectiologie Necker Pasteur, IHU Imagine

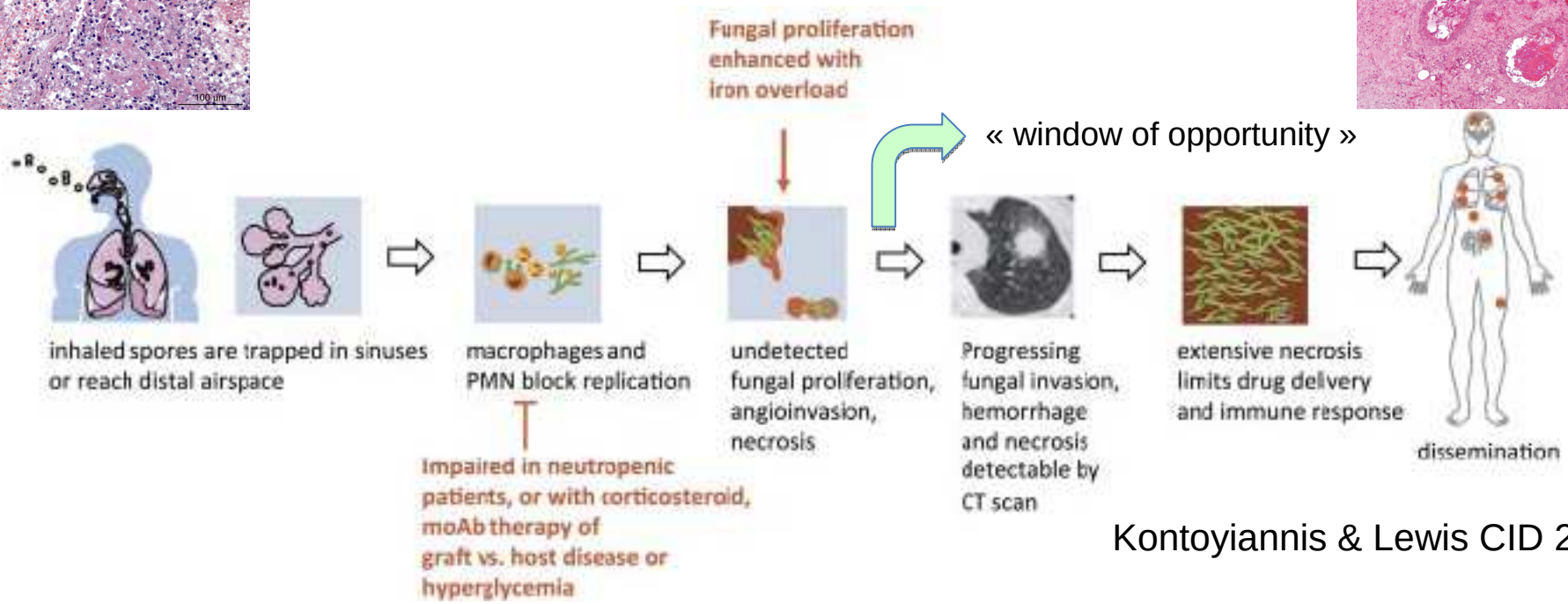
Hôpital Necker Enfants Malades, &

Institut Pasteur, CNRMA, CNRS URA3012, Paris, France.

Pathogenesis



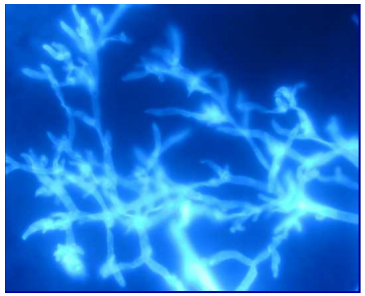




Kontoyiannis & Lewis CID 2012

Roilides CID 2012

Function	*Aspergillus fumigatus*	*Rhizopus oryzae*
PAMP recognition	TLR2 and TLR4	TLR2
MNC genes regulated only by organism, No.	4287	1142
IL-6 secretion	+	+++
IL-8 secretion	ND	++
TNF-α secretion	+	+++
Phagocytosis	++	+
O_2^- production	++	+
Hyphal damage	+++	++



Epidemiological trends

Population based study

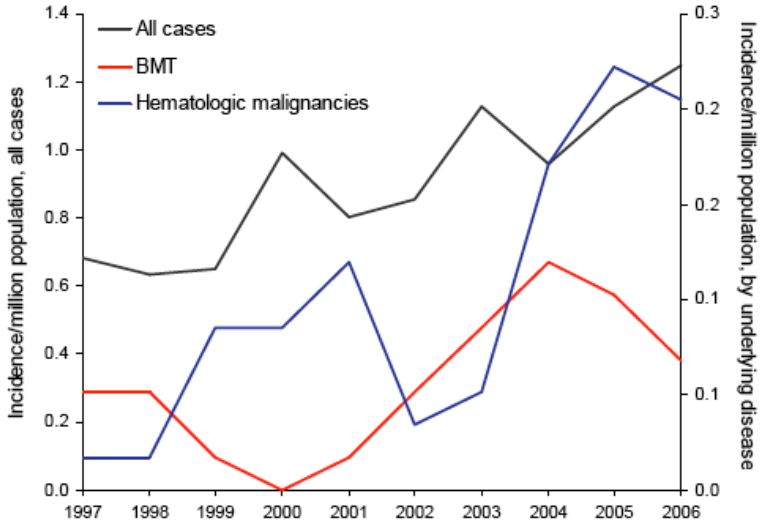
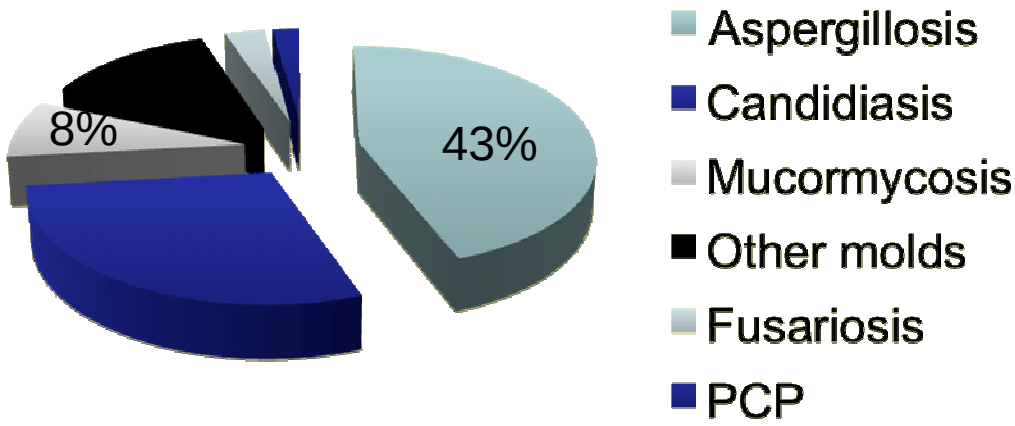


Figure 1. Evolution of the incidence of zygomycosis, France, 1997–2006. BMT, bone marrow transplantation.

Bitar et al. EID 2009

HSCT, Transnet, USA

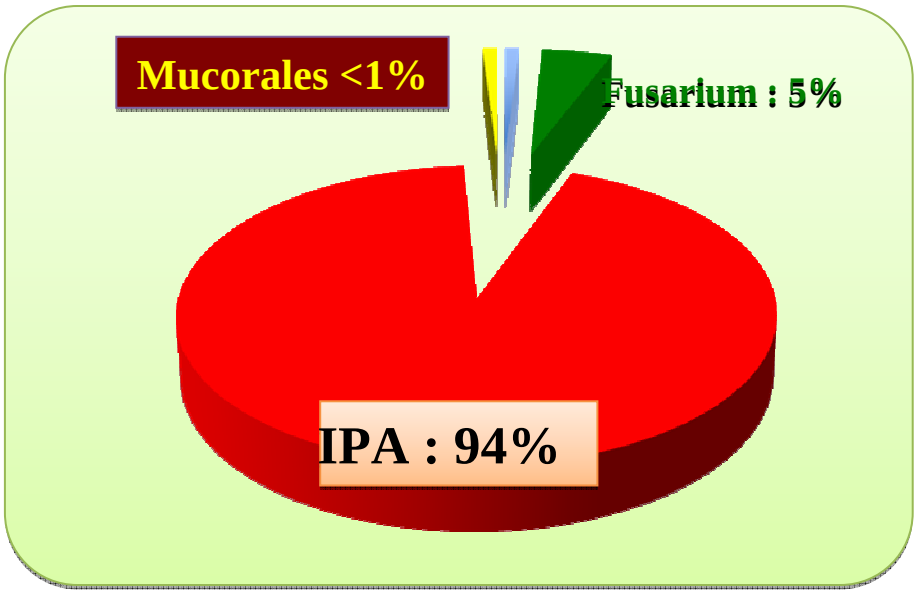


Kontoyiannis CID 2010

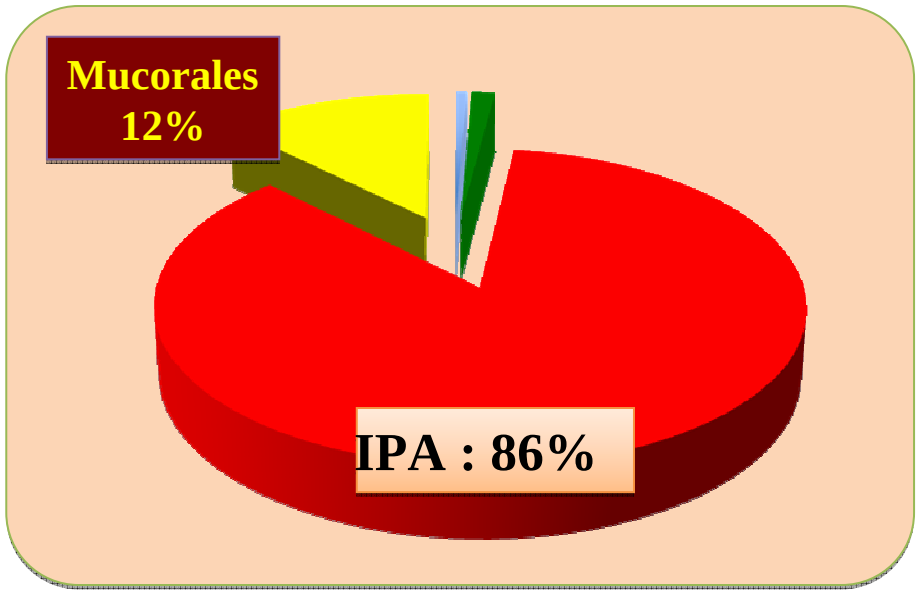
Mould pneumonia: 20 years survey

n=253, single center experience

1993 – 2003 (n = 107)



2004 – may 2012 (n = 146)



	1993 - 2003	2004 - May 2012	P
Others	1	1	ns
Fusarium	5	2	ns
Aspergillus	**100 (94%)**	**125 (86%)**	**0.05**
Mucorales	**1 (0.9%)**	**18 (12%)**	**< 0.001**

Courtesy D Caillot. Hematology, Dijon Univ Hosp

Current risk factors in mucormycosis

Reference	Count-ries	Period	Cases No.	HM (%)	DM (%)	SOM/ SOT	DFO (%)	HIV (%)	AI/ CO	Trauma/ no
Roden, 2005	Global	1885-2004	929	21	36	7	6	2	1	19
Bitar, 2009	France	1997-2006	63	17	16	7	-	5	-	54
Pagano, 2009	Italy	2004-2007	60	62	18	2	-	2	3	40
Saegeman, 2010	Belgium	2000-2009	31	77	6	13	-	3	-	13
Ruping, 2010	Global	2006-2009	41	63	17	10	-	-	-	-
Skiada, 2011	Europe	2005-2007	230	55	17	9	1	2	7	20
Chakrabarti, 2006	India	2001-2005	178	1	74	1	-	-	-	19
Chakrabarti, 2009	India	2006-2007	75	9	44	5	-	1	29	14
Lanternier, 2012	France	2005-2007	101	50	23	3	-	-	-	18

HM= Hematological malignancy, DM=Diabetes mellitus, DFO= Deferroxamine therapy, HIV= human immuno-deficiency virus, AI/CO= Autoimmune/corticosteroid therapy, SOM/SOT=Solid organ malignancy/transplant

Risk factors and clinical localization

Predisposing conditions	Pathogenetic Mechanism	Clinical Presentation
Haematological malignancy and HSCT	Prolonged neutropenia	**Pulmonary and Sinus** > Cutaneous > Sino-orbital
Uncontrolled **diabetes mellitus** (metabolic acidosis)	Impaired neutrophil activation, interference in Fe binding to transferrin, ↑Fe usage by Mucorales	**Rhinocerebral** > Pulmonary > Sino-orbital > Cutaneous
Prolonged **corticosteroids**, Autoimmune disease	Defects in macrophages and neutrophils, Corticosteroid induced diabetes, Hypocomplementemia	**Disseminated > Renal >** Cutaneous > Rhinocerebral > Gastrointestinal Tract
SOT and GVHD	Cellular Immune suppression, Corticosteroid induced diabetes	**Pulmonary > Sinus >** Cutaneous > Rhinocerebral >Disseminated
Intravenous drug abuse	Injection of spores contained in drugs	**Cerebral > Cutaneous >** Renal > Heart > Rhinocerebral > Disseminated

Petrikkos, CID 2012

« Retrozygo » cohort in France

Table 1. Characteristics of 101 Patients With Proven or Probable Mucormycosis in France, 2005–2007

	No (%) of Patients
Mean (SD) age, years	50.7 (±19.9)
Male sex	59/101 (58)
Main risk factor	
Hematological malignancy[a]	50/101 (50)
+ HSCT	12/50 (24)
+ GVHD	5/50 (10)
+ Diabetes mellitus	9/50 (18)
+ Corticosteroids	13/50 (26)
+ Neutropenia	41/50 (80)
Diabetes mellitus[b]	23/101 (23)
Type 1	10/23 (43)
Ketoacidosis	8/23 (35)
Solid organ transplantation	3/101 (3)
Trauma	18/101 (18)
Other[c]	7/101 (7)

Lanternier, CID, 2012

	No.(%) of Patients With Each Underlying Factor				
	Hematological Malignancy (n = 50)	Diabetes Mellitus (n = 23)	Trauma (n = 18)	SOT (n = 3)	Other (n = 7)
Lung	22 (44)	3 (13)	0	1	2
Rhinocerebral	6 (12)	16 (70)	1 (6)	0	2
Cutaneous	4 (8)	0	15 (83)	0	1
Disseminated	13 (26)	2 (9)	1 (6)	1	1
Other	5 (10)	2 (9)	1 (6)	1	1

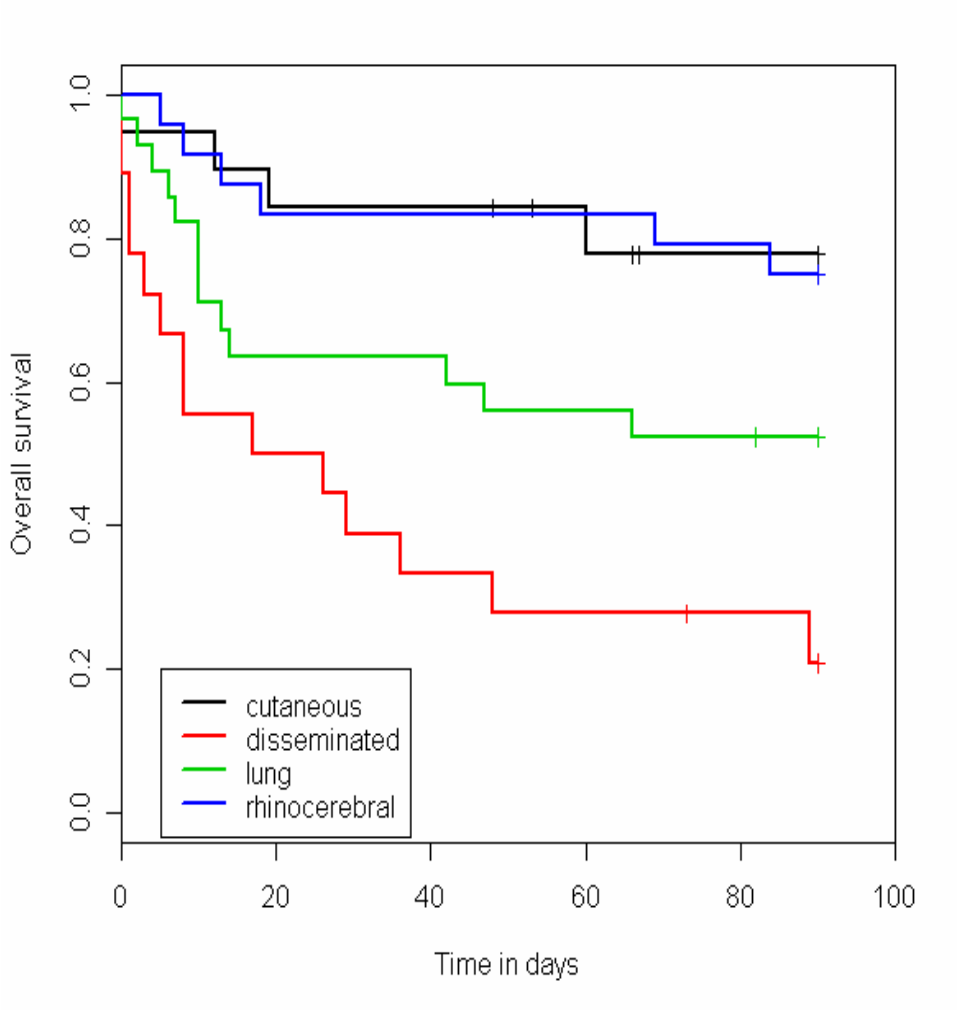
Pulmonary mucormycosis in SOT

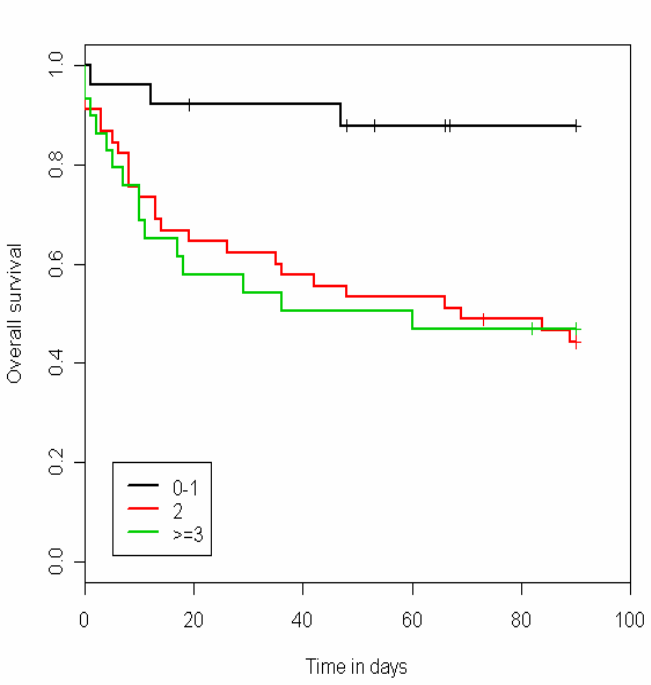
- **Clinical sites involved** (Transnet, Pappas, CID 2010):
  - Pulm: 56%
  - Sinus: 13%
  - Skin: 13%
  - Dissemination: 9%

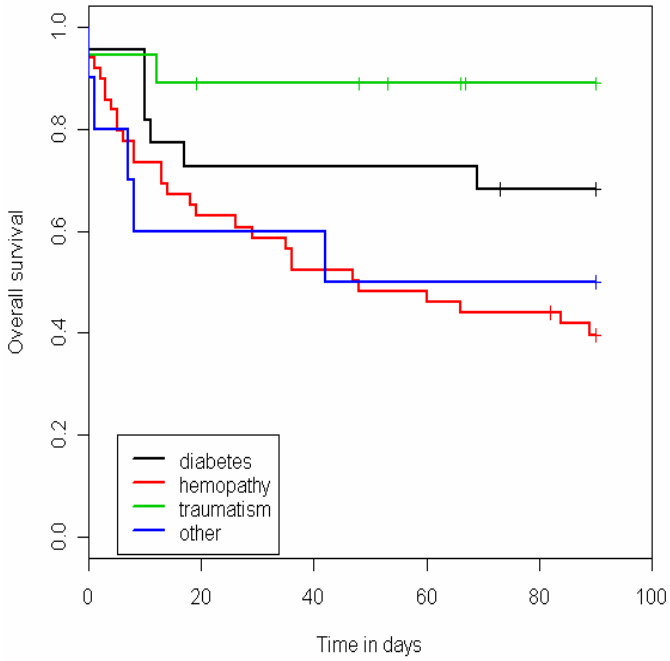
- **Pulmonary involvement (53% of 58 cases):**
  - 5.5 mo post-transplantation
  - 74.2% (23/31) limited to the lungs
  - consolidation/mass lesions (29%), nodules (25.8%) / cavities (22.6%)
  - 25.8% (8/31) dissemination (half skin involvement)
  - *Lichtheimia* spp. increased dissemination ($p<0.02$)
  - 45.2% mortality

Zygomycosis Transplant Study Group
Sun, Am J Transplant 2009

Increased mortality if pulmonary localization





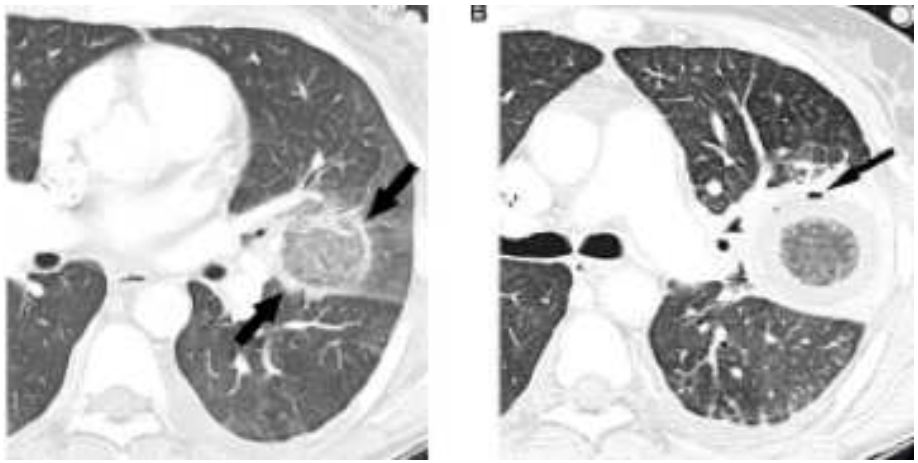


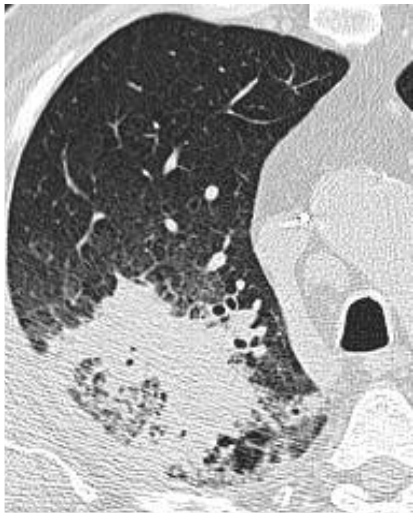
Lanternier, CID, 2012

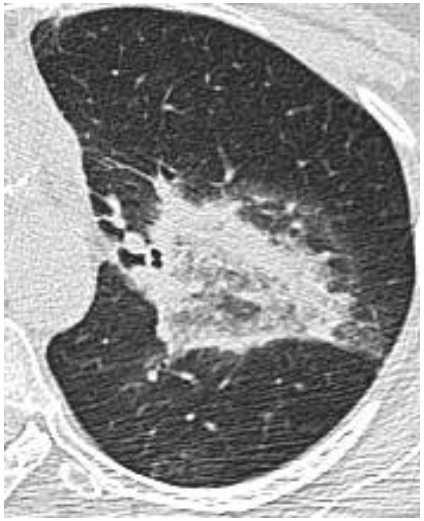
Pulmonary mucormycosis in hematology: « inverse halo sign »

Wahba H, CID 2008

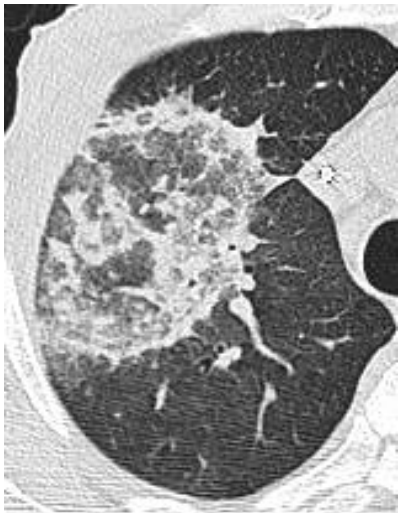
- IFI (n=189), revision of CTs
- 7 inverse halo sign (4%)
  - 1/132 aspergillosis
  - 6/37 mucormycosis (19%)







Courtesy Caillot D, Dijon



Reverse halo sign

- Single center experience
- Acute leukemia

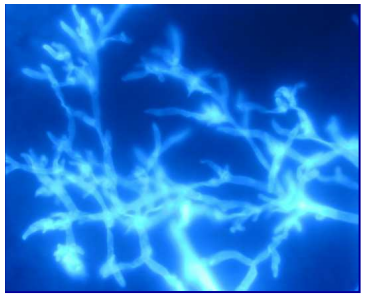
Table 3. Evolution of Computed Tomographic Scans of 16 Patients With Proven Pulmonary Mucormycosis

CT characteristics	Days 0–5	Days 6–14	Days 15–26
No. of patients with CT performed	16/16 (100)	11/16 (69)	11/16 (69)
No. of CTs performed	25	14	11
No. of patients with CT during neutropenia	15/16 (94)	9/11 (82)	4/11 (36)
Typical RHS	15/16 (94)	7/11 (64)	0/11 (0)
Diameter of lesion ≤3 cm	2/16 (12)	0/11 (0)	1/11 (9)
Diameter of lesion >5 cm	7/16 (44)	8/11 (73)	9/11 (82)
Micronodules	1/16 (6)	7/11 (64)	10/11 (91)
Pleural effusion	2/16 (12)	6/11 (55)	7/11 (64)
Air-crescent sign or cavitation	0/16 (0)	1/11 (9)	4/11 (36)

Data are presented as No. of scans with characteristic/No. of scans with available data (%). Day 0 corresponds to the day of the first CT scan. Micronodules are defined by diameter <1 cm.

Abbreviations: CT, computed tomography; RHS, reversed halo sign.

Legouge, CID, 2014



Lack of biomarkers

- Lack of **antigen detection**

a) β-glucan (Koo, CID 2009, Bellanger APMIS 2011)

b) Specific

- Lack of **antibody** detection

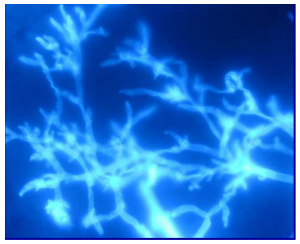
ESCMID 2014 recommendations for laboratory diagnosis

Population	Intention	Method/Finding	SoR	QoE
Any	To diagnose mucormycosis	Direct microscopy preferably using optical brighteners	A	IIu
Any	To diagnose	Culture	A	IIIr
Any	To diagnose	Histopathology	A	IIu
Any	To diagnose	Immunohistochemistry	C	IIu
Any	To diagnose	Galactomannan in blood or bronchoalveolar lavage	B	III
Any	To diagnose	1,3-β-D-glucan in blood	D	III
Haematological malignancy	To monitor treatment	ELISPOT	C	IIu
Any	To diagnose	Molecular based tests on fresh clinical material	B	IIu
Any	To diagnose	Molecular based tests on paraffin slides	B	IIu

→ **Reference methods remain**

Microscopic examination and culture

CMI 2014



Collection of specimen and specimen sampling

Tissue biopsies (do not crush) or specimen obtained aseptically from sterile site should be preferred for histopathology and culture

Mucormycosis localization	Specimen
Pulmonary	**Bronchoalveolar lavage** **Biopsy of pulmonary lesions (transbronchial or percutaneous CT- guided or surgical)**
Rhinocerebral	Sinus aspirate, tissue biopsy
Cutaneous	Skin biopsy

Histopathology of pulmonary mucormycosis

Table 1 Histopathologic findings according to immunologic background in 20 cases of pulmonary mucormycosis.

	Total ($N = 20$)	Neutropenic ($N = 13$)	Non-neutropenic ($N = 7$)[a]	Allogeneic HSCT ($N = 6$)[b]
Angioinvasion	20 (100)	13 (100)	7 (100)	6 (100)
Angioinvasion in >50% of vessels	12 (60)	10 (77)	2 (29)[c]	4 (67)
Intravascular thrombosis	16 (80)	12 (92)	4 (57)	5 (83)
Intra-alveolar hemorrhage	17 (85)	12 (92)	5 (71)	3 (50)[d]
Hemorrhagic infarction	18 (90)	12 (92)	6 (86)	5 (83)
Coagulative necrosis	17 (85)	10 (77)	7 (100)	6 (100)
Inflammatory necrosis	6 (30)	4 (31)	2 (29)	4 (67)[d]
Granuloma	2 (10)	1 (8)	1 (14)	0 (0)

[a] Five non-neutropenic patients had been treated with high-dose corticosteroids.
[b] All allogeneic HSCT recipients had significant GVHD, 3 were neutropenic, and 5 had been treated with high-dose corticosteroids.
[c] $P = 0.06$ for the comparison of neutropenic and non-neutropenic patients.
[d] $P < 0.05$ for the comparison of allogeneic HSCT recipients with non-allogeneic HSCT recipients.

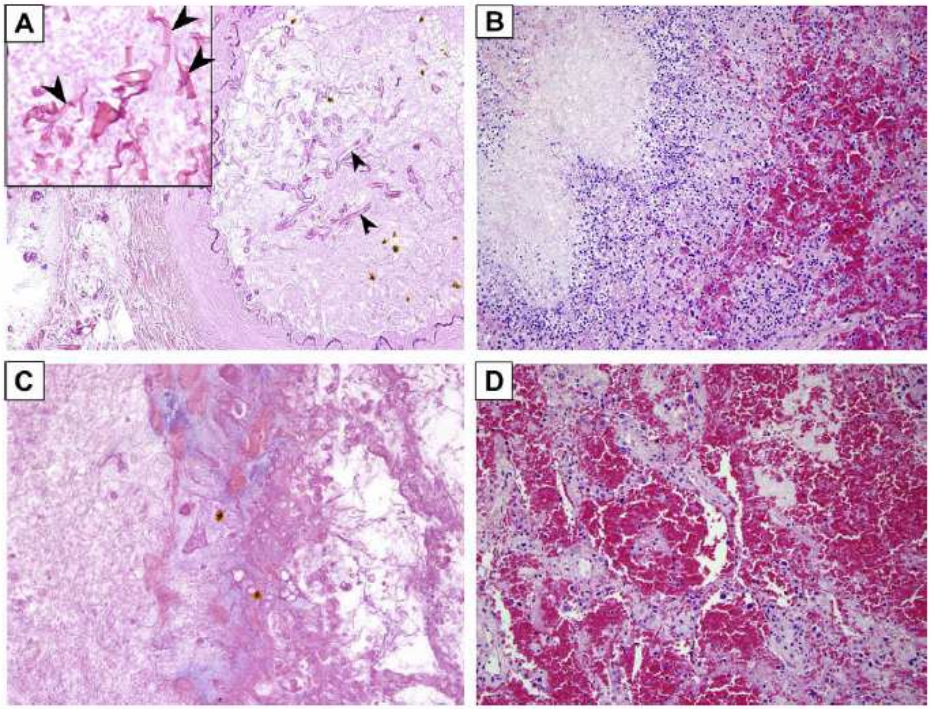
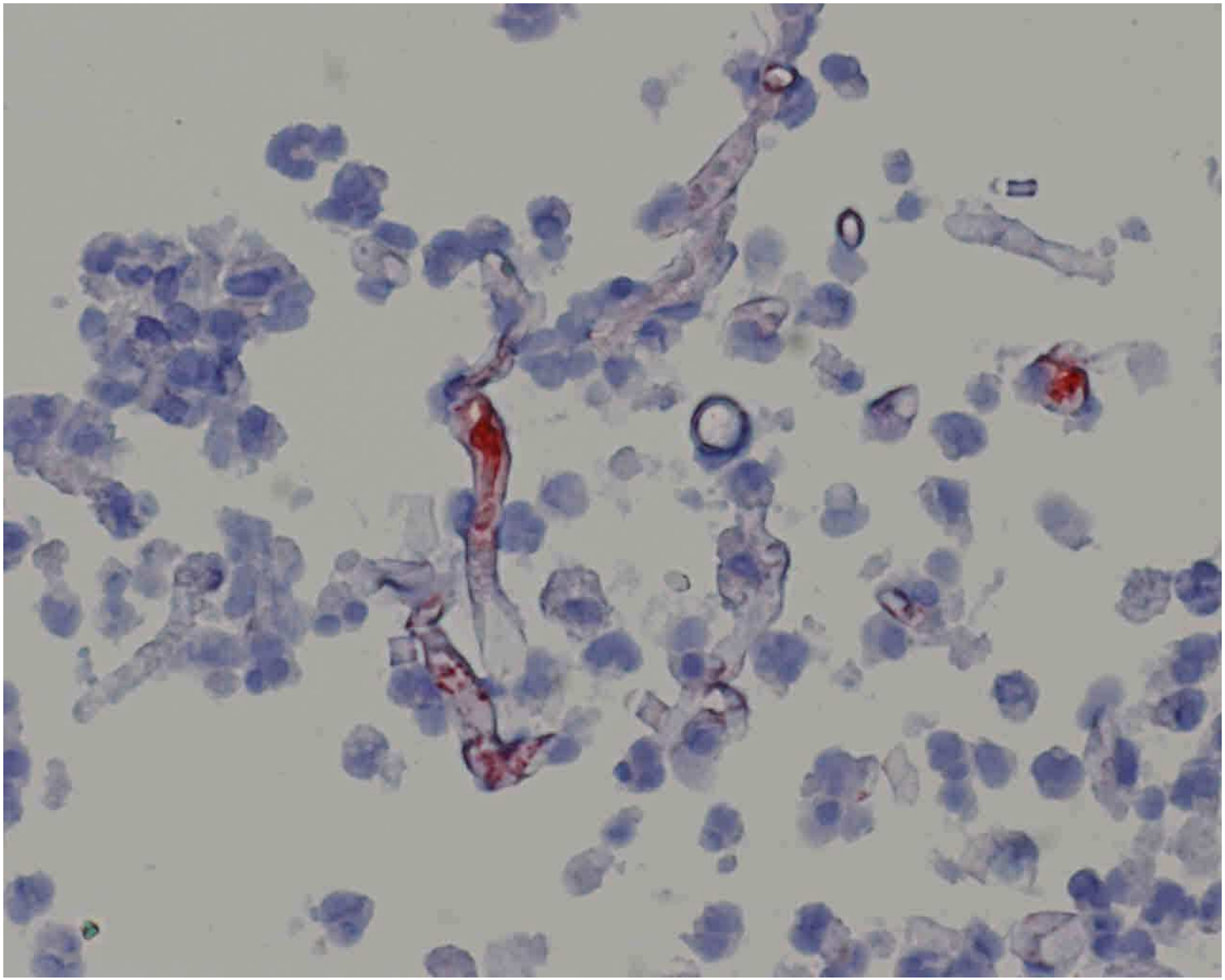


Figure 1 Histopathologic features of pulmonary mucormycosis in cancer patients. (A) Hyphae invading a blood vessel lumen (arrowheads). Inset: short pleomorphic, non-septated hyphae typical of *Mucorales* species (hematoxylin-and-eosin [H&E] stain ×400). (B) Inflammatory necrosis (H&E stain ×400). (C) Coagulative necrosis with no significant inflammatory infiltration (H&E stain ×400). (D) Intra-alveolar hemorrhage (H&E stain ×400).

Ben Ami J Infect 2009

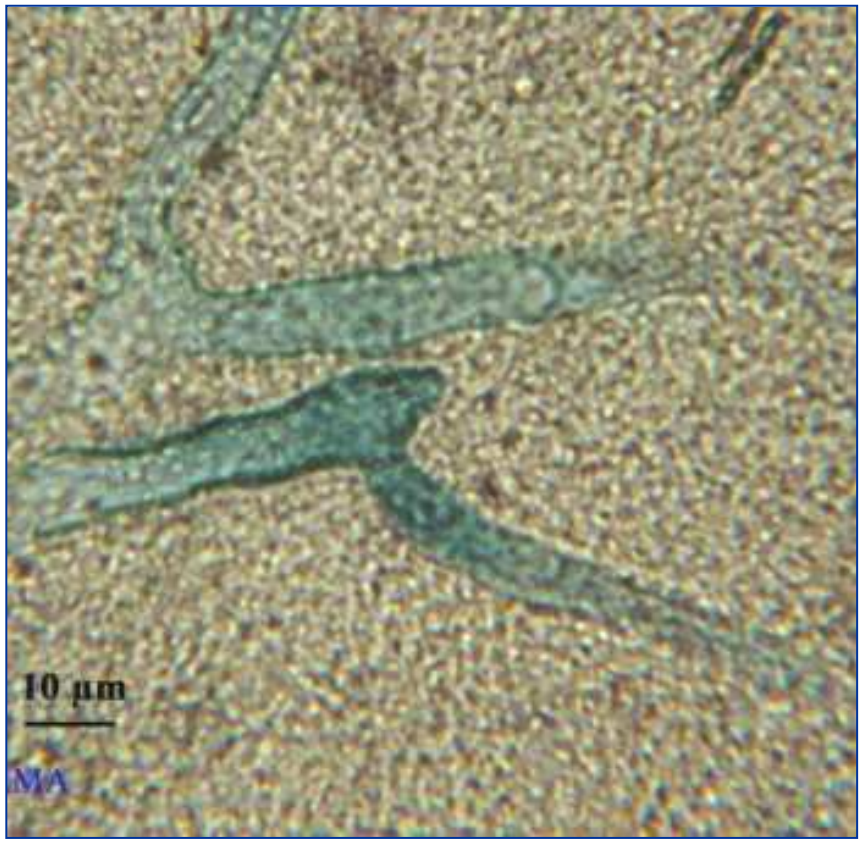
Immunohistochemistry during mucormycosis



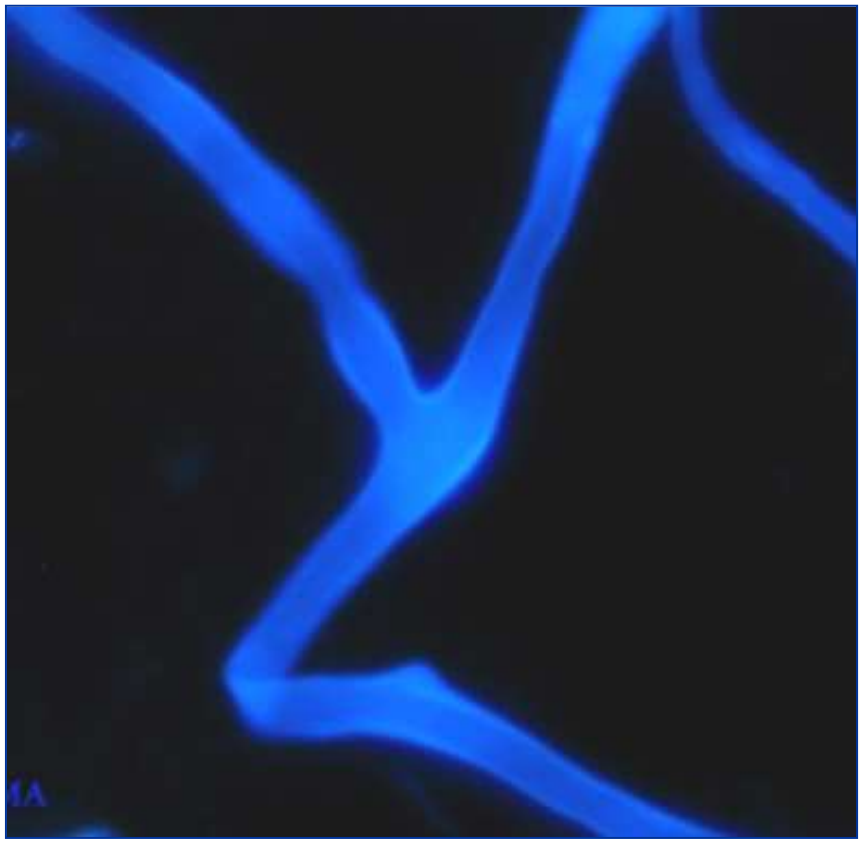
Courtesy F Chrétien, Inst Pasteur, Paris; Dako Ab.

Microscopic examination

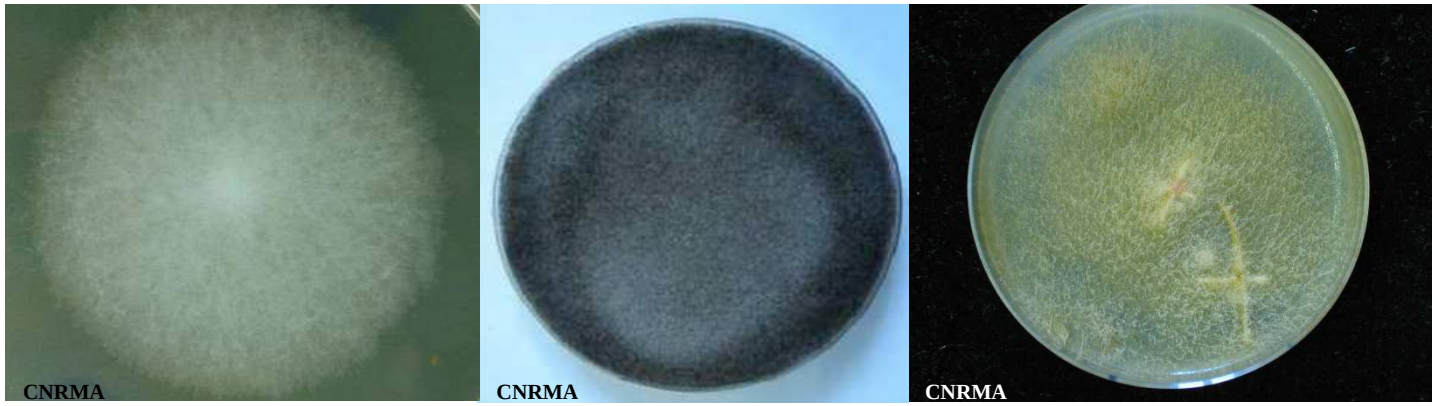
Black chlorazole



Calcofluor

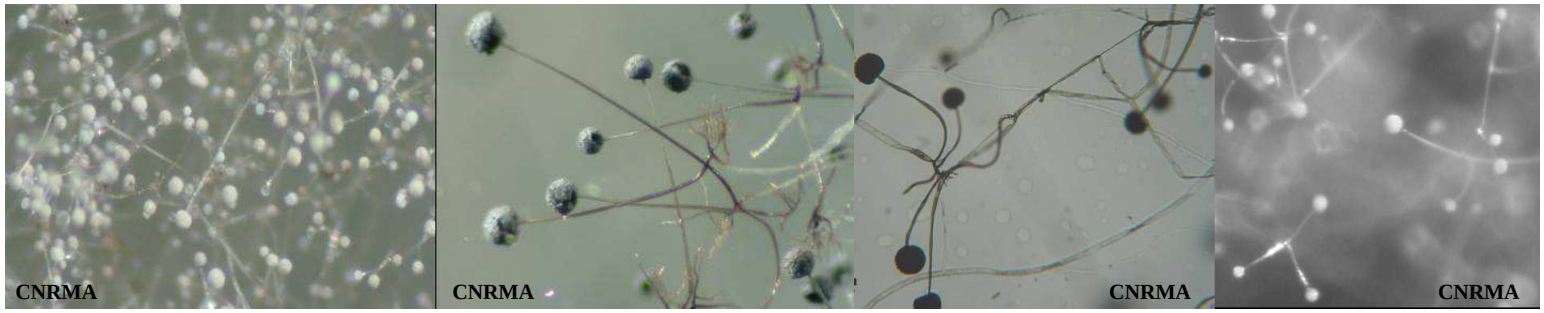


Culture: macroscopic examination



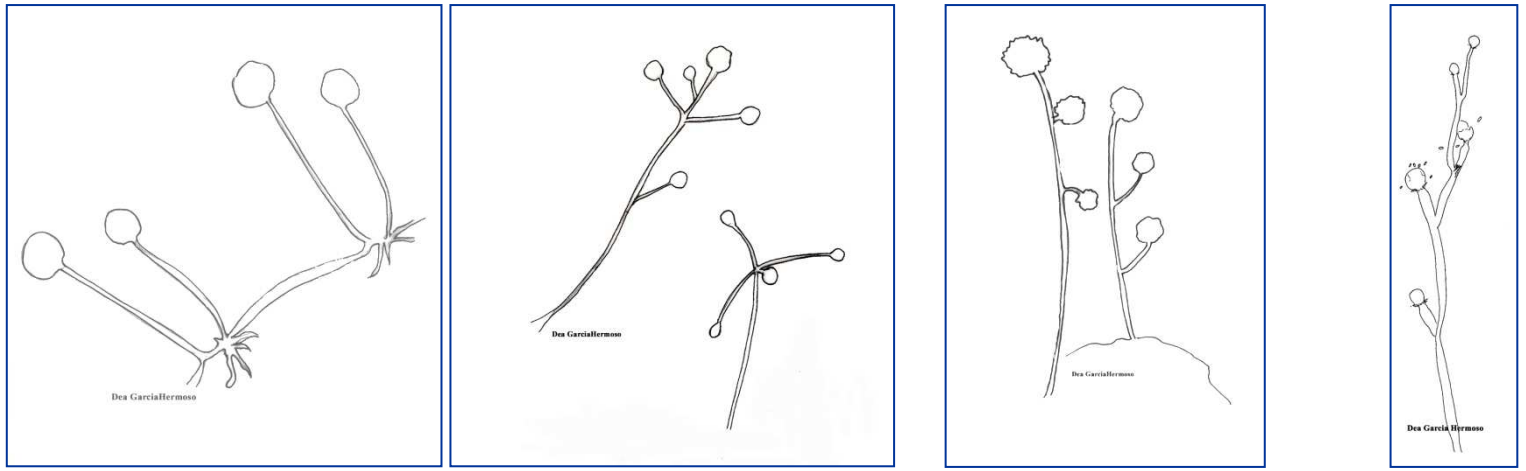
- Use of cycloheximide media with a high carbohydrate content to allow **sporulation** (Malt 2%, potato dextrose)
- Subculture at 27-30°C
- Rapid and extensive mycelial development

- Observation of fungal **architecture** (10 times objective)
  - Appearance of the colonies and branching pattern of sporangiophores



Culture: microscopic examination

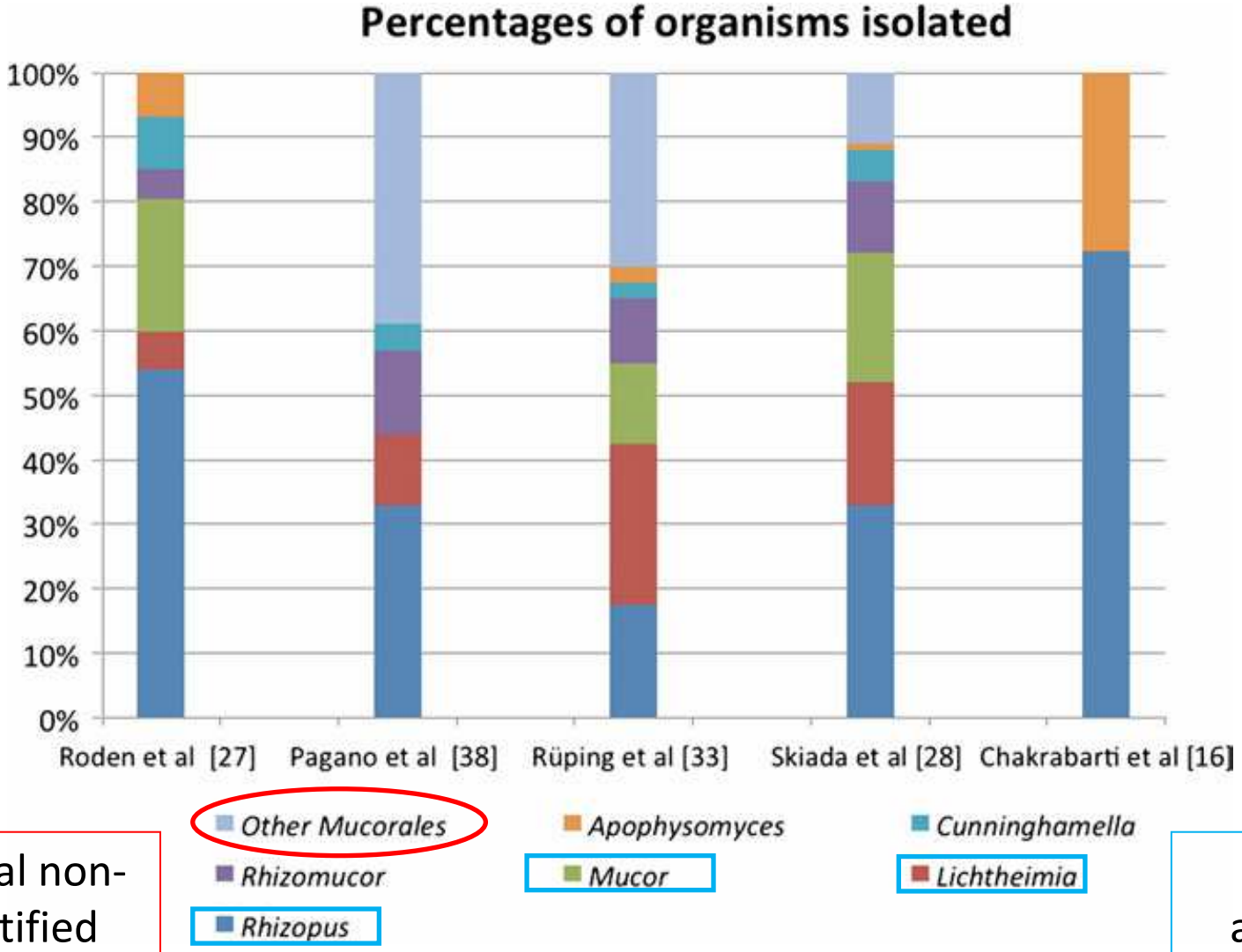
- **Characteristics of zygospores**



- **Presence/absence apophysis, rhizoids, columella, or chlamydospores**

Garcia-Hermoso D, Dannaoui E, Lortholary O and Dromer F. Chapter 119. Agents of Systemic and Subcutaneous Mucormycosis and Entomophthoromycosis .In ASM Manual of Clinical Microbiology ASM Press 2011 (Manual of Clinical Microbiology, 10th Edition May 2011)

Worldwide distribution of Mucorales

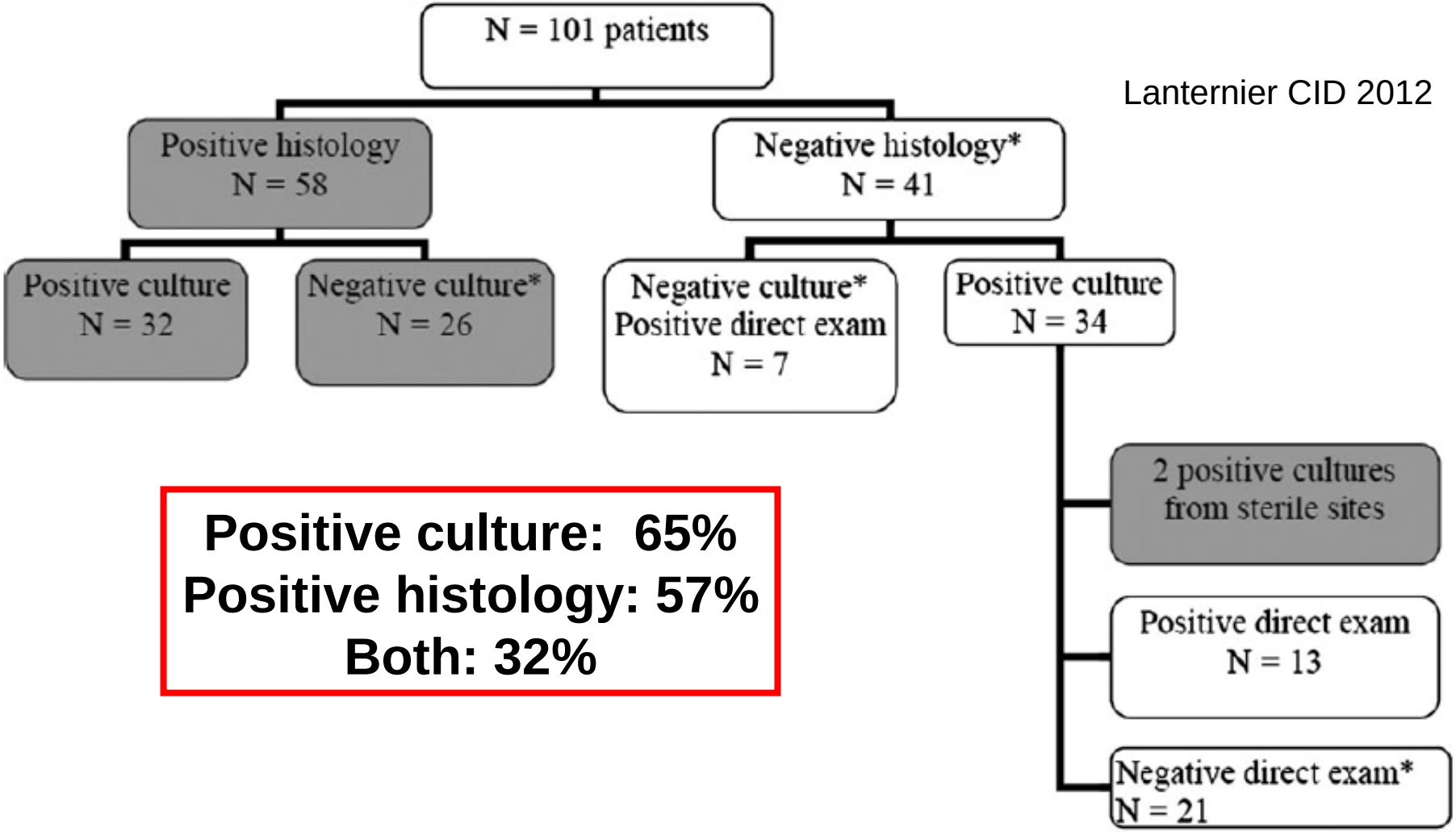


Several non-identified species

Species associated with diabetes

Petrikkos, CID 2012

Mucormycosis diagnosis: real life data



Lanternier CID 2012

Positive culture: 65%
Positive histology: 57%
Both: 32%

Proven mucormycosis (n = 60)

Probable mucormycosis (n = 41)

***: negative or not done**

205/361 cases (1958 -1985) : 57% histopathology alone [Espinel-Ingroff, Mycopathologia 1987]

Diagnosis of pulmonary mucormycosis

2003-2006, Innsbrück

- 61 hematology/SOT pts
- Suggestive images
- CT guided biopsy
- Direct Exam Calcofluor +49/61 (80%)
  - 36 (73%) septate hyphae
  - **13 (27%) non septate hyphae all confirmed by PCR or culture**

1993-2012, Dijon

- 18 hematology pts with pulmonary mucormycosis
- BAL +: 2/15 (13%)
- Pulmonary biopsy (CT or surgical): 11/11

Lass-Florl CID 2007

D Caillot, personal data

General therapeutic principles

1. Control host factors

Taper steroids

Hold immunosuppressive moAb (anti-TNF-α, alemtuzumab)

Control hyperglycemia (Rammaert, Diabetes Metabolism 2012)

2. Surgery

Any localisation when feasible, but rhino-oculo-cerebral and post-trauma +++

Extent and timing ± frequency of debridment remains unknown

Delineate margins of infected tissues (Reed CID 2008; Vironneau CMI in press; Wankentien CID 2012; Fanfair NEJM 2012)

Independent factor of decreased mortality (Roden CID 2005)

Subsequent complex reconstruction surgery

Before allogeneic SCT (Schneidawind, TID 2012)

3. Early appropriate antifungal therapy

means early differentiation of Mucorales from more common molds

In vitro susceptibility testing of *Mucorales* (1)

Species	MIC range, mg/mL (no. of isolates)				CAS, minimal effective concentration range in mg/mL (no. of isolates)
	ICZ	VCZ	PCZ	AMB	
Aspergillus lentulus	0.5–1 (8)	1–2 (8)	NA	1–2 (8)	2–16 (8)
Aspergillus ustus	1 to >8 (10)	4–8 (10)	2 (1)	0.25–8 (10)	2–8 (8)
Aspergillus terreus	0.03–8 (63)	0.25–4 (63)	0.06–0.25 (8)	0.12–16 (63)	0.06–0.5 (13)
Scedosporium apiospermum	0.03–2 (30)	≤0.03 to 0.5 (30)	0.125–1 (13)	0.5 to >16 (30)	0.25–4 (11)
Scedosporium prolificans	2 to >32 (55)	0.5–8 (55)	2 to >8 (55)	1 to >16 (55)	4–8 (2)
Fusarium solani	≥8 (15)	1–4 (10)	>8 (6)	0.25–8 (15)	≥8 (29)
Paecilomyces lilacinus	1 to >8 (3)	0.2–1 (3)	0.12–0.5 (3)	>8 (3)	3 to >100 (5)
Scopulariopsis brevicaulis	>8 (25)	>8 (25)	>8 (25)	8 to >16 (25)	4 to >16 (25)
Zygomycetes	0.03–32 (51)	2–64 (51)	0.06–2 (36)	0.03–2 (51)	>16 (15)
Trichosporon asahii	0.03–8 (15)	0.015–8 (15)	0.12–1 (5)	0.5–16 (15)	>16 (9)
Geotrichum capitatum	0.03–0.5 (23)	0.03–0.5 (23)	NA	0.06–0.25 (23)	0.5 (1)
Cladophialophora bantiana	≤0.03 to 0.25 (10)	≤0.03 to 1 (10)	<0.03 to 0.06 (5)	0.25–0.5 (10)	2–8 (5)

2 drugs: amphotericin B and its lipid derivatives + posaconazole

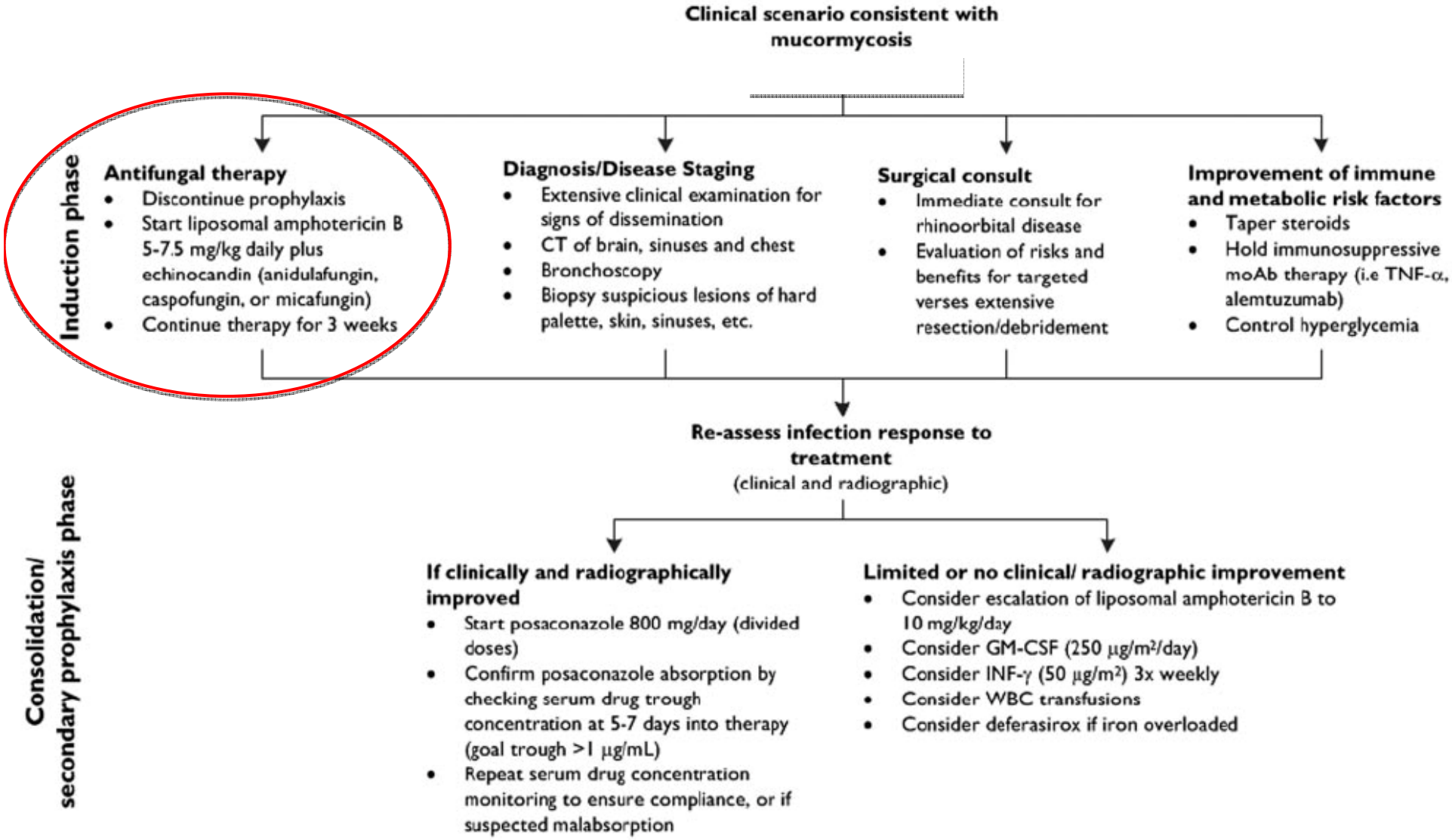
Kanafani & Perfect, CID 2008

In vitro susceptibility differs according to *Mucorales* species (2)

(n=217 Mucorales isolates)	Amphotericin B % with MIC ≤1µg/mL	Posaconazole % with MIC ≤0.5µg/mL	Itraconazole % with MIC ≤0.5µg/mL
Rhizopus* sp *(101)	100	80	62
Rhizopus arrhizus (20)	100	64	50
Rhizopus microsporus (12)	100	78	60
Mucor* sp. *(41)	94	70	57
Mucor circinelloides (6)	100	0	0
Rhizomucor* sp *(5)	100	67	67
***Lichtheimia* sp. (3)**	100	100	50
Lichtheimia corymbifera (9)	100	100	100
Cunninghamella* sp. *(13)	63	75	29
Apophysomyces elegans (6)	100	83	80

Almyroudis AAC 2007

Management of mucormycosis in hematology



Kontoyiannis & Lewis, Blood 2011

ECIL3 recommendations:

1st line therapy

Management should include antifungal therapy, control of underlying conditions and surgery	**A II**
Antifungal therapy	
AmB deoxycholate	**C II**
Liposomal AmB ≥ 5 mg/kg/d	**B II**
ABLC	**B II**
ABCD	**C II**
Posaconazole	**C III**
Combination therapy [but no data suggesting antagonism]	**C III**
Surgery	
Rhino-orbito-cerebral	**A II**
Soft tissue	**A II**
Localised pulmonary lesion	**B II**
Disseminated	**C III**

Skiada Haematologica, 2012

ESCMID/ECMM Joint Guidelines
First line strategy; « A » recommendations

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To increase survival rates	Surgical debridement	A	IIu	Tedder Ann Thor Surg 1994 Roden CID 2005 Chakrabarti PostMedJ 2009 Skiada CMI 2011 Lanternier CID 2012 Zaoutis PIDJ 2007	N=32 N=90 N=45 N=9 N=59 N=92, paediatric
Any	To cure and to increase survival rates	Surgical debridement in addition to antifungal treatment	A	IIu	Roden CID 2005 Greenberg AAC 2006 Skiada CMI 2010 Zaoutis PIDJ 2007	N=470 N=19 N=90 N=92, paediatric
Immunocompromised	To increase survival rates	Immediate treatment initiation	A	IIu	Chamilos CID 2008	N=70
Any	To cure and to increase survival rates	Amphotericin B, liposomal ≥ 5 mg/kg*	A	IIu	Pagano Haematologica 2004 Gleissner LeukLymph 2004 Cornely CID 2007 Rüping JAC 2010 Shoham Med Mycol 2010 Skiada CMI 2011 Lanternier ICAAC 2012 Ibrahim AAC 2003 Lewis AAC 2010	N=4 N=16 N=5 N=21 N=28 N=130 N=40 animal model animal model
CNS	To cure	Amphotericin B, liposomal 10 mg/kg, initial 28 days*	A	IIa	Groll JID 2000 Ibrahim AAC 2008	animal model animal model

Cornely, CMI 2014

ECIL3 recommendations:
2nd line and maintenance therapy

2nd line = salvage therapy (selection biases/difficulties in assessing therapy response)	
Combination lipid AmB and caspofungin	**B II**
Combination lipid AmB and posaconazole	**C III**
Combination with deferasirox	**C III**
Maintenance therapy [late recurrences if persistence of immunosuppression… thus careful decision to stop therapy]	
Posaconazole	**B III**

Skiada Haematologica, 2012

How best to manage pulmonary mucormycosis…?

- Increased clinical and microbiological awareness as epidemiology is changing !
- Mucormycosis should be early differentiated from other filamentous fungal infections: novel biomarkers
- Impact of better immunopathogenesis & Mucorales biology understanding/genome sequencing
- **Management in specialized centres**
  - Control of underlying disorders
  - Early administration of a lipid formulation of AmB (LAmB if brain)
  - Rationale for « high » dose LAmB (AmbiZygo Trial, Submitted for publication)
  - Controversial role of polyene/echinocandins 1st line combination
  - No role for iron chelation (in haematology patients); discuss surgery
  - Towards new clinical trials; dual or even triple therapies???