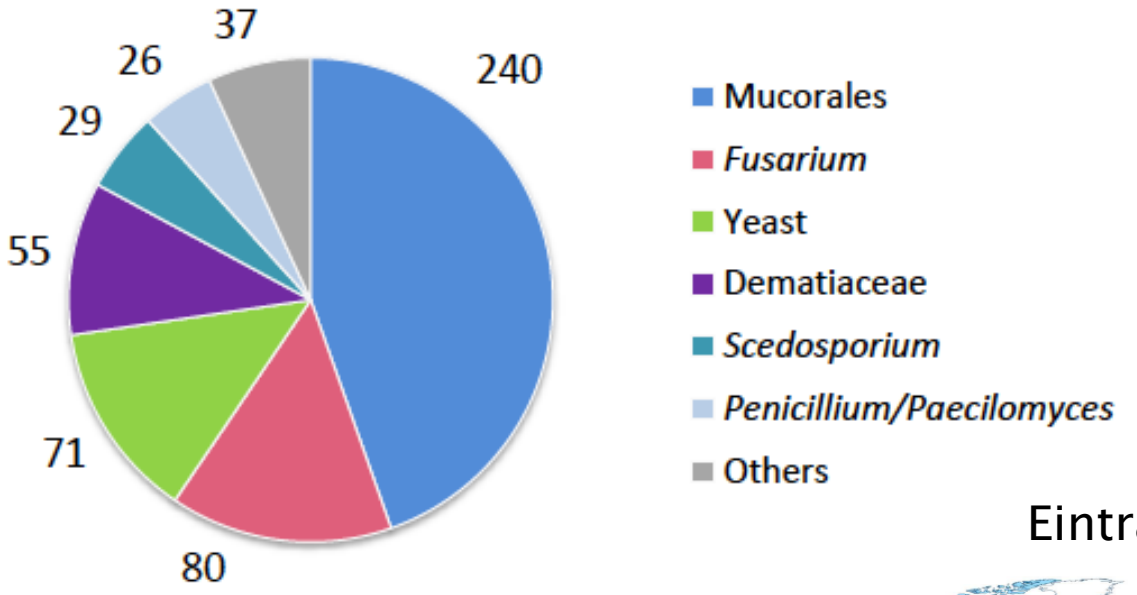
Neues von Mucor & Co

Birgit Willinger

Abteilung für Klinische Mikrobiologie

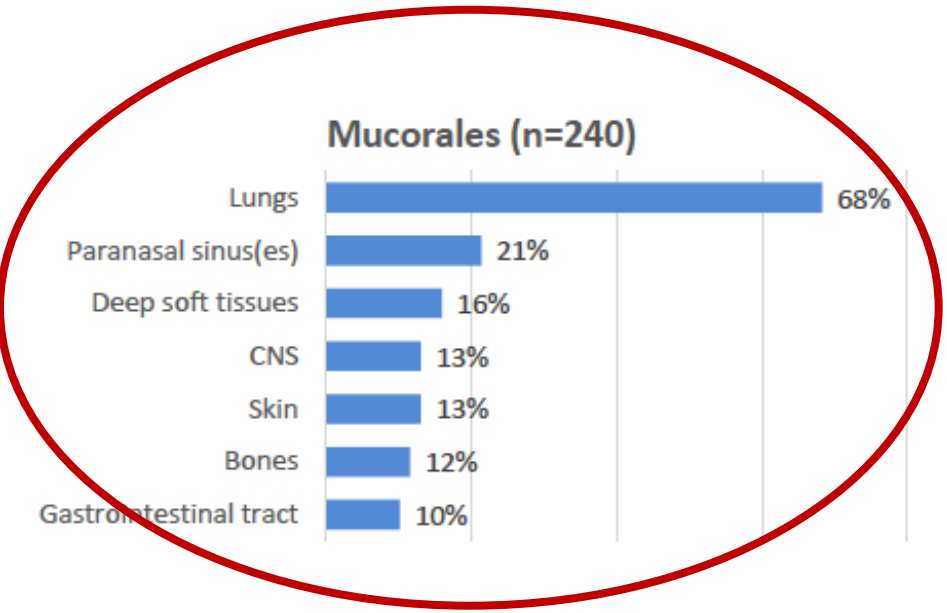
Klinisches Institut für Labormedizin

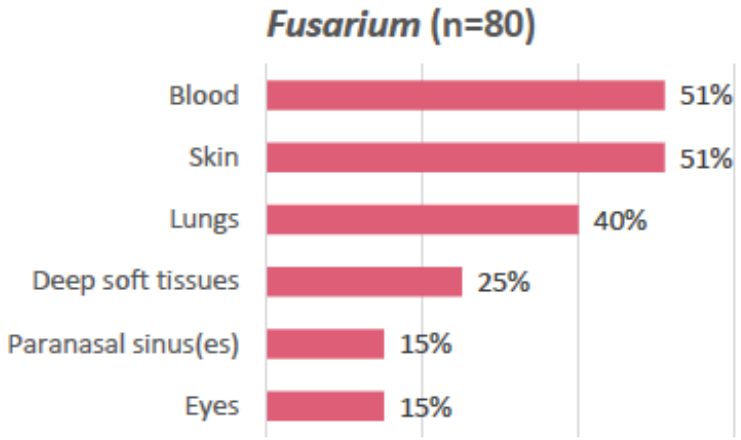
Emerging Pathogens 2006 - 2016

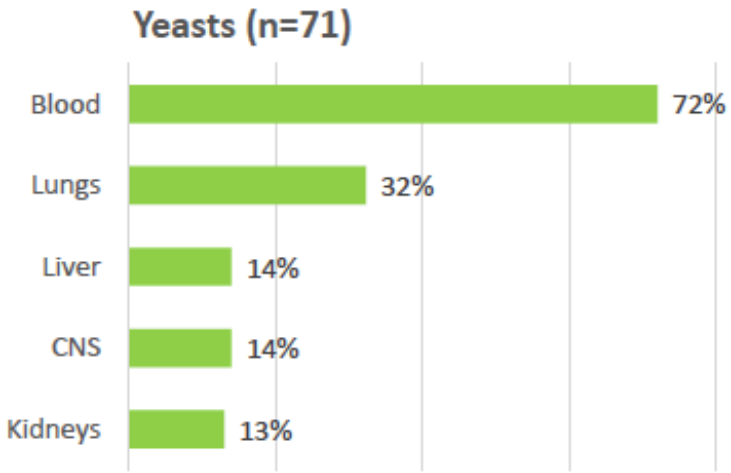


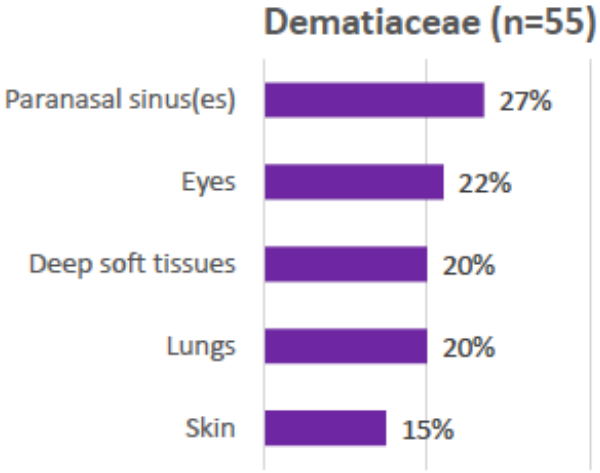
Einträge aus 64 Ländern

Emerging Pathogens 2006 - 2016









Mucormykosen im AKH Wien (2009 - 2014)

Mucorales: **16 Fälle**

5 x in Kombination mit Aspergillus Nachweis

m : w = 10 : 6

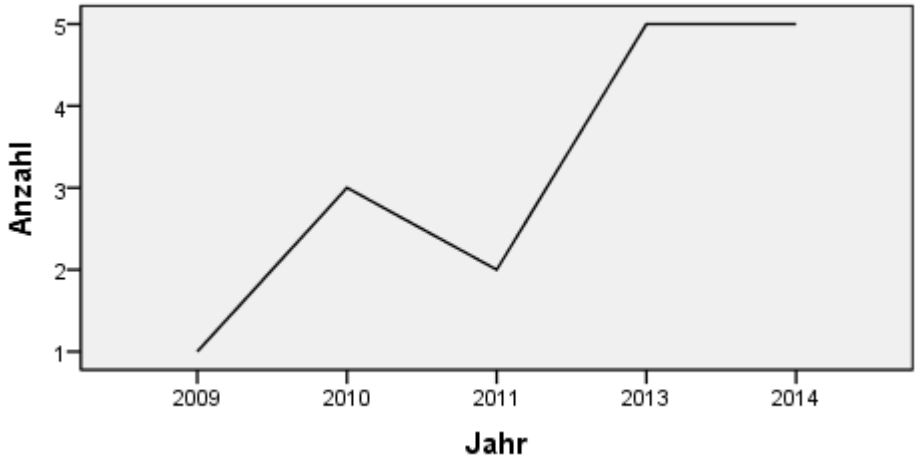
60 – 69a: n=7

Mucorales	n	%
Lichtheimia corymbifera	5	31.3
Rhizopus arrhizus [oryzae]	4	25
R. microsporus	2	12.5

Jahresverteilung:

2014: n=5

2013: n=5



Mucormykosen im AKH Wien (2009 - 2014)

Grunderkrankung	n	%
Organtransplantation	6	37.5
Hämatoonkologische Erkrankung	6	37.5
Solider Tumor	2	12.5
Diabetes mellitus	2	12.5

Lokalisation	n	%
Lunge	6	37.5
Haut/Weichteil	4	25
Dissemination	3	18.9

Signifikante Ergebnisse:

- Mucorales infizierten Haut-/Weichteilgewebe signifikant häufiger ($p < 0,05$) als *Aspergillus sp.*
- Mucorales entwickelten signifikant häufiger ($p < 0,05$) disseminierte Verläufe als *Aspergillus sp.*

Vgl. mit anderen Studien:

- HE, SOT und Diabetes mellitus gehören ebenfalls zu den häufigsten Grunderkrankungen
- Pulmonale, kutane und rhinozerebrale Infektionen zählen zu den häufigsten Manifestationen

Schwierige Diagnose

MEDIZINISCHE UNIVERSITÄT WIEN

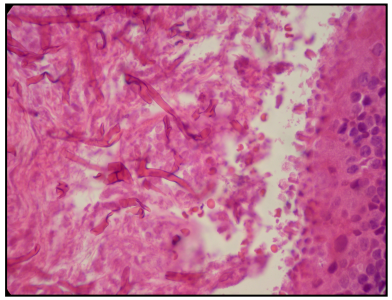
Birgit WIllinger

Abteilung für Klinische Mikrobiologie, Klin. Institut für Labormedizin

Diagnose von Mucormykosen

Biopsie - BAL

Histologie | Mikroskopie | Kultur



Mikroskopie

Sequenz-analyse

MS Maldi TOF

Biomarker

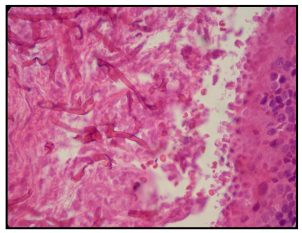
PCR | ~~Antigen~~

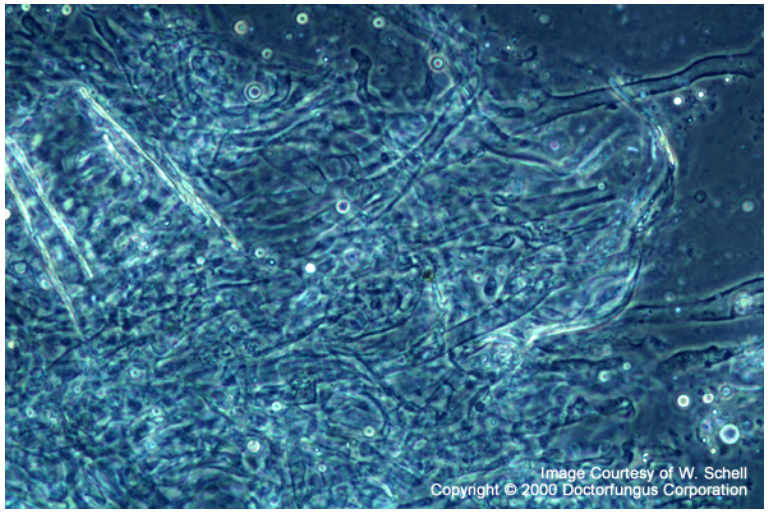
Schwierig:

- Nicht immer eindeutig
- Mischinfektionen

Diagnose von Mucormykosen

Biopsie - BAL			Biomarker	
Histologie	Mikroskopie	Kultur	PCR	~~Antigen~~

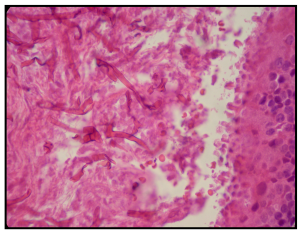


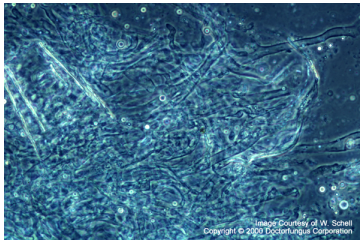


Diagnose von Mucormykosen

Biopsie - BAL

- Histologie
- Mikroskopie
- Kultur





- Mikroskopie
- Sequenz-analyse
- Maldi TOF

Biomarker

- PCR
- ~~Antigen~~

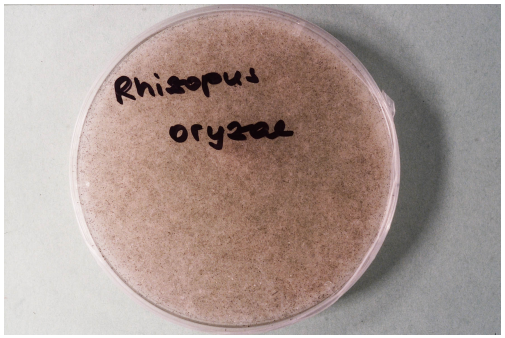
Diagnose von Mucormykosen

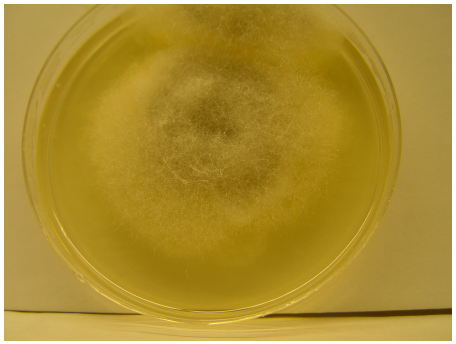
Biopsie - BAL

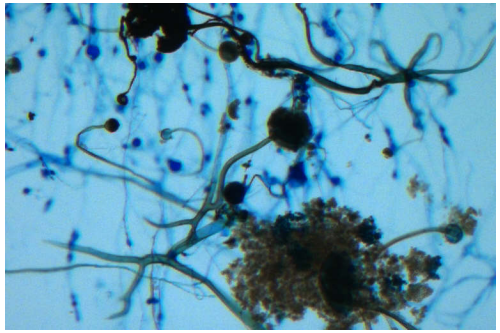
Kultur

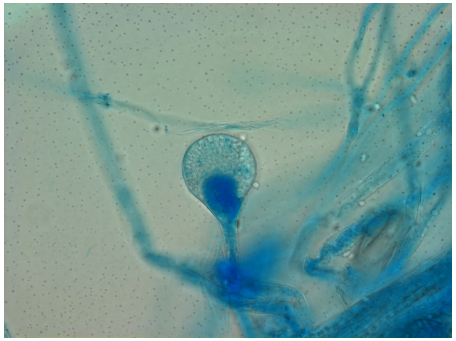
Makroskopie

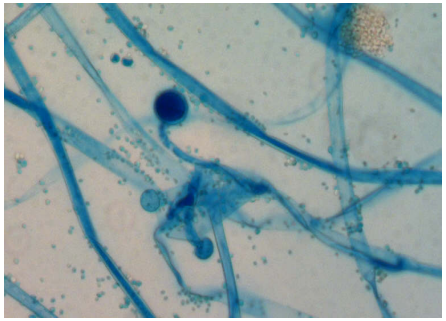
Mikroskopie











Diagnose von Mucormykosen

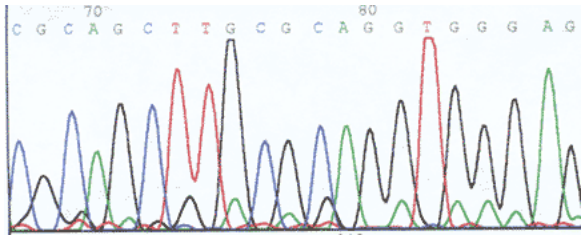
Biopsie - BAL

Kultur

Makroskopie

Mikroskopie

Sequenzanalyse



- ITS
- D1-D2-28S
- EF-1
- Calmodulin

Diagnose von Mucormykosen

Biopsie - BAL

Kultur

Makroskopie

Mikroskopie

Sequenzanalyse

MS Maldi TOF

- Sanguinetti et a. JCM 2017
- Shao J et al. 2018, JCM 2018

MALDI-TOF MS

- 4 Systeme
  - Bruker
  - Vitek MS (bioMérieux)
  - AXIMA-ID (Saramis, Shimadzu/AnagosTec) RUO
  - Andromas SAS

- ID schwieriger als bei Bakterien und Hefen
- Richtige Probenaufbereitung wichtig
  - Extrahierte Protein-Suspensionen besser als direktes Koloniematerial (Glucan und Chitin!)
- Gute Ergebnisse für Mucorales (hauptsächlich Rhizopus und Lichtheimia) besonders wenn in house-Datenbanken verwendet werden
- Einschränkung der Ergebnisse durch IVD-Datenbanken

Sanguinetti et al. 2017, JCM 55: 369 -379

Vergleich der verschiedenen Formate zur ID bei Schimmelpilzen

Extraction type	In-house database	No. moulds isolates tested	Moulds diversity	% of correct identification
MALDI Biotyper				
Complete extraction	Yes	177	33 species	87
Complete extraction	Yes	625	58 species	89
Complete extraction	Yes	200	38 species	87
Bead-beating followed by complete extraction	Yes	421	91 species	88.9
Liquid culture media followed by complete extraction	Yes	200	33 species	72
Complete extraction	Yes	206	20 species	85.9
Ethanol	Yes	103	Aspergillus, Fusarium and Mucor only	88.34
Complete extraction	Yes	1107	107 species	98.8
Andromas				
Fast formic acid	No	64	*Aspergillus* only	98.4
None	Yes		*Aspergillus* only	98.6
Saramis				
None study				
Vitek MS				
Direct smear deposit	No	36	Aspergillus only	81.8
Complete extraction	No	32	*A. fumigatus* and *A. lentulus* only	100

Cassagne et al. 2016; mycoses 59: 678 - 90

MALDI-TOF MS - Evaluierung

MALDI system[b]	Genus or group	Species studied (no. of species)	Acceptance criterion for ID[c]	No. of isolates with ID result/total no. of isolates identifiable in DB[d]	DB used for identification of isolates	Accuracy (%)	Comparat method(s)
Vitek MS	*Aspergillus*	*A. flavus*	≥60%	3/9	Vitek MS IVD	33.0	MB, MO
				6/9	SARAMIS	66.0	
		A. nomius	≥60%	0/3	Vitek MS IVD	0.0	MB, MO
				0/3	SARAMIS	0.0	
		A. tamarii	≥60%	0/2	Vitek MS IVD	0.0	MB, MO
				0/2	SARAMIS	0.0	
Bruker Daltonics	*Aspergillus*	*Aspergillus* spp. (23)[f]	≥2.0	20/21	Biotyper	95.2	MB, MO
				24/24	In-house	100	
Bruker Daltonics	*Fusarium*	*Fusarium* spp. (19)[g]	≥2.0	222/268	In-house	82.8	MB, MO
Bruker Daltonics	*Rhizopus*	*R. arrhizus*	NR	25/25	In-house	100	MB
		R. microsporus	NR	13/13	In-house	100	MB
Bruker Daltonics	*Talaromyces*	*T. marneffei*	≥2.0	39/39	In-house	100	MB
Bruker Daltonics	*Talaromyces*	*T. marneffei*	≥2.0	23/28	In-house (NTUH-3370)	82.1	MB, MO
	Paecilomyces	*Paecilomyces* spp. (3)	≥2.0	0/12	Biotyper (general library and Filamentous Fungi Library 1.0)	0.0	MB, MO
	Fusarium	*Fusarium solani*	≥2.0	1/6	Biotyper (general library and Filamentous Fungi Library 1.0)	16.6	MB, MO
	Rhizopus	*Rhizopus* spp. (3)	≥2.0	0/3	Biotyper (general library and Filamentous Fungi Library 1.0)	0.0	MB, MO

Sanguinetti et al. 2017, JCM 55: 369 -379

MALDI-TOF MS

Organism (no. of isolates)	No. (%) of isolates at the genus or species level identified by log(score) value							
	Bruker library				Bruker library plus BMU database			
	≥2.0	≥1.7	<1.7	Mis-ID	≥2.0	≥1.7	<1.7	Mis-ID
R. arrhizus (20)	19(95)	20(100)	0(0)	0(0)	19(95)	20(100)	0(0)	0(0)
R. microsporus (27)	24(88.9)	27(100)	0(0)	0(0)	24(88.9)	27(100)	0(0)	0(0)
R. stolonifer (1)	0(0)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)
R. pusillus (4)	4(100)	4(100)	0(0)	0(0)	4(100)	4(100)	0(0)	0(0)
S. racemosum (2)	2(100)	2(100)	0(0)	0(0)	2(100)	2(100)	0(0)	0(0)
L. corymbifera (4)	4(100)	4(100)	0(0)	0(0)	4(100)	4(100)	0(0)	0(0)
L. ramosa (6)	0(0)	3(50)	0(0)	3(50)[a]	0(0)	3(50)	0(0)	3(50)[a]
L. ornata(1)	0(0)	0(0)	0(0)	1(100)[b]	0(0)	0(0)	0(0)	1(100)[b]
M. irregularis (23)	0(0)	0(0)	23(100)	0(0)	23(100)	23(100)	0(0)	0(0)
M. hiemalis (5)	0(0)	0(0)	5(100)	0(0)	3(60)	5(100)	0(0)	0(0)
M. racemosus (4)	0(0)	0(0)	4(100)	0(0)	4(100)	4(100)	0(0)	0(0)
C. bertholletiae (3)	0(0)	0(0)	3(100)	0(0)	3(100)	3(100)	0(0)	0(0)
C. phaeospora (1)	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)
C. echimulata (1)	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)
Total (111)	55(49.5)	67(60.4)	37(33.3)	7(6.3)	90(81.1)	104(93.7)	0(0)	7(6.3)[d]

Shao et al. 2018, JCM 56

Daten aus der Routinediagnostik im Wiener AKH

	Cut-Off (1.9)		Cut-Off (1.8)		Cut-Off (1.7)	
Schimmelpilz-Isolate	Spezies	Miss ID	Spezies	Mis ID	Spezies	Miss ID
Mucorales (34)	**25**	**4**	**27**	**4**	**28**	**4**
L. corymbifera (11)	10	-	11	-	11	-
M. circinelloides (7)*	5	1	5	1	5	1
M. ramosissimus (1)*	1	-	1	-	1	-
Rhizomucor pusillus (1)	1	-	1	-	1	-
R. microsporus (6)	5	-	5	-	6	-
R. arrhizus (7)	2	3	3	3	3	3
Mucor sp. (1)	1	-	1	-	1	-

Diagnose von Mucormykosen

Biopsie - BAL

Biomarker

PCR

- Panfungal: ITS mit nachfolgender Sequenzierung
- Mucorales-spezifischer Ansatz
- PCR/ESI – MS (PCR Electrospray Ionization Mass spectometry)

- Frische Proben
- Paraffin-Schnitte

JOURNAL OF MEDICAL MICROBIOLOGY

RESEARCH ARTICLE

Zeller *et al.*, *Journal of Medical Microbiology* 2017;66:1383–1392

DOI 10.1099/jmm.0.000575

MICROBIOLOGY SOCIETY

Detection of fungal pathogens by a new broad range real-time PCR assay targeting the fungal ITS2 region

Iris Zeller, Claudia Schabereiter-Gurtner,† Verena Mihalits, Brigitte Selitsch, Wolfgang Barousch, Alexander M. Hirschl, Athanasios Makristathis and Birgit Willinger*

Methodology. Our in-house, HybProbe real-time PCR assay targets the ITS2 region of fungal DNA. The applicability was evaluated by testing 105 clinical samples from 98 patients with suspected fungal infection. Samples included tissue biopsies, paraffin embedded tissues, aspirates, EDTA-anticoagulated blood, cerebrospinal fluids and bronchoalveolar lavages.

Paraffin-Schnitte

Sample (Patient)	Specimen type	Other test results	Result of culture	Result of panfungal PCR	
				Cp	BLAST analysis
1 (1)	Paraffin embedded tissue (lung, bullous pemphigoid)	Postmortem histologically proven pulmonary aspergillosis	Not done	28.4	*A. fumigatus*
2 (2)	Paraffin embedded tissue (lung)	Histologically proven fungal infection	Not done	27.8	*A. fumigatus*
3 (3)	Paraffin embedded tissue (lung)	Postmortem histologically proven fungal infection of lung, meninges, myocardium	Not done	29.7	*A. fumigatus*
4 (4)	Paraffin embedded tissue (sinus)	Histologically proven fungal infection	Not done	28.6	*A. fumigatus*
5 (5)	Paraffin embedded tissue (sinus)	Histologically proven fungus ball	Not done	28.1	*A. fumigatus*
6 (6)	Paraffin embedded tissue (nasal passage)	Histologically proven fungal infection	Not done	30.1	*A. fumigatus*
7 (7)	Paraffin embedded tissue (liver)	Histologically proven fungal infection	Not done	30.4	*C. albicans*
8 (8)	Paraffin embedded tissue (retrocardiac fungal abscess)	Histologically proven fungal infection	Not done	31.0	*C. albicans*
9 (9)	Paraffin embedded tissue (lung)	Histologically proven purulent mucormycosis	Not done	36.1	*Lichtheimia corymbifera*
10 (10)	Paraffin embedded tissue (lung)	Histologically proven fungal infection	Not done	36.0	*Rhizomucor miehei*
11 (11)	Paraffin embedded tissue (lymph node)	Histologically proven mucormycosis	Not done	28.5	*Rhizopus microsporus*

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Frische Biopsien

Sample (Patient)	Specimen type	Other test results	Result of culture	Result of Fungi assay	
				Cp	BLAST analysis
21 (21)	Tissue (lung)	Histologically proven aspergillosis	*A. fumigatus*	27.8	*A. fumigatus*
22 (22)	Tissue (maxillary sinus)	Histologically proven fungus ball, KOH: filamentous fungus	*A. fumigatus*	24.5	*A. fumigatus*
23 (23)	Tissue (pancreas cyst)	Proven: Candidemia and Candida-peritonitis	*C. albicans*	23.0	*C. albicans*
24 (24)	Tissue (orbit)	Histologically proven rhinocerebral zygomycosis	*Rhizopus oryzae*	31.0	*Rhizopus oryzae*
25 (25)	Tissue (maxillary sinus)	Histologically proven zygomycosis of maxillary sinus	*Rhizopus microsporus*	31.4	*Rhizopus microsporus*
26 (26)	Tissue (lung)	Histologically proven fungal infection	*Fusarium oxysporum*	34.0	*Fusarium oxysporum* complex
27 (27)	Tissue (cornea)	Known colonization of contact lenses with *Fusarium solani complex*	No fungal growth	23.3	*Fusarium solani* complex

Nachweis von DNA aus dem Blut zum direkten Nachweis von Mucorales

- Nicht invasiv (Serum, Plasma)
- Sensitivität 90 – 100%
- Frühe Diagnose
  - Raschere Diagnose als aus Biopsien
  - 8 Tage vor histologischem/mykologischem Nachweis
  - 4 Tage vor „reverse halo sign“ (akute Leukämie)

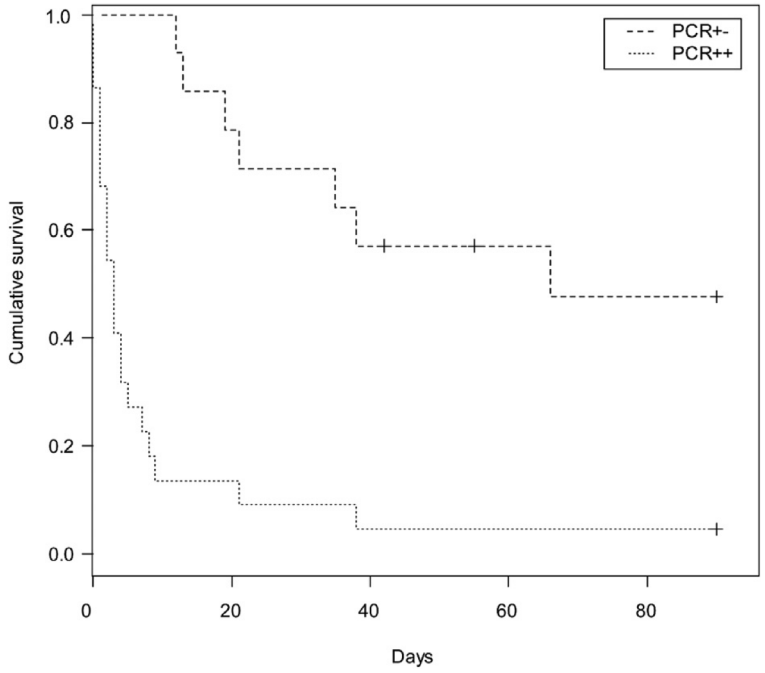
Millon, Clin Infect Dis 2013
Millon, Clin Microbiol Infect 2016
Springer J, J Med Microbiol 2016
Bourcier, Mycosis 2017
Caillod, Open Forum Infect Dis 2017

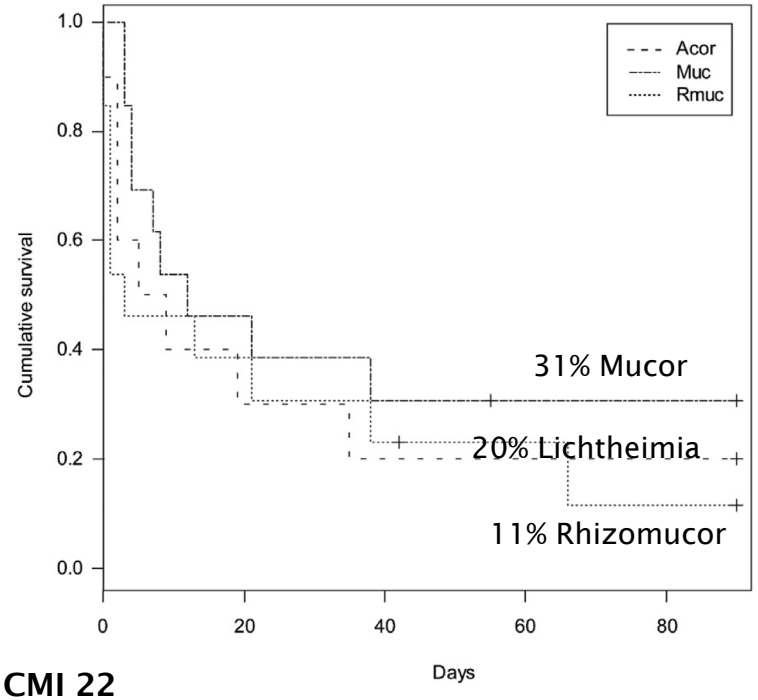
Unterschiedlicher Zugang

- Kombination dreier qPCRs (18S) - Millon, CID 2013
  - Rhizopus/Mucor
  - Rhizomucor
  - Lichtheimia
- Mucorales-spezifische qPCR mit nachfolgender Sequenzierung (Springer, JMM 2016)
- Cunninghamella spezifische qPCR (18S) - Bellanger

Bessere Prognose durch Einsatz einer spezifischen PCR?

- Persistenz einer positiven PCR mit schlechterem Outcome assoziiert
- Bessere Prognose, wenn PCR negativ wird





Millon 2016, CMI 22

Clinical evaluation of a Mucorales-specific real-time PCR assay in tissue and serum samples

Jan Springer,[1]† Michaela Lackner,[2]† Christian Ensinger,[2] Brigitte Risslegger,[2] Charles Oliver Morton,[3] David Nachbaur,[4] Cornelia Lass-Flörl,[2] Hermann Einsele,[1] Werner J. Heinz[1] and Juergen Loeffler[1]

- Mucorales spezifische real-time PCR (Muc 18S)für Gewebe und Serum
- Breites Spektrum
- **100%** Detektion bei gesicherten/wahrscheinlichen invasiven Mucormykosen
- **29%** Detektion bei möglichen invasiven Mucormykosen
- Sensitivität 91%, Spezifität 100%
- Bis zu 21 d früherer Diagnose im Vergleich zu konventionellen Methoden (Serumproben)

J Med Microbiol (2016), 65: 1414-21

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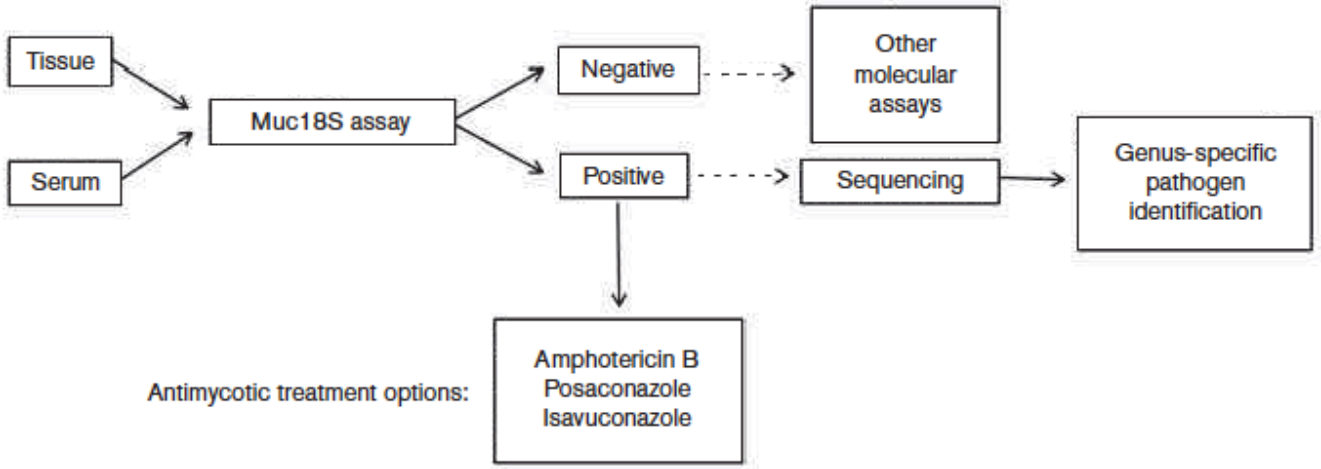

J Med Microbiol (2016), 65: 1414-21

Guidelines Mucorales

Population	Intention	Method/Finding	SoR	QoE	References	Comment
Any	To diagnose mucormycosis	Direct microscopy preferably using optical brighteners	A	IIu	30,31	Allows rapid presumptive diagnosis; non-septate or pauci-septate, irregular, ribbon-like hyphae, angle of branching 45–90°, identification to genus and species level not possible, hyphal diameter in aspergillosis 2–3 μm, in mucormycosis 6 to >16 μm
Any	To diagnose	Culture	A	IIIr	32,35	Avoid grinding, preferred temperature 37°C
Any	To diagnose	Histopathology	A	IIu	7,26,36–38	Features as in direct microscopy, does not allow for genus or species differentiation; perineural invasion commonly seen, if nerves sampled
Any	To diagnose	Immunohistochemistry	C	IIu	39	No commercial assay available Monoclonal antibodies commercially available
Any	To diagnose	Galactomannan in blood or bronchoalveolar lavage	B	III	41 43 192	$n = 2$ $n = 1$ $n = 2/8$ missed mucormycoses: consider mucormycosis, if galactomannan test negative, but radiology positive
Any	To diagnose	1,3-β-D-glucan in blood	D	III	44,45	Not a reliable marker
Haematological malignancy	To monitor treatment	ELISPOT	C	IIu	46	No commercial assay available
Any	To diagnose	Molecular based tests on fresh clinical material	B	IIu	30,47,193,194	No commercial assay available; fresh material preferred over paraffin-embedded
Any	To diagnose	Molecular based tests on paraffin slides	B	IIu	48,49, 51	No commercial assay available

Cornely et al. 2014, CMI 20, Supp. 3: 5 - 26

Neues von Mucor & Co

- Nachweis nach wie vor schwierig
- Verbesserungen durch MALDI-TOF
- Neue Ansätze mittel PCR in Gewebe und Serum
- Frühzeitige Diagnose von Mucormykosen ist dadurch näher gerückt

Vielen Dank für Ihre Aufmerksamkeit!