PARACELSUS KLINIKEN
Klinik Osnabrück

Diagnostik von Pilzinfektionen - offene Fragen und Kontroversen

PEG-Tagung - Sektion "Antimykotische Chemotherapie"

Bonn, 9.3.2018

Prof. Markus Ruhnke

GLOBAL CATASTROPHE: HOW 150 PEOPLE DIE EVERY HOUR FROM FUNGAL INFECTION WHILE THE WORLD TURNS A BLIND EYE

Neglected by policy makers and most international health agencies, the launch of Global Action Fund for Fungal Infections **(GAFFI), an international organisation set to highlight the plight of 300 million people worldwide will begin to reverse the unnecessary trend in death and suffering.**

Fungal infections kill at least 1,350,000 patients with or following AIDS, cancer, TB and asthma as well as causing untold misery and blindness to tens of millions more worldwide. Yet its symptoms are mostly hidden and occur as a consequence of other health problems. Diagnostic tests are not available to millions to worldwide, and they need to be.

JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

ylaxis of Fungal Diseases

Emerging Problems in Infectious Diseases

Estimated burden of fungal infections in Kenya

John A Guto[1,4], Christine C Bii[2], David W Denning[1,3,4]

Estimated burden of fungal infections in Germany

Markus Ruhnke,[1] Andreas H. Groll,[2] Peter Mayser,[3] Andrew J. Ullmann,[4] Werner Mendling,[5]
Herbert Hof,[6] David W. Denning[7] and The University of Manchester in association with the LIFE
program

in the

The burden of fungal disease in D

Klaus L. Mortensen,[1] David W

[1]Department of Infectious Disease
Centre and National Aspergill
In: Statens Seru

Journal of Medical Microbiology (2015), 64, 423–426

Burden of serious fungal infections in Spain

-Tudela[1], A. Alastruey-Iz
ole[7] and D. W. De

, M. Cuenca-Estrella[1,2], C. León[3], J. M. Miro[4], A. Nuñez Bolu
nchester in association with the LIFE program at http://

European Journal of Clinical Microbiology & Infectious Diseases
July 2016, Volume 35, Issue 7, pp 1115–1120

An estimate of the burden of serious fungal diseases in Greece

Apiranthitou, D. W. Denning, N. V. Sipsas

The burden of serious fungal diseases in Russia

N. Klimko,[1] Y. Kozlova,[1] S. Khostelidi,[1] O. Shadrivova,[1] Y. Borzova,[1] E. Burygina,[1] N. Vasilieva[1] and D. W. Denning[2]

[1]*I. Metchnikov North-Western State Medical University, St. Petersburg, Russia and* [2]*Manchester Academic Health Science Centre, The National Aspergillosis Centre, University Hospital of South Manchester, The University of Manchester, Manchester, UK*

Fungal Diseases in Israel

Ronen Ben-Ami MD[1] and David W. Denning FRCP[2,3]

[1]Infectious Diseases Unit, Tel Aviv Sourasky Medical Center and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[2]National Aspergillosis Centre, University Hospital of South Manchester, University of Manchester, UK
[3]Global Action Fund for Fungal infections, Geneva, Switzerland

Pilzdiagnostik in der Hämatologie

Prof. M. Ruhnke

Mykosen in Deutschland

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Table 1 Burden of fungal diseases in Germany according the selected underlying diseases.

	Number of infections per underlying disorder per year						
	Unknown	HIV/AIDS	Respiratory	Cancer/Tx	ICU	Total burden	Rate/100K[2]
Fungal skin diseases	6 721 000	n.a.[1]	n.a.	n.a.	n.a	6 721 000	8347
Oral candidosis	n.a.	15 600	n.a.	97 965	n.a.	113 565	141
De							
Ca							
Ca							
Re							
All							
Se							
Ch							
Inv							
Mu							
Cr							
Pn							
Histoplasmosis	5	10	n.a.	n.a.	n.a.	15	0.02
Fungal keratitis	32	n.a.	n.a.	n.a.	n.a.	32	0.04
Total burden estimated						9 610 789	

Invasive Aspergillose N = ca. 4280

Candidämien N = ca. 3700

Candida Peritonitis N = ca. 3700

Ruhnke et al., Mycoses, 2015, 58 (Suppl. S5), 22–28

Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group

Ben De Pauw,[a] Thomas J. Walsh,[a] J. Peter Donnelly,[a] David A. Stevens, John E. Edwards, Thierry Calandra, Peter G. Pappas, Johan Maertens, Olivier Lortholary, Carol A. Kauffman, David W. Denning, Thomas F. Patterson, Georg Maschmeyer, Jacques Bille, William E. Dismukes, Raoul Herbrecht, William W. Hope, Christopher C. Kibbler, Bart Jan Kullberg, Kieren A. Marr, Patricia Muñoz, Frank C. Odds, John R. Perfect, Angela Restrepo, Markus Ruhnke, Brahm H. Segal, Jack D. Sobel, Tania C. Sorrell, Claudio Viscoli, John R. Wingard, Theoklis Zaoutis, and John E. Bennett[b]

Ascioglu et al., CID (2002);34; De Pauw et al., CID (2008);46

EORTC/MSG - Definitionen

"Proven Invasive Fungal Infections/Disease"

Gesichert = proven

Histologisch: invasives Wachstum

oder

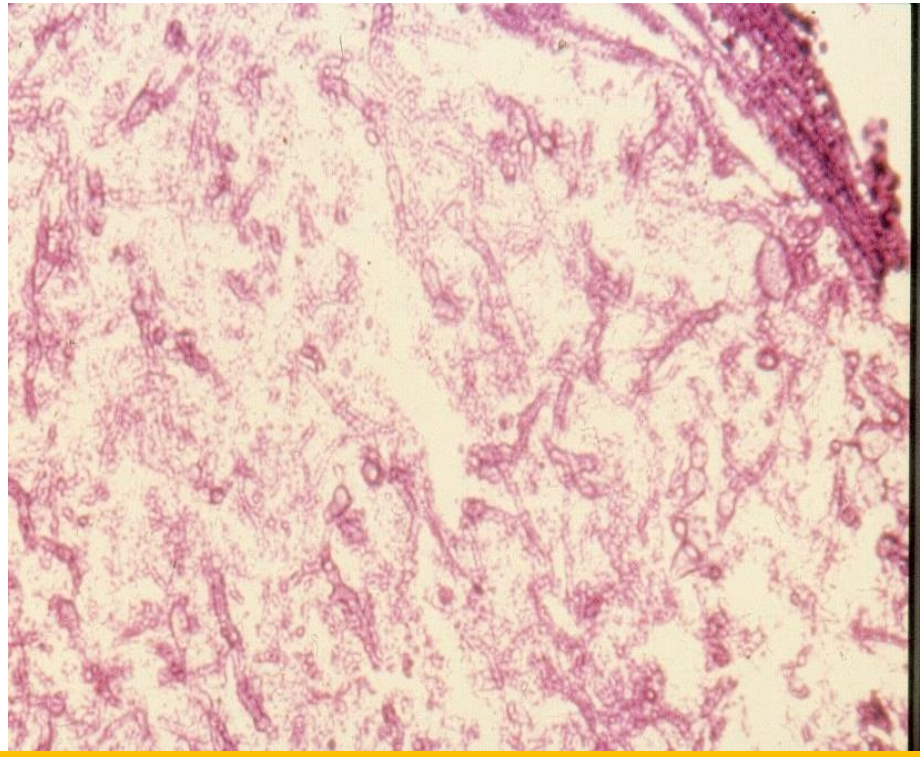
kultueller Nachweis aus primär sterilem Material

Wahrscheinlich = probable

Risikofaktor + Klinik + Mikrobiologie

Möglich = possible

Risikofaktor + Klinik oder Mikrobiologie



Histopathologie ist der „Goldstandard“

Ascioglu et al., CID (2002);34; De Pauw et al., CID (2008);46

Anwendung EORTC/MSG Kriterien

Table 1. Patterns of invasive fungal disease in pratice, based on 2008 EORTC-MSG criteria.

	A	B	C				D	E
	-	-	I	II	III	IV	-	
Radiological signs and clinical symptoms	No	Persistent febrile neutropenia	No	Clinical (any new infiltrate not fulfilling the EORTC/MSG criteria)		Radiological signs on CT (dense, well-circumscribed lesions(s) with or without a halo sign, air-crescent sign, or cavity)		Not considered necessary
Mycology results	Negative	Negative	Positive biomarker or microscopy or culture	Negative	Positive biomarker or microscopy or culture	Negative	Positive biomarker or microscopy or culture	Positive tissue or specimen from a sterile site
Clinical evidence of IFD	No	No	No	No	No	Yes	Yes	Yes
Mycological evidence of IFI	No	No	Yes	No	Yes	No	Yes	Yes
Final diagnosis	Unclassified					Possible IMD	Probable IMD	Proven IMD
Management	Prophylaxis	Empirical therapy	Diagnostic-driven (pre-emptive) therapy				Targeted therapy	

Maertens et al., Haematologica (2012)

Indications and outcomes of antifungal therapy in French patients with haematological conditions or recipients of haematopoietic stem cell transplantation

Methods: observational prospective study, children/adults with haematological conditions or HSCT were recruited upon start of non-prophylactic systemic AF treatment in 37 French haematological facilities (2007 -2008). I

FD episodes were classified according to the 2008 EORTC/MSG criteria.

Results: 419 patients (298 adults and 121 children): 88% haematological malignancies, 28% HSCT recipients and 68% neutropenic.

423 IFD episodes: 21% mycologically documented (59% probable /proven aspergillosis, 32% proven candidiasis and 9% probable/proven other IFD), 20% classified as possible IFD.

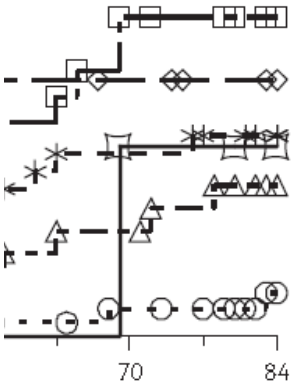
Herbrecht et al. JAC 2012

fungal therapy in ical conditions or m cell transplantation

Table 3. Species identified by day 7 of antifungal treatment (*n* = 48 patients with proven IFD)

Species	*n* (%)
Yeasts	
Candida albicans	14 (29)
Candida glabrata	5 (10)
Candida krusei	4 (8)
Candida parapsilosis	3 (6)
Candida kefyr	2 (4)
Candida guilliermondii	1 (2)
Candida lambica	1 (2)
Candida tropicalis	1 (2)
Candida spp.	1 (2)
Candida albicans and *Candida glabrata*	1 (2)
Candida albicans and *Candida parapsilosis*	1 (2)
Candida albicans, *Candida glabrata* and *Candida zeylanoides*	1 (2)
Rhodotorula mucilaginosa	1 (2)
unidentified yeast[a]	2 (4)
Moulds	
Aspergillus fumigatus	3 (6)
Aspergillus flavus	1 (2)
Fusarium solani	1 (2)
Fusarium spp.	2 (4)
Mucor spp.	2 (4)
unidentified mould[a]	1 (2)

[a]Diagnosis by microscopy on biopsy material, no culture growth.



l therapy according to classification at day 7 (*n* = 419

n aspergillosis, 15% for proven
; for possible IFD, 3% for febrile

Herbrecht et al. JAC 2012

Leitlinie "Diagnostik von Mykosen" der AG Infektionen der DGHO (AGIHO)

General recommendations	Strength
The highest level of evidence for the presence of IFI should be obtained before initiating systemic antifungal therapy	A
Most signs and symptoms related to IFI are unspecific and require further diagnostic procedures	A
A combination of various methods with regular screenings ismandatory for early diagnosis of IFD, as well as for monitoring response to antifungal treatment	A

Ruhnke et al. Ann Oncol 2012

Structure of the ESCMID/ ECMM Recommendations

Guideline Methodology – Two Independent Evaluations

1. Strength of Recommendation = SoR
2. Quality of Evidence = QoE

→ Allows strong recommendations in the absence of highest quality of evidence.

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Patient with fever	Diagnose fungaemia	Take blood cultures	A	II	Acme WJFD 2002	

Parachute use to prevent death and major trauma related to gravitational challenge: systematic review of randomised controlled trials

thebmj

Objectives To determine whether parachutes are effective in preventing major trauma related to gravitational challenge.

Design Systematic review of randomised controlled trials.

Data sources: Medline, Web of Science, Embase, and the Cochrane Library databases; appropriate internet sites and citation lists.

Study selection: Studies showing the effects of using a parachute during free fall.

Main outcome measure Death or major trauma, defined as an injury severity score > 15.

Results We were unable to identify any randomised controlled trials of parachute intervention.

Conclusions As with many interventions intended to prevent ill health, the effectiveness of parachutes has not been subjected to rigorous evaluation by using randomised controlled trials. Advocates of evidence based medicine have criticised the adoption of interventions evaluated by using only observational data. We think that everyone might benefit if the most radical protagonists of evidence based medicine organised and participated in a double blind, randomised, placebo controlled, crossover trial of the parachute.

BMJ 2003; 327 doi: https://doi.org/10.1136/bmj.327.7429.1459

Parachute use to prevent death and major trauma related to gravitational challenge: systematic review of randomised controlled trials

thebmj

Analysis And Comment » Controversy

Parachute approach to evidence based medicine

BMJ 2006 ; 333 doi: https://doi.org/10.1136/bmj.333.7570.701 (Published 28 September 2006)
Cite this as: *BMJ* 2006;333:701

Waiting for the results of randomised trials of public health interventions can cost hundreds of lives, especially in poor countries with great need and potential to benefit. If the science is good, we should act before the trials are done

In 2003 Smith and Pell published an entertaining but profound article titled: "Parachute use to prevent death and major trauma due to gravitational challenge."[1] They used the lack of randomised controlled trials in testing parachutes to show that situations still exist where such trials are unnecessary. We argue that the parachute approach, where policies are set based on good science but without randomised trials, is often more suitable in resource poor settings. We use the examples of oral rehydration therapy, male circumcision to prevent HIV infection, and misoprostol for postpartum haemorrhage to show how an overemphasis on randomised controlled trials in poor settings poses important ethical and logistic problems and may incur avoidable deaths.

BMJ 2006; 333 doi: https://doi.org/10.1136/bmj.333.7570.701

Diagnostik von Mykosen AGIHO - Leitlinie 2018

Was hat sich seit 2012 geändert?

- Mikroskopie
- Kultur (**Erregernamen, Namensänderungen**)
  - **Resistenztestung** von Aspergillus spp. und Resistenzentwicklung gegen Azole (u.a. molekulare Resistenztestung)
- Antigen - / Antikörper
  - Daten zur **kombinierten Diagnostik/Screening der IA mit Galactomannan + PCR +/-ß-D-Glucan**
  - **Neue Testsysteme** (LFD = „laminar flow device")
- **Molekulare Diagnostik**
  - Mehr Daten zur molekularen Diagnostik (neue Verfahren, z.B. „microarrays")
  - Kommerzielle PCR-Teste eingeführt

Diagnostik von Mykosen
AGIHO - Leitlinie 2016

- Bildgebung
  - **Dünnschicht-CT Thorax statt HR-CT**
  - **„reversed halo sign"** bei Zygomykosen
- Endoskopie
- Gewebediagnostik
- Allgemeine Vorgehensweisen
  - **„integrated pathways"**
  - „clinical driven" vs. „diagnostic driven"
  - **Diagnostischer Algorithmus unter Antimykotika-Prophylaxe**

Histopathologie Goldstandard?

Challenges and Pitfalls of Morphologic Identification of Fungal Infections in Histologic and Cytologic Specimens

A Ten-Year Retrospective Review at a Single Institution

Ankur R. Sangoi, MD,[1] William M. Rogers, MD,[1] Teri A. Longacre, MD,[1] Jose G. Montoya, MD,[2] Ellen Jo Baron, PhD,[1] and Niaz Banaei, MD[1]

- **Of the 47 of 338 positive mold and yeast cultures with concurrent surgical pathology evaluation without known history of a fungal infection,**
- **37 (79%) were correctly identified based on morphologic features in histologic and/or cytologic specimens.**
- **The 10 discrepant diagnoses (21%) included misidentification of septate and nonseptate hyphal organisms and yeast forms.**
- **Errors resulted from morphologic mimics, use of inappropriate terminology, and incomplete knowledge in mycology. The accuracy did not correlate with preceding antifungal therapy or use of special stains and was not operator-dependent.**

Sangoi et al., Am J Clin Pathol 2013

Histopathologie Goldstandard?

Diagnostic Accuracy of Histopathologic and Cytopathologic Examination of *Aspergillus* Species

*Akeesha A. Shah, MD, and Kevin C. Hazen, PhD**

Diagnostic accuracy was defined as the percentage of cases with culture proven* Aspergillus *spp divided by the number of cases diagnosed as Aspergillus spp on HCE that had growth on fungal cult

90 surgical/cytology cas concurrent fungal culture were reviewed, 58 of which grew a fungal organism. Of thes 45 grew an Aspergillus spp, whereas 13 grew an organism other than Aspergillus spp h common (Scedosporium, Fusarium, and Paecilomyces spp) and unc (Trichosporon loubieri), ***<u>resulting in a diagnostic accuracy of 78%</u>***

The low diagnostic accuracy indicates that several fungal organisms can morphologically mimic Aspergillus spp and can only be distinguished by fungal culture and DNA sequencing.

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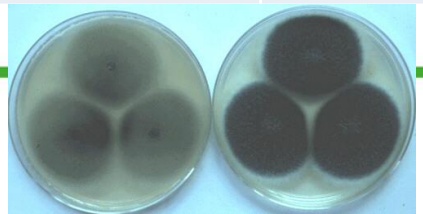
Wie nennen wir die Erreger ?

Nomenklatur von Pilzen Aspergillus Spezies

Teleomorph = geschlechtliche (perfekte) Vermehrung	Anamorph = ungeschlechtliche (imperfekte) Vermehrung
Eurotium amstelodami	**Aspergillus glaucus**
Emericella nidulans	**Aspergillus nidulans**
Fennellia terrei	**Aspergillus terreu**
Hemicarpenteles clavati	**Aspergillus clavat**
Neosartorya fumigata	**Aspergillus fumigatus**
Neosartorya fischeri	**Aspergillus fischerianus**
Petromyces flavi	**Aspergillus flavus**
Petromyces nigri	**Aspergillus niger**

= no sex!



Taxonomy of medically important fungi in the molecular era

Traditionally, fungi have been allowed to carry multiple names that describe different asexual and sexual morphological stages. This duplicated name system is because these phases can propagate independently and thus the shared identity is not always obvious. At the molec genetic level the two stages are identical, and the this system is becoming increasingly impractical, reason Article 59 regulating dual naming in fu Code of Botanical Nomenclat

This amendment has a potentially profound effect on linical mycology, because with this fundamental change established fungal names and many disease names ieopardised. Additionally, many well known, clinically rtant species, such as *Aspergillus fumigatus*, *Coccidioides* s, *Exophiala jeanselmei*, and *Sporothrix schenckii*, en found to consist of several molecular siblings. ecular diversity leads to an enormous increase in levant fungi and to changes of

This amendment has a potentially profound effect on clinical mycology, because with this fundamental change all established fungal names and many disease names are jeopardised…

deHoog et al. Lancet ID 2013

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EUKARYOTIC CELL, May 2009, p. 713–722 Vol. 8, No. 5
1535-9778/09/$08.00+0 doi:10.1128/EC.00272-08

Aspergillus alabamensis, a New Clinically Relevant Species in the Section *Terrei*▽

S. Arunmozhi Balaj hn W. Baddley,[2,3] Stephen W. Peterson,[4] David Nickle,[5]
János Varga,[Boey,[1] Cornelia Lass-Flörl,[8] Jens C. Frisvad,[9]
Robert A. [e ISHAM Working Group on *A. terreus*

Most members of this new cryptic species were recovered as colonizing isolates from immunocompetent patient populations, had decreased in vitro susceptibilities to the antifungal drug amphotericin B, and were morphologically similar to but genetically distinct from *Aspergillus terreus isolates…*

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Kommt man ohne die molekulare Diagnostik und Erregeridentifizierung heute noch aus?

Taxonomy of Fungi Causing Mucormycosis and Entomophthoramycosis (Zygomycosis) and Nomenclature of the Disease: Molecular Mycologic Perspectives

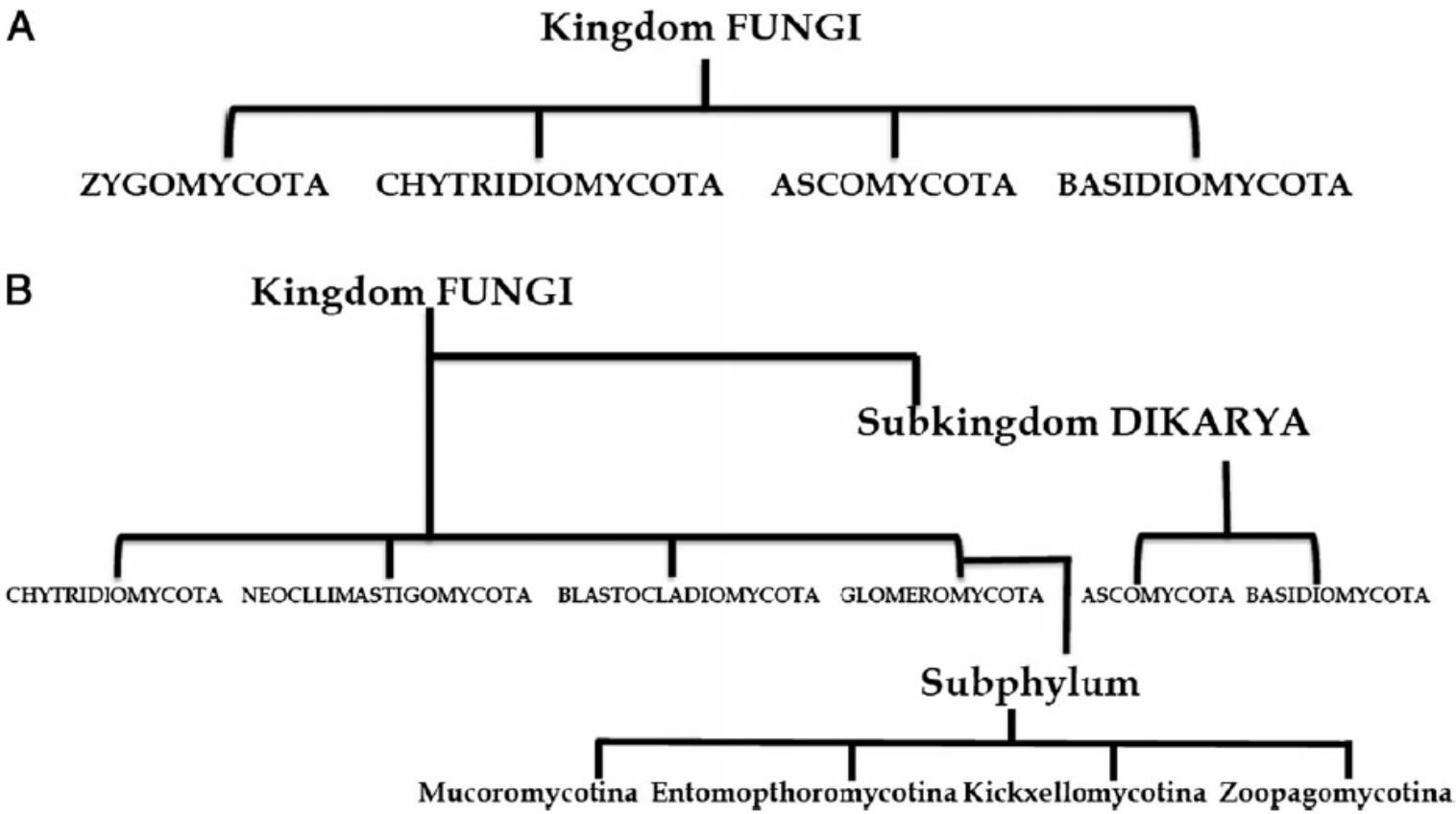

Figure 1. Old (*A*) and a proposed new (*B*) classification schemes of the kingdom Fungi.

Kyung J. Kwon-Chung; CID 2012

Der „molekulare" Spucktest!

High-frequency Triazole Resistance Found In Nonculturable *Aspergillus fumigatus* from Lungs of Patients with Chron

David W. Denning,[1,2,3] Steven
Jaclyn Smith,[2] Ahmed Buei

Methods. Using an
assessed respiratory fu
positive, culture negati
polymorphisms (SNPs) a

Results. *Aspergillus* DN
or microscopy confirmed
42 (71.4%) patients w
negative, PCR-pos
M220) within
with CPA had resistance markers present, some without prior triazole treatment, and in most despite adequate plasma drug concentrations around the time of sampling.

Six of 8 (75%) of those with ABPA and 12 of 24 (50%) with CPA had resistance markers present, some without prior triazole treatment…

Denning et al., CID 2011

und in Deutschland?

AAC
Journals.ASM.org

cyp51A-Based Mechanisms of *Aspergillus fumigatus* Azole Drug Resistance Present [illegible] Clinical Samples from Germany

Oliver Bader,[a] Michael Weig,[a] Utz R[illegible] Lugert,[a] Martin Kuhns,[a] Martin Christner,[b] Jürgen Held,[c] Silke Peter,[d] Ulrike Schumacher,[d]* Dieter Buchhe[illegible] Uwe Groß,[a] MykoLabNet-D Partners

Institute for Medical Microbiology and Germa[illegible] University Medical Center Göttingen, Göttingen, Germany[a]; Department for Medical Microbiology, Virology and Hygi[illegible] for Medical Microbiology and Hygiene, University Medical Center Freib[illegible] many[d]; 3rd Department of Internal Medicine—H[illegible] sitic Agents and Mycobacteri[illegible]

…From a total of 527 samples, 17 (3.2%) showed elevated MIC_0 values …for at least one of the three substances (itraconazole, voriconazole, and posaconazole) tested. The **highest prevalence of resistant isolates was observed in cystic fibrosis patients (5.2%).** Among resistant isolates, the TR/L98H mutation in cyp51A was the most prevalent, but isolates with the G54W and M220I substitutions and the novel F219C substitution were also found…

In Zukunft „fungal sequencing“?

Performance of Targeted Fungal Sequencing for Culture-Independent Diagnosis of Invasive Fungal Disease

Carlos A. Gomez,[1,2] Indre Budvytiene,[3] Allison J. Zemek,[1] and Niaz Banaei[1,2,3]

[1]Department of Pathology and [2]Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, and [3]Clinical Microbiology Laboratory, Stanford University Medical Center, Palo Alto, California

Methods: Between January 200… …imens, consisting of fresh an… …nd sterile body fluid… results (n = 11… microscopic… **amplicons f… ribosomal …**

Result… known diagnosis …

In patients with suspected IFD, the diagnostic yield of fungal sequencing was 62.9% (73/116) overall and 71.3% (57/80) in patients classified with proven IFD (EORTC/MSG criteria). Samples obtained by open biopsy had a significantly higher diagnostic yield (71.5%) compared with core-needle biopsy (50%) and fine needle aspiration…

Gomez et al., CID 2017

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Die Realität in der Hämatologie:

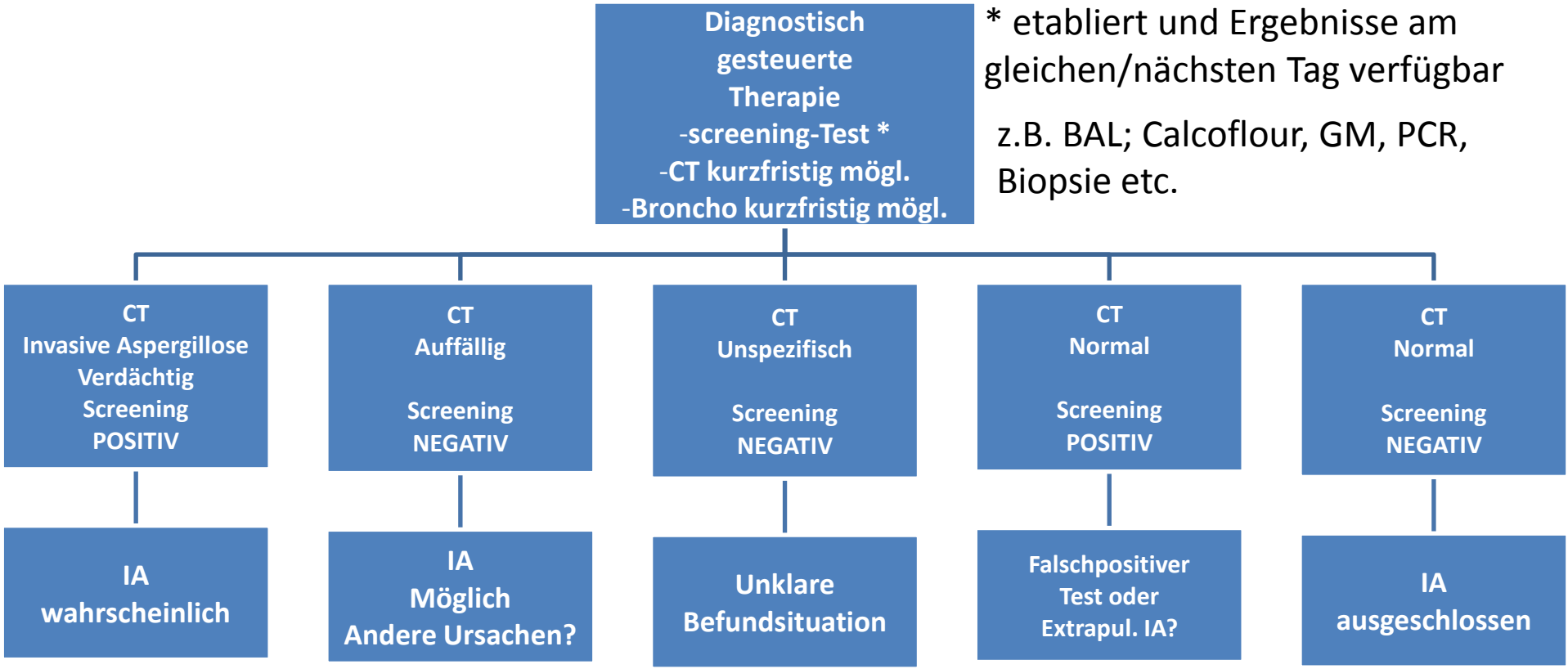
„integrated pathways"?

„clinical driven" vs. „diagnostic driven"

Diagnostischer Algorithmus unter

Antimykotika-Prophylaxe

Algorithmus zur "diagnostic driven" Behandlung der invasiven Aspergillose



Agrawal et al; JAC (2011)

INTERNAL MEDICINE JOURNAL

Internal Medicine Journal **44** (2014)

Consensus guidelines for the use of empiric and diagnostic-driven antifungal treatment strategies in haematological malignancy, 2014

Surveillance-driven diagnostic antifungal treatment strategy

Twice-weekly testing of serum with GM-ELISA and whole blood with *Aspergillus* PCR‡

Perform HRCT scan of chest for any of the following:

- At time-point of first (+) GM-ELISA or PCR result. Continue with GM-ELISA and PCR testing while awaiting HRCT results
- If GM-ELISA and PCR testing produces consistently negative results but patient has refractory fevers§ (+/– neutropenia)¶
- Clinical signs/symptoms of IFD††

Commence antifungal therapy if any of the following is fulfilled:‡‡

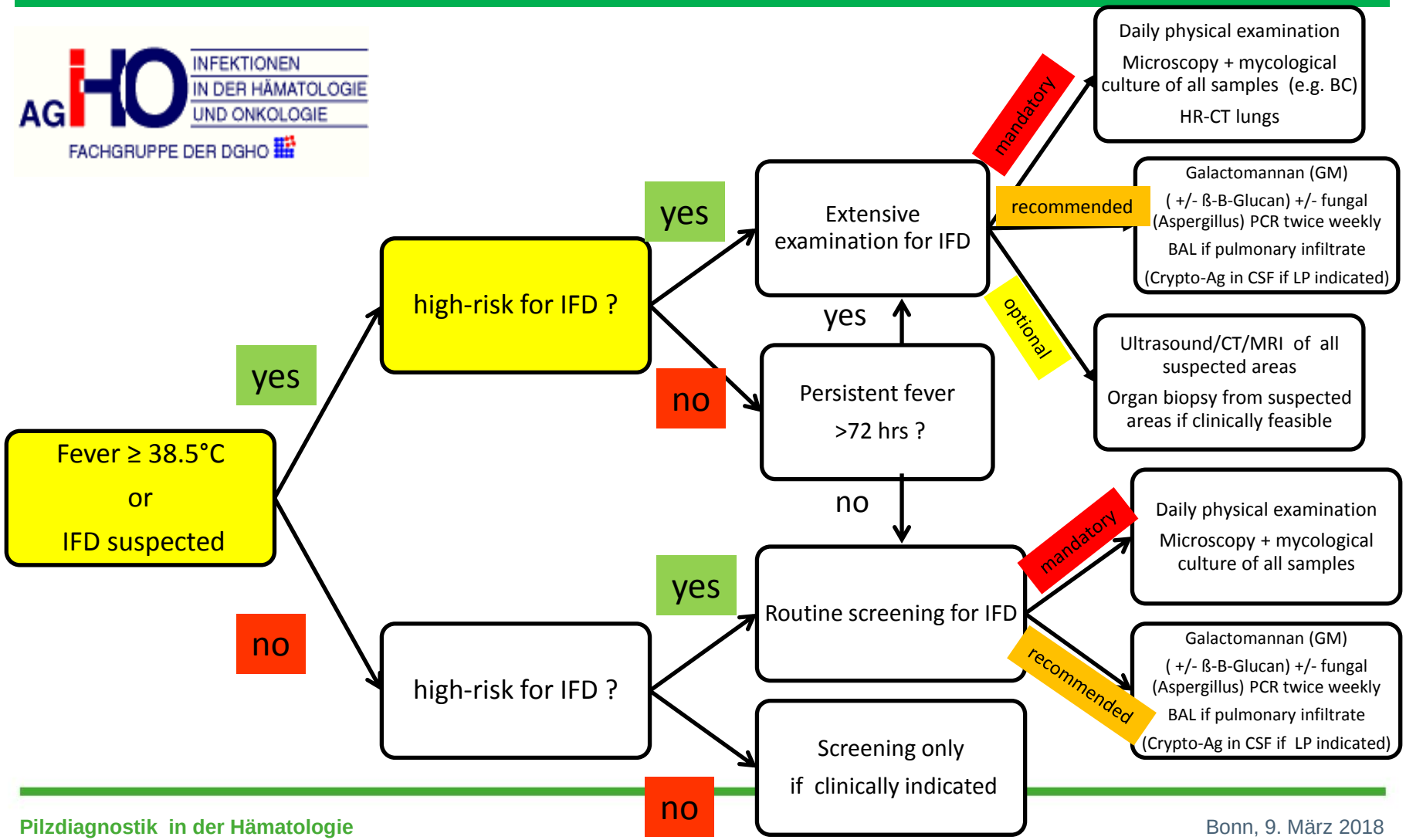
- 2 consecutively (+) GM-ELISA or PCR results
- >2 intermittently (+) GM-ELISA or PCR results in a 2-week period
- Single positive GM-ELISA or PCR and any lesion§§ on HRCT scan of chest
- Characteristic¶¶ lesion on HRCT scan of chest only

Antifungal therapy should not be given and investigations for other infections††† should be implemented for any of the following:

- Single positive GM-ELISA or PCR result alone
- Non-characteristic‡‡‡ lesion on HRCT scan of chest only
- All test results are negative

Overview on diagnostic procedures recommended in patients at risk for IFD before, during or after granulocytopenia (<500 cells/µL) with or without fever

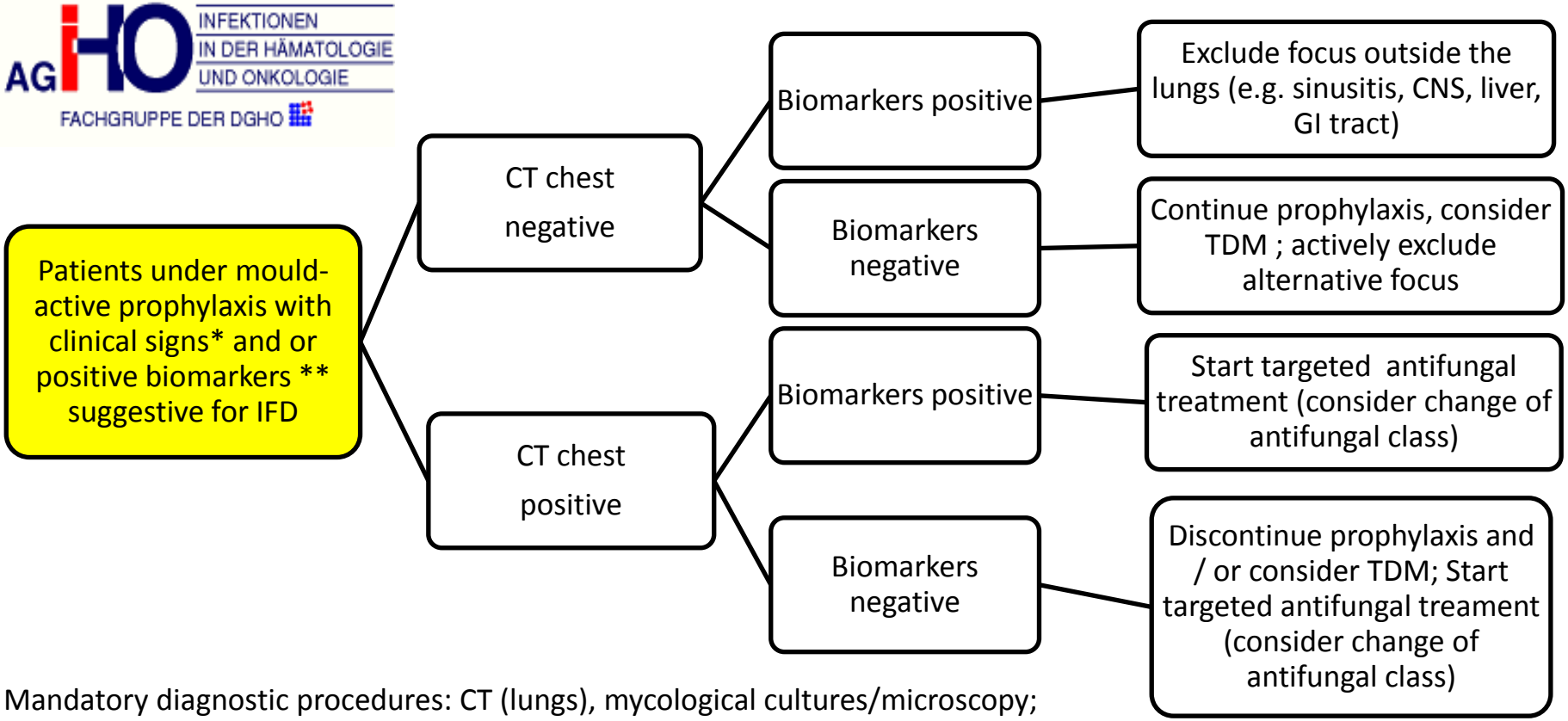
AGIHO INFEKTIONEN IN DER HÄMATOLOGIE UND ONKOLOGIE
FACHGRUPPE DER DGHO



Aktualisierung der Leitlinie Pilzdiagnostik der AGIHO 2018

Diagnostic algorithm for IFD in high-risk patients under mould active prophylaxis

AGIHO INFEKTIONEN IN DER HÄMATOLOGIE UND ONKOLOGIE
FACHGRUPPE DER DGHO



Mandatory diagnostic procedures: CT (lungs), mycological cultures/microscopy;
* clinical signs suggestive for IFD (e.g. lung infiltrate, brain abscess, liver/spleen lesions)
**Biomarkers (e.g. GM, ß-d-Glucan, PCR)

Zusammenfassung

- „laufende" Veränderung von Erregernamen macht den Biologen glücklich, aber nicht den Kliniker!
- „aggressive Diagnostik" (Biopsie) ist für die Wahl und Dauer der antimykotischen Therapie wichtig!
- Erregerdiagnostik geht nicht mehr ohne molekularbiologische Methoden!
- Diagnostisch/therapeutische Algorithmen haben insbes. bei Schimmelpilzmykosen eine hohe Bedeutung („diagnostic-driven „ bzw. „pre-emptive therapy" – aufgrund von Mängeln div. diagnostischer Methoden)!

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