Medical University of Graz

Diagnosis of invasive mould infection in patients receiving antifungal prophylaxis/treatment:

Galactomannan

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Structure of my Talk

Background
Serum GM
BALF GM
Other Materials
Neutropenia
Conclusion

- **Background**
- **GM as Biomarker for Aspergillosis and influence of antifungals:**
  - **Serum GM**
  - **BALF GM**
  - **GM in Urine and CSF**
  - **Neutropenia and GM**
  - **Suggested GM Testing Algorithm**
- **Conclusion**

In Vivo Diagnosis

Background
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BALF GM
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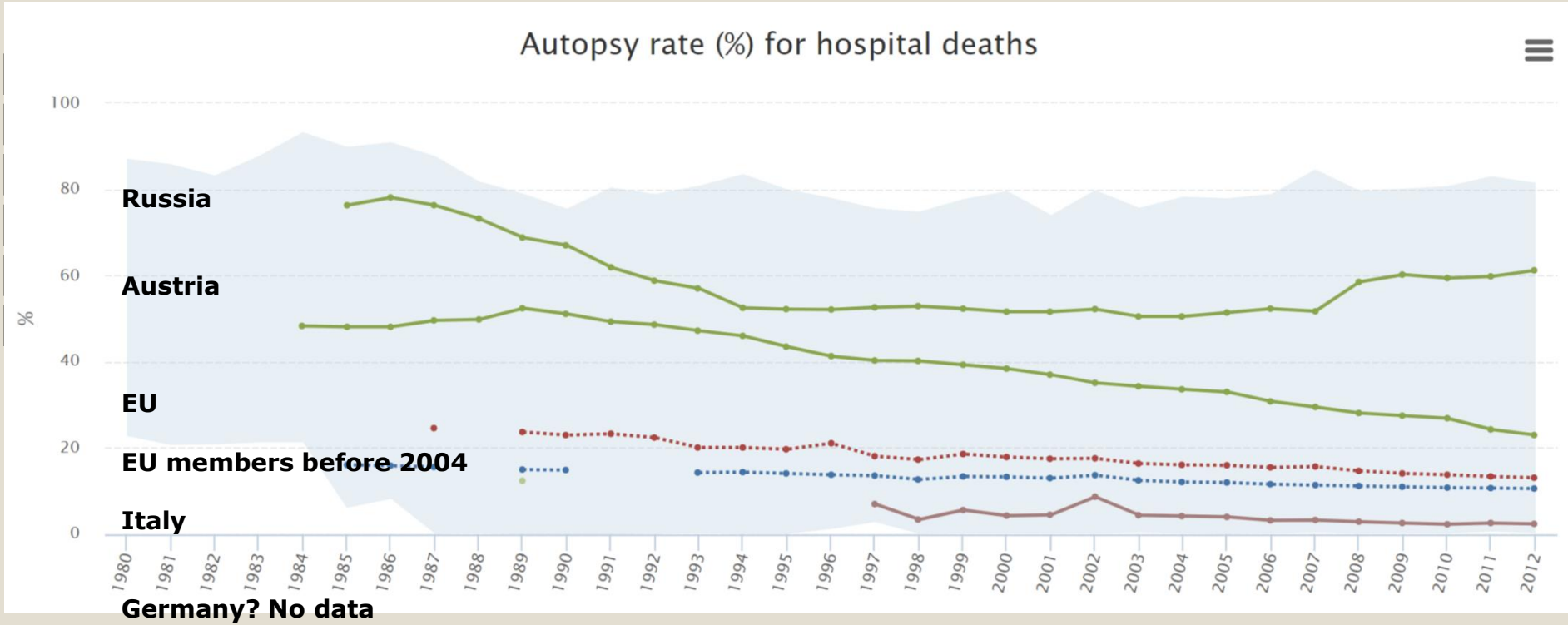
- **Invasive Aspergillosis (IA): Crude mortality of 80-90% in absence of adequate treatment**

Key factors in survival
- **Timely diagnosis!**
- **Early start of antifungal therapy**

- **In vivo diagnosis also essential to estimate Prevalence of Invasive Aspergillosis.**

Autopsy Rates in Europe



https://gateway.euro.who.int/en/visualizations/line-charts/hfa_544-autopsy-rate-for-hospital-deaths

Big Difference between countries. Overall Trend: DECREASE!

IA at Autopsy at MD Anderson, Texas

Background
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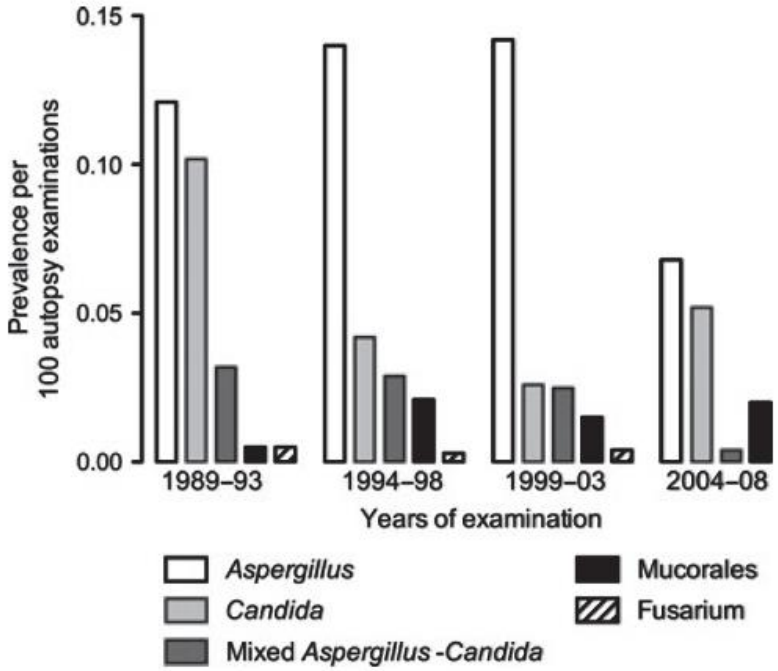

84 % of the IFIs diagnosed postmortem in the first 5 years of the study
the rate decreased to **49 %** in the last 4 years of the study

Characteristic	1989–1993 *n* = 145 (%)	1994–1998 *n* = 86 (%)	1999–2003 *n* = 81 (%)	2004–2008 *n* = 59 (%)	*P* value[1]
Autopsies per 100 deaths	0.63	0.35	0.27	0.06	<0.001
Prevalence of all invasive fungal infections (IFIs; per 100 autopsies)	0.32	0.30	0.31	0.19	<0.001

Lewis, R..Kontoyiannis, D. Mycoses 2013; 638-45

In Vivo Diagnosis

Background
Serum GM
BALF GM
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Conclusion

- **Early In vivo Diagnosis relies on early markers of IA:**
  - **GM from Serum / BALF**
  - **PCR**
  - **BDG, LFD**

Test (combination) and condition	Sensitivity (n = 16)	Specificity (n = 37)	IA: *proven/ probable vs. no IA*
PCR (BALF)	7/16 (43.8%)	37/37 (100%)	7/7
PCR (whole blood)	0/16 (0%)	37/37 (100%)	0/(
GM 0.5 ODI (BALF)	6/16 (37.5%)	34/37 (91.9%)	6/9
GM 0.5 ODI (serum)	5/16 (31.3%)	37/37 (100%)	5/5
GM (BALF) 1.0 ODI	5/16 (31.3%)	35/37 (94.6%)	5/7
Culture (BALF)	3/16 (18.8%)	37/37 (100%)	3/3

Eigl et al Med Mycol. 2017 Jul 1;55(5):528-534.

FIGURE 1 Results with the new *Aspergillus*-specific lateral-flow device test ranging (from the left to the right) from negative (–) to weak positive (+), moderate positive (++) and strong positive (+++)

DOI: 10.1111/myc.12704

Hoenigl et al Mycoses 2017

In Vivo Diagnosis

Background
Serum GM
BALF GM
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Neutropenia
Conclusion

Increasing number of studies indicating that diagnostic performance of these early biomarkers/tests for IA are impacted by Antimould prophylaxis/Treatment

Aspergillus Galactomannan

Background
Serum GM
BALF GM
Other Materials
Neutropenia
Conclusion

- **Galactomannan (GM): polysaccharide component of the cell wall of *Aspergillus* spp.**
  - **released into host's circulation by growing hyphae**

- **GM EIA test positive in IA**
  - **Also positive in *Rasamsonia argillacea*, *Penicillium* spp., *Blastomyces* spp. , *Fusarium* spp. (?)**
  - **Negative in e.g. Mucorales**

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Cummings et al. Diagn Microbiol Infect Dis. 2007.
Nucci et al. Clin Microbiol Infect 2014

Galactomannan: Serum

Background
Serum GM
BALF GM
Other Materials
Neutropenia
Conclusion

- **SERUM:**

Overall sensitivity 78% (61 – 89%), overall specificity 81%

•Cut-off 0.5 ODI

Patients	Sensitivity (%, IC 95%)	Specificity (%, IC 95%)
Hematological disease	70 (62-77)	92 (90-93)
HSCT	82 (70-90)	86 (83-88)
Allogeneic transplants	84 (69-91)	87 (82-89)

Leeflang et al. Cochrane Database Syst Rev. 2008.
Maertens CID 2009
Reinwald. EJHAEM 2012

Serum Galactomannan and AF

- **Four year study:**
  - **262 hematological malignancy patients at high risk for IPA receiving Posaconazole prophylaxis**
  - **GM screening twice a week:**
    - **2972 Serum GMs, 96.7% negative**

- **5 breakthrough IPA infections, all positive serum GMs results (1.9% of all episodes)**
  - **Prophylaxis highly effective (1.9% of all episodes versus 7-20% without prophylaxis)!**
- **30 episodes with false positive GM results**

PPV GM „diagnostic driven"=suspicion of breakthrough IPA **89.6%**

PPV GM screening during ongoing mouldactive AF prophylaxis **11.8%**

NPV in both scenarios near to 100%

Duarte R et al. CID 2014

Serum Galactomannan and AF Study 2

Background
Serum GM
BALF GM
Other Materials
Neutropenia
Conclusion

- 146 evaluable episodes in pts with hematological malignancies receiving micafungin prophylaxis & GM serum Screening AND diagnostic driven GM
- 4 episodes true-positive in the context of probable breakthrough IA (incidence of breakthrough IA, 2.7%); screening performed only in one of these
- 111/146 high-risk episodes (76%) true-negative
- 31/146 (21.2%) false-positive; No false-negative episodes
- Positive predictive and negative predictive values of screening GM: **3.2% (1/31) and 100% (110/110)**
- Of diagnostic GM: **75% (3/4) and 100% (1/1)**
- **What is earlier?**

Vena A...Munoz P. Clin Microbiol Infect. 2017 doi: 10.1016/j.cmi.2017.05.006

All depends on Prevalence

Background
Serum GM
BALF GM
Other Materials
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Conclusion

- Prevalence of IA/breakthrough IA decisive for PPV

Naturally prevalence of IA under mould active prophylaxis (1-4%) is lower, than in high risk settings without mould active prophylaxis (7%-20%)

Assuming GM Sensitivity 80%, Specificity 80%

IPA Prevalence 20%, positive GM: PPV 16/32 =50%

IPA Prevalence 10%, positive GM: PPV 8/26 =31%

IPA Prevalence 5%, positive GM: PPV 4/23 = 17%

Serum GM and AF: Sensitivity?

Not receiving antifungal therapy				
No. of samples	24	28	18	24
Index cutoff value				
1.5	8 (2–30)	30 (13–52)	56 ([illegible])	33 (9–71)
1.0	8 (2–30)	36 (18–58)	72 (37–92)	42 (12–79)
0.5	17 (6–39)	61 (41–77)	89 (65–97)	50 (12–88)
Receiving antifungal therapy				
No. of samples	8	30	73	49
Index cutoff value				
1.5	13 (2–55)	10 (2–35)	23 (12–41)	10 (3–28)
1.0	13 (2–55)	30 (16–50)	30 (16–49)	12 (5–29)
0.5	13 (2–55)	57 (38–73)	52 (32–71)	20 (9–39)

Targeted/Pre-emptive Antifungal Therapy reduces Sensitivity of serum GM; Marr, CID 2005

Patient group	Week -2	Week -1	Week 0 (day of diagnosis until day +7)	Week 1 (week 2 after diagnosis)	Week 2 (week 3 after diagnosis)
	n positive / n tested (percentage)				
All patients					
Serum >0.5	3/14 (21%)	10/18 (56%)	15/19 (79%)	9/13 (69%)	5/8 (63%)
BAL >1.0	2/2	1/1		0/1	
Not receiving antifungal therapy					
Serum >0.5	0/4				
BAL >1.0	1/1				
Receiving antifungal therapy					
Serum >0.5	3/10 (33%)	10/13 (77%)	15/19 (79%)	9/13 (69%)	5/8 (63%)
BAL >1.0	1/1	1/1		0/1	

Antifungal Prophylaxis may not reduce Sensitivity of serum GM for Breakthrough Infections; Hoenigl, Mycoses 2013 Duarte CID 2014; Vena CMI 2017

Proof of concept

Background
Serum GM
BALF GM
Other Materials
Neutropenia
Conclusion

- 100 episodes with diagnostic driven serum GM strategy during Posaconazole prophylaxis
- Only 39 out of 100 episodes required serum GM testing, and only 78 serum GM tests were performed overall with this approach (i.e. 6.9% of the total number of assays performed per 100 episodes with serum GM screening despite posa prophylaxis ($P<0.001$)
- 11 serum GM tests positive in 6 episodes; all false positive.
- 2 breakthrough infections: Candida glabrata and Fusarium solanii
- Costs for serum GM testing 3,147 € in the diagnostic driven strategy versus 45,757 € in the screening strategy

Duarte R et al. Bone Marrow Transplantation (2017) 52, 326–329.

Proof of concept

Background
Serum GM
BALF GM
Other Materials
Neutropenia
Conclusion

- Duarte et al: 100 episodes with diagnostic driven serum GM strategy during Posaconazole prophylaxis
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- [illegible]
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- Costs for serum GM testing 3,147 € in the diagnostic driven strategy versus 45,757 € in the screening strategy.

Serum GM Screening can be safely removed in patients receiving posaconazole prophylaxis and replaced by a diagnostic driven GM testing strategy!

Duarte R et al. Bone Marrow Transplantation (2017) 52, 326–329.

BAL Galactomannan and AF

- Hintergrund
- Serum GM
- BALF GM
- Other Materials
- Neutropenia
- Conclusion

Table 3. Diagnostic Performance of Galactomannan Determination in the Bronchoalveolar Lavage in 530 Cases of Immunocompromised Patients with Hematologic Malignancies

	Sensitivity
As compared with EORTC/MSG	
Proven	0.50 (0.12–0.8
Proven and probable	0.49 (0.35–0.6
Proven and probable*	0.73 (0.55–0.8
Proven and probable and possible	0.35 (0.29–0.4
As compared with clinical judgment	
Receiving empirical antifungal treatment	0.42 (0.34–0.5
Suspicion of IFD on radiologic studies	0.34 (0.27–0.4

Definition of abbreviations: EORTC/MSG = European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group; IFD = invasive fungal disease; NLR = negative likelihood ratio; NPV = negative predictive value; PLR = positive likelihood ratio; PPV = positive predictive value.
*Classification disregarding serum galactomannan as a mycologic criterion for defining probable disease according to the EORTC/MSG.

Hematology Patients:

- 62% Mould active Prophylaxis/therapy at the time of bronchoscopy
- BAL GM not considered for IPA grading
- Serum GM only in 1/3 the patients
- Goldstandard: BAL Culture (?)

Afolter et al AJRCCM 2014 vs. Prattes et al AJCCM 2014

Test Methods	Sensitivity	Specifi
BAL GM > 0.5 ODI	97 (30/31)	81 (170/
BAL GM > 1.0 ODI	97 (30/31)	93 (197/
BAL GM > 3.0 ODI	61 (19/31)	99 (208/

Pulmonology Patients:

- Nobody on AF
- BAL GM considered for IPA grading

BAL Galactomannan and AF

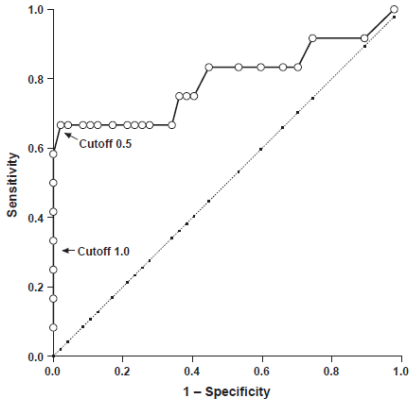

Figure 1 Receiver operating characteristic ROC analysis. ROC curve for BAL GM OD using only cases without inclusion of BAL GM for case definition purpose (n = 12, AUC 0.8). BAL, bronchoalveoloar lavage; OD, optical density.

Reinwald M eta al. EJHAEM 2012

In case of ongoing antimould treatment at time of bronchoscopy

0.5 ODI cutoff for BAL GM more sensitive and equally specific than 1.0 ODI cutoff

BAL GM:

- **Sensitivity might be reduced in case of mould active AF prophylaxis / treatment**
- **In patients receiving mould active AF 0.5 ODI cutoff preferable.**

GM cut-off 0.5:
Sensitivity without AF 95%
Sensitivity with AF 71%

GM cut-off 1.0:
Sensitivity without AF 81%
Sensitivity with AF 52%

Eigl et al. IJAA 2015

	BALF GM sensitivity (cut-off 0.5 ODI)	BALF GM sensitivity (cut-off 1.0 ODI)
Overall	87% (55/63)	71% (45/63)
Under mould active systemic antifungals	71% (15/21)	52% (11/21)
Voriconazole	90% (9/10)	70% (7/10)
Posaconazole	0% (0/4)	0% (0/4)
Caspofungin	80% (4/5)	60% (3/5)
Liposomal Amphotericin B	67% (2/3)	33% (1/3)
Without Antifungals	95% (40/42)	81% (34/42)

BAL Galactomannan

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Table 1 Galactomannan levels in native and pretreated bronchoalveolar lavage fluid samples.

	Number	Initial GM (median and range)	GM native from frozen sample (median and range)	GM pretreated from frozen sample (median and range)	*p*-Value[a]
Overall study population	15	0.73 (0.15–>16.79)	0.51 (0.08–>16.79)	0.01 (0–0.01)	0.001
Proven IPA	1	>16.79	>16.79	0.01	n.a.
Probable IPA	4	16.6 (15.53–>16.79)	>16.79 (all >16.79)	0.01 (all 0.01)	0.046
Possible IPA	1	0.73	0.51	0.01	n.a.
No IPA	9	0.19 (0.15–0.75)	0.22 (0.08–0.67)	0.01 (0–0.01)	0.008

Abbreviations: GM = galactomannan, IPA = invasive pulmonary aspergillosis, n.a. = not applicable.
[a] *p*-Values were calculated for GM native versus GM pretreated.

CAVE: BALF pretreatment with dithiothreitol (Sputasol AND Copan SLsolution; used for liquifying BALF) and N-Acetyl-L-Cystein agents reduce GM levels dramatically

SOLUTION: Liquillizer (Dithiothreitol-free and odorless) no impact on GM levels

Prattes J et al J Infect 2015
Gils S et al JCM 2016
Simon **J. Clin. Microbiol.** June 2017 55:6 1964-1965

Imperfect Diagnostics - SOLUTION

Test (combination) and condition	Sensitivity (n = 16)	Specificity (n = 37)
	IA: *proven/ probable vs.*	
PCR (BALF)	7/16 (43.8%)	37/37 (100%)
PCR (whole blood)	0/16 (0%)	37/37 (100%)
GM 0.5 ODI (BALF)	6/16 (37.5%)	34/37 (91.9%)
GM 0.5 ODI (serum)	5/16 (31.3%)	37/37 (100%)
GM (BALF) 1.0 ODI	5/16 (31.3%)	35/37 (94.6%)
Culture (BALF)	3/16 (18.8%)	37/37 (100%)
GM 1.0 ODI (BALF) and/or GM 0.5 ODI (serum)	7/16 (43.8%)	35/37 (94.6%)
PCR (BALF) and/or GM 0.5 ODI (serum)	10/16 (62.5%)	37/37 (100%)
PCR (BALF) and/or GM 1.0 ODI (BALF)	10/16 (62.5%)	35/37 (94.6%)
PCR (BALF) and/or GM 1.0 ODI (BALF) and/or GM 0.5 ODI (serum)	11/16 (68.8%)	35/37 (94.6%)
PCR (BALF) and/or GM 1.0 ODI (BALF) and/or culture (BALF) and/or GM 0.5 ODI (serum)	12/16 (75.0%)	35/37 (94.6%)

All diagnostic lab tests for IA have IMPERFECT SENSITIVITIES WHICH MAY BE FURTHER REDUCED UNDER MOULD ACTIVE AF!!

- **Blood samples more affected than BAL samples!**

SOLUTION: BIOMARKER/TEST COMBINATIONS!!

- **GM +/- PCR +/- LFD from BALF AND Blood**
  - **+/- BAL culture +/- Blood BDG**
- **Markedly increased sensitivity, specificity mosty unchanged**

REFS FOR BIOMARKER COMBINATIONS : Buchheidt, BOCH et al. CMI 2016; Hoenigl , Buchheidt et al JCM 2014; 52(6):2039-45; Eigl, Hoenigl, Boch et al. Med Mycol 2017; White et al JCM 2013; Reinwald et al Eur J Hematol 2012; Torrelli et al. JCM 2011

CSF and Urine Galactomannan

Background
Serum GM
BALF GM
Other Materials
Neutropenia
Conclusion

CSF: Future studies needed

- **CSF GM recommended in current guidelines for patients with CNS IA**
- **Based on case reports indicating promising performance**

Viscoli et al JCM 2002
Hoenigl, Cur Pharm Design 2012

Other materials: Urine

Background
Serum GM
BALF GM
Other Materials
Neutropenia
Conclusion

Advantages of biomarker detection from urine samples

- **Non-invasive collection**
- **May reduce blood drawings from (anemic) patients**
- **Large sample volume**
- **Point of care home testing???**

Current limitations

- **Low evidence for clinical use**
- **Only few biomarkers have been studied in urine samples (galactomannan, LFD, TAFC)**
- **Urine dilution as potential confounding factor**

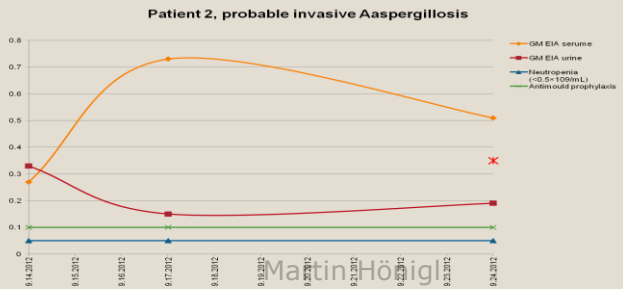
- Duettmann et al Med Mycol 2014
- Reischies et al JCM 2015
- Dufresne et al Plos ONE 2012

Urine Galactomannan

Background
Serum GM
BALF GM
Other Materials
Neutropenia
Conclusion

Urine: Future studies needed

- **GM in native urine: Significant positive correlation found between urine & serum GM results**
- **Urine GM/Urine creatinine index**
  - **takes into account urine dilution**



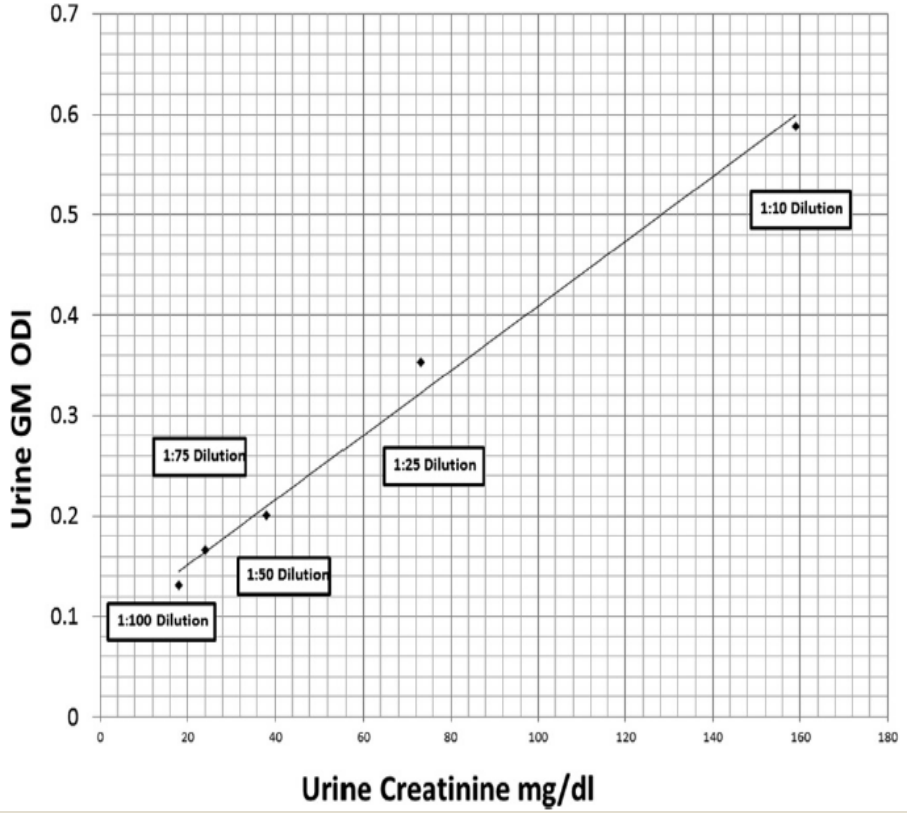
Urine GM Cut-off	Sensitivity	Specificity	PPV	NPV	DOR (95% CI)
0.2 ODI	0.19	0.937	0.222	0.924	3.48 (95% CI 1.03-11.74)
0.15 ODI	0.333	0.905	0.25	0.935	4.76 (95% CI 1.73-13.11)
0.1 ODI	0.476	0.86	0.244	0.945	5.57 (95% CI 2.18-14.22)

Duettmann et al Med Mycol 2014
Fisher et al J Paed Infect Dis Soc 2012
Reischies JCM 2016

Creatinine as a marker for dilution

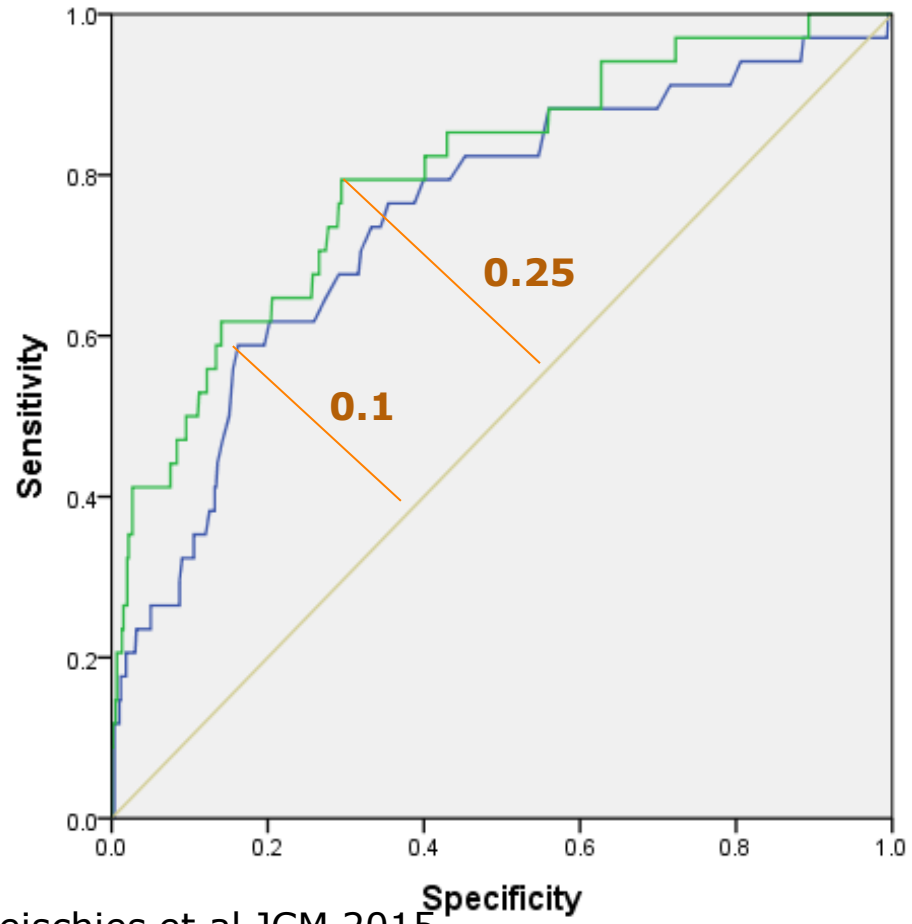
- Background
- Serum GM
- BALF GM
- Other Materials
- Neutropenia
- Conclusion

Serial dilution of urine samples: GM ODI levels reduced proportional to urine creatinine levels

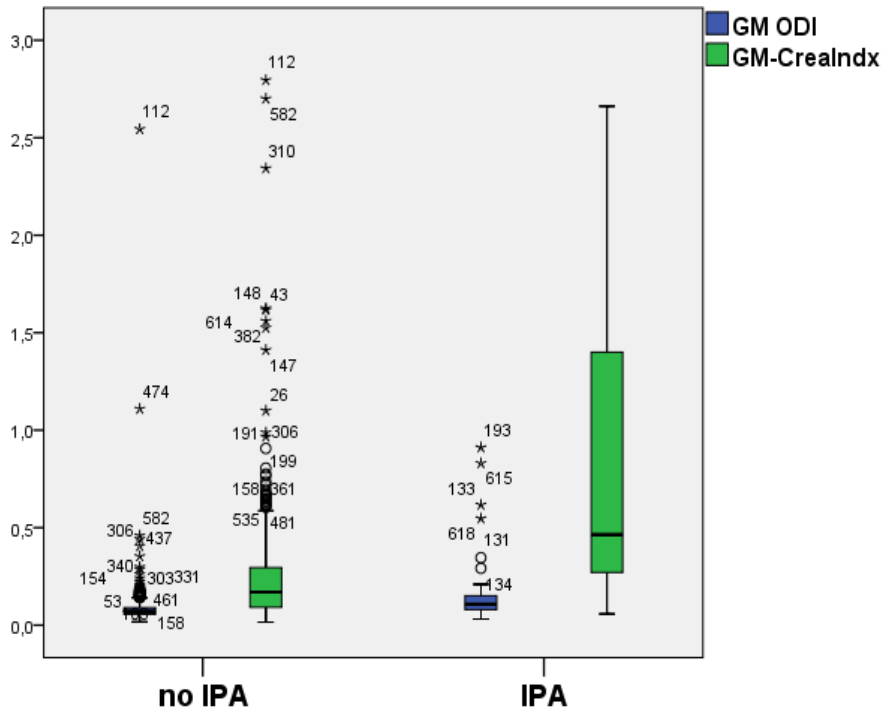


Reischies et al JCM 2016
Reischies ICAAC 2015, oral communication

GM/Creatinine Index - IPA vs no IPA



AUC
- **GM ODI 0.746**
- **GM-CreaIndx 0.801**



Reischies et al JCM 2015
Reischies ICAAC 2015, oral communication

Results

- Sensitivities, specificities, positive (PLR) and negative likelihood ratio (NLR) for TAFC/crea index for probable versus no IA

	Sensitivity	Specificity	PLR	NLR
Per Patient				
TAFC/crea (95% CI)	0.86 (0.49 – 0.97)	0.88 (0.64 – 0.97)	6.86 (1.81 – 25.96)	0.16 (0.03 – 1.01)
Per Sample				
TAFC/crea (95% CI)	0.81 (0.60 – 0.92)	0.90 (0.71 – 0.97)	8.5 (2.2 – 32.3)	0.21 (0.09 – 0.51)

Neutrophil count

Background
Serum GM
BALF GM
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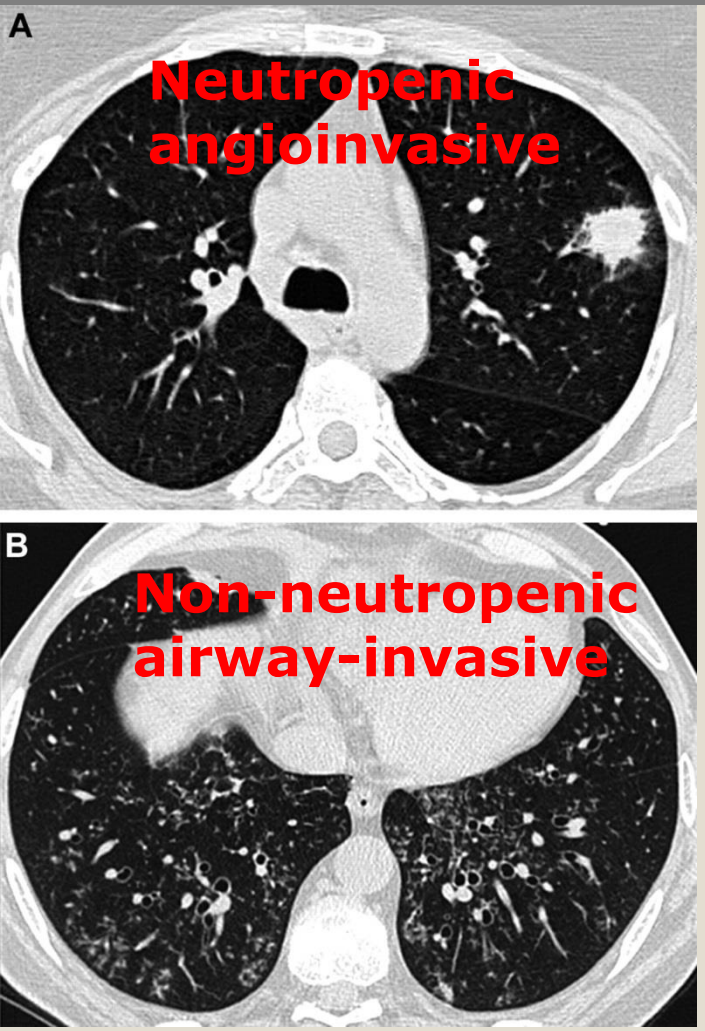
Neutropenia
Conclusion

Neutropenic Patients	Non-neutropenic Patients
Mouldactive Prophyaxis frequent	Mouldactive Prophylaxis rare (e.g., GVHD, secondary prophylaxis, lung transplant recipients...)
Angioinvasive growth of Aspergillus	Airway invasive growth of Aspergillus

Radiology versus Galactomannan (2)



IA Animal Model:

Serum

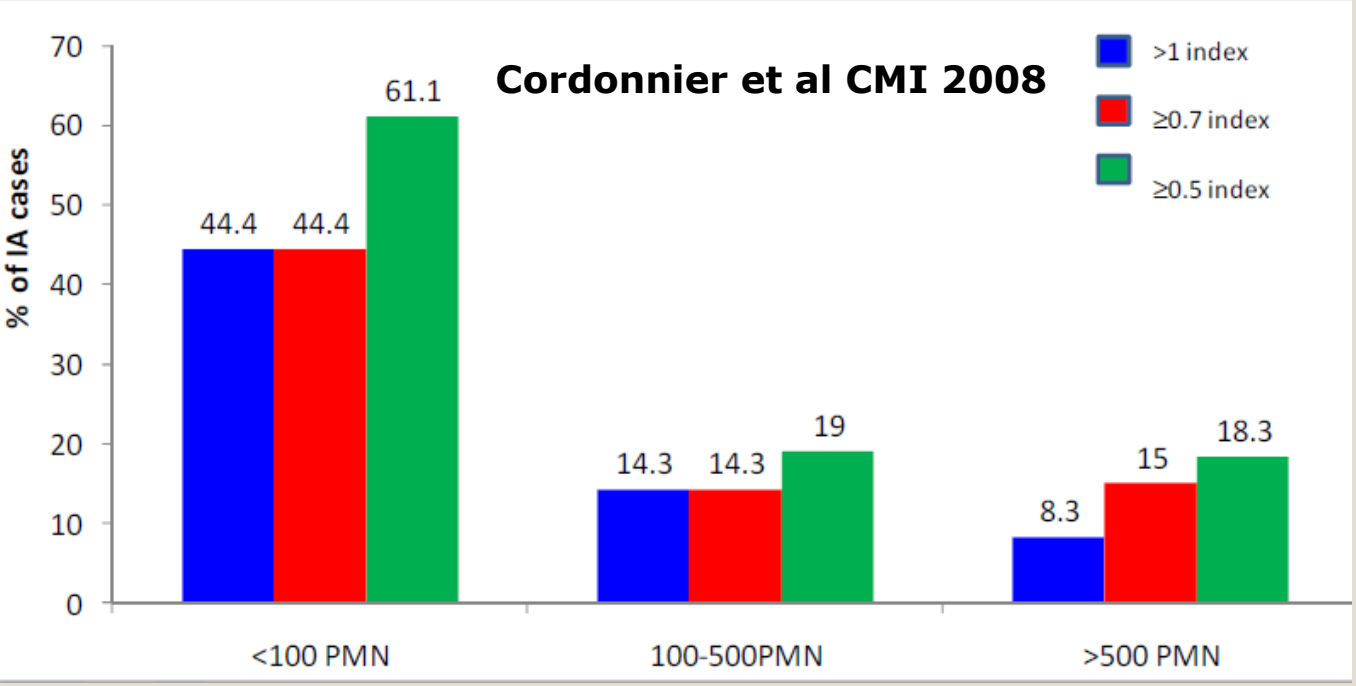
GM significantly higher in Ara-C induced Neutropenia *vs* immunosuppression with Cyclosporin/Prednislon (i.e. non-neutropenic)
Similar trend for beta-D-Glucan!

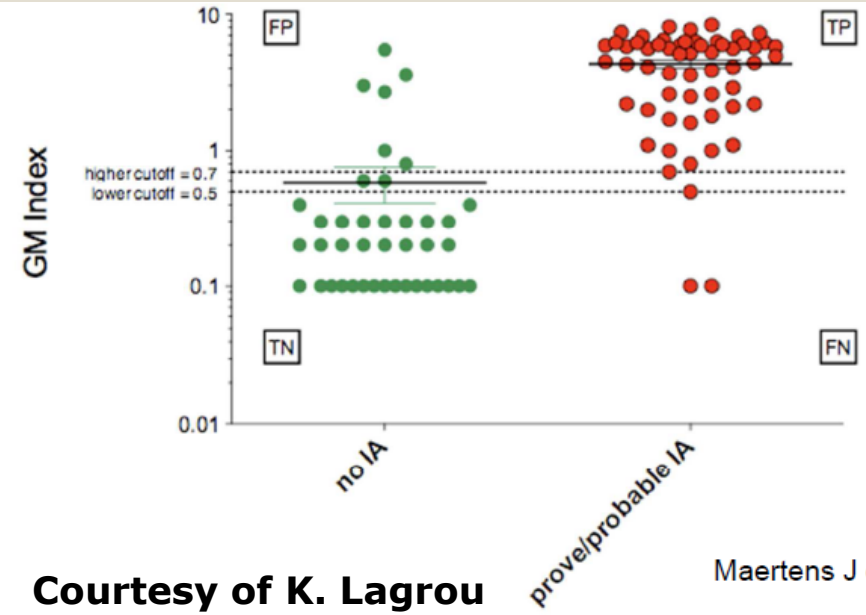
BAL

Increased in both: neutropenic and non-neutropenic; no difference

Bergeron et al.Blood 2012
Petraitiene et al Med Mycol 2014

Serum GM: Influence of Neutropenia





Jan 2005 – Sept 2008
58 proven/probable IA
41 controls

In non-neutropenic patients with invasive Aspergillosis Serum GM is less reliable!!

Bronchoscopy and BAL GM recommended!

	Neutropenic	non-Neutropenic	P-value
BAL, 1.0	100%	94.7%	0.99
Serum, 0.5	90%	36.8%	0.008

Maertens J et al., CID, 2009, 49: 1688-1693.

Courtesy of K. Lagrou

Galactomannan: When and where from?

In patients at "lower" risk for IA (e.g. COPD, solid tumours, liver cirrhosis, long time high dose corticosteroids)

GM testing in case of Clinical Suspicion

i.e. diagnostic driven GM testing

- **Preferably BAL** (mostly one time testing)
- **Additionally Serum GM (NPV??)** (at least twice)

Galactomannan: When and how often?

In patient groups at "high" risk for IA (e.g. alloSCT recipients, induction chemotherapy for AML)

Depends on whether patient is receiving mould active AF prophylaxis/treatment

Galactomannan: When and how often?

In patient groups at "high" risk for IA (e.g. alloSCT recipients, induction chemotherapy for AML, Lung TX)

If ongoing mould active prophylaxis / empirical therapy

- **Diagnostic Driven GM testing for breakthrough infection**

If no mould active prophylaxis / empirical therapy

- **GM screening 2-3 times a week**

Galactomannan: Treatment Startification

Background
Serum GM
BALF GM
Other Materials
Neutropenia
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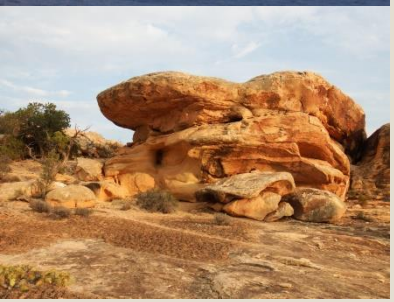
Serum GM for Treatment Stratification and Outcome prediction in those with preemptive/targeted AF Treatment:

Once serum GM is positive may test 2-3 times a week until GM is negative

Seeber K et al. Usefulness of the Serum Galactomannan Assay for Early Response Assessment and Treatment Stratifications of Invasive Aspergillosis Article in Current Fungal Infection Reports 6(3) · September 2012

Multiple studies Anaissie E AND Nucci M

Take home messages



GM important tool for diagnosis of breakthrough IA in neutropenic patients with AF prophylaxis

- **AF prophylaxis: diagnostic driven serum GM testing superior**
- **BAL (GM, LFD and PCR) or CT guided puncture whenever possible (discriminate from Mucorales etc.)**

BIOMARKER COMBINATIONS in case of AF treatment!!

Non-neutropenic patients at risk for IA

- **Blood Biomarkers – in particular serum GM – lower sensitivity**
- **BAL GM preferred**

Urine GM and other biomarkers: bigger role in the (near) future

Thank You For Your Attention!