MMTN
MEDICAL MYCOLOGY TRAINING NETWORK

How do I interpret ...
Serum galactomannan?

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ASIA FUNGAL WORKING GROUP
an ISHAM working group

HOW DO I INTERPRET

Galactommannan

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Galactomannan

- Long search circulating aspergillus antigens
- "extracellular galactomannan" discovered[#]
- EB-A2 monoclonal ab used to show presence of these ag
- EB-A2 ab detects galactofuran epitopes of "GM" molecule
- Several other glycoproteins, & lipopeptidoGM secreted by Aspergillus contain galactofuran epitope
- "GM" kit (ELISA) detects what are best called galactofuranose antigens

[#]Latge JP et al. Infect Immun 1994;62:5424

GM - early papers promising

French hospital – paediatric Haematology unit (347 pts), adult BMT unit (450 pts), serum GM 2x/week

53 pts with confirmed or probable IA; 48 + GM

Sensitivity = 90.6%

GM antigenemia preceded radiologic signs in 65% of the 48 pts

GM preceded ***radiologic signs by a mean of -8.4 days,*** and ***preceded clinical symptoms by a mean of -6 days***

Specificity = 94%

Sulahian A et al. Cancer 2001;91:311

Early use of GM, 2001

Analysis A1 – antemortem only	Analysis A2 – incl postmortem	Analysis B – incl post-mortem but
Proven: 7 true + No suggestion of IA & proven non-IA: true – Sensitivity: 100% Specificity: 99.1% PPV: 77.7% NPV: 100%	Proven: 30 true + No suggestion of IA & proven non-IA: true – Sensitivity: 100% Specificity: 98.1% PPV: 85.7% NPV: 100%	Proven and probable: assumed to be true + All others: assumed to be true – Sensitivity: 89.7% Specificity: 98.1% PPV: 87.5% NPV: 98.4%

Maertens J *et al*. Blood 2001;97:1604

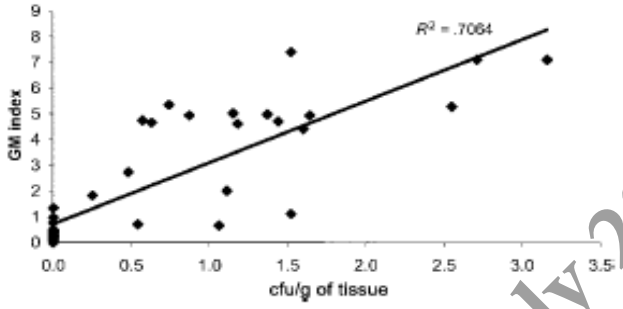
Early use of GM – Leuven - GM details

- GM + preceded development of pulmonary infiltrates in 19 of 28 evaluable patients (median 5d, range 1 – 27)
- No transient GM positivity among proven cases
- 24 patients had persistent or rising titres of GM – all died with or of IA
- 6 patient cleared GM – 4 survived*

* The other 2: died of fatal hemoptysis, bacterial pneumonia

Maertens J *et al*. Blood 2001;97:1604

GM and fungal burden



Correlation between GM and fungal burden in rabbits infected with 10^8 *A. fumigatus*

x axis: pulmonary fungal burden (quantitative culture of homogenized lung tissue)

y axis: GM level day before sacrifice

Marr KA *et al*. J Infect Dis 2004;190:641

Summary of GM data

Ref	Sens	Spec	Comments
Kawamura ('99)	100%	100%	Inadeq descrp of criteria, pt type; cut-off 1
Ulusakarya ('00)	80%; 50%*	96%	Prospec; FN; cut-off 1.5; consec pos
Salonen ('00)	100%* Susp 67%		Prospec; Haem & HSCT;cut-off 1.5
Sulahian ('01)	91%	94%	Prospec; Haem & HSCT (paed); cut-off 1.5; consec pos
Fortun ('00)	56%	94%	Retrospec; Li Tx; Cut-off 1; consec pos
Kami ('01)	58%	97%	Retrospec/prospec; Haem; cut-off 1.5

GM – just not good enough

Meta-analysis data

Haem Malignancy
- Sensitivity 58
- Specificity 95%

HSCT
- Sensitivity 65%
- Specificity 65%

Solid Organ Tx
- Sensitivity 41%
- Specificity 85%

Pfeiffer CD et al. Clin Infect Dis 2006;42:1417

Problems with GM

Inherent to the substance

Aspergillus ubiquitous in environment

Aspergillus may exist as commensals in pulm, GI tract

→ Absorbed antigens may circulate in blood with no actual invasive disease

GM positivity not the same as a positive HIV serology!!

GM positivity not necessarily indicative of infection; gives info on probability of disease

Problems with the GM studies

Insensitivity of current diagnostic standards

Negative TTNA → placement of patient in "control" group (yet neg TTNAs and + autopsies are well known!)

Many are retrospective reviews of their data: but they had used GM clinically to guide treatment! If anti-fungal therapy had aborted the infection – in "final analysis", the + result might have been re-classified as a false +!

Marr KA. Clin Infect Dis 2005;41(Suppl 6):S381

GM (ELISA) cross-reacts with fungal exo-antigens

Fungi taken out of storage, re-cultured.

Fixed biomass looped out, centrifuged, supernatant run on ELISA

	Reactivity wrt *A. fumigatus*
A. fumigatus	100
A. flavus	107
A. terreus	122
Paecilomyces variotii	106
Penicillium chrysogenum	128
Penicillium digitatum	107
Alernaria spp	39

Swanink CMA *et al*. J Clin Micro 1997;35:257

Interpretation of serum GM

Who's the host?
Neutropenic?
Other immunocompromised?

How is it being used?
Diagnostic tool? (like HIV serology)
Screening tool?
Monitoring tool?

Are there other diagnostic tools?
CT chest?
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Could this be a false-positive?

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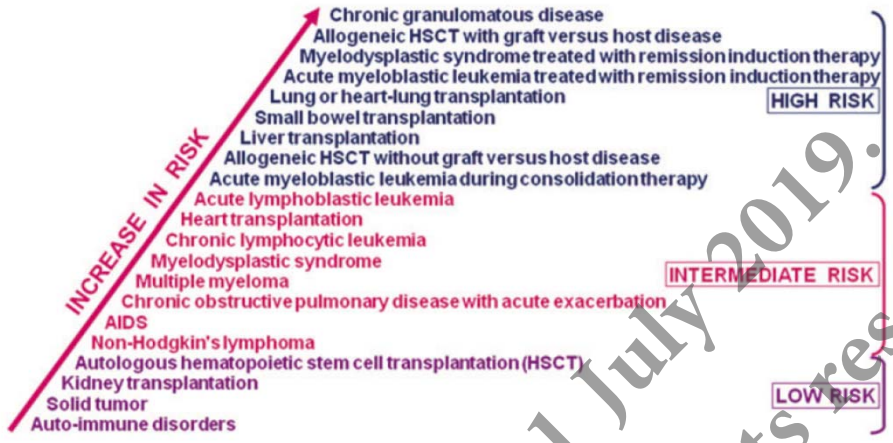

Figure 3. Risk of invasive aspergillosis based on the primary host factor.

Herbrecht R et al. Ann NY Acad Sci 2012;1272:23

The *Asp*ICU diagnostic criteria – "PUTATIVE"

❖ **Lower respiratory tract culture with Aspergillus** – ***entry criterion***

➢ **Compatible signs/symptoms** – (any 1 of 7)
- Fever refractory to at least 3 days of appr abx
- Fever recrudescent after 48hr afebrile hr, while still on abx, with no app cause
- Dyspnoea
- Hemoptysis
- Pleuritic chest pain
- Pleural rub
- Worsening resp status while on vent support, despite appr abx

➢ **Abnormal CXR or CT chest**

➢ **Either or both**
- Host (neutropenia, malig treated with chemo, steroids, congen/acqd immunodef)
- BAL with Aspergillus (+ or ++) with no bacterial growth AND hyphae seen on cytological smear

Taccone FS et al. Crit Care 2015;19:7

TABLE 1 Definitions of invasive pulmonary aspergillosis (IPA) in chronic obstructive pulmonary disease (COPD) patients

Proven IPA	Histopathological or cytopathological examination, from needle aspiration or biopsy specimen obtained from any pulmonary lesion present for <3 months, showing hyphae consistent with Aspergillus and evidence of associated tissue damage, if accompanied by any one of the following: 1) Positive culture of Aspergillus spp. from any LRT sample. 2) Positive serum antibody/antigen test for *A. fumigatus* (including precipitins). 3) Confirmation that the hyphae observed are those of Aspergillus by a direct molecular, immunological method and/or culture.
Probable IPA	As for proven IPA but without confirmation that Aspergillus is responsible (points 1, 2 and 3 are not present or tested). OR COPD patient, usually treated with steroids and severe according to GOLD (stage III or IV), with recent exacerbation of dyspnoea[#], suggestive chest imaging[¶] (radiograph or CT scan; <3 months[+]) and one of the following: 1) Positive culture[§] and/or microscopy for Aspergillus from LRT. 2) Positive serum antibody test for *A. fumigatus* (including precipitins). 3) Two consecutive positive serum galactomannan tests.
Possible IPA	COPD patient, usually treated by steroids and severe according to GOLD (stage III or IV), with recent exacerbation of dyspnoea[#], suggestive chest imaging[¶] (radiograph or CT scan; <3 months[+]), but without positive Aspergillus culture or microscopy from LRT or serology.
Colonisation	COPD patient with positive Aspergillus culture from LRT *without* exacerbation of dyspnoea, bronchospasm or new pulmonary infiltrate.

Data from references [23] and [38]. LRT: lower respiratory tract; *A. fumigatus*: *Aspergillus fumigatus*; GOLD: Global Initiative for Chronic Obstructive Lung Disease; CT: computed tomography. [#]: Exacerbation of dyspnoea and/or bronchospasm resistant to appropriate treatment including antibiotics; [¶]: pulmonary lesion(s) unresponsive to appropriate antibiotics (refers to dose, route, spectrum and activity against cultured bacteria); [+]: pulmonary lesions, especially cavitary, present for >3 months are better classified as chronic pulmonary aspergillosis (see text), unless direct tissue invasion is demonstrated; [§]: standard or sabouraud culture, or molecular detection test when licensed.

Bulpa P et al. Eur Resp J 2007;30:782

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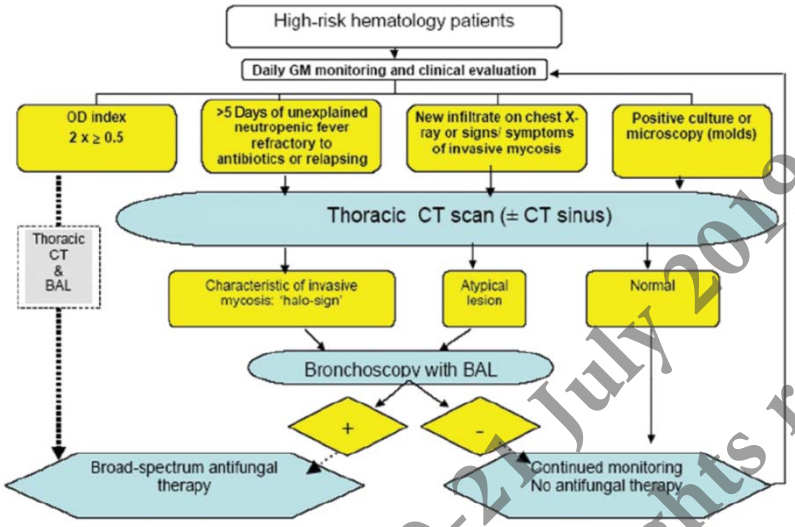
Combining GM with CT?



Maertens J *et al*. Clin Infect 2005;41:1242

Galactomannan and PCR versus culture and histology for directing use of antifungal treatment for invasive aspergillosis in high-risk haematology patients: a randomised controlled trial

C Orla Morrissey, Sharon C-A Chen, Tania C Sorrell, Samuel Milliken, Peter G Bardy, Kenneth F Bradstock, Jeffrey Szer, Catriona L Halliday,

Lancet Infect Dis 2013;
13: 519–28

Australian RCT – biomarker based strategy *vs* standard strategy

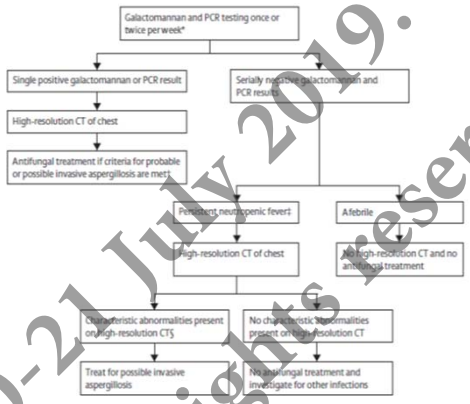
STANDARD

Fever 3 – 5 days despite broad-spectrum abx

✓Cultures of blood, urine, ± sputum, ± faeces, & HRCT thorax

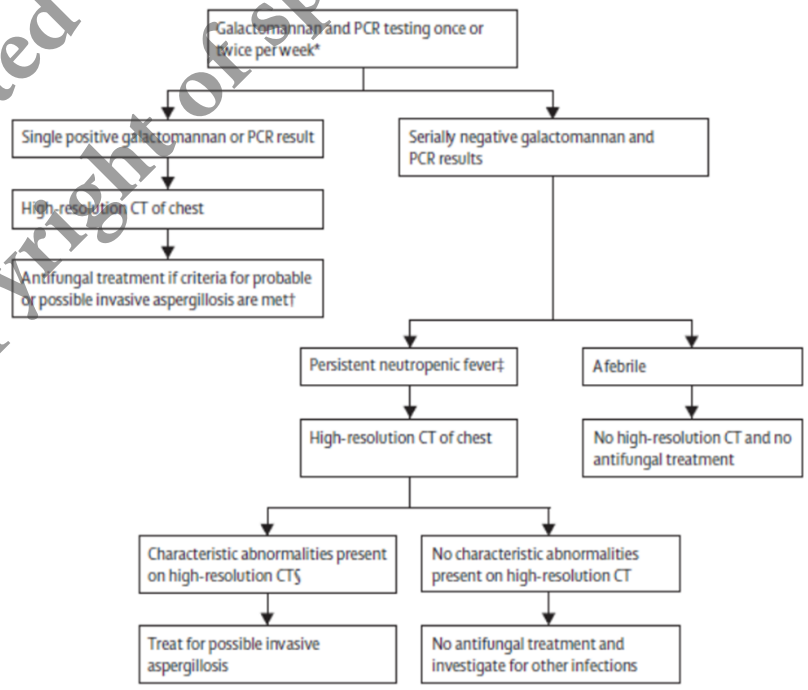
✓Empirical anti-fungals recommended while awaiting results

BIOMARKER



Prophylaxis – a/c to centre's usual practice

Morissey CO et al. Lancet Infect Dis 2013;13:519



Australian RCT – biomarker based strategy *vs* standard strategy

	Standard (n=122)	**Biomarker** (n=118)	***p***
Received AFT	32%	15%	0.002
Probable IA	0	14%	0.0001
Mortality	15%	10%	0.31
IA-related mort	5%	3%	0.3
Hepatotox	17%	10%	0.11
Nephrotox	43%	51%	0.20

No patient with serially neg GM & PCR had IA diagnosed by std c/s + histo

Morissey CO et al. Lancet Infect Dis 2013;13:519

Meta-analysis – combine with PCR?

Study inclusion criteria:

Involves patients at high-risk

Use both GM and PCR in diagnostic/screening strategy

Use EORTC/MSG criteria (2002/2008)

	Sensi %	Spec %	PPV %	NPV %
PCR	84	76	38	96
2 PCRs	57	93	59	92
GM	92	90	61	98
2 GMs	62	95	67	93
GM or PCR	99	64	33	10
GM & PCR	68	98	88	95

Arvanitis M et al. Clin Infect Dis 2015;61:1263

Let's look closely at the figures …

	Sensi %	Spec %	PPV %	NPV %
PCR	84	76	38	96
2 PCRs	57	93	59	92
GM	**92**	90	**61**	98
2 GMs	62	95	67	93
GM or PCR	99	64	33	10
GM **&** PCR	**68**	98	**88**	95

Arvanitis M et al. Clin Infect Dis 2015;61:1263

Combining GM & PCR – meta-analysis results

	PLR	NLR	DOR	AUROC
PCR	3.5	0.21	17	0.87
2 PCRs	8.4	0.46	18	0.87
GM	**9.3**	0.09	104	0.96
2 GMs	**12.1**	0.4	30	0.94
GM or PCR	2.8	0.02	128	0.99
GM & PCR	43.2	0.32	135	0.93

PLR – positive likelihood ratio (this result is __x more likely to happen in a patient with the condition than in a patient without
DOR – diagnostic odds ratio – ratio of odds being + if patient has the disease to odds being + if patient doesn't have the disease
AUROC – area under receiver operating characteristics curve

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Arvanitis M et al. Clin Infect Dis 2015;61:1263

Real-time PCR on the first galactomannan-positive serum sample for diagnosing invasive aspergillosis in liver transplant recipients

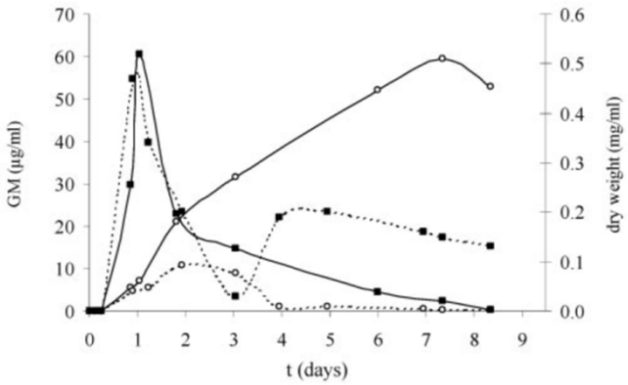
By final classification, PCR on 1st serum sample with + GM had

- ✓ 62% sensitivity
- ✓ 100% specificity
- ✓ 100% PPV
- ✓ 71% NPV for IA in Li Tx recipients

RR of prob/poss IA of a serum GM + was 3.4x higher if the PCR was also positive

Botterel F et al. Transpl Infect Dis 2008;10:333

GM, DNA released at different stages of growth of Aspergillus



Black square – Aspergillus mass; Open circle – GM
Solid vs dotted lines – different pH

hr	GM	Aspergillus DNA
16	3.70	Neg
24	4.85	Neg
48	5.89	Neg/Pos
168	0.01	Pos

- ✓ GM released during logarithmic phase, when glucose abundant
- ✓ When nutrients dry up, mycelium breaks down – GM continues to be released
- ✓ DNA also released after mycelial breakdown, but not always detectable
- ✓ In vivo, breakdown maybe related to cellular defenses

Menink-Kirsten MASH et al. J Clin Microbiol 2006;44:1711

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GM screening not useful in posaconazole prophylaxis

- 4-year study (Barcelona), AML (161 episodes of chemo), allo-HSCT (up to D100), allo-HSCT with GVH
- Twice-weekly screening in afebrile
- Part of diagnostic driven approach if febrile (incl CT thorax, BAL if pulm infiltrate)
- LamB or Caspofungin at clinician's discretion

Duarte RF et al. Clin Infect Dis 2014;59:1696

GM screening not useful in posaconazole prophylaxis

- 188 episodes (71.7%) were 'all-negative" GM episodes (no IA or IFI was diagnosed; 6 deaths non IFI related but no autopsies)
- 30 episodes (11.4%) were false-positive episodes
- 26 of these 30 FP episodes were surveillance results
- 5 (1.9%) true positive episodes

PPV of screening GM: 11.9%
PPV of diagnostic-driven GM: 86.7%

Duarte RF et al. Clin Infect Dis 2014;59:1696

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May be useful at high cut-off

Over 41 mths, followed non-haematological patients with Aspergillus grown from resp sample

Used EORTC 2002 criteria (good!)

- 10 patients (out of 75) were "proven" or "probable"
- Varied underlying conditions (steroids/li tx/COPD/solid-organ malignancy)

	Sensi	Spec	PPV	NPV
GM >0.5	60.0	89.2	46.1	93.6
GM .1	50.0	100	100	92.8

Guinea J et al. Med Mycol 2008;46:575

Persistently + GM bad

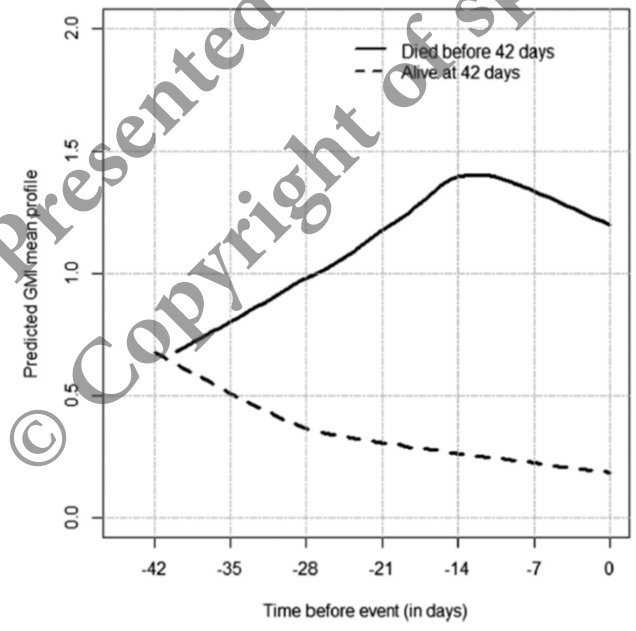
Haematology unit, Leuven; regular GM testing; rv of 70 patients (mortality 42%)

Response at Day 42	GMI Success	GMI Failure	Total
Favorable response	17	1	18
Unfavorable response	6	41	47
Total	23	42	65

GMI success – persistently neg GMI after 1st neg, in absence of new PUL/extrapulm lesions of IA
GMI failure – persistently + GMI

K = 0.8857 at 12 weeks

Maertens J et al. Cancer 2009;115:355



Data from SECURE study

Patients in mITT analysis with > 1 GM level measured by D7

Every unit ↑ in GM from baseline to D7 increased the likelihood of death by a factor of 3 (HR 3.008, 95CI1.99 – 4.54)

Every unit ↑ in GM from baseline to D14 increased the likelihood of death by a factor of 1.7 (HR 1.69 95CI 1.37 – 2.09)

Factors that models can't account for – persistent neutropenia, concomitant steroids, concomitant CMV, ongoing GVH …

Kovanda LL et al. Clin Infect Dis 2017;64:1557

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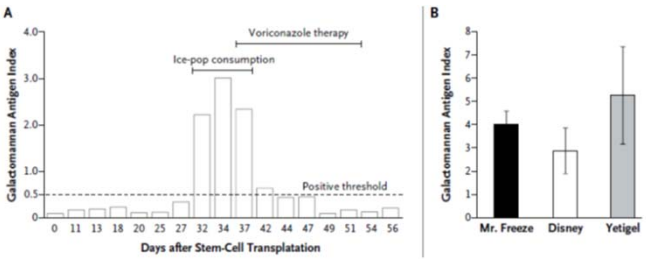
False-Positive Results by the Platelia *Aspergillus* Galactomannan Antigen Test for Patients Treated with Amoxicillin-Clavulanate▽

F. Zandijk,[1] A. Mewis,[2] K. Magerman,[2] and R. Cartuyvels[2]*

Clin Vacc Immunol 2008;15:1132

False Positive Galactomannan Test after Ice-Pop Ingestion



Gigue N et al. NEJM 2013;369:97

Case report: Enteral nutritional supplement as a likely cause of false-positive galactomannan testing☆

Tong-Yong Ng[a,*], Mei-Ling Kang[b], Ban-Hock Tan[b], Cecilia Cheng-Lai Ngan[c]

Med Mycol Case Rep 2014;3:11

Pip-Tazo: cause of false + GM

In Feb 03, changed from Ceftaz/Amik to Pip-Tazo/Amik

Pip-Tazo recipients with 2 GM+ 74%

Non Pip-Tazo recipients with 2 GM+ 11%

Multi-variate analysis: underlying disease & receipt of Pip-Tazo stat sig ($p<0.001$)

30 vials of Pip-Tazo from 15 batches subjected to GM: 12 batches + (GM 1.3 – 5.7)

Viscoli C *et al*. Clin Infect Dis 2004;38:913

Journal of Antimicrobial Chemotherapy Advance Access published April 11, 2012
J Antimicrob Chemother
doi:10.1093/jac/dks111
Journal of Antimicrobial Chemotherapy

Piperacillin/tazobactam (Tazocin™) seems to be no longer responsible for false-positive results of the galactomannan assay

M. Mikulska[1]*, E. Furfaro[1], V. Del Bono[1], A. M. Raiola[2], S. Ratto[1], A. Bacigalupo[2] and C. Viscoli[1]

Not on PipTazo: 25/1606 + for GM (1.6%)

On PipTazo: 10/394 + for GM (2.5%) [p = 0.18]

GMI of those on PipTazo was higher than those not on PipTazo (0.141 vs 0.122, p = 0.001)

All 90 randomly selected vials of GM negative for GM

Kinetics of false-positive GM

40 patients (haem malig) undergoing 42 episodes of β-lactam treatment

None with clinical or radiological suspicion of IA

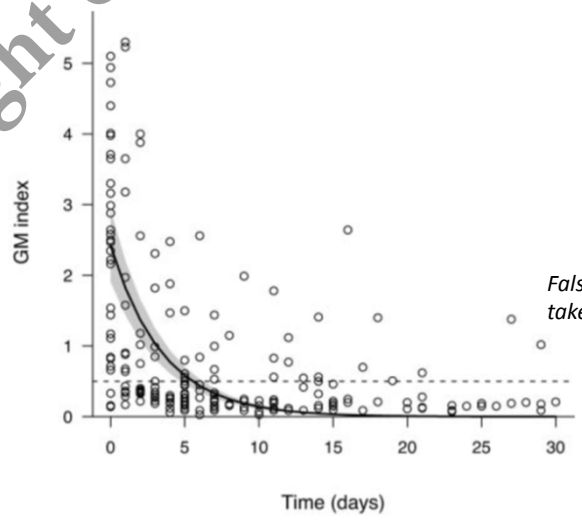
Time of test	No. of samples tested	No. (%) of samples with positive GM index[a]
Pretreatment	42	5 (12)*
Day 1	6	1 (17)
Day 2	12	9 (75)
Day 3	20	18 (90)
Overall	42	42 (100)[b]

[a] A GM index of ≥0.5 was considered positive.
[b] $P < 0.0001$ as compared to before treatment.

* These 5 were one-offs

Aubry A et al. J Clin Microbiol 2006;44:389

Follow-up in month after cessation of β-lactam treatment



False-positives secondary to β-lactams take time to decay

Aubry A et al. J Clin Microbiol 2006;44:389

False-positive *Aspergillus* galactomannan assay in solid organ transplant recipients with histoplasmosis

Vergidis P et al. Transpl Infect Dis 2012;14:213

Tends to occur with high levels of Histoplasma ag (>39)

Short Report: Serum *Aspergillus* Galactomannan for the Management of Disseminated Histoplasmosis in AIDS

Riviere S et al. Am J Trop Med Hyg 2012;87:303

Cross-Reactivity of *Fusarium* spp. in the *Aspergillus* Galactomannan Enzyme-Linked Immunosorbent Assay

Tortorano AM et al. JCM 2012;50:1051

Out of 11 pts with haem malig with Fusariosis, 9 were + for serum GM (0.5 – 7.7)

Isolates from 7 patients and from their collection – grown, centrifuged – supernatant tested + for GM

Evaluation of quantitative real-time PCR and Platelia galactomannan assays for the diagnosis of disseminated *Talaromyces marneffei* infection

Li X et al. Med Mycol 2019

In-house PCR: Sensi 100% (fungemic), 69% (non-fungemic)
GM sensi 81% (fungemic), 63% (non-fungemic)

Multiple myeloma as a major cause of false-positive galactomannan tests in adult patients with cancer

Samsung Medical Centre, over 4 mths

False-positive definition

- GM OD >0.5
- Did not fulfil EORTC 2008 criteria
- Did not require anti-fungals in mth after + result

Out of 30 false-positive cases, multiple myeloma observed 4x more often (20%) than in negative group (p=0.003)

Put PipTaz into multivariable analysis, mm turned out to be only factor independently assoc with false-pos GM

Reviewed an additional 173 mm pts over a 3-yr period: 25.5% had a false-pos GM

Only 6.3% of mm pts met EORTC "host" criterion

Ko JH et al. J Infect 2016;72:233

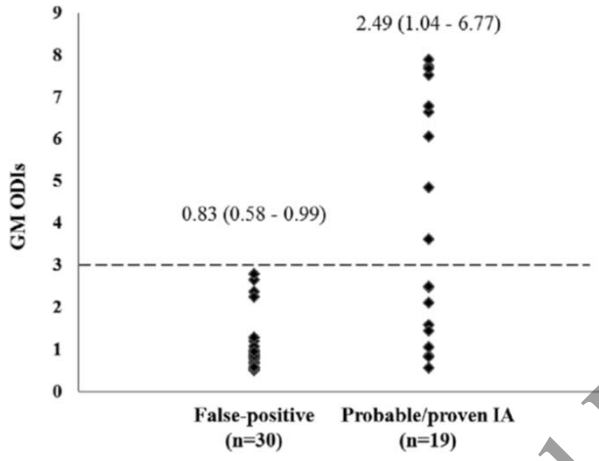
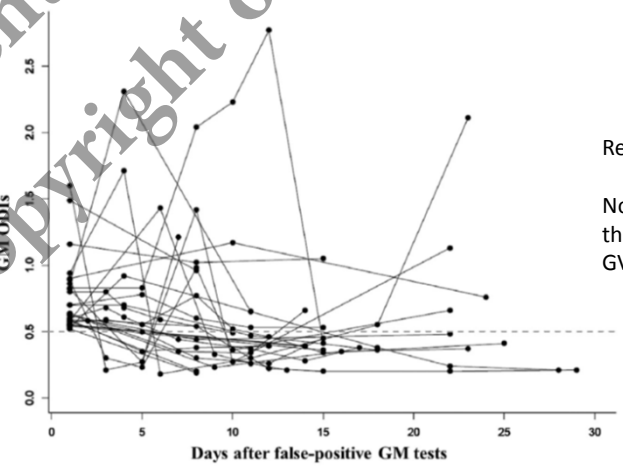


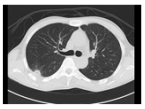
Figure 2 GM ODIs of false-positive and probable/proven IA cases. The median (IQR) ODI for each group is displayed above. None of the false-positive cases had a GM ODI higher than 3 in

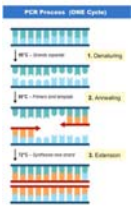
Ko JH et al. J Infect 2016;72:233



Repeats tend to be neg

No false-pos was >3 in this study, but none had GVH





Ko JH et al. J Infect 2016;72:233

Use the GM wisely

Develop departmental policy

You can use GM to screen febrile neutropenics if they're not on mold-active prophylaxis

If using GM as diagnostic tool

- Send > 1 sample
- Beware of causes of false-positive
- Look at other circumstances (host, CT)
- Consider combining with PCR