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Optimised Microbiological Diagnostics

Axel Hamprecht

Institute for Medical Microbiology, Immunology and Hygiene
University Hospital Cologne

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Content

- Consequences of missing diagnosis
- Invasive vs. non-invasive tests
- Value of BAL
- Performance characteristics

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LI in neutropenic patients with fever

- 13-60% of patients with neutropenia >10 days develop lung infiltrates (LI)
- Treatment mostly based on clinical/radiological signs
- No pathogen detected in majority of cases
  - LI of non-infectious origin (hemorrhage, toxicity, leukemic infiltration, edema etc.)
  - LI of infectious origin

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Consequences

- Often pre-emptive therapy
- Broad spectrum AB ±antifungal

Megapenem + Gigazolid + Ultrafungin

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What are we missing?

- Uncommon pathogen
- Multidrug-resistant pathogen
- Initiate appropriate therapy
- Stop inappropriate therapy
- Duration/intensity of therapy

→ Correct microbiological diagnosis is important

C. bertholletiae

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Diagnostics of LI

Annals of Oncology 26: 21–33, 2015
doi:10.1093/annonc/mdu192
Published online 15 May 2014

Diagnosis and antimicrobial therapy of lung infiltrates in febrile neutropenic patients (allogeneic SCT excluded): updated guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Medical Oncology (DGHO)†

G. Maschmeyer[1*], J. Carratalà[2], D. Buchheidt[3], A. Hamprecht[4], C. P. Heussel[5], C. Kahl[6], J. Lorenz[7], S. Neumann[8], C. Rieger[9], M. Ruhnke[10], H. Salwender[11], M. Schmidt-Hieber[12] & E. Azoulay[13]

[1]*Department of Hematology, Oncology and Palliative Care, Klinikum Ernst von Bergmann, Potsdam, Germany;* [2]*Department of Infectious Diseases, Bellvitge University Hospital, University of Barcelona, Barcelona, Spain;* [3]*Department of Hematology and Oncology, Mannheim University Hospital, Mannheim;* [4]*Institution for Medical Microbiology, Immunology and Hygiene, University Hospital Cologne, Cologne;* [5]*Department of Diagnostic and Interventional Radiology with Nuclear Medicine, Thoraxklinik, University Hospital, Heidelberg;* [6]*Department of Hematology and Oncology, Klinikum Magdeburg, Magdeburg;* [7]*Department of Pneumology, Infectious Diseases, Sleep Medicine and Intensive Care, Klinikum Lüdenscheid, Lüdenscheid;* [8]*Medical Oncology, AMO MVZ, Wolfsburg;* [9]*Department of Medicine III, University Hospital Großhadern, München;* [10]*Department of Medical Oncology and Hematology, Charité University Medicine Campus Mitte, Berlin;* [11]*Department of Hematology, Oncology, Stem Cell Transplantation, Asklepios Klinik Altona, Hamburg;* [12]*Department of Hematology, Oncology and Tumor Immunology, Helios-Klinikum Berlin-Buch, Berlin, Germany;* [13]*AP-HP, Hopital Saint-Louis, Service de Réanimation Médicale, Université Paris-Diderot, Sorbonne Paris-Cité, Faculté de Médecine, Paris, France*

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”Non-invasive“ techniques

- Blood culture
- Sputum, induced sputum
- Galactomannan (serum)
- Beta 1,3 D-glucan (serum)
- Antigen *L. pneumophila, S. pneumoniae* (urine)
- …

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Blood cultures

Pathogens isolated in patients with LI

- *S. pneumoniae, S. viridans, S. aureus*
- Enterobacteriaceae*, P. aeruginosa, H. influenzae*

- MALDI-TOF directly from positive BC bottles

Bruker Daltonics

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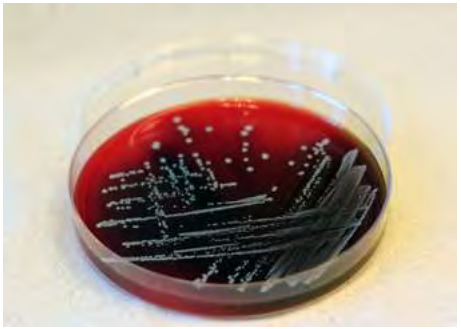
How good are BC in patients with LI?

- Mostly negative; often contaminants (CNS)
- BC were diagnostic in 25/219 patients [11.4%][1]



[1] Azoulay *et al*., Am J Respir Crit Care Med 2010, 182; 1038–1046

Online Lecture Library

Slide withheld at request of author

Sputum examination

Analyze for

- Bacteria, mycobacteria, fungi

Value of sputum?

- Diagnostic in 41/219 [18.7%] patients [1]
- Sputum↘ in neutropenic patients

[1] Azoulay *et al*., Am J Respir Crit Care Med 2010, 182; 1038–1046

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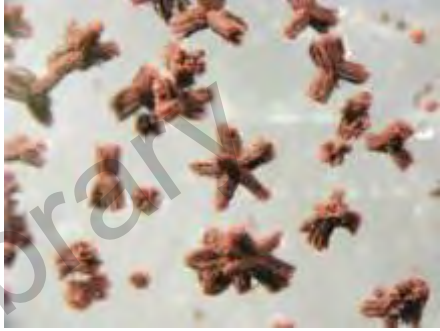
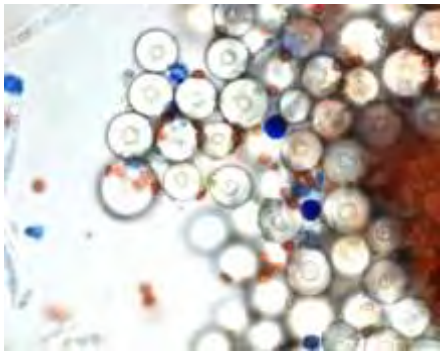
Galactomannan (serum)

- GM part of cell wall of Aspergillus
- Sensitivity of GM for IA depends on patient population[1]
  - 70% hematological patients
  - 82% BMT
  - 22% SOT
- Many reasons for false positives

[1]Pfeiffer *et al.*, CID 2006; 42, p 1417-1727





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GM and antifungal prophylaxis

- Antifungal prophylaxis/therapy
  → false-negatives
- False positives may outnumber true-positives in prophylaxis setting (posaconazole), when GM is used for screening [1]
- When used upon clinical suspicion in this setting, PPV 89.6%

[1] Duarte *et al.*, Clin Infect Dis. (2014) 59 (12): 1696-1702

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1,3 β-D-glucan

pbs.org

- part of the cell wall of most fungi
- *Candida, Aspergillus, Fusarium, P. jiroveci, H. capsulatum* can be detected
- *Cryptococcus* spp., Mucorales & many others → no detection by assay
- Sensitivity for PCP ~95%
- In hematology patients sensitivity 61.5%, specificity 90.8%, PPV 46.1%, NPV 97.1

Lamoth *et. al.*, CID 2012; 54(5):633-43

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Are non-invasive techniques enough?

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Advantages of BAL

- Focus of infection
- Recover difficult pathogens (moulds, especially mucormycetes)
- Variety of tests can be done from BAL fluid
- 30-50% therapy changes based on BAL findings

BAL- major issues

- BAL is a safe procedure in hematology pat.
- Standardised protocol recommended
- Diagnostic yield 25-81%; depends on
  - Risk profile of patients/population
  - Previous therapy
  - Analyses performed

Kahn *et al.*, JCM 1988 26; 1150-1155
Joos *et al.*, Respir Med. 2007; 101(1):93-7
Kim *et al.*, Ann Hematol. 2015 Jan;94(1):153-9

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BAL – major issues

- Early vs. late bronchoscopy
  - 2.5 fold higher within first 4 days[1]
  - Highest yield within 24h after presentation
- Radiological presentation
  - consolidated, ground-glass, or tree-in-bud infiltrates higher yield[2]

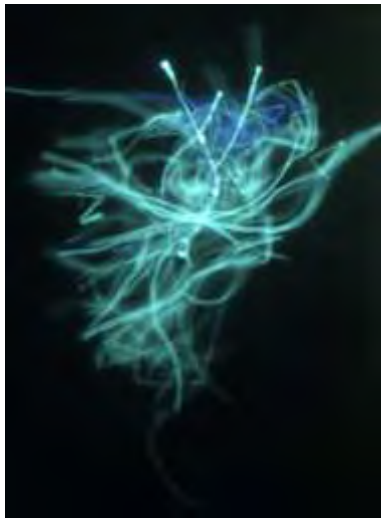
[1]Shannon *et al.*, Bone Marrow Transplant. 2010 45(4):647-55

[2]Brownback *et al.*, Ann Thorac Med. 2013 Jul;8(3):153-9

What tests should be done from BAL fluid?

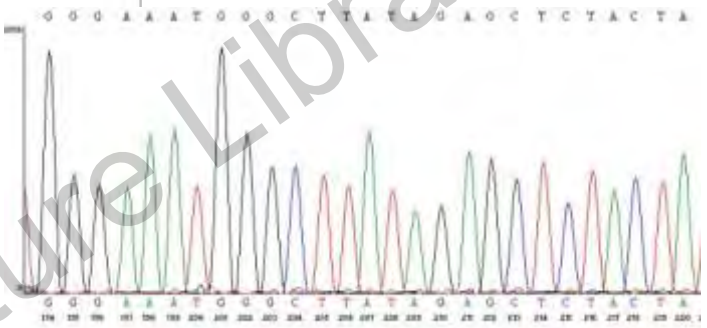
Microbiological tests from BAL fluid

- Stains: Gram-stain, cytospin preparation, Calcofluor white stain, direct IF *P. jirovecii*
- Cultures (semi-)quantitative (bacteria incl. *Legionella*, fungi, mycobacteria)
- Galactomannan



A. fumigatus, calcofluor white stain

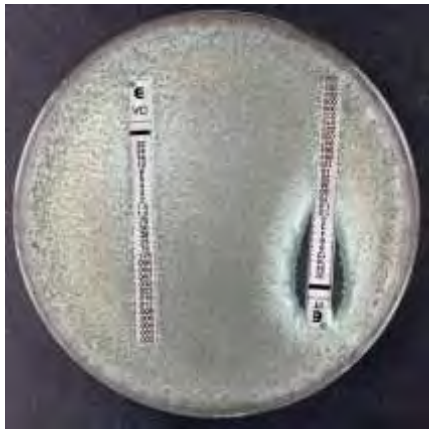
PCR from BAL



- *P. jirovecii*
- respiratory viruses
  - CMV, influenza, parainfluenza, RSV, coronavirus, rhinovirus and human metapneumovirus
- atypical bacteria (*M. pneumoniae, C. pneumoniae, L. pneumophila*)
- *M. tuberculosis*, MOTT

Culture of fungi & susceptibility testing

- Sensitivity of fungal culture modest (20-40%)
- Media matters – Inhibitory Mould agar probably better than Sabouraud agar[1]
- Susceptibility testing of moulds
- Increasing azole resistance in *A. fumigatus*
  - 8/27 (29.6%) azole resistant *A. fumigatus* from HSCT patients[2]



A. fumigatus TR46/Y121F/T289A genotype

[1]Scognamiglio *et al.*, JCM 2010, p. 1924–1925

[2]Steinmann *et al.*, JAC 2015 [in press]

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Galactomannan from BAL

- SEN 87%, SPE 89% for proven/probable IA[1]
- Best cut-off?
- SEN better than GM from serum, especially
  - in patients receiving prophylaxis/treatment
  - in non-BMT patients
- GM from BAL and serum complementary[2]

[1]Zou *et al.*, PLoS One. 2012;7(8):e43347

[2]Fisher *et al.*, Transpl Infect Dis. 2014;16(3):505-10

Aspergillus PCR

- PCR for *Aspergillus* spp.
  - culture independent, fast
  - sensitivity similar to GM; affected by antifungals[1]
  - standardisation
  - some assays can detect azole-resistant *A. fumigatus*[2,3]

[1]Avni *et al.*, JCM 2012; 50 no. 11 3652-3658

[2]Spiess *et al.,* AAC 2012;56(7):3905-10

[3]Chong *et al.*, JCM 2015; 53(3):868-74

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Aspergillus Lateral Flow Device

- monoclonal antibody (mAb JF5)[1]
- Serum+BAL
- Fast, easy to use
- Good concordance with GM
- SEN 70-80%, SPE 90-95%
- SEN 94% when used in combination with GM[2]

A) GM ELISA B) LFD

Johnson *et al.*, Biomark Med. 2014 Mar;8(3):429-51

[1]Thornton, Clin Vaccine Immun. 2008;15:1095-105

[2]Hoenigl *et al.,* JCM 2014, 52(6):2039

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P. jirovecii PCR from BAL

- sensitivity of direct IF for PcP low
- quantitative real-time PCR analysis sensitivity of ~99%, NPV 99%[1]
- threshold for PcP vs. colonization? [2,3]

[1]Lu *et al.*, JCM 2011, p. 4361–4363

[2]Mühletaler *et al.*, Eur Respir J 2012; 39: 971–978

[3]Robert-Gagneux *et al.*, JCM 2014; p. 3370–3376

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The future?

- Commercial multiplex-Assays (respiratory panels), different PCR platforms (fast-track respiratory 21/33 etc.)
- PCR/ESI-MS (abbott iridica)
  - PCR/electrospray ionization mass spectrometry
  - 750+ bacteria, 130+ virus, 200+ fungi
- no data from hematological patients with LI

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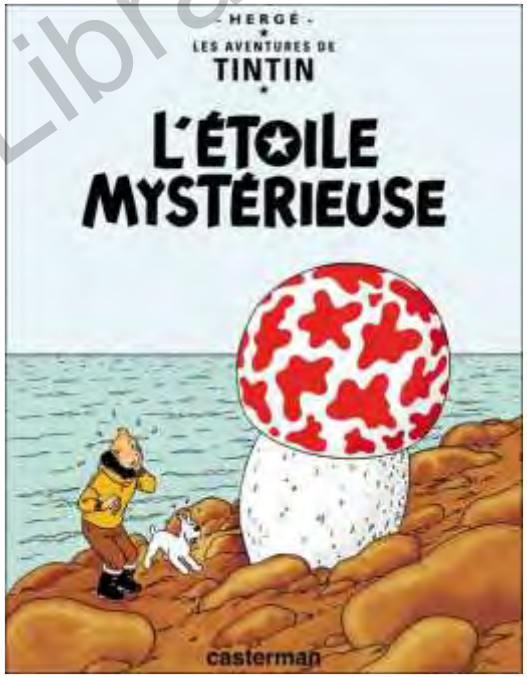
Conclusion

- Diagnosis of LI remains challenging - puzzle
- Combination of different methods offers best results
- Sample/choice of material decisive for success/failure of diagnosis
- Communication between clinician/ clinical microbiologist/pathologist essential



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Thank you for your attention