Fungal Biomarkers in Transplantation:
Distinguishing Colonization from Infection

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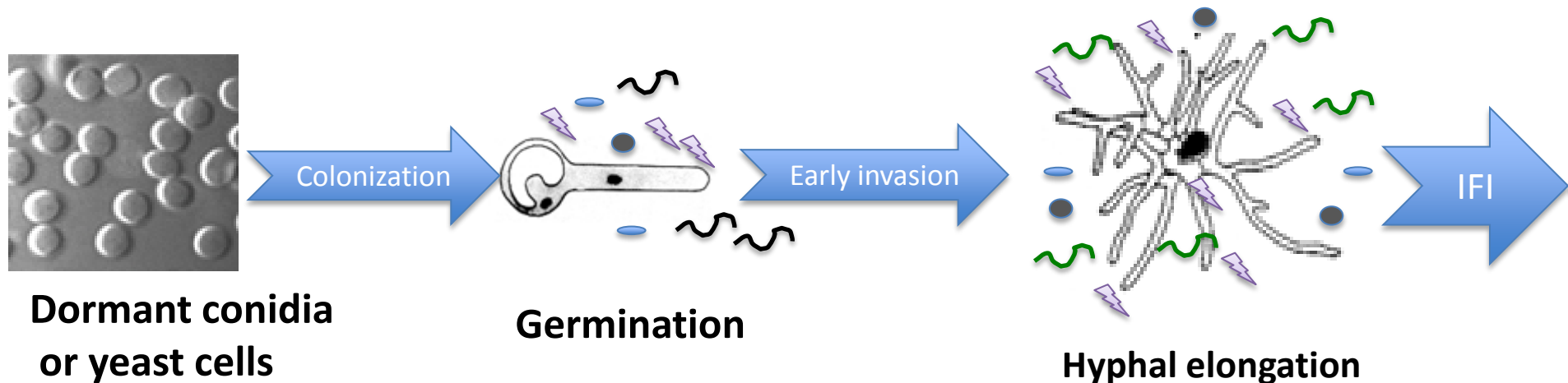University of Utah and ARUP Laboratories

Overview

- Differentiating colonization from invasive disease is essential for guiding the optimal use of available antifungal agents.

- Potential diagnostic strategies
  - Pathogen derived biomarkers
    - Cell wall components and nucleic acid
  - Measures of host response to infection
    - Antibodies, inflammatory markers, gene expression profiles

- Post-transplant scenarios:
  - Airway colonization vs. invasive pulmonary aspergillosis (IPA)
  - Skin/mucosal colonization vs. invasive candidiasis (IC)

Invasive Fungal Infections (IFI)

- Microbiologic factors
  - Fungal burden and organism virulence
- Host factors
  - Immunity, mucosal barriers, relative hypoxia/ischemia in grafted organ

Dormant conidia or yeast cells → Colonization → **Germination** → Early invasion → **Hyphal elongation** → IFI

Images adapted from www.aspergillus.org.uk/indexhome.htm?education/slides.htm~main

Invasive Pulmonary Aspergillosis (IPA) and Lung Transplantation

- Airway colonization is common and often transient
  - Airborne conidia (2.5–3.5 µm diameter) readily enter the lung alveoli
  - *Aspergillus* isolated from 45% of lung transplant recipients[1]
  - Invasive disease relatively rare (5-16%)[2-3]

1) Chest 1997; 112:1160-64; 2) Am J Transplant 2014; 14(6): 1328-33; 3) Med Mycol 2005; 43: S267-70;

Defining Fungal Colonization

Signs/Symptoms	Radiology	Microbiology	Path
● No fever *OR* hypothermia ● No change in secretions ● No new symptoms or exam findings ● Normal mucosa *OR* absence of endobronchial lesions	● No new or progressive abnormality	● Single (+) BAL culture *OR* ● Single (+) BAL PCR *OR* ● (+) BAL galactomannan *OR* ● ≥ TWO (+) sputum cultures/PCRs	● No histologic evidence of invasive disease

ISHLT Definitions. J Heart Lung Transplant. 2013;32:157-187

Patients may have other concomitant infections

IFIs have multiple CT appearances after lung transplantation

Biopsy may not be possible and is subject to sampling error

Galactomannan (GM)

TMB Substrate → HP → Colored Product

GM

Specimen well

✧ Sandwich enzyme-linked immunosorbent assay

- Antibody binds β-(1-5)-galactofuranosyl (gal*f*) side chains
- Result is an optical density (OD) index (≥0.5)
- Cross-reactivity with *Penicillium* and *Paecilomyces*
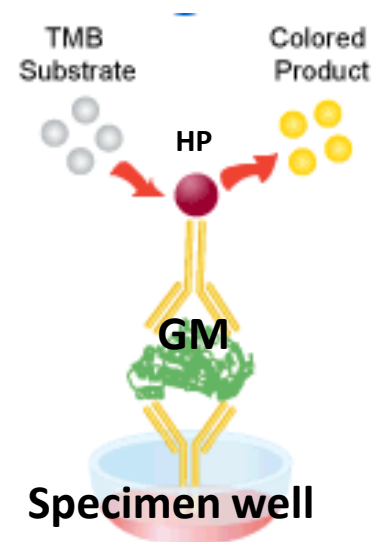
Serum

- 70 Lung transplants[1]
  - Detected 29% (2/8) IPA
  - Detected 0% (0/4) tracheobronchitis

- Meta-analysis[2] (SOT subgroup)
  - Sensitivity 22% (95% CI; 3-60%)
  - Specificity 84% (95% CI; 78-88%)
  - Better sensitivity in Heme/BMT

BAL

- Lung transplant (OD≥0.5) [3-5]

  - Sensitivity (range 60-100%)
  - Specificity (range 40-95%)
  - Colonization causes positives
    - OD index higher in IPA
    - ↑ cut-off may increase specificity

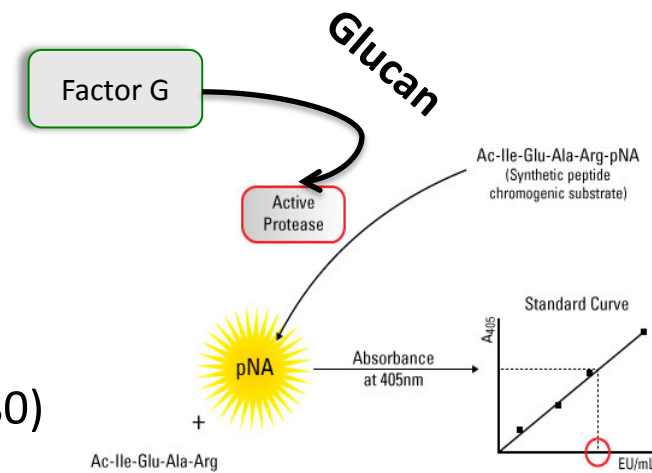1) Am J Transplant 2004; 4: 796-802; 2) CID 2006; 41:1417-27;
3) Transplantation 2007; 83: 1330-1336; 4) Transplantation 2010; 90: 306-311;
5) CID 2011; 52:1218-1226

1,3-β-D-Glucan (BDG)

◇ Limulus amebocyte lysate assay
- Pan-fungal marker
- Activation of horseshoe crab coagulation factor G
- Spectrophotometric readout reported in pg/ml (≥80)

Serum

- 73 Lung transplants[1]
  - 14 IFIs and 59 no IFI
    - >> Sensitivity 64%
    - >> Specificity 9%
  - Mold colonization associated with false positive BDGs

BAL

- Few studies[2-3]
  - Sensitivity for IPA similar to GM
  - Poor specificity
    - No mention of colonization
      - >> presumably yeast
  - Poor reproducibility

Based on limited data – BDG not recommended for lung transplant recipients or BAL

1) J Clin Microbiol 48:4083-4088; 2) J Infection 2014; 69: 278-283; 3) Mycopathologica 2013; 175: 33-41

Nucleic Acid Amplification Tests

✧ Quantitative detection of *Aspergillus* DNA or RNA

- Genus or species-specific designs (ribosomal targets), varying cross-reactivity
- Potential for contamination from the environment and reagents

Real-time 18S rDNA PCR

- 137 Lung transplant recipients[1]
  - BAL from 16 IPA, 26 colonized
    - >> Sensitivity 100% (95% CI; 79-100%)
    - >> Specificity 84% (95% CI; 78-88%)
  - 80% (13/16) false positive tests due to colonization
    - Lower Cq with IPA vs. colonization
    - Cross-reactivity with *Penicillium*

Real-time 28S rRNA NASBA

- Immunocomp rat IPA model[2]
  - BAL
  - Analytically sensitive < 1CFU/assay
  - Robust linear range x 5 log units
  - No theoretic benefit over rDNA for colonization discrimination

1) CID 2011; 52:1218-1226; 2) J Clin Microbiol 2010; 48: 1378-1383;

The *Aspergillus fumigatus* Volatome

- Volatile organic compounds (VOCs)
  - Secondary metabolites of fungi
    - Profile can be organism and nutrient specific[1]
      - Terpene and sesquiterpenes

  - Exhaled breath condensate
    - Gas chromatography-mass spectrometry (GC-MS)
      - VOC pattern discriminated IA from non-IA patients (51/54, 94%)[2]
        - Included 25 patients with nodular pneumonia cause by other pathogens
      - 2-pentylfuran found in CF lung disease and bronchiectasis[3]

  - Further studies required to establish clinical utility and assess confounding effects
    - Food, drugs, the environment, upper airway colonization

1) Eukaryotic Cell 2014; 13: 1014-1025; 2) ICAAC 2013: Abstract M-219; 3) Med Mycol 2009; 47: 468-476

Measures of Host Response to IPA

- Acute phase reactants and innate immunity in BAL
  - ⬆ Long Pentaxrin 3 (PTX3)
    - Secreted pattern-recognition receptor
    - 172 BALs from 76 lung recipients and 9 healthy controls[2]
      - » 3 IA, 20 colonization, 79 other organisms, 61 culture negative
      - » PTX levels measured by ELISA were highest in IPA
        - No overlap in levels between groups

  - ⬆Haptoglobin, ⬆C-reactive protein and ⬇annexin 1[2]
    - BAL from immunocompromised rabbits with and without IPA
      - » Host derived proteins predominantly found in IPA vs. uninfected controls
      - » Significant change in response to therapy
      - » Annexin potentially degraded by *A. fumigatus* proteases

1. IDSA 2013 Abstract # 1429
2. AAC 2014; 58: 3373-3378

The Spectrum of *Candida* Infection

Candida Colonization

Deep-seated candidiasis

Candidemia
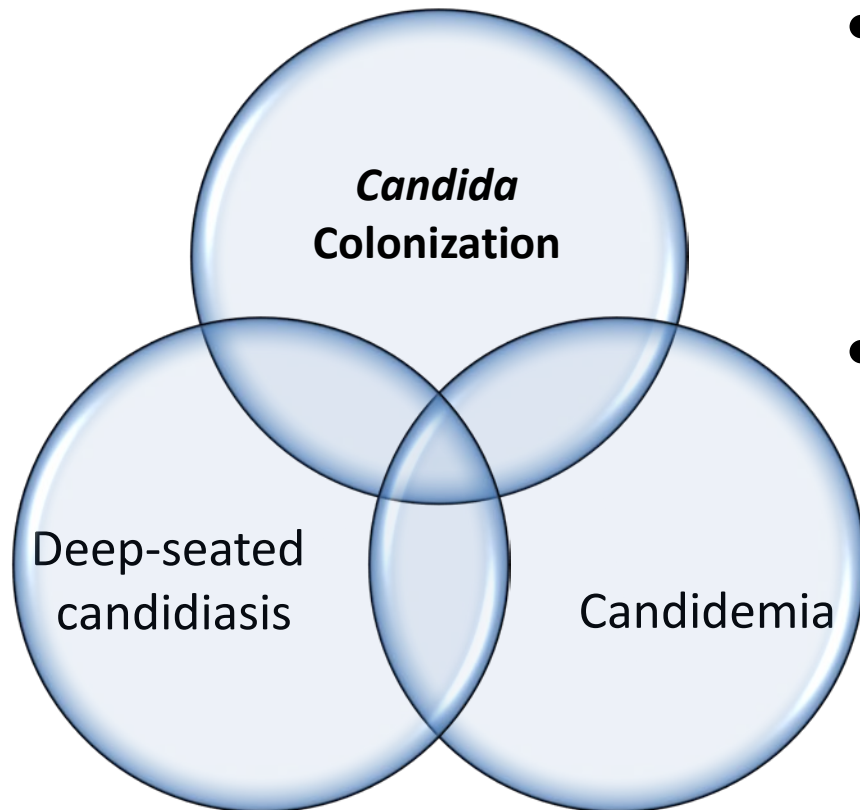
- Part of the normal flora
  - Colonization is common
    - 60 - 80% ICU patients colonized at multiple sites[1]
- Invasive candidiasis (IC)
  - Includes blood stream and deep-seated infections
    - Typical ICU pre-test probability 3%[2]
    - Clinical signs and symptoms non-specific

1.) Crit Care Med 2009; 37(5):1624-33 2.) Eur J Clin Microbiol Infect Dis 2007; 26: 271-6; 3) Diagn Microbiol Infect Dis 1993; 17: 103-109

1,3,-β-D Glucan (BDG)

- Meta-analysis (IC subgroup)[1]
  - Sensitivity 81% (95% CI; 77-85%)
  - Specificity 83% (95% CI; 80-83%)
  - No colonization analysis

- ICU studies[2-3]
  - No association of false (+) tests with colonization
  - When IC prevalence 3-10%
    - NPV may be the most useful

- False-positives:
  - Blood products
  - Gauze packing
  - Amoxicillin-clav acid
  - Hemodialysis
  - Bacteremia
  - Mucositis
  - Specimen manipulation
- **2 sequential tests**

1.) J Clin Microbiol 2012; 50: 7-15; 3. CID 2013; 56: 1284-922. 2.) Crit Care. 2014 Jun 29;18(3):R135; 3.) PLoS One. 2012;7(8):e42282

Candida antigens, antibodies and metabolites

- Mannan – immunogenic cell wall polysaccharide
  - Test characteristics improved when serum antibody/antigen assays used in combination[1]
    - \>> Sensitivity 83% (95% CI, 79-87)
    - \>> Specificity 86% (95% CI, 82-90)
  - Colonization
    - May cause detectable mannan antibodies[2]
    - No statistical effect on mannan antigen[3-4]
- D-arabinitol – *Candida* spp. metabolite
  - GC-MS of urine D-arabinitol/L-arabinitol ratio[5]
    - 17 neutropenic patients IC, 22 colonized, 22 uninfected
      - \>> Sensitivity 88%, Specificity 91%
      - \>> Differentiated most colonization (including bladder) from disseminated IC

1.) Crit Care. 2010;14(6):R222; 2.) Expert review Mol Diagn 2008; 8:315-325;
3.) J Clin Microbiol 2013; 51: 1158-1164; 4.) BMC Infect Dis 2010; 10: 292-297;
5.) J Clin Microbiol 1996; 34: 2175-3179

Candida PCR direct from Blood

- Rabbit models of disseminated candidasis[1-2]
  - DNA detectable in blood
- Patients with deep-seated disease and (-) blood culture
  - PCR sensitivity 88%[3]
- Meta-analysis laboratory developed tests (proven/prob IC)[3]
  - Sensitivity 95% (95% CI; 88-98%)
  - Specificity 92% (95% CI; 88-95%)
    - Few studies addressed colonization
      >> Trend toward ⬇ specificity
      >> Imperfect diagnostic standard
  - Heterogeneity

1.) J Clin Microbiol 1999; 37:925-30; 2.) J Clin Microbiol 2006; 44: 143-50; 3.) CID 2012; 54: 1240-8; 4.) J Clin Microbio 2011; 49: 665-70;
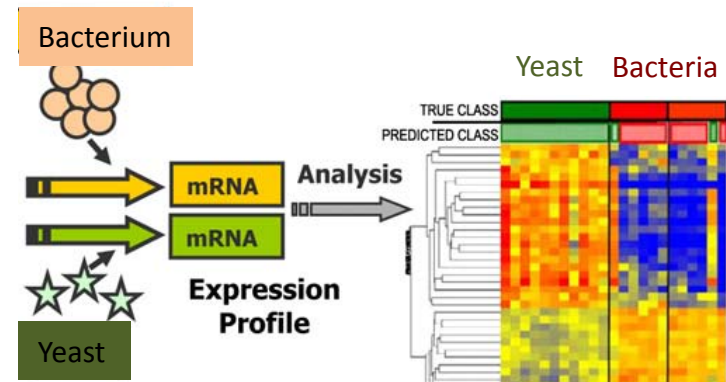
Host Gene Expression Analysis

- Mouse model of IC[1]
  - Transcription profile
    - Whole blood microarray
  - Immune effector gene signatures
    - Patterns differentiated *Candida* vs. *S. aureus* vs. uninfected controls
      - >> Sensitivity 98%
      - >> Specificity 96%

  1.) Sci Transl Med 2010; 2: 1-10

Modified from Cell Host & Microbe, 2009; 6: 199
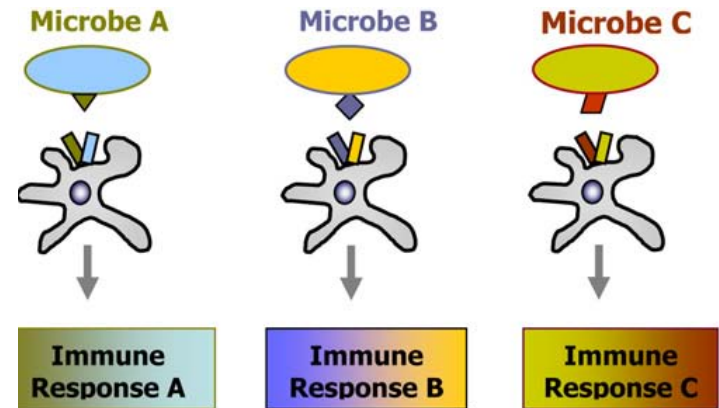
Conclusions

- Apsergillosis
  - GM and qPCR do not differentiate airway colonization from invasive disease
- Candidiasis
  - BDG not consistently affected by colonization in ICU, trend toward lower specificity with blood PCR
- Future directions
  - Evaluation of biomarker combinations
    - Need to assess assay performance in specific populations and the impact of colonization
  - Continued standardization of pathogen-specific NAAT
    - Develop diagnostics to target pathogen or host gene expression
  - VOC breath testing, host immune response in blood and BAL warrant further human study

Thanks and Questions?