A Statistical Model to Predict Invasive Fungal Disease in Pediatric Cancer and Hematopoietic Stem Cell Transplantation Patients

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Session: 297. Pediatric Viral and Fungal Diseases
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Background. Invasive fungal diseases (IFDs) are devastating opportunistic infections that result in significant morbidity and death in pediatric cancer and hematopoietic stem cell transplantation (SCT) patients. Identification of risk factors for IFD will help clinical decisions relevant to the diagnosis and management of IFD in a timely manner. Despite this, data evaluating predictive risk tools for IFD in pediatric cancer are limited.

Methods. We conducted a retrospective review of pediatric oncology patients with a diagnosis of febrile neutropenia (FN) at the UChicago Comer Children’s Hospital from July 2009 to December 2016. We analyzed 13 clinical, laboratory, and treatment-related risk factors for IFD including (age, gender, underlying diagnosis, SCT status, graft vs. host disease, chemotherapy in the last 2 weeks, temperature, height, fever duration, presence of hypotension, absolute neutrophil count, duration of neutropenia, absolute monocyte count, and the absolute lymphocyte count (ALC)). IFD was stratified as possible, probable, and proven according to the latest EORTC/MSG criteria (2020). Multivariable logistic regression risk prediction models were developed with separate analyses for all suspected IFD cases and only proven and probable cases.

Results. A total of 667 FN episodes (FNEs) were identified in 265 patients. IFD was diagnosed in 62 episodes (9.2%) of which 13 (1.9%) were proven, 27 (4%) probable, and 22 (3.3%) possible. Five variables obtained were significantly more common with IFD (P < 0.001). SCT receipt (< 0.01), neutropenia longer than 10 days (P < 0.03) were additional risk factors. The final model performs very well compared with other published models with an area under the curve (AUC) of 0.90 for all IFD, 0.94 for probable IFD, and AUC < 300 mm²/Creatinine < 0.02), and ALC <300 mm³/L.

Conclusion. Our findings showed important clinical markers for the development of IFD in pediatric oncology patients. A predictive regression model including identified significant factors has been created. Risk stratification with prospective external validation using this model can be used to refine the clinical approach.

2885. A Host Transcriptional Signature for Accurate Diagnosis of Candidemia in the Hospital Setting

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Session: 308. Fungi: Blood, Sweat, and Genes
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Background. Candidemia is one of the most common nosocomial bloodstream infections in the United States and causes significant morbidity and mortality in hospitalized patients. Improved rapid diagnostics capable of differentiating candidemia from other causes of febrile illness in the hospitalized patient are of paramount importance. Pathogen class-specific biomarker-based diagnostics such as those focusing on host gene expression patterns in circulating leukocytes may offer a promising alternative.

Methods. RNA sequencing was performed on peripheral blood samples from 27 hospitalized patients with blood culture positive invasive candidiasis. Samples from healthy controls as well as at-risk subjects with acute febrile illness and similar clinical backgrounds but other infections or noninfectious etiologies were used as comparator phenotypes (35 subjects with culture-proven bacterial infection, 49 with confirmed viral infection, and 17 with acute noninfectious illness). Bayesian techniques were utilized to develop infection-specific classifiers and leave one out cross-validation was used to estimate the predictive probability of each pathogen class.

Results. Candidemia triggers a unique, robust and conserved transcriptomic response in human hosts with 1,170 genes differentially upregulated compared with healthy controls. Based on this strength of signal, we developed a transcriptional classifier that was capable of identifying candidemia, viral, or bacterial infection with a high degree of accuracy (AUCROC = 0.93, Bacterial 0.98, Viral 0.99). The Candida component of this classifier (29 genes) was able to diagnose candidemia with a sensitivity of 88% and specificity of 100%.

Conclusion. The host transcriptomic response during candidemia in hospitalized adults is highly conserved and unique from the genomic responses to acute viral and bacterial infection. This approach shows promise for the development of clinical host response-based classifiers capable of differentiating multiple types of clinical illnesses at once in at-risk febrile patients.

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2886. Retrospective Case-Control Study of the Performance of the Karius Test, a Plasma Microbial Cell-free DNA Next-generation Sequencing test, to Detect Invasive Mold Infections in Hematopoietic Cell Transplant Recipients with Pneumonia

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Background. Pulmonary invasive mold infections (IMI) cause significant morbidity and mortality after hematopoietic cell transplant (HCT). Noninvasive diagnostic options are limited, particularly for non-Aspergillus (non-Asp) molds. Given differences in activity and toxicities of antifungals, early diagnosis and targeting of specific IMI is important. We evaluated the performance of the Karius Test, a plasma microbial cell-free DNA next-generation sequencing (NGS) test, for detecting IMI in HCT recipients.

Methods. We conducted a retrospective case–control study of 24 HCT recipients with proven non-Asp pulmonary IMI, 51 probable/proven Aspergillus pulmonary IMI, and 20 controls with nonfungal pulmonary infections. All subjects had plasma obtained within 14 days of diagnosis. Workup included bronchoalveolar lavage (BAL) and/or biopsy, with fungal stains/culture and galactomannan testing of BAL and serum. Plasma cell-free DNA was extracted and NGS performed (Karius, Redwood City, CA). Human reads were removed and remaining sequences aligned to a curated database including over 300 fungi. Organisms present above a predefined significance threshold were reported. A higher sensitivity research-use only pipeline was also used. Analysis of sequencing data was blinded to all clinical data.

Results. We identified pathogenic molds in 19/24 (79%) of subjects with proven non-Asp IMI, including Mucor, Rhizomucor, Scedosporium, Rhizopus, and Cunninghamella spp. In Aspergillus proven/probable IMI, A. fumigatus was identified in 13.7% (7/51) of subjects. In 3 other subjects with proven/probable aspergillosis, we also identified Rhizomucor miehei and R. pusillus, and Cunninghamella, consistent with clinical findings. The use of an optimal-sensitivity pipeline identified an additional 9 subjects with Aspergillus spp., and other pathogenic molds, increasing detection of molds to 37.3% (19/51). Specificity for molds in negative samples was 100% (20/20).
Conclusion. The Karius plasma NGS test is a noninvasive means of detecting IMI with high sensitivity for non-Aspergillus in HCT recipients with pulmonary disease. Further assay optimization may increase sensitivity for Aspergillus. This may be a useful adjunctive test for diagnosing IMI, and larger studies should be conducted.

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2887. Identifying Candida albicans Transcription Factors (TFs) That Regulate Pathogenesis of Intra-abdominal Candidiasis (IAC) by Screening a Deletion Mutant Library

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Background. IAC is a common manifestation of invasive candidiasis, but its pathogenesis is poorly understood. We developed a mouse model of C. albicans IAC, in which disease progresses from peritonitis to abscesses (IAA) in a manner that recapitulates human infection. Our goal was to use the model to identify C. albicans TFs that regulate virulence during IAC.

Methods. We screened a signature-tagged library (48 unique oligonucleotide markers) of homozygous deletion mutants for 165 C. albicans TF genes, created in duplicate in strain SC5314 (S. Noble). Mice were infected intra-peritoneally in triplicate with pools of 24 mutants and wild-type, and strains harvested at 72 hours in IAA.

Results. Twenty-one TF mutants were significantly attenuated for virulence in both libraries, and 2 TF mutants were significantly more virulent in both libraries, as measured by tissue burdens (figure). Biologic processes over-represented among attenuated mutants were regulation of pH responses, biofilm, hyphaal formation, echnocandin responses, and copper metabolism. pH responses are likely to be crucial to pathogenesis of IAC, as C. albicans transitions from pH 8 during peritonitis to pH 6.8 within IAA. 9 pH response regulators contributing to virulence included RIM101, STP2 (alkaline), ASH1, SFL1, SFL2 (neutral), MNL1, SKO1, PHO4 (weak acid), and CSR1 (acid). We created rim101 null mutant and reconstitution strains, and demonstrated that the gene was essential for complete virulence during peritonitis and IAA. Transcriptional profiling of strains by RT-PCR during peritonitis and in vitro showed both conserved and rewired Rim101 targets. SAP5, which encodes an aspartyl protease, is a major Rim101 target in vivo and in vitro; over-expression of SAP5 in rim101 restored virulence during IAA at 3, 7, and 10 days, but not during peritonitis. Other pH regulatory TF genes are currently being validated as virulence determinants, and pathway relationships between MNL1, SKO1, and PHO4 during IAA formation are being explored through epistasis approaches.

Conclusion. Screening of a C. albicans TF mutant library identified pH responses and other biologic processes as important during pathogenesis of IAC. Rim101, an alkaline pH response regulator, contributes to both peritonitis and IAA, the latter at least in part through its effects on Sap5.

2 >4 fold changes in mutants of both libraries

Disclosures. All Authors: No reported Disclosures.

2888. STAT4 Mutation in Three Generations with Disseminated Coccidioidomycosis (DCM) also Exhibits Increased Susceptibility to Coccidioidal Infection in Transfected Mice

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Background. Reported coccidioidomycosis has increased with case rates of 198/100,000 in Arizona (2012). In California alone, 2000–2011 hospitalizations were $2.2B. Dissemination occurs in 8% of reports with significant morbidity and occasional deaths. DCM was found in 3 generations: grandmother (skin), mother (skin), and son (bone). Whole exome sequencing identified a heterozygous (het) STAT4 mutation (p.E626G) in all three. This mutation alters the phosphotyrosine binding pocket and is predicted to impair STAT4 function, interfering with (i) receptor binding and phosphorylation, (ii) nuclear localization, and/or (iii) transcription. Expression profiling of antigen-stimulated peripheral blood mononuclear cells from one patient showed dampening of known STAT4 targets compared with controls.

Methods. STAT4 p.E626G was generated and confirmed in C57BL/6NJ (WT) mice using CRISPR-Cas9. With continued breeding, neither homozygous (hom) nor het mice had gross abnormalities. There were normal spleen and lung lymphoid cell numbers, Thymus and bone marrow had normal development of lymphoid subsets. We performed intranasal infection with reduced virulence C. posadasii strain 1038 or with F. tularensis live vaccine-strain (LVS). Naive or Δcps1-vaccinated mice were tested for resistance to C. posadasii strain Silveira.

Results. At day 21 post Cp 1038 infection, hom, het, and WT mice had similar lung fungal burdens (~10^7 cfu). All p.E626G mice died between days 31 and 39 with lung burden significantly higher (~9 × 10^6 cfu) than WT sacrificed on day 44 (7 × 10^5 cfu, P = 0.015). After LVS infection, p.E626G mice had increased lung bacterial cfu and all had dissemination to the spleen compared with WT lung bacterial burden and no splenic dissemination. Immunized het and WT mice all had significantly reduced lung cfu 14 days following C. posadasii infection compared with unvaccinated WT mice.

Conclusion. The STAT4 p.E626G mutated mouse recapitulated patients’ increased susceptibility to coccidioidal infection. The decreased fungal burdens seen in Δcps1-vaccinated mice suggest that vaccination may be effective in those persons genetically susceptible to DCM. Given the increasing frequency and economic burdens of coccidioidomycosis, pursuit of vaccination strategies should continue.