648. Baloxavir Resistance: qPCR Detection of Antiviral Resistance Markers in Influenza A Virus

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Session: 67. New Diagnostics
Thursday, October 3, 2019: 12:15 PM

Background. Influenza (flu) infections affect a large subset of the population every year and have significant impacts on the health of patients, especially those with weak or compromised immune systems such as the elderly, children, cancer patients, and transplant recipients. Baloxavir marboxil was approved in October 2018 as a novel antiviral therapeutic for treating flu. During clinical trials, mutations were identified at the I28 codon of the polymerase acidic (PA) protein that greatly increased the resistance of a flu strain to this novel drug. In this study, a qPCR was developed and validated to identify these resistance mutations, allowing for guided therapy based on the resistance profile of the strain.

Methods. Flu A sequences (6,175) of the PA gene from the NCBI Influenza Virus Database collected over the last 5 years were compiled and aligned. Primers and probes were designed to target the I38 codon of the PA gene, and specific probes for each codon yielding a resistant amino acid mutation (I38T, -M, and -F) were designed. Locked nucleic acid (LNA) bases were used to increase the specificity of the probes. A combination of clinical flu specimens, laboratory strains, and synthetic constructs of each potential resistance mutation were used to validate the precision, sensitivity, and accuracy of the assay in nasopharyngeal swabs.

Results. Precision of the cycle threshold (Ct) values for each detector was determined to have a standard deviation of less than 3 for inter-assay and less than 2 for intra-assay replicates. Sensitivity was determined to be 800 copies/mL in nasopharyngeal swabs. Accuracy was found to be 92.3%. A single laboratory strain from the H1N1 2009 epidemic showed cross-reactivity with both wild-type and resistant probes, but no circulating clinical H1N1 samples tested showed this response.

Conclusion. The precision, sensitivity, and accuracy of a qPCR for resistance mutations to baloxavir marboxil support this assay's utility as an aid in the treatment of flu in at-risk patient groups. This assay allows for rapid detection (<24 hours) of resistance markers to aid clinicians in improving flu case outcomes.

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649. Prospective Validation of an 11-mRNA Host Immune Signature as a Novel Blood Test for Acute Septic Arthritis

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Session: 67. New Diagnostics
Thursday, October 3, 2019: 12:15 PM

Background. Septic arthritis is an orthopedic emergency requiring immediate surgical intervention. Joint aspirations detect inflammatory cells within hours but often cannot distinguish between infections (e.g., bacterial) or other causes (e.g., gout). Cultures take days, so decisions about surgery are made with incomplete data. Aspirations carry risk and require technical skill and advanced imaging. Novel diagnostics are thus needed. An 11-mRNA host immune blood signature has been validated to distinguish between infectious and noninfectious acute inflammation. It is part of the 29-mRNA HostDx™ Sepsis test that can also distinguish between bacterial and viral infections and predicts severity (currently under development as a rapid point-of-care test). We studied whether the 11-mRNA signature can determine if acute arthritis was due to an infectious cause.

Methods. We conducted a blinded, prospective, noninterventional study on patients undergoing workup for a septic primary joint. Patients received standard-of-care including joint aspiration and lab tests: White Blood Cells (WBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and joint fluid analysis. Simultaneously, blood was drawn (PAXgene RNA tubes). mRNAs were measured on NanoString nCounter™ blinded to clinical results.

Results. 20 patients were included, of which 10 had infections based on positive synovial fluid cultures. The 11-mRNA blood signature had an area under the ROC curve (AUROC) of 0.87 for separating infectious from noninfectious conditions compared with 0.58 (ESR), 0.60 (CRP), and 0.50 (WBC); AUROC for synovial WBC was 0.54. At 100% sensitivity for infection, specificity of the signature was 40%; thus, a substantial fraction of nonseptic patients could have been ruled out for further surgical intervention.

Conclusion. The 11-mRNA signature showed markedly increased accuracy in predicting septic joints compared with routine diagnostic markers. As a quick point-of-care test this blood RNA signature may be an important tool for early, accurate identification of acute septic joints and need for emergent surgery, thereby improving clinical care and healthcare spending. These findings further add to the generalizability of results obtained in the HostDx Sepsis test.

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650. Relationship of a Multiplex Molecular Pneumonia Panel (PP) Results with Hospital Outcomes and Clinical Variables

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Session: 67. New Diagnostics
Thursday, October 3, 2019: 12:15 PM

Background. The Pneumonia Panel (PP) (BioFire Diagnostics, Salt Lake City, UT) detects 15 potentially pathogenic bacteria semiquantitatively (copy #/mL), 8 viruses and 7 resistance genes from the lower respiratory tract in ≈1 hour in the laboratory. Since identification and susceptibility take ≈ 2 days, this rapid result time is very attractive; however, the clinical significance of the PP copy #/mL as well as a predictable group of PP positive but culture negative patients is unknown. We retrospectively studied the relationship of 270 PP results to culture results, clinical data and outcomes.

Methods. Bronchoalveolar lavage fluid (N = 197) and endotracheal aspirates (N = 73) submitted to the UF Health Shands Hospital microbiology laboratory from June-September 2018 were frozen at −70°C, until tested on the PP. Patient data were extracted from the inpatient electronic medical record (Epic).

Results. Of 270 patients tested, 111/270 (41.1%) were PP bacteria negative/culture no growth or normal flora (Group 1), 59/270 (21.9%) were PP positive/culture negative (Group 2), and 100/270 (37.0%) were PP positive/culture positive (Group 3) for at least 1 concordant bacterial potential pathogen. Hospital length of stay (LOS), P = 0.0274, ANOVA; ICU LOS P = 0.0007 and BAL % Polys P < 0.0001 were significantly longer/higher in Group 3 than in Groups 1 and 2 (Table 1). Max daily temp on the day of culture in PP-positive groups 2 and 3 was significantly higher than the PP-negative group 1, P = 0.0260, ANOVA, (Table 1). Age, daily WBC, lowest paO2, max FiO2, % on antibiotics (≥80% for all groups), and % with viruses in the PP were not significantly different across groups. When all PP pathogens were grouped by copy # /mL, ICU LOS was significantly longer for 10(7) copies/mL (P = 0.0088), as was BAL % polys (P = 0.0006). Max daily temp was almost significantly higher for PP-positive groups 10(5), 10(6), and 10(7) combined compared with the PP-negative group (Table 2, P = 0.0608).

Conclusion. Hospital LOS was significantly longer and BAL %Polys higher in the bacterial pathogen culture-positive/PP-positive group vs. not positive groups. ICU LOS and BAL %Polys were significantly higher for the PP-positive groups vs. PP negative regardless of culture results. PP results (copy #/mL) independently correlated with outcome and clinical measures.

	Hospital LOS n=270 obs*	ICU LOS n=270 obs	BAL % Polys n=78 obs	Max Daily Temp n=267 obs
Group 1 BP negative/culture negative N=111	18±16	10.8±14	44±36	99.6±1.4
Group 2 BP positive/culture negative N=59	17±13	10.9±12	71±26	100.2±1.6
Group 3 BP positive/culture positive N=100	23±18	17.9±16	81±19	100.6±1.5
p-value (ANOVA, SAS)	0.0274	0.0007	<0.0001	<0.0001

*obs=observations, not all patients had %Polys done or Daily Temps electronically available

Copies/ml	Hospital LOS n=270 obs*	ICU LOS n=270 obs	BAL % Polys n=78 obs	Max Daily Temp n=267 obs
0 N=111	18±16	11±14	44±36	99.6±1.4
10(4) N=13	15±8	8±6	68±43	100.8±1.8
10(5) N=30	22±15	16±16	72±24	100.6±1.6
10(6) N=26	17±13	11±12	66±19	99.9±1.5
10(7) N=90	22±18	17±17	84±18	100.5±1.5
p-value (ANOVA,SAS)	0.2042	0.0088	0.0006	<0.0001

*obs=observations, not all patients had %Polys done or Daily Temps electronically available

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651. Multi-Center Evaluation of the BioFire® FilmArray® Blood Culture Identification 2 Panel for the Detection of Microorganisms and Resistance Markers in Positive Blood Cultures

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Session: 67. New Diagnostics
Thursday, October 3, 2019: 12:15 PM

Background. The BioFire® FilmArray® Blood Culture Identification 2 (BCID2) Panel is a diagnostic test that provides results for 26 bacterial, 7 fungal pathogens and 10 antimicrobial resistance (AMR) genes from positive blood culture (PBC) specimens in about an hour. The BCID2 Panel builds upon the existing BCID Panel with several additional assays that include *Candida auris* and an expanded AMR gene menu that provides methicillin-resistant *Staphylococcus aureus* (MRSA) results plus detection for *mcr-1*, carbapenem resistance, and ESBL. Here, we summarize studies conducted to establish clinical performance using an Investigational Use Only version of the BCID2 Panel.

Methods. Three studies were performed. The first involves prospective collection and testing of an expected ~1,000 residual PBCs at 7 US and 2 EU sites, which began in

October 2018 and will conclude in June 2019. BCID2 Panel performance is compared with reference methods of microbial culture as well as PCR/sequencing for AMR genes. In addition, BCID2 Panel MRSA results are compared with the FDA-cleared Xpert MRSA/SA BC system (Cepheid, Inc). Relevant bacterial isolates recovered from PBCs are also evaluated by various phenotypic antimicrobial susceptibility testing (AST) methods. The prospective evaluation is supplemented with a second study that involves testing of ~300 pre-selected, archived PBCs containing rare organisms. The third study includes over 500 seeded blood cultures containing very rare organisms with an evaluation of co-spiked samples.

Results. With over 1,200 samples tested to date (out of an anticipated 1,800 total), the BCID2 Panel has demonstrated an overall sensitivity of >98% and specificity of >99% for identification of microorganisms compared with culture. Concordance between the BCID2 Panel and the Xpert MRSA/SA BC test is >99% for identification of MRSA. Evaluation of BCID2 Panel AMR gene detection relative to AST and PCR is ongoing.

Conclusion. The FilmArray® BCID2 Panel appears to be a sensitive, specific, and robust test for rapid detection of microorganisms and MRSA in PBCs. With the use of this comprehensive test, improved antimicrobial stewardship is anticipated.

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652. Impact of FilmArray Meningitis Encephalitis Panel on HSV Testing and Acyclovir Use in Children Beyond the Neonatal Period

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Session: 67. New Diagnostics

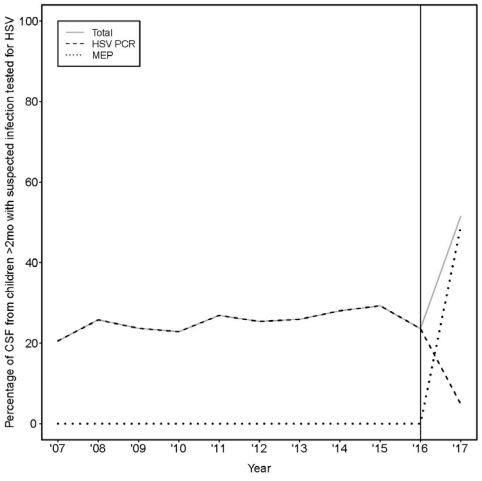
Thursday, October 3, 2019: 12:15 PM

Background. Testing and empiric use of acyclovir for herpes simplex virus (HSV) in children beyond the neonatal period undergoing lumbar puncture for suspected central nervous system (CNS) infection doubled in the past decade, while the incidence of HSV CNS infection is unchanged. A new syndromic multiplex PCR panel (FilmArray Meningitis Encephalitis Panel [MEP]) rapidly detects 14 pathogens in cerebrospinal fluid (CSF), including HSV. The impact of MEP implementation on HSV testing and acyclovir use is unknown.

Methods. We retrospectively compared CSF testing and acyclovir use in the pre-MEP era January 1, 2007–January 22, 2017 to post-implementation era of MEP January 23, 2017–December 31, 2017 amongst children >60 days with a CSF specimen sent to the Children's Hospital Colorado microbiology laboratory. HSV singleplex PCR testing was available in both the pre-MEP and MEP eras.

Results. The proportion of CSF specimens from children with suspected CNS infection undergoing HSV testing (MEP or HSV PCR) doubled from 25% in the pre-MEP era to 54% in the MEP era ($P < 0.01$; Figure 1). In the MEP era, HSV testing was conducted by MEP in 96% of cases and HSV PCR in 8% of cases. In both eras, a majority of CSF specimens undergoing HSV testing had no pleocytosis (63% vs. 59%, $P = 0.27$). Children with negative HSV testing by MEP were less likely to be started on acyclovir than those with negative HSV testing by singleplex PCR (18% vs. 50%, $P < 0.01$) and, amongst those started, acyclovir was discontinued sooner, after a median 3 vs 5 doses ($P = 0.05$). Overall, however, a similar proportion of children with suspected CNS infection received acyclovir in the MEP and pre-MEP eras (13% vs. 12%), despite a low rate of HSV positivity (0.5% vs. 0%).

Conclusion. Implementation of MEP for syndromic CSF testing in children >60 days with suspected CNS infection doubled HSV testing without affecting the rate of empiric acyclovir initiation. Patients with negative HSV testing on MEP were less likely to be started on acyclovir, and if started, received fewer doses than those who tested negative on HSV singleplex PCR, likely due to more rapid turnaround time. However, increased MEP testing offset this, suggesting increased use of newer rapid syndromic tests will not cure creeping empiricism. Diagnostic stewardship targeting MEP use toward children with pleocytosis to decrease unnecessary test utilization are warranted.



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653. Diagnosis of Burn Sepsis Using the FcMBL ELISA: A Pilot Study in Critically Ill Burn Patients

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Session: 67. New Diagnostics

Thursday, October 3, 2019: 12:15 PM

Background. Infection is the leading cause of death among burn survivors, with sepsis associated with more extensive burns. Conventional diagnostic criteria are insensitive in this population. We examined a novel diagnostic ELISA based on Mannose-Binding Lectin (MBL) linked to an immunoglobulin Fc domain, which measures the concentration of Pathogen-Associated Molecular Patterns (PAMPs) across a broad range of bacterial and fungal organisms, for diagnosis and antimicrobial management of sepsis in burn patients.

Methods. We prospectively enrolled burn patients with ≥15% Total Body Surface Area (TBSA) burns into groups of noninfected, sepsis, or incipient infection, and healthy volunteers. Sepsis was defined by clinical actions responsive to sepsis. The FcMBL ELISA was performed daily using fresh whole blood. Burn subjects were sampled daily until completing antimicrobials, for 14 days if noninfected, and once for healthy controls. Differences in median PAMP concentrations between groups were assessed with the Kruskal–Wallis test, including multiple comparisons between categories.

Results. 14 burn patients (3 noninfected, of whom 1 died prior to sampling, 4 Sepsis, 7 Incipient) were enrolled. The median (25–75% CI) PAMP concentration was 0.53 (0.12–1.34) ng/mL in healthy controls, 3.725 (2.53–5.94) ng/mL in noninfected, 2.22 (1.42–4.62) ng/mL in incipient, and 1.59 (0.83–2.29) ng/mL in sepsis groups. PAMP concentrations in sepsis were different ($P = 0.0057$) from noninfected, but incipient did not differ from noninfected ($P = 0.2025$). The dynamic range was lower in healthy controls (2.69 ng/mL) than incipient (4.57 ng/mL), sepsis (4.70 ng/mL), or noninfected (5.90 ng/mL). PAMP elevations correlated with clinical deterioration from infection, and were not associated with OR visits for debridement and grafting. 7 of 11 infected patients had declining PAMP levels at completion of antimicrobial therapy. 2 subjects had PAMP elevations associated with *Aspergillus* molds in their burn wounds.

Conclusion. The FcMBL ELISA assay may be useful for diagnosis of infection in burn patients, and may facilitate earlier discontinuation of antimicrobials. This assay may also have a novel utility for early diagnosis of Invasive Fungal Infection.

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654. Evaluation of the Febridx Host Response Point-of-Care Test to Differentiate Viral From Bacterial Etiology in Adults Hospitalized with Acute Respiratory Illness During Influenza Season

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Session: 67. New Diagnostics

Thursday, October 3, 2019: 12:15 PM

Background. Antibiotics are overused in patients hospitalized with acute respiratory illness (ARI). Diagnostic uncertainty regarding microbial etiology contributes to this practice and so a host response test that can distinguish between viral and bacterial infection has the potential to reduce unnecessary antibiotic use. The FebriDx is a low cost, rapid, host response POCT that uses fingerpick blood samples to distinguish between viral and bacterial infection but has not been evaluated in hospitalized adults with ARI.

Methods. We took fingerpick blood samples from adult patients with ARI, hospitalized during influenza season, and tested them on the FebriDx. Respiratory samples were tested for viruses on the FilmArray Respiratory Panel (FARP). The FebriDx was evaluated for ease of use, failure rate and accuracy of the results (Viral, Bacterial, Negative).

Results. 149 patients were approached and 10 patients declined fingerpick testing. A valid result was obtained from 124/139 (89%) overall. Common user comments included test failure due to difficulty of getting blood to fill the capillary tube and difficulty in interpreting the results lines due to the variability of color change. 111/124 (89%) were tested for viruses by FARP. 69/111 (62%) had viruses detected. Of 69 patients with viruses detected, 41 (59%) had influenza, 12 (17%) rhino/enterovirus and 16 (23%) other viruses. 44/69 (64%) had a viral FebriDx result. For influenza-positive patients 34/41 (83%) had a viral FebriDx result, 1/12(8%) of rhinovirus-positive patients had a viral FebriDx result and 9/16 (56%) of patients with other viruses detected had a viral FebriDx result. These are interim results. Full results for 200 patients will be available at presentation.

Conclusion. The use of the FebriDx POC was associated with a failure rate of ~10% and problems with the interpretation of result lines. FebriDx was not sufficiently accurate in differentiating viral and bacterial infection when using detection of virus by PCR as the definition of viral infection; however, FebriDx had a high PPV for all viral