2563. Clinical Metagenomic Next-Generation Sequencing for Diagnosis of Meningitis and Encephalitis

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Background. Metagenomic next-generation sequencing (mNGS) of CSF can identify nearly all pathogens in a single test. We previously validated a CSF mNGS assay in a licensed clinical laboratory. To date, the utility of mNGS for infectious disease diagnosis has been described in case reports and small case series, but not in a large-scale clinical trial.

Methods. The PDAID ("Precision Diagnosis of Acute Infectious Diseases") study was a 1-year nationwide prospective study across 8 tertiary care hospitals to evaluate the performance and utility of a clinical metagenomic sequencing assay for diagnosis of meningitis, encephalitis, or myelitis from cerebrospinal fluid (CSF) (ClinicalTrials.gov number NCT02910037). We recruited acutely ill hospitalized inpatients lacking a diagnosis at the time of enrollment. CSF samples were processed and analyzed by mNGS testing within 1 week of receipt in the clinical microbiology laboratory, with sequencing results reported in the patient medical record and used to make contemporaneous treatment decisions. Weekly clinical microbial sequencing boards were convened to discuss mNGS results with treating physicians, and clinical impact evaluated by surveys, chart review, and direct clinician feedback.

Results. A total of 204 patients were enrolled. Patients were severely ill (ICU 48%, average length of stay 26 days, overall 30-day mortality 7.4%). Fifty-nine neurologic infections were diagnosed in 57 patients (27.9%). mNGS identified 15 (25.4%) infections that were missed by all conventional microbiological tests, including emerging and/or uncommon pathogens such as St. Louis encephalitis virus, hepatitis E virus acquired by lung transplant, and *Nocardia farcinica*. Twelve of the 15 mNGS-only diagnoses (80%) had clinical impact, with 9 of 15 (60%) guiding appropriate treatment. For diagnosis of infections by direct detection CSF testing, mNGS had 79.1% sensitivity and 98.8% specificity, versus 65.1% sensitivity and 99.4% specificity by conventional testing.

Conclusion. A significant proportion of neurologic infections are missed despite extensive diagnostic testing performed in tertiary care hospitals. Clinical metagenomic CSF testing was found to be useful in increasing the number of diagnosed neurologic infections and providing actionable information for physicians.

Disclosures. **All authors:** No reported disclosures.

2564. Clinical Validation of a Commercial LAMP Test for Ruling Out Malaria in Returning Travelers: A Prospective Diagnostic Trial

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Background. The mainstay of malaria diagnosis relies on rapid diagnostic tests (RDT) and Giemsa-stained microscopy both of which lack analytical sensitivity. This leads to repeat testing to rule out malaria. Nucleic acid amplification (NAT) methods are more sensitive, but testing requires technical proficiency beyond the average clinical laboratory.

Methods. We conducted a prospective diagnostic trial of the Meridian *illumigene* Malaria assay (LAMP) compared with reference microscopy and RDT (BinaxNOW Malaria) in returning travelers in Western Canada between June 2017 and January 2018. Returning travelers with signs and symptoms of fever were enrolled into the study. RDT, microscopy, and LAMP assays were performed simultaneously. To increase the yield of positive specimens for all species of malaria, retrospective specimens of *Plasmodium vivax*, *P. ovale*, and *P. malariae* species were supplemented. Real-time (RT)–PCR testing was performed on all specimens to resolve discrepancies. A cost–benefit analysis was performed.

Results. A total of 296 consecutive patients (50.7% male, mean age 32.5) were enrolled, most visiting friends and relatives (43.2%), traveling to Asia (48.4%), presenting with fever (88.9%), not taking prophylaxis (82.8%), and treated as outpatients (84.3%). In the prospective arm, LAMP had a sensitivity of 98.1% (95% CI 89.9–99.9) and a specificity of 97.6% (95% CI 95.2–99.0) versus microscopy. After discrepant resolution with RTPCR, LAMP had a sensitivity of 100% (95% CI 93.9–100) and a specificity of 100% (95% CI 98.7–100) versus microscopy. When including retrospective specimens, LAMP had a sensitivity of 98.7% (95% CI 92.7–99.9) and a specificity of 97.6% (95% CI 95.2–99.1) versus microscopy, and after discrepant resolution of this set, LAMP had a sensitivity of 100% (95% CI 95.5–100) and a specificity of 100% (95% CI 98.7–100). The rate of invalid tests with LAMP was 3.05%. After discrepant resolution, RDT had a sensitivity of 83.3% (95% CI 58.6–96.4) and a specificity of 96.2% (95% CI 93.2–98.1) versus microscopy. A cost–benefit analysis of reagents and labor suggests up to USD 13 savings per specimen using a revised algorithm with LAMP screening.

Conclusion. A novel, highly sensitive testing algorithm for malaria screening with associated cost savings in the nonendemic setting is proposed.

Disclosures. **D. Pillai**, Meridian Biosciences: None, Diagnostic testing material for study.

2565. A Novel Prognostic Gene Set for the Prediction of Severe Dengue

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Background. There is an urgent need for the identification of biomarkers predictive of severe dengue. Single cohort transcriptomic studies have not yielded a parsimonious gene set predictive of severe dengue. We hypothesized that integration of gene expression data from heterogeneous patient populations with dengue infection would yield a set of conserved genes that is predictive of severe dengue and generalizable across cohorts.

Methods. Ten dengue gene expression datasets were identified in publicly available microarray repositories. A novel integrated multicohort platform was used to detect differentially expressed gene transcripts between uncomplicated and severe dengue patients and validate the identified putative signature *in silico* and prospectively in a new cohort of 34 dengue patients in Colombia. Dengue diagnosis was made by NS1 antigen and anti-DENV IgM antibody and confirmed by RT-PCR assays, ELISA, and IgG avidity measurements. The expression level of the signature genes was measured via microfluidic qRT-PCR assays in blood samples collected longitudinally during the course of illness.

Results. Using the multicohort analysis to analyze 446 peripheral blood samples of patients with dengue infection from 7 publicly available gene expression datasets, we identified a 20 gene set that predicts the development of severe dengue. We *in silico* validated the diagnostic power of this gene set to separate severe dengue from dengue with or without warning signs in 3 independent datasets composed of 84 samples with a global area under the ROC curve (AUC) of 0.80 [95% CI 0.68–0.88]. We prospectively validated the gene set in a new cohort composed of 34 dengue patients from Colombia with an AUC of 0.89 [95% CI 0.81–0.97]. The severity scores measured in patients with severe dengue progressively declined in longitudinal samples.

Conclusion. Our data indicate that the identified 20 gene signature predicts the development of severe dengue in patients prior to its onset and suggest that dengue infection itself triggers this host response. These findings may provide new insight into the pathogenesis of severe dengue and have implications for the development of a prognostic molecular assay to identify patients at risk to develop severe dengue.

Disclosures. **T. E. Sweeney**, Inflammatix, Inc.: Employee and Shareholder, Salary. **P. Khatri**, Inflammatix, Inc: Scientific Advisor and Shareholder, Licensing agreement or royalty.

2566. Diagnosis of *Pneumocystis jiroveci* Pneumonia in HIV-Negative Immunocompromised Patients: Is the Gomori-Methenamine Silver Stain of Bronchoalveolar Lavage Fluid the Gold Standard or Sub-Standard?

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Background. Direct visualization of *Pneumocystis jiroveci* on bronchoalveolar lavage (BAL) fluid using the Gomori-methenamine-silver (GMS) stain historically has been the mainstay of diagnosis for *P. jiroveci* pneumonia (PJP), with studies from the early HIV/AIDS era reporting sensitivities of 90–95%. However, the burden of *P. jiroveci* organisms in BAL fluid is significantly lower in HIV-negative immunocompromised patients compared with HIV-positive patients with PJP, raising concerns that the BAL GMS stain is less sensitive in this population.

Methods. We conducted a retrospective observational study at Yale-New Haven Hospital from 2012 to 2018, using electronic medical record chart reviews, to identify

patients who underwent bronchoscopy with BAL GMS stain for the diagnosis of PJP. We collected additional patient factors such as age, sex, HIV status, and immunosuppressed status. For patients with a negative BAL GMS stain, we collected data on other diagnostics, including positive GMS lung biopsies, positive PJP PCR or DFA, and elevated serum (1–3)-β-D-glucan levels. We defined BAL GMS-negative cases as proven or probable based on investigator generated criteria (see Figure 1).

Results. We identified 52 patients with PJP who received a BAL GMS stain, including 28 HIV-positive and 24 HIV-negative cases. Of 24 HIV-negative cases, 11 had BAL GMS-positive PJP and 13 had BAL GMS-negative PJP (9 proven and 4 probable). In the latter group, 6 had hematologic malignancies (HM), 2 had solid-organ transplants (SOT), 1 had hematopoietic stem cell transplant, 2 had SOT plus HM, and 2 received high-dose steroids. Proven diagnoses were made by GMS-positive lung biopsy ($n = 6$), DFA ($n = 2$), and PCR ($n = 1$). Elevated (1–3)-β-D-glucan was observed in 7 of 8 cases (median: >500 pg/mL; range 39 to >500). Three patients developed adverse outcomes (1 readmission due to untreated PJP and 2 treatment delays). BAL GMS sensitivity for HIV-negative patients was 11/24 (46%) vs. 28/28 (100%) in HIV-positive patients.

Conclusion. The sensitivity of BAL GMS for PJP is poor in HIV-negative immunocompromised patients. Missed cases or delayed treatment for PJP may lead to adverse outcomes. In HIV-negative patients with a clinical syndrome compatible with PJP, a negative BAL GMS does not rule out PJP and must be confirmed by supplementary diagnostics.

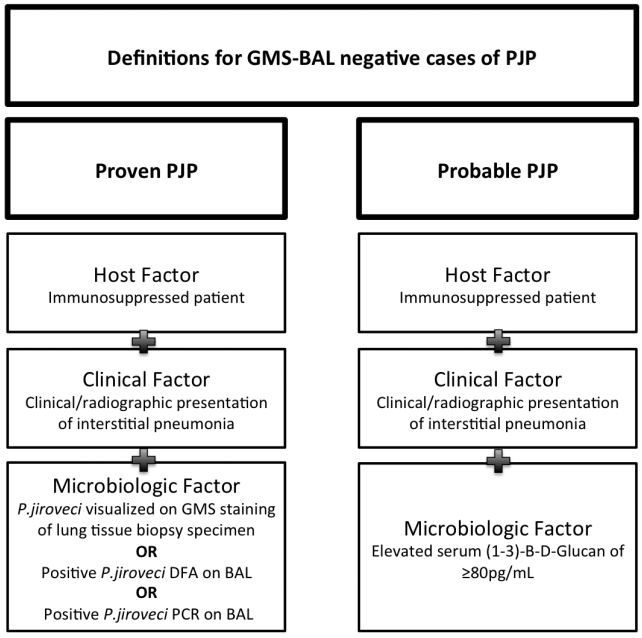

Figure 1

Disclosures. **All authors:** No reported disclosures.

2567. Diagnosis of Invasive *Aspergillosis* in Hematological Malignancy Patients Receiving Mold-Active Antifungals: Performance of Interleukin-6 and -8, *Asp* LFD, and *Aspergillus* PCR in Same-day Blood and Bronchoalveolar Lavage Fluid Samples

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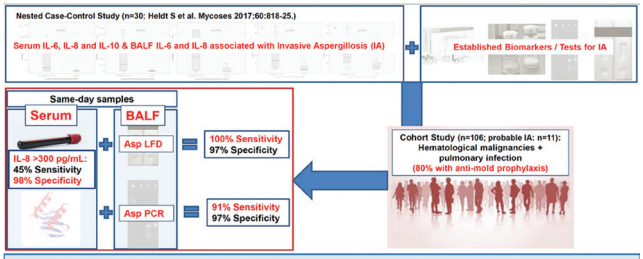
Background. *Aspergillus* spp. induce elevated levels of several cytokines, including Interleukin (IL)-6 and IL-8. It remains unknown whether these cytokines hold value for clinical routine and enhance diagnostic performances of established and novel biomarkers/molecular tests for invasive aspergillosis (IA) in patients receiving mold-active antifungals.

Methods. This cohort study included 106 prospectively enrolled (2014–2017) adult cases with underlying hematological malignancies and suspected pulmonary infection undergoing bronchoscopy. Serum samples were collected within 24 hours of bronchoalveolar lavage fluid (BALF) sampling. Both serum and BALF samples were used to evaluate diagnostic performances of the *Aspergillus*-specific lateral-flow device test (LFD), *Aspergillus* PCR, galactomannan, β-D-glucan, and cytokines that have shown significant associations with IA in our previous matched case–control analysis (including IL-6 and IL-8), for IA classified according to the revised EORTC/MSG criteria.

Results. Among the 106 cases, 11 had probable IA, 32 possible IA, and 63 no evidence for IA; 80% received mold-active antifungals at the time of sampling. Diagnostic tests and biomarkers showed significantly better performance in BALF compared with blood, with the exception of serum IL-8 which was highly specific for IA and proved to be the most reliable blood biomarkers. Combinations of serum IL-8 with either BALF LFD (sensitivity 100%, specificity 94%) or BALF PCR (sensitivity 91%, specificity 97%) were highly sensitive and specific for differentiating probable IA from no IA.

Conclusion. High serum IL-8 levels were highly specific, and when combined with either the BALF *Aspergillus*-specific LFD, or BALF *Aspergillus* PCR also highly sensitive for diagnosis of IA.



Performance of diagnostic tests in serum samples (IL-8, IL-6, LFD, *Aspergillus* PCR, and BDG) and in same-day BALF samples (IL-8, LFD, *Aspergillus* PCR), as well as combinations, for differentiating probable invasive aspergillosis (IA; n=11) versus no evidence for IA (n=63) ordered by Diagnostic Odds Ratios (DOR).

Test/test combination	Sensitivity	Specificity	PPV	NPV	DOR	Positivity in Possible IA / possible IMI cases	Positivity in possible IA / probable IMI cases
Serum IL-8 (>300 pg/mL)	45% (5/11)	98% (62/63)	83% (5/6)	91% (62/68)	51.7 (5.1-518)	8% (2/25)	0% (0/7)
Serum IL-8 (>60 pg/mL)	55% (6/11)	92% (58/63)	55% (6/11)	92% (58/63)	13.9 (3.1-62.2)	16% (4/25)	43% (3/7)
Serum IL-8 (>14 pg/mL)	82% (9/11)	63% (40/63)	28% (9/32)	95% (40/42)	7.8 (1.6-39.4)	48% (12/25)	57% (4/7)
Serum IL-6 (>40 pg/mL)	73% (8/11)	70% (44/63)	30% (8/27)	94% (44/47)	6.2 (1.5-25.9)	20% (5/25)	29% (2/7)
Serum LFD (45 min)	9% (1/11)	97% (61/63)	33% (1/3)	86% (61/71)	3.0 (0.3-36.9)	16% (4/25)	0
Serum BDG (>80 pg/mL)	45% (5/11)	75% (47/63)	24% (5/21)	89% (47/53)	2.4 (0.7-9.1)	4% (1/25)*	86% (6/7)
Blood Aspergillus PCR	0% (0/10)	100% (55/55)	-	85% (55/65)	-	0	0
Serum LFD (15 min)	0% (0/10)	98% (54/55)	-	84% (54/64)	-	4% (1/25)	0
BALF LFD (10 min)	73% (8/11)	95% (60/63)	73% (8/11)	95% (60/63)	53.3 (9.2-310)	0	0
BALF Aspergillus PCR	27% (3/11)	98% (58/59)	75% (3/4)	91% (58/64)	21.8 (2.0-235)	4% (1/24)	0
BALF LFD (15 min)	73% (8/11)	87% (55/63)	50% (8/16)	95% (55/58)	18.3 (4.0-83.8)	12% (3/25)	43% (3/7)
BALF IL-8 (>556 pg/mL)	91% (10/11)	48% (30/63)	23% (10/43)	97% (30/31)	9.1 (1.1-75.3)	52% (13/25)	71% (5/7)
BALF IL-8 (>1000 pg/mL)	73% (8/11)	67% (42/63)	28% (8/29)	93% (42/45)	5.3 (1.3-22.2)	36% (9/25)	43% (3/7)
Serum IL-8 (>300 pg/mL) AND/OR Serum IL-6 (>40 pg/mL)	73% (8/11)	70% (44/63)	30% (8/27)	94% (44/47)	6.2 (1.5-25.9)	20% (5/25)	29% (2/7)
Serum IL-8 (>300 pg/mL) AND/OR BALF LFD (10 min)	100% (11/11)	94% (59/63)	73% (11/15)	100% (59/59)	304 (15.3-6042)	8% (2/25)	0% (0/7)
Serum IL-8 (>300 pg/mL) AND/OR BALF LFD (15 min)	100% (11/11)	86% (54/63)	55% (11/20)	100% (54/54)	132 (7.2-2432)	20% (5/25)	43% (3/7)
Serum IL-8 (>300 pg/mL) AND/OR BALF Aspergillus PCR	91% (10/11)	97% (57/59)	83% (10/12)	98% (57/58)	285 (23.6-3447)	12% (3/25)	0

Disclosures. **G. Johnson**, OLM Diagnostics: Employee, Salary.

2568. Variability in Pediatric Antibiotic Prescribing for Upper Respiratory Illnesses by Provider Specialty

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Background. Antibiotic prescribing varies among providers, contributing to antibiotic resistance and adverse drug reactions.

Objective. To evaluate variation in antibiotic prescribing between pediatric and nonpediatric providers for common upper respiratory illnesses.

Methods. Patient encounters for children aged <18 years from a regional healthcare system were identified. Electronic medical records from 2011 to 2016 were extracted for diagnoses of upper respiratory infection (URI), pharyngitis, acute otitis media (AOM), and sinusitis. Encounters with competing medical diagnoses, recent hospitalization, and antibiotic prescriptions within 30 days were excluded. Adherence to antibiotic guidelines was assessed by provider training (pediatric, nonpediatric physicians, and advance practice providers [APP]). Additional factors assessed included clinic or urgent care setting, calendar year, and patient's age, gender, insurance status, and number of sick visits in the prior year.

Results. Across 6 years, 141,361 visits were examined: 43,914 for URI, 43,701 for pharyngitis, 43,925 for AOM, and 9,821 for sinusitis. Pediatricians were more likely than APPs and nonpediatric providers to have guideline-concordant prescribing for pharyngitis (pediatricians 66.7 (54.5, 77.0)%, nonpediatricians 49.1 (36.3, 62.0)%, APPs 52.2(39.4, 64.7)%, $P < 0.0001$) and sinusitis (pediatricians 70.8(53.8, 83.4)%, nonpediatricians 63.3(46.8, 77.2)%, APPs 62.1(45.1, 76.5)%, $P = 0.48$) and