Title: Metagenomic Sequencing Detects Respiratory Pathogens in Hematopoietic Cellular Transplant Patients

Running Title: mNGS Detects Respiratory Pathogens in HCT Patients

Authors: Langelier, C[1]*; Zinter, MS[2]*; Kalantar, K[3]; Yanik, GA[4]; Christenson, S[5]; Odonovan, B[3]; White, C[3]; Wilson, M[6]; Sapru, A[2]; Dvorak, CC[7]; Miller, S[8]; Chiu, CY[1,8]; DeRisi, JL[3,9]

* Langelier C and Zinter MS share first authorship.

Affiliations:

[1] Division of Infectious Diseases, Department of Medicine, University of California, San Francisco, CA, USA

[2] Division of Critical Care Medicine, Department of Pediatrics, University of California, San Francisco - School of Medicine, San Francisco, CA, USA

[3] Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA, USA

[4] Division of Hematology Oncology, Department of Pediatrics, University of Michigan, Ann Arbor – School of Medicine, Ann Arbor, MI, USA

[5]Division of Pulmonary, Critical Care, Sleep and Allergy, Department of Medicine, University of California, San Francisco, USA

[6] Weill Institute for Neurosciences, Department of Neurology, University of California, San Francisco, San Francisco, CA, USA

[7] Division of Allergy, Immunology, and Blood and Marrow Transplantation, Department of Pediatrics, University of California, San Francisco - School of Medicine, San Francisco, CA, USA

[8] Department of Laboratory Medicine, University of California, San Francisco - School of Medicine, San Francisco, CA, USA

[9] Chan Zuckerberg Biohub, San Francisco CA

Corresponding author:

Charles Langelier, MD, PhD

Division of Infectious Diseases

Department of Medicine

1700 4th St, Room 403

San Francisco CA, 94158

chaz.langelier@ucsf.edu

Funding:

NHLBI K12HL119997 (Langelier C), NICHD K12HD000850, the Pediatric Blood and Marrow Transplant Foundation, the National Marrow Donor Program Amy Strelzer Manasevit Grant (Zinter MS), NHLBI U10HL069330 (Yanik GA), NHLBI K23HL123778 (Christensen S), NINDS K08NS096117 (Wilson, M), NHLBI R01HL114484 (Sapru A), NIAID U54AI082973 (Dvorak CC), NHLBI R01HL105704 and NIAID R21AI120977 (Chiu, CY), NIAID P01AI091575 and the Chan Zuckerberg Initiative (DeRisi JL).

Author Contributions:

Study concept and design: Langelier, Zinter, Sapru, Dvorak, Yanik, DeRisi.

Acquisition of data: Langelier, Zinter.

Analysis and interpretation of data: Langelier, Zinter, Kalantar, White, O'Donovan, Christenson, DeRisi, Miller, Chiu.

Drafting of the manuscript: Langelier, Zinter.

Critical revision of the manuscript for important intellectual content: Langelier, Zinter, Miller, Chiu, DeRisi.

Statistical analysis: Langelier, Zinter, Christenson, Wilson, O'Donovan, Kalantar, DeRisi.

Administrative, technical, or material support: Langelier, Zinter, Sapru, Yanik, Dvorak, DeRisi.

Study supervision: DeRisi, Sapru, Yanik.

Approval of final manuscript: Langelier, Zinter, Kalantar, Yanik, White, O'Brien, Wilson, Miller, Chiu, Sapru, Yanik, Dvorak, DeRisi

Commentary: Lower respiratory tract infections are a leading cause of death in hematopoietic cellular transplant recipients, but the microbiologic etiology of these infections is frequently unknown. The limits of current diagnostics preclude targeted antimicrobial treatments, resulting in excess morbidity and mortality. Metagenomic next-generation sequencing may enable precision diagnosis of respiratory infections in hematopoietic cellular transplant patients by simultaneously detecting microbial pathogens, transcriptional biomarkers of the host response, and the pulmonary microbiome. This approach may facilitate targeted antimicrobial prescription and provide a new method for distinguishing infectious and non-infectious post-transplant respiratory illness.

Conflicts of Interest: None

Descriptor: 10.04 Diagnosis of Infections

Word Count: 3476

ABSTRACT

RATIONALE: Current microbiologic diagnostics often fail to identify the etiology of lower respiratory tract infections (LRTI) in hematopoietic cellular transplant recipients (HCT), which precludes the implementation of targeted therapies.

OBJECTIVES: To address the need for improved LRTI diagnostics, we evaluated the utility of metagenomic next generation sequencing (mNGS) of bronchoalveolar lavage (BAL) to detect microbial pathogens in HCT patients with acute respiratory illnesses.

METHODS: We enrolled 22 post-HCT adults ages 19-69 years with acute respiratory illnesses who underwent BAL at the University of Michigan between January 2012 and May 2013. mNGS was performed on BAL fluid to detect microbes and simultaneously assess the host transcriptional response. Results were compared against conventional microbiologic assays.

MEASUREMENTS & MAIN RESULTS: mNGS demonstrated 100% sensitivity for detecting respiratory microbes (human metapneumovirus, respiratory syncytial virus, *Stenotrophomonas maltophilia,* human herpesvirus 6 and cytomegalovirus) when compared to standard testing. Previously unrecognized LRTI pathogens were identified in six patients for whom standard testing was negative (human coronavirus 229E, human rhinovirus A, *Corynebacterium propinquum* and *Streptococcus mitis)*; findings were confirmed by independent PCR and 16S rRNA sequencing. Relative to patients without infection, patients with infection had increased expression of immunity related genes (p=0.022) and significantly lower diversity of their respiratory microbiome (p=0.017).

CONCLUSIONS: Compared to conventional diagnostics, mNGS enhanced detection of pathogens in BAL fluid from HCT patients. Furthermore, our results suggest that combining unbiased microbial pathogen detection with assessment of host gene biomarkers of immune response may hold promise for enhancing the diagnosis of post-HCT respiratory infections.

Abstract Word Count: 249

Keywords: next generation sequencing, pneumonia, lower respiratory tract infection, bone marrow transplant, transcriptome

INTRODUCTION:

Lower respiratory tract infections (LRTI) are a leading reason for hospitalization and mortality in hematopoietic cellular transplant (HCT) recipients (1, 2). This problem is underscored by autopsy studies showing that previously undetected pulmonary pathogens may contribute to death in 30% of HCT recipients (3, 4). Despite this, the etiologic pathogens remain unidentified in most cases of LRTI, due largely to the limitations of current microbiologic tests in terms of sensitivity, speed and breadth of assay targets (5).

Diagnosis of LRTI in HCT recipients is particularly challenging due to high rates of non-infectious inflammatory conditions such as graft versus host disease (GVHD) that can drive pulmonary inflammation, induce fever and mimic infection (6). Furthermore, the diagnostic yield of traditional tests is reduced in HCT patients due to antimicrobial prophylaxis, reduced antibody titers, and infections from uncommon opportunistic microorganisms (7).

The limitations of current microbiologic tests drive excessive use of empiric broad-spectrum antimicrobials, which potentiates the emergence of drug resistance and increases risk of *Clostridium difficile* infection (8). In some situations, empiric regimens may lack activity against an underlying microbe, leading to treatment failure, disease progression, and consequent adverse outcomes (5, 9). Furthermore, in transplant patients, concern for GVHD may compel clinicians to initiate empiric immunosuppressive agents that could inadvertently exacerbate disease in the presence of an unrecognized infection (10).

Previously, microarray approaches proved useful for broadening the scope of pathogens detectable in a single LRTI assay; some microarrays have even incorporated conserved sequences from all known viruses, allowing detection of novel viral strains (11). Metagenomic next-generation sequencing (mNGS) inherently offers enhanced diagnostic capabilities by providing a culture-independent, comprehensive measurement of the microbial composition of clinical samples (12, 13). This technology permits the simultaneous detection of bacterial, viral and fungal pathogens without introducing bias associated with fixed-target PCR or serologic

assays (11, 12, 14). By capturing both microbial and human RNA, mNGS also permits simultaneous transcriptional profiling of the host immunologic response, which can provide complementary insight regarding the presence and type of infection (15, 16). Due to the clear need for enhanced respiratory pathogen diagnostics in HCT recipients, we undertook this study examining the utility of host/pathogen mNGS for detection of LRTI pathogens in HCT patients hospitalized with acute respiratory illnesses.

MATERIALS AND METHODS:

Subjects:

This retrospective study evaluated sequentially enrolled adult HCT recipients who underwent bronchoscopy with bronchoalveolar lavage (BAL) at the University of Michigan Medical Center between January 25th, 2012 and May 20th, 2013. All patients met established clinical definitions of community-acquired or hospital-acquired pneumonia and had two or more of the following: cough, fever, chills, dyspnea, pleuritic chest pain, crackles, or bronchial breathing; in addition, an opacity, infiltrate or nodules on chest radiograph was required (17-19). In some cases, patients had other equally probable non-infectious explanations for respiratory symptoms. All subjects had routine microbiological testing of BAL fluid as part of standard of care diagnostic workup for respiratory illness. Patients consented to permit banking of surplus BAL specimens in a biorepository at -80°C in accordance with University of Michigan IRB protocol HUM00043287. De-identified samples were then transported to UCSF for mNGS.

Clinical Microbiologic Testing

During the period of study enrollment, standard of care clinical microbiologic diagnostic testing for respiratory pathogens included bacterial, mycobacterial and fungal cultures, CMV culture, *Aspergillus* galactomannan assay, multiplex PCR for influenza A/B, respiratory syncytial

virus (RSV) and human metapneumovirus (HMPV), human herpesvirus-6 (HHV-6) PCR, and silver stain for *Pneumocystis jiroveci*. Additional studies included nasophayngeal (NP) swab PCR for influenza A/B, RSV, and HMPV, blood cultures, serum PCR for herpes simplex virus (HSV) types 1 and 2, varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV) and HHV-6, *Clostridium difficile* toxin stool PCR, and select additional studies on BAL per the clinical judgment of the treating physicians.

Metagenomic Next-Generation Sequencing

Total nucleic acid was extracted from 250µl of patient BAL fluid using bead-based lysis and the Zymo Viral DNA/RNA Kit (Zymo Research). RNA and DNA were isolated separately using DNAse or RNAse, respectively, and the former was reverse transcribed to generate complementary DNA (cDNA). DNA and cDNA then underwent adapter addition and barcoding using the Nextera system (Illumina). Depletion of abundant sequences by hybridization (DASH) was employed to selectively deplete human mitochondrial cDNA, thus enriching for microbial transcripts (20). The final RNAseq and DNA sequencing (DNAseq) libraries underwent 135 nucleotide paired-end Illumina sequencing. Methodology is described further in the online data supplement.

Pathogen Detection Bioinformatics

Detection of microbial pathogens leveraged a custom bioinformatics pipeline to discriminate pathogens from background microbial sequences in clinical samples. This methodology is detailed in the Online Data Supplement (21). Briefly, this analytical approach involved first filtering for quality and complexity, next extracting sequences aligning to the human and Pan troglodytes genomes (NCBI GRC h38 and UCSC PanTro4) using STAR (22) and then subsequently removing reads aligning to non-fungal eukaryotes and phage using

Bowtie2 (23). The identities of the remaining reads were determined by querying the NCBI nucleotide (nt) and non-redundant protein (nr) databases using GSNAP-L.

To mitigate spurious taxonomic assignments due to laboratory background contaminants, we calculated a standard Z-score for each microbial genus relative to a control group of 17 "no-template" water-only controls (detailed in Table E1) derived from sequencing runs performed in our laboratory over the past 2 years (24). Z-scores were calculated based on unique reads aligned to either NCBI nt or nr, per million sequence input reads (rpM_{nt}, rpM_{nr}).

The respiratory tract is not a sterile environment, and as such, we devised a ranking score to aid in the stratification of the inherently complex alignment data. This metric involved multiplying the number of reads aligned per million reads sequenced by the sum of the nt and nr Z-scores for each microbial genus identified in each patient: score = rpM_{nt} x (Z_{nt} + Z_{nr}). To further improve the specificity of microbial assignments, we required 1) microbes to be detected in both nucleotide (nt) and protein (nr) database alignments; 2) microbes to have a Z_{nt} and Z_{nr} > zero and 3) bacteria and fungi detected by RNAseq to also have detectable genomic sequence by DNAseq. In addition, we permitted DNA viruses to be detected on only DNAseq and RNA viruses to be detected on only RNAseq. For each patient, any microbes that passed these requirements were then processed using the following decision tree, summarized in Figure 1.

Definitions

Microbes were considered **confirmed pathogens** if a) both clinical testing and mNGS identified the microbe, b) there existed literature evidence of microbial pathogenicity in the lungs, and c) the microbe score was as least two-fold greater than that of any other microbe of the same type (virus, bacteria or fungus) identified in the patient (17). Microbes were considered **new potential pathogens** if a) mNGS alone identified the microbe, b) there existed literature evidence of microbial pathogenicity in the lungs, and c) the microbe score was two-fold greater than any other microbe of the same type (virus, bacteria or fungus) in the patient. New

potential pathogens were confirmed by independent specific PCR testing for viruses and 16s rRNA gene Sanger sequencing for bacteria. Finally, microbes were considered **unlikely or uncertain pathogens** if they a) lacked robust literature evidence of microbial pathogenicity in the lungs, b) had a score that was less than two-fold that of the top-ranking microbe, suggesting a polymicrobial sample, c) determined to be clinically insignificant by the treatment team, or d) were a DNA virus of uncertain pathogenicity present in low abundance (< 5rpM).

Microbial diversity

Alpha diversity of the respiratory microbiome in each subject was assessed using the Simpson Diversity Index (SDI) and compared between patients with a) confirmed pathogens, b) unlikely or uncertain pathogens and c) new potential pathogens using the nonparametric Wilcoxon Rank Sum test (25).

Host gene expression analyses

RNA transcripts aligning to the human genome were captured by our computational pipeline as described above. Cumulative sum scaling normalization of protein-coding gene transcripts was carried out (26) and genes expressed in fewer than 30% of samples, or as outliers in only 10% of samples, were removed. Using a supervised approach, pathways related to immune functionality were selected *a priori* from the Molecular Signatures Database (27) and compared in terms of total normalized expression between subjects using the nonparametric Wilcoxon Rank Sum test as described in the online data supplement. These pathways contained gene biomarkers associated with antiviral response, interferons α, β, and γ, IL-6/JAK/STAT5 signaling and adaptive immunity, and have all been strongly associated with the immune response to infection (28, 29).

RESULTS*:*

Cohort characteristics and clinical outcomes

We enrolled 22 HCT recipients hospitalized for acute respiratory symptoms aged 19-69 years at a median 356 days post-transplant, as summarized in Table 1. Their most common transplant indications were leukemia (n=12) and lymphoma (n=8). The majority of patients received allogeneic HCT (n=20) and/or myeloablative conditioning (n=20), as summarized in Table E2. The median absolute neutrophil count (ANC) at the time of BAL was 3.8/μL (IQR 2.1-5.4) and engraftment was achieved at the time of BAL in all patients. Acute GVHD was present in three subjects and chronic GVHD was present in 13 subjects. At the time of BAL, 15 subjects were receiving immune modulating agents.

Fever was present in 13 of 22 (59%) of patients, all patients met systemic inflammatory response syndrome criteria, and no patients had septic shock. Twelve patients required supplemental oxygen, three required mechanical ventilation and all survived their illnesses except for one subject who died 10 days after BAL due to lymphoma relapse. Seven of the 22 (32%) subjects died between 51 and 3024 days post-BAL and their primary causes of death were GVHD (n=3), relapsed malignancy (n=2), idiopathic respiratory failure (n=1), and unknown (n=1).

Antimicrobial use

All study subjects received antimicrobial prophylaxis prior to symptom onset and bronchoscopy. Empiric broad spectrum antibiotics were used in 16 of the 22 (73%) patients including vancomycin (n=4), cefepime (n=4), vancomycin + cefepime (n=5), cefepime + tobramycin (n=1), and vancomycin + cefepime + tobramycin (n=1) (Table 1). Pathogen-targeted antimicrobials were used in three subjects (14%). Four patients (15%) received no antimicrobials aside from pre-existing prophylactic agents.

Clinical Microbiologic Findings

Standard of care clinical BAL diagnostics performed at the study hospital (described above in Methods) identified microbes in seven of 22 (32%) patients, of which six were thought to represent etiologic pathogens by the treating physicians. These included HMPV (n=2), RSV (n=2), HHV-6 (n=1) and *Stenotrophomonas maltophilia* (n=1). CMV was identified by shell vial culture in subject 37 but thought to represent incidental carriage because of symptom resolution in the absence of intervention prior to the return of testing.

Next Generation Sequencing Findings

An average of 49 million paired-end sequencing reads were generated from each BAL sample, of which <1% were microbial. Sequencing statistics and pipeline output for each patient are described in Table E3. mNGS identified all seven microbes found by standard clinical diagnostics, demonstrating 100% sensitivity for pathogen detection. In total, RNAseq identified 10 RNA viruses, all of which have established pathogenicity in the lungs, and the RNA intermediates of five DNA viruses that have uncertain pulmonary pathogenicity. DNAseq identified the genomes of these same five DNA viruses as well as five others including CMV and HHV-6, which have been associated with pneumonitis. mNGS captured entire viral genomes for five patients at an average read depth of 3500-fold, as shown in Figure E1. Bacteria known to exist contextually as either pathogens or commensals, including *Stenotrophomonas maltophilia*, *Streptococcus mitis*, and *Corynebacterium propinquum* were identified, as were genera not typically considered pathogenic (30-32). Specificity of mNGS could not be assessed because this cohort lacked patients with proven non-infectious respiratory illnesses.

Relationship Between Respiratory Microbial Diversity and Detection of Pathogens

Loss of diversity within respiratory tract microbial communities has been proposed as an ecological marker of infection (33, 34). We thus evaluated alpha diversity of actively replicating

microbes identified by RNAseq using the SDI and found that subjects with confirmed pathogens had significantly lower diversity relative to patients with only microbes of unlikely or uncertain pathogenicity (0.34, IQR 0.15-0.64, n=6 vs. 0.92, IQR 0.86-0.93, n=10, p=0.017, Figure 2 and Table E4). Reduced diversity was also observed if subjects with confirmed or potential new pathogens were compared together against those without (0.41, IQR 0.20-0.55, n=12 vs. 0.92, IQR 0.86-0.93, n=10, p<0.001).

Analysis of Host Gene Expression

We hypothesized that gene expression from respiratory fluids obtained at the site of infection would distinguish patients with LRTI from those with non-infectious respiratory diseases. To test this idea, we evaluated *a priori* selected gene biomarkers of innate and adaptive immune responses using the total normalized expression of all genes. Given suspected between-subject immunologic response heterogeneity due to differences in infection type and degree of immunologic compromise, we created a composite metric based on the sum normalized gene expression of all biomarkers. We found significantly increased expression in patients with confirmed LRTI pathogens versus those without (94.9, IQR 93.8-105.6, n=6 vs. 33.1, IQR 20.7-75.1, n=7, p=0.022), as shown in Figure 3 and Table E5. When patients with confirmed or potential new pathogens were compared together against all others, the relationship trended towards significance (94.6, IQR 76.5-105.6, n=11 vs. 33.1, IQR 20.7-75.1, n=7, p=0.0634), and reached significance (p = 0.014) if a subject with a relatively lower abundance of HRV-A transcripts was instead grouped with patients found to have microbes of unlikely or uncertain pathogenicity. Raw gene counts are provided in the additional online supplemental data table.

DISCUSSION:

In this proof of concept study, we demonstrate that mNGS can simultaneously detect pathogens and the host response in HCT patients with acute respiratory illnesses. When benchmarked against the conventional standard of care testing performed during the time of study enrollment, mNGS demonstrated 100% sensitivity for microbial detection and permitted identification of potential new viral and bacterial pathogens in six of 15 (40%) subjects with otherwise negative testing. We observed that the presence of respiratory pathogens was characterized by increased expression of host immune response genes and lower airway microbial diversity. Our results suggest that simultaneous mNGS detection of respiratory pathogens, the host's immunologic response and the airway microbiome may provide complementary data that could inform the clinical significance of a detected pathogen.

Amongst the cases with negative clinical testing, mNGS identified six patients harboring transcriptionally active microbes with recognized respiratory pathogenicity (35-40). With respect to viruses, HCOV-229E was identified in two patients and HRV-A was identified in three patients, one of whom was also culture-positive for *Stenotrophomonas maltophilia.* Notably, HRV and HCOV were not represented on the multiplex PCR panel used at the study hospital; similar limited panels are still in use at many hospitals today. Unlike rapid antigen or multiplex PCR assays, mNGS is not limited to a fixed number of pre-specified targets on a panel, obviating the need to order multiple independent diagnostic tests. Furthermore, mNGS can detect entire viral genomes, as illustrated in Figure E1, an attribute that can enable genotyping, detection of resistance mutations, and epidemiologic tracking of disease outbreaks (41).

With respect to previously unidentified bacterial pathogens, mNGS detected *Streptococcus mitis*, an oropharyngeal microbe known to cause bacteremia and acute respiratory distress in HCT recipients, as a potential pulmonary pathogen in one patient (40, 42). *Corynebacterium propriniquum*, one of the few virulent *Corynebacterium* species associated with LRTI, comprised the majority of microbial transcripts in another subject's BAL

fluid (43). Both patients received empiric vancomycin, which has activity against *Streptococcus and Corynebacteria spp.,* and recovered from their acute respiratory illnesses. Findings were independently confirmed at the genus level by 16s rRNA gene sequencing.

Ten patients with negative conventional testing were found to have microbes of unlikely or uncertain pathogenicity. Notably, each of these patients had potential alternative explanations for their respiratory symptoms. While one of these patients had bacteremia/sepsis, the remaining nine had acute and/or chronic GVHD, underlining the importance of non-infectious alloreactive inflammation in post-HCT pulmonary complications.

mNGS identified several viruses with DNA genomes, however only five of these also had well-defined evidence of active replication marked by detectable RNA transcripts (HSV, human papilloma virus and torque teno viruses (TTV, n=3). TTV was the most commonly detected DNA virus, a finding consistent with prior reports demonstrating an increased prevalence of this presumptively innocuous constituent of the human virome in immunosuppressed patients (44). Herpesviridae genomic DNA in the absence of viral transcripts was identified in five patients and included HHV-6 (n=1), CMV (n=2), HSV (n=1) and EBV (n=1). One subject, who died 10 days post-BAL due to relapsed lymphoma, had EBV DNA detected in the setting of EBV viremia. WU polyoma virus was detected by DNA sequencing another case, and while this virus has been associated with respiratory infections in immunocompromised patients, evidence supporting its role as a pathogen is lacking (45).

CMV was identified by both viral culture and mNGS in one subject, however this finding was considered unrelated to the patient's respiratory illness by the treating physicians. Notably, while CMV was detected by DNAseq, no RNA transcripts were identified, suggesting that it may have represented incidental carriage as opposed to a transcriptionally active pathogen (46). This patient was also found to harbor *Pseudomonas fluorescens*, a described colonizer of the human respiratory tract (47), as well as several other taxa representing common constituents of

the oropharyngeal or respiratory microbiome, including *Rothia*, *Prevotella*, and *Actinomyces spp.*, as detailed in Table E3.

Lung microbiome diversity and the host immune response as biomarkers of infection

Because asymptomatic carriage of respiratory pathogens is well described (48, 49), establishing biomarkers of genuine infection is critical for determining whether a given microbiologic finding is clinically significant. Our findings suggest that respiratory tract microbial diversity may be such a biomarker. Specifically, we found that patients with LRTI pathogens had significantly lower alpha diversity versus those without (p=0.017, Figure 2, Table E5), presumably reflecting dominance of actively replicating pathogens (33, 34).

Our results also demonstrate that both mNGS and conventional methods identify clinically significant and insignificant microbes, and emphasize the need to assess the impact of a given microbiologic finding in the context of a patient's immunologic response (5). Expression of a multi-gene immune response metric was significantly increased in patients with confirmed respiratory pathogens relative to those without, suggesting that despite significant and heterogeneous states of immune suppression, HCT recipients still exhibited immunologic biomarkers of active infection (p=0.022, Figure 3).

Amongst subjects with potential new pathogens, we observed that two of the three HRV-A positive subjects demonstrated the lowest expression of this immune response metric, while the remaining subject, who was co-infected with HRV-A and *Stenotrophomonas maltophilia*, had one of the highest values. This is consistent with prior reports demonstrating that HRV can induce a broad range of clinical disease severity, and that viral-bacterial co-infection can increase the severity of disease (48, 49). Amongst subjects with confirmed pathogens, the patient with clinically suspected HHV-6 pneumonitis was notably an outlier in terms of reduced immune metric expression and elevated airway diversity. The respiratory pathogenicity of HHV-6 is controversial, and our paired mNGS pathogen data demonstrating absence of viral

transcripts but detectable genomic DNA support a less virulent role for HHV-6 in this patient (50).

Broadspectrum antibiotic overuse is frequently driven by suspicion for occult pathogens missed by standard diagnostic testing. For instance, patient 1, whose conventional testing identified only HMPV, received empiric vancomycin and cefepime due to suspected occult bacterial infection and subsequently developed *C. difficile* colitis. mNGS confirmed the presence of HMPV and also demonstrated absence of bacterial pathogens in this patient. The theoretical availability of our mNGS findings during the actual period of patient hospitalization potentially could have informed more targeted antimicrobial use in the 18 study subjects who received broad spectrum antibiotics in the absence of detectable bacterial pathogens by mNGS.

While these results are highly encouraging, this proof-of-concept study suggests many routes for further improvement. First, this study was limited by a relatively small sample size, only one case of culture-positive bacterial LRTI, no fungal infections, and no subjects with clinically diagnosed non-infectious airway diseases. Future studies with larger cohorts will be needed to validate the sensitivity and specificity of mNGS for LRTI diagnosis in this population. Second, our limited sequencing depth did not yield the human transcriptome coverage that would be desired for robust differential gene expression analyses, although we were able to rigorously evaluate a composite metric of immunity genes. Future larger studies are needed to identify and validate gene classifiers that can distinguish LRTI from non-infectious airway diseases in HCT recipients.

CONCLUSIONS:

Here we leverage continued improvements in metagenomic sequencing to expand the capabilities of LRTI diagnostics in HCT recipients with acute respiratory illnesses. We demonstrate that compared to current microbial diagnostics, mNGS has a greater capacity for detecting microbes and an ability to couple pathogen detection with simultaneous profiling of the

host response and the airway microbiome. We suggest several ways in which future studies may improve on this proof-of-concept study, including validation in a larger prospective cohort of HCT recipients and other high-risk populations.

TABLE LEGENDS

Table 1: Clinical and Microbiologic Data. Abbreviations: Radiography: B/L, bilateral; LUL, left upper lobe; RUL, right upper lobe. Chest CT was obtained for all subjects except 1, 3, 4, 8, 9 and 14 who received chest X-rays. Antimicrobials: ACV, acyclovir; CIDV cidofovir; CPM, cefepime; FOSC, foscarnet, FLUC, fluconazole; GCV, ganciclovir; MICA, micafungin; POSA, posaconazole; RIBV, ribavirin; TMP-SMZ, trimethoprim-sulfamethoxazole; TOBR, tobramycin; vACV, valacyclovir; VANC, vancomycin; VORI, voriconazole. Respiratory Support: HFNC, high flow nasal cannula; IPPV; invasive positive pressure ventilation; NC, nasal cannula. *Clinicians concluded that CMV in subject 37 was not the principal cause of respiratory disease.

FIGURE LEGENDS

Figure 1. Overview of Pathogen Detection Workflow. Total nucleic acid extracted from BAL fluid of HCT recipients underwent DNA and RNA sequencing. A custom bioinformatics pipeline simultaneously identified pathogens and assayed the human transcriptome. Pathogens meeting inclusion and exclusion criteria were ranked by microbial significance score [Score = rpM_{nt} x (Z_{nt} + Z_{nr})]. To further improve the specificity of microbial assignments: we required 1) microbes to be detected in both nt and nr database alignments, 2) microbes to have a Z_{nt} and Z_{nr} greater than zero, and 3) bacteria and fungi detected by RNAseq to also have detectable genomic sequence by DNAseq. For each patient, any microbes that passed these requirements were then classified as either 1) confirmed pathogens, 2) new potential pathogens or 3) unlikely or uncertain pathogens.

Figure 2. BAL Microbial Diversity is Inversely Associated with Presence of a Transcriptionally Active Respiratory Pathogen. Each data point represents a single patient

for whom the Simpson Diversity Index (SDI) is plotted on the y-axis. Subjects are grouped according to confirmed pathogen (red triangles) vs. unlikely or uncertain pathogen (black circles). Patients with confirmed pathogens had significantly lower diversity relative to patients with only microbes of unlikely pathogenicity (0.34, IQR 0.15-0.64, n=6, vs. 0.92, IQR 0.86-0.93, n=10, p=0.017). Raw data are listed in **Table E4**.

Figure 3. Expression of a Host Immune Response Multi-Gene Metric Correlates with Detection of LRTI Pathogens. Each data point represents a single patient for whom the composite immune response gene metric is plotted on the y-axis. Subjects are grouped according to confirmed pathogen (red triangles) vs. or unlikely or uncertain pathogen (black circles). Patients with confirmed pathogens had significantly higher multi-gene metric relative to patients with only microbes of unlikely pathogenicity (33.1, IQR 20.7-75.1, n=7 vs. 94.9, IQR 93.8-105.6, n=6, p=0.022). Raw data are listed in Table E5.

References

(1) Center for International Blood and Marrow Transplant Research (CIBMTR), National Marrow Donor Program (NMDP), European Blood and Marrow Transplant Group (EBMT), American Society of Blood and Marrow Transplantation (ASBMT), Canadian Blood and Marrow Transplant Group (CBMTG), Infectious Disease Society of America (IDSA), Society for Healthcare Epidemiology of America (SHEA), Association of Medical Microbiology and Infectious Diseases Canada (AMMI), Centers for Disease Control and Prevention (CDC). Guidelines for preventing infectious complications among hematopoietic cell transplant recipients: a global perspective. *Bone Marrow Transplant* 2009;44:453-558.

(2) Zinter MS, Dvorak CC, Spicer A, Cowan MJ, Sapru A. New Insights Into Multicenter PICU Mortality Among Pediatric Hematopoietic Stem Cell Transplant Patients. *Crit Care Med* 2015;43:1986-1994.

(3) Roychowdhury M, Pambuccian SE, Aslan DL, Jessurun J, Rose AG, Manivel JC, Gulbahce HE. Pulmonary complications after bone marrow transplantation: an autopsy study from a large transplantation center. *Arch Pathol Lab Med* 2005;129:366-371.

(4) Kontoyiannis DP, Reddy BT, Torres HA, Luna M, Lewis RE, Tarrand J, Bodey GP, Raad II. Pulmonary candidiasis in patients with cancer: an autopsy study. *Clin Infect Dis* 2002;34:400-403.

(5) Zaas AK, Garner BH, Tsalik EL, Burke T, Woods CW, Ginsburg GS. The current epidemiology and clinical decisions surrounding acute respiratory infections. *Trends Mol Med* 2014;20:579-588.

(6) Panoskaltsis-Mortari A, Griese M, Madtes DK, Belperio JA, Haddad IY, Folz RJ, Cooke KR, American Thoracic Society Committee on Idiopathic Pneumonia Syndrome. An official American Thoracic Society research statement: noninfectious lung injury after hematopoietic stem cell transplantation: idiopathic pneumonia syndrome. *Am J Respir Crit Care Med* 2011;183:1262-1279.

(7) Gartner BC, Kortmann K, Schafer M, Mueller-Lantzsch N, Sester U, Kaul H, Pees H. No correlation in Epstein-Barr virus reactivation between serological parameters and viral load. *J Clin Microbiol* 2000;38:2458.

(8) Brown K, Valenta K, Fisman D, Simor A, Daneman N. Hospital ward antibiotic prescribing and the risks of Clostridium difficile infection. *JAMA Intern Med* 2015;175:626-633.

(9) Fridkin S, Baggs J, Fagan R, Magill S, Pollack LA, Malpiedi P, Slayton R, Khader K, Rubin MA, Jones M, Samore MH, Dumyati G, Dodds-Ashley E, Meek J, Yousey-Hindes K, Jernigan J, Shehab N, Herrera R, McDonald CL, Schneider A, Srinivasan A, Centers for Disease Control and Prevention (CDC). Vital signs: improving antibiotic use among hospitalized patients. *MMWR Morb Mortal Wkly Rep* 2014;63:194-200.

(10) Wilson MR, Naccache SN, Samayoa E, Biagtan M, Bashir H, Yu G, Salamat SM, Somasekar S, Federman S, Miller S, Sokolic R, Garabedian E, Candotti F, Buckley RH, Reed KD, Meyer TL, Seroogy CM, Galloway R, Henderson SL, Gern JE, DeRisi JL, Chiu CY. Actionable Diagnosis of Neuroleptospirosis by Next-Generation Sequencing. *N Engl J Med* 2014.

(11) Kistler A, Avila PC, Rouskin S, Wang D, Ward T, Yagi S, Schnurr D, Ganem D, DeRisi JL, Boushey HA. Pan-viral screening of respiratory tract infections in adults with and without asthma

reveals unexpected human coronavirus and human rhinovirus diversity. *J Infect Dis* 2007;196:817-825.

(12) Yozwiak NL, Skewes-Cox P, Gordon A, Saborio S, Kuan G, Balmaseda A, Ganem D, Harris E, DeRisi JL. Human enterovirus 109: a novel interspecies recombinant enterovirus isolated from a case of acute pediatric respiratory illness in Nicaragua. *J Virol* 2010;84:9047-9058.

(13) Chiu CY. Viral pathogen discovery. *Curr Opin Microbiol* 2013;16:468-478.

(14) Greninger AL, Runckel C, Chiu CY, Haggerty T, Parsonnet J, Ganem D, DeRisi JL. The complete genome of klassevirus - a novel picornavirus in pediatric stool. *Virol J* 2009;6:82-422X-6-82.

(15) Tsalik EL, Henao R, Nichols M, Burke T, Ko ER, McClain MT, Hudson LL, Mazur A, Freeman DH, Veldman T, Langley RJ, Quackenbush EB, Glickman SW, Cairns CB, Jaehne AK, Rivers EP, Otero RM, Zaas AK, Kingsmore SF, Lucas J, Fowler VG,Jr, Carin L, Ginsburg GS, Woods CW. Host gene expression classifiers diagnose acute respiratory illness etiology. *Sci Transl Med* 2016;8:322ra11.

(16) Zaas AK, Chen M, Varkey J, Veldman T, Hero AO,3rd, Lucas J, Huang Y, Turner R, Gilbert A, Lambkin-Williams R, Oien NC, Nicholson B, Kingsmore S, Carin L, Woods CW, Ginsburg GS. Gene expression signatures diagnose influenza and other symptomatic respiratory viral infections in humans. *Cell Host Microbe* 2009;6:207-217.

(17) Jain S, Self WH, Wunderink RG, Fakhran S, Balk R, Bramley AM, Reed C, Grijalva CG, Anderson EJ, Courtney DM, Chappell JD, Qi C, Hart EM, Carroll F, Trabue C, Donnelly HK, Williams DJ, Zhu Y, Arnold SR, Ampofo K, Waterer GW, Levine M, Lindstrom S, Winchell JM,

Katz JM, Erdman D, Schneider E, Hicks LA, McCullers JA, Pavia AT, Edwards KM, Finelli L, CDC EPIC Study Team. Community-Acquired Pneumonia Requiring Hospitalization among U.S. Adults. *N Engl J Med* 2015;373:415-427.

(18) Musher DM, Thorner AR. Community-acquired pneumonia. *N Engl J Med* 2014;371:1619-1628.

(19) Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, Dowell SF, File TM,Jr, Musher DM, Niederman MS, Torres A, Whitney CG, Infectious Diseases Society of America, American Thoracic Society. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis* 2007;44 Suppl 2:S27-72.

(20) Gu W, Crawford ED, O'Donovan BD, Wilson MR, Chow ED, Retallack H, DeRisi JL. Depletion of Abundant Sequences by Hybridization (DASH): using Cas9 to remove unwanted high-abundance species in sequencing libraries and molecular counting applications. *Genome Biol* 2016;17:41-016-0904-5.

(21) Doan T, Wilson MR, Crawford ED, Chow ED, Khan LM, Knopp KA, O'Donovan BD, Xia D, Hacker JK, Stewart JM, Gonzales JA, Acharya NR, DeRisi JL. Illuminating uveitis: metagenomic deep sequencing identifies common and rare pathogens. *Genome Med* 2016;8:90-016-0344-6.

(22) Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 2013;29:15-21.

(23) Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012;9:357-359.

(24) Strong MJ, Xu G, Morici L, Splinter Bon-Durant S, Baddoo M, Lin Z, Fewell C, Taylor CM, Flemington EK. Microbial contamination in next generation sequencing: implications for sequence-based analysis of clinical samples. *PLoS Pathog* 2014;10:e1004437.

(25) Simpson E. Measurement of Diversity. *Nature* 1949;163:688.

(26) Paulson JN, Stine OC, Bravo HC, Pop M. Differential abundance analysis for microbial marker-gene surveys. *Nat Methods* 2013;10:1200-1202.

(27) Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst* 2015;1:417-425.

(28) McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. *Nat Rev Immunol* 2015;15:87-103.

(29) Jones SA. Directing transition from innate to acquired immunity: defining a role for IL-6. *J Immunol* 2005;175:3463-3468.

(30) Diez-Aguilar M, Ruiz-Garbajosa P, Fernandez-Olmos A, Guisado P, Del Campo R, Quereda C, Canton R, Meseguer MA. Non-diphtheriae Corynebacterium species: an emerging respiratory pathogen. *Eur J Clin Microbiol Infect Dis* 2013;32:769-772.

(31) Deletoile A, Decre D, Courant S, Passet V, Audo J, Grimont P, Arlet G, Brisse S. Phylogeny and identification of Pantoea species and typing of Pantoea agglomerans strains by multilocus gene sequencing. *J Clin Microbiol* 2009;47:300-310.

(32) Maraki S, Papadakis IS. Rothia mucilaginosa pneumonia: a literature review. *Infect Dis (Lond)* 2015;47:125-129.

(33) Abreu NA, Nagalingam NA, Song Y, Roediger FC, Pletcher SD, Goldberg AN, Lynch SV. Sinus microbiome diversity depletion and Corynebacterium tuberculostearicum enrichment mediates rhinosinusitis. *Sci Transl Med* 2012;4:151ra124.

(34) Dickson RP, Erb-Downward JR, Prescott HC, Martinez FJ, Curtis JL, Lama VN, Huffnagle GB. Analysis of culture-dependent versus culture-independent techniques for identification of bacteria in clinically obtained bronchoalveolar lavage fluid. *J Clin Microbiol* 2014;52:3605-3613.

(35) Ison MG, Hayden FG, Kaiser L, Corey L, Boeckh M. Rhinovirus infections in hematopoietic stem cell transplant recipients with pneumonia. *Clin Infect Dis* 2003;36:1139-1143.

(36) Hakki M, Rattray RM, Press RD. The clinical impact of coronavirus infection in patients with hematologic malignancies and hematopoietic stem cell transplant recipients. *J Clin Virol* 2015;68:1-5.

(37) Bernard K. The genus corynebacterium and other medically relevant coryneform-like bacteria. *J Clin Microbiol* 2012;50:3152-3158.

(38) Motomura K, Masaki H, Terada M, Onizuka T, Shimogama S, Furumoto A, Asoh N, Watanabe K, Oishi K, Nagatake T. Three adult cases with Corynebacterium propinquum respiratory infections in a community hospital. *Kansenshogaku Zasshi* 2004;78:277-282.

(39) Furumoto A, Masaki H, Onidzuka T, Degawa S, Yamaryo T, Shimogama S, Watanabe K, Oishi K, Nagatake T. A case of community-acquired pneumonia caused by Corynebacterium propinquum. *Kansenshogaku Zasshi* 2003;77:456-460.

(40) Marrie TJ. Bacteremic community-acquired pneumonia due to viridans group streptococci. *Clin Invest Med* 1993;16:38-44.

(41) Sherry NL, Porter JL, Seemann T, Watkins A, Stinear TP, Howden BP. Outbreak investigation using high-throughput genome sequencing within a diagnostic microbiology laboratory. *J Clin Microbiol* 2013;51:1396-1401.

(42) Tunkel AR, Sepkowitz KA. Infections caused by viridans streptococci in patients with neutropenia. *Clin Infect Dis* 2002;34:1524-1529.

(43) Bernard K. The genus corynebacterium and other medically relevant coryneform-like bacteria. *J Clin Microbiol* 2012;50:3152-3158.

(44) Focosi D, Maggi F, Albani M, Macera L, Ricci V, Gragnani S, Di Beo S, Ghimenti M, Antonelli G, Bendinelli M, Pistello M, Ceccherini-Nelli L, Petrini M. Torquetenovirus viremia kinetics after autologous stem cell transplantation are predictable and may serve as a surrogate marker of functional immune reconstitution. *J Clin Virol* 2010;47:189-192.

(45) Babakir-Mina M, Ciccozzi M, Perno CF, Ciotti M. The human polyomaviruses KI and WU: virological background and clinical implications. *APMIS* 2013;121:746-754.

(46) Machado CM, Dulley FL, Boas LS, Castelli JB, Macedo MC, Silva RL, Pallota R, Saboya RS, Pannuti CS. CMV pneumonia in allogeneic BMT recipients undergoing early treatment of pre-emptive ganciclovir therapy. *Bone Marrow Transplant* 2000;26:413-417.

(47) Scales BS, Dickson RP, LiPuma JJ, Huffnagle GB. Microbiology, genomics, and clinical significance of the Pseudomonas fluorescens species complex, an unappreciated colonizer of humans. *Clin Microbiol Rev* 2014;27:927-948.

(48) Langelier C, Christenson SA. An Expression of Clinical Significance: Exploring the Human Genome to Understand the Variable Response to Rhinovirus. *Am J Respir Crit Care Med* 2016;193:710-712.

(49) Heinonen S, Jartti T, Garcia C, Oliva S, Smitherman C, Anguiano E, de Steenhuijsen Piters WA, Vuorinen T, Ruuskanen O, Dimo B, Suarez NM, Pascual V, Ramilo O, Mejias A. Rhinovirus Detection in Symptomatic and Asymptomatic Children: Value of Host Transcriptome Analysis. *Am J Respir Crit Care Med* 2016;193:772-782.

(50) Zerr DM. Human herpesvirus 6 (HHV-6) disease in the setting of transplantation. *Curr Opin Infect Dis* 2012;25:438-444.

Table 1. Clinical and Microbiologic Data

Patient ID	Symptoms and Duration	Chest Radiography	Standard BAL Microbiology	Top Scoring Microbes by mNGS	Non-BAL Microbiology	Antimicrobial Prophylaxis	Antimicrobial Treatment	GVHD	Respiratory Support	Cause of death
Pathogens identified by conventional and NGS diagnostics										
1	Fever, hypoxia	x 3 days	RLL, LLL infiltrate	*HMPV*	*HMPV*	HMPV (NP), C. *difficile* (stool)	ACV, LEVO, MICA, TMP-SMZ	CFPM, VANC	aGVHD, cGVHD	NC	Relapse, day 253
5	Cough, dyspnea, fever	x 3 days	Multifocal nodules, bronchiectasis	*Stenotrophomonas maltophila*	*S. maltophila* + *HRV-A*	Negative	ACV, LEVO, TMP-SMZ, VORI	TMP-SMZ, VANC	aGVHD, cGVHD	NC	GVHD, day 462
8	Dyspnea	x 12 days	B/L GGO	*RSV*	*RSV*	CMV (blood)	ACV, LEVO, PENT, POSA	CIDV,FOSC, GCV, VANC, (IVIG)	No	HFNC	Relapse, day 265
9	Cough	x 3 days	RUL, RML, LUL GGO, nodules	*Negative*	*HMPV*	HMPV (NP)	ACV, TMP-SMZ	None	cGVHD	None	alive
10	Cough, fever	x 4 days	Bibasilar airspace disease	*RSV*	*RSV*	RSV (NP)	FLUC, LEVO, vACV	CFPM, RIBV, (IVIG)	aGVHD, cGVHD	NC	GVHD, day 3024
36	Dyspnea, fever	x 1 day	Diffuse septal thickening, GGO	*HHV-6*	*HHV-6*	HHV-6 (blood)	ACV, FLUC, LEVO, VORI	FOSC	No	None	alive
New potential pathogens identified by NGS										
6	Cough, dyspnea	x 3 days	B/L nodules	*Negative*	*HCOV-229E*	Negative	TMP-SMZ, vACV, VORI	CFPM, TOBR, VANC	cGVHD	None	alive
7	Dyspnea	x 1 day	B/L perihilar opacities	*Negative*	*HCOV-229E*	Negative	ACV, FLUC, LEVO, TMP-SMZ	CFPM, VANC, (IVIG)	aGVHD, cGVHD	IPPV x 4 days	GVHD, day 96
13	Cough, dyspnea	x 2 days	LLL opacities, air trapping	*Negative*	*Corynebacterium propiniquum*	Negative	ACV, FLUC, LEVO, TMP-SMZ	CFPM, VANC	No	IPPV x 3 days	Idiopathic respiratory failure, day 51
14	Cough, fever	x 2 days	LLL opacities	*Negative*	*HRV-A*	Negative	TMP-SMZ	None	cGVHD	None	alive
18	Cough, fever	x 2 days	RML, RLL atelectasis	*Negative*	*HRV-A*	*S. epidermidis* (blood)	FLUC, LEVO, vACV	CFPM, VANC	n/a	None	alive
19	Dyspnea	x 4 wks	RUL, LUL reticular opacities	*Negative*	*Streptococcus mitis*	Negative	ACV, LEVO, VORI	VANC	aGVHD, cGVHD	None	alive
Microbes of uncertain or unlikely pathogenicity										
3	Fever	x 2 days	B/L infiltrates	*Negative*	*Flavobacterium sp.*	Gram positive rod (blood)	ACV, LEVO, TMP-SMZ, VORI	VANC	No	None	GVHD, day 267
11	Cough, fever	x 2 days	LLL opacities	*Negative*	*Negative*	*Negative*	ACV, FLUC, LEVO, TMP-SMZ	CFPM	aGVHD, cGVHD	None	alive
22	Dyspnea, rigors	x 1 day	RUL, LUL peribronchial thickening, GG	*Negative*	*Torque teno virus*	Negative	ACV, LEVO, TMP-SMZ, VORI	CFPM	cGVHD	NC	alive
23	Cough , dyspnea, fever	x 3 wks	B/L GG, lymphadenopathy	*Negative*	*Negative*	EBV viremia	ACV, LEVO, MICA, TMP-SMZ, VORI	CFPM	aGVHD	IPPV x 10 days (until death)	No Autopsy, day 10
24	Dyspnea, fever	x 1 wk	B/L perivascular and airspace GG	*Negative*	*Pyrenophora sp.*	Negative	ACV, TMP-SMZ, VORI	CFPM, TOBR	aGVHD, cGVHD	NC	alive
25	Cough, dyspnea, fever	x 4 wks	Diffuse bronchiectasis, multifocal GG nodules	*Negative*	*WU Polyoma Virus*	*Negative*	LEVO, VORI, TMP-SMZ	VANC	aGVHD, cGVHD	NC	alive
31	Dyspnea	x 2 wks	B/L GG, bronchiectasis	*Negative*	*Prevotella*	Negative	ACV, FLUC, LEVO, TMP-SMZ	CFPM	cGVHD	NC	alive
34	Cough, dyspnea	x 2 wks	Multifocal nodules, opacities, air trapping	*Negative*	*Torque teno virus*	Negative	ACV, TMP-SMZ	None	cGVHD	None	alive
35	Cough, dyspnea	x 2 days	Multifocal GG, tree-in-bud opacities	*Negative*	*Negative*	Negative	ACV, FLUC, LEVO	VANC	aGVHD, cGVHD	NC	alive
37	Cough, dyspnea	x 8 days	B/L GG	*CMV**	*CMV**	Negative	TMP-SMZ	None	aGVHD, cGVHD	None	Alive

Figure 1

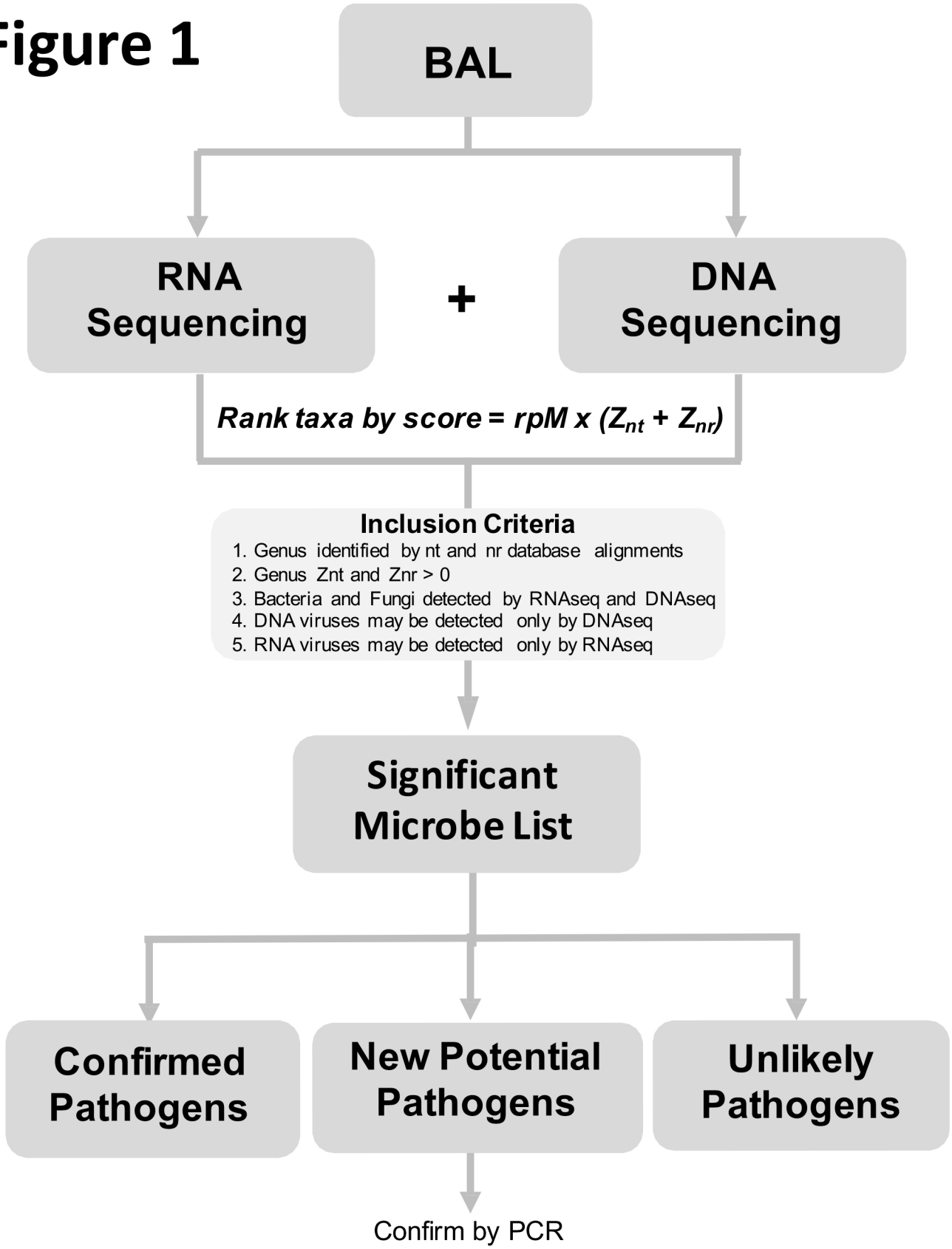

BAL
RNA Sequencing
+
DNA Sequencing
Rank taxa by score = rpM x (Z_{nt} + Z_{nr})
Inclusion Criteria
1. Genus identified by nt and nr database alignments
2. Genus Znt and Znr > 0
3. Bacteria and Fungi detected by RNAseq and DNAseq
4. DNA viruses may be detected only by DNAseq
5. RNA viruses may be detected only by RNAseq
Significant Microbe List
Confirmed Pathogens
New Potential Pathogens
Unlikely Pathogens
Confirm by PCR

Figure 2

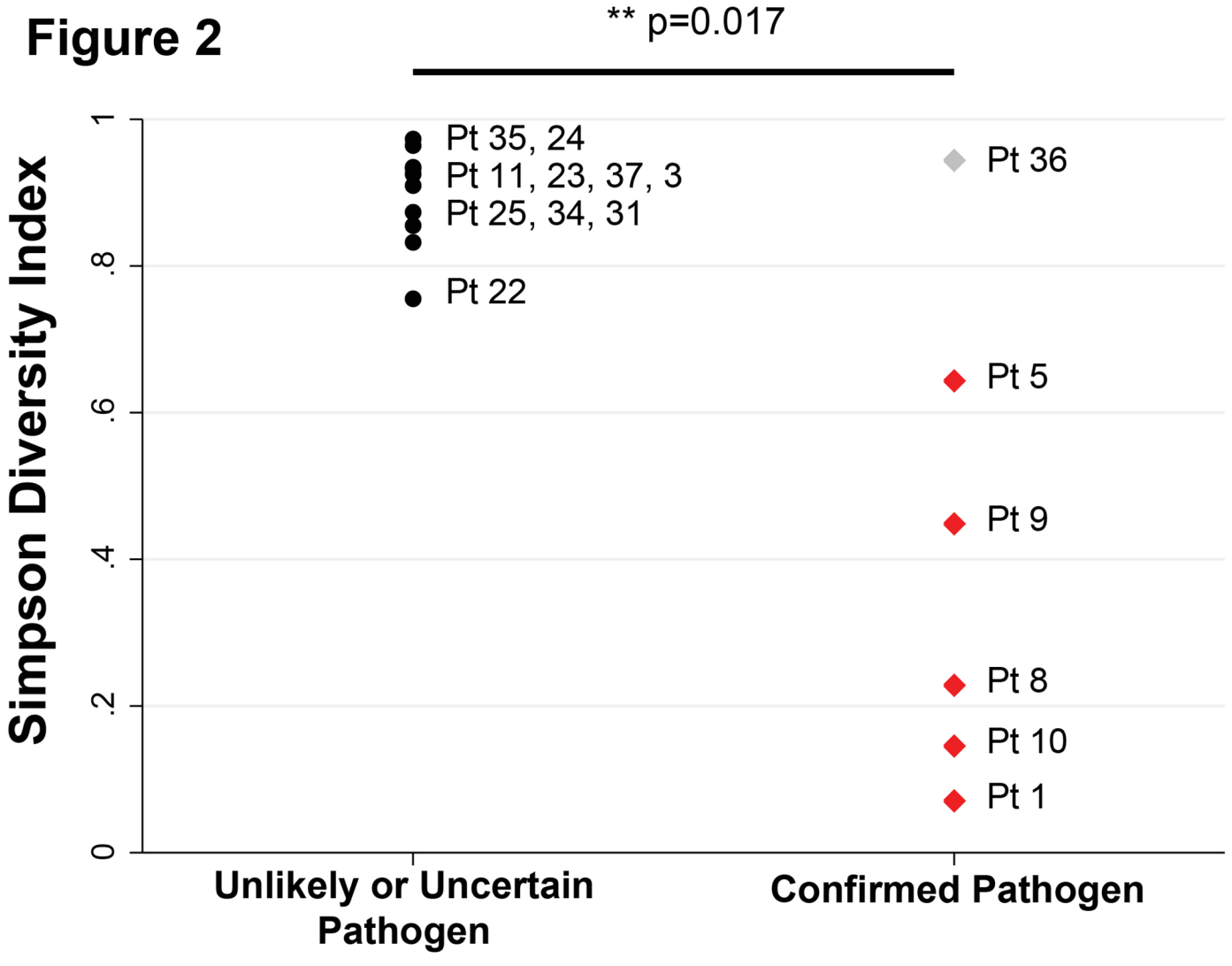
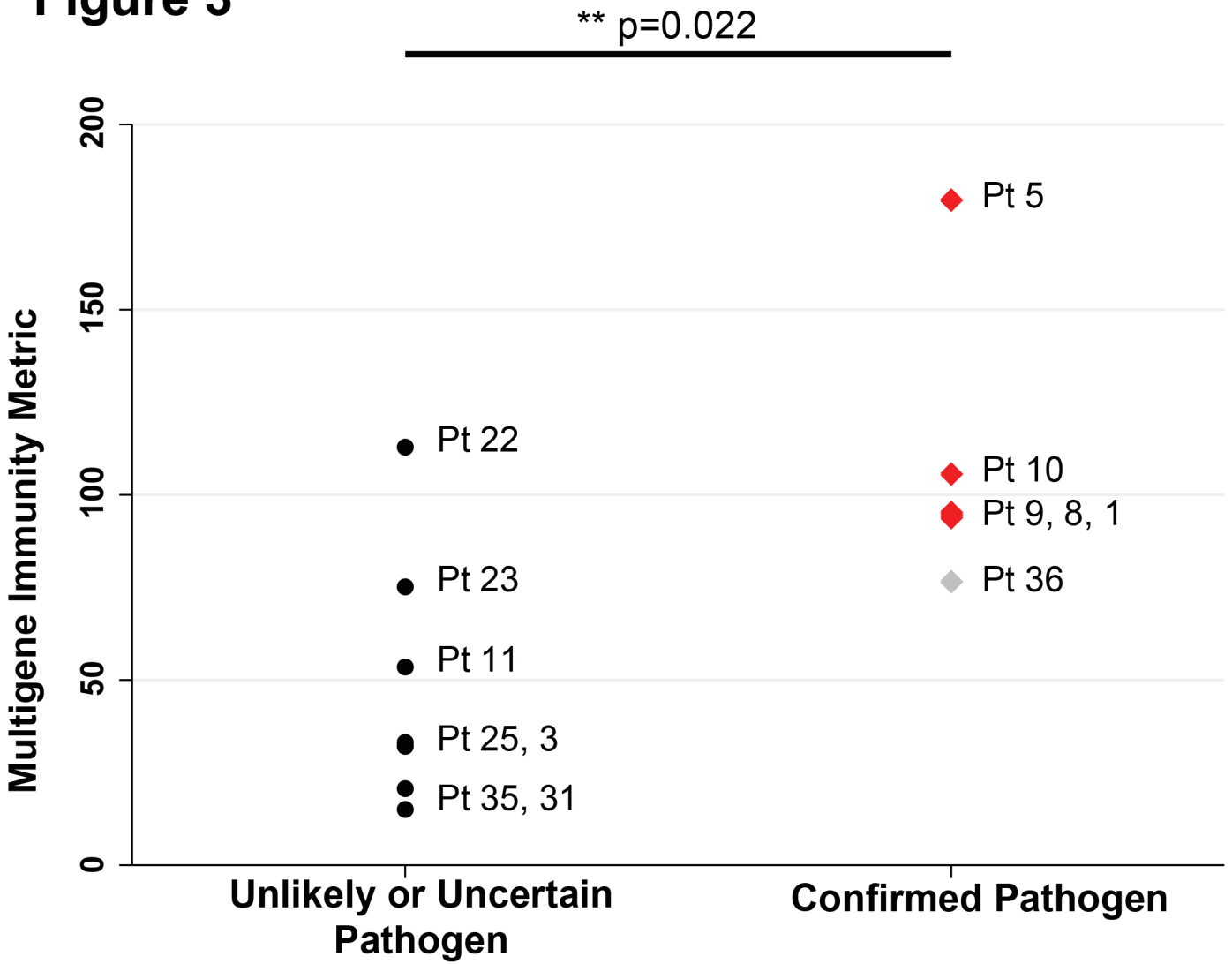

** p=0.017
Simpson Diversity Index
0
.2
.4
.6
.8
1
Pt 35, 24
Pt 11, 23, 37, 3
Pt 25, 34, 31
Pt 22
Pt 36
Pt 5
Pt 9
Pt 8
Pt 10
Pt 1
Unlikely or Uncertain Pathogen
Confirmed Pathogen

Figure 3


** p=0.022
Multigene Immunity Metric
200
150
100
50
0
Pt 5
Pt 22
Pt 10
Pt 9, 8, 1
Pt 36
Pt 23
Pt 11
Pt 25, 3
Pt 35, 31
Unlikely or Uncertain Pathogen
Confirmed Pathogen