Basic Category
IFI35 Modulates Early Inflammatory Responses During H5N1 Influenza Infection.

Anshu P. Gounder1,2, Christine C. Yokoyama2,3, Herbert W. Virgin1,2,3, Adrianus C. Boon1,2,3

1Department of Molecular Microbiology, Washington University School of Medicine; 2Department of Medicine, Washington University School of Medicine; 3Department of Pathology and Immunology, Washington University School of Medicine

**Introduction:** The outcome of Influenza A virus (IAV) infection depends on a complex set of interactions between the virus and the host response to infection. Induction of interferon and cytokine/chemokine responses by infected cells aids in limiting viral replication and spread. However, uncontrolled interferon or inflammatory cytokine responses can have potent immuno-modulatory effects that may exacerbate disease. We previously identified interferon-induced protein 35 (IFI35) as a candidate host gene associated with increased disease severity and susceptibility to highly pathogenic H5N1 IAV using a genome-wide linkage analysis of resistant and susceptible mouse strains.

**Methods:** To understand the role of IFI35 in influenza infection and disease progression, we intranasally challenged congenic strains of IFI35−/− and wild-type C57Bl/6N (WT) mice with H5N1 IAV and assessed changes in disease parameters such as morbidity, viral burden, immune cell infiltration, and cytokine and chemokine induction.

**Results:** For the first time, we show that IFI35 deficient mice have reduced morbidity and increased recovery following peak weight loss compared to WT mice, confirming that IFI35 expression exacerbates influenza disease. At early time-points following infection, IFI35−/− mice had reduced immune cell infiltration into the lungs compared to WT mice, despite similar viral burden during the entire course of infection. This suggests that IFI35 is not acting as an anti-viral molecule during H5N1 infection *in vivo*, but may act on host innate immune responses to IAV infection. We found a significant reduction in specific inflammatory cytokines and chemokines (IL-12p40, CXCL1, CCL2, and G-CSF) in IFI35−/− mice at early time-points post-infection, indicating that IFI35 modulates early inflammatory responses. Interestingly, a majority of the cytokine IL-12p40 in bronchoalveolar lavage fluid from H5N1 infected mice was found to be in a homodimer (IL-12p402) form, which has not been previously reported for IAV infection. IL-12p402 is a potent chemoattractant for macrophages, dendritic cells, and inflammatory monocytes and stimulates nitric oxide production, which can exacerbate disease during H5N1 infection.

**Conclusions:** Our *in vivo* data suggests that IFI35 acts to exaggerate the innate immune response leading to increased immune cell infiltration into the lung and inappropriate induction of cytokine and chemokine responses, which both contribute to morbidity and increased disease severity during H5N1 IAV infection. We are currently characterizing how IFI35 modulates IL-12p40 production after IAV infection. These initial studies are the basis for further investigation into the mechanism of IFI35 mediated regulation and enhancement of inflammatory cytokines during H5N1 infection.
Abstract B2

**Influenza A Virus Causes Chronic Inflammatory Lung Disease Linked to Acute but not Chronic Viral Clearance**

Shamus P Keeler\(^1\), Eugene Agapov\(^1\), Rose Tidwell\(^1\), Michael E. Hinojosa\(^1\), Anand C. Patel\(^{1,2}\), Slobodan Paessler\(^3\), Jeffrey J. Atkinson\(^1\), and Michael J. Holtzman\(^{1,3}\)

\(^1\)Pulmonary and Critical Care Medicine, Department of Medicine, \(^2\)Department of Pediatrics, and \(^3\)Department of Cell Biology, Washington University School of Medicine, St. Louis, MO, and \(3\)Galveston National Laboratory, Department of Pathology, and Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX

**Abstract:** Clinical and experimental observations suggest that the development of chronic lower respiratory disease is linked to respiratory viral infection but there is no experimental model that establishes the long-term aspect of this relationship using a potent human pathogen. Here we use influenza A virus (IAV) to develop a model for chronic lung disease that progresses long after initial infection. We show that IAV (A/WS/33 strain) triggers chronic lung disease that is dependent on the severity of infection and viral level during the acute illness and is manifested by long-term airway mucus production and hyperreactivity along with persistently transcribed IAV in wild-type mice (C56BL/6 strain). The chronic mucus and IAV are localized to focal areas of residual inflammation within the lung. These inflammatory foci and airway hyperreactivity persist for at least 26 weeks after viral inoculation, whereas IAV is no longer detectable by this time. In mice that were reconstituted for deficient Mx1 function and given an increased IAV inoculum to match the acute illness manifested in wild-type mice, we found marked attenuation of chronic disease that was associated with lower lung levels of IAV at 12 days but similar levels at 49 days post-inoculation. Infection with a separate IAV strain (CA/2009) and species (ferrets) also resulted in long-term inflammatory lung disease. The results establish the capacity of a respiratory virus that causes acute illness in humans to also trigger chronic lower respiratory disease. The disease process features acute severity and chronic airway inflammation, hyperreactivity, and mucus production that are linked to acute viral level and clearance and therefore appears applicable to humans with chronic lower respiratory diseases such as asthma and COPD.
Abstract B3

**Structural Determinants of CLCA1 Activity In Inflammatory Lung Disease**

Kayla N. Berry\(^1,2\), Michael J. Holtzman\(^2,3\), and Tom J. Brett\(^2,3,4\)

Medical Scientist Training Program and Immunology Program\(^1\), Pulmonary and Critical Care Medicine, Department of Internal Medicine\(^2\), Cell Biology and Physiology\(^3\), and Biochemistry and Molecular Biophysics\(^4\), Washington University School of Medicine, St. Louis, Missouri 63110

**Introduction:** CLCA1 (calcium-activated chloride channel regulator 1) is a secreted regulator of the calcium-activated chloride channel TMEM16A. CLCA1 expression in airway epithelial cells drives mucus overproduction through unknown mechanisms. CLCA1 contains an N-terminal metalloprotease domain that is responsible for self-cleavage into two fragments (N-terminal fragment and C-terminal fragment). It is unknown how the metalloprotease domain activity is regulated and how self-cleavage contributes to mucus overproduction.

**Methods:** To understand the role of CLCA1 self-cleavage in mucus overproduction and how CLCA1 metalloprotease activity is regulated, we have initiated cellular and structural studies. In functional studies, NCI H-292 lung mucoepidermoid carcinoma cells were transiently transfected with wild-type CLCA1, a protease mutant (E157Q) and a cleavage site sequence mutant. Transcript levels of MUC5AC, the major mucin expressed in the lung, and TMEM16A were then assessed. In structural studies, we have employed disulfide mapping to determine the specific disulfide linkages in the N-terminal fragment of CLCA1. The N-terminal fragment of the protease mutant (N-terminal E157Q) was purified from HEK-293Fs and prepared for mass-spectrometry, either in oxidizing or reducing conditions. Comparison of the spectra from both conditions will allow us to determine the disulfide bonds in the cysteine-rich region.

**Results:** We have initiated studies using an airway epithelial cell line to understand the role of self-cleavage in CLCA1 driving mucus overproduction, to identify which fragment drives mucus overproduction, and to determine whether CLCA1 activation of TMEM16A is involved. Preliminary results indicate that, compared to wild-type CLCA1, protease and cleavage sequence mutants induce fewer MUC5AC transcripts. In addition, we have initiated structural analysis of the CLCA1 metalloprotease domain by mass spectroscopy disulfide mapping to develop a model to understand how metalloprotease activity is regulated and to guide structure determination studies by X-ray crystallography.

**Conclusions:** The current preliminary results suggest that CLCA1 protease activity and self-cleavage is necessary for driving MUC5AC expression. We are currently assessing whether the N-terminal or C-terminal fragment, is required
Secreted Human Clca1 Activates Calcium-Dependent Chloride Currents Through Direct Binding Of Its Vwa Domain With An Extracellular Loop Of Tmem16a/Anoctamin 1.

Zeynep Yurtsever1,2, Monica Sala-Rabanal3,4, Colin G. Nichols3,4 and Tom J. Brett2,3,5,6

Biochemistry Program1, Departments of Internal Medicine2, Cell Biology and Physiology3, Center for Investigation of Membrane Excitability Diseases4, Division of Pulmonary and Critical Care3, and Department of Biochemistry and Molecular Biophysics5, Washington University School of Medicine, St. Louis, Missouri 63110

Introduction: The calcium-activated chloride channel (CaCC) regulator (CLCA) proteins were so named because their expression leads to generation of calcium-dependent chloride currents (I_{CaCC}) in mammalian cells; however, the molecular identity of the channel(s) mediating these currents, and the mechanisms through which CLCA1 regulates their activity, have remained unknown. Previously, we showed that 1) human CLCA1 is a secreted self-cleaving zincin metalloprotease, 2) the resulting N-terminal fragment increases I_{CaCC} density in HEK293T cells in a paracrine fashion, 3) these I_{CaCC} are carried by TMEM16A/Anoctamin 1, and 4) CLCA1 physically interacts with, and drives the surface expression of, TMEM16A (J. Biol. Chem. 2012;287:42138, and eLife 2015;4:e05875).

Methods: To gain deeper mechanistic insight into how CLCA1 activates I_{CaCC} through TMEM16A, we undertook functional studies using live cell imaging, direct interaction analysis by BLI, and whole cell patch clamp assays.

Results: Here, we demonstrate that a Von Willebrand factor type A (VWA) domain within the CLCA1 N-terminus is the minimum requirement for activation of TMEM16A-mediated currents. Thus, I_{CaCC} with the biophysical hallmarks of TMEM16A were measured in HEK293T cells transfected with N-terminal CLCA1 (N-CLCA1) protein constructs containing the VWA domain, and in the same cells the surface expression of TMEM16A was notably increased. Those effects were not observed in mock-transfected cells, or in cells transfected with VWA-less N-CLCA1 constructs. Our live-cell flow cytometry binding assays, confocal microscopy data and whole-cell patch clamp recordings indicate that there is a rapid, direct interaction between the VWA domain of CLCA1 and the last extracellular loop of TMEM16A, and that the conserved metal-ion-dependent adhesion site (MIDAS) motif within the VWA domain, usually responsible for stabilizing protein-protein interactions, is not involved in CLCA1-TMEM16A interactions.

Conclusions: CLCA1 is the first secreted direct mediator of TMEM16A activity, and our studies suggest that CLCA1 and TMEM16A operate together to generate I_{CaCC} in multiple tissues.
Normal autophagy activity is maintained in human airway epithelial cells from severe asthmatics

A.C. Burks, A.D. Naylor, J. Haspel, M. Castro, J.D. Dickinson, T.S. Stappenbeck, M.J. Holtzman, S.L. Brody

1Washington University School of Medicine, St. Louis, MO, US, 2University of Nebraska School of Medicine, Omaha, NE, US

Background: Asthma is characterized by abnormal airway epithelium, with heightened inflammation, goblet cell metaplasia, and increased mucus secretion. These elements are intensified during exacerbations often due to respiratory virus infection and are driven by IL-13. We previously showed that IL-13 activates autophagy and is required for mucus secretion in normal human airway epithelial cells. Studies have linked autophagy to the pathogenesis of asthma but the precise mechanisms are unknown. Autophagy directs targeted cytosolic material via double-membrane autophagosomes to lysosomes for degradation, and is increased in lung tissues affected by COPD and cystic fibrosis. We hypothesized that autophagy is altered in airway epithelial cells from severe asthmatic compared to non-asthma subjects.

Methods: Human tracheobronchial epithelial cells obtained from normal lung transplant donors and bronchial brushings of airways from well phenotyped, severe asthmatic individuals (n=5) were cultured using air-liquid interface (ALI) conditions. Differentiated preparations (ALI d 21-28) were confirmed by immunostaining for cell-specific markers. Following treatment with media alone, IL-13 (50 ng/mL), or influenza virus (A/WS33) for 48 h, autophagy activity was determined by LC3 flux assay using chloroquine (100mM for 2 h) for autophagosome-lysosome inhibition. Autophagy protein LC3BII levels were assayed by immunoblot and densitometry values normalized to actin.

Results: We found no propensity of asthma derived progenitor cells to have increased autophagy activity compared to normal progenitor cells, suggesting that progenitor cells from severe asthmatics are not reprogrammed to have altered autophagy responses. Differentiation of airway epithelial cells from asthmatics resulted in significantly more MUC5AC positive cells compared to those from non-asthma subjects, however there was no inducible autophagic flux in response to short term (48 h) IL-13 exposure, suggesting that longer IL-13 exposures are required. Influenza virus is known to increase autophagy, however autophagy activity was not changed in preparations from normal donors or those from asthmatic subjects at 48 h post infection.

Discussion: These data are consistent with our prior finding that IL-13 induces autophagy in the differentiated goblet cell after prolonged stimulation. Although higher percentages of goblet cells are present in cell cultures from asthmatics compared to normals, the numbers may be insufficient to reliably detect significant autophagy changes by techniques used. Alternatively, the goblet cells from asthmatic subjects may require longer IL-13 exposures to induce autophagy. Failing to identify altered autophagy responses in airway epithelial cells from asthmatics does not exclude potential differences in autophagy of airway immune cells active in asthma.

Funding Source: NIH, NIAID, Asthma and Allergic Diseases Cooperative Research Centers (U19AI070489)
Porcine Tracheal Epithelial Cells (pTECs) as an in vitro model for IL-13 Stimulated Mucus Production

Benjamin J. Gerovac, Yael G. Alevy, Jennifer Tucker, Jian Xu, Rowena Grainger, Steven L. Brody and Michael J. Holtzman
Pulmonary and Critical Care Medicine, Washington University of Medicine, St. Louis, Missouri

Abstract: Mucin hypersecretion and mucous cell metaplasia are characteristics of respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD). Previous reports have shown the cytokine interleukin 13 (IL-13) plays a major role in both mucus cell metaplasia and mucus hypersecretion. Furthermore, studies using primary human tracheal epithelial cells revealed calcium activated chloride channel 1 (CLCA1) was necessary for IL-13 dependent stimulation of mucin production. Interestingly, this dependence is not conserved in mice, as prior data showed chloride channel accessory 3, the homologous CLCA1 gene in mice, was not required for IL-13 stimulated mucus production. Therefore, the mouse is not a good animal model system for studying mucus upregulation by IL-13. Genetic analysis elucidated similar porcine and human CLCA gene loci. Therefore, we set forth to determine if the pig recapitulated the reliance of CLCA for the IL-13 dependent increase of mucus production. Using porcine tracheal epithelial cells in vitro, the data demonstrate both porcine Muc5AC and CLCA are upregulated by porcine IL-13 by ELISA. In addition, immunofluorescent staining also shows an increase in Muc5AC and CLCA, and also shows colocalization between the two. Overall, preliminary data suggest the pig may be a suitable model for investigating aspects of human respiratory disease.
Abstract B7

**Disease mutations in TREM2 reveal a functional surface and two distinct loss-of-function mechanisms**

Daniel L. Kober¹, Jennifer M. Alexander-Brett², Michael J. Holtzman²,³, and Tom J. Brett²,³,⁴,⁵

Molecular Biology and Microbial Pathogenesis¹, Department of Internal Medicine², of Cell Biology & Physiology³, Biochemistry & Molecular Biophysics⁴, and Drug Discovery Program in Pulmonary and Critical Care Medicine⁵ Washington University School of Medicine, St. Louis, MO

**Introduction:** Triggering receptor expressed on myeloid cells-2 (TREM2) is an innate immune receptor that regulates myeloid cell activation in a variety of pathologies including inflammatory pulmonary disease such as COPD. Despite its importance, the endogenous ligand for TREM2 is unknown and there is a deficiency of information regarding its structure and mechanism. Recently, distinct coding variants in the TREM2 Ig domain have been linked to the severe dementia known as Nasu-Hakola disease (NHD) or Alzheimer’s disease (AD). It is unknown how different mutations to the same protein domain result in different pathologies. We hypothesized understanding how these variants alter TREM2 structure and function would shed light on this key protein.

**Methods:** We performed structural, biophysical, and functional studies to elucidate the molecular mechanisms underlying TREM2-associated pathologies. We developed a novel mammalian-cell expression system for TREM2, crystallized the Ig domain, and determined its structure at 3.1 Å.

**Results:** Analysis of the structure revealed the NHD residues are buried while the AD residues are solvent exposed, suggesting the NHD mutations destabilize the protein while AD mutations impact ligand binding. Extensive biochemical and biophysical experiments demonstrate the NHD mutations disrupt protein stability and reduce surface expression of folded protein while the AD mutations are not grossly altered in structure or stability. Next, we addressed the nature of the TREM2 ligand and asked whether AD mutants disrupt binding. We found TREM2 binds a specific protein ligand on mammalian cells through a glycosaminoglycan-dependent mechanism. AD mutations, including R47H, disrupt binding to this ligand while a T96K variant (which has unclear disease risk) increases binding. These variants epitope map a functional ligand-binding surface on the TREM2 protein that is unique within the overall TREM family. Our ongoing work is addressing the full identity of the TREM2 ligand complex.

**Conclusions:** Our findings reveal two distinct loss-of-function mechanisms for disease-linked mutations. NHD mutations cause misfolding while AD mutations do not grossly impact structure or stability. TREM2 binds a protein-dependent ligand on mammalian cells and AD-linked mutations disrupt this interaction. These novel findings will inform patient-specific molecular therapies targeting TREM2 for the treatment of inflammatory pulmonary and neurodegenerative disorders.
Abstract B8

*Mycobacterium tuberculosis* phenolic glycolipids induce Interleukin-17 to limit hypoxia and necrosis in the host during tuberculosis

Racquel Domingo-Gonzalez¹, Kristin Griffiths¹, June Treerat¹, Javier Rangel-Moreno², Joaquin Zuñiga³, Shabaana A. Khader¹

¹Department of Molecular Microbiology, Washington University School of Medicine; ²Division of Allergy/Immunology and Rheumatology, University of Rochester School of Medicine and Dentistry; ³Department of Immunology, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas

Despite implementation of anti-tuberculosis therapies and the Bacillus Calmette-Guerin (BCG) vaccine, *Mycobacterium tuberculosis* (Mtb) persists as a serious global threat to human health and is compounded by the emergence of multidrug- and extensively drug-resistant strains. A hallmark of pulmonary tuberculosis (TB) is the tubercle granuloma, which during disease state can develop hypoxic necrotic or caseous centers resulting in the disintegration of the granuloma structure and release of infectious Mtb. In a mouse model of Mtb infection, we show that Interleukin (IL)-17A (IL-17) plays a critical role in limiting the development of necrotic granulomas with hypoxic centers, following infection with the hyper-virulent W-Beijing Mtb strain, HN878. Importantly, phenolic glycolipids (PGLs) on Mtb HN878 drive IL-1β and IL-17 production in vitro, and in vivo infection with HN878 lacking PGLs results in increased inflammation. Human genetic analyses reveal a link between the single nucleotide polymorphism (SNP) rs2275913 in the IL-17 promoter and increased susceptibility to pulmonary TB (PTB). Together, our data provide evidence that IL-17 plays an early and important role in limiting hypoxia and necrosis during infection with clinical hyper-virulent Mtb strains.
**Interleukin (IL)-22: A role in host immune responses during the progression of tuberculosis.**

Puthayalai Treerat¹, Oliver Prince¹, Alfredo Cruz-Lagunas², Marcela Muñoz-Torrico², Miguel Ángel Salazar-Lezama³, Moises Selman², Kristin Griffiths¹, Beth Fallert-Junecko³, Todd Reinhardt³, John F. Alcorn⁴, Deepak Kaushal⁵, Joaquin Zuñiga², Javier Rangel-Moreno⁶, Jay K. Kolls⁷ and Shabaana A. Khader¹.

¹Department of Molecular Microbiology, Washington University in St. Louis, St. Louis, MO, 63110 USA; ²Instituto Nacional de Enfermedades Respiratorias "Ismael Cosio Villegas", Mexico City, Mexico; ³Department of Infectious Diseases and Microbiology, University of Pittsburgh, Pittsburgh, PA, USA; ⁴Division of Pulmonology, Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; ⁵Divisions of Bacteriology and Parasitology, Tulane National Primate Research Center, Covington, LA 70434; ⁶Division of Allergy, Immunology and Rheumatology, Department of Medicine, University of Rochester Medical Center, Rochester, NY, USA; ⁷Richard King Mellon Institute for Pediatric Research, Department of Pediatrics and Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Tuberculosis (TB), a serious infection caused by *Mycobacterium tuberculosis* (*Mtb*), remains a major health challenge. Upon inhalation, *Mtb* is rapidly phagocytozed by resident lung macrophages in which can become their niche. The exact mechanisms defining the control of *Mtb* infection and disease outcomes remain not entirely understood. However, certain inflammatory responses have been shown to facilitate TB progress. Of the cytokines produced during infection and inflammation, IL-22 is a key proinflammatory cytokine being produced upon specific conditions and inductions. Although a role of IL-22 in host immunity has been extensively studied, the molecular mechanism and exact role during TB progress has not yet been fully revealed. So far, an increase in IL-22 produced from CD4+ T cells in active TB patients has been reported. A single nucleotide polymorphism (SNP) in the IL-22 gene promoter region (rs2227473) has also been found to be associated with a decrease in TB susceptibility, indicating a role of IL-22 during TB progress. Moreover, our recent data have suggested that IL-22 plays an important role in a strain-dependent manner, particularly with a hypervirulent strain HN878. *Il-22/-/-* mice showed an increase in bacterial burden in the lungs and spleens. Several key cytokines/chemokines were found to be altered in *Mtb* HN878 infected *Il-22/-/-* mice. Thus we conclude that IL-22 plays a role in host immune responses regulation during TB progress by regulating other cytokines production.
Lymphocytic Follicle Formation Induced By *Mycobacterium tuberculosis* Mutant During Acute Tuberculosis Infection

Oliver A. Prince¹, Deepak Kaushal², Javier Rangel-Moreno³, Jordi B. Torrelles⁴, Shabaana A. Khader¹

¹Department of Molecular Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri; ²Division of Bacteriology, Tulane National Primate Research Center, Covington, Louisiana; ³Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, Louisiana; ⁴University of Rochester Medical Center, School of Medicine and Dentistry, Rochester, New York; ⁵Center for Microbial Interface Biology Division of Infectious Diseases, Ohio State University, Columbus, Ohio

**Introduction:** *Mycobacterium tuberculosis* (*Mtb*) is the causative agent of the pulmonary tuberculosis (TB). The emergence of multi-drug resistant and extensively drug resistant strains of *Mtb* thwarts current drug therapy. In order to fully maximize drug therapy we need to better understand the basic immune mechanisms that mediate immune control of TB. Granuloma formation is the hallmark of TB and is characterized by immune cell aggregation. The pathway towards granuloma formation is not well understood. However, recent developments published by the Khader lab have shed light on some of the structural and functional markers of TB control within the granuloma. The formation and organization of the lymphoid follicle is consistent with control of TB induced by vaccination in mouse and primate models. A transposon screen conducted in primates has identified a mutant enriched in iBALT structures, ΔmmpL7, which is part of a group of inner membrane proteins involved with coupled lipid biosynthesis and transport. Preliminary data indicate that ΔmmpL7 interactions with mouse epithelium modulate cytokine expression of granulocyte colony stimulating factor (G-CSF) as well as chemokine expression via CXCL9 and CXCL10 and perhaps subsequent neutrophilic and lymphocytic recruitment, respectively. We hypothesize that *Mtb* induce iBALT structures through epithelial derived G-CSF and CXCL9/10 expression altering early neutrophil and CXCR3+ lymphocyte trafficking during the acute TB.

**Methods/Results:** Histological analysis demonstrates that wild-type *Mtb* exhibits decreased lymphoid follicle formation during aerosol infection of B6 mice (in vivo), which correlates with increased epithelial (mouse cell line, in vitro) induction of G-CSF, CXCL5/9/10 (RNA analysis), increased neutrophil accumulation in the lung and concurrent decreased dendritic cell accumulation in the lymph node (flow cytometry).

**Conclusion:** *Mtb* influence lymphoid follicle formation through early infection of the epithelial barrier resulting in subsequent immune responses mediated in part by recruited neutrophils which drive decreased dendritic cell accumulation and decreased lymphoid follicle formation. Thus early epithelial interactions determine late lymphoid follicle formation and control of TB mediated by the host.
Abstract B11

Presence of a novel intracellular C3-C3aR system in human lung epithelial cells.

Hrishikesh S Kulkarni, M. Kathryn Liszewski, Michelle E. Elvington, Steven L. Brody, John P. Atkinson
Divisions of Pulmonary and Critical Care Medicine and Rheumatology, Department of Medicine, Washington University School of Medicine, St Louis, MO

Introduction: The complement system forms a first line of defense against pathogens, principally via proteolytic cleavage-induced activation of complement component C3. One product, the anaphylatoxin C3a, is tonically generated within cell lines of lymphoid origin and is required for CD4+ T cell survival. However, its intracellular presence and role in lung epithelial cell (LEC) function has not been defined.

Methods: BEAS-2B, a transformed human bronchial epithelial cell line (CRL-9609; ATCC, VA) and A549, a lung adenocarcinoma cell line were employed for preliminary analyses. Key findings were assessed using human tracheal epithelial cells (hTEC) obtained from the WUSM Pulmonary Epithelial Cell Core. Western blots were performed using polyclonal rabbit anti-C3a and goat anti-C3 Abs (CompTech). Flow cytometry was utilized to monitor surface staining as well as the intracellular presence of C3, its fragments and C3aR. Subcellular fractions were obtained using a Subcellular Protein Fractionation Kit (Thermo Scientific). C3 uptake from blood by LECs was studied using serum obtained from healthy controls as a source, and compared to purified C3 and methylamine-treated C3, which has a hydrolyzed thioester bond.

Results: Both cell lines contain as well as secrete C3 in the resting (non-stimulated) state. These cell lines also expressed the receptor for C3a, C3aR. These findings were also observed in primary hTECs isolated from normal human lungs (n=4). Using Western blotting, we identified C3a-containing fragments in these resting cells, suggesting constitutive C3-cleavage in LECs. Using flow cytometry, we observed that both C3 and C3aR were predominantly present intracellularly in BEAS-2B and A549 cell lines, as well as in hTECs. On analyzing subcellular fractions, C3 and C3aR in the BEAS-2B cell lines localized to intracellular membranes. Novel C3a-containing fragments were identified in the chromatin extract. LECs rapidly took up C3 from serum in the form of C3-methylamine, and this exogenous C3 was restricted to the cytoplasm and intracellular membranes.

Conclusion: C3 is secreted by LECs and is also present intracellularly in distinct sub-cellular compartments. C3aR is present on intracellular membranes, possibly in association with C3a-containing proteins. Intracellular C3 appears to produced by LECs, but at least a part of it and its fragments are derived from C3 taken up from blood. These findings establish the presence of an intracellular C3-C3aR system that is constitutively active in unstimulated human LECs. These findings form the basis of our current work on identifying the mechanism(s) of C3 and C3aR production and their intracellular shuttling, activation and function.
Clinical Category
Abstract C1

**C4d-negative Antibody-Mediated Rejection after Lung Transplantation**

P. R. Aguilar¹, R. D. Yusen¹, C. A. Witt¹, D. E. Byers¹, D. Kreisel², E. P. Trulock¹, T. Mohanakumar³, R.R. Hachem¹.

¹Pulmonary/Critical Care Medicine, Washington University, St. Louis, MO, ²Cardiothoracic Surgery, Washington University, St. Louis, MO, ³Surgery, Washington University, St. Louis, MO

**Purpose:** The definition of antibody-mediated rejection (AMR) after lung transplantation (LT) has undergone revision as recognition of this entity has increased. Although considered a key criterion for the diagnosis of AMR, C4d deposition has been an inconsistent finding. We compared outcomes of LT recipients who developed C4d-negative AMR to those who developed C4d-positive AMR.

**Methods:** We performed a retrospective cohort study of patients transplanted between 1/2006 and 7/2015, and identified those who developed AMR defined as acute allograft dysfunction, DSA, and a lung injury pathology. We excluded those who did not have C4d staining at the time of diagnosis (n=20). We grouped those who developed AMR into a C4d-positive cohort and a C4d-negative cohort and compared outcomes of the 2 groups using Kaplan-Meier method and the log rank test.

**Results:** 37 patients developed AMR during the study period; 15 developed C4d-positive AMR and 22 developed C4d-negative AMR. All patients with AMR were treated with IVIG. Of these, 36 were treated with rituximab, 7 with bortezomib, and 9 with plasma exchange (PLEX) (Table 1). Survival and CLAD-free survival after the diagnosis of AMR were poor in both groups, and there was no significant difference in either survival or CLAD-free survival between the 2 groups (Figure 1).

**Conclusion:** A large proportion of patients with AMR in this cohort did not have C4d deposition. However, there was no significant difference in outcome between those who had C4d-negative and those who had C4d-positive AMR. These findings suggest that C4d deposition may not be a necessary criterion for the diagnosis of AMR.

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Anellovirus can be used to predict acute rejection in pediatric lung transplant recipients

Joshua Blatter\textsuperscript{1}, Stuart Sweet\textsuperscript{1}, David Wang\textsuperscript{2}\textsuperscript{1}\textit{Washington University School of Medicine, Department of Pediatrics; \textsuperscript{2}Washington University School of Medicine, Department of Pathology & Immunology}

\textbf{Introduction:} Anelloviruses are single-stranded DNA viruses that are assumed to be commensal. Adult lung transplant recipients have been noted to have increased levels of anellovirus in blood and in bronchoalveolar lavage fluid. Due to its predominance in immunosuppressed patients, anellovirus has been proposed as a functional marker of immune status. We hypothesized that low levels of anellovirus, reflecting relative immunocompetence, would be associated with episodes of rejection in pediatric lung transplant recipients.

\textbf{Methods:} We used a total of 458 blood samples collected as part of the Clinical Trials in Organ Transplantation in Children (CTOT-C) multicenter study, collected at multiple time points within three years post transplant from 61 pediatric lung transplant recipients aged 1 to 18 years. We constructed a standard curve from a quantitative polymerase chain reaction (PCR) TaqMan assay selective for alphatorquevirus, an anellovirus genus. Nucleic acid was extracted from samples, and the quantitative PCR and standard curve were used to quantify an anellovirus copy number in each extracted blood sample. Data analysis was conducted with Stata/SE 13.1.

\textbf{Results:} Patients who experienced an episode of acute cellular rejection (ACR) within the first three months of transplant had lower average anellovirus levels (p=0.04) and higher average anellovirus count variability (p=0.04) than patients who did not experience ACR over this period. Each patient’s first post-transplant anellovirus level (averaging 10.6 days after transplant) was categorized as “high” or “low” based on being above or below median copy number at this time point. Log-rank test for equality of survival function in the first three months revealed that low anellovirus patients were significantly more likely than high anellovirus patients (p=0.004) to experience an episode of ACR in the first three months following transplant.

\textbf{Conclusion:} There is an association between low anellovirus levels and the development of ACR in pediatric lung transplant patients. This finding is consistent with existing literature identifying anellovirus as a potential marker of immune status, with less immunosuppressed patients having lower levels of anellovirus, and being more likely to develop rejection. Increased variability in anellovirus levels among patients who develop ACR could suggest lapses in adherence to immunosuppressive regimens. Future research will be needed to explore whether anellovirus is exclusively a biomarker or has a causative role in establishing the post-transplant immune milieu.
Towards Individualization: The Role of Pharmacogenetics in Lung Transplant Outcomes

Laneshia Tague MD¹, Derek E. Byers MD PhD¹, Ramsey Hachem MD¹, Andrew Gelman PhD²
¹Washington University in St. Louis, Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine; ²Washington University in St. Louis, Department of Surgery, Division of Cardiothoracic Surgery

Introduction: Immunosuppression plays a key role in graft function. Single nucleotide polymorphisms (SNPs) result in variable activity/response of key proteins and enzymes necessary in the processing and metabolism of these medications. Identification of key polymorphisms would help better predict response to certain therapies and allow for a more individualistic, approach to immunosuppression. It is known that certain polymorphisms in the UGT1A9 protein decreases mycophenolic acid exposure in kidney transplant recipients and increase risk of rejection. Recently, we used SNP analysis to identify autophagy gene polymorphisms associated with BOS-free survival in the lung transplant population. Now, we plan to apply this technology to examine SNPs in key metabolic enzymes as they relate to survival and long-term clinical outcomes in our lung transplant population.

Methods: We will conduct a retrospective cohort study of all adult lung transplant recipients at Washington University in St. Louis between 1991 and 2013. Demographic, clinical and genetic data will be collected via review of the medical record and maintained in a secure REDCAP database. Genetic data will be collected via saliva sample. We will conduct SNP analysis via the Taqman assay. Our primary outcome will be survival. Our secondary outcomes will be cumulative acute rejection, chronic rejection/development of BOS, and infections.

Results: A total of 534 adult lung transplant recipients from 1991 to 2013 are to be included in our cohort. Table 1 shows the SNP frequencies in our patient population.

Conclusion: Our database will be the only known comprehensive clinical lung transplant databases to also include genetic data. We hope to correlate our SNP data with clinical outcomes. This has the potential to lead to individualized immunosuppression that is more efficient and less toxic.

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Abstract C4

**Multidimensional Analysis of Human Lung Myeloid Cells Reveal Unique Subpopulations in COPD and IPF**

Washington University School of Medicine, Internal Medicine Department, Division of Pulmonary and Critical Care

**Introduction:** Human alveolar macrophages (AM) have been described as following cannonical macrophage activation pathways that are similar but distinct from those established in mouse models. Autofluorescence seen in human AM isolated from tissue limits the dynamic range for interpretation of differences among subsets of AM and between AM and interstitial macrophages (IM). We have utilized mass cytometry to overcome historic autoflourescence limitations and now demonstrate a broad dynamic range of all common AM surface markers to better define subsets seen in digested tissue from diseased lungs. Isolation of novel human AM subsets by surface marker identification can now be utilized to reveal activation states that have been previously oversimplified due to reliance on in vitro techniques.

**Results:** Digested human lungs obtained at explant for lung transplantation were evaluated using a 30 antibody panel that includes 22 surface markers known to be expressed on AM was performed as a single antibody cocktail on a Cytof2 (Fluidigm) mass cytometer. All analysis was performed using Cytobank software with unbiased clustering based on the multidimensional surface marker panel. COPD and IPF samples reveal distinct densities of unique IM and AM populations that are more disease than individual specific. COPD specimens demonstrate an enhancement of a CD36+ IM subset with a variable loss of CD36+ AM that is not seen in IPF. Further analysis of CD36+ AMs does not suggest an M1 or M2 macrophage activation pathway but does demonstrate enhancement of MMP-9 production by qPCR of magnetic bead enriched bronchoalveolar lavage cells.

**Summary:** Unique AM and IM macrophage subsets can be demonstrated utilizing surface expression of common macrophage proteins. These subsets differ significantly between individuals with end-stage COPD and IPF. At least one subset of CD36+ AM may be significantly diminished in end stage COPD and may reflect a shift to accumulation of this subset in the interstitium. Although CD36+ macrophages do not reflect known in vitro activation states they do demonstrate increased MMP-9 expression.

**Conclusions:** Mass cytometry is a novel technique that generates an unprecedented opportunity to examine human lung myeloid cell subsets. Although this work may be limited by disease severity and current sample size, reproducible unique AM subsets can now be identified from frozen tissue samples of any lung disease.
Abstract C5

Translational Potential for Mass Cytometry in Lung Transplantation with Limitations from Medicinal Iodine

Washington University School of Medicine, Department of Medicine, Division of Pulmonary and Critical Care

Introduction: A novel flow cytometry technique called mass cytometry has been developed that utilizes a mass spectrometer to detect rare metal-tagged antibodies by single cell analysis. Advantages of this technique include simultaneous evaluation of 30+ epitopes and no need for compensation because each metal has a specific non-overlapping atomic mass. Lung transplantation is the only alternative for many cases of severe emphysema, pulmonary fibrosis or cystic fibrosis. Routine bronchoscopy in the post-transplant period generates a rich source of samples for translational research, however the very high autofluorescence of the alveolar macrophages (AM) limits the translational potential of this clinically relevant and available cell type. We report information on the robust translational potential of mass cytometry after lung transplantation and important caveats unique to this patient population due to interference of medicinal iodine in the form of Amiodarone.

Results: Post-lung transplant AMs were evaluated using a 30 antibody panel that includes 22 surface markers known to be expressed on AM. Cells were analyzed on a Cytof2 (Fluidigm) mass cytometer. For comparator analysis M0, M1 and M2 activated monocyte-derived macrophages and mononuclear cell preparations were evaluated utilizing the same antibody cocktail. All analysis was performed using Cytobank software with unbiased clustering based on the multidimensional surface marker panel. After successful initial runs several samples were found to have sufficient excess iodine that mass detector overheating and damage might occur in the 127I channel. Retrospective analysis revealed both iodinated contrast and post-operative Amiodarone as possible sources with samples from one subject prior to and after Amiodarone initiation confirming the interference of this medication with mass cytometry. Summary: Mass cytometry reveals a broader dynamic range of protein expression than standard fluorophore based flow cytometry with a simplified workflow that is conducive to translational research. However as with any novel technique application to patient samples will reveal unexpected clinical scenarios that interfere with broad application of the technique to complex subjects like the post-lung transplant population.

Conclusion: Severe emphysema, pulmonary fibrosis and cystic fibrosis are currently treated with lung transplantation when possible. Early detection of markers that predict long-term outcomes may be derived from novel techniques that can evaluate existing clinical samples. Mass cytometry shows strong potential as a novel research tool to overcome the limitations of current techniques to evaluate cells collected by BAL but has some limitations in the immediate post-lung transplantation period due to iodine.
A Novel Classification for Pneumonia in Mechanically Ventilated Patients

Kristen Fisher¹, Tracy Trupka¹, Marin Kollef¹
¹Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Washington University, St. Louis, MO

Introduction: The current definitions for pneumonia classify patients based upon risk for pathogen resistance. These definitions include the terms Community Acquired Pneumonia (CAP) for patients at less risk for resistance and Health-Care Associated Pneumonia (HCAP), Ventilator Associated Pneumonia (VAP), and hospital acquired pneumonia (HAP) for patients who have risk factors for more resistant pathogens. However, these definitions have received much criticism. Some of the criticisms are that with the current classification, the ability to predict multi-drug resistance (MDR) has not been replicated consistently in clinical studies nor have these definitions been consistently predictive of outcomes. Also, factors that promote potential variability including geographic location and immunosuppressive status are not accounted for in these definitions. Viral pneumonia which in the modern era has become more easily identifiable is also not included in the current definitions. Retrospective analyses classifying hospitalized patients with pneumonia based upon pathogen and resistance have shown more consistent correlation with outcomes including 90 day readmission. However, no prospective studies have been done to date.

Methods: A prospective cohort study is being performed in the medical intensive care units at Barnes-Jewish Hospital over 12 months from January 2016 to January 2017. All mechanically ventilated patients admitted to the MICU with a diagnosis of pneumonia are identified prospectively. Included patients meet a clinical diagnosis of pneumonia as defined by a radiographic infiltrate in addition to two of the following criteria 1) WBC >10,000 or <4,000 (2) temperature >38.6 °C or <36 °C , (3) purulent secretions from the lower respiratory tract (4) PaO2/FiO2 ratio less than 300. Baseline characteristics including demographics, prior hospitalization or use of antibiotics, co-morbid conditions, immunosuppressive status, Clinical Pulmonary Infections Score (CPIS) and severity of illness based upon APACHE II scores are collected. Based upon culture and respiratory viral panel data, patients are classified as antibiotic-susceptible, antibiotic resistant, pathogen negative, or viral pneumonia. Antibiotic resistance is defined as resistance to ceftriaxone. Under this classification, patients will be compared in terms of outcomes including mortality, length of stay (ICU and hospital days), vasopressor days, days on mechanical ventilation, treatment failures, and 90 day readmission.

Results: 69 patients have been enrolled during the first 8 weeks of the study with culture data completion for 58 patients. Of these patients, 13.8% have been classified as antibiotic-susceptible, 20.7% as antibiotic resistant, 31% as pathogen negative, and 34.5% as viral pneumonia. Multivariate analysis will be performed comparing outcomes based upon this classification.
Repeatability of CT Airway Measurements in Severe Asthma

Chase Hall¹, Jim Kozlowski¹, Rosalia Alcoser¹, Charles Goss¹, Ken Schechtman¹, Brad Wilson¹, David Gierada¹, Sally E Wenzel², Joseph K Leader², Eugene Bleecker³, E.A. Hoffman⁴, David Mauger⁵, Mario Castro¹

¹Washington University, St. Louis, MO; ²University of Pittsburgh, Pittsburgh, PA; ³Wake Forest University, Winston-Salem, NC; ⁴University of Iowa, Iowa City, IA; ⁵Penn State University, Hersey, PA

**Background:** Computer-aided analysis of airway dimensions on CT scans has demonstrated that automated image analysis software applied to the same images by different users results in highly repeatable measurements. The variation in measurements related to repeating the entire CT scan is unknown and may affect the ability to monitor patients for changes over time.

**Methods:** In the Severe Asthma Research Program (SARP) Ancillary Imaging Study, 40 subjects to date were characterized clinically (15 severe asthma, 3 moderate asthma, 16 mild asthma, 6 normal controls) and underwent two volumetric lung CT scans (supine, full inspiration). Using Pulmonary Workstation software (VIDA Diagnostics), the cross-sectional wall area percent (WA%) and wall thickness percent (WT%) were measured at the segmental (generation 3) and more distal generations in 6 segments: 1, 4, and 10 of the right and left lungs. Repeatability was assessed by determining the difference between pairs of measurements with 95% confidence intervals (CI) and by intraclass correlation.

**Results:** The WA% ICC was moderate to strong (0.5-1.0) for the mild and severe asthma groups with the ICC decreasing in the more distal generations beyond generation 3. Similar results were seen in the ICC for WT% in mild and severe asthma cohorts with the highest reliability being in generation 4. The ICC analysis for the normal controls and moderate asthma cohorts demonstrated wide 95% confidence intervals. This was likely due to the small sample size. There was no statistically significant difference in either mean WT% or WA% in segments 1, 4, and 10 generations 3-6 of both the right and left lungs between the initial and repeat volumetric CT scans.

**Conclusions:** The repeatability of quantitative CT airway measurements on separate scans is substantial but may vary over different segments and generations. Such data may be useful to estimate the amount of change that can be reliably detected in longitudinal studies.
The Effect of Imatinib on Airway Remodeling in Patients with Severe Refractory Asthma

Abhaya P. Trivedi, Li Zhou, Mario Castro

Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Washington University, St. Louis, MO

Introduction: Mast cells are a source of pro-inflammatory cytokines and vasoactive mediators that are involved in the pathophysiology of asthma. Stem cell factor is one of the primary growth factors for mast cells and signals through the cKit tyrosine kinase receptor. Imatinib inhibits cKit and its effects on inflammation and airway hyper-responsiveness in patients with severe asthma were studied. In addition, serum tryptase levels and spirometry were assessed. The effect of imatinib on airway remodeling measured by multidetector CT (MDCT) was also evaluated in a subset of these patients.

Methods: A randomized, double blind, placebo-controlled, multi-center trial of imatinib was conducted over 24 weeks in patients taking a high dose inhaled corticosteroid and additional controller with persistent airway hyper-responsiveness and poor asthma control. Of the 62 patients that were randomized, 48 patients had MDCT scans completed before and after the treatment period. We analyzed the imaging with Apollo software (VIDA Diagnostics, Iowa City). Airway geometry measurements such as diameter, wall thickness, wall area percentage, and wall thickness percentage were obtained in the 3rd generation airways. We evaluated lung density by obtaining total volume, tissue volume, air volume, and emphysema like lung (percentage of lung below -950 Hounsfield units). The pre and post treatment MDCTs were compared to determine if there was a reduction in airway wall thickness and emphysema like lung after treatment with imatinib.

Results: The data is currently being analyzed for comparison.

Conclusion: Severe asthma represents a heterogeneous group of phenotypes and several biologic agents that target particular pathways are showing efficacy in specific severe asthma phenotypes. Inhibition of mast cell activation through imatinib is being studied in patients with severe asthma. Because biologic agents have the potential to reverse the airway remodeling process, it can be useful to quantify the possible improvement using imaging modalities such as MDCT to provide objective measures of assessment.
Abstract C9

**Case Based Lectures vs. Simulation Based Training: Which is Better for Resident Education?**

Abhaya P. Trivedi, Marin Kollef

Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Washington University, St. Louis, MO

**Rationale:** Acute care teams (ACT) were created to improve outcomes of acutely decompensating patients in an effort to prevent cardiopulmonary arrest. The ACT at our hospital is led by a medicine resident and is comprised of an intensive care nurse, a respiratory therapist, and other medicine house staff members. Residents are expected to participate in the ACT team at the start of their residency training, and to lead the team in their second and third years of residency. Formal training was not mandated initially, but was later created through the simulation center. Because one training session was deemed insufficient, a case based lecture was added prior to the simulation training.

**Methods:** Interns and residents in the internal medicine program attended an interactive, case based lecture that discussed several ACT scenarios. A survey was distributed prior to the session, pre-test or P0. The survey assessed comfort level and incorporated 10 knowledge-based questions. After the lecture, all house staff completed a survey, post-test #1 (P1). In addition, interns participated in simulation center training and then completed a survey, post-test #2 (P2). P0 and P1 were compared to measure the benefit of the case based lecture. P0 and P2 were compared to determine the benefit of the simulation training for interns.

**Results:** All residents agreed that case based lectures and simulation center education were more applicable than didactic lectures. From P0 to P1 interns’ and residents’ knowledge-based average score improved from 6.89 to 8.68 (p value < 0.001). As a separate group, interns’ knowledge-based average score also improved significantly from P0 (6.11) to P1 (8.3) with a p value < 0.001. There was no significant difference in knowledge-based scores between P0 (6.11) and P2 (6.55) for the intern class (p value 0.40).

**Conclusion:** The addition of one case-based teaching session improved intern and resident education in management of ACT scenarios as evidenced by their increase in knowledge-based scores. Interns felt that the simulation training session helped them feel more comfortable being a part of and leading an ACT than the lecture. However, their knowledge-based scores did not improve after their participation in the simulation session. Although there is some perceived benefit to simulation training by house staff, it may not be feasible to have multiple sessions throughout residency given the resources required. Case based lectures can be implemented frequently throughout residency and can provide at least an equivalent benefit to simulation training.

**Funding source:** None
Abstract C10

Impact of Point of Care Ultrasound in the MICU.

Adam Anderson, Warren Isakow
Division of Pulmonary and Critical Care Medicine, Department of Medicine, Washington University School of Medicine, St. Louis MO

Introduction: The majority of admissions to a medical intensive care unit are associated with respiratory failure or shock; however, the underlying etiology is frequently difficult to define. Bedside point of care ultrasonography (POCUS) has been shown to be an invaluable tool in the emergency department and critical care medicine. We hypothesize that a universal bedside ultrasound screening protocol can be implemented with accurate image capture and interpretation resulting in a change in medical management.

Methods: To improve critical care ultrasound training and ensure fellows’ competence, a curriculum has been created that includes readings, didactics, and an ultrasound conference. As part of each unique medical intensive care unit (MICU) admission for a patient with respiratory failure or shock, a fellow driven POCUS is performed focusing on limited echocardiography, lungs, abdomen and a vascular deep venous thrombosis (DVT) screen. A worksheet, that becomes part of the medical record, is completed at the time of the examination documenting the differential diagnosis both before and after the ultrasound, image interpretation and limitations, as well as any changes in the diagnostic or therapeutic plan. Examples of image acquisition, techniques and analysis are reviewed in the educational ultrasound conference. Chart review was performed to obtain clinical information, the ultrasound worksheet, and saved ultrasound images. Descriptive statistics were used to assess the impact.

Results: Using a convenience sampling, 414 patient charts were reviewed over 12 months. An ultrasound was indicated in 285/414 patients (68%; 95% CI ± 4.5%). POCUS was performed and documented in 88/285 (31%; 95% CI ± 5.4%), resulting in 28 total diagnostic changes and 57 total therapeutic changes. POCUS impacted decisions about volume or hemodynamic management in 35/88 (40%; 95% CI ± 10.2%). After censoring incomplete data sets, the differential diagnosis was changed in 27/79 patients (34% 95% CI ± 10.5%). POCUS impacted the differential diagnosis and/or management in 59/88 (67% 95% CI ± 9.8%).

Conclusion: As the critical care population becomes more complex and difficult to categorize, additional tools are needed to supplement clinical care. POCUS has growing evidence to support its use in the critically ill population. In our cohort, POCUS influenced management of volume status in 40% of patients. The overall impact influencing diagnostics or management was evident in ~2/3 of patients. POCUS, despite all other data, adds additional information to the care of patients admitted to a MICU in respiratory failure or shock. Further studies, however, are necessary to fully evaluate the impact on morbidity and mortality.
Clinical effectiveness of pairing an antibiotic de-escalation protocol based on a novel new pneumonia classification system with clinical reminders in reducing total antibiotic administration: a prospective, observational study

Tracy Trupka, Marin Kollef, Kristen Fisher, Paul Jung, Mollie Gowan
Pulmonary and Critical Care Medicine, Pharmacy

Introduction: Studies have shown inappropriate initial antibiotic regimens to be an independent risk factor for death in sepsis. Thus, initial interventions now include administration of broad spectrum antibiotics. As a result, over the past several decades, increasing numbers of multi drug-resistant (MDR) pathogens have emerged with an associated increase in the rate of mortality in critically ill patients. Newer studies have looked at antibiotic usage after implementation of a standardized de-escalation protocol or antibiotic stewardship tool such as the ADVISE program utilized in Australia. These protocols have been proven to reduce antibiotic usage and also demonstrate stabilization/decrease of previously up-trending rates of MDR pathogens.

In the era of the now widely available viral multiplex PCR and pending bacterial DNA and RNA PCRs, new questions arise regarding antibiotic stewardship and the optimal methods of antibiotic de-escalation. It has yet to be addressed how the results of this diagnostic data will fit into our current de-escalation algorithms. Andruska et al recently published a retrospective review which compared hospital readmission rates after reclassifying pneumonias with a novel qualitative system based on respiratory culture data. This established that patients with antibiotic-resistant bacterial pneumonia have a higher 90 day readmission rate and patients with culture negative pneumonia have a lower 90 day mortality. Lack of de-escalation in pathogen negative and viral pneumonias may be an additional target for early de-escalation therapy in order to limit unnecessary antibiotic exposure.

Methods: Our study will include all patients admitted to the 8300 and 8400 MICU teams with respiratory failure requiring invasive mechanical ventilation and placed on empiric antibiotics for lower respiratory tract infection.* An enhanced antibiotic stewardship team will manually review microbial and viral specimen and patient charts for clinical condition on a daily basis. Antibiotic recommendations regarding de-escalation and course duration will be given for patients in the MICU with the active stewardship team based on a pre-defined algorithm. Rather than basing continued antibiotic course on the initial likelihood of MDR pathogens at the time of presentation (HCAP, CAP, aspiration pneumonia), recommendations will be based on clinical suspicion of pneumonia and current culture data. Patients will be re-classified as either non-infectious cause of respiratory failure, pathogen negative lower respiratory tract infection (LRTI), antibiotic-sensitive LRTI, or antibiotic-resistant LRTI. After 6 months, the enhanced antibiotic stewardship team will transition to the opposite MICU. Data from the enhanced stewardship MICU will be compared with that from the MICU without enhanced stewardship.

* Excluding BMT patients, post-lung transplant, CF/bronchiectasis, AIDS, neutropenic malignancy, and patients with other non-pulmonary presumed or proven source of infection.
Using the Early Warning System (EWS) to Identify and Establish Goals of Care/Advanced Directives in a High Risk Patient Population: A Randomized, Prospective Trial

David Picker, MD¹; Maria Dans, MD²; Kevin Heard³; Thomas Bailey, MD⁴; Yixin Chen, PhD⁴; Chenyang Lu, PhD⁴; Marin H. Kollef, MD¹

¹Division of Pulmonary and Critical Care Medicine, Washington University School of Medicine, St. Louis, MO; ²Palliative Care Services, Barnes-Jewish Hospital, St. Louis, MO; ³Center for Clinical Excellence, BJC HealthCare, St. Louis, MO; ⁴Division of Infectious Diseases, Washington University School of Medicine, St. Louis, MO; ⁵School of Engineering and Applied Sciences, Washington University in St. Louis, St. Louis, MO

**Introduction:** A significant proportion of our population dies in hospitals, with nearly twenty percent spending time in Intensive Care Units (ICU). Data suggests this is incongruent with patient preference. Identifying and capturing even a small fraction of individuals who would prefer to stay out of the ICU would not only help align patient goals and outcomes, but also have a tremendous impact on the utilization of healthcare resources. Barnes-Jewish Hospital (BJH) uses an Early Warning System (EWS) based on objective laboratory and vital sign criteria to notify healthcare providers to patients at risk for clinical deterioration, with higher risk of death and transfer to ICU. In this pilot study, we examined an intervention to help determine whether discussion at time of EWS activation regarding goals of care (GOC) and transfer to ICU impacted the rates of transfer to ICU, and presence of advanced directives.

**Methods:** Single center, prospective randomized control trial of patients from general inpatient medicine service from January 2015 to January 2016. Oncology, transplant, and cystic fibrosis patients excluded from study. Study team notified of randomly selected patients who generate alert from the EWS. Goals of care discussion performed with intervention group. Rates of transfer to ICU, proportional change in presence of advance directives, and code status changes compared with the control group using standard statistical methodology. All subjects consented to this study under a protocol approved by the institutional review board.

**Results:** At the final analysis, there have been 89 patients in the intervention group and 117 patients in the control group. Baseline demographics (age, race, and medical comorbidities) between groups are similar. There is a statistically significant difference in rate of transfer to the ICU between groups; 27% for the control group, and 12% for the intervention group (p=0.009). Additionally, 18% of the intervention group created new advanced directives, compared to control group rate of 0% (p < 0.001).

**Conclusion:** Intervention at time of Early Warning System alert allows opportunity for intervention to discuss goals of care at a critical time during hospitalization. Interim analyses of data suggest this leads to decreased rates of transfer to the ICU, and greater proportion of patients creating advance directives. This intervention not only helps align patient care goals with outcomes, but also has a tremendous potential impact to improve healthcare resource utilization.