PULMONARY RESEARCH DAY

Thursday, March 15, 2018

Division of Pulmonary and Critical Care Medicine

Washington University in St. Louis
School of Medicine
B1. A Novel Acoustofluidic Platform for Precision Medicine in Airway Disease

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Chronic respiratory disease, most often due to COPD, is the third leading cause of death in the U.S., and mortality is tightly linked to excess mucus production. Unfortunately, there are no specific therapies to control mucus production, in part because there are no precise methods to monitor this abnormality in patients. We have identified chloride channel accessory 1 (CLCA1) as a key regulator and marker of mucus production and have proceeded to develop a point-of-care microfluidic device that incorporates sample preparation and CLCA1 detection. Here, we describe a detection module that is based on ultrasound-confined microcarrier particles held in a longitudinal standing bulk acoustic wave (LSBAW). In this type of trap, the local pressure field is augmented using a pillar array oriented orthogonal to the flow direction, and particles are retained at acoustic pressure minima (nodes). Antigen-coated microspheres are immobilized and incubated with fluorescent antibodies to allow for contactless, in-channel immunoassays. Polystyrene (PS) beads (1x10\textsuperscript{6} beads/ml) were incubated for 24 h at 37 °C in bronchoalveolar lavage (BAL) fluid from pigs that were IL-13-challenged to stimulate mucus production and increase CLCA1 levels. For detection, a mixture of two proprietary anti-CLCA1 monoclonal antibodies (mAbs) was modified with a red fluorescent dye, sulfo-cyanine3 (Cy3) NHS ester dissolved in pH 8.0 buffer solution to produce a final mAb concentration of 580 µg/mL. Antigen-coated PS beads were locally focused using a PZT-8 piezoceramic transducer (f = 980 kHz, 250 mV\textsubscript{pp}) within the pillar array before introduction of the mAb mixture at 7 µL/min. The channel was left at 25°C for 1.5 h and then washed for 10 min with a solution of phosphate-buffered saline at a flowrate of 7 µL/min. Beads were collected at the outlet and analyzed using flow cytometry. The experimental samples were compared to untreated PS beads and antigen-coated PS beads modified with Cy3 dye under equivalent conditions. Increased fluorescence over the control solutions demonstrated that our LSBAW device can serve as a functional immunoassay platform. We can therefore proceed to optimizing the assay system and then coupling it to an on-chip acoustic-based module that homogenizes mucus-containing samples. The resulting device will provide the first automated micro-technology to monitor mucus (or other protein) levels in patients with airway disease and to select and track these patients for treatment and response to therapy, particularly with our concomitantly developed anti-mucus drugs.
B2. Towards a comprehensive single-cell atlas of gene expression in idiopathic pulmonary fibrosis
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Despite decades of active research and recent advances in therapy, idiopathic pulmonary fibrosis remains a devastating diagnosis with a median survival under 3 years, and much of the pathophysiology underlying the disease is poorly understood. Recent advances in transcriptomics allow gene expression to be monitored at unprecedented resolution, with simultaneous profiling of each cell type in an organ. We sought to apply these single cell (DropSeq) and single nucleus (sNucDropSeq) sequencing approaches to whole lung tissue, to develop a technique that can later be applied in animal models and diseased human lung. For the single cell approach, mouse lung was dissociated through a combination of enzymatic and mechanical approaches, and cells isolated through differential centrifugation. Yields of viable nucleated cells were sufficient for DropSeq analysis. Single nucleus analyses are advantageous as they can be applied to banked, frozen tissues. In mouse lung, a nuclear isolation protocol again resulted in adequate yield for sNucDropSeq. These approaches will ultimately be applied in a novel mouse in which Sonic Hedgehog (Shh) is inducibly expressed in alveolar epithelium, to identify downstream targets of this signaling cascade which has previously been implicated in pulmonary fibrosis. FoxD1-expressing cells in lung are thought to represent a pericyte population that gives rise to effector myofibroblasts in lung. Transgenic FoxD1-Cre-TdTomato mice will enable further exploration of this pathway in fibrosis; in preliminary studies tissue sections were obtained demonstrating TdTomato fluorophore labeling in lung. Future work will complement data generated from mouse models of lung fibrosis with single cell RNA-seq analysis of healthy and diseased human lung tissue.
**B3. An Epithelial Autophagy Factor is Required to Prevent Spontaneous Cell Death and Intestinal Atrophy**

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**Introduction:** Regulation of cell death is a key component of epithelial turnover in the intestine. This balance can be perturbed by various extrinsic factors including the cytokine TNFα, a cell signaling protein that is responsible for inflammation as well as cell death. It has been suggested that autophagy regulates TNF-mediated cell death in the intestinal epithelium, but only under conditions of infection and damage; thus a direct connection between autophagy and intestinal epithelial cell death is lacking.

**Methods:** Here, we generated mice lacking an autophagy inducer, ATG14 in the intestinal epithelium (Atg14<sup>−/−</sup> Villin-Cre (VC)+). We present the initial phenotypic characterization of this mouse model. We measured body weights after weaning and used histologic and immunohistochemical approaches to measure intestinal structure and cell viability. Lastly, to test whether Atg14<sup>−/−</sup> VC+ intestinal epithelial cells undergo TNFα-mediated apoptosis in-vivo, we delivered TNFα blocking antibody to Atg14<sup>−/−</sup> VC+ mice, and additionally generates Atg14<sup>−/−</sup> VC+; Tnfrsflα−/− mice.

**Results:** Atg14<sup>−/−</sup> VC+ mice consistently failed to gain weight in comparison to littermate controls consistent with the conclusion that these animals fail to thrive (Panel A). Histologically, there was progressive loss of small intestinal villi accompanied by crypt hypertrophy and subsequent crypt dropout (Panel B). Based on marker studies, the increased cell death in these mice was due to apoptosis (Panel C). In-vitro analysis of Atg14<sup>−/−</sup> VC+ intestinal epithelial cells showed increased apoptosis specifically when treated with TNFα. Both TNFα blocking antibody and genetic deletion of Tnfr1 rescued villus loss and cell death phenotype in Atg14<sup>−/−</sup> VC+ mice (panel D).

**Conclusion:** These findings suggests an indispensable role of ATG14 in regulating epithelial cell homeostasis in the intestine. Atg14<sup>−/−</sup> VC+ mice developed spontaneous villus loss and small intestinal epithelial cell death. We demonstrate that ATG14 is required for the postnatal survival of the intestinal epithelium and the homeostasis of the small intestinal mucosa. This work lays a foundation for future studies of the interaction between autophagy and TNF signaling in the intestine.
B4. Quantifying ciliary dynamics during assembly reveals step-wise waveform maturation in human airway cells
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Introduction: Motile cilia protrude from the apical surface of specialized epithelial cells in the airway, oviduct, and brain. In the airway, multi-ciliated cells are required to effectively remove particulates and pathogens that become trapped in mucus secreted by neighboring cells. Dynein motor proteins along the length of the cilium induce bending and a rhythmic, coordinated waveform. Genetic mutations in proteins required for motile cilia can cause primary ciliary dyskinesia (PCD). While cilia are sometimes visualized with high-speed video microscopy and described qualitatively as part of a multi-faceted approach to diagnosing disease, quantitative assessments of healthy cilia to understand the normal waveform have rarely been performed. Additionally, the development of the waveform during ciliogenesis has not been studied.

Methods: We first sought to define the characteristics of a healthy cilia waveform and the variation within samples or across cells from excess tracheobronchial tissues obtained from lungs donated for transplantation. We examined cilia ex vivo from tracheobronchial epithelial cells scraped from freshly obtained tissues and in preparations of primary-culture cells over the course of ciliogenesis that was induced using air-liquid interface conditions. We scraped cells from tissues and in vitro preparations and mounted cells on slides to record cilia motion by side view. Using high-speed video microscopy, we analyzed cilia motion and quantified cilia parameters to describe the shape and forces in each waveform. We also examined the localization of three dynein outer arm proteins with immunofluorescence to observe when dyneins are incorporated during ciliogenesis, relative to waveform maturation.

Results: We found that there was a large variation in measurements such as cilia length and cilia beat frequency in both ex vivo and in vitro cilia. Waveform shape depended on variables such as cilia length and stage of differentiation. While the cultured cells matured and captured many of the characteristics of ex vivo cilia, some differences remained between the two groups. The maturation of the waveform was associated with a stage-dependent localization of the dynein arms within the proximal and distal regions of the cilia during ciliogenesis.

Conclusion: Our observations suggest a step-wise maturation of the waveform during ciliogenesis in airway cells that depends on cilia length.
In the airway, proper activity of the anion channel CFTR contributes to innate immune defense by maintaining a hydrated and alkaline mucus layer. This allows potentially pathogenic microorganisms to be trapped, quickly killed, and cleared via mucociliary clearance, thus preventing microbial colonization of the lungs. In cystic fibrosis (CF), this activity is impaired, resulting in repeated pulmonary infections that damage the lung and, if severe and prolonged enough, lead to early death without lung transplantation. Available therapies remain focused on targeted rescue of the CFTR mutation. However, given the thousands of mutations found in this patient population, individualized rescue of each would be difficult. An alternative and potentially universal strategy may involve activation of a different chloride channel in lung epithelium to bypass CFTR dysfunction. Toward that end, we recently demonstrated that the vWA domain of CLCA1 (calcium activated chloride channel regulator 1) directly engages the calcium activated chloride channel TMEM16A and stabilizes its surface expression on the order of minutes, thereby increasing anion currents through the channel. Further supporting a trafficking mechanism, we find that CLCA1 rescues TMEM16A from a late endosomal fate. We are currently pursuing a structural model of this interaction, which would be used to inform future design of therapies based on the CLCA1/TMEM16A interaction. We have made significant progress by determining the structure of the CLCA1 vWA domain to 2.05 Å, the first structure of any part of CLCA1. Since CLCA1 directly engages TMEM16A, we hypothesized that this molecular recognition could be utilized to specifically activate anion currents in airway epithelia through TMEM16A to compensate for dysfunctional CFTR channels. In whole cell patch clamp experiments, we demonstrate that CLCA1, and in particular its vWA domain, is able to activate TMEM16A currents in primary CF airway epithelial cells from three distinct CF genotypes (ΔF508/2789+5G>A, ΔF508/ΔF508 and ΔF508/2184insA). We furthermore show that purified vWA domain is able to sustain currents through TMEM16A in polarized CF airway epithelia of another genotype (ΔF508/621+1G>T) in Ussing chamber experiments. Together, these studies highlight the exciting potential for universal CF treatment modeled after the CLCA1 vWA domain/TMEM16A interaction, and future work will examine the ability of the interaction to restore healthy mucous properties.
Rationale: Excess mucus production and consequent airway obstruction is a major factor in the morbidity and mortality of acute and chronic respiratory diseases, including asthma and COPD. However, the precise basis for increased mucus formation and effective means to specifically and safely down-regulate mucus to physiological levels remain uncertain. In that context, we discovered that IL-13-induced mucus production in primary-culture human airway epithelial cells was associated with selective MAPK13 activation and was blocked with MAPK13 inhibition using siRNA specific for MAPK13 mRNA or small-molecules designed to target the MAPK13 ATP-binding site. In addition, we found increased levels of activated MAPK13 in lung tissue from patients with excess mucus due to COPD. Therefore, we further studied MAPK13 to define activation and localization in relation to mucus production in primary-culture human airway epithelial cells.

Methods: Primary-culture human tracheobronchial epithelial cells (hTECs) were generated under air-liquid interface (ALI) conditions and were treated with or without IL-13 for 0-21 d. The levels of phospho-MAPK13 were monitored using phospho-MAPK antibody array, and the cellular level and location of MAPK13 and mucin MUC5AC were monitored using confocal laser scanning microscopy.

Results: Levels of phospho-MAPK13 were increased by 0.5 h and were maximal at 2 d of IL-13 treatment compared to vehicle-treated controls. MAPK13 immunostaining was detectable at near background levels at 0-21 d without IL-13 treatment but was markedly increased to intense cytoplasmic staining in a subset of cells at 7-21 d with IL-13 treatment. These MAPK13-positive cells were also selectively and intensely stained positive for MUC5AC. Further, the pattern of MAPK13 and MUC5AC immunostaining in the cytoplasm was identical, consistent with co-localization of these proteins to mucin granules.

Conclusions: These data show that MAPK13 activation (based on phospho-MAPK array) develops quickly (within 0.5 h) in response to IL-13 in human airway epithelial cells. This activation is followed by prolonged induction (lasting at least 21 d) of MAPK13 expression that co-locates precisely with MUC5AC in mucous cells and therefore likely to mucin granules within those cells. Together with our previous work showing that MAPK13 and CLCA1 blockade can inhibit excess mucus production and MUC5AC and CLCA1 induction also co-locates after IL-13 treatment, the results suggest that MAPK13, CLCA1, and MUC5AC might associate at the level of mucin granules to regulate mucus formation during inflammatory airway disease.
B7. Type 2 immune cells exhibit selective interactions with sensory nerve fibers in atopic dermatitis
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Introduction: Reciprocal interactions between the immune and nervous systems at barrier surfaces are increasingly recognized as key components of host defense. These interactions are also implicated in pathologic sensory responses that are common in atopic disorders exemplified by chronic itch in atopic dermatitis (AD). Although the type 2 cytokine IL-4 has recently been shown to be a critical molecular mediator of chronic itch, the dynamics and cellular organization of neuroimmune interactions in atopic skin inflammation are not understood. In this study, we hypothesized that immune cells that produce IL-4 would demonstrate specific and dynamic interactions with sensory nerve fibers in the skin.

Methods: We employed intravital two-photon imaging of a dual reporter mouse in which sensory nerve fibers express tdTomato under a sensory neuron-specific (Nav1.8) Cre recombinase and immune cells express IL-4-specific enhanced GFP (eGFP) to visualize and measure potential neuroimmune interactions. We also reconstituted Nav1.8-tdTomato⁺ reporter mice with IFNγ-eYFP⁺ IL-17-eGFP⁺ reporter bone marrow to characterize interactions of type 1 and type 3 immune cells with sensory nerve fibers.

Results: We found that IL-4-eGFP⁺ immune cells interact directly with sensory nerve fibers in AD-like skin lesions. At the single cell level, we observed that a subset of IL-4-eGFP⁺ cells markedly reduced their instantaneous speed upon close contact with sensory nerve fibers but accelerated to a rapid velocity after moving away from the fibers. At the population level, IL-4-eGFP⁺ type 2 immune cells interfacing with sensory nerve fibers had a significantly lower mean speed than those that never formed connections with nerve fibers. In contrast, type 1 and type 3 immune cells did not demonstrate similar neuroimmune interactions in AD-like disease.

Conclusions: Collectively, we have identified a previously unrecognized and selective behavior of type 2 immune cells towards sensory nerve fibers in the skin. The observation that type 2 immune cells physically interact with sensory fibers in vivo lends further support to previous studies demonstrating that neuronal type 2 cytokine signaling directly regulates sensory responses and provides a platform to identify key mediators of neuroimmune synapses that occur in atopic disorders.
Identification of Cx3cr1+ microglia-like cells in the peripheral nervous system

Whereas microglia are recognized as fundamental players in central nervous system (CNS) development, homeostasis, disease progression and injury response, less attention has been paid to macrophages of the peripheral nervous system (PNS). By comparing PNS macrophage populations from the dorsal root ganglia, sciatic, vagus, and subcutaneous nerves to conventional macrophage and microglia populations, we identified over 100 mRNA transcripts that were selectively enriched in all PNS macrophages at steady state, including mRNAs for monoamine oxidase A that catabolizes norepinephrine, ceruloplasmin that serves a key role in iron metabolism and has been implicated in Parkinson’s disease, and Adam33 which has been associated with asthma susceptibility. Other mRNA transcripts distinguished each PNS macrophage from the others. Finally, steady state PNS macrophages expressed several core signature genes found selectively in microglia, including Trem2, Tmem119, P2ry12, and Olfm13. Suggesting further that PNS macrophages shared key features of microglia, PNS macrophages were distinguished as CD45lo CX3CR1hi by flow cytometry and exhibited notable radioresistance. Since myelin loss is a fundamental feature in nerve trauma and neurodegenerative diseases, we also examined a mouse model of Schwann cell demyelination in which TSC2 is constitutively knocked out in Schwann cells, resulting in a severe lack of myelination in peripheral nerves. This model was characterized by a 20-fold expansion of PNS macrophages. Surprisingly, these expanding macrophages did not resemble monocytes or M1 or M2 polarized macrophage activation states, but instead further shifted their gene expression profile towards an even more microglia-like identity, coupled with a neuroprotective signature. We conclude that PNS macrophages exhibit a range of genetic programs geared towards nerve support in the periphery, including programs that resemble microglia. Injury in the PNS is, at least in some models, associated with the onset of a neuroprotective, rather than inflammatory, phenotype in these macrophages.
B9. C-C motif chemokine receptor 2 drives protective immunity by mediating alveolar macrophage localization in tuberculosis granulomas

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Introduction: C-C motif chemokine receptor 2 (CCR2) is a major chemokine axis that recruits myeloid cells including monocytes and macrophages. Thus far, CCR2−/− mice have not been found to be more susceptible to infection with Euro-American lineage 4 strains of Mycobacterium tuberculosis (Mtb). Here, using a prototype W-Beijing family lineage 2 clinical Mtb strain, HN878, we show that CCR2−/− mice exhibit increased susceptibility to tuberculosis (TB). In prior work, CCR2+ monocytes have been the focus of study, but here, we show that alveolar macrophages (AMs) express CCR2 and are critical for protection against TB disease.

Methods: Low dose aerosol infection of mice with Mycobacterium tuberculosis was employed for all in vivo studies. Separate lung lobes were harvested for flow cytometry, histology, RNA, bacterial burden and/or multiplex analysis from each mouse. A novel technique was used to label cells present in the airway by intratracheally administering fluorophore-conjugated antibody directly into the lungs prior to harvest.

Results: Following exposure to Mtb HN878, alveolar macrophages (AMs) are amongst the earliest cells infected. Using a novel labeling technique, we show that AMs accumulate early in the airways following Mtb HN878 infection and express CCR2. This axis is specifically upregulated in HN878 infection when compared to other strains such as the standard H37Rv. During disease progression, CCR2-expressing AMs exit the airways and localize within TB granulomas to mediate protective immunity. We show in vitro that HN878 infected, but not H37Rv infected, dendritic cells (DCs) and epithelial cells produce CCL2, suggesting a source for AM homing. RNA-sequencing of sorted airway and non-airway AMs show distinct gene expression profiles, suggesting that upon exit from airways, AMs become classically activated. Furthermore, absence of CCR2+ cells specifically at the time of AM egress from the airways resulted in enhanced susceptibility to Mtb infection, increased accumulation of neutrophils, and loss of Mtb control.

Conclusions: Together, our data provide novel evidence for a critical protective role for CCR2 in AM localization within the TB granulomas to mediate protective immunity against clinically relevant and emerging Mtb infections. Here we show that CCR2 is required for AMs to egress from the airway and home into forming granulomas, guided by signals from epithelial cells and DCs. Furthermore, upon leaving the airway, these AMs take on a classically activated phenotype, suggesting that they have compartmentally distinct roles within the lung during TB infection.
Figure: CCR2 expression is required for AMs to egress from airways and localize within TB granulomas

Single cell lung suspensions from uninfected and infected mice (n=5) were prepared and (a) the gating strategy for airway and non-airway AMs is shown. CD11c<sup>-</sup>CD11b<sup>-</sup>SiglecF<sup>-</sup>CD45.2<sup>+</sup> cells were gated as airway AMs, while CD11c<sup>-</sup>CD11b<sup>-</sup> SiglecF<sup>-</sup> CD45.2<sup>-</sup> cells were gated as non-airway AMs. (b) The total number of AMs over the time course of HN878 in B6 mice was determined by flow cytometry (n=5 per time point). (c) From total airway labelled cells (CD45.2<sup>+</sup>), the percentage of each myeloid cell type was determined in HN878-infected B6 mice by flow cytometry. (d) Total CCR2<sup>+</sup> AMs, CCR2<sup>+</sup> airway (CD45.2<sup>-</sup>) AMs, and CCR2<sup>+</sup> non-
airway (CD45.2) AMs over the course of HN878 infection in B6 mice were determined by flow cytometry. AMs=Alveolar Macrophages, Neuts=Neutrophils, Monos=Monocytes, RMs=Recruited Macrophages, mDCs=Myeloid Dendritic Cells, MMPs=matrix metallopeptidases, PRRs=pattern recognition receptors. (c) Z-score Pearson correlation-based clustering of differentially expressed genes of interest (all significantly differentially expressed genes in airway macrophages, and 29 genes of functional interest that were higher in non-airway macrophages). (b-d) Each time point was compared to baseline d0 counts using Student’s t-test.
Mycobacterium tuberculosis (Mtb) is the causative bacterial pathogen for the disease tuberculosis (TB). Approximately one third of the world's population is latently infected with Mtb. Despite the use of M. bovis Bacillus Calmette–Guérin as a vaccine, TB continues to cause 1.8 million deaths each year. The immune responses that provide humans with protection against Mtb infection and disease are not clearly understood. Thus, more research is needed to further identify and understand protective host mechanisms. Previously, S100A8/A9 was demonstrated to mediate neutrophilic inflammation and lung pathology during tuberculosis. The objective of this study was to further understand the contribution of S100A9 on Mtb infection and disease. The impact of S100A9 deficiency was determined by using Mtb infected S100A9 deficient mice to assess molecular and immunological changes that occurred during infection. We demonstrate that the absence of S100A9 is protective during the chronic stages of Mtb infected mice. S100A9 deficiency was also shown to impact cellular recruitment by decreasing neutrophil influx to the lung. Though no differences in inflammation we observed, we did find that the B cell follicle area increased, a characteristic of protection, during infection. Using transgenic mice expressing S100A9GFP, both innate and adaptive cellular populations were shown to express S100A9, specifically the CD11b^+ cellular populations. Adoptive transfer of CD11b^+ cellular population into S100A9 deficient Mtb-infected mice and reversed the protection in the S100A9 deficient mice. Our results demonstrate that the presence of S100A9 protein promotes Mtb infection and influences cellular recruitment of CD11b^+ cells, specifically neutrophils and recruited macrophages. Together, our results suggest that targeting specific molecules, such as S100A8/A9 proteins, can potentially enhance host protection against Mtb.
B11. Host immune modulation by multidrug resistant *Mycobacterium tuberculosis*

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Tuberculosis is a significant global health threat, with one-third of the world’s population infected with its causative agent, *Mycobacterium tuberculosis* (*Mtb*). Additionally, approximately 4% of new and 21% of previously treated tuberculosis cases are rifampicin resistant or multidrug resistant (MDR), which require treatment with less effective and toxic second-line drugs. However, the immune response to multi-drug resistant (MDR) *Mtb* the molecular host-pathogen interactions mediating the world-wide spread of MDR *Mtb* strains remain poorly understood. Therefore, we aimed to determine if the immune requirements of protection between drug sensitive and MDR *Mtb* strains were similar. We found that while infection with the drug susceptible *Mtb* strain HN878 induces interleukin-1β to mediate *Mtb* control, loss of the IL-1 receptor type I (IL-1R1) signaling pathway does not result in enhanced susceptibility during infection with the MDR *Mtb* strain W_7642. Instead, this MDR *Mtb* strain drives high induction of interferon-β and reduced induction of interleukin-1β. After screening other related MDR *Mtb* clinical isolates for their requirement for IL-1 signaling, we used whole genome sequencing to determine single nucleotide polymorphisms (SNPs) that could contribute to this phenomenon. In particular, we were interested in SNPs identified in the gene encoding the beta subunit of RNA polymerase (*rpoB*), which are known to confer rifampicin resistance. These SNPs are also implicated in altering the expression of *Mtb* cell wall lipids, and therefore we hypothesized they may modulate the way in which MDR *Mtb* is sensed by the host immune system. We generated an HN878 strain carrying the identified *rpoB*-H445Y SNP, and show that in a murine model of infection, IL-1R1 deficient mice had no enhanced susceptibility to infection with this mutant when compared to wildtype mice. These findings transform our understanding of how MDR *Mtb* strains can acquire drug resistance SNPs which alter *Mtb* surface lipid expression, and modulate host immune responses to benefit MDR *Mtb* pathogenesis.
B.12 *Aspergillus fumigatus* pre-exposure worsens pathology and improves control of *Mycobacterium abscessus* pulmonary infection in mice

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**Introduction:** Cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator gene which cause to mucus accumulation, recurrent pulmonary infection, and inflammation; and in turn chronic lung disease. Recently, rates of nontuberculous mycobacteria (NTM) infections in CF patients have been increasing (Esther CR et al. J Cyst Fibros 9:117-123). Once such NTM is *M. abscessus*, which can cause life-threatening pulmonary infections. Also, an increased prevalence of NTM infections associated with worsening lung function has been reported in CF patients that are also co-infected with *A. fumigatus* (Esther CR et al. J Cyst Fibros 9:117-123). The interactions between *A. fumigatus* and NTMs such as *M. abscessus* in CF patients are not well-understood.

**Methods:** We used a mouse model to investigate the relationship between *A. fumigatus* and *M. abscessus* pulmonary infection. Mice were infected with *M. abscessus* alone, or pre-exposed to *A. fumigatus* and co-infected with *M. abscessus*. C57BL/6 (B6) mice, as well as *Tbx21*−/−, *Rorc*−/− and *Tbx21*−/− *Rorc*−/− mice, which lack Type 1, Type 17, or both Type 1 and Type 17 immune responses, respectively, were infected. Harvested lungs were analyzed using flow cytometry, ELISA, for bacterial burden, and morphometric analysis. Bone marrow derived macrophages (BMDMs) were generated from mice and used for in-vitro assays with *M. abscessus*.

**Results/Conclusions:** Mice co-infected with *A. fumigatus* and *M. abscessus* exhibited increased lung inflammation on pathology and flow cytometry, and decreased mycobacterial burden compared to mice infected with *M. abscessus* alone. Additionally, BMDMs exhibited improved in-vitro killing of *M. abscessus* in the presence of fungal antigens. This increased control of *M. abscessus* infection was dependent on the presence of both transcription factors, T-box 21 (*Tbx21*) and RAR-related orphan receptor gamma t (*Rorc*), indicating a role for both Type 1 and Type 17 immune responses in *M. abscessus* control. Our results demonstrate that *A. fumigatus* can worsen pulmonary pathology and impact *M. abscessus* control in mice. We hypothesize that *A. fumigatus* and *M. abscessus* co-infection in a CF patient with impaired clearance of infection could lead to a prolonged cycle of inflammation and lung damage, contributing to the decline in pulmonary function observed in clinical studies.
Clinical Category
C1. Impact of Neutropenia on Survival in Lung Transplant Recipients Receiving Mycophenolate for Immunosuppression

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Purpose: Mycophenolic acid is the most commonly used anti-proliferative agent post-lung transplantation. One of its major, therapy-limiting side effects is neutropenia. Our aim was to determine the impact of neutropenia on clinical outcomes including acute rejection and infection in the first year after transplant as well as development of CLAD and overall survival in lung transplant recipients receiving mycophenolate for immunosuppression.

Methods: We performed a single center retrospective cohort study of adult lung transplant recipients between 2008 and 2013. Neutropenia was defined as mild (ANC<1500), moderate (<1000) or severe (ANC<500) and classified as early (within one year of transplant) and late (after one year post-transplant). Overall survival was analyzed using Kaplan Meier and Cox Regression. Rates of acute rejection and infection were analyzed using the chi-square test.

Results: Of the 228 patients included in the cohort, 101 (42.1%) developed neutropenia. Of those patients who developed neutropenia, 42 had mild neutropenia, 34 had moderate neutropenia and 25 had severe neutropenia. Late severe neutropenia was associated with poorer overall survival when compared to patients with mild (p=0.002), moderate (p=0.008) or no neutropenia (p=0.018) (figure 1). Additionally, patients with severe neutropenia had significantly higher rates of gram-positive infections (56% vs 35% for moderate, 26.2% for mild and 26% for none, p=0.002) and higher rates of gram-negative bacterial infections (68% vs 67.6% for moderate, 45.2% for mild and 55% for none, p=0.149). Interestingly, there was no difference among the groups in either acute rejection or the development of CLAD.

Conclusion: Late severe neutropenia is associated with decreased survival after lung transplantation in patients receiving mycophenolate. This appears to be unrelated to graft rejection and is likely due to increased rates of infection, but further study is warranted.
C2. Risk factors in development of AMR in patients with lung transplantation

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In a recent analysis of 73 patients who underwent bilateral lung transplantation (BLTx) who developed antibody mediated rejection (AMR) showed that there was an association that trended towards significance between having cystic fibrosis (CF) and the development of AMR when compared to patients undergoing lung transplantation due to other reasons. Animal models have shown that respiratory infections can break immunosuppression-mediated lung allograft tolerance can either precipitate acute rejection (AR) or chronic lung allograft dysfunction (CLAD). We plan on investigating this association between CF and AMR. Our hypothesis is that chronic infections, such as those in CF patients, predispose these patients in developing AMR. We plan on evaluating patients who under lung transplantation between 2005-2016 and analyzing infection data and the risk of developing AMR.
C3. Risk factors for the development of donor-specific antibodies and their impact on outcomes after lung transplantation
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¹Pulmonary/Critical Care, Washington University, St Louis, MO, ²Cardiothoracic Surgery, Washington University, St Louis, MO,

Purpose: We hypothesized that lung allograft injury promotes the development of DSA, which increases the risk of CLAD and death. The purpose of this study is to identify risk factors for the development of DSA after lung transplantation (LT).

Methods: We conducted a retrospective single-center cohort study of 419 LT recipients between 1/1/09 and 12/31/15; follow up was complete through 12/31/16. Patients were screened for DSA at 1, 2, 3, 6, 12 months then every 3 months after LT. Patients were also screened for DSA in the case of allograft dysfunction. We constructed univariate Cox regression models and included variable with p<0.1 in a multivariate model. We evaluated events that occurred after LT as time-dependent variables.

Results: During the study period, 112 (40%) recipients developed DSA and 307 (60%) did not. Univariate Cox models identified PRA (HR 1.009, CI 1.003-1.015, p=0.003), cardiopulmonary bypass (CPB) (HR 1.562, CI 1.154-2.115, p=0.004), delayed chest closure (DCC) (HR 1.515, CI 1.103-2.081, p=0.01), and PGD grade 3 at any time (HR 1.452, CI 1.048-2.011, p=0.025) as significant risk factors for DSA. Episodes of ACR, LB, and respiratory infection were not significant risk factors for DSA. The final multivariate regression model included PRA (HR 1.009, CI 1.003-1.015, p=.003) and PGD 3 at any time (HR 1.458, CI 1.053-2.020, p=0.023). CPB and DCC were excluded from the multivariate model because of interactions with PGD grade 3 at any time. DSA as a time-dependent variable was a significant risk for the development of CLAD (HR 1.848, CI 1.362-2.509, p=<0.001) and death (HR 2.237, CI 1.58-3.168, p=<0.001). All DSA cleared in 113 (67%) patients, and DSA clearance was associated with significantly better allograft survival (HR = 0.4, 95%CI: 0.25 - 0.64, p < 0.0001) although this was not associated with freedom from CLAD (HR = 0.76, 95%CI: 0.48 - 1.20, p = 0.243).

Conclusion: PGD grade 3 at any time and PRA are independent significant risk factors for the development of DSA, and DSA are associated with a higher risk of CLAD and death after LT.
C4. Outcomes After Lung Transplantation in the Elderly
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\textsuperscript{1}Division of Pulmonary and Critical Care Medicine, Washington University School of Medicine, St. Louis, MO, \textsuperscript{2}United Network for Organ Sharing, Richmond, VA, \textsuperscript{3}Division of Cardiothoracic Surgery, Washington University School of Medicine, St. Louis, MO,

Purpose: Previous data have shown reduced survival in older (≥65 years) recipients after lung transplantation (LTx). We aimed to evaluate short- and long-term outcomes and causes of death (COD) in older LTx recipients. We hypothesized that older patients are more likely to die from infection than rejection because of immune senescence.

Methods: The ISHLT Transplant Registry data were reviewed for adult LTx recipients transplanted in 2005-6/2015. The primary outcome was 1- and 5-year patient survival. Secondary outcome was COD within 1 and 5 years post-transplant.

Results: 18,701 patients (20.1% ≥65 years) transplanted between 2005-6/2015, and 10,175 patients (16.6% ≥65 years) transplanted from 2005-6/2011 were included in the analysis of 1- and 5-year mortality, respectively. For 2005-6/2015 transplants, patients ≥65 were less likely to have a diagnosis cystic fibrosis (0.1% vs 17.4%; p<0.001) and were more likely to have idiopathic interstitial pneumonia (56.6% vs 29.3%; p<0.001), a history of malignancy (13.2% vs 5.7%; p<0.001), and receive single LTx (56.8% vs 22.5%; p<0.001). One (84.2% vs 88.95%; p<0.001) and 5-year survival (45.0% vs 61.8%; p<0.001) were significantly lower in the age ≥65 group. Age ≥65 was independently associated with 1- (HR 1.40; 95%CI 1.26 -1.54) and 5-year mortality (HR 1.44; CI 1.33-1.56). Other factors associated with mortality at 5 years included single LTx (HR 1.26; 95% CI 1.17 -1.35), and recipients needing some (HR 1.12; 95% CI 1.03 -1.22) or total assistance (HR 1.35; 95% CI 1.23 -1.48). The COD within 1 and 5 years after LTx were different between the two age groups [Table 1]. There was no difference in non-CMV infection and graft failure as a COD within 5 years between the two groups. Malignancy was a more common COD in the age >65 group but BOS was a more common COD in the age <65 group.

Conclusion: Recipient age ≥65 was independently associated with short- and long-term mortality. Causes of death in older patients were different than in younger patients. Malignancy was more common as a COD in the older group within 5 years.

<table>
<thead>
<tr>
<th>Recipient Cause of Death</th>
<th>Deaths Within 1 Year After Transplant (N=2,212) 07/01/2005-06/30/2015 transplants</th>
<th>Deaths Within 5 Years After Transplant (N=4,026) 07/01/2005-06/30/2011 transplants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18 – 64 (N=1,624)</td>
<td>65 + (N=588)</td>
</tr>
<tr>
<td>Missing</td>
<td>149</td>
<td>39</td>
</tr>
<tr>
<td>Acute rejection</td>
<td>37 (2.5%)</td>
<td>8 (1.5%)</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>111 (7.5%)</td>
<td>59 (10.7%)</td>
</tr>
<tr>
<td>CMV</td>
<td>18 (1.2%)</td>
<td>5 (0.9%)</td>
</tr>
<tr>
<td>Infection, Non-CMV</td>
<td>448 (30.4%)</td>
<td>167 (30.4%)</td>
</tr>
<tr>
<td>Graft Failure</td>
<td>272 (18.4%)</td>
<td>104 (18.9%)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>58 (3.9%)</td>
<td>32 (5.8%)</td>
</tr>
<tr>
<td>Multiple organ failure</td>
<td>191 (12.9%)</td>
<td>49 (8.9%)</td>
</tr>
<tr>
<td>OB/BOS</td>
<td>58 (3.9%)</td>
<td>21 (3.8%)</td>
</tr>
<tr>
<td>Technical</td>
<td>16 (1.1%)</td>
<td>9 (1.6%)</td>
</tr>
<tr>
<td>Other</td>
<td>266 (18.0%)</td>
<td>95 (17.3%)</td>
</tr>
</tbody>
</table>

Table 1. Causes of death within 1 and 5 years of transplants by age group
\textsuperscript{1} Chi-square
C5. Longitudinal evaluation of airway remodeling in asthma over time by airway biopsy
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*Division of Pulmonary and Critical Care Medicine, Washington University School of Medicine,
#University of Texas MD Anderson Cancer Center, †Cleveland Clinic

Introduction: There is limited data evaluating the progression of airway remodeling in asthma
over time. To our knowledge, this is the first study comparing airway remodeling on biopsy
among subjects with severe and non-severe asthma and healthy controls over time.

Methods: A prospective cohort of subjects with severe and non-severe asthma and healthy
controls from the Severe Asthma Research Program (SARP) underwent bronchoscopy with
endobronchial biopsies at baseline and approximately 3 years later. Airway biopsy remodeling
measurements included epithelial area (EA), lamina reticularis area (LRA), smooth muscle area
(SMA), the ratios of these measurements corrected for basement membrane length (EA/BML
and LRA/BML) or the ratio of smooth muscle area corrected for total tissue area (%SMA). Data
were analyzed using an analysis of variance ANOVA.

Results: 45 subjects (23 severe asthma, 15 non-severe asthma, 7 healthy controls, ages
33.2±11.9, 38.4±9.6 and 32.4±7.8 years, respectively; 67% women, 51% African American, 42%
Caucasian) underwent baseline and follow up biopsies which were performed approximately
34.0±7.4 months apart. Compared to non-severe asthma and healthy subjects, severe asthmatics
had significantly lower lung function and asthma control/quality of life scores at baseline. Lung
function did not significantly change over time for any group. Additionally, severe asthmatics
had significantly higher rates of positive skin atopy tests, increased inhaled corticosteroid use
and higher immunoglobulin E (IgE) levels at baseline (p=0.013, p<0.001, p=0.03, respectively).

On baseline biopsy samples, there was no significant difference for any measurements among
the three groups. However, on follow-up, severe asthmatics had significantly higher EA/BML,
LRA/BML and [EA+LRA]/BML compared to the non-severe asthma and healthy subjects
(p=0.03, p=0.01, and p=0.01 respectively). There was no significant difference for %SMA
among the groups at baseline or at follow-up. Comparing the change between baseline and
follow-up biopsies (ΔEA/BML, ΔLRA/BML, Δ[EA+LRA]/BML and Δ%SMA), there was no
significant difference among the groups.

Conclusions: Subjects with severe asthma have significantly increased epithelial and lamina
reticularis remodeling on airway biopsy compared to non-severe asthma patients and healthy
controls on follow-up. This suggests airway remodeling progresses over time despite treatment
though conclusions are limited to the relatively small sample size and variability in the biopsy
measures. Further longitudinal studies are needed to determine whether certain phenotypes have
more extensive remodeling over time.

Financial Disclosures: This project is supported by the National Institutes of Health, National
Heart, Lung, Blood Institute Severe Asthma Research Program (RFA-HL-01-012) and grant
(UL1 TR000448).
Table 1: Airway biopsy remodeling changes in asthma patients and healthy controls over time

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls (n=7)</th>
<th>Non-severe Asthmatics (n=15)</th>
<th>Severe Asthmatics (n=23)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1: Baseline</td>
<td>3.37 ± 0.72</td>
<td>2.55 ± 0.75</td>
<td>2.20 ± 0.79</td>
<td>0.007</td>
</tr>
<tr>
<td>Post-BDR FEV1: Baseline</td>
<td>3.57 ± 0.79</td>
<td>2.91 ± 0.88</td>
<td>2.71 ± 0.66</td>
<td>0.041</td>
</tr>
<tr>
<td>AQLQ Score: Baseline</td>
<td>7.0 ± 0.1</td>
<td>5.1 ± 1.6</td>
<td>3.9 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EA/BML: Baseline</td>
<td>23.5 ± 6.0</td>
<td>29.8 ± 13.0</td>
<td>28.7 ± 8.1</td>
<td>0.417</td>
</tr>
<tr>
<td>EA/BML: Follow-up</td>
<td>24.8 ± 3.9</td>
<td>28.9 ± 6.7</td>
<td>32.4 ± 7.4</td>
<td>0.032</td>
</tr>
<tr>
<td>ΔEA/BML</td>
<td>1.3 ± 8.1</td>
<td>-1.5 ± 14.1</td>
<td>2.8 ± 7.7</td>
<td>0.505</td>
</tr>
<tr>
<td>LRA/BML: Baseline</td>
<td>6.9 ± 2.3</td>
<td>8.8 ± 2.6</td>
<td>8.9 ± 2.9</td>
<td>0.264</td>
</tr>
<tr>
<td>LRA/BML: Follow-up</td>
<td>5.8 ± 1.3</td>
<td>7.8 ± 1.6</td>
<td>8.0 ± 1.8</td>
<td>0.013</td>
</tr>
<tr>
<td>ΔLRA/BML</td>
<td>-1.3 ± 2.4</td>
<td>-1.2 ± 2.5</td>
<td>-1.2 ± 2.7</td>
<td>0.997</td>
</tr>
<tr>
<td>[EA+LRA]/BML: Baseline</td>
<td>30.4 ± 6.1</td>
<td>38.6 ± 12.9</td>
<td>37.7 ± 9.1</td>
<td>0.245</td>
</tr>
<tr>
<td>[EA+LRA]/BML: Follow-up</td>
<td>30.6 ± 4.2</td>
<td>36.7 ± 6.7</td>
<td>40.4 ± 8.1</td>
<td>0.010</td>
</tr>
<tr>
<td>Δ [EA+LRA]/BML</td>
<td>-0.1 ± 8.6</td>
<td>-2.7 ± 14.4</td>
<td>1.7 ± 9.0</td>
<td>0.541</td>
</tr>
<tr>
<td>%SMA: Baseline</td>
<td>16.6 ± 6.8</td>
<td>18.2 ± 11.6</td>
<td>18.4 ± 12.9</td>
<td>0.941</td>
</tr>
<tr>
<td>%SMA: Follow-up</td>
<td>11.7 ± 10.3</td>
<td>20.5 ± 9.4</td>
<td>17.2 ± 10.4</td>
<td>0.183</td>
</tr>
<tr>
<td>Δ%SMA</td>
<td>-4.9 ± 12.7</td>
<td>2.2 ± 14.8</td>
<td>-1.2 ± 14.0</td>
<td>0.537</td>
</tr>
</tbody>
</table>

Post-BDR= post bronchodilator. AQLQ= asthma quality of life questionnaire. EA= epithelial area. BML= basement membrane length. LRA= lamina reticularis area. %SMA= smooth muscle area percent.
C6. Regional Ventilation Changes in Severe Asthma After Bronchial Thermoplasty

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2Cincinnati Children’s Hospital Medical Center, Cincinnati, OH
3University of Virginia School of Medicine, Charlottesville, VA
4Xemed LLC, Durham, NH

Financial Disclosure: This project is supported by NIH grants R44 HL112397, UL1 TR000448, T32 HL007317-39, T32 AI106688-03

Purpose: Hyperpolarized noble gas magnetic resonance imaging is a novel imaging modality that can provide safe and high-resolution ventilation images. Studies have demonstrated that patients with severe asthma have more and larger ventilation defects when evaluated by hyperpolarized gas MRI than those without severe asthma. Preliminary morphology studies of the airways leading to the ventilation defects demonstrate increased airway wall thickness. Bronchial thermoplasty (BT), which decreases airway smooth muscle, may decrease the ventilation abnormalities in bronchopulmonary segments when assessed by hyperpolarized gas MRI. We sought to quantify regional lung ventilation before and after BT.

Methods: Thirty patients with uncontrolled severe asthma (meeting ATS/ERS Workshop criteria) were imaged once with multi-detector CT (MDCT) and twice with hyperpolarized gas MRI (129Xe, MagniXene™, Xemed) at baseline, 3 weeks apart, prior to BT. A segmental airway mask was generated from the CT images using Apollo (VIDA). The ventilation images were then segmented using Advanced Normalization Tools (ANTS/ITK) into 4 ventilation categories (non-ventilated, poorly-ventilated, normal, and well-ventilated) and registered to the segmental airway mask. Twenty-seven patients to date underwent BT, 129Xe MRI was repeated 12 weeks after the third BT and compared to the baseline MRIs. Linear mixed model analysis was used to examine the mean differences in ventilated volume percentage for each ventilation category in all segments and for the whole lung before and after BT.

Results: Our cohort was predominantly Caucasian (90%) females (79%) with uncontrolled severe asthma (PC20 of 1.33 +/- 3.04 mg/mL and ACT score 9.60 +/- 3.64) with 52% on biologic therapy and reduced FEV1 (73.1 +/- 26.2% predicted) at baseline. There were no significant differences in segmental or whole lung ventilation within each ventilation category between the two baseline 129Xe MRI scans. There was a trend towards reduction in the percentage of poorly-ventilated lung (-4% p=0.078) and an increase in the percentage of well-ventilated lung (+5% p=0.051) 12 weeks after BT. Results at individual bronchopulmonary segments varied, but most trended towards improved ventilation.

Conclusion: Hyperpolarized 129Xe MRI can be reliably used to determine segmental and whole lung ventilated volume percentages. Regions of poorly ventilated lung may be improved with BT, a novel treatment for severe asthma that reduces smooth muscle in the airway. This information may be useful in targeting only segments with poor ventilation for treatment, potentially eliminating the need for multiple treatment sessions and decreasing adverse effects.
Figure 1: Mean Difference in Poorly-Ventilated & Well-Ventilated Volume Percentage after BT Compared to Baseline
95% CI of differences from analysis of paired differences between: Post BT - Baseline
C7. Use of Enteral Phenobarbital in ICU Patients with Refractory Agitation
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¹Division of Pulmonary/Critical Care Medicine, ²Division of Internal Medicine, ³St. Louis College of Pharmacy, ⁴Division of Pulmonary/Critical Care Medicine

Introduction: A high prevalence of patients in the adult intensive care unit (ICU) with respiratory failure requiring mechanical ventilation experience agitation and delirium. This leads to adverse effects such as unintended medical device removal that cause self-harm. In a subset of patients, the agitation and delirium are refractory to standard sedation practices, including utilization of opioids, propofol, benzodiazepines, and dexmedetomidine. Furthermore, increased amounts of continuous intravenous sedation (CIVS) have been shown to be associated with increased mechanical ventilator days, ICU length of stay (LOS), hospital LOS, tracheostomy requirement and incidence of delirium. Use of enteral sedative medications is a strategy frequently used to limit CIVS. Our purpose in this project is to describe in case series 13 patients requiring the use of enteral phenobarbital for indication of refractory agitation to achieve desired level of sedation while decreasing the requirement of CIVS, and report the prevalence of agitation and delirium utilizing this strategy of sedation.

Methods and Results: IRB approval ID# 201802025. Electronic medical records were retrospectively reviewed for patients who received phenobarbital for the indication of refractory agitation while on mechanical ventilation in the medical ICU at Barnes-Jewish Hospital from 2/1/2016 - 1/31/2018, which included 13 patients. Data collection is on-going. As the nature of this research is exploratory, we plan to use summary statistics in analysis. The following data points will be reported: age, gender, race/ethnicity, BMI, co-morbidities (including history of psychiatric conditions, alcohol or substance abuse), severity of illness scoring based upon APACHE II score within 24 hours of ICU admission, indication for ICU admission/mechanical ventilation, presence of agitation and delirium measured by RASS and CAM-ICU, respectively; cumulative dosing of psychoactive medications, mechanical ventilation days, ICU length of stay, hospital length of stay, ICU mortality, hospital re-admission at 30 days, need for chemical paralysis, unintended medical device removal, need for re-intubation, need for tracheostomy, occurrence of ventilation-associated pneumonia/condition, vasopressor requirement, and acquired organ system derangement measured via delta SOFA score.

Conclusions: The analysis is on-going, however we hypothesize that enteral phenobarbital therapy was able to provide alternative means to CIVS in patients with significant agitation in the ICU.

Clinical implications: This case series provides indication for future research opportunity investigating statistically significant reduction in CIVS and associated adverse outcome, as well as incidence of delirium and agitation.
Patrick G Lyons, a Lekshmi Santhosh, b Juan C Rojas, c Thomas M Ciesielski, a Vineet M Arora c

 a Washington University in St. Louis School of Medicine. b University of California, San Francisco. c University of Chicago Hospitals

Introduction: Patient complexity and less frequent monitoring increase patient risk after transfer from the intensive care unit (ICU) to the wards. We previously found that residents perceived handoff communication failures to be related to adverse patient events peri-transfer.1 In a 3-site survey, we aimed to identify the handoff elements involved in communication failures and to describe patient consequences and physician workload related to ICU-ward handoff communication failures.

Methods: From August 2015 to January 2018, we surveyed Internal Medicine residents at the University of Chicago (UofC), University of California-San Francisco (UCSF), and Washington University in St. Louis (WUSTL) regarding written handoff note quality and patient outcomes related to handoffs at ICU-ward transfer. Respondents completed a 30-item anonymous, self-administered questionnaire. We asked respondents to estimate the frequency of missing or erroneous information in handoff notes, to recall near-miss or adverse patient events related to handoff communication failures, and to estimate time spent recovering information resulting from communication failures.

Results: Of 295 residents approached, 175 (59%) completed the survey (61/73 UofC, 68/123 UCSF, 46/99 WUSTL). Rehabilitation needs, intravenous access and hardware, and risk assessments for readmission to the ICU were most frequently omitted or incorrectly communicated elements of handoff notes (Figure 1A). More than 60% of respondents reported that at least twice per month notes omitted or miscommunicated pending study results, active subspecialty consultants, nutrition and intravenous fluids, antibiotics, and healthcare decision-maker information. Over 40% of respondents recalled missed critical results, medication errors, and ICU readmissions related to incorrect or missing information (Figure 1B). Handoff elements at risk for miscommunication and the relative frequencies of adverse outcomes both differed significantly between institutions. Over 80% of respondents recalled spending 30 minutes or more per patient to recover information due to handoff communication failures, and almost 70% reported calling the ICU for clarifying information after transfer.

Conclusions: In this first multicenter survey of resident perceptions of ICU-ward patient handoffs, respondents described frequent written handoff errors, numerous miscommunication-related adverse patient events, and time wasted repeating previously-completed patient care tasks. Significant site differences were observed in both handoff elements at risk for error and relative frequency of adverse events, which may reflect institutional structure or care process differences (e.g. open versus closed ICU team structure). Future work should investigate whether structured interventions targeted at more effective handoff communication practices can improve patient outcomes.