

Example #1

Studies of autophagy induction by *Francisella tularensis* capsule/O-antigen mutants and their fate following infection of human macrophages

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Background and significance. *Francisella tularensis* is the causative agent of tularemia, with as few as 10 colony forming units sufficient to cause disease in humans^{4,6,7}. The virulence of *F. tularensis* is attributed to its ability to initially evade the innate immune response, escape phagosomal degradation, delay apoptosis, and replicate within the cytosol of phagocytes^{1,2,8}. Recently, several capsule and O-antigen mutants of *F. tularensis* have been isolated that exhibit significantly reduced virulence and, in contrast to wild-type bacteria, the ability to induce autophagy, a cellular degradation process, within infected macrophages^{3,5,9}. As the bacterial capsule and O-antigen components exist at the interface between *F. tularensis* and the innate immune response, characterizing the fate of these mutants and their relation to autophagy will generate a greater understanding of the role bacterial capsule and O-antigen play during macrophage infection.

Preliminary data. Transposon-based mutagenesis of the *wbt* gene cluster of *F. tularensis* resulted in the relevant bacterial capsule and O-antigen mutants. These mutants do not grow in mouse macrophages, but exhibit near wild-type growth in primary human macrophages during the first 24 h of infection. Interestingly, beginning at ~24 hours of infection the mutant bacteria begin to colocalize with ubiquitin and the autophagy marker LC3B, whereas the wild-type bacteria do not. Preliminary biochemical analyses and microscopy data suggest that the mutant bacteria may not be captured in autophagosomes or delivered to lysosomes for destruction.

In view of these data we **hypothesize** that certain capsule/O-antigen mutants stimulate initiation of autophagy, but the pathway does not go to completion. This will be tested with the **Specific Aim:**

1. Assess the extent of autophagy induction and progression by immunoblotting and confocal microscopy after infection of human macrophages with either *F. tularensis* Live Vaccine Strain (LVS) or *wbtA* mutant *F. tularensis*.

Methods. 1) Macrophages and bacteria: Human monocytes will be isolated from the peripheral blood of healthy volunteer donors using a protocol approved by the University of Iowa IRB. Monocytes will be differentiated into macrophages using established methods. *F. tularensis* strains will be cultured on modified Mueller-Hinton broth/agar plates with antibiotics to maintain strain homogeneity. 2) Immunoblot analysis of autophagy pathway flux: Infected macrophages will be harvested and processed to determine the extent of LC3B-I to LC3B-II conversion (a marker of autophagy initiation), as well as the degradation of p62 (an indicator of pathway flux). 3) Confocal microscopy to characterize colocalization with autophagy markers: Infected macrophages will be prepared for microscopy to image the extent of *F. tularensis* colocalization with ubiquitin, p62 and LC3 and other autophagy markers. At the same time, colocalization with rab5, lamp-1, and cathepsin D will be used to determine the extent of autophagosome maturation and fusion with lysosomes. 4) Transmission electron microscopy will be used to directly demonstrate if mutant bacteria remain free in the cytosol or are partially or completely sequestered inside autophagosomes at late stages of infection.

References:

- [1] Checroun C., Wehrly T.D., Fisher E.R., Hayes S.F., and Celli J. 2006. Autophagy-mediated reentry of *Francisella tularensis* into the endocytic compartment after cytoplasmic replication. *Proc Natl Acad Sci USA*. **103(29)**: 14578-83.
- [2] Jones C.L., Napier B.A., Sampson T.R., Llewellyn A.C., Schroeder M.R., and Weiss D.S. 2012. Subversion of Host Recognition and Defense Systems by *Francisella* spp. *Microbiol Mol Biol Rev*. **76(2)**: 383-404.
- [3] Maier T.M., Casey M.S., Becker R.H., Dorsey C.W., Glass E.M., Maltsev N., Zahrt T.C., and Frank D.W. 2007. Identification of *Francisella tularensis* *Himar1*-based transposon mutants defective for replication in macrophages. **75(11)**: 5376-89.
- [4] Oyston P.C. 2008. *Francisella tularensis*: unravelling the secrets of an intracellular pathogen. *J. Med. Microbiol*. **57**: 921-30.
- [5] Raynaud C., Meibom K.L., Lety M.A., Dubail I., Candela T., Frapy E., and Charbit A. 2007. Role of the *wbt* locus of *Francisella tularensis* in lipopolysaccharide O-Antigen biogenesis and pathogenicity. *Infect Immuno*. **75(1)**: 536-41.
- [6] Saslaw S., Eigelsbach H.T., Wilson H.E., Prior J.A., and Carhart S. 1961. Tularemia vaccine study. I. Intracutaneous challenge. *Arch. Intern. Med*. **107**: 689-701.
- [7] Saslaw S., Eigelsbach H.T., Prior J.A., Wilson H.E., and Carhart S. 1961. Tularemia vaccine study. II. Respiratory challenge. *Arch. Intern. Med*. **107**: 702-14.
- [8] Schwartz J.T., Barker J.H., Kaufman J., Fayram D.C., McCracken J.M., and Allen L.A. 2012. *Francisella tularensis* inhibits the intrinsic and extrinsic pathways to delay constitutive apoptosis and prolong human neutrophil lifespan. *J Immunol*. **188(7)**: 3351-63.
- [9] Tanida I. 2011. Autophagy basics. *Microbiol Immunol*. **55(1)**: 1-11.

Example #2

Prevalence and Risk Factors of Cardiomyopathy in Patients with FKR_P Gene Mutations

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Problem

Cardiomyopathy is known to be prevalent in patients with mutations in the fukutin-related protein gene (*FKRP*).^{3,6} The frequency of cardiac involvement in *FKRP* patients has been analyzed in small published cohorts native to different geographic locations, but the reported values have varied widely, from 10-60%.² Furthermore, reports of correlations between cardiomyopathy in *FKRP* patients and factors such as genotype, sex, and age lack consistency in published literature. Such contradictions limit clinical management of this patient population. Our primary objective is to identify the frequency of cardiomyopathy in the largest to date cohort of North American patients with *FKRP* mutations. A secondary objective is to determine if cardiomyopathy onset in our novel cohort is correlated with age, sex, genotype, and stage of skeletal muscle disease, and to compare our descriptive statistics to existing data. Our work is significant because it will contribute to a more complete understanding of *FKRP* patients' risk for developing cardiomyopathy. This will enhance clinicians' ability to monitor and counsel them with regard to cardiac disease.

Hypothesis

We hypothesize that the frequency of cardiomyopathy in patients with *FKRP* mutations increases with increasing age. We also predict that those heterozygous for the common mutation (thus, those with more rapidly progressive skeletal muscle disease) will have earlier onset of cardiomyopathy.

Aims

1. To establish the frequency of cardiomyopathy in a North American cohort of *FKRP* patients.
2. To describe the relationship between onset of cardiomyopathy in our novel cohort and clinical characteristics (age, sex, genotype, and motor and respiratory function).

Background

The dystrophin-glycoprotein complex (DGC) is structurally critical to the plasma membranes of skeletal muscle fibers and cardiac myocytes.¹ Dystroglycan is one component of the DGC and has two subunits: an integral membrane subunit, β -dystroglycan; and an extracellular subunit, α -dystroglycan.¹ α -DG's affinity to binding extracellular matrix proteins (such as laminin) is dependent on glycosylation.¹ Hypoglycosylation of α -dystroglycan characterizes a subset of muscular dystrophies known as the dystroglycanopathies (DGs)¹ and is caused by mutations in one of 18 identified genes. Mutations in the most commonly affected gene, *FKRP*, manifest in a spectrum of phenotypes, from MDC1C, a severe congenital muscular dystrophy (CMD), to the milder LGMD2I, which may be one of the more common types of limb girdle muscular dystrophies in North America.² The most common mutation of *FKRP* is c.826C>A, p.L276I.² Homozygosity for c.826C>A results in later onset and more slowly progressive skeletal muscle weakness.

Sveen, et. al., suggests a higher prevalence of cardiac involvement in LGMD2I patients homozygous for this common mutation, without relationship to strength,⁵ while others have suggested the reverse relationship between genotype, strength, and cardiac dysfunction.^{4,6} Variability in age of cardiomyopathy onset has been reported among patients with the same

FKRP mutations, even among siblings.³ A male predominance of cardiac involvement in LGMD2I patients was reported, but that finding was confounded by genotype.⁴

Proposed Methods

Subjects: All participants in the University of Iowa Wellstone Center's ongoing dystroglycanopathy natural history study (NIH NCT00313677, "Clinical Trial Readiness for the Dystroglycanopathies") with documented *FKRP* mutations are eligible for inclusion. Informed consent has already been obtained for all subjects (IRB 200510743).

Preliminary work: Echocardiogram reports and films were requested from participants' medical centers. Two UI cardiologists (blinded to genetic diagnosis) independently reviewed a subset of 41 echocardiogram films to determine ejection fraction (EF), shortening fraction (SF), and left ventricle (LV) size. If disagreement on EF or SF values affected the classification of an echocardiogram as normal or abnormal, a third cardiologist reviewed the echocardiogram. Statistical analysis with kappa statistic was used to measure agreement between local review of films and the original reports. A kappa statistic of 0.83 validated the review of external records as an acceptable surrogate for independently reviewing all films.

Data collection: A battery of clinical information is collected annually on all study participants. This data includes standardized measures of motor function and interval medical history. Clinical reports, including echocardiograms, are collected as available. We will extract and summarize descriptive data from the study's current database, to include sex, genotype, age of first abnormal echocardiogram, forced vital capacity (FVC), six-minute walk test (6MWT), and timed 4-stair climb. We will review all 128 echocardiogram reports available from 40 individuals and collect data including EF, SF, and comments on wall motion and LV function. Abnormal echocardiograms will be defined as those with EF < 50%, SF < 25%, and/or descriptions of abnormal wall motion and/or LV function.

Analysis: We will use descriptive statistics to show relationships between clinical features (genotype, sex, motor and respiratory function) and risk for cardiomyopathy at different ages. A Kaplan-Meier curve will be generated to show predicted age at onset of cardiomyopathy for the whole group and for different genotypes. M. Bridget Zimmerman, Ph.D., UI Professor of Biostatistics, will be available for consultation in the statistical analysis of all data.

References

1. Kabaeva, Z., Meekhof, K.E., and Michele, D.E. 2011. Sarcolemma instability during mechanical activity in Large^{myd} cardiac myocytes with loss of dystroglycan extracellular matrix receptor function. *Hum Mol Genet* 20: 3346-3355.
2. Kang, P.B., Feener, C.A., Estrella, E., et. al. 2007. LGMD2I in a North American population. *BMC Musculoskeletal Disorders* 8: 115.
3. Kefi, M., Amouri, R., Chabrak, S., et. al. 2008. Variable Cardiac Involvement in Tunisian Siblings Harboring *FKRP* Gene Mutations. *Neuropediatrics* 39: 113-115.
4. Poppe, M., Bourke, J., Eagle, M., et. al. 2004. Cardiac and Respiratory Failure in Limb-Girdle Muscular Dystrophy 2I. *Ann Neurol* 56: 738-741.
5. Sveen, M.-L., Schwartz, M., and Vissing, J. 2006. High Prevalence and Phenotype-Genotype Correlations of Limb Girdle Muscular Dystrophy Type 2I in Denmark. *Ann Neurol* 59: 808-815.
6. Wahbi, K., Meune, C., Hamouda, E. H., et. al. 2008. Cardiac assessment of limb-girdle muscular dystrophy 2I patients: An echography, Holter ECG and magnetic resonance imaging study. *Neuromuscular Disorders* 18: 650-655.

Example #3

Patient Experiences and Priorities in Rural Inter-hospital Transfer

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SIGNIFICANCE

Regionalization is a strategy of matching patient needs to medical resources, and regionalization has been shown to improve clinical outcomes in trauma, stroke, and ST-elevation myocardial infarction care^{1,2,3}. Regionalization often requires transfer to tertiary medical centers, but unfortunately, inter-hospital transfer can be a significant burden in caring for patients and their families. For example, a prior study done by this research team showed that inter-hospital transfer often delays appropriate treatment for severe sepsis and septic shock patients⁴. Rural emergency department transfer patients 6 times more than the national average^{5,6}, suggesting that inter-hospital transfer is an important consideration for developing health systems to serve rural populations. A prior mixed-methods study conducted by the same study team identified that both proximity to home and care in a comprehensive medical center are patient priorities, and they influence how patients make decisions about transfer to a tertiary hospital⁷. Further, the prior study showed that many emergency physicians overestimate the patient-oriented influence of proximity to home and underestimate their desire to seek tertiary care. Because all patients in the prior study had been transferred to a tertiary center, the sample selection may have been prone to selection bias, underestimating the patient-oriented value of care at home (patients who elected to remain in their communities for care were not interviewed). This study, however, will focus on patients being treated outside of an academic center in order to further clarify rural patient priorities through sampling of a more inclusive population.

In addition to inter-hospital transfer, telemedicine is another tool for regionalization that is growing in importance and in use. Telemedicine pairs a tertiary emergency physician with a local provider, and can provide tertiary expertise and specialty coverage to rural hospitals without requiring expensive and sometimes unnecessary inter-hospital transfer. Although providers feel that this service has value, there remain barriers to its more widespread implementation, and patient perceptions are one hypothesized barrier (also borne out in the prior mixed-methods study). This study will utilize existing telemedicine technologies in rural communities to elicit patient views on telemedicine-enabled emergency care, including a better understanding of barriers and the impact of telemedicine on community-hospital perception.

¹ Feazel L, Schlichting AB, Bell GR, Shane DM, Ahmed A, Faine B, Nugent A, Mohr NM. Achieving regionalization through rural inter-hospital transfer. *Am J Emerg Med*. 2015 Sept; 33(9):1288-96. PMID: 26087707.

² Andersen HR, Nielsen TT, Rasmussen K, Thuesen L, Kelbaek H, Thayssen P, et al. A comparison of coronary angioplasty with fibrinolytic therapy in acute myocardial infarction. *N Engl J Med* 2003;349(8):733-42.

³ Garwe T, Cowan LD, Neas B, Cathey T, Danford BC, Greenawalt P. Survival benefit of transfer to tertiary trauma centers for major trauma patients initially presenting to nontertiary trauma centers. *Acad Emerg Med* 2010;17(11):1223-32.

⁴ Faine B, Noack J, Wong T, Messerly J, Ahmed A, Fuller BM, Mohr NM. Inter-hospital transfer delays appropriate treatment for severe sepsis and septic shock patients: A retrospective cohort study. *Crit Care Med*. 2015;43(12):2589-96. PMID 26491865.

⁵ Kindermann, D. R., Mutter, R. L., Houchens, R. L., Barrett, M. L., & Pines, J. M. (2015). Emergency Department Transfers and Transfer Relationships in United States Hospitals. *Acad Emerg Med Academic Emergency Medicine*, 22(2), 157-165.

⁶ Nacht, J., Macht, M., & Ginde, A. A. (2013). Interhospital Transfers from U.S. Emergency Departments: Implications for Resource Utilization, Patient Safety, and Regionalization. *Acad Emerg Med Academic Emergency Medicine*, 20(9), 888-893.

⁷ Mohr NM, Wong TS, Faine B, Schlichting AB, Noack H, Ahmed A. Discordance between patient and clinician priorities in rural inter-hospital transfer: A mixed methods study. *J Rural Health* [in press – Epub ahead of print]. 2015. PubMed PMID: 26174410

The objective of this study is to understand the priorities and opinions of rural patients scheduled for hospital admission, especially as it relates to inter-hospital transfer and telemedicine use. Little is known about the role of transfer and telemedicine in improving health care utilization and access in rural communities. This study will build on the research team's prior studies to understand how inter-hospital transfer changes patients' opinions about their care, and to estimate the effects of telemedicine in influencing the patient-oriented value of local emergency care. In this era of increased patient-centered care and accountable care organizations, more clearly understanding the barriers to care that rural residents face is critically important to developing regionalization networks.

SPECIFIC AIMS OF STUDY

We will explore our objectives through these specific aims:

Aim 1. To understand rural patient values, priorities, and tradeoffs in the inter-hospital transfer process, with a focus on how patients decide whether they would like to be transferred. *Hypothesis:* Inter-hospital transfer is least desirable for those who place high value on proximity to home, and patient-oriented care balances these competing priorities.

Aim 2. To understand how the transfer experience shapes patient perceptions about the value of local healthcare resources. *Hypothesis:* The need for transfer will not influence patient perceptions of the adequacy or importance of local care (this hypothesis contradicts conventional wisdom).

Aim 3. To measure whether engaging in tertiary consultation using a telemedicine medium will improve patients' confidence in their healthcare, and to measure how they will subsequently view the value of local healthcare in their own communities. *Hypothesis:* Telemedicine will improve patients' confidence in their care and will make it more likely that they will subsequently seek local care.

APPROACH

We will conduct semi-structured qualitative interviews with patients being admitted from 3 rural emergency departments (Keokuk Area Hospital, Van Buren County Hospital, and Jefferson County Health Center). Questions for the interview will be developed by consensus by three content experts in regionalization and rural health care, including at least one physician who practices in a rural hospital. Any consenting patient being admitted to the hospital (at a participating rural hospital, whether admission is to the local hospital or planned after transfer) will be connected with the study team using a telemedicine connection for an interview that includes both quantitative (visual analog scale, dichotomous outcomes) and qualitative questions (using expanded interview questions based on the prior mixed methods study). Interviews will be recorded and transcribed, and two investigators will use the transcripts to identify themes in qualitative questions using inductive content analysis. Themes will be coded, and interviews will be conducted in groups of 10 patients until theme saturation is achieved on all objectives (required 80 patients in prior study). Quantitative data will be reported using summary statistics, and priorities will be compared for those being transferred vs. those admitted locally using chi-squared, t-test, or rank sum test, as appropriate. Qualitative data will be reported in themes with summary quotations, as reported previously.

IMPACT

The results of this study will be used to inform the design of a planned cluster-randomized trial to test the patient-oriented effects (mortality, health care utilization, satisfaction) of two strategies of rural sepsis care supported by telemedicine: rapid transfer to a tertiary care center vs. delayed transfer after rural resuscitation.