Anderson, Leslie

Preceptors: Adrienne Morgan, Ph.D
University of Rochester School of Medicine and Dentistry
The Center for Advocacy, Community and Health, Education and Diversity

Influences on Parental Feeding Practices in Low-Income African American Families at Three Parish Churches in New Orleans, Louisiana

Introduction

Childhood obesity is a growing epidemic in the United States. What's even more alarming is that the percentage of children between the ages of 6-11 years who meet the guidelines for obesity has drastically increased from 7% in 1980 to nearly 18% in 2010 (Adolescent and School Health,2013). Moreover, the U.S. Department of Health and Human Services reports that the percentages of overweight children and adolescents between the ages of 6-11 years of age in the years 2007-2010, were 1.8 times higher among Non-Hispanic Blacks (African-American) than Non-Hispanic White girls within the same age range (Obesity and African Americans, 2014).With such disproportionately high percentages in the rates of obesity one may question what are the underlying causes that have led to the various health abnormalities in the African American diet in particular? While there may be genetic predispositions to obesity as well as many other health abnormalities that are prevalent within African-American culture, studies have suggested that "obesity-related" genes can be combatted by eating a healthy- diet and getting enough exercise (Obesity and Prevention Source, 2014). Possible explanations of this growing obesity epidemic, and related chronic diseases affecting individuals of the low-income African- American population in the U.S.may be attributed to the lack of resources, such as access to stores with healthy food selections. Although one's food environment may serve as a strong proponent in the various health conditions that affect the low-income African-American community, there is a strong nurture component of the poor nutrition within African-American families that may also contribute to poor diets and associated health complications. Feeding is a central aspect of parenting that involves intense interactions between the parent and his/her child and such interactions may shape the child's eating behavior during a sensitive period of brain development, which may have a lifelong impact on one's diet and appetite regulation (Baughcum, 2001). Perhaps, there are underlying factors impacting parental feeding practices which have been perpetuated throughout time and contributes to the current state of many African- American's diet and health. Such factors include knowledge, culture, accessibility, affordability, and lifestyle, which may all play an integral role in determining what meal options a caregiver will provide for his or her family.

Objective:

The purpose of this project was to learn more about how parental feeding practices influence the nutrition choices of their children.

Methods and Approach:

Between July 1, 2014 and July 20, 2014, 45 individuals were recruited from the following three predominantly African- American church communities in New Orleans, Louisiana: The City of Love Church, St. Joseph Baptist Church, and Greater Morning Star Missionary Baptist Church. An anticipated total of 60 participants were expected to complete a paper copy survey amongst the three locations. However, only 45 participants turned in a completed survey. Two of the three churches, Greater Morning Star Baptist Church and St. Joseph Baptist Church, are located within the community of Algiers in New Orleans in a predominately African- American neighborhood. The City of Love Church is located within the predominately African-American New Orleans's uptown community. Based on the completed surveys, 17 individuals were recruited from the Greater Morning Star Baptist Church and St. Joseph Baptist Church. 13 individuals were recruited from The City of Love church. The following requirements had to be met in order for an individual to participate in the study:1) Caregivers had to be 18 years or older; 2) Participants had to self-identify as African American; 3) Participants had to speak English; and they had to care for at least one child between the ages of 6-11 years old, with the child also self-identifying as African- American. A 14 question survey using a Lickert scale was administered and each question represented one of the five hypothesized categories for the influences on parental feeding practices: culture, knowledge, lifestyle, affordability and accessibility. The survey was self-crafted. The first four questions focused on the culture of the participants. Questions 5-8 focused on the knowledge that one has concerning healthy food choices. Questions 9-12 focused on the effects lifestyle may have on his/her food selections and meal preparations. Question 13 was targeted to the concerns of individuals enrolled in the Supplemental Nutrition Assistance Program (SNAP) and if they received enough benefits to feed their families on a monthly basis. Lastly, question fourteen addressed the concerns of having reliable transportation to a local supermarket. An information table was set up in each churchs' atrium, and members voluntarily inquired and completed a survey after church services. Each participant was handed a de-identified hard copy of the survey to complete on-site. As compensation, the participants were asked to provide a contact number and mailing address as an entry to a $50 raffle ticket drawing. All identifying information was properly disposed upon the drawing of the winner. Each caregiver participant was also given handouts regarding USDA recommendations on daily exercise and child nutrition.

Results

Participants were asked to complete a 14 question survey, of which 45 surveys were completed. Through direct observation, primarily women participants identified as the primary caregivers compared to men. Participants were not asked to identify their gender within the survey.

Table 1.

QUESTION	RESPONSE (n= responder percentage)
CULTURE	

1. Food is the main part of my family gatherings.	Never : n= 0 Rarely: n=2.3% Sometimes: n= 4.5% Often: n= 15% Always: n= 77%
2. Healthy diets are for people trying to lose weight.	Never: n= 15.6% Rarely: n=8.9% Sometimes: n= 23% Often: n= 23% Always: n= 20% No answer: n= 8.9%
3. My child is healthy as long as they are not too thin or fat.	Never: n=20 % Rarely: n= 13.3% Sometimes: n= 38% Often: n= 15.6% Always: n= 13.3%
4. I like to try new ways to cook for my family.	Never: n=2.2% Rarely: n= 6.7% Sometimes: n= 22.2% Often: n= 24.4% Always: n=41% No answer: n= 2.2%
KNOWLEDGE	
5. You are able to choose healthy foods at the grocery store.	Very confident : n= 60% Somewhat confident: n= 31.1% Not confident at all: n= 6.6% Not sure: n=0%
6. Do you believe that making healthy food choices will help keep you and your family healthy?	Yes it will: n= 82% It will help a little: n= 16.6% No it will not : n=0% Not sure if it will help: n= 0% No answer: 2.2%
7. Growing up, I was taught that it was important to have a healthy diet.	Never: n= 11.1% Rarely: n= 40% Sometimes: n 4.4% Often: n= 17.8% Always: n= 24.4%

8. I read the nutrition labels before I buy food for my family.	Never: n= 13.3% Rarely: n= 13.3% Sometimes: n= 37.8% Often: n= 17.8% Always: n= 17.8%
LIFESTYLE	
9. Do you think it is difficult to eat a healthy meal?	Yes: n= 17.8% No: n= 68.8% Not sure: n= 11.1%
10. For question 9, if answered yes, please tell me why it is difficult for you to eat a healthy meal.	a) It takes too much time to prepare: n= 2.2% b) Healthy foods cost too much: n= 40% c) My family does not eat healthy foods d) It is difficult to find healthy foods that my family likes. (n = 2.2%) e) I do not know how to buy healthy foods. (no response) f) I do not know how to use food labels. (no response) h) I do not know what foods are healthy. i) Other. * (see below)
11. During mealtime my family eats together.	Never: n= 0% Rarely: n= 4.4% Sometimes: n= 13.3% Often: n= 13.3% Always: n= 62.2% No Response: 4.4%
12. Within my household, there are other individuals who can help me take care of my children.	Never: n= 17.8% Rarely: n= 17.8% Sometimes: n= 26.7% Often: n= 8.9% Always: n= 24.4% No response: n=4.4%
AFFORDABILITY	
13. I receive enough assistance from the SNAP benefits to feed my family.	Yes: n= 20% No: n= 60% I'm not sure: n= 13.3% No answer: n= 6.7%
Accessibility	

14. I have reliable transportation to buy my groceries from a supermarket.	Yes: n=78% No: n= 6.7% I'm not sure: n= 8.9% No answer: n= 4.4%

* Question 10 " other" response: Four individuals responded in total. Three individuals provided a written answer. The responses focused on not having healthy restaurants nearby, healthy foods tasted nasty, and few healthy choices available. One individual stated that they did but healthy foods.
*** Participants were asked to match their thoughts on each question with the provided answers.

Discussion:

Understanding the demographics and cultural background of one's patients can provide insight into delivering optimal care. Obesity and its associated medical problems has become a part of many individual's lives within American culture, particularly the low-income African-American culture. In order to understand the reasoning behind the prevalence of childhood obesity within minority African- American children, it is important to assess the environment of the child, including his/her parental guardianship. It is not enough for one to merely suggest monitoring one's dietary intake to maintain a healthier lifestyle. Looking at social factors and how it influences his/ her decision to eat certain foods plays a major role in one's dietary intake. Upon surveying low-income African- American caregivers, it was concluded that social factors do in fact play a role in one's decision-making; furthermore, such decisions may have a direct influence on the children of these caregivers. Food and culture are closely tied, and the value one places on food has a correlation to the cultural environment that he or she was immersed in. Food did prove to be a focal point of family gatherings, which may lead one to state that food is valued for the emotional bonding it brings rather than mere subsistence. Thus the type of food one consumes may not be monitored for health purposes, but, rather, selected for the emotional stimulus it brings. Cultural attitudes of healthy eating being seen as a means to diet and to lose weight, as well as health being judged by physique can also shape one's attitude towards certain food selections. Interestingly, there was a spectrum of individuals who viewed healthy diets as a means for trying to lose weight. Such a belief may dissuade individuals from choosing healthier options because he/she is comfortable with his/her body type. Another point to mention is that one's definition of health can vary greatly. Perhaps, the participants, and many others within the African American community, view health as being at an ideal weight size, and thus, may not find a need to actively choose healthier food options. Having the proper knowledge concerning one's health is also reflective of the choices one will make regarding his/ her family nutrition. If one is never properly taught the standards of health, including one's diet, then it cannot be expected that the values conveyed to their children would differ. Factors such as lacking the time and proper financial resources also helps to shape the meals that a caregiver provides for his/ her family. Thus, social factors such as socioeconomic status can play a role in individuals planning meals based upon affordability as opposed to the effects that it has on one's health. Interestingly, 60% of the participants stated that SNAP benefits were not enough to feed their families. Having financial access to food may not seem as a barrier to purchasing nutritional food items; however, if the food stipend is not enough, perhaps individuals are still restricted to buying foods based on cost rather than the health benefits. Considering other aspects of the caregiver's life such as assistance in caring for his/her children or reliable transportation to the supermarket were also important in assessing the influences of his/her family's food selections. While proper transportation to a grocery store that provided healthy food selections did not appear as an issue, the availability of help within the household could have potentially been a concern for the

primary caregivers. Having the burden of caring for children in all aspects of their lives, including meal preparations, can be stressful. Thus, one may tend to serve meals that are convenient rather than nutritious. Having additional help within the household can lessen such burden and perhaps give the caregiver more time to prepare meals that are more beneficial to their family. Possible limitations/ biases to this study could include not having an equal representation of male and female participants. The results could have possibly differed with a greater representation of male participants who may or may not have identified with the specific barriers outlined within the survey.

Overall, this project showed the importance of seeing the various factors that can influence one's decision making process as it relates to food and health. Various social factors did influence one's ability to properly provide a healthy lifestyle for his/her family. Moreover, the example that one set's for proper food nutrition can shape the attitudes of the children whom they care for, which may perpetuate cycles of healthy or non-healthy eating habits. These results suggests that the issues of obesity and poor dieting among African-Americans extend beyond poor judgment and lack of will-power. Rather, one's social/cultural makeup appear to play an integral role in one's decision making process. The prevalence of obesity within the African-American community, especially within children, can thus be related to one's culture and environment. As future leaders of the healthcare field, one can realize the various factors that may influence the lifestyle of his/her patients. One can use this knowledge to understand the patient's perspective and tailor healthcare in a way to maximize the patient's health.

Resources

Baughcum, Amy. "Maternal Feeding Practices and Beliefs and Their Relationships to Overweight in Early Childhood." *Development and Behavioral Pediatrics* . 22.6 (2001): n. page. Print.

Centers for Disease Control and Prevention . *Adolescent and School Health*. Atlanta : 2013. Print. <http://www.cdc.gov/healthyyouth/obesity/facts.htm>.

Obesity and Prevention Source. *Harvard School of Public Health* . N.p.. Web. <http://www.hsph.harvard.edu/obesity-prevention-source/obesity-causes/genes-and-obesity/>.

Office of Minority Health . *Obesity and African Americans* , 2013. Print. <http://minorityhealth.hhs.gov/>.

Bevan, Graham

Jean-Philippe Couderc, PhD and Dr. Arthur Moss, M.D.
University of Rochester
Heart Research Follow-up

Heart rate dependency of T-wave morphology in symptomatic and asymptomatic patients with type-1 Long QT Syndrome

Background:

The Congenital Long QT syndrome Type 1 (LQT1) is associated with syncope, life-threatening cardiac arrhythmias, and sudden cardiac death [1]. While heart-rate corrected QT interval (QTc) is predicative of cardiac events, not all symptomatic patients with LQT1 have a prolonged QTc [2]. The T-wave of the ECG may be valuable in risk-stratification of LQT1 patients. The T-wave represents ventricular repolarization and it can capture the repolarization heterogeneity that is arrhythmogenic in LQT1 [3, 4]. Patients with LQT1 have abnormal ventricular repolarization that is heart-rate dependent [5].

Objectives:

We examined heart rate dependent differences in T-wave morphology between symptomatic and asymptomatic LQT1 patients using Holter ECGs. We will determine the efficacy of these parameters in discriminating between symptomatic and asymptomatic patients to improve risk-stratification.

Methods:

We investigated the following descriptive parameters of T-wave morphology: QT interval, T-wave peak to T-wave end (TpTe), T-wave amplitude (Tamp), and complexity of repolarization ($\lambda 2/\lambda 1$)[6]. We examined 12-lead Holter ECGs (H12 recorder, Mortara, Milwaukee, WI) from 93 LQT1 patients: 42 asymptomatic (ASym) and 51 symptomatic (Sym) collected by Lane *et. al* [7]. We used the V2 lead because differences between types of Long QT may be greater here [8]. Using the University of Rochester-developed software, Comprehensive Analysis of Repolarization Signal (COMPAS). These measurements were based on both beat-to-beat ECG and representative median beats from 10 consecutive cardiac cycles. Through RR bin analysis, we categorized median measurements into 25 ms bins from RR intervals of 600ms to 1200ms [6, 9]. We compared the clinical characteristics and T-wave morphology parameters between the ASym and Sym groups using Wilcoxon Rank Sum. We used binary logistic regression to determine odds ratios for each t-wave morphology parameter at slow RR intervals (900-1200ms) and fast RR intervals (600-900ms).

Results:

Symptomatic patients had a greater QT in lead V2 at RR intervals > 925 ms, a greater TpTe at RR intervals > 875 ms, and a greater Tamp at RR intervals > 875 ms (p value <.05). There were no differences in $\lambda 2/\lambda 1$ between ASym and Sym at any bin intervals (p value >.05). Binary logistic regression of model showed goodness of fit wald chi-square p values<.05 for QT and Tamp at slow heart rate and all T-wave morphology parameters at fast heart rates. At fast heart rates odds ratios for QT, Tamp, TpTe, and $\lambda 2/\lambda 1$ (95% Wald confidence intervals) were 1.006 (1.003, 1.009), 9.627 (5.658, 16.379), .975 (.965, .984), and 7.152 (1.972, 25.938),

respectively. At slow heart rates odds ratios for QT, Tamp, TpTe, and λ2/λ1 (95% Wald confidence intervals) were 1.017 (1.013, 1.020), 33.441 (17.182, 65.084), 1.006 (.997, 1.015), and .713 (.208, 2.439), respectively. The percent concordant and percent disconcordant are 76.9% and 22.9% respectively.

Discussion:

Each of these T-wave morphology patterns fits the binary logistic regression model at fast heart rates but only Tamp and QT fit at slow heart rates. At fast heart rates, decreased TpTe and increased λ2/λ1 are associated with increased odds of being symptomatic. At slow heart rates Tamp and QT are directly associated with increased odds of being symptomatic. Further analysis may demonstrate how well these parameters can risk-stratify.

References

1. Moss, A.J., *Long QT Syndrome.* JAMA, 2003. **289**(16): p. 2041-4.
2. Priori, S.G., et al., *Risk stratification in the long-QT syndrome.* N Engl J Med, 2003. **348**(19): p. 1866-74.
3. Kuo, C.S., et al., *Characteristics and possible mechanism of ventricular arrhythmia dependent on the dispersion of action potential durations.* Circulation, 1983. **67**(6): p. 1356-67.
4. Extramiana, F. and C. Antzelevitch, *Amplified transmural dispersion of repolarization as the basis for arrhythmogenesis in a canine ventricular-wedge model of short-QT syndrome.* Circulation, 2004. **110**(24): p. 3661-6.
5. Couderc, J.P., et al., *Genotype- and Sex-Specific QT-RR Relationship in the Type-1 Long-QT Syndrome.* J Am Heart Assoc, 2012. **1**(2): p. e000570.
6. Vaglio, M., et al., *A quantitative assessment of T-wave morphology in LQT1, LQT2, and healthy individuals based on Holter recording technology.* Heart Rhythm, 2008. **5**(1): p. 11-8.
7. Lane, R.D., et al., *Changes in ventricular repolarization duration during typical daily emotion in patients with Long QT syndrome.* Psychosom Med, 2011. **73**(1): p. 98-105.
8. Struijk, J.J., et al., *Classification of the long-QT syndrome based on discriminant analysis of T-wave morphology.* Med Biol Eng Comput, 2006. **44**(7): p. 543-9.
9. Badilini, F., et al., *QT interval analysis on ambulatory electrocardiogram recordings: a selective beat averaging approach.* Med Biol Eng Comput, 1999. **37**(1): p. 71-9.

Basic Science, Clinical & Translational Research

Bragg, Andrew

Preceptor:
Adit Ginde, MD, MPH
University of Colorado School of Medicine
Department of Emergency Medicine

Use of Proton Pump Inhibitors for the Prevention of Septic Acute Kidney Injury

Abstract:

Sepsis is a syndrome of infection-related systemic inflammation that is responsible for over 200,000 annual deaths and $16.7 billion in direct medical costs in the United States alone.[1] Among individuals in septic shock (the most severe degree of sepsis) as many as 60% will develop acute kidney injury (AKI), an abrupt loss of renal function that can lead to electrolyte imbalance, metabolic acidosis, and death.[2-4] Not surprisingly, AKI is associated with significantly higher mortality for septic patients.[5-8] Despite the prevalence and severity of this condition, the etiology of septic AKI remains poorly understood, and treatment is limited to supportive care.[9,10]

Recent research has shown that suppression of the ATP4A proton pump by genetic deletion and by use of proton pump inhibitors (PPIs) strongly preserves kidney function in murine models of ischemic and septic AKI (publication pending). Because PPIs are commonly used for stress ulcer prophylaxis in critically ill patients, it is relatively simple to compare changes in kidney function across septic patients with and without PPI treatment.[11-15] In addition to kidney function, we will also compare mortality, length of hospital stay, and other outcomes across the exposure and control groups. Furthermore, PPI treatment in critically ill patients has been shown to slightly increase the risk of acquiring *Clostridium difficile* colitis, which is associated with significantly worse outcomes for patients.[18,19] As a result, we will also be comparing incidence of *C. difficile* infection across both groups, in order to better quantify the risks associated with PPI treatment. We are in the process of reviewing medical records for patients admitted to University of Colorado Hospital with septic shock, and we expect to begin data analysis within the next 6 months.

Objectives:

We hope to demonstrate that early PPI treatment is associated with decreased incidence and severity of AKI among patients in septic shock. We will also evaluate potential adverse effects, especially *Clostridium difficile* infection. Although the design of this study does not allow us to demonstrate any causal relationships, we hope that it will provide the basis for a more robust trial to evaluate the efficacy of PPIs in preventing septic AKI.

Methods:

This is a retrospective cohort study of adult patients at the University of Colorado Hospital Emergency Department, admitted between October 1, 2011 and December 31, 2013. We screened all patients with an admitting or discharge diagnosis of septic shock (ICD-9 code 785.52). In addition, patients who received antibiotics in the Emergency Department and were admitted to the intensive care unit will be screened to determine if inclusion criteria were met (to capture cases who did not receive the ICD-9 diagnosis). During chart review, septic shock was defined as the presence of all of the following criteria in the Emergency Department, based on standard guidelines for diagnosis of septic shock.[16,17]

a. Suspected or confirmed infection, and receiving antimicrobial therapy
b. Systemic inflammatory response syndrome (SIRS) defined by two or more of the following:

- White blood cell count <4000 or >12000 cells/ml
- Heart rate >90 beats/minute
- Respiratory rate >20 breaths/minute or $PaCO_2$ < 32 mmHg
- Temperature <36°C or >38°C

c. Shock, deemed new or due to infection, as defined by at least one of the following after administration of at least 40 cc/kg of intravenous fluid:
   - Systolic blood pressure <90 mmHg
   - Mean arterial pressure <60 mmHg
   - Vasopressor therapy required for >1 hour

Patients meeting these criteria are grouped based on PPI treatment early in the hospital course. The primary exposure group consists of patients who received PPIs within 12 hours of Emergency Department presentation. Secondary exposure groups consist of 1) patients who received PPIs 12-36 hours after ED presentation and 2) patients who received PPIs 36-72 hours after presentation. The primary comparison group consists of patients meeting septic shock criteria who did not receive PPIs within 72 hours of ED presentation. Based on preliminary results, we anticipate that the final sample will include 300-350 patients.

The primary outcome we are interested in is the change in serum creatinine from baseline (first measurement in ED) to 72 hours (+/- 6 hours). Secondary outcomes will include change in serum creatinine from baseline to 7 days, need for hemodialysis, GI bleeding events, new diagnosis of *Clostridium difficile* colitis, ICU and hospital length of stay, and mortality.

Results:

Due to the tedious nature of chart abstraction, we do not have enough data to begin analysis yet.

Conclusions:

We have not collected enough data to draw any meaningful conclusions at this time. Data collection is ongoing, and we hope to begin analysis within the next 6 months. While the study design prevents us from inferring any causal relationships from this study, a positive result would provide the basis for a more robust trial in the future. However, if we find that PPI treatment increases the risk of *Clostridium difficile* colitis within this cohort, we will need to carefully consider the potential risks and benefits before any further trials begin.

References

1. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med. 2001;29(7):1303-10. PMID: 11445675
2. Bagshaw SM, Uchino S, Bellomo R, Morimatsu H, Morgera S, Schetz M, Tan I, Bouman C, Macedo E, Gibney N, Tolwani A, Oudemans-van Straaten HM, Ronco C, Kellum JA. Septic acute kidney injury in critically ill patients: clinical characteristics and outcomes. Clin J Am Soc Nephrol. 2007;2(3):431-9. PMID: 17699448
3. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, Schetz M, Tan I, Bouman C, Macedo E, Gibney N, Tolwani A, Ronco C. Acute renal failure in critically ill patients: a multinational, multicenter study. JAMA. 2005;294(7):813-8. PMID: 16106006
4. Plataki M, Kashani K, Cabello-Garza J, Maldonado F, Kashyap R, Kor DJ, Gajic O, Cartin-Ceba R. Predictors of acute kidney injury in septic shock patients: an observational cohort study. Clin J Am Soc Nephrol. 2011;6(7):1744-51. PMID: 21734090
5. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol. 2005;16(11):3365-70. PMID: 16177006
6. Bagshaw SM, Lapinsky S, Dial S, Arabi Y, Dodek P, Wood G, Ellis P, Guzman J, Marshall J, Parrillo JE, Skrobik Y, Kumar A. Acute kidney injury in septic shock: clinical outcomes and impact of

duration of hypotension prior to initiation of antimicrobial therapy. Intensive Care Med. 2009;35(5):871-81. PMID: 19066848
7. Oppert M, Engel C, Brunkhorst FM, Bogatsch H, Reinhart K, Frei U, Eckardt KU, Loeffler M, John S. Acute renal failure in patients with severe sepsis and septic shock--a significant independent risk factor for mortality: results from the German Prevalence Study. Nephrol Dial Transplant. 2008;23(3):904-9. PMID: 18065435
8. Silvester W, Bellomo R, Cole L. Epidemiology, management, and outcome of severe acute renal failure of critical illness in Australia. Crit Care Med. 2001;29(10):1910-5. PMID: 11588450
9. Wan L, Bagshaw SM, Langenberg C, Saotome T, May C, Bellomo R. Pathophysiology of septic acute kidney injury: what do we really know? Crit Care Med. 2008;36(4 Suppl):S198-203. PMID: 18382194
10. Benes J, Chvojka J, Sykora R, Radej J, Krouzecky A, Novak I, Matejovic M. Searching for mechanisms that matter in early septic acute kidney injury: an experimental study. Crit Care. 2011;15(5):R256. PMID: 22030145
11. Cook DJ, Fuller HD, Guyatt GH, Marshall JC, Leasa D, Hall R, Winton TL, Rutledge F, Todd TJ, Roy P, et al. Risk factors for gastrointestinal bleeding in critically ill patients. Canadian Critical Care Trials Group. N Engl J Med. 1994;330(6):377-81. PMID: 8284001
12. Faisy C, Guerot E, Diehl JL, Iftimovici E, Fagon JY. Clinically significant gastrointestinal bleeding in critically ill patients with and without stress-ulcer prophylaxis. Intensive Care Med. 2003;29(8):1306-13. PMID: 12830375
13. Cook DJ, Griffith LE, Walter SD, Guyatt GH, Meade MO, Heyland DK, Kirby A, Tryba M. The attributable mortality and length of intensive care unit stay of clinically important gastrointestinal bleeding in critically ill patients. Crit Care. 2001;5(6):368-75. PMID: 11737927
14. Alhazzani W, Alenezi F, Jaeschke RZ, Moayyedi P, Cook DJ. Proton pump inhibitors versus histamine 2 receptor antagonists for stress ulcer prophylaxis in critically ill patients: a systematic review and meta-analysis. Crit Care Med. 2013;41(3):693-705. PMID: 23318494
15. Barkun AN, Bardou M, Pham CQ, Martel M. Proton pump inhibitors vs. histamine 2 receptor antagonists for stress-related mucosal bleeding prophylaxis in critically ill patients: a meta-analysis. Am J Gastroenterol. 2012;107(4):507-20; quiz 21. PMID: 22290403
16. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb SA, Beale RJ, Vincent JL, Moreno R. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med. 2013;41(2):580-637. PMID: 23353941
17. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G, Sccm/Esicm/Accp/Ats/Sis. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med. 2003;31(4):1250-6. PMID: 12682500
18. Janarthanan S, Ditah I, Adler DG, Ehrinpreis MN. Clostridium difficile-associated diarrhea and proton pump inhibitor therapy: a meta-analysis. Am J Gastroenterol. 2012;107(7):1001-10. PMID: 22710578
19. Lagu, et al. The impact of hospital-onset *Clostridium difficile* infection on outcomes of hospitalized patients with sepsis. J Hosp Med. 2014;9(7). PMID: 24715578

Eric Chang, MS2

Preceptor:
Scott E. Liebman, M.D., M.P.H.
University of Rochester
Department of Nephrology

Characterizing end stage renal disease patients starting hemodialysis with a catheter

Introduction

Hemodialysis (HD) is a treatment option for patients with end stage renal disease (ESRD)[1]. Optimal dialysis can prevent a variety of uremic complications and can also extend the lifespan of patients with ESRD[1,2]. Access to circulation is essential for HD and may be achieved in three ways: via arteriovenous fistulas (AVFs), arteriovenous grafts (AVGs) and catheters[3]. All three methods are usable for dialysis, but AVFs are preferred[4,5,6]. When compared to catheter use, AVFs have been shown to have half the rate of septicemia[7], 43% lower risk for cardiovascular-related mortality[6], reduced rates of infection[8] and reduced rates of all-cause mortality[5]. AVGs are considered suboptimal when compared to AVFs but are still safer than catheters[4].

Despite AVFs being the preferred vascular access, approximately 30% of patients undergoing HD at URMC use a catheter as their permanent dialysis access. In addition, there are also patients using a catheter as they transition to an AVG or AVF (which typically takes two to three months to mature). Investigating why incident HD patients started with a catheter may reveal the obstacles to AVF/AVG placement and use. If identified, strategies to address these barriers would allow us to further optimize ESRD care at URMC.

Objective:

Our goal is to identify the reasons for why patients start HD with a catheter.

Methods:

Patients who began HD with a catheter in a URMC-network hospital/outpatient setting during 1/1/2012 to 4/15/2014 were identified through the Department of Nephrology's records. Patients dialyzing for fewer than 90 days, were under 18 years of age, or were being managed by Pediatric Nephrology but initiated HD after age 18 were excluded.

Charts of qualifying patients were accessed from Epic Systems eRecord and demographic information and lab values were collected. Physician notes were used to identify the cause(s) of CKD and ESRD and to recreate the history between the first nephrology consult and HD initiation. When possible, collected data were corroborated with patients' dialysis registration forms (Form 2728).

All data points were recorded and percentages, means, medians and standard deviations calculated in Microsoft Excel.

Additional analysis was done specifically for patient groups that constituted the top two reasons for catheter starts.

Results:

A total of 136 patients began HD with a catheter in this study. 58 (42.65%) of all HD catheter-starts suffered an acute kidney injury (AKI) on chronic kidney disease (CKD), while 19 patients (13.97% of all HD catheter-starts) refused to have an AVF/G put in place. When combined, these patients accounted for over half (56.62%) of all the 136 HD catheter-start patients.

28 of the 58 AKI on CKD patients were regularly seen by a nephrologist in an outpatient setting and were not considering a kidney transplant/on a transplant list. The cause of their CKD, last office visit GFR before initiating HD, and stage of CKD before initiating HD are summarized in Table 1.

Table 1. Patients with AKI on CKD being seen by a nephrologist and not considering/on kidney transplant list

		Number	% of Total (n=28)
Cause of CKD	Diabetes	14	50.00%
	Hypertension	10	35.71%
	Cardiorenal syndrome	5	17.86%
		Median	**SD**
Last office visit GFR before HD start		20.00	8.93
CKD Stage before HD start		4.00	0.66

Conclusions:

Our data show that AKI on CKD and patient refusal of AVF/G placement are major reasons for why ESRD patients at URMC start HD with a catheter. These results highlight the difficulty of managing CKD patients who are prone to AKI; patients with stable CKD could have similar laboratory values to the AKI on CKD patients in our study (GFR in the 20's) and not need referral to a vascular surgeon. However, superimposing an AKI on their CKD would potentially preclude fistula/graft planning altogether by necessitating an emergency HD start. Which CKD patients are susceptible to AKI and could benefit from an earlier referral is unclear and could be the focus of further study. Additionally, exploring why patients refused AVF/G placement could yield new insights that would aid clinicians in counseling and supporting similar patients in the future.

References:

1. Himmelfarb J, Ikizler TA. Hemodialysis. N Engl J Med. 2010 Nov 4; 363: 1833-1845.
2. Hemodialysis Adequacy 2006 Work Group. Clinical practice guidelines for hemodialysis adequacy: update 2006. Am J Kidney Dis 2006; 48 (Suppl 1): S2–90.
3. NIDDK, Web. 31 Jan 2014. http://kidney.niddk.nih.gov/kudiseases/pubs/hemodialysis/.
4. Dhingra RK, Young EW, Hulbert-Shearon TE et al. Type of vascular access predicts mortality in US hemodialysis patients. Kidney Int 2001; 60: 1443–1451.
5. Pastan S, Soucie JM, McClellan WM. Vascular access and increased risk of death among hemodialysis patients. Kidney Int 2002; 62: 620–626.
6. Wasse H, Speckman B, McClellan WM. Arteriovenous fistula use is associated with lower cardiovascular mortality compared with catheter use among ESRD patients. Semin Dial 2008; 21: 483–488.
7. Powe NR, Jaar B, Furth SL et al. Septicemia in dialysis patients: incidence, risk factors, and prognosis. Kidney Int 1999; 55: 1081-90.
8. Nassar GM, Ayus JC. Infectious complications of the hemodialysis access. Kidney Int 2001; 60: 1–13.

Dauphin, Leah

Preceptor:
Julia A. Margenthaler, MD
Washington University School of Medicine, St. Louis, Missouri
Department of Surgery

CANCER ANXIETY AND PATIENT SELECTION OF MASTECTOMY OVER BREAST CONSERVATION THERAPY

Objectives: Breast conservation therapy (BCT) provides equivalent survival outcomes to mastectomy for women with early-stage breast cancer. Despite this, recent studies have reported increases in the rate of mastectomy and contralateral prophylactic mastectomy. We investigated the indications for mastectomy in a cohort of women. We sought to determine specific patient and clinical characteristics impacting this decision-making pro

Methods: A questionnaire was administered to 349 patients who had undergone previous unilateral or bilateral mastectomy for breast cancer during the years 2006 to 2010. The survey queried on demographics, surgical treatment received, and the rationale for those decisions. A retrospective chart review collected clinical characteristics and details surrounding the treatment decision-making process. Descriptive statistics were utilized for data summary.

Results: Of 349 patients surveyed, 326 had complete clinical data. Of those, 206 (63%) were not offered BCT and mastectomy was recommended by their physician. Of 206 not offered BCT, clinical data demonstrated BCT contraindications for 171 (83%) with multicentric disease or extent of disease prohibitive of BCT, 25 (12%) who failed BCT secondary to positive margins, and 10 (5%) with recurrence following BCT. The remaining 120 (37%) patients were offered BCT but chose mastectomy. Reasons provided for this decision (patients were allowed to choose more than one reason) included "felt mastectomy would reduce recurrence risk" in 85 (71%), "felt mastectomy would improve survival" in 44 (37%), "avoidance of radiation therapy" in 22 (18%), "felt mastectomy was a better option cosmetically" in 6 (5%), "avoidance of future surveillance imaging" in 3 (3%), and "encouragement by friends/family" in 2 (2%).

Conclusions: Nearly two-thirds of the patients undergoing mastectomy for breast cancer in our study were not offered BCT secondary to absolute and/or relative contraindications. For those patients electing mastectomy despite BCT eligibility, the predominant reason for their choice was anxiety over future cancer risk. Prospective studies are needed to determine whether patient education regarding perceived versus actual recurrence risk would alter this decision-making process.

References

NIH Consensus conference. Treatment of early-stage breast cancer. JAMA 1991;265(3):391.

Poggi MM, Danforth DN, Sciuto LC, et al. Eighteen-year results in the treatment of early breast carcinoma with mastectomy versus breast conservation therapy. Cancer 2003;98(4):697.

McGuire KP, Santillan AA, et al. Are mastectomies on the rise? A 13-year trend analysis of the selection of mastectomy versus breast conservation therapy in 5865 patients. Ann Surg Oncol 2009;16(10):2682.

Fisher CS, Martin-Dunlap T, et al. Fear of recurrence and perceived survival benefit are primary motivators for choosing mastectomy over breast-conservation therapy regardless of age. Ann Surg Oncol 2012;19:3246.

DeHority, Rebecca

Preceptor:
John Elfar, MD
University of Rochester Medical Center
Center for Musculoskeletal Research
Department of Orthopedics

Characterization of Peripheral Nerve Crush Injury

Background and Introduction:

Peripheral nerve injury is a key feature of trauma to the extremities and the recovery of the nerve takes longer than other types of injury. The severity and location of a peripheral nerve injury determine the optimal clinical intervention and prognosis. A mild peripheral nerve injury has the ability to regenerate without invasive treatment, whereas a severe nerve injury requires surgical intervention. It is critical to assess the severity of the injury to determine the optimal course of treatment, however current methods of distinguishing type and severity of injury are delayed for weeks post-injury.[1,2]

The staging of peripheral nerve injury according to the currently accepted classification is suboptimal. This classification system was developed by Seddon and Sunderland and applies characteristics of individual neurons to entire nerves, defining injuries in binary terms such as neuropraxia, axonoetmesis, or neuroetmesis[3,4]. However, the reality of peripheral nerve injuries is much more complex. In a large, injured peripheral nerve severed axons exist alongside intact axons and demyelinated axons, creating a lot of variability in structural integrity and functional outcome[5]. The difficulty in effectively classifying peripheral nerve injuries creates a diagnostic dilemma.

Different degrees of injury result in different time courses of recovery and improvement in function[6.] Our lab has previously found that 4-aminopyridine (4-AP) can be used to prolong the action potential of demyelinated axons and transiently improve the function of crushed peripheral nerves[7]. We hypothesize that this transient improvement represents the portion of the nerve that retains it architecture and thus want to determine what percent of the nerve must be intact to support the level of functional improvement that is seen with the administration of 4-AP.

Objectives:

To describe the architecture of the nerve that underlies the transient functional improvement seen in systemic administration of 4-AP to a mouse with a peripheral nerve crush injury. Furthermore, to characterize the changes in the amount myelin and neurofilament at the site of the crush injury, and proximal and distal to the injury.

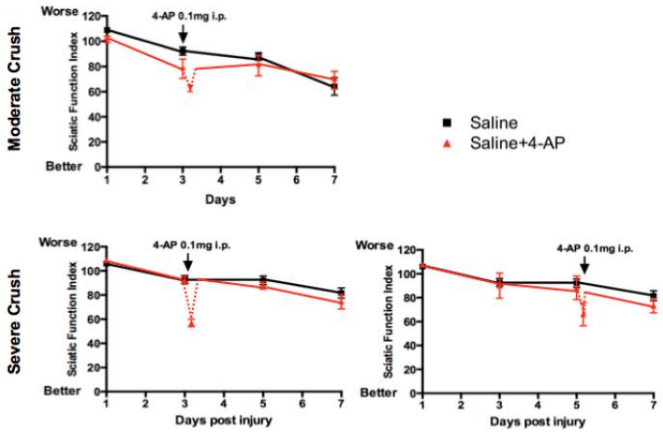
Methods:

We performed a standard moderate and severe crush injury to the sciatic nerves of mice. 4-AP was administered to a portion of the mice and Sciatic Functional index completed using standardized procedures. In another subset of mice the crushed nerves were harvested at day 3 and day 5 for immunohistochemical analysis. Cross sections of the nerve at sites proximal, at the site of the crush, and distal to the injury were stained for P0 (a marker of myelination) and

neurofilament (NF). Quantitative analysis of stained cross-sections of the nerve was completed using FIJI software.

Results:

A transient, but significant improvement in SFI is seen when 4-AP is administered systemically. The functional improvement was more significant for the severe crush injuries than the moderate crush injuries (30% vs. 17%) at day 3. The improvement is less profound at day 5. This suggests that a critical window of available, but dysfunctional nerves exist following a crush injury.



The quantitative analysis of nerves with immunofluorescent staining for myelin and NF reveal that the injury propogates both proximally and distally. At 3 days post injury, all three positions (proximal, crush, and distal) show significantly decreased levels of myelin and NF compared to control nerves. Furthermore, this injury progresses so at day 5 there is even further demyelination and NF loss. Interestingly the demyelination that occurs is much more significant than the NF loss. This progressive demyelination at all positions around a crush injury has not been previously described in the context of the functional improvement seen with 4-AP.

Conclusions:

The overlay of the functional data with the quantitative analysis of the nerve architecture suggests that there is a critical window of available, but dysfunctional nerves following a crush injury. 4-AP is able to "awaken" these dysfunctional nerves and allow for a transient improvement in function. Analysis of the nerve architecture underlying this improvement suggests that the progressive demyelination is responsible for the decreased effect of 4-AP at day 5 post injury. This highlights the possible role of progressive demyelination in the early pathophysiology of peripheral nerve crush injury.

References:

1. Campbell WW. Evaluation and management of peripheral nerve injury. Clinical Neurophysiology. Sep 2008;119(9):1951-1965.
2. Grant GA, Goodkin, R, Kliot M. Evaluation and surgical management of peripheral nerve problems. Neurosurgery. Apr 1999;44(4):825-839.
3. Sunderland S. A classification of peripheral nerve injuries producing loss of function. Brain. 1951; 74(4):491-516.
4. Sunderland S. The anatomy and physiology of nerve injury. Muscle & nerve. Sep 1990;13(9):771-784.
5. Elfar JC, Jacobson JA, Puzas JE, Rosier RN, Zuscik MJ. Erythropoietin accelerates functional recovery after peripheral nerve injury. Journal of Bone and Joint Surgery. 2008;90:1644-1653.
6. Zingman A, Elfar JC. Erythropoietin mediated neuroprotection in relation to nerve crush injury. In press.
7. Tseng KC, Elfar JC. The potential diagnostic approach to characterize peripheral nerve injury using 4-AP. Neurology. April 2014; 82(10): Supplement P3.057.

Kwak, Stephen

Joseph Miano, Ph.D.
Associate Professor
University of Rochester Medical Center
Department of Medicine, Aab Cardiovascular Research Institute

Potential Role of Novel MyolncRNA-11 in Cardiac and Skeletal Muscle Growth and Differentiation

Introduction:

Non-coding RNA, once considered "junk genomic material", have been shown to be important in several developmental processes[1,2,]. This discovery has shifted our paradigm on genomic material outside the exome and has created excitement to explore regions of human genome that contain 98% of the genomic single nucleotide polymorphisms (SNPs). Consequently, many labs have collectively discovered and documented tens of thousands of non-coding RNA sequences[3]. With so many documented non-coding RNAs (ncRNAs), two broad classes have been created to classify them: short ncRNA (processed transcript length of <200 nucleotides) and long ncRNA (processed transcript length of >200 nucleotides) [4,5,6]. However, the vast majority of the documented non-coding RNAs remain poorly characterized. As more non-coding RNAs are discovered and their functions uncovered, our perspective of genome content and the way we think of genes will change. Furthermore, we will gain new insight into disease-causing mutations associated with non-coding RNA function.

Objectives:

This study aims to further expand our understanding of long ncRNAs (lncRNAs) in the context of vascular smooth muscle cell (VSMC) and endothelial cell (EC) biology. More specifically, this study aims to further characterize some of the lncRNA discovered by the Miano lab, many of which are not annotated in any public database. By acquiring a better understanding of how lncRNAs fine tune gene expression in VSMC and EC, this study helps to aid in gaining insight into how non-coding RNA mutations might cause disease.

Methods:

This study used a systematic approach to studying lncRNAs, which was developed by the Miano lab (revised manuscript submitted). First, 3 lncRNAs of a subset of novel lncRNAs from novel RNA-seq screens were chosen for RNA expression validation using conventional (gel) and quantitative RT-PCR on panels of 15 human cell lines and 12 human tissues, including dated plasma from the URMC Medical Center Blood Bank. The latter tissue panel is of importance from a clinical standpoint as circulating lncRNAs are increasingly being reported as biomarkers of disease and the Miano lab has already discovered 4 novel lncRNAs abundantly present in plasma. No protein experiments were done for these genes since they are, by definition, of low protein-coding potential. .Then, RNA fractionation studies were performed to begin elucidating the localization (nucleus versus cytoplasmic). Dicer substrate RNAs (dsRNA) from Integrated DNA Technologies were ordered and tested for knockdown efficacy in *in vitro* studies using RT-PCR. After testing knockdown of lncRNA, effects of lncRNA knockdown on neighboring gene expression examined to determine whether the lncRNAs under investigation have any *cis*-acting effects on local gene expression.

Results:

Of the three lncRNAs examined, two (myolnc-11 and myolnc-14) were validated using conventional (gel) and quantitative RT-PCR on panels of 15 human cell lines and 12 human tissues. Myolnc-14 was expressed in 14 of 15 human cell lines and 2 of 12 human tissues (heart and skeletal muscle). Myolnc-11 was expressed in 8 of 15 human cell lines and 2 of 15 human tissues (heart and skeletal muscle).

Myolnc-11 was further investigated as it has been shown to play a role in hypertrophic cardiomyopathy. Cell fractionation studies showed myolnc-11 is expressed equally in nucleus and cytoplasm. Knockdown of myolnc-11 with dsRNA showed a 2-fold decrease in nuclear myolnc-11 expression in RD cells. Conversely, stimulation of myolnc-11 with myocardin showed a 2-3 fold increase in nuclear myolnc-11 expression in HCASM cells. Cytoplasmic myolnc11 remained constant in dsRNA and myocardin stimulation studies.

Investigating the effects of knockdown and stimulation showed no change in expression of one of the neighboring genes (myoz-2).

Conclusions:

This study shows that myolnc14 may be a poor candidate to be a house keeping lncRNA, which was its hypothesized function, as it lacks ubiquitous expression across human cell lines and human tissues However, it may serve a role in heart and skeletal muscle tissues since both tissue samples highly expressed myolnc-14.

This study also showed that myolnc-11 may serve a role in heart and skeletal tissues as it was highly expressed in both tissues. Furthermore, this study showed that myolnc-11 is localized in both the cytoplasm and nucleus. Additionally, this study showed that myolnc-11 is inducible by myocardin and that myocardin specifically induces nuclear myolnc-11. However, the effect of myolnc-11 on neighboring genes is unknown since knockdown and stimulation did not affect myoz-2 expression.

References:

1. Geisler S, Coller J. RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. Nature Reviews Molecular Cell Biology 14, 699-712 (2013)
2. Lee JT. Epigenetic regulation by long noncoding RNAs. Science 338:1435-1439 (2012)
3. The ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* 489, 57-74 (2012)
4. Okazaki Y, Furuno M, Kasukawa T, et al. Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. Nature 420:563-573 (2002)
5. Kapranov P, Cawley SE, Drenkow J, Bekiranov S, Strausberg RL, Fodor SPA, Gingeras TR. Large-scale transcriptional activity in chromosomes 21 and 22. Science 296:916-919 (2002)
6. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science 294:853-858 (2001)

Magno, Jinno, MS2

Preceptor:
Jessica Shand, M.D., M.H.S.
Assistant Professor of Pediatrics
University of Rochester Medical Center
Pediatric Hematology/Oncology

Increased TLR4 Expression in MLL-Rearranged Infant Acute Lymphoblastic Leukemia

Introduction: Relapse of acute lymphoblastic leukemia (ALL) is the leading cause of cancer death in children[1-3]. Mixed lineage leukemia (MLL)-rearranged infant ALL (diagnosed <12 months) represents a high-risk subset of disease, in part because it is more resistant to chemotherapy than standard-risk childhood ALL[4-6]. The role that the immune system plays in chemotherapy resistance, specifically in modulating local responses to dying leukemia cells in the bone marrow, is poorly understood. We propose that chemotherapy treatment of ALL cells may alter the immune environment, by releasing damage associated molecular proteins (DAMPs) that activate the innate immune system[7, 8] . Toll-like receptors, particularly Toll-like receptor 4, may play an important role in recognition of DAMPs by generating downstream signals that affect local cytokine production by innate immune cells in the bone marrow[7, 8].

Objective: To assess whether TLR4 is upregulated in ALL cells following doxorubicin treatment, and to assess whether there is a difference in TLR4 expression between high-risk MLL-rearranged ALL (MLL-ALL) and standard-risk (SR-ALL) ALL after treatment with doxorubicin, an anthracycline chemotherapy used in high-risk ALL therapy and recognized to induce immunogenic cell death

Methods: Primary human leukemia cells were isolated from either diagnostic bone marrow or pheresed peripheral blood (n=5; 1 MLL-ALL, 4 SR-ALL) of pediatric patients with ALL (RSRB #0024477) and placed in culture. Next, they received either no treatment, or 2 nM doxorubicin for 3 hours. Cells were then washed, cultured for an additional 24 hours and analyzed for mRNA expression by qPCR and TLR4 cell surface expression by flow cytometry. Fold-increase in TLR4 mRNA was calculated using the ddCt method using beta-glucuronidase as an endogenous reference gene. TLR4 expression, measured by mean fluorescence intensity (MFI) of a phycoerythrin-conjugated anti-TLR4 antibody, was measured using the gating strategy: 1) gating for lymphoblast populations using forward and side scatter, 2) isolating live cell populations by gating on 7AAD 3) isolating pre-B leukemia cells by gating on CD19+. Compensation was performed using beads and IgG isotype controls were used as a negative control for nonspecific antibody binding while Thp1+LPS (human macrophage cell line) and SupB15 (pre-B ALL cell line) were used as positive staining controls for TLR4 and CD19 respectively.

Results: Both qPCR and flow cytometry showed some basal level of TLR4 expression in all ALLs tested. (qPCR: MLL-ALL, 0.050, SD±0.015; SR-ALLs, 0.013-0.150, SD±0.060). Of the 5 ALL cells tested, the high-risk MLL-rearranged infant ALL was the only ALL to consistently show an increase in TLR4 mRNA level by qPCR after doxorubicin treatment (4.22 fold increase, $p < 0.05$). The other, standard-risk ALLs showed no significant changes in TLR4 message level

after treatment. Although initial flow cytometry experiments showed an increase in TLR4 expression (ΔMFI = 1555) in MLL-ALL cells after doxorubicin treatment, live/dead analysis showed that chemotherapy treatment produced an autofluorescence artifact, confirmed by the use of an IgG PE isotype control against live MLL-ALL (ΔMFI = 1359, IgG PE isotype; 1328, MLL-ALL).

Conclusions and Future Directions: The increase in TLR4 mRNA expression in MLL-ALL may provide a receptor mechanism in the pathogenesis of high-risk ALL. Downstream effects of TLR4 activation may result in increased cell survival or promote inflammation through either a MyD88-dependent or independent mechanism and activation of NF-kB pathways[9, 10]. Studies are underway to address whether chemotherapy treatment produces differences in NFkB expression in our primary leukemia cells. If so, this has future implications as a prognostic marker and/or potential therapeutic target. MyD88 signaling is now being recognized as potential component of immune escape and prognostic marker in human cancers[11], specifically chronic lymphocytic leukemia[12]. Future studies required measurement of TLR4 and it's downstream receptors in at least 5 MLL-ALL's to address the limited sample size. In addition, time course experiments to determine TLR4 surface expression between 0-72 hours after doxorubicin therapy are planned.

References:

1. SEER Cancer Statistics Review, 1975-2010. Bethesda: National Cancer Institute; 2013.

2. Hunger SP, Lu X, Devidas M, et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. J Clin Oncol. 2012;30(14):1663-1669.

3. Pui CH, Campana D, Pei D, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med. 2009;360(26):2730-2741.

4. Hilden JM, Dinndorf PA, Meerbaum SO, et al. Analysis of prognostic factors of acute lymphoblastic leukemia in infants: report on CCG 1953 from the Children's Oncology Group. Blood. 2006;108(2):441-451.

5. Pieters R, Schrappe M, De Lorenzo P, et al. A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. Lancet. 2007;370(9583):240- 250.

6. Behm FG, Raimondi SC, Frestedt JL, et al. Rearrangement of the MLL gene confers a poor prognosis in childhood acute lymphoblastic leukemia, regardless of presenting age. Blood. 1996;87(7):2870-2877.

7. Fang H, Ang B, Xu X, et al. TLR4 is essential for dendritic cell activation and anti-tumor T-cell response enhancement by DAMPs released from chemically stressed cancer cells. Cell Mol Immunol. 2013 Dec 23.

8. Ehrchen JM, Sunderkötter C et al. The endogenous Toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer. J Leukoc Biol. 2009 Sep;86(3):557-66. doi: 10.1189/jlb.1008647.

9. Akira S. Toll-like receptor signaling. *J Biol Chem* 2003; 278: 38105–8.

10. Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol* 2004; **4**: 499–511.

11. Kelly M, Alvero A, Chen R, et al. TLR-4 Signaling Promotes Tumor Growth and Paclitaxel Chemoresistance in Ovarian Cancer. Cancer Research. April 1, 2006 66; 3859

12. Barcellini W, Imperiali FG, Zaninoni A, et al. Toll-like receptor 4 and 9 expression in B-chronic lymphocytic leukemia: relationship with infections, autoimmunity and disease progression. Leuk Lymphoma. 2013 Oct 21.

13. Children's Oncology Group protocol AALL1131 (Current Protocol for High- and Very-High Risk Pre-B cell ALL, 2014).

Marini, Thomas

Preceptors: Kevin Fiscella, MD and Mechelle Sanders, BA
University of Rochester
Department of Family Medicine

Phase I of National Library of Medicine Research Study on Patient Use of Internet Resources

Abstract:

Access to reliable and easy-to-understand health information continues to be a problem in poor and minority communities. This creates obstacles in informed decision making and may be problematic for managing medical conditions appropriately. One possible avenue in addressing this deficit in information is through technology and the internet. For example, MedlinePlus (a website full of tools to educate patients on health conditions) was created specifically in the hope of increasing access to medical information. Patient portals are another means of using technology to empower and educate patients. However, in underserved communities, it is unknown how best to increase awareness of these important resources. This research project attempts to address the concern over a lack of health information in underserved communities by studying attitudes and practices related to internet usage among the patient population at three federally qualified health centers in Rochester. The ultimate goal of this project is to discover if resources like MedlinePlus or patient portals can have a positive impact on patient care in these settings. In order to intervene meaningfully, baseline knowledge of how this population uses the internet is necessary. Based on this information, it can be elucidated how best these technological resources may be able to serve patient needs. In this phase of the project, 304 surveys were collected from patients attending appointments at the three health centers on their practices and attitudes related to the internet. Preliminary analysis of the collected data suggests that while awareness of these resources is lacking, interest in them is substantial. Furthermore, it seems training programs to assist those who are not technologically competent are justified as many in the population lack the technical skills necessary to avail themselves of these invaluable resources.

Objectives:

1) To better understand how patients at three federally qualified health centers use the internet and their attitudes about internet usage. 2) To apply this information to increasing awareness of MedlinePlus and usage of the patient portal. 3) To address the fundamental lack of medical information in underserved communities.

Methods:

A survey instrument was constructed to collect baseline information on this patient population's attitudes and practices related to internet resources and patient portals. The survey was given on site at three separate federally qualified health centers in the Rochester area. Data were collected by research assistants using RedCap by interviewing patients in waiting rooms. Data were secured using password protections and was completely anonymous. 304 surveys were collected during the period of study.

Results:

Surveys were collected and analyzed on RedCap. Internet usage in this population was mixed. Among the sample 29.1% never used the internet while 44.8% used the internet every day. Among all participants, 54.2% had never used the internet to look up information about their health (27.1% report having no access to a computer) while 33% report using the internet at least monthly to look up information. Among those who had used the internet to look up information about their health 59.4% were either "interested" or "very interested" in using the internet as a tool in understanding information about their health and 81.9% reported the internet to be "useful" or "very useful" in this regard. However, among those who had used the internet to look up information about their health, 80.3% had never heard of MedlinePlus and only 6 participants reported using it. 76.2% of those surveyed had never heard of the patient portal. However, 64% of those interviewed were "interested" or "very interested" in using the portal, and 57.7% were "interested" or "very interested" in accessing the portal on a smart phone.

Conclusions:

1) Increased awareness of the resources of MedlinePlus and patient portals may be a key area in helping to provide health information to underserved groups. 2) For a large segment of this patient population, implementation of training programs may assist in the increased usage of these resources as many patients in this population lack experience with computers and the internet.

References

Calman N, Kitson K, & Hauser D. Using information technology to improve health quality and safety in community health centers. *Progress in Community Health Partnerships*. 1: 83-88 (2007).

Gibbons CM. Use of health information technology among racial and ethnic underserved communities. *Perspect Health Inf Manag*. 8: 1-13 (2011).

Ketcham JD, Lutfey KE, Gerstenberger E, Link CL, & McKinlay JB. Physician clinical information technology and health care disparities. *Medical Care Research and Review*. 66: 658–81 (2009).

Sheets L, Gavino A, Callaghan F, & Fontelo P. Do language fluency and other socioeconomic factors influence the use of PubMed and MedlinePlus? *Appl Clin Inform*. 4: 170-184 (2013).

Szczepura A. Access to healthcare for ethnic minority populations. *Postgrad Med J*. 81: 141-147 (2005).

Lalita Movva, MS2

Preceptors:
Jingbing Xue, M.D.
Assistant Professor
University of Rochester Medical Center
Department of Imaging Sciences

Adam Fang, M.D.
Radiology Resident, PGY-5
University of Rochester Medical Center
Department of Imaging Sciences

Clinical Efficacy, Safety, and Feasibility of Using Video Glasses During Interventional Radiologic Procedures

Introduction: Many patients have anxiety regarding medical procedures (1,2). This increase in anxiety can have detrimental effects on the health of a patient, since anxiety can lead to physiologic stress, which can then cause a decreased immune response (3,4). Furthermore, patients who experience anxiety regarding surgical procedures tend to have more complications after the procedure, tend to need more pain medication after the procedure, and tend to need more anesthesia during the procedure (5,6). Patients undergoing interventional procedures who have high anxiety levels often also require more medication and longer procedure times (7).

Because of these negative effects that anxiety can have for patients undergoing various procedures, different strategies to reduce patient anxiety have been investigated. Examples of such strategies include listening to music before surgery or using audiovisual technology with pediatric patients undergoing magnetic resonance imaging (MRI). Studies investigating interventions such as these have found various benefits, such as decreased patient anxiety and reduced needs for sedation (8,9,10). Video glasses enable patients to watch movies or other programming in order to serve as a distraction from the procedure, and may also serve as a way to reduce patient anxiety.

Objectives: The purpose of this project is to evaluate the safety, feasibility, and clinical efficacy of using video glasses in a variety of interventional radiologic procedures.

Methods: From August 2012 to August 2014, 86 patients undergoing various outpatient interventional radiologic procedures successfully completed the study at the University of Rochester Medical Center Department of Imaging Sciences at Strong Memorial Hospital, University Imaging at Highland Hospital, or University Imaging at Science Park. The patients were randomized to either a control (no video glasses, n=43) or intervention (video glasses, n=43) group. A State-Trait Anxiety Inventory (STAI) was given to the patient before and after the procedure. Doses of sedation (midazolam) and analgesia (fentanyl), as well as length of procedure, were also recorded. Average mean arterial pressure (MAP), heart rate (HR), respiratory rate (RR), and pain score, were recorded before, during, and after the procedure. Post-procedure complications and any adverse events related to using video glasses were recorded. Attending physicians, residents, and nurses completed post-procedure surveys

evaluating the safety and feasibility of using video glasses. Post-procedure satisfaction surveys were filled out by a subsection of the patients in the intervention group.

Results: A total of 86 patients successfully completed the study. Overall, women had higher pre-procedure STAI scores compared to men (p=0.0104). Patients with high state pre-procedure anxiety (STAI scores $\geq$ 43, n = 22) required slightly higher amount of sedation and significantly higher analgesia during the procedure compared to patients with low state pre-procedure anxiety (STAI scores < 43, n = 64). Patients using video glasses had significantly reduced levels of anxiety compared to the control group (17.1% vs. 8.3%; p=0.0424). Patients using the video glasses also had significantly reduced mean arterial pressures compared to the control group (p=0.0128). There was not a significant difference in amount of sedation and analgesia, nor a significant change in heart rate, respiratory rate, pain score or procedure time, between the intervention group and the control group. None of the patients experienced any adverse events related to use of video glasses. Post-procedure surveys filled out by the patients in the majority of cases showed that the video glasses were not distracting and did not interfere or pose a safety issue during the procedure. Overall, most patients stated they enjoyed the video content and use of video glasses, and would use the video glasses again for a future procedure.

Conclusion: Video glasses can be safely used during many interventional radiologic procedures without disturbing the work of physicians and nurses. These glasses can be used to reduce patient anxiety and improve the overall experience that patients have while undergoing these procedures.

References:

1. Horne DJ, Vatmanidis P, Careri A. Preparing patients for invasive medical and surgical procedures 1: adding behavioral and cognitive interventions. Behav Med 1994; 20:5–13.
2. Mueller PR, Biswal S, Halpern EF, Kaufman JA, Lee MJ. Interventional radiologic procedures: patient anxiety, perception of pain, understanding of procedure, and satisfaction with medication-a prospective study. Radiology 2000; 215:684-688.
3. Grieve RJ. Day surgery preoperative anxiety reduction and coping strategies. Br J Nurs 2002; 11(10):670–678.
4. Starkweather AR, Witek-Janusek L, Nockels RP, Peterson J, Mathews HL. Immune function, pain, and psychological stress in patients undergoing spinal surgery. Spine 2006; 31(18):E641-E647.
5. Stirling L, Raab G, Alder EM, Robertson F. Randomized trial of essential oils to reduce perioperative patient anxiety: feasibility study. J Adv Nurs 2007; 60(5):494–501.
6. Spaulding NJ. Reducing anxiety by pre-operative education: make the future familiar. Occup Ther Int 2003; 10(4):278–293.
7. Schupp JS, Berbaum K, Berbaum M, Lang EV. Pain and anxiety during interventional radiologic procedures: effect of patient's state anxiety at baseline and modulation by nonpharmacologic analgesia adjuncts. J Vas Interv Radiol 2005; 16:1585-1592.
8. Cooke M, Chaboyer W, Schluter P, Hiratos M. The effect of music on preoperative anxiety in day surgery. J Adv Nurs 2005; 52(1):47–55.
9. Arsian S, Ozer N, Ozyurt F. Effect of music on preoperative anxiety in men undergoing urogenital surgery. Aust J Adv Nurs 2008; 26(2): 46–54.
10. Harned RK 2nd, Strain JD. MRI-compatible audio/visual system: impact on pediatric sedation. Pediatr Radiol 2001; 31(4):247-50.

Piazza, Nina

Preceptors:
Andrew Aligne, MD
University of Rochester Medical Center
Department of Pediatrics, Hoekelman Center

Katherine Greenberg, MD
University of Rochester Medical Center
Department of Pediatrics, Adolescent Medicine Division

Knowledge and Awareness of Long-Acting Reversible Contraception (LARC) Among City of Rochester Young Women

Introduction:

Teen pregnancy leads to a number of critical health and social issues including school dropout, poverty, child illness, etc. Substantial public costs are associated with adolescent pregnancy. Despite efforts to reduce teen pregnancy in the United States, adolescent birth rates remain among the highest of the developing world at 34.3/1000. The problem is even more prominent in Rochester, NY, as shown by a rate of 56/1000.

Inconsistent or incorrect contraceptive use is an important contributor to teen pregnancy. LARC includes the intrauterine device (ParaGard or Mirena), which lasts 5-10 years, and the implant (Nexplanon or Implanon), which lasts up to three years. These passive prevention methods eliminate the adherence issues associated with common methods like the oral contraceptive pill, which requires active daily administration. LARC is currently recommended as first-line contraception for teens by WHO, CDC, ACOG, AAP, etc.

Despite its benefits, LARC usage remains very low. Factual knowledge about LARC is low, as is awareness of where to obtain it free and confidentially. Promotion of LARC usage among Rochester teens is an appropriate strategy to reduce unintended or adolescent pregnancy. The Hoekelman Center at the URMC Pediatrics Department is beginning a three-year community-level health education project to raise LARC awareness amongst community leaders, health care providers, and young women.

Objectives: The goal for this project was to collect information about the current knowledge and attitudes that young women in Rochester have towards LARC. This project took place before the Hoekelman Center's health education project began, to get a sense for the current level of LARC knowledge in Rochester. In the coming months and years, other researchers will conduct similar projects, to evaluate the impact that the Hoekelman Center's health education project is having on LARC awareness in Rochester.

Methods: This study involved four focus groups that took place in the summer of 2014. With the help of local health educators, young women between the ages of 15 and 19 were recruited to participate in an hour-long focus group, in which they were shown simple images of some of the most popular contraception methods (intrauterine device, birth control shot, birth control pills, birth control implant, condoms, emergency contraception, birth control ring, and birth control patch), and asked to discuss what they knew and thought about these different options. They

were also asked to quantitatively rate how likely they would be to recommend the different options to a friend.

Results: Based on the quantitative data from eleven participants in their answer to the question "How likely would you be to recommend this birth control method to a friend, on a scale of one to ten?", it was found that the birth control implant was the most highly recommended option, followed by condoms, the intrauterine device, and then the birth control shot. The birth control patch and ring were the two least popular methods. The qualitative data showed that many myths remain about LARC methods in this population, some of the most common being that the birth control implant requires a surgery for placement, and that the intrauterine device can perforate the uterus and travel to other areas of the body.

Conclusions: The young women that were recruited for our study were all involved in pro-social youth groups, which had given them some previous exposure to LARC methods. Therefore, the data reflects a subset of the population of young women in Rochester who are well educated and excited about LARC. Even though the results did not match the original expectation of evaluating baseline awareness, it is a positive sign for the future of the longitudinal health education project to see that young women are welcoming of LARC methods. Future directions of research may involve focus groups with a subset of young women not involved in pro-social groups to get baseline data for the health education initiative, as well as focus groups with the young women who have expressed excitement and early adoption of LARC methods, to gain insight on how to best disseminate this information to the rest of their peer group.

References:

1. ACTRochester. (2013). Youth risk behavior. Retrieved, 2013, from http://www.actrochester.org/children-youth/youth-risk-behavior/rate-teen-pregnancy
2. Centers for Disease Control and Prevention. (April 2, 2013). Teen pregnancy prevention. Retrieved February/15, 2013, from http://www.cdc.gov/teenpregnancy/
3. Committee on Adolescent Health Care, Long-Acting Reversible Contraception Working Group. (2012). *Adolescents and long-acting reversible contraception: Implants and intrauterine devices.* (No. 539).American College of Obstetricians and Gynecologists.
4. Kost, Kathryn, et al. Estimates of contraceptive failure from the 2002 National Survey of Family Growth. *Contraception* 77.1 (2008): 10-21.
5. Mestad, R., Secura, G., Allsworth, J. E., Madden, T., Zhao, Q., & Peipert, J. F. (2011). Acceptance of long-acting reversible contraceptive methods by adolescent participants in the contraceptive CHOICE project. *Contraception, 84*(5), 493-498.
6. Metro Council for Teen Potential. (2010). *Teen birth and teen pregnancy statistics for Rochester, New York.* Unpublished manuscript.
7. Secura, G. M., Allsworth, J. E., Madden, T., Mullersman, J. L., & Peipert, J. F. (2010). The contraceptive CHOICE project: Reducing barriers to long-acting reversible contraception. *American Journal of Obstetrics and Gynecology, 203*(2), 115. e1.
8. Ventura, S. J., Curtin, S. C., & Abma, J. C. (2012). National vital statistics reports. *National Vital Statistics Reports, 60*(7)

9. Whitaker, A. K., Johnson, L. M., Harwood, B., Chiappetta, L., Creinin, M. D., & Gold, M. A. (2008). Adolescent and young adult women's knowledge of and attitudes toward the intrauterine device. *Contraception, 78*(3), 211-217.
10. Winner, Brooke, et al. Effectiveness of long-acting reversible contraception. *New England Journal of Medicine* 366.21 (2012): 1998-2007.

Pitch, Michelle

Preceptors:
Muriel Laine, Ph.D.
University of Chicago
Ben May Department for Cancer Research

Geoffrey Greene, Ph.D.
University of Chicago
Ben May Department for Cancer Research

An analysis of the estrogen receptor-alpha LXXLL motif in estrogen-mediated protein co-recruitment and downstream signaling pathways

Introduction: The mechanism behind Estrogen Receptor-alpha (ERα) and its subsequent activation is important in understanding the basis behind estrogen-mediated signaling in breast cancer, as well as for developing new, safe therapies to treat cancer and symptoms of menopause. Upon binding to an agonist, ERα dimerizes and undergoes a conformational change that exposes a LXXLL amino acid motif on helix 12 of the protein. Exposure of this motif allows other coregulatory proteins, such as steroid receptor coactivator 1 (SRC-1), to bind and lead to downstream target gene expression and proliferation. This occurs primarily through a genomic pathway, in which the newly formed complex acts as a nuclear transcription factor.[1,2,3]

Recently, the use of Tissue Selective Estrogen Complexes (TSECs) has been shown to provide the therapeutic benefits of an agonist without the dangerous side effects of aberrant ERα activation as encountered with previous therapies.[4] TSECs consist of a mixture of estrogen, a true agonist, and a Selective Estrogen Receptor Modulator (SERM). As ERα functions in its dimerized state, the question of mixed occupancy - one monomer occupied by an agonist, the other by a SERM - is an important one. While past studies have looked at profiles of agonists and SERMS alone, the coactivator recruitment and functional consequences for these mixed occupancy dimers remain to be fully characterized. Understanding the mechanism behind these complexes is crucial to our comprehension of the differential regulation of ERα in therapies and treatment.[5]

Objectives: We aim to study the effect of a mixed ERα by using the presence or absence of a mutation to mediate inactivation or activation of the transcription complex, respectively. This project examines the effect of a mutated LXXLL motif on coregulatory protein binding and downstream signaling effects. We hypothesized that due to the mutation in the protein binding region of helix 12, an altered protein recruitment profile would lead to an altered downstream target gene expression and signaling profile.

Methods: Tandem ERα plasmid constructs were cloned to contain a mutation in one, both, or none of the monomers in the dimer (WT/mut, mut/mut, WT/WT) by site-directed mutagenesis and restriction enzyme digestion. Prior to assay, plasmids were transfected into the endogenously ERα-negative cell line C4-12 (established cell line) and treated with varying amounts of either an endogenous agonist (estradiol) or a SERM (bazedoxifene).

Co-immunoprecipitation: Cells were collected 48 hours post treatment with estradiol. Cells were lysed under non-denaturing conditions to keep protein-protein interactions intact. Protein lysates were incubated

with an antibody directed against SRC-1, protein complexes pulled down by magnetic beads containing protein G, and analyzed via standard western blotting techniques.

Gene Expression: RNA was collected and purified from cells 24 hours post-treatment with estradiol. cDNA from purified RNA was assayed using quantitative PCR for known ERα target genes TFF1, GREB1, PTGS2, PDZK1.

Results: By co-immunoprecipitation, it is evident that the mutated ERα tandem plasmid does not bind SRC-1 – a well-established coregulatory protein. Transfection in C4-12 cells does not affect the absolute levels of SRC-1 available in the cells, validating our findings to be those of a difference in binding rather than absolute SRC-1 expression. Furthermore, the mutated tandem appears to give a different target gene profile, with a significant inhibitory effect on the expression of known target gene TFF1.

Conclusions: Altering the LXXLL motif in just one of the two monomers in the ERα dimer complex is sufficient to impact substantial differences in the protein-protein interactions and downstream target gene expression in the ERα signaling pathway. Future directions would include assaying a wider range of known coregulatory proteins and target genes. In addition, proliferation assays would elucidate the functional significance of these mutations with respect to estrogen-mediated growth.

References

1. Hall, Julie M., et al. The Multifaceted Mechanisms of Estradiol and Estrogen Receptor Signaling. Journal of Biological Chemistry. October 2001; 276: 36869-36872.

2. Bjornstrom, L. and Sjoberg, M. Mechanisms of Estrogen Receptor Signaling: Convergence of Genomic and Nongenomic Actions on Target Genes. Molecular Endocrinology. 2005 Apr;19(4):833-842.

3. Kong, E.H., et al. Structure and Mechanism of the Oestrogen Receptor. Biochemical Society Transactions. 2003; 31(1) 56-59.

4. Pickar, JH., and Mirkin, S. Tissue-selective agents: selective estrogen receptor modulators and the tissue-selective estrogen complex. Menopause Int. 2010 Sep;16(3):121-8.

5. Greene, G. and Laine, M. Unpublished data: personal correspondence.

6. Dong, D.D., et al. Functional Analysis of the LXXLL motifs of the human glucocorticoid receptor: association with altered ligand affinity. J Steroid Biochem Mol Biol. 2006 Oct;101(2-3):106-17.

Rudofker, Eric

Preceptors:
Jeffrey D. Alexis, MD, FAAC, Saadia Sherazi, MD, MS
University of Rochester School of Medicine & Dentistry, Rochester, NY
Division of Cardiology

Gastrointestinal bleeding and risk of subsequent thrombosis with continuous-flow left ventricular assist device

Background: Left ventricular assist devices (LVADs) offer an innovative treatment option for patients with advanced heart failure. HeartMate II (HMII), has become the most widely used LVAD. It is a continuous-flow non-pulsatile device that requires chronic anticoagulation. Gastrointestinal (GI) bleeding and thrombosis are common complications of continuous-flow LVADs.

Objectives: We aimed to identify predictors of a thromboembolic (TE) event among HeartMate II (HMII) patients who have already had one or more GI bleed. We hypothesized that patients who have had multiple GI bleeds are at higher risk of subsequent TE events.

Methods: This was a retrospective review of 126 patients who had HMII implantation between January 2011 and February 2014 at the University of Rochester Medical Center, Rochester, NY. GI bleeding was defined as a GI bleed requiring admission, transfusion ≥2 units of blood products, or intervention via endoscopy or interventional radiology, occurring ≥7 days from LVAD implant. Clinical data were retrieved for each GI bleeding event, including etiology of bleed, GI procedures, INR, LDH, medication changes, administration of blood products, and subsequent GI bleeds and/or TE events. A TE event was defined as confirmed or suspected pump thrombosis leading to explant of LVAD and/or death, or ischemic stroke.

Results: We identified 42 patients with 71 GI bleeding events. There were 6 subsequent TE events among 5 patients, which included 4 patients with pump thrombosis and 2 with stroke. The TE events occurred an average of 105±167 days post-implant. Patients with TE events had an average of 3.2±1.8 total GI bleeding events versus 1.5±1.2 total GI bleeding events in patients without TE events (p=0.098). A TE event occurred after an average of 1.7±0.8 bleeds. In 1 (20%) case, a patient with a TE event had exactly one GI bleeding event whereas in 26 (70%) cases, patients without a TE event had exactly one GI bleeding event (p=0.047). There were 2 (40%) and 4 (11%) females in the TE event and GI bleeding-only groups, respectively (p=0.14). The mean ages at implant of the TE event group and GI bleeding-only group were 63±6 and 61±12, respectively (p=0.49). There were 23 (55%) patients with ischemic cardiomyopathy and 29 (69%) with history of smoking. At least one GI procedure was performed in 67 (94%) cases. The etiology of GI bleed was an AVM in 27 (40%) cases, unknown in 22 (33%) cases, and non-AVM in 18 (27%) cases. At the time of bleeding, patients were taking aspirin in 61 (86%) cases, warfarin in 59 (83%) cases, heparin in 6 (9%) cases, dipyridamole in 5 (7%) cases, and enoxaparin in 2 (3%) cases. Risk factors for having a gastrointestinal bleed were African-American race (HR: 4.16; 95% CI: 2.02 to 8.57; p=0.0001) and age over 60 years at implant (HR: 2.70; 95% CI: 1.39 to 5.26; p=0.0034).

Conclusions: GI bleeding was common among patients supported with HMII. Many patients who had GI bleeds had subsequent GI bleeds and in some cases had subsequent TE events. Patients with TE events had more GI bleeding events overall, and were more likely to be female. Groups more likely to have a bleed included African-Americans and patients over age 60 at time of implant. While older age is a well-known risk factor for having a GI bleed after LVAD implantation, race has never been shown to significantly impact risk. Further studies should be done to confirm this finding. This supports the hypothesis that the etiology of GI bleeding on LVAD support is multi-factorial and includes a genetic component.

Steiner, Jack

Preceptor:
Craig A. Mullen, MD, PhD
Department of Pediatric Hematology/Oncology
University of Rochester Medical Center

Development of a High-Throughput Assay for Identification of Bone Marrow Stromal-Derived Factors That Enhance Acute Lymphoblastic Leukemia Cell Survival

Introduction:
Childhood B-lineage acute lymphoblastic leukemia (ALL) is the most common form of malignancy in children. While cure rates for newly diagnosed ALL are high, 25% of these patients relapse, and ALL still accounts for a large proportion of cancer-associated deaths in children each year. ALL cell survival is poor in the absence of bone marrow stromal cells (BMSC), and thus it is thought that BMSC provide necessary trophic signals to leukemia cells. Our lab is developing a conceptually simple screening system to identify these factors that support ALL survival. We have observed that BMSC prevent apoptosis of primary ALL cells in serum-free conditions, and we reason that interfering with the production of key stroma-derived trophic factors will lead to increased ALL cell apoptosis *in vitro*. If anti-apoptotic signals from stromal cells to leukemia cells were identified, novel molecular targets for ALL therapy could be developed.

Objectives:
Acute lymphoblastic leukemia (ALL) cells die in the absence of bone marrow stromal cells (BMSC) *in vitro.* Our lab is focusing on identifying BMSC-derived factors that support ALL survival. By co-culturing ALL and BMSC, we have shown previously that manipulation of key anti-apoptotic factors from stroma results in decreased ALL cell viability when measured by flow cytometry. We hypothesize that a single-well ATP-luminescence assay could also be used to assess ALL viability, making our system more amenable to high-throughput screening.

Methods:
The assay has 3 components: (1) human bone marrow stromal cells (BMSC) and (2) primary ALL cells (not established cells lines); and (3) G418, a compound that interferes with global protein synthesis in stromal cells. We employ a mesenchymal stromal cell line immortalized with a human TERT gene, which has been shown to be representative of primary human stroma. 20,000 BMSC are placed into 96-well plates. After 48 hours, cells are treated with G418 and washed. 30,000 primary human ALL cells are then added to the wells in serum-free media. 5 days later, viable ALL cells are counted either by flow cytometry or an ATP-luminescence assay (CellTiter-Glo®, Promega).

Results:
(1) Interference of stromal cell protein synthesis significantly increases ALL cell apoptosis.
BMSC were treated for 6 hours with 25µg of G418, an irreversible inhibitor of protein synthesis. Wells were then washed with serum-free medium. ALL cell apoptosis was higher on G418-treated stroma (flow cytometry: 6178±215 viable ALL cells on treated stroma vs. 10923±1733

on un-manipulated stroma, p-value=0.001). The results were replicated in the ATP-luminescence assay (0.24 ± 0.46 RLU on treated stroma vs. 1.84 ± 0.47 RLU on un-manipulated stroma, $p<0.0005$).

(2) Flow cytometry and ATP-luminescence detect similar reductions in ALL cell viability on G418-treated stroma. We have previously used flow cytometry to quantitate viable ALL cells, and we hypothesize that measurement of intracellular ATP is a potential alternative. BMSC were again treated with 25µg G418 and washed with serum-free medium. ALL cell viability was assessed using flow cytometry and ATP-luminescence assay. Numbers of viable ALL cells were extrapolated from luminescence data using a standard curve. G418-treated stroma yielded a 42.20±9.77% reduction in ALL cell viability when measured by flow cytometry, and a 50.01±15.65% reduction when measured by ATP-luminescence assay (p=ns).

Conclusions:

The ATP-luminescence assay could be used to assess ALL cell viability in our BMSC-ALL co-culture system. Furthermore, its quick and simple procedure makes the assay a potential high-throughput alternative to flow cytometry. Additional experiments must be conducted to determine if the ATP-luminescence assay can detect changes in ALL viability after siRNA knockdown of single stromal genes.

References:

1) Manabe A, Coustan-Smith E, Behm FG, Raimondi SC, Campana D. Bone marrow-derived stromal cells prevent apoptotic cell death in B-lineage acute lymphoblastic leukemia. Blood. 1992;79:2370-2377.

2) Mihara K, Imai C, Coustan-Smith E, et al. Development and functional characterization of human bone marrow mesenchymal cells immortalized by enforced expression of telomerase. Br J Haematol. 2003;120:846-849.

3) Petty RD, Sutherland LA, Hunter EM, Cree IA. Comparison of MTT and ATP-based assays for the measurement of viable cell number. J Biolumin Chemilumin. 1995;10:29-34.

4) Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA-Cancer J Clin. 2014; 64:9-29.

St.John, Matthew

Preceptor
Dr. John C. Elfar, MD
Associate Professor
Department of Orthopedics
University of Rochester Medical Center

Time to Efficacy for Corticosteroid Injection for Subacromial Impingement

Introduction: Corticosteroid injections are commonly used by a variety of medical specialties for a range of musculoskeletal issues. Current reports of the procedure's 'use' and 'effectiveness' are largely driven on physician-to-physician anecdotal evidence[2]. Various formulations and dosages regimes exist with little evidence on the duration of symptomatic relief or time to efficacy.

Objectives: Our aim is to collect clinical data from individuals receiving first time subacromial corticosteroid injections with the goal of evaluating the time to efficacy. This efficacy will be analyzed with regard to the patient's perception of the treatment, as well as through a standardized measure.

Background: Studies completed regarding such injections varied in their reported effectiveness and duration of relief anywhere from none at all to one week to a year[2, 3,4]. Further, reviews have been mixed in its analysis of the effectiveness of injection results[4]. One meta-analysis did demonstrate a significant difference in symptom relief between corticosteroid injections and placebo at the two-week mark[1]. A review of nonsurgical care for subacromial impingement syndrome demonstrated a marked average reduction in pain following a corticosteroid injection for most patients for up to one year[3]. Patients for whom nonsurgical injection options for subacromial impingement syndrome are not successful, arthroscopic surgical options usually yield positive results that can last well over ten years, and can therefore be a viable further treatment option[3].

Methods: Patients in the University Sports Medicine Clinical Center receiving a subacromial injection of mixed corticosteroid and anesthetic injection of celestone and lidocaine for relief of impingement symptoms were offered an opportunity to enroll in this study. Only individuals who had never before received a subacromial corticosteroid injection were included in enrollment. Twenty-nine patients were enrolled, five of which were unable to complete the data collection process. After receiving informed consent, patients were asked to rate their pre-injection pain levels on a scale of 1-10, to complete a questionnaire regarding their expectation of relief, and finally to complete a standardized QuickDASH survey. The higher the QuickDASH score, the lower the patient's functionality. Patients were contacted on a daily basis for two weeks following the injection, by email or phone, and were asked to respond to four questions, regarding their current level of pain, their remembered pre-injection level of pain, the degree to which they felt relief from their injection and whether or not they felt their injection was a success. QuickDASH scores were also collected on a weekly basis for six weeks after initial enrollment.

Results: At the present stage of data collection, thirteen of the patient responses were ready for analysis, and it is on these that we are reporting. At the two-week mark, nine patients referred to the injection as a success and saw at least moderate relief of their impingement symptoms. Three patients were unsure at the two-week mark if their injection was a success and one believed the injection was not a success. Both groups, successful and not, saw a decrease in average QuickDASH score over the two-week period. The QuickDASH scores decreased with significance within the successful group ($P< 0.01$) but not in the unsuccessful group ($P>0.5$) over the initial two-week period. There was a significant difference in QuickDASH scores for the thirteen individuals in aggregate between the initial reading and the two-week mark ($P<0.05$) as well as a significant decrease in reported pain levels during the same interval ($P<0.01$). There was no significant difference in QuickDASH levels between the two-week mark and the six-week mark ($P>0.5$).

Conclusions: While the rest of our patient data must be analyzed and used in conjunction with a patient data sample collected in the same manner last year, a few general trends arose in this data sample regarding time to efficacy and expectation of relief for a subacromial injection. According to our preliminary results, there is no significant difference in functionality between the two-week and six-week mark and therefore physicians might have a good idea, soon after injection, how much longer term relief a patient will likely experience. Further, clinicians may be able to quickly delineate between patients for whom the injection will be a success relative to those who will experience little eventual symptomatic and functional relief. Those who did not feel that their injection was a success at the two-week point also did not have a significant difference in QuickDASH scores between the initial and two-week time period. Further analysis with the collective data will need to be done on the difference in functionality for those who experience relief versus those who do not, relative perceived pain scales, and precise timeframes for successful systematic relief.

References

1. Arroll B, Goodyear-Smith F. Corticosteroid Injections for Osteoarthritis of the Knee: a Meta-Analysis. BMJ. 2004 Apr 10;328(7444):869

2. Cole BJ, Schumacher R. Injectable Corticosteroids in Mondern Practice. J Am Acad Orthop Surg 2005; 13:37-46

3. Harrison A, Flatow E. Subacromial Impingement Syndrome: a review article. J AM Acad Orthop Surg 2011; 19:701-708

4. Koester M, Dunn W, Kun J, Spindler K. The Efficacy of Subacromial Corticosteroid Injection in the Treatment of Rotator Cuff Disease: A Systematic Review. J Am Acad Orthop Surg 2007; 15:3-11

5. Thiem U, Lamsfub R, Gunther S, Schumacher J, Baker C, Endres H, Zacher J, Burmester G, Pientaka L. Prevalence of Self-Reported Pain, Joint Complaints and Knee or hip Complaints in adults Aged ≥ 40 Years: A Cross-Sectional Survey in Herne, Germany. 2013; DOI: 10.1371/journal.pone.0060753

Seth Tobolsky, MS-2

Preceptors:
Dean Schillinger, M.D.
Chief of General Internal Medicine/Professor of Medicine, San Francisco General Hospital
University of California, San Francisco

Courtney Lyles, Ph. D.
Assistant Professor, Division of General Internal Medicine, San Francisco General Hospital
University of California, San Francisco

Facilitating Diabetes Self-Management in Limited Health Literacy Populations: Barriers and Implementation

Background:

The Diabetes Literacy Project is a global, multi-center study funded by the European Commission's 7th Framework Programme, working to develop and implement best-practice paradigms for facilitating self-management of Type 2 diabetes mellitus in patients identified as low or limited health literacy. Effective management of chronic disease, unlike acute disease, requires continuous and extensive patient self-management and high levels of patient self-efficacy, and this is a particular challenge in low health literacy populations, defined as patients with lower ability to comprehend and accurately utilize health information. Within this large, multi-phase project examining multiple modalities for designing and implementing diabetes self-management interventions, the UCSF site has partnered with the University of Southampton in the United Kingdom on Work-Package 8, developing (UK site) and implementing (UCSF, UK, and Ireland sites) a web-based intervention educating diabetes patients on the benefits of physical activity for managing their condition.

Objectives:

-Implement the UK-designed physical activity intervention website in a setting with 1) a large proportion of diabetic patients and 2) a large proportion of low health literacy patients.
-Measure the efficacy of the website intervention in its two forms, a static version and an interactive version: is this an effective method for promoting physical activity as a self-management strategy for low health literacy patients with diabetes? Does emphasis of audiovisual and interactive components increase engagement for patients with low health literacy?
-Analyze recruitment and intervention efficacy for confounding factors: health literacy is highly multifactorial—are outcomes different for patients with limited English proficiency (LEP)? For patients of various race/ethnicity backgrounds? For patients of various computer literacy backgrounds?

Methods:

-*Setting for Recruitment*: San Francisco General Hospital, General Medicine Clinic. This is an outpatient internal medicine clinic that serves mostly uninsured and underinsured (MediCal), with a high proportion of low SES, low-income patients, with a large proportion of Latino/a and Asian-American patients, most from immigrant backgrounds. Approximately 1/3 of GMC patients have a standing diagnosis of Type 2 diabetes mellitus, and the vast majority of GMC patients have 2 or more concurrent chronic disease diagnoses.
-*Recruit eligible patients*: (a) must speak English to be able to successfully comprehend intervention content, (b) have a standing diagnosis of Type 2 diabetes mellitus
-*Recruitment Protocol*:

1) Appointment List Generation: for each clinical shift, algorithm generated excluding patients with no English proficiency and did not have diabetes.

2) Systematic communication with providers: approach providers on a shift-by-shift basis, discuss eligibility of each patient (secondary exclusion criteria: documented English proficiency incongruent with functional English proficiency, patient experiencing psychosocial distress or is too acutely ill to participate).

3) Session with patients identified in both stages as eligible: patients given option to engage with website intervention.

-*Participation:*

Participation option of accessing intervention website at home (via URL and instructions), or option of being escorted to SFGH Library with free computers available for use.

-*Analysis*:

-Analysis of recruitment, and barriers/facilitators to patient engagement with the website intervention.

Results:

Results are at this point preliminary, as my role in the project was to create a protocol for recruitment, and initiate the process of implementation. An n of ~360 patients for the first month of recruitment were selected by the appointment list generator as eligible, but an average of only 4-5 patients per clinic shift were identified by providers as eligible to be interviewed. Out of each cohort of 4-5 patients fulfilling all primary and secondary eligibility criteria, an average of 3-4 were able to be interviewed, with an average of 0-1 agreeing to participate in the study. For the four weeks I recruited for Work-Package 8 at SFGH, 18.8% of patients interviewed agreed to participate. However, it is notable that this represents only 16 patients approached, 14 interviewed, and 3 agreeing to participate.

Conclusions:

Given that the Diabetes Literacy Project, and its component Work-Package 8, are global studies, with the majority of development and implementation occurring in EU member states, it is clear from the preliminary evidence that crucial barriers exist to effective outreach to the target patient cohort. The vast majority of patients at the GMC are part of low-income, vulnerable communities in the City of San Francisco, and definitions of "low health literacy" are clearly different across national boundaries. The objective for Work-Package 8 is to perform an effectiveness study comparing intervention completion rate and physical activity knowledge between the static and interactive arms of the website trial; however, this is, as of now, infeasible given critical barriers that have surfaced within the first month of recruitment. Using Russ Glasgow's RE-AIM Framework to analyze the preliminary results of study recruitment from an implementation science framework, specific barriers to recruitment have manifested in the "Reach" and "Adoption" realms of the framework. In analyzing the "reach" of the study, all but 4 interviewed patients reported their average weekly computer/Internet usage as "not at all", and reported minimal to no computer literacy, with the rest of the patients declining to participate reporting being too acutely ill/recently hospitalized or a sense of distrust for research infrastructure. In analysis of patients listed under the initial cohort of 360 selected by the appointment list generation algorithm, the discrepancy between documented English proficiency and functional English proficiency was the single most important factor in ruling out candidates for the study—English proficiency as documented in their chart was based on very basic interactions with front desk staff, and actual English proficiency was found to be too low in the majority of patients upon review for secondary eligibility criteria. Given this preliminary evidence, the "adoption" component of intervention implementation seems to be playing a large role in recruitment outcomes between the UCSF site and study sites in the UK and Ireland, further complicating the question of establishing best-practice paradigms for patient self-management of diabetes in low health literacy populations. Overall, though the results are preliminary and there has not yet been opportunity for statistical analysis, clear trends for significant barriers to recruitment have surfaced amongst SFGH GMC's population of very limited English proficiency, low to nonexistent computer literacy, and low SES patients, who are highly representative of the true "low health literacy" populations of California and the United States at large—the populations who continue to be the most vulnerable to detrimental diabetes mellitus outcomes.

Waroich, Jozal

Holly Hindman, MD
University of Rochester
Flaum Eye Institute

Subbasal corneal nerve changes following exposure to desiccating environment

Introduction: Aqueous tear deficient dry eye syndrome is known to be associated with a number of subbasal corneal nerve changes, including increased tortuosity and branching. Exposure to desiccating environments is a risk factor for clinically reported dry eye syndrome and may also precipitate the development of such abnormal nerve fiber patterns.

Objective: To investigate the development of abnormal corneal nerve morphology consistent with aqueous tear deficient dry eye in mice exposed to a desiccating environment.

Methods: C57BL/6J and DBA/2J mice were placed in a low-humidity, increased air-flow environmental chamber for 14 days. Control mice were placed in a normal environment for the same duration. Corneal whole mounts were stained immunohistochemically to reveal nerve patterns using neuronal β-tubulin specific Tuj1 as a nerve marker. Fluorescent microscopy was used to assess the parameters of interest.

Results: Nerve branching and tortuosity consistent with that reported in aqueous tear deficient dry eye patients in previous literature (Zhang, et al) was observed in 4 out of 18 experimental mice. Morphologic assessment of the remaining experimental mice and all control mice was not possible due to failure to visualize nerve structures following the staining protocol.

Conclusions: Exposure to a desiccating environment may induce changes in the subbasal corneal nerves consistent with those seen previously in patients with aqueous tear deficient dry eye syndrome. Inconsistent staining results suggest that further refinement of immunohistochemistry protocols and/or consideration of alternate nerve visualization methods is necessary for future study in corneal whole mounts.

References:

1. American Academy of Ophthalmology Cornea/External Disease Panel. Preferred Practice Pattern® Guidelines. Dry Eye Syndrome, 2013, American Academy of Ophthalmology: San Francisco, CA.

2. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf, 2007. 5(2): p. 93-107.

3. Gayton, J.L., Etiology, prevalence, and treatment of dry eye disease. Clin Ophthalmol, 2009. (3): p. 405-412.

4. Barabino S, Shen LL, Chen L, Rashid S, Rolando M, Dana MR. The controlled-environment chamber: a new mouse model of dry eye. Invest Ophthalmol Vis Sci, 2005. 46(8): p. 2766-2771.

5. Barabino S, Dana MR. Animal models of dry eye: a critical assessment of opportunities and limitations. Invest Ophthalmol Vis Sci, 2004. (45):1641-1646.

6. Suzuki M, Massingale ML, Ye F, Godbold J, Elfassy T, Vallabhajosyula M, et al. Tear osmolarity as a biomarker for dry eye disease severity. Invest Ophthalmol Vis Sci, 2010. (51): p. 4557-4561.

7. Khanal S, Tomlinson A, Diaper CJ. Tear physiology of aqueous deficiency and evaporative dry eye. Optom Vis Sci, 2009. 86(11):1235-1240.

8. Sullivan BD, Crews LA, Sonmez B, et al. Clinical utility of objective tests for dry eye disease: variability over time and implications for clinical trials and disease management. Cornea, 2012. 31(9): p. 1000-1008.

9. Labbe A, Alalwani H, Van WC, Brasnu E, Georgescu D, et al. (2012) The Relationship between Subbasal Nerve Morphology and Corneal Sensation in Ocular Surface Disease. Invest Ophthalmol Vis Sci (53): p. 4926-4931.

10. Labbe, A., et al., Corneal nerve structure and function in patients with non-sjogren dry eye: clinical correlations. Invest Ophthalmol Vis Sci, 2013. 54(8): p. 5144-5150.

11. Mantelli, F., et al., The cellular mechanisms of dry eye: from pathogenesis to treatment. J Cell Physiol. 2013. 228(12): p. 2253-2256.

12. Dartt, D.A., Neural regulation of lacrimal gland secretory processes: relevance in dry eye diseases. Prog Retin Eye Res, 2009. 28(3): p. 155-177.

13. Esquenazi, S., et al., Comparative in vivo high-resolution confocal microscopy of corneal epithelium, sub-basal nerves and stromal cells in mice with and without dry eye after photorefractive keratectomy. Clin Experiment Ophthalmol, 2007. 35(6): p. 545-9.

15. Arany S, Catalan MA, Roztocil E, Ovitt CE. Ascl3 knockout and cell ablation models reveal complexity of salivary gland maintenance and regeneration. Dev Biol, 2001. 353(2): p. 186-193.

16. Zhang M., et al., Altered corneal nerves in aqueous tear deficiency viewed by in vivo confocal microscopy. Cornea, 2005. 24(7): p. 818–824.

Meng, Yifan

Marc Schermerhorn, MD
Beth Israel Deaconess Medical Center
Vascular Surgery

The Relationship between the Society for Vascular Surgery Lower Extremity Threatened Limb Classification System and Patient Outcomes in Tibial Angioplasty

Introduction:

Critical limb ischemia (CLI) describes a subgroup of patients with a threatened lower extremity due to chronic ischemia. The numerous existing classification systems, like the Fontaine and Rutherford systems, characterize perfusion but do not adequately categorize extent of tissue loss and infection. A new framework, the Society of Vascular Surgery (SVS) Lower Extremity Threatened Limb Classification System, was developed by Mills et al in 2014 to include three major factors known to impact the threat to a limb: Wound, Ischemia and foot Infection (WIfI). Each domain is graded on a 0 to 3 scale, following which the scores can be aggregated to determine risk of amputation and likelihood of benefit from revascularization. Due to its recent conception, the SVS WIfI scale still requires rigorous validation. We sought to evaluate the relationship between WIfI grades and postoperative outcomes in patients who underwent tibial angioplasty.

Objectives:

We sought to evaluate the relationship between WIfI grades and postoperative outcomes in patients who underwent tibial angioplasty.

Methods:

We examined perioperative and long-term mortality and complications in 672 patients who had tibial angioplasty performed at Beth Israel Deaconess Medical Center from 2004 to 2013. Patients were retrospectively graded according to the WIfI system, and follow-up data were obtained via medical records. Chi-squared analysis, Fishers' Exact Test and multivariable logistic regression were performed for data analysis.

Results:

Preliminary results were only available for wound grade at time of abstract publication. Of 672 patients who underwent tibial angioplasty, 41% had a wound grade of 2 (Table 1). No significant difference was found in thirty-day mortality among the four wound grades, but three-year mortality was significantly higher in wound grade 3 than in wound grade 1 (44% vs. 27%, $P<0.001$). History of diabetes mellitus (DM) was associated with higher wound grade (60%, 79%, 82% and 90% for wound 0, 1, 2 and 3 respectively, $P<0.001$). Congestive heart failure (CHF) and chronic renal insufficiency (CRI) were also associated with a higher wound grade (CHF 12%, 27%, 27% and 44% for wound 0, 1, 2 and 3, respectively, $P < .001$; CRI 11%, 20%, 31% and 34% for wound 0, 1, 2 and 3, respectively, $P < .001$).

Conclusions:

Though preliminary results are only available for wound grade at this time, there is a clear correlation of wound grade with risk factors, mortality and complications. We anticipate that further analysis of ischemia and foot infection grades will reveal more such correlations. The WIfI system shows promise in its ability to accurately characterize CLI, although further research is needed before wide adoption of this system as a clinical decision-making tool.

References:

Mills, J. L., Conte, M. S., Armstrong, D. G., Pomposelli, F. B., Schanzer, A., Sidawy, A. N., & Andros, G. (2014). The Society for Vascular Surgery Lower Extremity Threatened Limb Classification System: risk stratification based on wound, ischemia, and foot infection (WIfI). Journal of Vascular Surgery, 1, 220-34.e1-2.

Wound Grade	#	%	Ischemia Grade	#	%	Foot Infection Grade	#	%
0	155	23	0	39	6	0	425	63
1	190	28	1	330	49	1	163	24
2	277	41	2	200	30	2	73	11
3	50	7	3	103	15	3	11	2

Table 1: WIfI grades on 672 patients who underwent tibial angioplasty from 2004 - 2013 (n=672)

Baman, Jayson

Preceptor:
Anthony K.L. Leung, Ph.D.
Assistant Professor
Department of Biochemistry and Molecular Biology
Bloomberg School of Public Health
Johns Hopkins University

Studying PARP-13 localization and formation of cytoplasmic processing bodies and/or stress granules under stress conditions

Abstract:

Stress granules are non-membrane bound cytoplasmic structures that form in response to cellular stress, and their presence has been linked in neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration[1]. The current understanding of their composition, dynamics and role in cellular processes is incomplete, although studies have suggested that these and other similar cytoplasmic ribonucleoprotein complexes may play roles in a diverse set of developmental and cellular functions[2-4]. Previous studies have shown that stress granules contain many of the initiation factors classically associated with protein translation (eIF3, eIF4A and others), hence they are considered sites of translation stalling and mRNP degradation[1, 5, 6]. Additionally, cytoplasmic processing bodies (P-bodies), which are constitutively present in human cells, are involved in the degradation of mRNA. There is a dynamic exchange between P-bodies and stress granules during translation stalling/degradation, and it is hypothesized that disruption of this relationship may contribute to disease pathology[1].

Significant evidence, including immunostaining studies and genetic analyses, have shown that poly(ADP-ribose) polymerase 13 (PARP-13) localizes to stress granules. There are two isoforms of the protein, PARP-13.1 and PARP-13.2, with the former containing a catalytically inactive PARP domain on its C-terminus that the latter lacks; otherwise, the two isoforms share the same conserved functional domains[7]. In fact, previous studies show that PARP-13 plays an integral role in the mediation of miRNA silencing under stress conditions[8]. The enclosed abstract and project therein is not a functional assay, but rather analyzes the degrees of PARP-13 localization and the formation of P-bodies and/or stress granules under a variety of conditions in human cell lines, including oxidative stress and transfection with a viral double-stranded RNA mimic. In addition, a HeLa cell line that contains a knockout of the PARP-13 gene was analyzed in plasmid-mediated restoration assays for the reconstitution of P-bodies and stress granules.

Objectives:

This study sought to contribute to understanding the localization of PARP-13 to stress granules and/or P-bodies in response to stress applied to HeLa cells. In addition, one of the

objectives was to study the restoration of stress granule formation in PARP-13 knockout cells by transfecting the cells with PARP-13.1 and PARP-13.2 plasmids.

Methods:

HeLa cell lines were utilized for the in vivo studies of cellular responses. To study the effects of oxidative stress on P-body and stress granule formation in both wild-type and PARP-13 knockout HeLa cell lines, cells plated at $1x10^5$ cells/mL were treated with sodium arsenite (250 μM for 30 min). Following treatment, an immunostaining protocol was carried out that used different fluorescently-conjugated secondary antibodies to detect AT-rich regions of DNA (for nucleus localization), PARP-13 and a canonical stress granule or P-body marker; antibodies against eIF3 and 4ET were used in stress granule and P-body studies, respectively. Immunofluorescence images of fixed cells were attained, and the presence of stress granules or P-bodies and the localization patterns of PARP-13 were noted.

Similar experiments were conducted to study responses to viral infection by utilizing poly(I:C), a known mimic of viral double-stranded RNA. Cells plated at $1x10^5$ cells/mL were treated with 1μg/mL poly(I:C) for 6 hours. Additionally, samples were treated with different dilutions of the poly(I:C) stress for 8 or 16 hours, and Western blotting analysis was carried out to observe any differences in PARP-13 expression due to varying dilutions and exposure times.

To assess the restoration of PARP-13 expression in PARP-13 knockout cells, $1x10^5$ cells/mL were treated with an approximately 1.1μg/mL DNA plasmid cocktail that contained 12.5% PARP-13.1 plasmid, 37.5% PARP-13.2 plasmid and 50% control plasmids; these proportions were based off of previous unpublished data from Dr. Yoshinari Ando, a postdoctoral fellow in the lab. As with the oxidative stress and viral mimic experiments, immunostaining protocols were carried out and Western blotting was conducted to analyze protein levels.

Results:

Preliminary results affirm previous studies in showing arsenite treatment is sufficient to induce stress granule formation in both wild-type and PARP-13 knockout cells, and it is insufficient to induce significant P-body formation in either cell line. Additionally, data indicate that PARP-13 significantly localizes to stress granules following arsenite treatment. Treatment with poly(I:C) was able to upregulate stress granule formation in wild-type cells but not in PARP-13 knockout cells, supporting previous indications that different pathways promote stress granule formation dependent on the nature of the stress.

Additionally, during the analysis of PARP-13 knockout cells transfected with various restoration plasmids, it was noted that both PARP-13.1 and -13.2 localize to stress granules, but calculations using the Pearson's correlation coefficient suggest that PARP-13.2 localizes more strictly to stress granules. Transfection with the PARP-13.2-containing plasmid (alone or in conjunction with the PARP-13.1-containing plasmid) was able to induce stress granule formation, but transfection with the PARP-13.1-containing plasmid alone was unable to induce stress granules in knockout cells.

Conclusions:

Although the conclusions from these studies need to be verified by reproducing their data with continued experimentation, the preliminary results show that PARP-13.2 alone is sufficient to induce stress granule formation, and that the PARP-13 seems to be necessary to upregulate stress granule formation in cells treated with poly(I:C). Oxidative stress was sufficient to induce

a pathway that increases stress granule formation even in the absence of PARP-13, further supporting studies that show that oxidative stress and viral double-stranded RNA constructs induce stress granules by different mechanisms. Investigation into the functional and clinical significance of these conclusions is necessary. These studies alone contribute to a growing body of knowledge regarding the formation and composition of stress granules in human cells.

References:

1. Ramaswami, M., Taylor, J.P. & Parker, R. Altered ribostasis: RNA-protein granules in degenerative disorders. *Cell* **154**, 727-36 (2013).
2. Weil, T.T. et al. Drosophila patterning is established by differential association of mRNAs with P bodies. *Nat Cell Biol* **14**, 1305-13 (2012).
3. Thedieck, K. et al. Inhibition of mTORC1 by astrin and stress granules prevents apoptosis in cancer cells. *Cell* **154**, 859-74 (2013).
4. Dougherty, J.D., White, J.P. & Lloyd, R.E. Poliovirus-mediated disruption of cytoplasmic processing bodies. *J Virol* **85**, 64-75 (2011).
5. Anderson, P. & Kedersha, N. Stress granules: the Tao of RNA triage. *Trends Biochem Sci* **33**, 141-50 (2008).
6. Buchan, J.R. & Parker, R. Eukaryotic stress granules: the ins and outs of translation. *Mol Cell* **36**, 932-41 (2009).
7. Leung, A.K. et al. Poly(ADP-ribose) regulates stress responses and microRNA activity in the cytoplasm. *Mol Cell* **42**, 489-99 (2011).
8. Leung, A., Todorova, T., Ando, Y. & Chang, P. Poly(ADP-ribose) regulates post-transcriptional gene regulation in the cytoplasm. *RNA Biol* **9**, 542-8 (2012).

Basic Science, Clinical & Translational Research

Asselin, Patrick

Preceptors:
Jeff Bazarian, MD, MPH
Associate Professor
Department of Emergency Medicine

Weighted Helmet Impact Measures Correlate with Brain White Matter Changes After One Football Season of Repetitive Head Hits.

Background:

Repetitive head hits (RHH) incurred during sports like football produce acute changes in brain white matter (WM) that may contribute to Chronic Traumatic Encephalopathy (CTE) many years later. More precisely defining the relationship between RHH and acute WM changes is a necessary first step in developing efforts to reduce the long-term risk of CTE. The WM changes are difficult to visualize and quantify with current clinical imaging studies. Diffusion tensor imaging (DTI) allows a quantitative measurement of changes in white matter based on diffusion of water along the axons in the brain (1). We specifically examined changes in fractional anisotropy (FA), which is a scalar measurement (0-1) describing water's diffusion, with zero being equal diffusion in all directions, and one being water diffusing along a single axon. Even with the ability to quantify these changes as indicators of injury severity, it is difficult to predict or estimate these changes with current metrics or other non-DTI measurements that would be useful in a clinical setting. Prior studies report an inconsistent relationship between acute WM changes and the cumulative number and magnitude of head impacts incurred over a sport season (2). These studies did not account for the interval of time between head impacts (TBH), nor for the period of time between head impacts and DTI scanning (TUD), both of which are likely to influence the appearance of WM at the end of the football season (3). In order to address these gaps, we developed several new head impact metrics weighted for TBH and TUD.

Objectives:

1. To determine if the weighted cumulative head impact metrics correlate with changes in brain WM after a single season of collegiate football
2. To determine if weighted cumulative metrics correlate better than an unweighted metrics with WM.

Methods:

In the 2011 football season, 10 University of Rochester football players wore helmets equipped with the head impact telemetry system (HITS) (2). The helmets recorded impacts at every practice and game during the season. The HITS measures linear acceleration, rotational acceleration, Gadd Severity Index (GSI), Head Impact Criterion 15 (HIC15), and HIT severity profile (HITsp) with each head impact. The WM changes were measured using DTI, which were performed at the beginning and end of the football season. DTI changes in each subject were defined as the percent of all WM voxels with a significant increase in FA as well as a significant decrease in FA from the beginning to end of the football season

One unweighted metric and three weighted metrics were analyzed. The unweighted metric simply summed all the values for a single HITS impact measure. The first weighted

metric determined a value for each impact measure based on the current hit and the time since prior hits, and was known as the time between hits metric (TBH). The second metric known as the time until DTI (TUD) determines an impact value for each hit based on the number of days between the hit and the date of DTI scan. The final metric combined the TBH and TUD into a single mathematical equation, known as the TBH-TUD metric. The three above weighted metrics were summed in the same fashion as the unweighted metric.

Results:

Unweighted cumulative, TBH, and TUD values show a statistically significant direct relationship with fractional anisotropy (FA) decrease. The highest r^2 values for FA decrease are seen when the TUD metric was used to weight HIC15 and GSI. Both linear and logistic regressions for all time-weighted metrics show statistically significant relationships with FA increase. The significant relationships are seen most consistently with FA increase. However, the TBH-TUD metric shows the highest r^2 for FA increase for all impact measures. In summary, linear models correlate better with changes in FA decrease, while logistic models are better at predicting changes in FA increase.

Conclusions:

The use of time-weighted impact estimates correlate with the changes in white matter seen on DTI. Weighting the forces allows for better predictability of FA changes in DTI in comparison to a simple unweighted cumulative force.

References

1. Shenton, M. E., Hamoda, H. M., Schneiderman, J.S., Bouix, S., Pasternak, O, Rathi, Y., …Zafonte, R. (2012) A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. Brain imaging and Behavior, 6(2), 137-92. doi: 10.1007/s11682-012-9156-5

2. Funk, J. R., Rowson, S., Daniel, R. W., & Duma, S. M. (2012). Validation of concussion risk curves for collegiate football players derived from HITS data. Annals of Biomedical Engineering, 40(1) 79-89. doi: 10.1007/s10439-011-0400-8

3. Fujita, M., Weig, E. P., & Povilshock, J.T. (2012) Intensity- and interval specific repetitive traumatic brain injury can evoke both axonal and microvascular damage. Journal of Neurotrauma, 29(12), 2172-80. doi: 10.1089/neu.2012.2357

Walia, Ritika, MS2

Preceptor:
Robert Sackstein, MD.,Ph.D.
Brigham and Women's Hospital, Department of Dermatology
Harvard Skin Disease Research Center, Harvard Medical School
Program of Excellence in Glycosciences

Molecular Basis of Colon Cancer Metastasis: Effectors of E-Selectin Binding

Background

Colorectal cancer is the second leading cause of cancer-related death among both men and women in the United States, claiming more than fifty-thousand lives each year[1]. The danger lies in its virulent capacity to metastasize[2]. Tumor metastasis is a multi-step process nearly identical to that which mediates leukocyte trafficking to sites of tissue injury under shear blood flow conditions. The first step of this process is the engagement of E-selectin, a C-type lectin expressed on activated endothelium, to E-selectin ligands expressed on the cancer cells[3,4]. E-selectin ligands are sialofucosylated structures presented on protein or lipid scaffolds. The prototypes of these structures are the glycoforms known as sialylated Lewis^x (sLe^x) and sialylated Lewis^a (sLe^a); these consist of a lactosamine backbone made up of alternating N-acetylglucosamine (GlcNAc) and galactose (Gal) units, which is decorated with a terminal sialic acid (NeuAc) and a fucose (Fuc) in an α(1,3) or α(1,4) linkage, respectively, to the GlcNAc residues[4]. Previous studies have suggested that O-sialofucosylated variant isoforms of the ubiquitous protein CD44, termed hematopoietic cell E-/L- selectin ligand (HCELLv), function as E-selectin ligands on the colon cancer cell line LS174T[5,6]. Sialofucosylated carcinoembryonic antigen (CEA) has also been identified as an E-selectin ligand (CEA-EL) on CD44 knockdown LS174T cells[7], although the degree to which its functions complement or oppose those of CD44 is yet to be elucidated. Both CD44 and CEA are extremely pleiotropic molecules, and often, the splice variant isoforms of CD44 and CEA that are expressed on cancer cells are different from those isoforms expressed physiologically. Thus, further insight into the structure and function of these glycoproteins is warranted as they could serve as targets of directed therapy against highly metastatic, circulating cancer cells.

Objective:

The objective of my research was to characterize the E-selectin ligands expressed on cancer cells, primarily in terms of the protein scaffolds, (CD44 or CEA), on which they are presented.

Methods:

Colorectal cancer cell lines *LS174T, HCT-8,* and *HT 29* were obtained from the American Type Culture Collection (ATCC) and maintained as described. Twenty-seven flash frozen tissue samples were obtained courtesy of Dr. Wells Messersmith and Dr. John Arcaroli of the University of Colorado at Denver, consisting of a mixture of human-in-mouse xenotransplanted colorectal cancer (*CRC*) specimens and human biopsy *CRC* specimens. Several of these specimens were paired; i.e., the biopsy specimen and the xenotransplant specimen were derived from the same patient. Still other paired specimens consisted of primary tumor samples and liver metastases derived from the same patient, enabling this comparison as well. Lysates of all tissue samples and cell lines were prepared using an appropriate EDTA-free detergent buffer, along with sonication. The samples were then assessed for their relative CD44/HCELLv or CEA/CEA-

EL expression using immunoprecipitation, SDS-PAGE/western transfer, and immunoblotting using various antibodies as probes.

Results:

At the present stage of data collection, it appears that HCELLv and CEA-EL have equal potential to contribute to the E-selectin ligand activity of colorectal cancer cells. HCELLv (i.e. the E-selectin binding glycoform of CD44) is predominantly observed as a 160 kDa band on SDS-PAGE gels. CEA-EL (the E-selectin binding glycoform of CEA) is predominantly observed as a 180-200 kDa band on SDS-PAGE gels. Lower molecular weight isoforms of CD44 and CEA, while observed in the tumor samples and cell lines, have no E-selectin ligand activity. On native colon tissue, defined as normal (non-cancerous) colon tissue adjacent to a tumor, E-selectin ligand activity was observed only on a 90 kDa isoform of CD44 and on a 250 kDa isoform of CEA, with no E-selectin ligand activity on the 160 kDa and 200 kDa isoforms as in the tumors. There was no native CEA activity in the liver, but HCELL was again expressed as the 90 kDa standard isoform in native hepatocytes adjacent to a *CRC* liver metastasis. Significant pleiotropism was observed in the xenotransplanted *CRC* specimens, with both CEA-EL and HCELLv being presented on higher and lower molecular weight than those observed in the primary biopsy samples. It is probable that the selective pressures exerted by the process of xenotransplantation caused these cells to alter their E-selectin ligand expression. No tangible differences were observed in the E-selectin ligand activity of primary colon tumors and their liver metastases.

Conclusions/Future Directions:

While more data is required to draw absolute conclusions, a few general trends were repeatedly observed in the data collected thus far:

1. A 160 kDa isoglycoform of HCELLv is an E-selectin ligand on colon cancer cells derived from biopsy tissue. This is a significant observation because all prior work characterizing HCELLv in colon cancer has been done on immortalized cell lines, which are far removed from primary tissue and often grown in very artificial conditions.
2. A 200 kDa isoglycoform of CEA-EL is an E-selectin ligand on colon cancer cells derived from biopsy tissue. Again, all prior work characterizing CEA-EL has been done on cell lines, and moreover, on CD44 knock down subclones of cell lines. Our results show that even just-isolated colon cancer cells have significant CEA-EL expressivity.
3. Xenotransplantation of human colon cancer specimens results in pleiotropism of E-selectin ligand expression compared to that in identical, non-xenotransplanted colon cancer specimens. As mentioned, it is possible that different selective pressures exerted on these cells as a byproduct of the xenotransplantation result in this pleiotropism. However, it is also possible that these selective pressures enable only the most virulent cancer cells in the biopsy sample to survive, thus leading to enrichment of the sample for E-selectin ligand expressing cells, and enabling even minor E-selectin ligands to be detected.

Overall, this research project was valuable because it explored colorectal cancer biology extremely proximal to the native conditions in which colorectal cancer develops, i.e., using direct biopsy samples. It also analyzed the credibility of pre-clinical studies using xenotransplanted mouse specimens. Further exploration of the emerging trends is likely to yield results with interesting translatable potential.

References:

1. *Colorectal Cancer Detailed Guide.* Atlanta, Ga: American Cancer Society;2012.
2. *Colorectal Cancer Facts and Figures 2011-2013.* Atlanta, Ga: American Cancer Society;2011.
3. Jacobs PP, Sackstein R. CD44 and HCELL: preventing hematogenous metastasis at step 1. *FEBS Lett.* Oct 20 2011;585(20):3148-3158.
4. Sackstein R. Glycosyltransferase-programmed stereosubstitution (GPS) to create HCELL: engineering a roadmap for cell migration. *Immunol Rev.* Jul 2009;230(1):51-74.
5. Burdick MM, Chu JT, Godar S, Sackstein R. HCELL is the major E- and L-selectin ligand expressed on LS174T colon carcinoma cells. *J Biol Chem.* May 19 2006;281(20):13899-13905.
6. Hanley WD, Burdick MM, Konstantopoulos K, Sackstein R. CD44 on LS174T colon carcinoma cells possesses E-selectin ligand activity. *Cancer Res.* Jul 1 2005;65(13):5812-5817.
7. Thomas SN, Zhu F, Schnaar RL, Alves CS, Konstantopoulos K. Carcinoembryonic antigen and CD44 variant isoforms cooperate to mediate colon carcinoma cell adhesion to E- and L-selectin in shear flow. *J Biol Chem.* Jun 6 2008;283(23):15647-15655.

Kantorowska, Agata

Preceptors:
Henry C. Lee, MD, MS
Stanford University
Assistant Professor, Department of Pediatrics (Neonatology)
Director of Research, California Perinatal Quality Care Collaborative

Donor Human Milk Availability Promotes Breast Milk Feeding Among VLBW Infants in California, Lowers Hospital Rates of NEC

Introduction: Breastfeeding is widely considered the optimal form of nutrition for all infants, but it is especially important for babies born prematurely. In preterm infants, human milk feeding is associated with a lower risk of necrotizing enterocolitis (NEC), retinopathy of prematurity, and sepsis.[1,2,3] Human milk is one of the only known protective agents against NEC.[4] Studies have shown that enteral feeding containing at least 50% human milk in the first 14 days of life is associated with a six-fold decrease in the odds of NEC.[1] Because of the challenges associated with breastfeeding infants in the NICU, donor human milk is often used as a substitute for a mother's own milk.[5] However, the majority of currently available data on donor human milk and NEC rates in preterm infants comes from studies conducted over 20 years ago.[6] There has also been some debate about whether access to donor human milk could actually lead to decreased breastfeeding—the idea being that availability of an alternate human milk source could lead to attenuated efforts to promote lactation among mothers of preterm infants.[7] Data collected by the Italian Association of Human Milk Banks shows that donor human milk is actually associated with an increased rate of exclusive breastfeeding in very low birth weight (VLBW, birth weight < 1500 grams) infants[7], but it is hard to analyze data about human milk banks in the United States. Data from human milk banks in the U.S. is not standardized and there is a lack of a central depository.[5] The Human Milk Banking Association of North America (HMBANA) has stated that this could be hindering research, quality improvement initiatives, and implementation of donor milk programs in NICUs.[5]

Objectives: Our project had several aims. Our overarching purpose was to link data from the California Perinatal Quality Care Collaborative (CPQCC) with data from the Mothers' Milk Bank of San Jose. The CPQCC is an organization that collects data from 132 NICUs in California, gathering information on the care of over 90% of California's NICU admissions of VLBW infants. This provides a sizeable and meaningful database from which very real information about NICU infant care in California can be extracted. The Mothers' Milk Bank of San Jose is the largest human milk bank in the United States (in terms of distribution) and the only human milk bank that distributes donor human milk to NICUs in California. By combining these two datasets, we were hoping to get a pretty clear picture of donor human milk use and benefits in California. Our first aim was to see what has been happening to the availability of donor milk in California over the period of 2007-2013. In addition, we wanted to see if the availability of donor human milk in a hospital may have had an effect on the rates of NEC and breastfeeding at discharge for VLBW infants at that hospital.

Methods: We used data from the CPQCC and the Mother's Milk Bank of San Jose. In order to track donor human milk availability over time, we calculated and plotted the percentage of NICU

births that occurred in a hospital with donor human milk available over the course of 2007-2013. This plot was stratified according to NICU level. In California, Regional NICUs take care of the sickest patients who may require subspecialty and/or surgical care, Community NICUs can care for VLBW infants who may require prolonged respiratory support, and Intermediate NICUs care for infants who have less need for intensive respiratory support.
22 hospitals were identified that underwent a clear transition from not having donor milk to having donor human milk available at some point during the course of 2007-2013. Paired t-test analyses were performed to compare rates of breastfeeding and rates of NEC among VLBW infants before and after these hospitals acquired donor human milk.
A multivariable logistic regression model was devised to examine which hospital, medical, and sociodemographic factors were associated with breast feeding among VLBW infants.

Results: (1) Donor human milk availability in California NICUs is increasing overall. Over the course of 2007-2013, the percentage of NICU infants that had donor human milk available to them increased, regardless of NICU level. There seems to be a greater push to have donor human milk available among Regional and Community NICUs. In 2007, 38.2% of premature infants in Regional NICUs had donor milk available to them and in 2013, 81.3% of premature infants in Regional NICUs had donor milk available to them.
(2) The availability of donor human milk in a hospital is correlated with an increase in the rate of breastfeeding at discharge among VLBW infants. The mean difference before/after donor human milk for the 22 hospitals that underwent a clear transition over the course of 2007-2013 was a +10.0% absolute increase in rate of breastfeeding at discharge.
(3) The availability of donor human milk in a hospital is correlated with a decrease in the rate of NEC among VLBW infants. The mean difference before/after donor human milk for the 22 hospitals that underwent a clear transition over the course of 2007-2013 was a -2.6% absolute decrease in rate of necrotizing enterocolitis. This translated to a change from an average hospital NEC rate of 6.6% before acquiring donor human milk to an average hospital NEC rate of 4.3% after donor human milk was available.
(4) The availability of donor human milk in the hospital where a VLBW infant was being treated is a strong positive predictor of breastfeeding at discharge. A multivariable logistic regression model for breastfeeding at discharge found that the presence of donor human milk yielded an odds ratio of 1.47 with a 95% confidence interval of [1.41, 1.54].

Conclusions: The availability of donor human milk in NICUs in California has increased since 2007. The potential consequences of this influx of donor human milk seem to be favorable. Paired t-test analyses found that introduction of donor milk led to a decrease in hospital rates of necrotizing enterocolitis and an increase in breastfeeding at discharge among VLBW infants. In a multivariable logistic regression for breastfeeding at discharge, availability of donor milk was a strong positive predictor.

References

1. Sisk PM, Lovelady CA, Dillard RG, Gruber KJ, O'Shea TM. Early human milk feeding is associated with a lower risk of necrotizing enterocolitis in very low birth weight infants. *J Perinatol* 2007, **27**: 428-433.
2. Hylander MA, Strobino DM, Pezzullo JC, Dhanireddy R. Association of human milk feedings with a reduction in retinopathy of prematurity among very low birthweight infants. *J Perinatol* 2001, **21**(6): 356-362.
3. Hanson LA, Korotkova M. The role of breastfeeding in prevention of neonatal infection. *Semin Neonatol* 2002, **7**(4): 275-281.

4. Stevenson DK, Blakely ML. Necrotizing enterocolitis: an inherited or acquired condition? *NeoReviews* 2006, **7**(3): e125-e134.
5. Brownell EA, Lussier MM, Herson VC, Hagadorn JI, Marinelli KA. Donor human milk bank data collection in North America: an assessment of current status and future needs. *J Hum Lactation* 2014, **30**(1): 47-53.
6. McGuire W, Anthony MY. Donor human milk versus formula for preventing necrotising enterocolitis in preterm infants: systematic review. *Arch Dis Child Fetal Neonatal Ed* 2003, **88**: F11-F14.
7. Arslanoglu S, Moro GE, Bellu R, Turoli D, De Nisi G, Tonetto P, Bertino E. Presence of human milk bank is associated with elevated rate of exclusive breastfeeding in VLBW infants. *J. Perinat. Med.* 2013, **41**(2): 129-131.