Premier Nutrition and Metabolism Research Paper Session and Vars Award Competition

The Harry M. Vars award is given annually to the person presenting the highest-scoring qualified abstract for the ASPEN Nutrition Science and Practice Conference. The candidates are evaluated on a manuscript based on their abstract, as well as on their expertise and knowledge of the science as demonstrated during their oral presentation at the Premier Paper Session. ASPEN18 is January 22-25, 2018 at Caesars Palace in Las Vegas, Nevada.

VARS CANDIDATE
2834423 - No gut no gain! Enteral bile acid treatment preserves gut growth but is not protective for TPN associated liver injury in a novel extensive short bowel resection animal model.

Gustavo Villalona, MD; Amber Price, BS; Keith S. Blomenkamp, BA, BS; Chandrashekhara Manithody, PhD; Matthew Westrich, BS; Vindhya Kakarla, MBBS; Shruthika Pochampally, BS; William Phillips, BS; Nicole Heafner, BS; Niraja S. Korremla, MBBS; Miguel Guzman, MD; Jose Greenspon, MD; Ajay K. Jain, MD

1Pediatrics, Saint Louis University, Saint Louis, MO; 2Surgery, Saint Louis University, Saint Louis, MO; 3Pathology, Saint Louis University, Saint Louis, MO; 4Pharmacology and Physiology, Saint Louis University, Saint Louis, MO

Purpose: Total Parenteral Nutrition (TPN) involves providing all nutrition intravenously in patients with bowel resection or impaired gut function. Unfortunately, this life saving therapy is associated with significant liver disease and mortality. New evidence points to gut derived signaling secondary to luminal contents that regulates the gut-liver cross talk (GLCT) essential for hepatic health. We have published improvement in TPN associated injury in animals with intact gut treated enterally by chenodeoxycholic acid (CDCA), a gut Farnesoid X Receptor (FXR) agonist. CDCA a bile acid (BA) causes secretion of the hepa-protection fibroblast growth factor 19 (FGF19), a signaling molecule mediating GLCT as part of the FXR-FGF19 axis. Leveraging these results we hypothesized that similar improvement could be translated to the clinically relevant condition of short bowel syndrome (SBS), which results from extensive bowel resection and requires long term TPN.

Methods: Utilizing our ambulatory TPN piglet model, we developed a novel 90% small bowel resected SBS model. To test our hypothesis, 12 SBS animals were randomly allocated to receive TPN+enteral CDCA or TPN+vehicle control for 2 weeks. Liver, bowel and serum were collected post euthanasia. Histology, quantitative PCR, ELISA and serum analysis were performed. Pairwise t-test for normally distributed data, otherwise pairwise Mann Whitney U test were conducted. All tests were 2 tailed using a significance level of 0.05.

Results: CDCA potency was confirmed by demonstrating FXR activation in HepG2 cell lines. TPN resulted in significant gut atrophy. CDCA preserved gut mass and villous/crypt ratio (V/C), demonstrated
The median and interquartile range for gut density (g/cm) and V/C was 8.25 (7.65-8.85), 1.92 (1.67-2.18) for TPN and 9.86 (9.3-11.8), 2.67 (2.37-2.82) for CDCA, p=0.041 and p=0.026 respectively. Crypt enterocyte proliferation calculated using the Bromodeoxyuridine index (BrDUI) was preserved with CDCA (mean 23.3%) vs TPN (mean 30.34%), p=0.028. As expected, CDCA activated gut FXR and apical sodium dependent BA transporter (qPCR p=0.02, p=0.04 vs TPN; respectively). TPN resulted in significant hepatic cholestasis with elevated GGT and high serum bilirubin, however no hepatic improvement was noted with CDCA (p=0.74, p=0.81 respectively). No differences in key hepatic GLCT regulators (FXR, FGF19 and Cholesterol 7 alpha-hydroxylase) were noted in either group (p=0.93, p=0.37 and p=0.58 respectively).

Conclusions: This study establishes a new ambulatory SBS animal model recapitulating the very important clinical SBS. We note with 90% bowel resection, there is inadequate CDCA induced activation of gut derived signaling thus precluding hepatic improvement. Therefore, modulating GLCT is intimately related to the existence of some small bowel, highlighting the important role of gut in modulating systemic disease. Thus no gut no gain! This is clinically very relevant as it provides novel information cautioning the recent excitement that BA therapy has generated for a variety of liver disorders targeting the GLCT. Thus in SBS, instead of enteral therapy, aggressive trialing of intravenously administered downstream signaling molecules along the GLCT pathway might prove more prudent. Interestingly gut preservation with CDCA despite extensive resection calls for further studies to ascertain mechanistic links.

Financial support received from: Jain AK received funding from NIH (K08DK098623) and internal funds from Saint Louis University

VARS CANDIDATE
2834917 - Morphomic Malnutrition Score: A Standardized Approach to Diagnosing Severe Malnutrition in Adults
Christopher S. Lee, BS 2; Erica Raymond, RD 3; Rebecca Goulson, BS 2; Brian Derstine, MS 2; Avinash Rajasekaran, BS 2; Jill R. Cherry-Bukowiec, MD, MS, PNS, FACS 1; Grace Su, MD 2; Stewart Wang, MD, PhD, FACS 2
1Surgery, Univ Michigan, Ann Arbor, MI; 2Morphomic Analysis Group, Department of Surgery, Michigan Medicine, Ann Arbor, MI; 3Michigan Medicine, Ann Arbor, MI

Purpose: Granular and objective diagnostic criteria for adult malnutrition are lacking. The availability of such criteria can help assist in the screening of large clinical populations and add clarity to the link between malnutrition and poor clinical outcomes and increased cost of care. This study uses analytic morphomics to define the Morphomic Malnutrition Score (MMS), a robust screening and diagnostic tool for severe malnutrition.

Methods: The study population (n=643) consisted of 2 cohorts: 1) 124 adults admitted through the University of Michigan emergency department between 2014 and 2016 and subsequently diagnosed with severe malnutrition by a registered dietician (RD) and an available computed tomography (CT) scan within 2 days of RD evaluation, 2) 519 adult kidney donor candidates between 2000 and 2010 with an appropriate CT scan. The kidney donors were chosen to represent a generally healthy (normal) cohort. The combined cohort was then randomly divided into two sets – one for the development of the score (n=453) and the other for validation (n=190). Body composition markers of muscle area and abdominal adiposity were measured at multiple vertebral levels from patient CT scans using analytic morphomic assessment (Figure 1), and then converted to gender and age-adjusted percentiles using the Reference Analytic Morphomics Population (RAMP). RAMP consists of 6000 adult trauma patients chosen to be representative of the general population and includes both healthy and unhealthy individuals. MMS was derived using multivariate logistic regression with nutrition diagnoses as the outcome. The resulting model coefficients were used to generate a score that was scaled from 0 to 10, based on ascending severity of malnutrition.

Results: Severely-malnourished patients had lower RAMP values when compared with kidney donors (Table 1), specifically for dorsal muscle group area at T12 (p<0.001), psoas muscle area at L4 (p<0.001), and subcutaneous fat area at L3 (p<0.001), which were included in the model used to derive MMS. MMS
for severely-malnourished patients was higher than kidney donors (8.045±2.146 vs. 4.329±2.023, respectively; p-value<0.001). An MMS>6.4 was identified as the optimal cut-off score (Figure 2) to distinguish severe patients from kidney donors in the validation set, resulting in a sensitivity of 82.1% and a specificity of 88.3% (Table 2).

**Conclusions:** MMS provides an evidence-based, granular assessment to distinguish severely-malnourished adults from a healthy patient population. MMS utilizes patient-specific data measured from CT scans performed for medical indications, making implementation simple and scalable for screening to identify patients who would benefit from RD assessment and intervention for malnutrition. Furthermore, MMS uses objective granular data that can inform diagnostic guidelines for severe malnutrition. Given that malnutrition is a modifiable risk factor for mortality, morbidity, and readmission, efforts to further validate MMS will better define malnutrition and encourage nutrition intervention, thereby improving outcomes and lowering cost of care.

Financial support received from: N/A

Table 1 - MMS Features

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Severely-Malnourished (n=96)</th>
<th>Kidney Donors (n=357)</th>
<th>p-value</th>
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<tr>
<td>Dorsal Muscle Group Area T12 RAMP Percentile</td>
<td>21.865 ± 23.728</td>
<td>59.649 ± 24.771</td>
<td>p &lt; 0.001</td>
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<tr>
<td>Psoas Muscle Area L4 RAMP Percentile</td>
<td>27.331 ± 24.055</td>
<td>58.335 ± 26.615</td>
<td>p &lt; 0.001</td>
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<tr>
<td>Subcutaneous Fat Area L3 RAMP Percentile</td>
<td>30.688 ± 28.844</td>
<td>62.012 ± 22.695</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>MMS</td>
<td>8.045 ± 2.146</td>
<td>4.329 ± 2.023</td>
<td>p &lt; 0.001</td>
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Table 2 - MMS Scoring of Validation Cohort (n=190)

<table>
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<tr>
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<th>RD Diagnosis</th>
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<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>MMS ≤ 6.4</td>
<td>Normal</td>
</tr>
<tr>
<td>MMS &gt; 6.4</td>
<td>Severe</td>
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Sensitivity: 82.1%; Specificity: 88.3%

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Figure 1:
1) CT scan used in analytic morphomic assessment. Individual vertebrae were labeled and imaging slices were generated to measure 2) cross-sectional area of dorsal muscle group at T12, 3) cross-sectional area of fat in the subcutaneous region at L3, and 4) cross-sectional area of the psoas muscles at L4.
Density curves of MMS for severely-malnourished adults and kidney donors. MMS>6.4 was the optimal score cut off to distinguish between the two populations.

**Purpose:** The provision of greater amounts of protein and energy to critically ill patients is associated with improved outcomes (1); however there is a lack of randomized control trials to establish causality. The primary aim of this pilot study was to determine whether an enteral feeding protocol using a volume target, with additional protein supplementation, delivers a greater amount of protein and energy to mechanically ventilated critically ill patients when compared to standard care.

**Methods:** This prospective, single centre, open label, randomized control trial was registered at the Australian New Zealand Clinical Trials Registry (U1111-1172-8563). Following informed consent, participants were allocated using a randomization algorithm in a 1:1 ratio to standard care (hourly rate-based feeding protocol providing 25kcal/kg and 1g/kg protein) or intervention (daily volume-based feeding protocol with supplemental enteral protein, providing 25kcal/kg and 1.5g/kg protein) while enterally fed in the intensive care unit (ICU) censored at day 15. The co-primary outcomes were the mean daily protein and energy delivered. Secondary outcomes included change in quadriceps muscle layer thickness (QMLT) measured using ultrasound, nutritional status (Subjective Global Assessment) at baseline and...
ICU discharge and other nutritional and patient-centred outcomes.

**Results:** Thirty participants were in each group. Baseline demographics were similar. Mean (SD) protein delivery over the study period for the standard and intervention groups were 0.75 (0.11) g/kg/day and 1.20 (0.30) g/kg/day respectively [mean difference of 0.45g/kg/day (95%CI 0.33-0.56, p<0.001)]. Mean (SD) energy delivered was 18.2 (2.7) kcal/kg/day in the standard group and 21.0 (5.2) kcal/kg/day in the intervention group [mean difference of 2.8 kcal/kg/day (95%CI 0.67 – 4.9, p= 0.01)]. The number of participants in each group who developed diarrhea [16 (53%) in both groups] and feeding intolerance [standard 8 (27%) and intervention 9 (30%)] were similar. Adjusting for baseline QMLT and independent of age, APACHE III Score and admission category muscle loss was attenuated in the intervention group when compared to control (DQMLT standard -0.42 (0.39) cm vs. intervention -0.23 (0.33) cm [mean difference 0.23cm (95%CI 0.04 – 0.41, p=0.02)]. There was significantly more malnourished patients at ICU discharge in the standard group (8 (28%) vs. 2 (7%); p=0.04). Duration of ICU and hospital stay and mortality were similar between groups.

**Conclusions:** In this single centre pilot trial a volume-based enteral feeding protocol with supplemental protein increased protein and energy delivery to critically ill mechanically ventilated patients. These increases were associated with attenuation of quadriceps muscle layer thickness and reduced prevalence of malnutrition at ICU discharge as quantified using the Subjective Global Assessment. This trial supports the feasibility and rationale for further studies using this intervention.


Financial support received from: This project is supported by the Mary Elizabeth Watson Early Career in Allied Health Research Fellowship provided by Melbourne Health, which provides $30,000 per year over two years.

**VARS CANDIDATE**

2830065 - Low muscle mass estimated by calf circumference is a significant risk factor for 30-day hospital readmission.

Gustavo G. Real, MD, MSc1; Inara R. Frühau, Medical student2; José Henrique K. Sedrez, Medical student2; Eduarda Jaine F. Dall’Aqua, Medical student2; Maria Cristina Gonzalez, MD, PhD1

1Postgraduate Program in Health and Behavior, Universidade Católica de Pelotas, Pelotas, Brazil; 2Medical School, Catholic University of Pelotas, Pelotas, Brazil

**Purpose:** Hospital readmissions are common, potentially preventable, and also a marker of poor quality of health services. Knowledge of associated risk factors involved in hospital readmission is essential to establish preventive strategies. This study aimed to identify risk factors for hospital readmission, with emphasis on nutritional aspects, in clinical patients admitted to a university hospital in Southern Brazil.

**Methods:** A prospective cohort was conducted to evaluate predictive factors of hospital readmission in patients admitted to the Clinic Medical ward. Demographic, socioeconomic, comorbidities, number of medications in use, and a short form of Patient-generated Subjective Nutritional assessment (PG-SGA) were performed in the first 24 hours of hospitalization. Nutritional risk was defined by a PG-SGA score of 9 or greater. In addition, nutritional assessment was supplemented with bioelectrical impedance to estimate phase angle, calf circumference as a surrogate of muscle mass, and handgrip strength as a marker of strength. Validated cut-offs for the local population were used for the calf circumference (33 cm for female and 34 cm for male) and handgrip strength for the patients with less than 60 years (20.8 kg for females and 36.7 kg for males). The usual strength cut-offs were adopted for patients with 60 years or older (20 kg for females and 30 kg for males). Charlson Comorbidity Index was used to assess the severity of the comorbidities. Re-assessments were made every seven days of hospitalization until discharge and the last assessment was considered to define the status in the moment of discharge. After 30 days of hospital discharge, the patients were contacted by phone in order to evaluate readmission.

**Results:** From 186 patients assessed, 25 could not perform one of the tests or did not know the current
weight or weight loss. One hundred and sixty one subjects had all the assessments and were, therefore, included in the statistical analysis. Most of the patients were male (54.6%) and the mean age of the participants was 59.2 ± 17.8 years. The median Charlson Comorbidity Index was 2.76 (IQR: 1;4). There were 27 hospital readmissions (16.8%) after a period of 30 days. Nutritional risk was identified in 77.6% of the sample and 46.0% of the patients had a low calf circumference at hospital discharge. After controlling for sex and age, a Charlson's Comorbidity Index greater than two (OR: 3.29, 95% CI: 1.21;8.97), the presence of cancer (OR: 4.52; 95% CI: 1.11;18.42), nutritional risk (OR: 9.53, 95% CI: 1.16;77.9), and low calf circumference (OR: 3.89, 95% CI: 1.34;11.31) were significantly associated with 30-day hospital readmission in the multivariate logistic analysis.

Conclusions: Calf circumference seems to be a practical, inexpensive and highly replicable method for estimating muscle mass in the hospital environment. The aforementioned results suggest that it can also be a good predictor of 30-day hospital readmission, even after controlling for other well-known risk factors, such as Charlson's Comorbidity Index, the presence of cancer and nutritional risk.

Financial support received from: N/A

VARS CANDIDATE

2834017 - Gut microbiota as a modulator of Paneth cells during total parenteral nutrition in mice

Jiwei Wang, PhD; Feng Tian, PhD; Li Zhang, PhD; Xuejin Gao, PhD; Xinying Wang, PhD

Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, Nanjing, China

Purpose: Intestinal barrier dysfunction plays an important role in gut-derived infections and sepsis during total parenteral nutrition (TPN). Our previous research showed that TPN lead to decrease in Paneth cell antimicrobial peptides, accompanied by gut microbiota dysbiosis. However, whether the altered microbiota results in or is the product of Paneth cell dysfunction has never been answered. Here, we investigated the role of gut microbiota in antimicrobial peptides of Paneth cells during TPN.

Methods: Experiment 1: Eight-week-old specific pathogen-free (SPF) mice (n=20) were treated with an antibiotic cocktail (4Abx) in drinking water for 2 weeks. The efficacy of antibiotics was evaluated by RT-PCR. Afterwards, these Abx mice were surgically implanted with rubber catheters in the right jugular vein, and then were randomly divided into two groups: control group received a sterile diet of standard laboratory chow and infused intravenously with 0.9% saline (Abx-Chow), while TPN group received continuous infusion of isocaloric and isonitrogenous PN solution (Abx-TPN). After 7 days, during which antibiotics were administered continuously, all Abx mice were sacrificed and whole small intestines were removed.

Experiment 2: Ten-week-old SPF mice (n=20) were randomly divided into control group and TPN group. After 7 days, the entire small intestine was removed, and enteric effluent from control and TPN mice was sterilely extracted and immediately gavaged (250μL intragastric) into 8-week-old germ-free (GF) mice under sterile conditions. Each treatment group (n=5) was housed in separate sterile isolators, namely Chow→GF and TPN→GF. All mice received a standard sterile diet for 2 weeks. The length of the small intestines was measured. Ileum mucosal morphology was assessed by H&E staining. Lysozyme, RegIIIγ and IL-22 were measured by RT-PCR, western blot, ELISA or immunohistochemistry. Small bowel effluent from control, TPN, and GF mice underwent untargeted Liquid Chromatography-Mass Spectrometric (LC/MS) analysis.

Results: Experiment 1: Antibiotics treatment efficiently decreased the number of the small intestinal microbes, and cecum of Abx mice expanded obviously. Antibiotics treatment decreased the expression of lysozyme, while TPN did not downregulate its expression further. Moreover, levels of lysozyme in Abx-TPN were higher than Normal-TPN mice (did not received antibiotics treatment) (Fig 1).

Experiment 2: Gut microbiota from chow or TPN was transferred to GF mice for 2 weeks. TPN→GF showed lower body weight, shorter intestinal length, severe atrophy of ileum villus, compared to Chow→GF. Besides, in TPN→GF group, protein level of lysozyme and RegIIIγ were both lower. Immunohistochemistry for lysozyme showed relative sparse. IL22 and IL-17 mRNA levels in ileum tissues also declined (Fig 2). Principal component analysis (PCA) on the basis of metabolites composition revealed major differences between Chow and TPN, also between Chow→GF and TPN→GF.
Hierarchical clustering analysis found certain tryptophan metabolites were differentially expressed, including kynurenic acid and indole derivatives (Fig 3). Pathway analysis showed aberrant tryptophan metabolism existed in Chow vs TPN, and Chow→GF vs TPN→GF simultaneously (Fig 4).

**Conclusions:** Gut microbiota plays a vital role in TPN-related Paneth cell dysfunction. Dysbiosis during TPN might alter the production of microbial metabolites, thereby influences antimicrobial peptides of Paneth cells.

Financial support received from: This work was supported by the National Natural Science Foundation of China (81470797).

Fig1. Elimination of gut microbiota effectivity rescues lysozyme in a mouse model of TPN. (A)&(B) Pictures of abdomen after saline(A) or 4-abx treatment(B) for 2 weeks. (C)&(D) Antibiotics treatment efficiently decreases the number of the gut microbes. qPCR was performed and the fold changes of 16S rDNA was normalized to host genomic Actb(C) and 18s RNA(D), respectively. (E) Body weight of Abx-Chow and Abx-TPN. (F) Protein levels of lysozyme in ileum tissues of Normal-chow, Normal-TPN, Abx-chow and Abx-TPN, relative to GAPDH as internal control.
Fig 2. Dysbiosis of gut microbiota mediates dysfunction of Paneth cells. Germ-free mice were colonized by gut microbes from chow or TPN for 2 weeks. (A) Body size of TPN→GF(upper) and Chow→GF(below). (B) Intestine and colon of TPN→GF(upper) and Chow→GF(below). (C) Body weight of TPN→GF(upper) and Chow→GF(below). (D) Intestinal length of TPN→GF(upper) and Chow→GF(below). (E)&(F) Ileum tissue HE staining and immunohistochemistry of lysozyme in TPN→GF(upper) and Chow→GF(below). (G) Protein levels of lysozyme in ileum tissues of TPN→GF and Chow→GF. (H) IL22 and IL-17 mRNA levels in ileum tissues of TPN→GF and Chow→GF.
Fig 3. Principal component analysis and heatmap of hierarchical clustering analysis. (A) & (B) Principal component analysis on the basis of metabolites composition in Chow vs TPN (A), and Chow→GF vs TPN→GF (B). (C) Heatmap of hierarchical clustering analysis, showing partial differentially expressed metabolites in Chow vs TPN (upper), and Chow→GF vs TPN→GF (below).
Fig4. Pathway analysis. (A) Pathway analysis for Chow vs TPN. (B) Pathway analysis for Chow→GF vs TPN→GF.