The Role the of the Gastrointestinal Microbiome in Foregut Leaks

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Introduction/Background

Leaks of the gastrointestinal tract are a devastating complication that can occur after foregut operations. It has been suggested that the pathogenesis of foregut leaks is directly influenced by the gut microbiome. The purpose of this study was to evaluate the composition of the microbiome within and between patients with gastrointestinal leaks to better understand the pathogenesis of these leaks.

Methods:

Patients undergoing interventions for gastrointestinal leaks from October 2021 to October 2022 were included in this study. During endoscopic and surgical interventions for gastrointestinal leaks, both microbial and host samples were collected. Genomic DNA of microbial samples were extracted and amplified. PCR products were sequenced using Illumina Nextera protocol. Effective sequence of bacterial 16S-rRNA gene was clustered into OTUs for analysis.

Results:

A total of 196 samples were collected from 16 patients (13 females; 3 males) with 49 samples used for the 16S analysis. The majority (56.2%) of patients required multiple interventions for their leaks, while a smaller portion (43.8%) underwent a single intervention. 42/49 samples (85.7%) included in the 16S analysis were from patients requiring multiple interventions with a mean of 4.6 interventions performed per patient in this group. In the entire cohort, Firmicutes was consistently the most abundant bacteria present. For patients that required multiple interventions, the microorganism composition changed over the course of treatment. At the index procedure, Firmicutes and Actinobacteria were on average the most abundant phyla present. By the end of treatment, Firmicutes remained dominant. However, abundances of Bacteroidetes and Proteobacteria increased, and the abundance of Actinobacteria decreased. Notably, there was a significant reduction in the Firmicutes to Bacteroidetes ratio by the end of treatment. In one patient who was not progressing well clinically, they were noted to have an increase in their Firmicutes to Bacteroidetes ratio and a much higher abundance of Proteobacteria when compared to other patients.

Conclusion

In conclusion, data from our study indicates that the Firmicutes to Bacteroidetes ratio of the gut microbiome significantly changed throughout the treatment of gastrointestinal leaks. A better understanding of this ratio and its role in gastrointestinal leaks could allow for more effective prevention and treatment strategies.

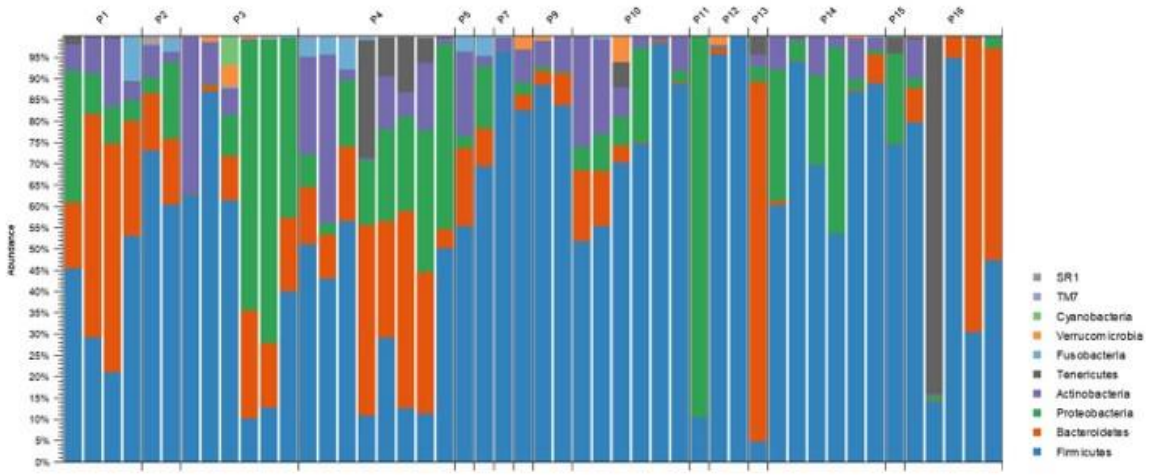

Figure 1. Relative abundance of phyla in a cylindrical accumulative graph of bacteria in the microbial samples. The horizontal ordinate (P1-P16) represents the samples correlating with each patient. The longitudinal ordinate represents the relative abundance of each phyla.

Presidential Session | Breast

Cryoablation combined with immune checkpoint inhibition in a murine metastatic breast cancer model increased T-cell activation providing support for clinical trials.

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Introduction/Background

Breast cancer cryoablation, a non-surgical procedure, uses rapid freeze-thaw cycles to kill the tumor and preserve tumor-associated antigens (TAAs) while conserving the surrounding healthy tissue and breast cosmetics. The immune system identifies these TAAs inducing an anti-tumor response that can target both the primary tumor and metastatic cancer cells - in vivo cancer vaccine. To enhance the abscopal effect - distant tumor targeting, immune checkpoint inhibitors are being explored. Check-point inhibitors work by blocking T cell inhibitor mechanisms taken advantage of by tumor cells. A clinical pilot study of preoperative single-dose ipilimumab (an anti-CTLA4) and cryoablation in women with early-stage breast cancer was safe and showed favorable intra-tumoral and systemic immunologic responses. Using a murine model of high-risk metastatic breast cancer, we investigated cryoablation in conjunction with anti-CTLA4 to enhance the anti-tumor T-cell immune response. We are investigating this translational approach to validate future clinical trials which may lead to effective minimally invasive and cost-effective treatment for high-risk metastsic breast cancer.

Methods:

BALB/c mice were bilaterally transplanted in the mammary fat pad with 4T1-12b-luc metastatic breast cells. Mice were treated with 100 µg anti-CTLA4 24-hrs pre/post-cryoablation vs control mice receiving cryoablation alone. Left tumors were cryoablated while right untreated tumors served as proxy for distant metastatic tumor for abscopal immune readout. One-week post cryoablation, mice were sacrificed for tissue analysis. Cryoablated and treatment naive tumors were analyzed for tumor-infiltrating lymphocyte (TIL) scores by hematoxylin and eosin (H&E) and cytotoxic T lymphocytes (CTLs) by CD8/ICOS immunofluorescence for immune activation and tumor location. For systemic immune response, the peripheral blood, tumors and spleens were analyzed by flow cytometry for immune cell populations and T cell activation status.

Results:

Mouse necropsies showed cryoablated tumors undergoing coagulative necrosis. Mice treated with cryoablation and anti-CTLA4 had a significant increase in TILs compared to controls. Immunofluorescence analysis revealed significant increase of CD4+ and CD8+ T-cells in the cryoablated tumors compared to the abscopal tumors - primarily at the tissue-tumor periphery. Flow cytometry analysis showed increased T-cell activation in anti-CTLA-4 treated mice with increased effector and effector memory CD4+ and CD8+ T cells in both the abscopal tumors and peripheral blood.

Conclusion

Cryoablation combined with anti-CTLA4 increased T-cell activation systemically and at the treated tumor compared to cryoablation treatment alone. The next step is to evaluate whether combinational therapy increases the abscopal effect in controlling metastasis in long-term survival studies in our breast cancer murine model before proceeding to clinical trials.

Hernia Prevention Utilizing Biologic Mesh and/or Small Bites: A Multi-Specialty 2x2 Factorial Randomized Controlled Trial

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Introduction/Background
Ventral incisional hernias (VIH) are the most common surgical complication following abdominal surgery. Randomized trials have shown efficacy of prophylactic synthetic mesh and small bites. Adoption of these practices has been limited due to concerns with placement of synthetic mesh in contaminated cases and small bites in an overweight population. We sought to assess the effectiveness of prophylactic biologic mesh and small bites to prevent post-operative major complications (VIH, surgical site infection, reoperation, death).

Methods:
High-risk patients (overweight/obese, current smoker) undergoing abdominal surgery with a midline incision (≥5cm) were randomized (2x2 factorial trial) to receive either sublay biologic mesh or no mesh and either small bites (0.5x0.5cm) or large bites (1x1cm) fascial closure. The primary outcome measure was major complications at one-year post-operative. CONSORT guidelines were followed. Assuming α=0.05, β=0.20, Δ=20%, it was estimated that 105 patients were needed. Primary outcome was assessed using Fisher's exact test.

Results:
107 patients were randomized: 52(49%) to mesh, 55(51%) to no mesh, 55(51%) to small bites, 52(49%) to large bites. 16% were smokers, 31% overweight, 55% obese. Most procedures were for colorectal diseases (75%), contaminated (96%), and performed open (58%). At one-year post-operative, there were no differences in major complications between study groups (mesh vs no mesh 21% vs 16%, p=0.62; small vs large bites 18% vs 19%, p=1.00).

Conclusion
In this trial, biologic mesh and small bites appear to have no benefit. Further randomized trials are needed among high-risk patients prior to widespread adoption of prophylactic biologic mesh or small bites.

Mechanical Tension Induced Changes in Dermal Fibroblasts PKM2 and Hsp27 Phosphorylation Regulates Fibrotic Responses

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Introduction/Background

During wound healing, fibroblasts (FB) respond to changes in the microenvironmental stiffness by reorganizing their actin cytoskeleton to anchor, proliferate, and produce an extracellular matrix. This is an energy-intensive process that is fueled by oxidative phosphorylation and glycolysis to regulate ATP. Emerging evidence shows aerobic glycolysis drives pathologic fibrosis in stiff environments, but its role in physiologic wound healing is unknown. Pyruvate Kinase M2 (PKM2) is a rate-limiting enzyme of glycolysis, whose activity is phosphorylation (p) dependent. Similarly, Heat shock protein (Hsp27) is known to regulate actin cytoskeleton rearrangement under tension. We hypothesize that PKM2/Hsp27-dependent alterations in FB energy metabolism are influenced by wound biomechanical forces to drive fibrotic responses.

Methods:

FB were isolated from 8wk C57BL/6J mice, then cultured on either flexible silicone membranes (930 kPa Young's modulus) that provide a softer substrate for cell growth, or standard plastic culture dishes (TCP - 1x107 KPa). Cells were stimulated with TGFb (10ng/ml) for 24hr +/- TEPP (100uM) which is a PKM2 tetramer activator. Total and phosphorylated PKM2 and Hsp27 were analyzed using immunoblotting. Hsp27 intracellular expression and alignment with actin was assessed by staining. Lactate, ATP/ADP balance, and a-SMA (qRT-PCR; immunoblotting) were analyzed by n=3 biologic replicates/group, p-values by ANOVA.

Results:

The expression of both total PKM2 and p-PKM2 were lower in cells on flex membrane than TCP at baseline. TGFb significantly increased p-PKM2/PKM2 ratio in cells on TCP (2 fold) compared to 1 fold in flex and TEPP treatment reduced the effect of TGFb more in cells on flex membrane. In contrast, total Hsp27 was more in cells on flex membrane at baseline by 1.4 fold and p-Hsp27 was less by 0.5 fold compared to cells on TCP. p-Hsp27 was more induced by TGFb treatment in cells on flex membrane than on TCP (6- vs. 3-fold). There was no effect of TEPP on p-Hsp27 in TCP, but it lowered p-Hsp27 in cells on flex. Lactate was similar at baseline in both conditions, but TGFb had a more pronounced induction of lactate in cells on TCP compared to flex, and TEPP treatment was effective in reducing the effect of TGFb similarly in both conditions. ADP/ATP ratio in cells on TCP was reduced by TGFb, but not on flex and TEPP treatment was ineffective on TCP, but it increased the ratio in cells on flex membrane. Lastly, the cells on the flex membrane had reduced a-SMA than those on TCP, and TGFb induced more a-SMA in cells on flex than on TCP (2- vs. 1.5-fold). TEPP treatment reduced a-SMA in cells on flex but not TCP.

Conclusion

Fibroblasts display distinct bioenergetic metabolism profiles and produce distinct scarring in different microenvironmental stiffness conditions. Our data suggests that cells are more amenable to treatment to regulate aerobic glycolysis and fibrosis markers in less stiffer environments.

Social Vulnerability and Survival in GI Cancers

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Introduction/Background

Social vulnerability is a federal metric used to assess a community's resilience in facing external stressors from disease or disaster. Socially vulnerable populations include the disabled, elderly, and minorities, amongst others. The social vulnerability index (SVI) uses 4 themes – socioeconomic status, household composition and disability, minority status & language, and housing/transportation - to rank 15 social factors for census tracts within counties. Social vulnerability has never been explored at the census tract level in any malignancy and the Texas cancer registry provides granular detail at both the patient and census block group level not available in national datasets. We sought to characterize the relationship between social vulnerability and survival in gastrointestinal cancers, as well as its potential to identify themes for focused interventions that may mitigate disparities.

Methods:

We retrospectively reviewed 196,651 patients with colorectal (CRC), gastric (GC), pancreatic (PDAC), and hepatocellular cancer (HCC) in the Texas Cancer Registry from 2004-2019. Patient demographics, social vulnerability (SVI), poverty index (PI), and clinicopathologic factors were analyzed for their impact on survival at 2 - and 5- years. Unadjusted and covariable- adjusted cox proportional hazards were used for survival analysis. All values reported herein are significant at p

Results:

Of 196,651 patients, 61% were CRC, 16% PDAC, 9% GC, and 14% HCC. The majority of HCC were localized (51.4%) while most CRC was regional (38%) and PDAC (55%) and GC (40%) were metastatic at diagnosis.

60% of patients in the cohort received surgery and 34% received chemotherapy. ½ of PDAC/HCC patients did not receive any therapy.

Percentiles for SVI themes were consistently near the national average except for GC which was in the 80th percentile for the minority/language theme.

At 2 years, poverty (5-9% - HR 1.06, 95%CI 1.03 - 1.09; 10-19% - HR 1.09, 95%CI 1.06 - 1.12; 20-100% - HR 1.13, 95%CI 1.10 - 1.17), Medicaid (HR 1.41, 95%CI 1.36 - 1.47), socioeconomic status (HR 1.18, 95%CI 1.13 - 1.24), household composition (HR 1.06, 95%CI 1.02 - 1.10), and the minority theme (HR 0.85, 95%CI 0.82 - 0.89) were associated with survival.

Findings were consistent at 5 years with the housing theme (HR 1.03, 95%CI 1.00 - 1.06) becoming significant.

Conclusion

Across 4 GI cancers, social vulnerability and poverty independently predicted survival at 2- and 5- years. Among SVI themes, socioeconomic status was the strongest predictor of worse survival and the minority theme was associated with improved survival, potentially reflecting the Hispanic paradox. Much like the federal government uses social vulnerability to direct resources in emergencies, SVI shows promise as a

tool for identifying where resources can be targeted at a local level to mediate the survival disparity for vulnerable populations with GI malignancies.

Presidential Session | Trauma/Burn/Critical Care

Recent Changes in Prehospital Interventions in Trauma Patients are Associated with Decreased Mortality

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Introduction/Background

Optimal prehospital management in trauma is hotly debated, with many studies arguing that aggressive prehospital treatment does not improve outcomes. However, no studies have assessed how EMS practices have changed over time in response to new evidence. The aim of this study is to quantify the frequency of prehospital interventions (PHI) performed by EMS over time. We hypothesize that the frequency of PHI has increased.

Methods:

We performed a retrospective chart review of adult patients transported by EMS to our ACS verified Level 1 trauma center from January 1, 2014, to December 31, 2020. PHI were manually recorded and changes in their frequency over time were assessed via year-by-year trend analysis and multivariate regression.

Results:

2,501 patients were included, of which 21% were transported by air EMS and 79% were transported by ground EMS. Over the 7-year study period, male gender (74% vs. 79%, p=0.005) and age (41 vs. 43, p=0.02) increased, while the proportion of blunt trauma (73% vs. 59%, p

Conclusion

PHI in trauma patients have changed significantly over the past six years. These changes were associated with improvements in ED and hospital mortality. PHI for trauma should be further refined to optimize outcomes.

Table 1: Changes in Prehospital Interventions Over Time

Interventions That Increased Over Time			Interventions That Decreased Over Time		
Variable	**% Increase***	**P-value+**	**Variable**	**% Decrease***	**P-value+**
Thoracostomy	↑ 50%	0.003	Fluid Administration	↓ 32%	<0.001
Tourniquet	↑ 175%	<0.001	Advanced Airway	↓ 38%	<0.001
Blood Transfusion	↑ 390%	<0.001	Cervical Spine Collar	↓ 38%	<0.001
Pelvic Stabilization	↑ 700%	<0.001	Backboard	↓ 60%	<0.001

* % Change from beginning to end of study period
+ P-value for trend by regression