Increased Variance in Oral and Gastric Microbiome Correlates With Esophagectomy Anastomotic Leak

Rishindra M. Reddy, MD, William B. Weir, MD, Shari Barnett, RTT, BS, Brendan T. Heiden, BS, Mark B. Orringer, MD, Jules Lin, MD, Andrew C. Chang, MD, Philip W. Carrott, MD, William R. Lynch, MD, David G. Beer, PhD, J. Christopher Fenno, PhD, and Yvonne Kapila, DDS, PhD

University of Michigan Medical School, Ann Arbor, Michigan; Department of Surgery, Section of Thoracic Surgery, University of Michigan, Ann Arbor, Michigan; University of Michigan School of Dentistry, Ann Arbor, Michigan; and Division of Periodontology, Department of Orofacial Sciences, University of California, San Francisco, San Francisco, California

Background. **Anastomotic leak after esophagectomy remains a significant source of morbidity and mortality. The gastrointestinal (GI) microbiome has been found to play a significant role in tumor oncogenesis and postoperative bowel anastomotic leak. We hypothesized that the GI microbiome could differentiate between esophageal cancer histologies and predict postoperative anastomotic leak.**

Methods. **A prospective study of esophagectomy patients was performed from May 2013 to August 2014, with the collection of oral saliva, intraoperative esophageal and gastric mucosa, and samples of postoperative infections (neck swab or sputum). The presence and level for each bacterial probe as end points were used to analyze correlations with tumor histology, tumor stage, and presence of postoperative complications by unequal variances *t* tests for multiple comparisons and principal coordinate analysis.**

Results. **Esophagectomy was successful in 55 of 66 patients who were enrolled. Among those, the diagnosis was adenocarcinoma in 44 (80%) squamous cell carcinoma in (13%), and benign disease in 4 (7%). The 30-day mortality was 1.8% (1 of 55). Complications included anastomotic leak requiring local drainage in 18% (10 of 55) and postoperative pneumonia in 2% (1 of 55). No correlation was noted between GI microbiome flora and tumor histology or tumor stage. A significant difference ($p = 0.015$) was found when the variance in bacterial composition between the preoperative oral flora was compared with intraoperative gastric flora in patients who had a leak but not in patients with pneumonia.**

Conclusions. **Patients with anastomotic leaks had increased variance in their preoperative oral and gastric flora. Microbiome analysis could help identify patients at higher risk for leak after esophagectomy.**

(Ann Thorac Surg 2018;105:865–70)

Esophageal cancer continues to be one of the fastest growing solid-organ tumors in the United States and in the world [1]. Curative treatment relies on surgical resection (esophagectomy) being part of the treatment paradigm for most tumors. Esophagectomy continues to be a morbid operation, with high complications rates (up to 50%) and high 30-day mortality rates (0% to 22%). Complications include pneumonia (16% to 33%), anastomotic leak (9% to 16%), and atrial arrhythmias (6% to 20%) [2]. These complications add significantly to the risk of death, to the length of hospitalization, and to the need for further treatments [3].

The study of the microbiome, the "unculturable" bacterial flora covering different surfaces, has grown exponentially in the past few years, and it appears that the microbiome may contribute to the development of a

Accepted for publication Aug 18, 2017.

Presented at the Poster Session of the Fifty-third Annual Meeting of The Society of Thoracic Surgeons, Houston, TX, Jan 21–25, 2017.

Address correspondence to Dr Reddy, University of Michigan, Section of Thoracic Surgery, 2120 Taubman Center, 1500 E Medical Center Dr, Ann Arbor, MI 48109; email: reddyrm@med.umich.edu.

The Supplemental Table can be viewed in the online version of this article [https://doi.org/10.1016/j.athoracsur.2017.08.061] on http://www.annalsthoracicsurgery.org.

0003-4975/$36.00
https://doi.org/10.1016/j.athoracsur.2017.08.061

number of disease processes, including periodontal disease, gastrointestinal (GI) autoimmune diseases, and even GI cancers [4].

The microbiome has been defined as the community of microbes in a specific ecosystem. The phylogenetic and genetic composition of the different species present can be identified by stratifying the different 16S ribosomal RNAs. The function related to the varying composition of microbes is poorly understood, but the microbiota is being evaluated for potential targeted treatments in cancer prevention [5] and cancer therapeutics [6] and also as a potential biomarker for more aggressive tumors [7]. In the colorectal literature, the type of bacterial flora has been implicated as the primary cause of postsurgical anastomotic leak [8].

There is limited understanding of the effect of the oral and upper GI microbiome on the development of esophageal cancer or on complications after an operation. We hypothesized that the oral and GI microbiome could differentiate between esophageal cancer histology and predict postoperative anastomotic leak after transhiatal esophagectomy.

Patients and Methods

The University of Michigan Institutional Review Board reviewed and approved this study (HUM00071631). The study prospectively consented and enrolled 66 patients undergoing evaluation for esophagectomy from May 2013 to August 2014. For microbiome analysis, oral saliva samples were taken before the operation (after induction chemoradiation therapy, if performed) in the clinic or in the preoperative holding area (sample A) and 1 to 3 days after the operation (sample D). Esophageal (sample B) and gastric (sample C) mucosal samples were collected intraoperatively after the esophagogastrectomy specimen was removed from the field. Perioperative antibiotics were routinely cefazolin or vancomycin for patients with a penicillin allergy.

Primary surgical complications, including postoperative pneumonia and postoperative anastomotic leak, were documented and analyzed. Leaks were defined by a positive leak on barium esophagogram or by clinical changes requiring opening of the neck wound. Pneumonia was defined by clinical changes (shortness of breath, leukocytosis, changes on roentgenogram) resulting in antibiotic treatments. The neck wounds of patients with cervical anastomotic leaks were swabbed (sample E). Induced sputum samples (sample F) were collected when pneumonia developed before antibiotics were initiated. Samples were flash frozen for later DNA analysis. Figure 1 shows the sequence and source of sample collection. Inadequate storage of 12 samples resulted in 208 analyzable samples.

DNA Isolation and Amplification

DNA was isolated from 208 samples with a PowerMag Microbiome RNA/DNA Isolation Kit (Mo Bio Laboratories, Inc, Carlsbad, CA) using an epMotion 5075 liquid handling system (Eppendorf, Hamburg, Germany). The V4 region of the 16S rRNA gene was amplified by standard polymerase chain reaction (PCR) as described previously [9] or by touchdown PCR as described (https://www.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf; Illumina, San Diego, CA). Amplicons were processed and sequenced on the Illumina MiSeq platform as described previously [9]. Standard PCRs used 1 μL or 5 μL DNA (undiluted), and touchdown PCR used 2 μL DNA (undiluted; see the Supplemental Table for details).

Sequence Processing and Analysis Overview

The 16S rRNA gene sequence data was processed and analyzed using mothur 1.36.1 software and the most recent MiSeq SOP [10, 11]. After sequence processing and alignment to the SILVA reference alignment [12, 13], sequences were binned into operational taxonomic units (OTUs) based on 97% sequence similarity using the average neighbor method [14, 15]. By calculating the Yue and Clayton dissimilarity index (θ_{YC}) distances (a metric that takes relative abundances of both shared and nonshared OTUs into account) [16] between communities and using analysis of molecular variance [17], we could determine whether there were statistically significant differences between the microbiota of different groups. Principal coordinates analysis was used to visualize the θ_{YC} distances between samples. We also investigated the taxonomic composition of the bacterial communities by classifying sequences within mothur using a modified version of the Ribosomal Database Project (RDP) training set (version14) [18, 19].

Samples Included

We subsampled 2,619 sequences per sample. We eliminated 9 samples because of low sequence counts: 13E (contains 626), 15C (contains 1,185), 15E (contains 2,026), 1C (contains 48), 25C (contains 786), 28C (contains 67), 28F (contains 62), 64C (contains 280), and 66C (contains 1,547). The analysis included 199 samples.

Statistical Analysis

Using presence and level for each bacterial probe as end points, we analyzed correlations with tumor histology, tumor stage, and the presence of postoperative complications by unequal variances *t* tests for multiple comparisons and principal coordinate analysis. DNA isolation, sequence processing, and statistical analysis were performed with the assistance of the University of Michigan Microbiome Core.

Results

Of the 55 patients who completed the survey and underwent successful transhiatal esophagectomy, the diagnosis was adenocarcinoma in 44 patients (80%), squamous cell carcinoma in 7 (13%), and benign disease in 4 (7%; Table 1). Patients were a mean age of 60.6 years, with 30 patients (54.5%) aged older than 60. All patients except 1 reported oral intake preoperatively, and 3