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Evaluation and management of patients with symptoms after anti-reflux surgery

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SUMMARY. Over the past two decades, there has been an increase in the number of anti-reflux operations being performed. This is mostly due to the use of laparoscopic techniques, the increasing prevalence of gastroesophageal reflux disease (GERD) in the population, and the increasing unwillingness of patients to take acid suppressive medications for life. Laparoscopic fundoplication is now widely available in both academic and community hospitals, has a limited length of stay and postoperative recovery time, and is associated with excellent outcomes in carefully selected patients. Although the operation has low mortality and postoperative morbidity, it is associated with late postoperative complications, such as gas bloat syndrome, dysphagia, diarrhea, and recurrent GERD symptoms. This review summarizes the diagnostic evaluation and appropriate management of such postoperative complications. If a reoperation is needed, it should be performed by experienced foregut surgeons.

KEY WORDS: anti-reflux surgery, fundoplication, gastroesophageal reflux disease.

INTRODUCTION

Gastroesophageal reflux disease (GERD), one of the most common gastrointestinal (GI) disorders, ranges from non-erosive reflux disease to erosive disease and complications, such as strictures and Barrett’s esophagus. Patients with GERD have symptoms, such as heartburn, regurgitation, dysphagia, chest pain, and epigastric pain, which can have a significant impact on their quality of life.1,2 Most patients with GERD are managed medically using proton pump inhibitors and lifestyle modifications, but a small group of individuals eventually undergo anti-reflux surgery (ARS, fundoplication).3–5

Despite strong support in favor of medical therapy alone, surgical management has remained attractive, particularly after the introduction of laparoscopic anti-reflux surgery (LARS). The popularity of LARS lies in shorter recovery periods and fewer complications when compared with open procedures, as well as high rates, of up to 90%, of long-term symptom improvement.6–9

Four possible outcomes may be observed after ARS: (i) a complete response, in which no residual symptoms of GERD are reported; (ii) a partial response, in which residual symptoms of GERD are reported; (iii) new symptoms, in which the preoperative symptoms disappear and new symptoms occur; and (iv) no response, in which there is no change in preoperative GERD symptoms (Fig. 1). There is no standardized definition of ‘failed anti-reflux surgery’; thus, we suggest this term be used to describe the three latter scenarios. In this review, we discuss evaluation and management of patients with recurrent symptoms after ARS.

MAGNITUDE OF THE PROBLEM

Studies evaluating long-term outcomes after primary ARS, ranging from 5 to 12 years of follow-up, have shown that 2–30% of patients may experience new, recurrent, or persistent GERD-related symptoms.10–13 The most common cause for ARS failure is an anatomic abnormality, followed by an incorrect primary diagnosis.14 Between 3% and 9% of patients with failed primary ARS undergo a revisional operation, most commonly for relief of dysphagia and recurrent reflux symptoms.12,15–17

In general, redo ARS has lower rates of success in symptom relief and patient satisfaction when
compared to primary fundoplication.\textsuperscript{12–14} In addition, revision fundoplications typically have higher rates of morbidity, longer operative times, higher rates of conversion from laparoscopic to open, and higher rates of postoperative complications.\textsuperscript{12–14} Thus, patients should not be referred for revision ARS without a comprehensive preoperative evaluation.

EVALUATION

Our framework used to approach a patient after failed ARS is symptom-based. The postoperative evaluation can be approached by two distinct categories of reported symptoms: (i) esophageal and (ii) GI (Fig. 2). Although there may be some overlap, the presence of primarily esophageal versus GI symptoms dictates the initial diagnostic and therapeutic pathway. Furthermore, atypical symptoms may be the primary symptom or a co-reported symptom, and these patients may respond differently to ARS.

Esophageal assessment

Esophageal evaluation begins with further subdivision into symptoms of (i) reflux and (ii) dysphagia. In one study, the prevalence rates of reflux, in the short term (2 years) and long term (>5 years), were 8.2% and 10.1%, respectively.\textsuperscript{17} Similarly, for dysphagia, the prevalence rates were 7.5% and 5.1%, respectively.\textsuperscript{17} Comparatively, in the subset of patients undergoing revisional surgery, dysphagia and reflux symptoms accounted for 48% and 33% of postoperative symptoms, respectively.\textsuperscript{18} Of note, in patients after revision, fundoplication continues to have a relatively high rate of dysphagia at 33%.\textsuperscript{19} These data demonstrate that the vast majority of patients undergoing revisional surgery have primarily esophageal symptoms rather than GI symptoms.

Patients who present with dysphagia should be further subdivided into two groups: (i) those with a twisted or overly tight wrap and (ii) those with a normal wrap. A twisted or overly tight wrap represents a suboptimal surgical procedure, and this
finding accounts for approximately 5% of failed anti-reflux procedures. In contrast, a normal wrap may represent (i) undiagnosed esophageal pathology and (ii) too tight or too loose hiatus. The former includes eosinophilic esophagitis, achalasia or other esophageal motility disorder, and cancer. Regarding the latter, the too tight hiatus often presents as crural stenosis, and the too loose hiatus often presents as intrathoracic migration of the wrap. In one study, 40% of patients with post-fundoplication dysphagia had signs of obstruction at or above the gastroesophageal junction (GEJ) suspicious of crural stenosis, 50% of patients had signs of intrathoracic wrap migration, and 10% of the patients radiographic stenosis of the wrap. The ability to predict postoperative dysphagia could allow providers to assess for successful outcomes after ARS. Dysphagia has been significantly associated with lower peak peristaltic pressure in the distal esophagus and the presence of a hiatus hernia preoperatively, as well as higher residual pressure on GEJ relaxation postoperatively. In a cohort of post-ARS patients, a higher preoperative dysphagia score and delayed esophageal emptying time by barium swallow predicted long-term postoperative dysphagia. Additionally, age, gender, manometry variables, pH-metry variables, and type of fundoplication did not predict postoperative dysphagia.

If a patient presents with reflux symptoms, the following three diagnoses must be considered: (i) poor initial wrap; (ii) a subsequently disrupted fundoplication; or (iii) undiagnosed esophageal pathology, like eosinophilic esophagitis. It may be difficult to separate between malfunction of the initial wrap and a subsequently disrupted fundoplication, as both may present with a decreased lower esophageal sphincter (LES) pressure. Earlier timing of symptoms may suggest the former. Subsequently disrupted fundoplication most commonly manifests as intrathoracic wrap migration, wrap disruption, and telescoping. Although heartburn is the most specific symptom for recurrent acid reflux in the postoperative period, it is important to remember that heartburn after ARS cannot always be attributed to reflux disease. This is particularly applicable as some of the aforementioned diagnoses, including achalasia and eosinophilic esophagitis, may also present with symptoms of heartburn and dysphagia.

When assessing the underlying pathology in symptomatic patients, it is important to consider the prevalence of possible causes of failed ARS. In one systematic review, the following anatomical abnormalities and their prevalence were reported: intrathoracic wrap migration (27.9%), wrap disruption (22.7%), telescoping (14.1%), paraesophageal hiatal herniation (6.1%), hiatal disruption (5.3%), and tight wrap (5.3%). Furthermore, a wrong primary diagnosis was seen in 1.2% of patients.

The timing of symptoms may provide further insight into the cause of failed ARS. New symptoms occurring during the postoperative period or the following weeks suggest complications from the surgery or postoperative symptoms, many of which resolve in the span of months. Beyond that immediate time frame, persistent symptoms without a period of symptom relief may represent (i) poor initial patient selection; (ii) misdiagnosed underlying condition; or (iii) failure of the fundoplication. Patients with a history of poor response to medical therapy are usually poor candidates for ARS. If no objective evidence of an organic cause of GERD-type symptoms was identified on preoperative evaluation, functional heartburn is a probable diagnosis. A misdiagnosed underlying condition includes the aforementioned eosinophilic esophagitis, motility disorder, or cancer. New or recurrent symptoms after a period of symptom relief postoperatively raise the possibility of wrap malfunction.

Other less likely but important to consider diagnoses include paraesophageal hernia and postoperative esophagogastric fistula. Notably, patients presenting with chest or epigastric pain, dysphagia, retching, anemia, and failure to pass gas should be suspected as having an incarcerated paraesophageal hernia, which is a medical emergency. These patients can be quickly diagnosed with computed tomography scan or barium swallow, and treated surgically.

**Tests for esophageal assessment**

Evaluation of esophageal symptoms should consist of a comprehensive clinical assessment, barium swallow, endoscopy with biopsies, esophageal motility, and pH/impedance monitoring.

**Barium esophagography** can be used to assess the anatomic placement and integrity of the fundoplication, particularly for excessive tightness or laxity, as well as other non-ARS-related causes, such as achalasia. As described by Hinder et al., there exist four radiographic patterns of failed fundoplication. Type I describes recurrence of the hiatal hernia due to disruption of the fundoplication. Type II and type III are visualized as a classic ‘hourglass defect’ caused by either intraoperative malpositioning or postoperative slippage of the wrap, resulting in a portion of the stomach slipping above the diaphragm or through the wrap, respectively. The latter is referred to as ‘slipped Nissen’ or intrathoracic wrap migration. It can be associated with reported symptoms of dysphagia, reflux, or both. Type IV describes paraesophageal herniation of the wrap. Common causes of such postoperative hernia are failure to identify a short esophagus and intraoperative failure to adequately dissect and reduce an existing hiatal hernia.

Once the integrity of the fundoplication has been assessed and presumed to be normal, the barium
esophagraphy may provide clues to suggest other etiologies for dysphagia or reflux symptoms. Of these alternate diagnoses, achalasia, although a rare entity, may often be confused with reflux disease. Although the gold standard for this diagnosis is manometry, achalasia can be suspected if barium esophagram shows smooth tapering in the distal esophagus (bird’s beak). In some instances of patients with eosinophilic esophagitis, the classic concentric esophageal rings, as well as complications of strictures or narrow caliber esophagus, may also be seen on barium studies. Finally, obstruction from malignancy can be appreciated as an intraluminal filling defect. It is important to note that LARS is not a cancer-preventing intervention, and cancer should always be in the differential diagnosis of patients with repeated symptoms after LARS, particularly those with Barrett’s esophagus.

Studies suggest that barium esophagraphy may play an important role in patients with refractory GERD and esophageal manometric dysmotility, a group where the efficacy of fundoplication has been controversial. A study has shown that patients who are able to clear a food bolus at contrast esophagography obtain similar symptomatic results after fundoplication as those with normal motility. Furthermore, other studies have demonstrated that delayed transit times of food bolus have high sensitivity and specificity, 80% and 95%, respectively, for postoperative dysphagia. The role of nuclear scintigraphy as a less invasive alternative to assess esophageal and gastric function in patients undergoing fundoplication remains unclear. In a cohort of patients, scintigraphic assessment of emptying scores remained unchanged in the majority of patients undergoing ARS. Although in patients with baseline abnormal preoperative transit times, esophageal and gastric emptying times improved in one-third and two-thirds of patients, respectively. Other studies have suggested an improvement in esophageal emptying for solid meals after fundoplication, whereas no improvement was appreciated after medical therapy alone. Despite these observations, emptying times did not correlate significantly with preoperative or postoperative symptoms or manometric findings. Current data do not support the use of scintigraphy in the evaluation of these patients.

Endoscopy with biopsies can identify 10–50% of abnormalities missed on a previous barium esophagram. Again, changes seen may represent failure of the fundoplication as well as other organic causes of the reported symptoms. In terms of ARS-related changes, a prospective cohort study identified five key endoscopic features consistent with failed ARS: (i) esophagitis; (ii) resistance to endoscope passage; (iii) abnormal location of wrap relative to diaphragmatic hiatus; (iv) location of squamocolumnar junction; and (v) the appearance of the wrap. These features will allow distinction between a normal post-fundoplication appearance (Fig. 3) and the abnormal (Figs 4–7). Additionally, evidence of continued acid reflux may be appreciated including esophagitis and mucosal ulcerations, such as Cameron’s lesions at the site of herniation. In contrast, an intact fundoplication is demonstrated by a snug endoscope upon retroflexion and elongated intra-abdominal portion of the LES.

Eosinophilic esophagitis may be suggested if concentric rings, exudates, plaques, and furrows are present on endoscopy in the setting of biopsies demonstrating more than 15 eosinophils per high power field. Lesions suspicious for malignancy should always be biopsied for pathological diagnosis (Fig. 8).

If the fundoplication appears intact, esophageal manometry is useful in assessing a mechanical cause of recurrent or new symptoms, especially in patients presenting with dysphagia. LES pressure can provide clues to wrap dysfunction, as the LES may be high if the wrap is too tight, or conversely, it may be low if the wrap is too loose. The integrated relaxation

![Fig. 3](image_url) Normal post-anti-reflux surgery (post-ARS) appearance Left: Nissen fundoplication revealing the tightness of the gastroesophageal (GE) junction and the length of the intra-abdominal portion of the lower esophageal sphincter. Right: Toupet fundoplication revealing tightness of the GE junction but with less length of the intra-abdominal portion of the lower esophageal sphincter.

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pressure is a key determinant of the diagnostic algorithm, as disorders will be divided into those with and without evidence of outflow obstruction at the esophagogastric junction (EGJ). The length of the wrap can also be assessed, as wraps that are too long or too narrow may cause symptoms. Analysis of esophageal motility is critical to the diagnosis of an underlying motility disorder, such as diffuse esophageal spasm, ineffective esophageal peristalsis, or achalasia. Notably, it may be difficult to distinguish wrap failure due to a too tight wrap, leading to EGJ obstruction from achalasia. Although lower esophageal pressure and sphincter strength tends to increase significantly after ARS, they remain significantly lower than controls without reflux symptoms.

Ambulatory pH/impedance monitoring is the gold standard for the evaluation of the competence of the fundoplication or esophageal stasis. If pH monitoring detects an alkaline environment in the distal esophagus, gastric pH monitoring can be used to differentiate between stasis of alkaline saliva...
and duodenogastroesophageal reflux.\textsuperscript{42,43} In patients with endoscopy-negative reflux disease, ambulatory 24-hour pH monitoring has a sensitivity and a specificity of 0–71\% and 85–100\%, respectively.\textsuperscript{44} With impedance-pH monitoring, a sensitivity and a specificity of greater than 90\% can be obtained.\textsuperscript{45} The best parameter to evaluate for and determine whether acid reflux is the cause of symptoms is the correlation between time of esophageal acid exposure and the timing of patient’s symptoms. A correlation is suggested if >75\% of the time symptoms occur within 2 minutes of reflux events.\textsuperscript{46,47}

Only in the absence of endoscopic, histological, pH, manometric, or radiographic evidence of an organic cause of symptoms can functional disorders be considered, such as functional heartburn, reflux-like dyspepsia, esophageal hypersensitivity, esophageal mechanosensitivity, and psychiatric conditions.\textsuperscript{48}

**Gastrointestinal assessment**

GI symptoms after ARS can be divided into symptoms related to (i) gastroparesis and (ii) gas bloat syndrome. The former is an impairment of gastric motility in the absence of a structural or mechanical obstruction, often associated with nausea, vomiting, and early satiety. The latter refers to the compromised ability to eliminate swallowed air by belching, leading to gas accumulation and symptoms of bloating. Gastroparesis is less commonly seen beyond the immediate postoperative period; one study reported a post-ARS prevalence at 3 months of 0.9\%.\textsuperscript{17} Of a cohort of patients undergoing revisional surgery, less than 2\% of patients had surgical indications related to symptoms of gastroparesis.\textsuperscript{17} The prevalence rates of gas bloat syndrome at 2 and 5 years are 8.7\% and 7.5\%, respectively.\textsuperscript{18} In the subset of patients undergoing revision surgery, the prevalence of atypical symptoms (including gas-related symptoms, delayed gastric emptying) accounted for only 4.6\% of the cohort.\textsuperscript{18} This demonstrates that most patients undergoing revision surgery complain of esophageal rather than GI symptoms.

Patients presenting with suspected gastroparesis should be further characterized by whether symptoms were (i) pre-existing or (ii) subsequent to the
fundoplication. The three most common causes of gastroparesis are idiopathic (33%), diabetic (24%), and post-surgical (19%).

Fundoplication, which can be complicated by vagal injury or vagotomy, accounts for 52% of iatrogenic gastroparesis. As an operative complication is only one possible etiology for delayed gastric emptying, evaluation and management should be comprehensive, and it is important to distinguish whether symptoms are, in fact, related to the procedure. Key causes of gastroparesis symptoms in the postoperative patient include medications, especially narcotics, or mechanical gastric outlet obstruction.

Patients with potential gas bloat symptoms can be subdivided into two groups: (i) those with irritable bowel syndrome and (ii) those with small intestinal bacterial overgrowth (SIBO). Of note, the particular LARS procedure performed, Toupet (270 degrees) fundoplication versus a Nissen (360 degrees) fundoplication, may influence gas bloat prevalence as patient undergoing the former suffer less gas bloat, flatulence, and postprandial fullness. Diarrhea may occur in up to 15% of post-fundoplication cases and is typically mild, low in volume, and worse after meals. It is thought to be a result of vagal injury, small bowel bacterial overgrowth, or exacerbation of underlying irritable bowel syndrome. The diarrhea resolves spontaneously, with the use of antimotility drugs, antibiotics for small bowel overgrowth, or cholestyramine.

Tests for gastrointestinal assessment
Evaluation of GI symptoms should consist of a comprehensive clinical assessment, upper GI series, endoscopy with biopsies, gastric emptying tests, and hydrogen breath tests.

Gastric emptying scintigraphy, a quantitative and non-invasive procedure, is the gold standard in the diagnosis of gastroparesis, but it carries a poor correlation between transit time and symptom severity. Delayed gastric emptying can diagnosed if gastric retention of the radiolabeled meal is >90% at 1 hour, greater than 60% at 2 hours, or greater than 10% at 4 hours.

Wireless capsule motility (SmartPill® GI Monitoring System, Given Imaging, Inc. Duluth, GA, USA) and antroduodenal manometry may be considered when there is a high clinical suspicion for an underlying gastric or intestinal motility disorder. The SmartPill utilizes pH and pressure to further characterize gastric and intestinal transit times, respectively. Intestinal motor patterns are better delineated using antro-duodenal manometry, which helps distinguish myopathic disorders (low amplitude contractions) from neuropathic disorders (poorly coordinated but normal amplitude contractions).

Extrinsic neuropathic gastroparesis, diabetic, or post-vagotomy can be suggested by the following: (i) increased frequency of migrating motor complexes during fasting; (ii) reduced frequency of distal antral contractions postprandially; and (iii) poorly developed intestinal fed pattern with return of migrating motor complex activity within 2 hours of ingestion of meal. There is a strong correlation between delayed gastric emptying and impaired postprandial antral contractility.

Hydrogen breath testing after the ingestion of 13C-lactulose may show an early rise in breath hydrogen production, suggestive of the presence of excessive amounts of bacteria in the small intestine, suggestive of SIBO.

Atypical symptoms
Atypical symptoms, including cough, sore throat, wheezing, hoarseness, and chest pain, are often reported with concurrent typical reflux symptoms. A long-term, prospective study of GERD patients demonstrated that atypical reflux symptoms, present in 92% of the cohort, improved as significantly as typical reflux symptoms after fundoplication. A randomized study has shown that both partial and total fundoplication have similar improvement in preoperative atypical symptoms. Others have reported that patients with atypical symptoms achieve symptom resolution in less than half of cases, as opposed to typical symptoms, where symptom resolution occurs in the majority of patients. Patients with atypical throat symptoms and concurrent typical reflux symptoms had symptomatic improvement after fundoplication, although this was not seen in patients with solely atypical throat symptoms. In summary, atypical symptoms seem to benefit from ARS, although the extent to which remains unclear.

MANAGEMENT
Esophageal manifestations
Dysphagia
Dysphagia is the most common indication for revisional ARS. Nearly all patients experience some mild, immediate postoperative dysphagia secondary to postoperative edema of the lower esophagus or transient esophageal hypomotility. These patients should be reassured, treated symptomatically, and put on a liquid diet, slowly progressing to solids as tolerated over 6–12 weeks postoperatively. Dysphagia that does not resolve by 3–4 months, termed persistent postoperative dysphagia (PPD), occurs in up to 12% patients after ARS. Using the diagnostic framework we have proposed, the particular management for dysphagia is focused toward the specific cause.
Reoperation is only required in a few patients presenting with PPD. In a prospective study of 233 patients, 12.4% required dilation for post-fundoplication dysphagia, and the first dilation was effective in 65% of patients. Similarly, a retrospective study showed that esophageal dilation was effective in relieving dysphagia in the majority of patients after LARS, and redo fundoplication was only indicated in cases refractory to esophageal dilation. Pneumatic dilation and wrap revision have been shown to be successful in treating dysphagia secondary to a tight or long wrap.

Reflux symptoms
Similar to management of dysphagia, the therapy focuses on whether symptoms reflect failure of the fundoplication (poor initial correction, subsequently disrupted warp) versus an intact fundoplication in the setting of another disease process (i.e. eosinophilic esophagitis). Only the former requires invasive intervention. After ARS, 39–43% of patients continued to take anti-reflux medications, and this subgroup reported up to a 95% symptom response rate. Only about half of the patients used medications due to GERD-related symptoms, and approximately half of the patients completed diagnostic testing to confirm the recurrence of GERD. These data suggest that medical management of reflux symptoms can be effective without the need for further surgical re-intervention. Reflux symptoms are the most commonly reported symptoms after ARS, and a second reoperation occurs in 1.2% of patients.

Gastrointestinal manifestations
Like postoperative esophageal symptoms, post-ARS abdominal bloating and early satiety tend to resolve over time without intervention. One study found that 94% of all reported dyspeptic symptoms in the first 3 months postoperatively resolved over the course of the first postoperative year.

Gas bloat syndrome
Most of these patients can be symptomatically managed with diet modifications, reassurance, and symptomatic treatment, such as simethicone tablets or charcoal caps. The treatment should focus on the underlying etiology. In cases of small intestinal bowel overgrowth, the management focuses on antibiotics. Options include rifaximin, amoxicillin and clavulanate plus metronidazole, trimethoprim and sulfamethoxazole plus metronidazole, or norfloxacins. If the diagnosis is irritable bowel syndrome, management should focus on dietary modifications, medications (stool softeners, laxatives, anti-diarrheal agents, antidepressants, and antispasmodic agents), or psychotherapy. Once these other etiologies have been ruled out, more invasive therapies may be employed, such as wrap revision, pyloroplasty, and subtotal gastrectomy with Roux-en-Y gastrojejunostomy.

Gastroparesis
The primary objectives in gastroparesis treatment are decreasing symptoms and improving nutrition. The severity of gastroparesis dictates management. Mild to moderate gastroparesis can be managed medically with dietary modifications, nutritional supplements, pro-motility agents, anti-emetics, and proton pump inhibitor therapy. Dietary recommendations include having three to four small, low-fiber, low-fat meals during the day and increasing the liquid to solid ratio during meals. Prokinetic agents that have been shown to improve gastric emptying are metoclopramide, domperidone, and erythromycin, but chronic use of these agents can result in tolerance and increased risk of side effects.

Treatment options for severe or refractory gastroparesis include pyloric botulinum toxin (BoTox) injection, gastric electrical stimulation (Enterra), gastro-jejunal enteral nutrition, and finally, subtotal gastrectomy with Roux-en-Y gastrojejunostomy. Endoscopic injection of BoTox into the pylorus is thought to prevent pyloric sphincter dysfunction, thus improving gastric emptying. Open-label studies performed on patients with gastroparesis suggest that BoTox treatment is successful in relieving symptoms and improving gastric emptying times. However, randomized clinical trials conducted comparing BoTox injections with placebo failed to find a significant difference in either symptom improvement or the rate of gastric emptying.

Gastric electrical stimulation is a therapeutic option for patients with refractory post-surgical gastroparesis. A recent single-surgeon study of 221 patients with severe gastroparesis demonstrated significant subjective and objective improvement up to 10 years after device placement. In comparison to a cohort of patients with idiopathic gastroparesis, post-ARS and diabetic patients experienced the greatest benefit in symptom reduction and nutritional improvement. Given the scant and conflicting data in the literature, more studies are needed in patients with postoperative gastroparesis to delineate the benefit of gastric electrical stimulation in this subset of patients. Nutrition is a key factor in patients with severe, refractory gastroparesis. A percutaneous endoscopic gastrostomy (PEJ) can be placed laparoscopically to allow access for nutrition, hydration, and medications. Additionally, a venting percutaneous endoscopic gastrostomy (PEG) tube can be inserted for gastric decompression. Evidence for the efficacy of PEJ and PEG tubes is scant, but one retrospective review of patients with refractory diabetic gastroparesis showed a 39% improvement in symptoms and a 56% improvement in nutritional...
status.77 Evidence for the efficacy of complete gastrectomy is inconsistent and this operation should not be considered, except in the most refractory cases.

Of note, once organic etiologies are ruled out, postoperative symptoms of heartburn, dysphagia, diarrhea, bloating, and nausea may be attributed to functional etiologies. These patients may benefit from treatment with tricyclic antidepressants.79 A prospective study evaluated 196 patients with quality of life surveys and manometry, and suggested a role for stress in the perceived outcome of ARS.80 For this reason, psychological services may be useful to optimize quality of life.

CONCLUSIONS

Post-fundoplication syndromes are multifaceted and require a thorough and comprehensive evaluation. Having a diagnostic framework to approach these patients is vital for management. Revisional surgery may be needed if symptoms are refractory to less invasive, conservative therapies and it should be performed by experienced foregut surgeons.

References

Nutcracker esophagus: demographic, clinical features, and esophageal tests in 115 patients

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SUMMARY. Nutcracker esophagus (NE) is a common esophageal motility disorder characterized by high amplitude peristaltic contractions in the distal esophagus. While previous studies have examined selected aspects of this condition (e.g. pathogenesis and treatment), there is a paucity of data regarding demographic and clinical features in large cohorts of patients. The aim of this study was to describe demographics, clinical features, comorbidities, time to diagnosis, source of patient referral by specialty, and medication use in a large cohort of patients with NE. We retrospectively analyzed consecutive cases of NE diagnosed from 2008–2010. The electronic medical records of these patients were reviewed, and relevant information was extracted. We identified 115 patients with NE. The median age was 62 years (range 25–87 years), and 63% were female. The median time patients experienced symptoms prior to diagnosis was 24 months (0–480 months). Most patients presented to an internal medicine consultant (42%) or to a gastroenterologist (35%). Presenting symptoms were chest pain (31%) and dysphagia (21%). Gastroesophageal reflux disease (GERD) symptoms were common: heartburn occurred in 51% of patients, 77% had a prior history of GERD, and 78% were receiving acid suppressive medications. GERD was confirmed by testing in at least 35%. Psychiatric comorbidity occurred in 24% with half the patients receiving psychotropic medications. Irritable bowel syndrome (IBS) and fibromyalgia co-existed in 15% and 12% of patients, respectively. Surprisingly, opioids were prescribed to 26% of patients. No statistically significant correlation was found between esophageal motility parameters and symptoms. In this study, NE patients were more commonly middle-aged females experiencing a considerable amount of time between symptom onset and diagnosis. Many were initially evaluated by internists for dysphagia or chest pain and had a history of GERD. Medication prescribed prior to diagnoses frequently involved acid suppression, but narcotic and psychotropic prescriptions were also commonly used. Central sensitization syndromes (fibromyalgia and IBS), psychiatric comorbidity, and reflux commonly coexisted. Our study suggests that future investigations should address the role and interaction of GERD and psychiatric disorders in NE.

KEY WORDS: comorbidity, diagnosis, electronic medical record, esophageal motility disorder, Nutcracker Esophagus.

INTRODUCTION

High-amplitude peristaltic contractions of the distal esophagus in patients with noncardiac chest pain (NCCP) were first reported by Brand and associates in 1977.1 This motility disorder was subsequently confirmed in 1979 by Benjamin et al. in seven patients with chest pain and/or dysphagia. Given the unusually high amplitude of peristaltic contractions, these authors termed this condition nutcracker esophagus (NE).2 NE is characterized by intact peristalsis with a mean pressure of ≥180 mmHg (after 10 wet deglutitions) in the distal esophagus at traditional line pressure manometry.3 It is one of the most common abnormalities found in patients with NCCP and has been reported in as many as 48% of these patients.4

Although it was first recognized over 30 years ago, NE remains a baffling condition. There is a significant amount of controversy regarding whether NE is a true esophageal motility disorder or a manometric marker in NCCP.5 The debate focuses on whether the abnormal motility found in patients with this disorder is responsible for producing symptoms of chest pain and/or dysphagia. A recent study, found
no significant correlation between the patient’s symptoms and the amplitude of esophageal peristalsis. Other investigators have also demonstrated a lack of association between high-amplitude contractions and chest pain. For instance, Richter et al. observed that while the calcium blocker nifedipine induced normalization of the high-amplitude pressures that characterize this disorder, there was no symptomatic remission after therapy. On the other hand, acid inhibition induces symptomatic improvement in patients with NE but has no impact on the motility pattern.

Other recent studies suggest that the high-amplitude peristaltic contractions are caused by abnormal autonomic innervation of the esophagus and a resultant hypercholinergic state. Yet, other investigators have reported a structural defect in the esophagus in NE, such as thickening of the muscularis propria. Mujica et al. found a stiffer esophageal wall and an increased visceral sensitivity in NE.

Until now, most of the previous studies on NE have focused on selected aspects of this disorder, such as the etiology or pathophysiology of this condition, or the efficacy of different treatment modalities, and its relation to noncardiac chest pain. There are few studies that adequately describe the NE patient population, time to diagnosis, and associated comorbidities. Therefore, the goal of this study was to provide a comprehensive description of a large cohort of patients receiving the diagnosis of NE. We specifically sought to address these patient’s demographics, clinical characteristics, comorbid conditions, time from onset of symptoms to diagnosis, and results of other esophageal tests (endoscopy, pH testing, and barium swallow).

MATERIALS AND METHODS

Population

After obtaining IRB approval, we retrospectively reviewed the esophageal motility records of patients receiving manometric testing in the motility laboratory at the Mayo Clinic in Jacksonville, Florida from the years 2008–2010. All consecutive individuals referred to our motility laboratory for esophageal symptoms typical and/or atypical (chest pain, dysphagia, and/or gastroesophageal reflux) with confirmed diagnosis of NE (defined below) were selected for the study.

The electronic medical records of these patients were reviewed to extract demographics, date of diagnosis, time elapsed from symptom onset prior to diagnosis, source of referral for evaluation (internal medicine physician, family physician, gastroenterologist, cardiologist, surgeon, other), potentially relevant past medical history, medication use at the time of evaluation, esophageal symptoms (grouped into main symptom and associated symptoms), esophageal motility testing results, endoscopy findings, and when available, results of esophageal pH testing and barium swallow tests. Psychiatric records and consultations were also verified. We entered the existence of a psychiatric diagnosis only if patients had an available formal structured consultation.

Motility testing

The studies included in this paper were performed using the traditional pressure line motility technique and prior to the implementation in our laboratory of high resolution technique. The technique used for motility testing at our institution has been previously published. Briefly, an 8-lumen, polyvinyl catheter (Arndorfer Specialties, Greendale, WI) was used. The four distal openings were 1 cm apart, and the four proximal openings were 5 cm apart. Both sets of channels were located at 90-degree angles. Each channel was perfused with 0.5 mL/min of distilled water with a system of low compliance pneumohydraulic capillary perfusion. The four distal and the four proximal transducers were connected to external transducers with output to a personal computer-based analysis system (Medtronic, Minneapolis, MN).

The patients were instructed to fast overnight and were evaluated in the supine position. The catheter was passed nasally into the stomach. After a brief adjustment period, the catheter was gradually removed in 1 cm intervals to record the lower esophageal sphincter (LES) pressure. LES pressure was measured in each of four channels during a station pull through. The measurement was taken as an average of the mid-expiratory pressure (relative to the gastric baseline) for three or more respiratory cycles. This average was taken from pressure at the highest level before the respiratory inversion point. Six wet swallows at 30-second intervals were administered to evaluate the percentage of LES relaxation and the residual LES pressure compared with the gastric baseline. The catheter was then positioned so that esophageal peristalsis could be evaluated at 3, 8, 13, and 18 cm above the LES in response to 10 5 mL water swallows. The esophageal amplitude was calculated as an average of the pressures obtained at 3, 8, 13, and 18 cm above the LES. Each contraction sequence was judged as peristaltic, non-transmitted, simultaneous, or ineffective (amplitude less than 30 mmHg in two or more transducers).

Diagnosis of NE

Patients were diagnosed with NE when the average amplitude of 10 wet swallows at the 3 cm and 8 cm transducers above the LES was ≥180 mmHg, which represented the mean two standard deviations (SD) from the normal pressures observed in healthy volunteers. We designated these patients as having...
‘Diffuse Nutcracker’. When the average pressure (10 wet swallows) was greater than or equal to two standard deviations from the mean at individual locations of the esophagus, such as at 3 cm, 8 cm, or 13 cm above the LES, we designated these patients as having ‘Segmental Nutcracker Esophagus’. Therefore, mean individual pressures of ≥199 mmHg (at 3 cm), ≥172 mmHg (at 8 cm), and ≥120 mmHg (at the 13 cm) at locations above the LES, respectively, were considered abnormal.18 We calculated the proportion of patients with each diagnosis.

**pH testing**

Our methods for both ambulatory, catheter-based pH testing, and wireless (Bravo) pH monitoring have been previously described.17,19 Specifically, ambulatory pH wire studies were performed with a 2.1-mm dual electrode antimony catheter (Medtronic, Minneapolis, MN), which was inserted through the nose. The catheter was placed so that the distal electrode monitored 5 cm above the manometrically determined LES. Intraesophageal pH was monitored by an electronic device (Medtronic, Minneapolis, MN) connected to the catheter. During the study period, the patients were instructed to perform their routine activities and observe no particular dietary restrictions. Proton pump inhibitors and H2 receptor antagonists were discontinued 7 and 3 days, respectively, before the study by the referring physician as clinically deemed appropriate. For Bravo pH testing, the pH monitoring capsule was applied in a standardized fashion in each patient. Esophagogastroduodenoscopy was performed, using titrated doses of fentanyl, meperidine, or midazolam for sedation. During the procedure, the location of the squamo-columnar junction, in terms of the distance from the incisors, was determined. Subsequently, the endoscope was removed, and the self-contained delivery system was passed trans-orally into the patient’s esophagus. The catheter was advanced until the capsule was located at a point 6 cm above the squamo-columnar junction. Suction was then applied through the suction channel to the catheter for at least 30 seconds, causing the adjacent mucosa to be drawn into the well of the capsule. pH data were obtained at 6-second intervals and transmitted via radiotelemetry to a small, pager-sized receiver worn by the patient. Patients were encouraged to go about their usual activities, including work and exercise. They were also instructed to consume their usual diet without restrictions. While showering, the patients were instructed to place the receiver on the bathroom floor or lavatory to keep the receiver as close to them as possible. At the end of the recording period (usually 48 hours), the patients returned the receivers, and the data were uploaded to a personal computer, analyzed using software provided by Medtronic and interpreted by a physician. Patients also kept a diary recording food intake, symptoms and activity, including position changes, and this information was used in the interpretation of the pH data, as during traditional pH testing.19

**Diagnosis of GERD**

Patients were classified as having a history of GERD if they complained of either heartburn, regurgitation, or both for at least two days a week the year prior to presenting for motility testing. GERD was diagnosed (objective confirmation) when there was at least erosive esophagitis (Los Angeles Grade A) identified during endoscopy, specialized Barrett’s metaplasia confirmed by pathology, an abnormal pH test, or any combination of the above. The decision to perform endoscopy and/or pH testing on or off proton pump inhibitor (PPI) medications was left at the discretion of the ordering physician given the restrospective nature of this study. pH tests were considered abnormal if any or all of the following parameters exceeded normal percent pH values: total time more than 5.5,1, upright time ≥8.1, or supine time ≥3.4.20

**Barium swallow**

We reviewed the available radiographic reports on these patients and classified these studies into groups using the following criteria: Normal test: no abnormalities observed; Abnormal – test, relationship to NE undetermined (failure of primary peristalsis). Abnormal – test, relationship to NE possible (tertiary contractions resulting in partial lumen obliteration), and other findings: Schatzki ring, hiatal hernia. Esophagograms at our center are performed and interpreted by general radiologists. The radiologists were aware of the symptom being investigated, but not the manometric results.

**Statistical analysis**

Numerical variables were summarized with the sample median, minimum, 25th percentile, 75th percentile, and maximum. Categorical variables were summarized with number and percentage. Mean distal amplitude and LES mean pressure were compared between patients with and without various symptoms using a Wilcoxon rank sum test. *P*-values of 0.05 or less were considered as statistically significant. All statistical analyses were performed using SAS (version 9.2; SAS Institute, Inc., Cary, North Carolina).

**RESULTS**

During the study period, there were 1220 studies performed in our laboratory. One hundred fifteen
(10.6%) met diagnostic criteria consistent with NE and were selected for this analysis. A summary of patient demographics and symptoms is provided in Table 1. Median age was 62 years (range: 25–87 years), and the majority of patients were female (63%). The median length of time that patients experienced esophageal symptoms prior to diagnosis of NE was 24 months (Range: 0–480 months). Eleven patients (10%) had at least one esophageal motility study prior to referral to our institution. Only one had a history of documented nutcracker esophagus with the remaining studies showing either a normal motility test or other non-achalasia spastic motility disorder. In three patients, motility results were unknown.

Patients first presented for their esophageal symptoms to the following specialties: internal medicine and its subspecialties (42%), gastroenterology (35%), cardiology (10%), surgery (9%), and other 3% (emergency department, physical medicine, and rehabilitation). The chief complaint among the patients was most commonly chest pain (31%), dysphagia (21%), GERD (17%), or multiple esophageal symptoms (20%). When patients with multiple symptoms were examined, the most common symptoms were dysphagia (55%), chest pain (53%), heartburn (51%), regurgitation (37%), and epigastric pain (34%).

The past medical history of each patient is summarized in Table 2. GERD was by far the most common patient comorbidity (77%), followed by hypertension (37%) and psychiatric comorbidities (24%). A history of IBS and fibromyalgia were reported by 15% and 12% of patients, respectively.

Patient medications at the time of diagnosis are displayed in Table 3. PPI use was noted in 70% of patients. Seventeen patients (15%) were taking an

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### Table 1 Patient demographics and symptoms

<table>
<thead>
<tr>
<th>Variable</th>
<th>Summary (n = 115)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62 (25, 50, 72, 87)</td>
</tr>
<tr>
<td>Gender (Male)</td>
<td>42 (37%)</td>
</tr>
<tr>
<td>Time with esophageal symptoms prior to diagnosis (months)</td>
<td>24 (0, 12, 55, 480)</td>
</tr>
<tr>
<td>Previous motility testing prior to diagnosis</td>
<td>11 (10%)</td>
</tr>
<tr>
<td>Department first referred to</td>
<td></td>
</tr>
<tr>
<td>Internal medicine</td>
<td>48 (42%)</td>
</tr>
<tr>
<td>Gastroenterology</td>
<td>40 (35%)</td>
</tr>
<tr>
<td>Family medicine</td>
<td>14 (12%)</td>
</tr>
<tr>
<td>Cardiology</td>
<td>11 (10%)</td>
</tr>
<tr>
<td>Surgery</td>
<td>10 (9%)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>Chief complaint</td>
<td></td>
</tr>
<tr>
<td>Chest pain</td>
<td>36 (31%)</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>24 (21%)</td>
</tr>
<tr>
<td>Epigastric pain</td>
<td>20 (17%)</td>
</tr>
<tr>
<td>Multiple chief complaints</td>
<td>23 (20%)</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>63 (55%)</td>
</tr>
<tr>
<td>Heartburn</td>
<td>61 (53%)</td>
</tr>
<tr>
<td>Regurgitation</td>
<td>43 (37%)</td>
</tr>
<tr>
<td>Epigastric pain</td>
<td>39 (34%)</td>
</tr>
<tr>
<td>Odynophagia</td>
<td>6 (5%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>30 (26%)</td>
</tr>
<tr>
<td>Belching</td>
<td>14 (12%)</td>
</tr>
<tr>
<td>Unintentional weight loss</td>
<td>15 (13%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>10 (9%)</td>
</tr>
</tbody>
</table>

The sample median (minimum, 25th percentile, 75th percentile, maximum) is given for numerical variables. Information was unavailable regarding time with esophageal symptoms prior to diagnosis (n = 9) and number of previous motility studies (n = 1).

### Table 2 Patient medical history

<table>
<thead>
<tr>
<th>Variable</th>
<th>Summary (n = 115)</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of GERD</td>
<td>88 (77%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>43 (37%)</td>
</tr>
<tr>
<td>Psychiatric comorbidities</td>
<td>27 (24%)</td>
</tr>
<tr>
<td>Depression</td>
<td>21 (18%)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>10 (9%)</td>
</tr>
<tr>
<td>Bipolar disorder</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>Somatiform disorder</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Irritable bowel syndrome</td>
<td>17 (15%)</td>
</tr>
<tr>
<td>Fibromyalgia</td>
<td>14 (12%)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>21 (18%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>19 (17%)</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>13 (11%)</td>
</tr>
<tr>
<td>COPD</td>
<td>8 (7%)</td>
</tr>
</tbody>
</table>

The sample median (minimum, 25th percentile, 75th percentile, maximum) is given for numerical variables. Information was unavailable regarding total number of psychotropic medications taken for 1 patient. CAD, coronary artery disease; HTN, hypertension; PPI, proton pump inhibitor; SSRI, selective serotonin receptor inhibitor.

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Comparison of pH testing information between patients with a normal and abnormal pH result

Table 5

<table>
<thead>
<tr>
<th>Variable</th>
<th>Summary (n = 115)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude 18 cm above LES (mmHg)</td>
<td>56 (15, 38, 90, 308)</td>
</tr>
<tr>
<td>Amplitude 13 cm above LES (mmHg)</td>
<td>90 (21, 60, 140, 244)</td>
</tr>
<tr>
<td>Amplitude 8 cm above LES (mmHg)</td>
<td>186 (73, 158, 236, 345)</td>
</tr>
<tr>
<td>Amplitude 3 cm above LES (mmHg)</td>
<td>227 (99, 198, 270, 461)</td>
</tr>
<tr>
<td>Mean distal amplitude (mmHg)</td>
<td>204 (136, 185, 237, 352)</td>
</tr>
<tr>
<td>Diffuse nutcracker esophagus</td>
<td>97 (84%)</td>
</tr>
<tr>
<td>Segmental nutcracker esophagus</td>
<td>18 (16%)</td>
</tr>
<tr>
<td>LES residual pressure (mmHg)</td>
<td>2.3 (0.0, 0.0, 5.3, 52.3)</td>
</tr>
<tr>
<td>% Relaxation</td>
<td>94 (26, 80, 100, 100)</td>
</tr>
<tr>
<td>LES mean pressure (mmHg)</td>
<td>24.0 (1.9, 16.4, 33.7, 110.8)</td>
</tr>
</tbody>
</table>

The sample median (minimum, 25th percentile, 75th percentile, maximum) is given for numerical variables. Information was unavailable regarding amplitude 18 cm above LES (n = 9), amplitude 13 cm above LES (n = 1), residual pressure (n = 7), % relaxation (n = 13), LES mean pressure (n = 6). DeMeester score (n = 2), upright pH < 4 (n = 1), and supine pH < 4 (n = 2). LES, lower esophageal sphincter.

Table 4 shows that 8% were on an H2 receptor blocker. In all, 90 patients (78%) were on some form of acid suppression therapy. Fifty percent of patients were taking psychotropic medications, with the most common reasons for the use of these medications being depression and anxiety (Table 3 provides the breakdown of the actual indications for medication use). Specifically, 36% of patients were taking antidepressants, and 35% were taking anxiolytics. Among these patients, the median number of psychotropic agents used per patient was 2 (range: 1–4). Thirty patients (26%) were taking opioid analgesics. Few patients were taking a calcium antagonist (14%) or nitrates (11%).

Esophageal motility information is provided in Table 4. ‘Diffuse’ nutcracker esophagus was observed in 97 patients (84%), with the remaining 18 patients (16%) having ‘segmental nutcracker’ esophagus. Examination of the amplitude of esophageal peristalsis and LES mean pressure found no statistically significant relationship with esophageal symptoms (all P ≥ 0.098).

Ambulatory pH testing was performed in 61 patients (53%) and 33 (54%) had an abnormal test. Table 5 displays reflux scores in patients with normal and abnormal pH results for the entire population. GERD was confirmed in 40 (35%) patients defined by either abnormal pH testing, erosive changes at endoscopy (n = 9), or Barrett’s metaplasia (n = 6) confirmed by pathology, or any combination of the above.

A summary of esophagogastrroduodenoscopy (EGD) and barium swallow information is given in Table 6. EGD was done in 100 patients (87%). Erosive changes were seen in 9% of patients, Barrett’s esophagus was observed in 6%, hiatal hernia was present in 24%, and irregular z-lines (small islands of intestinal metaplasia macroscopically suspicious for short segment intestinal metaplasia) were noted in 19%. Seventy patients (61%) had a barium swallow test.

Table 5

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal pH result (n = 27 (44%))</th>
<th>Abnormal pH result (n = 33 (54%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH testing on PPI</td>
<td>9 (33%)</td>
<td>6 (18%)</td>
</tr>
<tr>
<td>DeMeester score</td>
<td>4.6 (0.3, 2.1, 9.3, 14.1)</td>
<td>30.1 (6.1, 21.6, 41.9, 83.2)</td>
</tr>
<tr>
<td>Total pH &lt; 4</td>
<td>0.8 (0.0, 0.4, 2.1, 4.3)</td>
<td>7.3 (0.4, 5.2, 11.2, 23.6)</td>
</tr>
<tr>
<td>Upright pH &lt; 4</td>
<td>1.3 (0.0, 0.7, 2.6, 7.1)</td>
<td>9.9 (0.6, 7.5, 14.0, 25.2)</td>
</tr>
<tr>
<td>Supine pH &lt; 4</td>
<td>0.0 (0.0, 0.0, 0.3, 2.3)</td>
<td>5.4 (0.0, 0.5, 14.6, 42.1)</td>
</tr>
</tbody>
</table>

The sample median (minimum, 25th percentile, 75th percentile, maximum) is given for numerical variables. Information was unavailable regarding DeMeester score (n = 2), supine pH < 4 (n = 1).

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done. An abnormal barium swallow showing some form of dysmotility was observed in 41%; 24 patients (34%) tertiary contractions and 12 (17%) motor incoordination at fluoroscopy, 28 patients (40%) had hiatal hernia, and in 16 patients (23%) reflux was noted during fluoroscopic exam. No evidence of a ‘cork-screw’ appearing esophagus was observed in this series.

**DISCUSSION**

Over the past thirty years, research on NE has focused on selected aspects of this disorder, such as pathogenesis and treatment.7–16 In this report, we chose to address other less described areas of this condition, and in so doing, have provided a comprehensive description of a large cohort of the NE population from a single center.

We found that the majority of patients with NE were females (63%) with a mean age of 62 years. Our data are similar to two previous studies from surgical centers. Herbella et al. and Tsuboi et al.6,21 reported a female prevalence of 66% and 69% respectively; although the ages in their studies were younger than in ours (mean age of 55 years and 50, respectively). It is unclear why there are differences in age distribution between our series and those of other authors. We speculate that the older patients in our series maybe related to the large distribution of retiree population to the state of Florida (in comparison to California and Nebraska).

NE has been traditionally associated with NCCP and dysphagia.24 Our study confirms that both chest pain and dysphagia are common in NE. Chest pain was observed in 31% of those patients reporting it as chief complaint and as part of the spectrum of esophageal symptoms in 53%. Dysphagia occurred in 21% (chief complaint) and in 55% (as part of the group of symptoms). In addition, our study findings amplify the clinical spectrum of symptoms occurring in NE. We found that GERD symptoms such as heartburn (51%) and regurgitation (37%) were also common in this population. In fact, a prior history of GERD was described by 77% of our patients, and all of those (78%) were receiving an acid suppressive medication (PPI in 70%) at the time of initial consultation. Previous series from surgical centers have also found that symptoms of reflux, such as heartburn and regurgitation are common in NE ranging between 22–65%.6,21–23 These findings underscore the need to consider the diagnosis of NE among patients with GERD symptoms.

The importance of GERD in NE patients deserves additional comment. We found objective evidence of GERD in at least 40 patients (35%) by pH testing or endoscopy (minimum erosions or Barrett esophagus). The prevalence of GERD in NE is significantly higher when compared to the observed prevalence of GERD in a population survey that involved over 2000 subjects in the United States, 6% (acid regurgitation) and 18% had heartburn at least once a week or more.26 Previous studies in a smaller series of patients with NE have also noted coexisting GERD in 33–70% of patients with NE.6,21,23,25,26 Taken together, the findings of frequent GERD symptoms in 77% of our population and the objective confirmation of GERD in over one third of our cohort indicate that either GERD is a very common coincidental disorder, or it plays a role in the pathogenesis of this condition. Small therapeutic studies of acid suppression therapy in NE have yielded conflicting results.8,25,26 Larger trials need to be done to determine the impact of acid inhibition on symptom and motility resolution.

Another finding of this study was that nearly a quarter of the patients had a coexisting psychiatric diagnosis, and 50% were taking psychotropic medications. Moreover, patients who were taking psychotropic medications were frequently taking more than one of these agents concurrently. It has been shown that NE patients score significantly higher on instruments that measure somatization than healthy controls,27 they are and are more likely to be hypochondriacal and susceptible to minor illnesses. These patients are also more likely to react to psychological stress with an increase in the frequency and severity of gastrointestinal symptoms compared to healthy controls.26 It has also been shown that hypochondriasis is strongly associated with the perpetuation of pain in the absence of a significant organic disease. Central sensitization syndromes such as IBS and fibromyalgia were noted in 12–15% of our NE population. Based on the findings of our study and the literature summarized above, we speculate that neuropsychiatric factors may play a role in the development and maintenance of symptoms in NE. Moreover, due to the very high prevalence of psychotropic medication usage, even when a psychiatric diagnosis had not been established, we also suspect that the actual prevalence of psychiatric disorders in this population is much greater than we determined in the present study. This hypothesis is supported by an earlier study that found that 84% of a group of 25 patients with distal esophageal contraction abnormalities had a coexisting psychiatric diagnosis.29 It would be beneficial for future investigations to more rigorously examine the prevalence of psychiatric disorders in the NE population specifically and also to determine whether there is any correlation between the degree of psychiatric health and the severity of symptoms experienced by individuals with NE. This finding also raises the question of whether or not treatment for NE should be more focused on improving the mental health of these patients as opposed to using medication to control contraction amplitude.
Also of note, over a quarter of the study population was utilizing opioid pain medications upon diagnosis of NE, and 27% of these individuals were on more than one type of opioid analgesic. As far as we know, this finding has not previously been reported in the NE population. These observations suggest that despite the lack of published evidence for narcotic use or efficacy in NE, physicians seem to be resorting to potentially addicting medications to treat these patients, either because of an insufficient understanding of NE or the failure of more traditional therapies. We believe that studies need to be done to understand the meaning and importance of this finding and prevent potentially iatrogenic therapeutic interventions in NE.

In the present study, we found that 11 patients received esophageal motility testing prior to diagnosis, and 55% of these individuals showed no evidence of NE on this test. In the first study to assess the long-term course of the esophageal motility in NE patients, Dalton et al. found that only 23% of the study population consistently had NE on serial manometric testing. Achem et al. later found that among 15 patients with NE, only eight showed evidence of the same motility pattern on subsequent manometric testing. We also failed to identify a correlation between esophageal motility pressure abnormalities and symptoms. These results provide additional evidence of the instability of the motility pattern seen in NE and support the theory that this motility pattern is marker but not the underlying cause of NCCP.

The majority of individuals with NE (42%) first visited general internists or physicians certified in subspecialties of internal medicine for their esophageal symptoms excluding gastroenterology and cardiology. This information has not been previously described, and it emphasizes the need for primary care and internal medicine physicians to be familiar with the diagnosis of NE, the treatment of this disorder, and when to refer patients to a gastroenterologist for further evaluation.

Our study has potential limitations such as the retrospective nature of the design, the potential absence of information from medical records, the lack of systematic evaluation of all patients, including formal psychiatric testing in some patients. In addition, our study was done prior to the introduction of high-resolution manometry. It will be of great interest to determine whether this new technique sheds further light into the understanding of NE. Our study lacked a control population which would have also clearly strengthen the specificity of our observations. Despite these inherent weaknesses, this study represents one of the largest cohort studies from a single center specialized in the evaluation of esophageal disorders and offers additional insight into the characteristics of these patients.

In summary, our study of a large cohort of NE patients indicates that these patients are more commonly middle-aged females with chest pain or dysphagia. A history of GERD and prescription of acid suppressive agents is commonly reported by over 75% of these patients, and objective confirmation of GERD is noted in at least 35%; psychiatric comorbidities were prevalent with nearly one in every four patients receiving psychotropic medications. Thus, the contributing role of GERD and psychiatric comorbidity in this population requires further study. There is an average delay of 2 years to reach a diagnosis of NE. Most patients are evaluated by internal medicine subspecialists. Despite lack of published evidence, 27% of these patients were treated with potentially addicting opioid medications. The findings of our study underscore the need to educate physicians about the nature of this disorder and caution them about the potentially deleterious effects of these compounds in this setting.

References
Comparison of clinical features in patients with eosinophilic esophagitis living in an urban and rural environment

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SUMMARY. Eosinophilic esophagitis (EoE) has been associated with exposure to aeroallergens. Living in different locations (urban vs. rural) could potentially expose individuals to different environmental factors. Currently, there is limited data on the matter, and all was based on small population studies that did not exclude proton pump inhibitor (PPI)-responsive esophageal eosinophilia in their cohort. The primary aim of this study was to determine the prevalence of EoE in an urban versus rural population and compare demographic and clinical characteristics in patients that had been treated with high-dose PPI prior to diagnosis. Esophageal biopsies were obtained from a cohort of patients who presented with symptoms of dysphagia, odynophagia, globus sensation, and heartburn during a 10-year period. Only patients who had biopsies from the mid and distal esophagus with ≥20 eosinophils per high-power field while on high-dose PPI treatment during endoscopy were included. Urban population was defined as >1000 people/square mile, and rural population was defined as ≤1000 people/square mile (U.S. Census Bureau). Demographic data from each group was analyzed for age, sex, body mass index, duration of symptoms, and tobacco use. Chi-square analysis was used for frequencies with statistical significance defined as P ≤ 0.05. A total of 20,718 patients were identified and their records evaluated. From this cohort, 57 (0.28%) symptomatic patients (male/female: 39/18, mean age = 29.5 years) had biopsy-proven EoE (≥20 eosinophils/hpf) while on PPI treatment. Of those EoE patients, 29 (50.9%) reported living in rural area versus 28 (49.1%) living in the urban area. The most common medical history components included asthma (12.3%), and the most common presenting symptoms included dysphagia (50.9%), heartburn (26.3%), and nausea/vomiting (22.8%). The average duration of symptoms, body mass index, and smoking habits did not differ between the groups. Dysphagia was significantly more prevalent in the urban population (37.9% vs. 64.3% P = 0.047), while heartburn and reflux were more prevalent in the rural population (37.9% vs. 14.3 P = 0.043). Asthma was prevalent in both populations without a significant difference (P = not significant). There is no residential variation in the incidence of EoE among patients with non-PPI-responsive esophageal eosinophilia. Dysphagia was more prevalent in the urban population, while heartburn and reflux symptoms were more prevalent in the rural environment. Further exploration of environmental factors and specific allergens may help explain the varying symptoms and causes of EoE.

KEY WORDS: aeroallergen, environment, migration, PPI-responsive esophageal eosinophilia.
INTRODUCTION

Eosinophilic esophagitis (EoE) was primarily reported by Landres et al. in 1978 and since then has been increasingly recognized as one of the major etiologies for dysphagia, food impaction, and food regurgitation.1–3 Its prevalence is estimated to be 0.4% in an asymptomatic adult population.4 Among patients undergoing outpatient upper endoscopy for any indication, the prevalence of EoE varies between 5% and 16%, and is highest in patients with dysphagia.5,6 The proposed diagnostic criteria for EoE includes demonstration of ≥15 eosinophils per high-power field (HPF) in the esophageal epithelium.7 Although true pathophysiology of EoE remains unknown, the literature has attributed the main etiology of EoE to allergic and genetic causes.8–10

Many studies have investigated epidemiologic and endoscopic characteristics of the disease and suggest that EoE is more prominent in male children and young adults commonly associated with a personal and family history of allergic conditions.11–15 EoE is also reported to be more prevalent in urban areas.16 The reported geographic prevalence of EoE appears to be worldwide including the United States, Europe, Asia, and Middle East, and is increasing likely because of both increased recognition and incidence.17–25 However, there is insufficient data regarding specific factors that contribute to the development of EoE. Immune and allergic disorders such as asthma as well as exposure to Aeroallergens have been reported to be associated with the development of EoE. There is limited data exploring the effects of regional variation such as urban versus rural environment on EoE incidence. Our hypothesis was that residing in different environments may affect prevalence and demographic characteristics of EoE by potentially exposing individuals to different factors. In recent years, studies have demonstrated the effectiveness of proton pump inhibitors (PPIs) in reducing symptoms and histopathology in a subset of patients with esophageal eosinophilia and suspected EoE. Patients with a density of eosinophils <15/hpf after PPI therapy are categorized as PPI-responsive esophageal eosinophilia (PPI-REE).26,27 As a tertiary referral center and main pathology laboratory of the state of Iowa, the aim of our study was to investigate prevalence of EoE in both rural and urban non-PPI-REE cohort and compare demographic characteristics in both population groups.

MATERIALS AND METHODS

Study design

We retrospectively evaluated electronic records using the current procedural terminology code for upper endoscopies performed for patients for due to dysphagia, odynophagia, globus sensation, and heartburn at the Digestive Disease Center at the University of Iowa Hospitals Clinics from May 2003 through January 2013. The study was approved by the University of Iowa Hospitals and Clinics Institutional Review Board.

Previous studies have defined EoE as the presence of eosinophil numbers of ≥20/hpf in squamous epithelium at any of the levels, and current guidelines adapted ≥15/hpf in multiple levels as sufficient for diagnosis.28,29 This definition was adopted by our pathology laboratory, and we undertook this study from the starting point of the histopathologic finding of ≥20 eosinophils/hpf in a biopsy from esophageal squamous epithelium when we searched our patient data.

Our pathology laboratory has coded for the last decade all esophageal biopsies from the distal and mid-esophagus with ≥20 eosinophils/hpf as EoE. A single microscopic field with the highest eosinophil infiltration was used for diagnostic evaluation at ×400 power field of microscopic analysis equals 0.24 mm². The diagnosis of EoE was defined by the presence of the following histological features: (i) peak eosinophil counts ≥15–20/hpf; (ii) eosinophil microabscess; (ii) superficial layering of eosinophils; (iv) basal cell hyperplasia; (v) extracellular eosinophil granules; and (vi) subepithelial or lamina propria fibrosis. Demographic data from each group were analyzed for age at diagnosis, gender, body mass index (BMI), duration of symptoms, past medical history, presenting symptoms, tobacco use (including both current and/or former use), and use of PPI prior to endoscopy. All presenting symptoms and allergy history were obtained from the initial primary care or gastroenterology clinic note. The use of PPI therapy was based upon prescriptions in our electronic medical record or documentation of such therapy in clinic notes, and only patients treated with high-dose PPI (omeprazole 40 mg every day, or equivalent) ≥4 weeks prior to endoscopy were included in the study. Patients were excluded if there was inadequate chart information from which to extrapolate the aforementioned data. Date of diagnosis was the date of first esophageal biopsy showing EoE features.

Demographics

Patients were assigned to the urban or the rural group based on the primary address for the patient available in the electronic medical records. Rural versus urban area was defined using the U.S. Census Bureau 2010 Urban and Rural Classification System. The system defines urban population as >1000 people/square mile and defines rural population as ≤1000 people/square mile.30 We excluded patients that moved in and out of their county within 4 years prior to diagnosis. BMI categories were further divided into three groups (BMI <25, BMI 25–30, and BMI >30) at diagnosis.
Statistical analysis

Patient characteristics were analyzed as follows: continuous variable of age using t-test and analysis of variance, and categorical/frequency variables (gender, race, rural/urban locale, presenting symptoms, medical history, and BMI) with chi-square test or Fisher’s exact test in the case of having cells with small frequencies. SAS 9.3 was used for statistical analysis (2011, SAS Institute, Cary, NC, USA).

RESULTS

A total of 20,718 patients were identified and their records evaluated. From this cohort, 57 (0.28%) symptomatic patients had biopsy proven EoE (≥20 eosinophils/hpf) while on ≥4 weeks of high-dose PPI treatment. A total of 29 (50.9%) patients reported living in rural area versus 28 (49.1%) in urban area, demonstrating no significant difference in the incidence of EoE between these populations. The male: female ratio was 2:1, with 39 males (68.4%) and 18 females (31.6%). The mean age of diagnosis was 26.7 years (standard deviation = 17.2). Our population included 91.2% Caucasians. There was no significant difference between the monthly endoscopy rates and incidence of EoE among rural and urban population (Fig. 1). The average duration and treatment of symptoms prior to diagnosis did not significantly differ among the groups (Table 1). Both rural and urban adult groups did not differ in BMI (24 ± 8.2 vs. 27 ± 11.7, P = 0.271) or smoking habits (13.8% vs. 18.5%, P = 0.725, respectively). We did not find a significant difference between the mean ages at diagnosis and different BMI categories (<25, 25–30, and >30). In addition, there was no significant difference in age at diagnosis between rural and urban population (24.7 ± 20 vs. 28.7 ± 13.8, P = 0.388) (Table 1).

Dysphagia was significantly more prevalent in the urban population (37.9% vs. 64.3% P = 0.047), while heartburn and reflux were more prevalent in the rural population (37.9% vs. 14.3% P = 0.043). The most common medical history component in the rural and urban population included asthma (6.9% vs. 17.9%, P = 0.253) (Table 2). Other presenting

Table 1 Patient demographics and characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total cohort</th>
<th>Rural</th>
<th>Urban</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>57</td>
<td>29 (50.9%)</td>
<td>28 (49.1%)</td>
<td>0.631</td>
</tr>
<tr>
<td>Male</td>
<td>39 (68.4%)</td>
<td>19 (65.5%)</td>
<td>20 (71.4%)</td>
<td>0.670</td>
</tr>
<tr>
<td>Age at diagnosis, mean (SD)</td>
<td>26.7 (17.2)</td>
<td>24.7 (20.0)</td>
<td>28.7 (13.8)</td>
<td>0.388</td>
</tr>
<tr>
<td>Smokers</td>
<td>9 (15.7%)</td>
<td>4 (13.8%)</td>
<td>5 (17.9%)</td>
<td>0.725</td>
</tr>
<tr>
<td>BMI (SD)</td>
<td>25.5 (10.1)</td>
<td>24.0 (8.2)</td>
<td>27.0 (11.7)</td>
<td>0.271</td>
</tr>
<tr>
<td>Caucasian</td>
<td>52 (91.2%)</td>
<td>27 (93.1%)</td>
<td>25 (89.3%)</td>
<td>0.670</td>
</tr>
<tr>
<td>Average PPI treatment duration in months (SD)</td>
<td>19.7 (31.4)</td>
<td>20.4 (27.1)</td>
<td>18.9 (35.8)</td>
<td>0.854</td>
</tr>
</tbody>
</table>

BMI, body mass index; PPI, proton pump inhibitor; SD, standard deviation.

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symptoms like regurgitation, nausea, food impaction, and globus sensation did not differ between the groups (Table 3).

DISCUSSION

Our study shows a 0.28% prevalence of EoE in our cohort, which is less than the estimated prevalence of EoE in asymptomatic patients or with dysphagia (∼4%). In view of current data, the consensus is to include only non-PPI-REE patients in any EoE study cohort; hence, a trial of PPI therapy remains an important prerequisite to the diagnosis EoE. Our study is unique by the fact we are the first to evaluate the effect of residential variation on the incidence of a non-PPI-REE cohort.

Our study did not find any statistical difference in the incidence of EoE in a rural versus an urban environment (50.9% vs. 49.1%). Our study population encompassed a large, homogenous, mainly Caucasian (90.4%) upper Midwest cohort of patients with a low migratory profile, similar to the state of Iowa general population. According to the 2010 Census, 91.3% of the population of Iowa was Caucasian, 5.0% was of Hispanic or Latino origin, 2.9% Black or African-American, and all the rest were American Indian and Alaska Native, Asian, Native Hawaiian, and other Pacific Islander. Our study confirmed that EoE tends to occur in younger populations (median age 27) and is more frequent in men (68.2%) than women (31.8%) as seen in previous studies. However, we did not find any significant gender or age differences among the different populations.

The nature of our study merits comparison with the previous study done by Franciosi et al., which investigated sociodemographic and geographic characteristics of children with EoE. Their study included only pediatric population as opposed to adult population in our study and also showed that EoE patients were predominantly Caucasian and male. One of differences in our study was that we did not use a matched control population group. In addition, the Franciosi study demonstrated that EoE subjects were more affluent, educated, and resided in suburban areas. This is a contrast to our finding that showed no residential variation in the incidence of EoE. Furthermore, the Franciosi study noted that Caucasian race was a significant confounding variable that accounted for geographic differences among EoE subjects. However, given the homogeneity of Iowan population with above 90% of the population being Caucasian, the race was not considered to be a confounder in our study.

Another major difference from the Franciosi study is that we examined associated presenting symptoms of EoE in our cohorts (Table 3). Interestingly, dysphagia was significantly more prevalent in the urban population ($P = 0.047$), while heartburn and reflux were more prevalent in the rural population ($P = 0.043$). This difference could be possibly explained by a relative lack of access to gastroenterologists in the rural area. As heartburn and reflux are relatively less of alarming symptom compared with dysphagia, patients presenting with heartburn and reflux in the rural area are likely managed by their primary care doctors for a longer duration of time before they are referred to gastroenterologists. In contrast, patients in the urban area who have easier access to subspecialists are likely referred to gastroenterologists earlier.

Other presenting symptoms like regurgitation, nausea, and globus sensation did not defer between the groups. Food bolus impaction is more commonly seen in adult patients, and EoE is becoming a more common etiology. However, although the age of presentation is becoming younger with an increased prevalence of EoE, thus far most studies did not differentiate the groups to PPI-REE and non-PPI-REE. In our study, food bolus impaction was prevalent in only 17.5% of our population. This is low compared with previous studies, and we hypothesize that the low prevalence is due to a long average duration of PPI treatment prior to diagnosis, hence decreasing the severity of symptoms.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Medical history</th>
<th>Total</th>
<th>Rural</th>
<th>Urban</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>7 (12.3%)</td>
<td>2 (6.9%)</td>
<td>5 (17.9%)</td>
<td>0.253</td>
<td></td>
</tr>
<tr>
<td>Food allergy</td>
<td>2 (3.5%)</td>
<td>0 (0.0%)</td>
<td>2 (7.1%)</td>
<td>0.237</td>
<td></td>
</tr>
<tr>
<td>Seasonal allergy</td>
<td>1 (1.8%)</td>
<td>0 (0.0%)</td>
<td>1 (3.6%)</td>
<td>0.991</td>
<td></td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>1 (1.8%)</td>
<td>0 (0.0%)</td>
<td>1 (3.6%)</td>
<td>0.991</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Presenting symptoms</th>
<th>Total</th>
<th>Rural</th>
<th>Urban</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysphagia</td>
<td>29 (50.9%)</td>
<td>11 (37.9%)</td>
<td>18 (64.3%)</td>
<td>0.047*</td>
<td></td>
</tr>
<tr>
<td>Heartburn/reflux</td>
<td>15 (26.3%)</td>
<td>11 (37.9%)</td>
<td>4 (14.3%)</td>
<td>0.043*</td>
<td></td>
</tr>
<tr>
<td>Regurgitation</td>
<td>8 (14.0%)</td>
<td>3 (10.3%)</td>
<td>5 (17.9%)</td>
<td>0.470</td>
<td></td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>13 (22.8%)</td>
<td>8 (27.6%)</td>
<td>5 (17.9%)</td>
<td>0.382</td>
<td></td>
</tr>
<tr>
<td>Food impaction</td>
<td>10 (17.5%)</td>
<td>5 (17.2%)</td>
<td>5 (17.9%)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Globus sensation</td>
<td>5 (8.8%)</td>
<td>3 (10.3%)</td>
<td>2 (7.1%)</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

* is clinically significant, less than 0.05.
As demonstrated in previous studies, the prevalence of asthma, food, and/or seasonal allergens appears to be associated with EoE in our population as well. This supports an allergy-mediated pathophysiology for EoE. We did not find a difference in the prevalence of asthma, food allergy, seasonal allergy, or allergic rhinitis between the groups.

We evaluated BMI and prevalence of smoking in our adult population (age ≥18 years). BMI was evaluated only in the adult populations because of the fact that the weight in the pediatric population has to be adjusted to percentile scales, hence hampering any statistical analysis when combined with adult population as a uniform group. In our adult cohort, the median BMI was 25.5, defined as overweight. We did not find a significant difference between the mean ages at diagnosis and different BMI categories (<25, 25–30, and >30). Because of the fact that the average age of diagnosis was similar among all BMI subgroups, we assume that there is no correlation between obesity and the development of EoE. This finding is in contrast to gastroesophageal reflux disease (GERD), where there is a known correlation between obesity and disease development. The prevalence of smokers was the same among both populations (11.4% vs. 12.5%, P = not significant) and is lower than the average U.S. prevalence (∼19%). This fact implies that smoking probably does not attribute to development of EoE.

One weakness of our study is that we may have not captured all of the patients with EoE if they were referred to other surrounding medical centers. Another potential weakness of our study is the fact that our cohort encompassed upper gastrointestinal symptoms including globus sensation and GERD that may have influenced our relatively low prevalence. Although most of Iowa habitants reside in urban areas, the vast majority of the state is rural environment. Thus far, most of the population studies in the literature were conducted mostly on urban area populations, and contrasting incidence and prevalence of EoE in rural areas possibly could have been overlooked until now. Despite our non-PPI-REE study, we are aware of the emerging difficulties in the differentiation between true EoE and esophageal eosinophilia. Thus far, the interaction among EoE, GERD, and PPI therapy has not been totally understood. Whether these patients represent a subphenotype of GERD, EoE, or a combined mechanism of both disorders remains unknown. Currently, no histological specific features, pH esophageal monitoring, nor quantitative immuno-histochemistry for mast cells are capable to differentiate between the groups. Although current guidelines do suggest a PPI trial prior to diagnosis, there is a lack of consensus on the assumption that a positive response to PPI therapy distinguishes EoE from GERD.

The unique strength of our study is that our cohort had low migration profiles from their original childhood residential area, which stabilizes a patient’s exposure to the same allergens confined to their environment prior to and during the development of EoE. As evidence, Iowa’s net migration rate change in year 2000–2009 ranged from –2.8% to 1.2% per U.S. Bureau of the Census. Hence, there may be specific allergens that trigger EoE in distinct environments.

A complete patient’s profile assessment analyzing the sensitization to common inhalant and food allergens and patch test for food allergens demonstrated an increase of sensitization to aeroallergens, in particular ages ≥4 in patients with EoE, and a decrease of sensitization to food allergens with increasing age. We tried to differentiate zones by their agricultural products (wheat vs. corn), but could not find distinctive patterns.

Currently, we are trying to specify local allergens that lead to development of EoE of varying severities and symptoms. Further exploration of environmental factors and specific allergens may help to understand the causes of EoE. Despite our findings of lack of residential variation, we still do believe that different allergens in distinct environments play a role in predispensing patients to developing EoE of varying severity and complications, and patients may need different treatment approaches based on their living environment. These aspects of our hypothesis merit further investigation.

References


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et al

28 Spechler S J, Genta R M, Souza R F. Thoughts on the complex


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Simultaneous use of endoscopic resection and radiofrequency ablation is not safe in an esophageal porcine model

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1Department of Gastroenterology and Hepatology, St Antonius hospital, Nieuwegein and Department of 2Gastroenterology and Hepatology, 3Pathology, 4Surgery, Academic Medical Center, Amsterdam, The Netherlands

SUMMARY. Radiofrequency ablation (RFA) is safe and effective for eradication of Barrett’s esophagus after endoscopic resection (ER) of neoplasia. Widespread ER, however, is likely to induce stenosis, hampering subsequent circumferential RFA. A ‘single step’ procedure with ER and circumferential RFA in the same session may avoid this problem. Two variants are possible: circumferential RFA of Barrett’s esophagus including the lesion followed by ER of the ablated lesion (‘RFA→ER’), or ER of the lesion directly followed by circumferential RFA of remaining Barrett’s esophagus including the resection wound (‘ER→RFA’). First aim was to evaluate perforation risk of ‘ER→RFA’ using increasing RFA energies. Second aim was to compare stenosis rate after ‘ER→RFA’ versus ‘RFA→ER’. In Experiment 1, 24 areas in six pigs underwent widespread ER directly followed by circumferential RFA with increasing energies (2 x 10, 2 x 12-6 x 12 J/cm²) in the esophagus. In Experiment 2, eight pigs each had four treatment areas randomized: ‘ER→RFA’, RFA alone, ER alone, and ‘RFA→ER’. No acute perforations occurred when ablating ER wounds. Two delayed perforations occurred: one in experiment 1, another in experiment 2 at the ‘ER→RFA’ area. The remaining seven pigs in experiment 2 showed stenosis in all ‘ER→RFA’ and ‘RFA→ER’ areas versus 5/7 RFA alone areas, and 0/7 ER alone areas. In conclusion, the ‘single step’ variant ‘ER→RFA’ is not safe in this porcine model and seems therefore not ethical to evaluate in humans at this stage. Given the high rate of stenosis after ‘RFA→ER’ and RFA alone, one might question the validity of the porcine model for this type of experiments.

KEY WORDS: Barrett esophagus, esophageal neoplasm, endoscopic resection, radiofrequency ablation, swine.

INTRODUCTION

Radiofrequency ablation (RFA) has proved to be a safe and effective ablation technique for eradication of remaining Barrett’s epithelium after endoscopic resection (ER) of neoplasia.1–4

In this treatment, protocol patients undergo ER of the neoplastic lesion, followed by circumferential RFA of the remaining Barrett’s epithelium at least 6 weeks later to allow the resection wound to heal. After the first circumferential RFA, subsequent RFA sessions are scheduled every 2–3 months until complete eradication of all visible Barrett’s epithelium is achieved. Most studies on the combination of ER and RFA, however, have limited the extent of the ER to 2 cm in length and including 50% of the circumference. By limiting the extent of the resected area, the risk of developing a severe stenosis was reduced.5

Stenosis is not only a burden for the patient, but also makes circumferential RFA technically more difficult and comprises a higher risk of laceration or even perforation when performing RFA. As a consequence, Barrett’s mucosa might be left untreated, and subsequently, new neoplastic lesions may occur that are inaccessible for endoscopic therapy. Although a limited ER is possible in most patients, a minority may harbor larger visible abnormalities (i.e. longer than 2 cm in length and/or involving >50% of the circumference) that need to be resected despite the high risk of stenosis. These patients may therefore benefit from a ‘single step’ procedure in which ER
and RFA are performed in the same treatment session. In this way, problems associated with stenosis and circumferential RFA are overcome. Additional benefits of a ‘single step’ procedure might consist of a reduction in number of treatment sessions which causes less burden to the patient and reduces costs.

Two variants for a ‘single step’ procedure are possible, both having different drawbacks. One variant is ER of the neoplastic lesion directly followed by circumferential RFA of the remaining Barrett’s epithelium including the resection wound: ‘ER→RFA’. The major drawback of this variant is the direct delivery of RFA at the resection wound. As a result, the muscularis propria may be seriously damaged resulting in severe stenosis or even acute perforation.

The other variant of a ‘single step’ procedure is circumferential RFA of the whole Barrett’s segment including the neoplastic lesion followed by ER of the ablated neoplastic lesion: ‘RFA→ER’. In this sequence, delivering RFA on a resection wound is prevented. One major drawback of the ‘RFA→ER’ variant however is the increase in complexity of the subsequent ER. Because of edema and increased vulnerability of the mucosa after RFA, the endoscopic view is impaired, hampering both the recognition of the electrocoagulation marks as well as the assessment of the quality of the submucosal lifting. Another drawback of the ‘RFA→ER’ variant is the histological evaluation of the resection specimen. As the neoplastic lesion is ablated immediately before ER, coagulation artifacts may impair proper histological assessment of the lesion. In humans, ‘RFA→ER’ has shown to be feasible and relatively safe, but the procedure was found to be technically demanding despite being performed by highly experienced endoscopists.6

‘ER→RFA’ is technically easier to perform but may result in complications. Therefore, we aimed to explore the safety of ‘ER→RFA’ and compare it to the safety of ‘RFA→ER’ in a porcine model. The aim of the first experiment was to evaluate the perforation risk of ‘ER→RFA’ using supratherapeutic RFA energy applications. The aim of the second experiment was to compare the number and severity of stenoses after ‘ER→RFA’ and ‘RFA→ER’ in a porcine model.

**METHODS**

**Animal handling**

A total of 14 female pigs of 45–50 kg were included. Experiments were performed at the Animal Research Institute AMC after protocol approval by the Animal Ethical Committee at the Academic Medical Center in Amsterdam, the Netherlands. Animal care was in accordance with European Union guidelines. ER was performed either with the multiband mucosectomy technique (MBM, Duette kit, Cook Medical, Limerick, Ireland) or the cap technique with submucosal lifting (EMR Kit, Olympus Europe, Hamburg, Germany). Both techniques have been described previously in humans.7–10

**RFA system**

The RFA system and endoscopic procedure have also been described previously in humans.1–4 Circumferential RFA was performed with the balloon-based HALO360 system (BÂRRX Medical Inc., Sunnyvale, CA, USA). The balloon-based HALO360 system was chosen for several reasons. First, it is the easiest and less time-consuming method of performing circumferential RFA, and it is standard practice, especially in longer Barrett’s segments. Second, with focal devices, it is still possible to ablate the resection wound itself at the edges, therefore still being necessary to evaluate the risks of RFA on the resection wound.

**Experiment 1: short-term safety of ‘ER→RFA’**

In six pigs, a total of 24 treatment areas were marked in the esophagus: in two immediately euthanized pigs, six treatment areas, and in four surviving pigs, only three treatments areas for ethical reasons. After sizing, ER (3 cm in length and 50% of circumference) with MBM of the targeted resection area within the treatment area was performed. Subsequently, each treatment area, including the resection wound, was ablated with the balloon indicated by the previous sizing. Treatment areas were ablated with different RFA energy applications without cleaning in between (Table 1). A top-down strategy was used to evaluate if supratherapeutic RFA doses were safe (i.e. assessing safety margin). In addition, these different doses helped selecting the RFA dose for experiment 2.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>RFA energy applications and aimed survival in experiment 1</th>
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<tbody>
<tr>
<td>Pig 1</td>
<td>6; 6 x 10; 5 x 12; 4 x 12; 3 x 12; 2 x 12; 2 x 10</td>
</tr>
<tr>
<td>Pig 2</td>
<td>6; 2 x 10; 2 x 12; 3 x 12; 4 x 12; 5 x 12; 6 x 12</td>
</tr>
<tr>
<td>Pig 3</td>
<td>3; 6 x 12; 5 x 12; 4 x 12</td>
</tr>
<tr>
<td>Pig 4</td>
<td>3; 4 x 12; 5 x 12; 6 x 12</td>
</tr>
<tr>
<td>Pig 5</td>
<td>3; 4 x 12; 2 x 12; 2 x 10</td>
</tr>
<tr>
<td>Pig 6</td>
<td>3; 2 x 10; 2 x 12; 4 x 12</td>
</tr>
</tbody>
</table>

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Two pigs were immediately euthanized after the experiment; two pigs were aimed to be euthanized after 1 day and two pigs after 3 days.

Experiment 2: comparison of both ‘single step’ procedures

In each of the eight pigs, four treatment areas were marked in the esophagus: ‘ER→RFA’, RFA alone, ER alone, and ‘RFA→ER’. Randomization of the treatment areas was performed prior to any treatment and resulted in: distal ‘ER→RFA’, mid-distal RFA, mid-proximal ER, proximal ‘RFA→ER’; or: distal ‘RFA→ER’, mid-distal RFA, mid-proximal ER, proximal ‘ER→RFA’.

After sizing, ER with the cap technique of the targeted resection area was performed within the ‘ER→RFA’ and the ER alone treatment areas. Resection specimens were collected for histology. Subsequently, ‘ER→RFA’, RFA alone, and ‘RFA→ER’ treatment areas (including the ER wound of the ‘ER→RFA’ area) were ablated with the balloon indicated by the previous sizing and using the 10 J/cm²-clean-10 J/cm² regimen. Next, ER with the cap technique of the targeted area within the ‘RFA→ER’ was performed, and resection specimens were collected. At the end of the procedure, all treatment areas were marked just proximal by placing two small tattoos (SPOT, GI Supply, Camp Hill, PA, USA). All pigs were aimed to be euthanized 42 days after the experiment.

Follow-up

Pigs were euthanized with an intravenous overdose of pentobarbital after which the esophagus was harvested for histology.

Surviving pigs received a semiliquid diet and were placed on a grid for 3 days in order to prevent sawdust perforating the esophageal wounds. After 3 days, pigs progressively received a more solid diet. In case of regurgitation, i.e. stenosis, pigs were offered again a semiliquid diet that was supplemented with milk protein if additional caloric intake was necessary.

Histology

After harvesting the esophagus, the treatment areas were identified by opening the esophagus in longitudinal direction. The treatment areas were cut out and stretched on paraffin with pins.

In addition, in experiment 2, the mucosal circumference of each treatment area was measured with a ruler at the center of the treatment area and at the upper edge of the treatment area. ER specimens in experiment 2 were retrieved immediately after resection, pinned down on paraffin, and fixed in 10% formalin solution. After fixation, specimens were processed and stained with hematoxylin and eosin.

Histological evaluation was performed by a gastrointestinal expert pathologist (M.V.). Specimens were evaluated for the presence and depth of inflammation, fibrosis, and necrosis. The deepest layer with damage due to inflammation, fibrosis, and/or necrosis was recorded.

Endpoints

Primary endpoint for experiment 1 was:
1 Number of acute and delayed perforations after ‘ER→RFA’ using supratherapeutic RFA energy applications;
Secondary endpoint for experiment 1 was:
1 Depth of inflammation and/or necrosis in the esophageal wall at day 0, 1, and 3 on histology after ‘ER→RFA’ using supratherapeutic RFA applications.
Primary endpoint for experiment 2 was:
1 Number and severity of stenosis after ‘ER→RFA’, ‘RFA→ER’, RFA, and ER.
Secondary endpoints for experiment 2 were:
1 Depth of fibrosis in the esophageal wall on histology after ‘ER→RFA’, ‘RFA→ER’, RFA, and ER.
2 Depth of necrosis on histology of resection specimens after ‘ER→RFA’, ‘RFA→ER’, and ER;
3 Number of acute and delayed perforations after ‘ER→RFA’, ‘RFA→ER’, RFA, and ER.

Statistical analysis

Statistical analysis was performed with the Statistical Software Package version 16.0.2 for windows (SPSS, Chicago, IL, USA). For descriptive statistics, mean with standard deviation was used for variables with a normal distribution. One-way analysis of variance was used to compare the multiple treatment regimens in experiment 2.

RESULTS

Experiment 1: short-term safety of ‘ER→RFA’

Perforations

No acute perforations occurred when ablating the ER wounds. During the MBM procedure, prior to RFA, acute perforation occurred in 2 of the 24 treatment areas; both perforations were located in the distal esophagus. One delayed perforation occurred at the 5 x 12 J/cm² treatment area in one of the four pigs of the survival experiment.

Depth of damage in esophageal wall

Depth of damage on day 0, 1, and 3 are shown in Table 2. Although damage was present on day 0, no
inflammation or necrosis was seen. At day 1 and 3, clear inflammation and necrosis was seen. Exact location of deepest inflammation and necrosis within the treatment areas was not possible to determine.

**Experiment 2: comparison of both ‘single step’ procedures**

**Perforations and stenosis**

No acute perforations occurred. One delayed perforation occurred in one pig at the ‘ER→RFA’ treatment area causing a severe infection that required premature euthanasia at day 8. The remaining seven pigs developed severe stenosis causing regurgitation and impeding proper caloric intake, therefore being prematurely euthanized after a mean of 23 days (range 16–30 days) (Table 3). In these seven pigs, number and severity of stenosis was evaluated. All ‘ER→RFA’ and ‘RFA→ER’ showed severe stenosis at the treatment area. RFA alone treatment areas showed stenosis in five of the seven pigs. None of the ER alone treatment areas showed stenosis (Fig. 1). The severity of the stenosis based on the ratio of the circumference of the mucosa at the center and the edge of the treatment area was not significantly different between ‘ER→RFA’ and ‘RFA→ER’ (Table 4).

**Depth of damage in esophageal wall**

Inflammation and/or fibrosis was present in all treatment areas. It reached the muscularis propria in 63% (5/8) of the ER and RFA alone treatment

---

**Table 2** Perforations and damage in experiment 1

<table>
<thead>
<tr>
<th>Survival (days)</th>
<th>RFA energy dose (J/cm²) of ‘ER→RFA’ treatment areas</th>
<th>Acute perforation</th>
<th>Delayed perforation</th>
<th>Deepest layer with damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6 x 12</td>
<td>No</td>
<td>NA</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td>5 x 12</td>
<td>No</td>
<td>NA</td>
<td>Muscularis propria</td>
</tr>
<tr>
<td></td>
<td>4 x 12</td>
<td>No</td>
<td>NA</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td>3 x 12</td>
<td>No</td>
<td>NA</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td>2 x 12</td>
<td>No</td>
<td>NA</td>
<td>Muscularis propria</td>
</tr>
<tr>
<td></td>
<td>-†</td>
<td>Yes at ER</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Pig 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2 x 10</td>
<td>No</td>
<td>NA</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td>2 x 12</td>
<td>No</td>
<td>NA</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td>3 x 12</td>
<td>No</td>
<td>NA</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td>4 x 12</td>
<td>No</td>
<td>NA</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td>5 x 12</td>
<td>No</td>
<td>NA</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td>6 x 12</td>
<td>No</td>
<td>NA</td>
<td>Muscularis propria</td>
</tr>
<tr>
<td>Pig 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6 x 12</td>
<td>No</td>
<td>No</td>
<td>Transmural</td>
</tr>
<tr>
<td></td>
<td>5 x 12</td>
<td>No</td>
<td>No</td>
<td>Transmural</td>
</tr>
<tr>
<td></td>
<td>4 x 12</td>
<td>No</td>
<td>No</td>
<td>Transmural</td>
</tr>
<tr>
<td>Pig 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4 x 12</td>
<td>No</td>
<td>No</td>
<td>Transmural</td>
</tr>
<tr>
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<td>5 x 12</td>
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<td>Yes</td>
<td>Transmural</td>
</tr>
<tr>
<td></td>
<td>6 x 12</td>
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<td>No</td>
<td>Transmural</td>
</tr>
<tr>
<td>Pig 5‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4 x 12</td>
<td>No</td>
<td>No</td>
<td>Transmural</td>
</tr>
<tr>
<td></td>
<td>2 x 12</td>
<td>No</td>
<td>No</td>
<td>Muscularis propria</td>
</tr>
<tr>
<td></td>
<td>2 x 10</td>
<td>Yes at ER</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Pig 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 x 10</td>
<td>No</td>
<td>No</td>
<td>Muscularis propria</td>
</tr>
<tr>
<td></td>
<td>2 x 12</td>
<td>No</td>
<td>No</td>
<td>Muscularis propria</td>
</tr>
<tr>
<td></td>
<td>4 x 12</td>
<td>No</td>
<td>No</td>
<td>Transmural</td>
</tr>
</tbody>
</table>

†This area was not treated with 2 x 10 J/cm² as a perforation occurred during the endoscopic resection. ‡This pig aimed to survive 3 days was euthanized at day 1 due to a severe infection. ER, endoscopic resection; RFA, radiofrequency ablation; ER→RFA, single step procedure with first endoscopic resection followed by circumferential radiofrequency ablation; NA, not applicable.

<table>
<thead>
<tr>
<th>Survival (days)</th>
<th>Type of treatment per treatment area from proximal to distal</th>
<th>Reason for euthanasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig 1</td>
<td>‘RFA→ER’ / ER alone / RFA alone / ‘ER→RFA’</td>
<td>perforation</td>
</tr>
<tr>
<td>Pig 2</td>
<td>‘ER→RFA’ / ER alone / RFA alone / ‘RFA→ER’</td>
<td>stenosis</td>
</tr>
<tr>
<td>Pig 3</td>
<td>‘RFA→ER’ / ER alone / RFA alone / ‘ER→RFA’</td>
<td>stenosis</td>
</tr>
<tr>
<td>Pig 4</td>
<td>‘ER→RFA’ / ER alone / RFA alone / ‘RFA→ER’</td>
<td>stenosis</td>
</tr>
<tr>
<td>Pig 5</td>
<td>‘ER→RFA’ / ER alone / RFA alone / ‘RFA→ER’</td>
<td>stenosis</td>
</tr>
<tr>
<td>Pig 6</td>
<td>‘ER→RFA’ / ER alone / RFA alone / ‘RFA→ER’</td>
<td>stenosis</td>
</tr>
<tr>
<td>Pig 7</td>
<td>‘RFA→ER’ / ER alone / RFA alone / ‘ER→RFA’</td>
<td>stenosis</td>
</tr>
<tr>
<td>Pig 8</td>
<td>‘RFA→ER’ / ER alone / RFA alone / ‘ER→RFA’</td>
<td>stenosis</td>
</tr>
</tbody>
</table>

ER, endoscopic resection; RFA, radiofrequency ablation; ER→RFA, single step procedure with first endoscopic resection followed by circumferential radiofrequency ablation; RFA→ER, single step procedure with first circumferential radiofrequency ablation followed by endoscopic resection.

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areas and 75% (6/8) of both ‘single step’ procedures. Transmural inflammation and/or fibrosis in the muscularis propria was seen in 1/8 ER alone treatment areas and in 3/8 ‘ER→RFA’ treatment areas. Exact location of deepest inflammation and/or necrosis within the treatment areas was not possible to determine.

**Depth of damage in resection specimens**

All resection specimens of ‘RFA→ER’ were damaged: epithelium and lamina propria were missing, and in one case, the muscularis mucosae was damaged as well. In all resection specimens of ‘ER→RFA’ and ER alone, all layers were present except the muscularis propria.

### SUMMARY OF BOTH EXPERIMENTS

Table 5 summarizes all endoscopic and histological endpoints for both experiments. Acute perforations were only seen during experiment 1 when using MBM in the distal esophagus leading to the use of the cap technique in experiment 2. Delayed perforations were only seen with ‘ER→RFA’. In this porcine model, the majority of the areas treated with RFA alone or in combination with ER lead to severe stenosis. Damage in the esophageal wall reached the muscularis propria in the majority of all treatment modalities. Transmural damage was only seen after ER or ‘ER→RFA’. Damage to resection specimens reached at deepest the muscularis mucosae in one case after the ‘RFA→ER’.

### DISCUSSION

This porcine model study suggest that the ‘single step’ variant ‘ER→RFA’, i.e. ER directly followed by circumferential RFA including the resection wound, is not safe. Immediate ablation of the fresh resection wound did not cause any acute perforations, but two delayed perforations did occur several days after the procedure. These delayed perforations not only occurred in a pig treated with a supratherapeutic RFA energy dose (≥ 3 x 12 J/cm²) in experiment 1, but also in a pig treated with a low therapeutic RFA energy dose (10 J/cm²-clean-10 J/cm²) in experiment 2. These delayed perforations probably reflect the depth of the damage caused by RFA on the resection

![Fig. 1](image)

This esophagus was harvested 30 days after experiment 2 (A) and opened in length (B); on the right the proximal part, on the left the distal part. The blue markings are placed just proximal to the treatment areas: ‘ER→RFA’ (C), RFA alone (D), ER alone (E), and ‘RFA→ER’ (F). Both ‘single step’ procedures have resulted in stenosis (C and F) as well as the area only treated with RFA (D), while no stenosis is seen in the area only treated with ER (E).

<table>
<thead>
<tr>
<th>Mucosal circumference at treatment area</th>
<th>Center (cm)</th>
<th>Edge (cm)</th>
<th>Ratio center/edge</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>4.6 (± 1.8)</td>
<td>6.3 (± 1.5)</td>
<td>0.72 (± 0.15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RFA</td>
<td>3.3 (± 2.7)</td>
<td>5.8 (± 1.5)</td>
<td>0.52 (± 0.30)</td>
<td>0.013</td>
</tr>
<tr>
<td>‘ER→RFA’</td>
<td>1.4 (± 0.3)</td>
<td>5.6 (± 1.5)</td>
<td>0.27 (± 0.14)</td>
<td>0.154</td>
</tr>
<tr>
<td>‘RFA→ER’</td>
<td>1.2 (± 0.3)</td>
<td>6.6 (± 1.5)</td>
<td>0.18 (± 0.06)</td>
<td></td>
</tr>
</tbody>
</table>

*P values calculated with one-way ANOVA. ER, endoscopic resection; RFA, radiofrequency ablation; ER→RFA, single step procedure with first endoscopic resection followed by circumferential radiofrequency ablation; RFA→ER, single step procedure with first circumferential radiofrequency ablation followed by endoscopic resection.

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wound. When evaluated immediately (day 0) after the procedure the damage seemed limited, however, much deeper damage caused by necrosis and inflammation became visible after 1 day. Clear transmural damage of the muscularis propria was seen in all supertherapeutic RFA energy applications in experiment 1 and in a considerable number of the low therapeutic RFA energy dose in experiment 2.

Number and severity of stenosis of both ‘single step’ variants were compared in experiment 2. ‘ER→RFA’ as well as ‘RFA→ER’ resulted in severe stenosis in all cases. We tried to quantify the severity of stenosis by calculating the ratio of the mucosal circumference at the center of the treatment area and that at the upper end of the treatment area. No significant differences in the severity of stenosis were seen between both ‘single step’ procedures. As expected, ER alone did not result in stenosis of any treatment area as the resections were confined to 3 cm length and 50% of the circumference. Surprisingly, RFA alone resulted in stenosis in the majority of treatment areas. This in contrast to previous pioneering work on RFA in a porcine model showing no stenosis when using 9.7 or 10.6 J/cm² once, although stenosis was seen in up to 50% when using 11.5 or 13.3 J/cm² once. As we ablated twice with 10 J/cm², one might argue that this results in a higher dose; however, from studies in human esophagi, it is known that ablating twice with 10 or 12 J/cm² does not result in significantly deeper damage than ablating once.

When comparing the depth of the damage of both ‘single step’ variants, ‘ER→RFA’ resulted in transmural inflammation and fibrosis of the muscularis propria in 3/8 cases, while ‘RFA→ER’ did not result in transmural damage. Surprisingly, ER alone also resulted in transmural damage. The same observation has been made in dogs; data in humans are lacking. The damage of ‘ER→RFA’ might thus be partly explained by the effect of ER. Although RFA alone did not result in transmural damage, damage was as deep as the muscularis propria. The relatively deep damage seen after RFA in our experiments might be explained by several features. First, the mucosal layer of the porcine squamous esophagus is relatively thin compared with the human Barrett’s esophagus. Second, pigs were relative small and might therefore have had a thinner esophageal wall as well. Finally, the differences in wall architecture with a thick muscularis mucosae and almost absent submucosa in the distal esophagus and an almost absent muscularis mucosae with abundance of submucosal glands in the proximal esophagus of the porcine esophagus may also have resulted in the deep damage. All these factors may have contributed to the deep damage seen with RFA alone and might explain the high number of stenosis in our porcine model.

ER specimens of ‘RFA→ER’ showed superficial damaged because of the preceding RFA: epithelium and lamina propria were missing, and in one case, even de muscularis mucosae was damaged. Nevertheless, the majority of specimens showed no deeper damage than the muscularis mucosae. In humans, less deeper damage has been found in resection specimens after RFA which is probably a consequence of the thicker Barrett’s epithelium. As muscularis mucosae and submucosa remain present in ER specimens despite RFA, evaluating infiltration depth, differentiation grade, lymphangio invasion and therefore subsequent therapy are still adequately possible.

The major limitation of this study is that the porcine esophagus and the human esophagus have some distinct differences. Although all layers present in a human esophagus are present in the porcine esophagus, the layers are much thinner. In addition, the muscularis mucosae is almost as thick as the muscularis propria in the distal part of the porcine esophagus, while the proximal part has almost no muscularis mucosae and a very thick submucosa. The middle part of the porcine esophagus where both muscularis mucosae as well as submucosa are present seems thus the most comparable to the human situation. Because of these differences in the esophageal wall architecture, our study results cannot be completely extrapolated to the human Barrett’s situation.

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Nevertheless, our results suggest that ‘ER→RFA’ should not be performed in humans. ‘RFA→ER’ is, although less practical, likely to be safer. A series of 24 patients treated with the regimen has shown that this is a feasible approach in selected patients. The procedure is, however, technically more complicated. Although our study did not evaluate the increased complexity of the subsequent ER, subjectively, this complexity was experienced during the ‘RFA→ER’ procedures. In the future, we might benefit more from measures that can prevent stenosis formation after extensive ER, allowing the conventional treatment sequence of ER followed by ablation after healing of the resection wound. Further research focusing on prevention of stenosis formation should be encouraged.

In conclusion, in this porcine model study, the ‘single step’ procedure with ER immediately followed by circumferential ablation of the esophagus including the resection wound was associated with delayed perforation and transmural damage. Although this cannot be completely extrapolated to humans, it seems not ethical to evaluate this ‘single step’ variant further in humans. RFA of the esophagus including the neoplastic lesion immediately followed by ER of the ablated lesion, although cumbersome, seems safer and might have a place in the endoscopic treatment of patients with large neoplastic lesions in the Barrett’s esophagus.

Acknowledgments

The authors thank Nico Attevelt, Harut Avsaroglu, Sanne Hackmann, and Harry van Herck for their biotechnical assistance. The project was financially supported by an unrestricted grant from Astra Zeneca Netherlands.

DISCLOSURES

RFA catheters used in this study were provided without charge by BÂRRX medical. Snares used in this study were provided without charge by Olympus Netherlands.

References

Columnar metaplasia in the esophageal remnant after esophagectomy: a systematic review

L. J. Dunn, J. Shenfine, S. M. Griffin

Northern Oesophago-Gastric Cancer Unit, Royal Victoria Infirmary, Newcastle upon Tyne, UK

SUMMARY. Barrett’s metaplasia is a well-recognized risk factor for esophageal adenocarcinoma. It is believed to develop in response to the injurious effects of gastroesophageal reflux. Following subtotal esophagectomy and reconstruction with a gastric conduit, many patients experience profound reflux into the remnant esophagus. Barrett’s-like epithelium has been described in these patients, and they have been identified as a potential human model in which to study the early events in the development of metaplasia. This phenomenon also raises clinical concerns about the long-term fate of the esophageal remnant following surgery and the potential for further malignant change. This systematic review summarizes the literature on the prevalence and timing of Barrett’s metaplasia occurring after esophagectomy, reviews the evidence regarding risk factors and malignant progression in such patients, and considers the implications for clinical practice.

KEY WORDS: Barrett’s esophagus, esophageal remnant, esophagectomy.

INTRODUCTION

Patients who undergo esophagectomy and reconstruction with a gastric conduit are prone to reflux of both acid and duodenal contents. In addition to the impact this has on the quality of life of such patients, there is increasing recognition that this problem has pathological consequences. Barrett’s-like columnar metaplasia can occur in the remnant esophagus, and there are concerns about the long-term fate of the esophageal remnant following surgery.

The majority of patients who have undergone this type of surgery report reflux symptoms, and physiological monitoring studies have confirmed pathological levels of reflux in the esophageal remnant in over 80% of cases.1–3 The high prevalence of reflux is a consequence of the disruption of normal anatomical anti-reflux mechanisms. The lower esophageal sphincter, angle of His, and diaphragmatic sling are all resected or disrupted. Reflux is further promoted by the position of the gastric tube between the positive pressure environment of the abdominal cavity and the negative pressure of the thoracic cavity.

Many surgeons perform routine pyloroplasty that facilitates gastric emptying but has the adverse effect of promoting duodenal reflux.4

Barrett’s-like columnar metaplasia was first recognized in the esophageal remnant of post-esophagectomy patients in the 1970s.5 In recent years, larger studies have reported on this phenomenon, and interest has renewed.6,7 Endoscopic techniques have developed rapidly, and non-surgical treatment of early esophageal malignancy is expanding. A thorough understanding of the long-term fate of the esophageal remnant is essential if surgery is to be effectively compared with these newer treatment modalities.

This paper summarizes the literature on the prevalence and timing of Barrett’s metaplasia occurring after esophagectomy, reviews the evidence regarding risk factors and malignant progression in such patients, and considers the implications for clinical practice.

METHODS

The search strategy outlined in Table 1 was used to search both Medline 1946–2012 and Embase 1980–2012. The search strategy was adapted for the Web of Science and Scopus databases (Fig. 1). Bibliographies
of relevant papers were hand-searched for other relevant cited articles. The studies identified by the search were assessed for the quality of their data according to the following criteria:

- Number of patients included
- Prospective nature of study
- Requirement for histological corroboration of metaplasia
- Requirement for histological exclusion of residual metaplasia at time of surgery
- Inclusion criteria, e.g. routine follow up of all patients, investigation of research volunteers, or investigation of symptomatic patients

Studies on Barrett’s esophagus are difficult to compare as different definitions of the condition are used. The presence of specialized intestinal metaplasia (SIM) (columnar epithelium with goblet cells) is required for the diagnosis according to American definitions, but other types of columnar epithelium are included in the British definition.8,9 In the esophageal remnant following surgery, it is clear that the normal epithelial type is squamous epithelium, and any other phenotype here represents metaplasia. This review includes all types of columnar metaplasia occurring in the remnant esophagus. All types of surgery resulting in esophageal reconstruction with a gastric conduit were considered.

PREVALENCE OF POST-ESOPHAGECTOMY BARRETT’S IN THE ADULT SURGICAL POPULATION

Twelve studies were identified, which evaluated the prevalence of post-esophagectomy Barrett’s. All are from single centers; they are discussed in chronological order and summarized in Table 2.

The first study identified was published by Oberg and colleagues in 2002.3 All 60 surviving patients who had undergone surgery in the unit were invited to participate. Thirty-two underwent prospective endoscopic evaluation with 15 cases of columnar metaplasia detected (47%). The study required histological confirmation of squamous mucosa at the resection margin at the time of surgery, and areas of apparent esophagitis were routinely biopsied to exclude unrecognized metaplasia. The authors recognize that the inclusion of only a subset of patients who volunteered for investigation may give rise to a degree of bias toward those who were more symptomatic for reflux disease. They do state however that the major reason for decline of the study invitation was old age or medical comorbidities rather than the absence of symptoms.

A similar study, published the following year, included 20 of 51 identified surviving patients along with a further 20 from a cohort of 57 who underwent surgery during the study period.2 Nineteen cases of columnar metaplasia were identified (48%), including nine (23%) with SIM. As in the Oberg study, endoscopy was prospective by an endoscopist with a specialist research interest, and there was routine exclusion of residual Barrett’s and histological confirmation of metaplasia. There is the same potential selection bias toward symptomatic patients. Both studies included 24-hour acid and bilirubin monitoring in addition to endoscopy, and it is possible that asymptomatic patients were less willing to undergo this degree of monitoring.

The same year Franchimont and colleagues reported a 13.5% prevalence rate of metaplasia among a cohort of 66 patients who had undergone subtotal esophagectomy.10 This study was based on retrospective review of medical records. Patients were drawn from a group of 87 patients with 21 excluded because of missing data, no upper gastrointestinal endoscopy, or residual Barrett’s esophagus (one case). The indication for postoperative endoscopy is not stated, and it is therefore difficult to assess if there might be a selection bias. It is not clear whether the endoscopist specifically assessed for the presence of columnar metaplasia. The study employed routine biopsy sampling, but the location of biopsies was not standardized and they are described as having been taken from ‘around the esophagogastric anastomosis’. Clearly biopsies taken from below the surgical anastomosis are of no use in confirming the presence Barrett’s type metaplasia.

In 2004, four published studies assessed the prevalence of post-esophagectomy Barrett’s. The largest was a prospective series from the Dublin group.11 Consecutive patients were invited to participate; 48 were recruited with no refusals recorded. Strengths of
Table 2  Summary of published literature relating to post-esophagectomy Barrett’s

<table>
<thead>
<tr>
<th>Author, year</th>
<th>No. of patients</th>
<th>Follow-up period months, median (range)</th>
<th>Inclusion criteria</th>
<th>Histological corroboration of Barrett’s</th>
<th>Residual Barrett’s excluded</th>
<th>Incidence of CM</th>
<th>Incidence of SIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oberg et al., 2002</td>
<td>32</td>
<td>58 (36-125)</td>
<td>Research volunteers</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes 15 (47%)</td>
</tr>
<tr>
<td>Dreiser et al., 2003</td>
<td>40</td>
<td>38 (13-118)</td>
<td>Research volunteers</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes 19 (48%)</td>
</tr>
<tr>
<td>Franchimont et al., 2003</td>
<td>66</td>
<td>16 (1-39)</td>
<td>Not stated</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes 15 (23%)</td>
</tr>
<tr>
<td>O’Riordan et al., 2004</td>
<td>48</td>
<td>26 (12-67)</td>
<td>Consecutive patients in a research setting</td>
<td>Yes (in 13 of 14 patients)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes 24 (50%)</td>
</tr>
<tr>
<td>Peitz et al., 2004</td>
<td>14</td>
<td>27 (3-88)</td>
<td>Consecutive patients with clinical indication for endoscopy</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes 13 (93%)</td>
</tr>
<tr>
<td>Wolfsen et al., 2004</td>
<td>36</td>
<td>42 (7-90)*</td>
<td>Not stated</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes 8 (22%)</td>
</tr>
<tr>
<td>Lord et al., 2004</td>
<td>20</td>
<td>36 (9-504)*</td>
<td>Clinical indication for endoscopy and available tissue</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes 10 (50%)</td>
</tr>
<tr>
<td>Bax et al., 2007</td>
<td>45</td>
<td>59 (6-148)</td>
<td>Patients with clinical indication for endoscopy</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes 18 (40%)</td>
</tr>
<tr>
<td>da Rocha et al., 2008</td>
<td>101</td>
<td>Mean 126 ± 106</td>
<td>All patients</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes 36 (36%)</td>
</tr>
<tr>
<td>D’Journo et al., 2009</td>
<td>84</td>
<td>35 (1-295)</td>
<td>Not stated</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes 42 (50%)</td>
</tr>
<tr>
<td>Nishimura et al., 2010</td>
<td>100 (subgroup of 58 patients)</td>
<td>12 (24)</td>
<td>Not stated</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes 14 (14%)</td>
</tr>
<tr>
<td>Tsiouris et al., 2011</td>
<td>151</td>
<td>Average not stated (6 months to 10 years)</td>
<td>Not stated</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No 23 (40%)</td>
</tr>
<tr>
<td>Total</td>
<td>737</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>223 (38% of those assessed)</td>
</tr>
</tbody>
</table>

* Indicates that the follow up period is given only for patients diagnosed with post-esophagectomy Barrett’s.
this study include prospective evaluation by one of two experienced surgical endoscopists with routine biopsies 1–2 cm above the anastomosis. Additional biopsies were taken from suspected areas of Barrett’s and esophagitis. All resection specimens were reviewed to exclude residual Barrett’s esophagus. Interestingly, this study reports 10 patients who had histological evidence of columnar metaplasia but no associated endoscopically visible metaplasia. The reasons for this are not explored by the authors.

The other prospective study from 2004 includes only 14 patients.12 These appear to be a consecutive series of patients undergoing endoscopy for a variety of clinical indications. Again, the study benefits from histological review to exclude residual Barrett’s esophagus, and the authors describe being able to clearly identify the anastomosis during endoscopy. In one case, the patient did not undergo biopsy of a visible area of metaplasia because of concerns about bleeding. The main focus of this study was the anastomosis itself rather than the remnant esophagus above it. In all cases, the anastomosis is described as being covered by columnar epithelium, a finding confirmed histologically in the 13 patients suitable for biopsy with 10 cases of cardiac mucosa and three of oxyntic mucosa. Ten cases of endoscopically visible columnar metaplasia above the anastomosis were identified with histological confirmation in nine cases.

Two further retrospective case series were published in 2004. The first involved 36 patients who had undergone postoperative endoscopy and biopsy identified from a series of 45 patients who had undergone subtotal esophagectomy.13 Surgical specimens were reviewed to exclude residual Barrett’s metaplasia. Indications for endoscopy are not stated, and this series included a high proportion of patients who required dilatation of an anastomotic stricture during endoscopy (16/36, 44%). The concern here is that examination of the esophageal remnant may have been less meticulous if the primary aim was therapeutic dilatation. Eight cases of Barrett’s are described (18%) at a median time from surgery of 42 months, the follow-up period for those without Barrett’s is not reported.

The other retrospective series published that year involved the review of records for 100 patients who had undergone subtotal esophagectomy and gastric tube reconstruction.14 Only 20 patients with subsequent endoscopic biopsy of the esophageal remnant were identified. In 10 cases, columnar metaplasia was identified. Endoscopic follow up was not routine in this unit, and all patients were symptomatic at the time of investigation for regurgitation, dysphagia, chest pain, or weight loss, giving rise to potential selection bias as seen in many such studies. As described earlier, patients were only included if biopsies were available. The authors state that ‘biopsies were performed to conduct studies such as the present one’, but what is not clear is whether all patients undergoing postoperative endoscopy underwent biopsy sampling. This makes the true denominator for this series very difficult to determine; if biopsies were only taken when there was a suspicion of mucosal abnormality, the true denominator might be much larger and the prevalence of postoperative Barrett’s much lower.

A retrospective case series from 2007 includes 45 patients who had undergone endoscopy 6 months or more following esophagectomy and gastric tube reconstruction.15 There were 18 cases of columnar metaplasia giving a stated prevalence of 40%. All had biopsy samples available for histological confirmation. Seven cases demonstrated SIM, with no cases of dysplasia. This study has a number of weaknesses. There is no evidence that residual Barrett’s esophagus at the time of surgery was excluded. Patients primarily underwent endoscopy for dilatation of strictures or because there was a suspicion of recurrent malignancy. The level of experience of the endoscopist is not recorded, and there is no record of whether the examination included any specific assessment for the presence of columnar metaplasia. Location of biopsies and the macroscopic findings at the time of endoscopy are poorly recorded.

One of the largest published studies reports prevalence rates of post-esophagectomy Barrett’s of 11% at 5 years, 30% at 5–10 years, and 58% over 10 years.6 This study from Brazil involved a very different patient group to the others identified by this literature search. All patients underwent surgery for advanced achalasia secondary to Chaga’s disease. In addition to its size (101 patients), the study has a number of strengths. Endoscopic follow up every 2 years with multiple biopsies was routine, and the presence or absence of esophagitis or columnar metaplasia in the esophageal remnant was apparently routinely recorded. The study has the longest follow-up periods for any of its type, but it is not without some weaknesses. There is no indication that residual Barrett’s esophagus at the time of surgery was excluded, although this is unlikely given the indication for surgery. Patients with follow-up periods of up to 40 years are included. Given that this precedes the first description of post-esophagectomy Barrett’s and modern high-definition endoscopes, evaluation of patients early in this series may not be as reliable as that for later patients.

The following year the largest prospective study of metaplasia in the esophageal remnant was published.7 Eighty-four patients underwent endoscopic evaluation, 21 cases of visible columnar metaplasia were identified (25%) with 42 cases of histologically evident metaplasia (50%). The significantly higher histological prevalence of metaplasia is inadequately explained, and this degree of discrepancy is reported in only one other series.11 Significant numbers of
patients with endoscopic evidence of ulceration and erosions are reported, and whether these actually represent unrecognized columnar metaplasia is unclear. Indication for endoscopy for patients in this study is not clearly stated. The majority of patients (64%) admitted to reflux symptoms, but it is unclear if endoscopy was undertaken for this reason. Despite these issues, this study has a number of high-quality features. A meticulous examination and biopsy sampling technique is described, and residual Barrett’s esophagus was excluded by review of surgical resection specimens. The examining pathologist was blinded to the endoscopic findings, and there was subsequent correlation between endoscopic and histological findings.

A Japanese study published in 2010 predominantly considered reflux esophagitis in the esophageal remnant, but some data on columnar metaplasia is included. Data were available for 100 patients at 1 year, and a 14% prevalence of metaplasia is quoted. Fifty-eight patients had 2-year follow-up data, and 23 cases of metaplasia were identified (40%). This study is one of the largest, but the data on metaplasia are of poor quality. It is based on retrospective review of endoscopy records, 289 post-surgical patients were identified, only 100 are included in the study, and no inclusion criteria are stated. Given that the vast majority of esophageal tumors in Japan are squamous, coexisting and residual Barrett’s is extremely unlikely, but no evidence is presented to confirm this. The definition used for columnar-lined esophagus is not stated, and it is unclear if histological correlation was required.

The most recent paper identified was a retrospective review of the endoscopy records of 151 patients who had undergone surgical resection of the gastroesophageal junction. Patients were drawn from a total surgical cohort of 179 patients; indication for endoscopy is not stated. The study aimed to compare outcomes following standard surgical techniques with a new novel technique developed by the authors for concomitant fundoplication. Thirteen cases of Barrett’s in the esophageal remnant were identified, but in one case, there was evidence of residual Barrett’s esophagus when the original resection pathology was reviewed. The study used an American definition of Barrett’s esophagus, and therefore, only patients with SIM were counted. No data is presented on whether other types of metaplasia were observed or the incidence of endoscopic Barrett’s esophagus.

The present literature search identified 12 studies that assessed the prevalence of Barrett’s-type metaplasia following esophagectomy with a total of 737 patients included. The size and quality of the studies are somewhat limited. Not all studies report the presence of columnar metaplasia with and without SIM. In 586 patients, there was assessment for the presence of columnar metaplasia of any type, 223 cases were identified, giving a prevalence of 38%. In 637 patients, there was assessment for the presence of SIM; 108 cases were identified, a prevalence of 17%.

### PREVALENCE OF POSTOPERATIVE BARRETT’S IN THE PEDIATRIC SURGICAL POPULATION

Esophageal surgery in children is almost exclusively performed for benign diseases including congenital abnormalities and strictures. Surgical techniques are varied, but esophageal reconstruction with a gastric conduit is common meaning that such children experience the same propensity to reflux as adult patients who have undergone esophagectomy. Pediatric patients have a significantly longer life expectancy following surgery, and development and possible progression of Barrett’s metaplasia in these patients is of particular concern. Five studies were identified, which assessed the prevalence of Barrett’s following pediatric surgery (Table 3).

The first description of postoperative Barrett’s esophagus in a pediatric surgical population was published in 1990. Eighteen long-term survivors (>2 years) following gastric tube reconstruction were identified, 14 had undergone subsequent endoscopy. Ten patients had endoscopic evidence of Barrett’s, columnar metaplasia was confirmed on histology in eight cases, but there were no cases of intestinal metaplasia.

The largest study of children who have undergone esophageal surgery is a retrospective case series from Egypt. Surgery was undertaken for caustic

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Total patients</th>
<th>Patients who had endoscopy</th>
<th>Follow-up period</th>
<th>Endoscopic Barrett’s</th>
<th>Incidence of CM</th>
<th>Incidence of SIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lindahl et al., 1990</td>
<td>18</td>
<td>14</td>
<td>&gt;2 years</td>
<td>10</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Hamza et al., 2003</td>
<td>75</td>
<td>Not stated</td>
<td>‘Long term’</td>
<td>10</td>
<td>Not stated</td>
<td>Not stated</td>
</tr>
<tr>
<td>Borgnon et al., 2004</td>
<td>21</td>
<td>19</td>
<td>Median 2.5 years (range 1 month to 12.5 years)</td>
<td>2</td>
<td>Not stated</td>
<td>Not stated</td>
</tr>
<tr>
<td>Spitz et al., 2004</td>
<td>173</td>
<td>Not stated</td>
<td>Not stated</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Deurloo et al., 2005</td>
<td>92</td>
<td>49</td>
<td>Not stated</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

CM, columnar metaplasia; SIM, specialized intestinal metaplasia.

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strictures, and reconstruction utilized a gastric ‘pull-up’ technique. Long-term follow up of 75 patients is described with 10 cases of Barrett’s and one case of carcinoma. Duration of follow up is not stated, and the authors do not state the definition of Barrett’s esophagus used or if histological corroborated was obtained. It is unclear whether the reported case of carcinoma was of adeno or squamous type, or if there was any evidence of Barrett’s esophagus in the patient concerned.

A small case series describes 21 children who underwent esophageal replacement with an isoperistaltic gastric tube. Nineteen subsequently underwent endoscopy, and two cases of Barrett’s are reported. All patients had an anastomosis between the stomach and the cervical esophagus, but the surgery differed from that undertaken in adults as the injured esophagus was left in situ in three cases of caustic stricture. Again, the diagnostic criteria used for Barrett’s are not described, and the timing and indications for endoscopic follow up are not stated.

Spitz and colleagues state that they have encountered no cases of postoperative Barrett’s in a series of 173 children with a gastric ‘pull-up’ reconstruction, the majority of whom had surgery for esophageal atresia. The follow-up protocol for these patients is not described, and it is unclear how many of these children underwent endoscopy following surgery. This information is of critical importance given that Barrett’s esophagus can only be diagnosed or excluded by endoscopic examination.

The most recent study of children following esophageal surgery is from the Netherlands. Ninety-two potentially eligible patients were identified, and 86 questionnaires regarding reflux symptoms were returned at a median follow-up period of 17 years. Forty-nine patients underwent endoscopy, and two cases of columnar metaplasia were identified, both of gastric type. The surgical techniques are not described, and it is therefore not possible to assess if the anatomy of these patients is similar to that of adults who have undergone esophagectomy.

TIMESCALE FOR THE DEVELOPMENT OF POST-ESOPHAGECTOMY BARRETT’S

The earliest case of post-esophagectomy Barrett’s identified by the literature search occurred only 43 days after surgery. The authors describe review of the resection specimen to exclude residual Barrett’s and histological confirmation of postoperative columnar epithelium with SIM. There are several other reported cases of Barrett’s occurring less than a year after surgery but no others at this very early stage. This case is important, suggesting as it does that Barrett’s metaplasia can develop very quickly rather than being the result of years of reflux induced damage. An alternative explanation might be inadvertent sampling of the gastric conduit, but one would not routinely expect to find intestinal metaplasia within a healthy gastric conduit.

Four studies evaluated the association between time from surgery and the presence of Barrett’s-like metaplasia. Nishimura and colleagues, in their study of 100 patients, describe no cases of columnar metaplasia at 1 month, 14% prevalence at 1 year, and 40% prevalence in a subgroup of 58 patients who were followed up for 2 years. The other study which reports a significant association between the time from surgery and the presence of columnar metaplasia is that of da Rocha and colleagues. As outlined above the prevalence of columnar metaplasia in this study increased from 11% at 1–5 years to 58% for those more than 10 years post surgery.

Conversely, two studies report no significant association between time from surgery and presence of columnar metaplasia. Both were based on a single endoscopy for patients and comparison of time from surgery for those with and without columnar metaplasia. Interestingly in one study, median time from surgery for patients with columnar metaplasia was almost twice that of those with no metaplasia (39 months vs. 20 months), yet this difference failed to reach statistical significance. The concern here is that these small studies (with less than 50 patients in each) may be underpowered to detect a difference in time from surgery.

Three studies address the relationship between time from surgery and the presence of Barrett’s with SIM, all reporting a significant positive association. Dresner et al. found the time to first detection of SIM to be greater than the time to development of non-specialized cardiac metaplasia (median 27 months vs. 14 months, \(P = 0.011\)). Oberg et al. found that the median postoperative period was significantly longer in patients with SIM compared with those without (9.5 vs. 4.2 years, \(P = 0.004\)), although this study included only three patients with SIM. The third study to describe an association between time from surgery and the presence of SIM is that by da Rocha and colleagues, but no statistical data is provided to support this claim.

The studies listed earlier suggest that SIM may represent a later stage in the development of Barrett’s esophagus. Further evidence for this comes from four studies that describe progressive histological changes in individual patients. Lord et al. describes one case where biopsies from the esophageal remnant showed squamous epithelium at 15 months and cardiac type mucosa 9 months later. Unfortunately, this study was based on retrospective evaluation of biopsy material, and it is not clear how carefully the esophageal remnant was examined for the presence of metaplasia at the first endoscopy. A similar case is described by Gutschow et al. with progression from

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squamous mucosa at 8 months to SIM at 15 and 20 months, and adenocarcinoma at 28 months. More robust evidence of histological progression comes from the study by Dresner et al. In this prospective study, progression from squamous mucosa to cardiac type mucosa was demonstrated in 10 patients. In all cases, metaplasia was preceded by esophagitis. The final study that reports histological progression is that by D’Journo and colleagues. This is the only study to provide evidence that gastric type metaplasia might be a precursor to cardiac type metaplasia. The authors describe progression to cardiac type metaplasia in 4 of 12 patients who initially had gastric type metaplasia and progression to SIM in 8 of 16 patients with cardiac type metaplasia.

PREDISPONING FACTORS FOR THE DEVELOPMENT OF POSTOPERATIVE BARRETT’S

Association with preoperative histology

The most widely studied potential predisposing factor for postoperative Barrett’s esophagus is the preoperative histology. Both tumor type and the presence of preoperative Barrett’s esophagus have been assessed.

Only one study includes multivariate analysis of potential predisposing factors. This study considered gender, age, previous Barrett’s esophagus, adenocarcinoma versus squamous cell carcinoma, neo-adjuvant therapy, thoracic anastomosis versus cervical anastomosis, anastomatic complications, proton pump inhibitor (PPI), and prokinetic medication use as potential predisposing factors. Previous Barrett’s esophagus was associated with a significantly increased risk of developing postoperative Barrett’s on univariate analysis (odds ratio 2.667), but on multivariate analysis, the threshold for statistical significance was not reached ($P = 0.064$).

Other studies have assessed preoperative Barrett’s esophagus as a risk factor for postoperative Barrett’s, but all are based on univariate analysis. Oberg et al. found the prevalence of columnar metaplasia was significantly higher in patients with a preoperative diagnosis of Barrett’s esophagus compared with others (69% vs. 25%), but there were only 16 patients in each group. Two further studies found no significant association between the presence of preoperative Barrett’s esophagus and the development of postoperative Barrett’s. One involved only 14 patients and 10 cases of postoperative Barrett’s, and it could be argued that statistical comparison of groups of this size is inappropriate. The third study to evaluate this issue reported seven cases of postoperative Barrett’s in nine patients with a preoperative diagnosis of Barrett’s (77%) and 24 cases in 57 patients with no preoperative diagnosis of Barrett’s esophagus (42%), a difference that was not statistically significant. It is important to note that this study included 35 patients with esophageal adenocarcinoma, and at least 26 patients in the group with no preoperative Barrett’s therefore had disease on the Barrett’s metaplasia-dysplasia-adenocarcinoma spectrum that may be a marked confounding factor.

Three studies were identified, which evaluated association between the original tumor type and the development of postoperative Barrett’s. On univariate analysis only, and all found no statistically significant association between tumor type and postoperative Barrett’s.

Association with route of reconstruction

The type of surgical reconstruction and site of anastomosis following esophagectomy has been identified as a potentially important factor. It has been suggested that a cervical anastomosis is associated with less reflux than an anastomosis in the chest, and one might therefore expect the risk of postoperative Barrett’s to be lower in patients with a cervical anastomosis. Two studies were identified, which assessed the route of reconstruction.

D’Journo et al. compared patients who had undergone an Ivor–Lewis procedure and thoracic anastomosis ($n = 36$) with those who had undergone a three-stage procedure with cervical anastomosis ($n = 48$). On multivariate analysis, thoracic anastomosis was associated with a significantly greater risk of developing postoperative Barrett’s (odds ratio 3.05, $P = 0.018$). The second study to consider the route of reconstruction as a risk factor was that undertaken by Nishimura and colleagues. This study compared the subcutaneous, retrosternal and posterior mediastinal routes of reconstruction, but all patients are described as having a cervical esophagogastrotomy and the surgical methods are not described in detail. No significant difference in the prevalence of Barrett’s related to the route of reconstruction was detected.

ASSOCIATION WITH PYLOROPLASTY

Esophagectomy with vagotomy leaves a denervated stomach and a dysfunctional pylorus. Concomitant pyloroplasty aims to facilitate gastric emptying and may reduce volume reflux, but it has the potential to allow duodenal contents to enter the stomach and consequently reflux into the esophageal remnant. There is some evidence that mixed acid and bile reflux are more damaging than pure gastric reflux alone. A retrospective study by Palms et al. compared outcomes for patients who had undergone esophagectomy with or without pyloric drainage. Esophagitis was a more common endoscopic finding at 1 year in those who had undergone pyloric drainage, but whether this might translate into more cases of

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Barrett’s in the long term is not clear. No studies were identified which assessed pyloroplasty as a risk factor for post-esophagectomy Barrett’s.

ASSOCIATION WITH GASTRIC ACID SECRETION AND PPI USE

Preoperative and postoperative gastric acid secretion has been identified as a potential influence on the development of post-esophagectomy Barrett’s. A single study that addressed this issue was identified.³⁷ Thirty-eight patients with end-stage achalasia underwent presurgical and post-surgical measurement of basal and stimulated gastric acid secretion. Patients who developed postoperative Barrett’s (n = 14) had significantly higher levels of gastric acid secretion compared with those who did not develop Barrett’s (n = 24). Postoperative gastric acid secretion was also higher in the group that developed Barrett’s but not significantly so.

The use of PPIs is common following esophagectomy. They have been found to reduce the risk of reflux symptoms, but there is very limited evidence with regards to their role in the prevention of post-operative Barrett’s.²⁷ Both D’Journo and colleagues, and Franchimont and colleagues assessed PPI use as a potential protective factor against the development of Barrett’s.⁷,¹⁰ No significant association was demonstrated in either case, but numbers of patients were small and indications for PPI use were not stated. It is clear that PPI use cannot prevent all cases of post-esophagectomy Barrett’s, but there are no randomized trials to assess if they reduce the risk.

MALIGNANT PROGRESSION IN POSTOPERATIVE BARRETT’S

The importance of Barrett’s esophagus lies in its association with esophageal adenocarcinoma. Should postoperative Barrett’s have the same association, it would have clinical implications for the patients involved.

Five papers were identified, which described cases of dysplasia and adenocarcinoma arising within the esophageal remnant following subtotal esophagectomy. Two cases occurred more than 40 years after surgery for benign stricture in childhood. In one case, progression was demonstrated from dysplastic Barrett’s through to invasive adenocarcinoma.²⁹ In the second case, intramucosal adenocarcinoma was present within the area of postoperative Barrett’s at the time of diagnosis.¹⁴

Three further papers describe malignant progression in Barrett’s following surgery in adults. da Rocha et al. describes two cases of high-grade dysplasia (HGD) occurring 13 and 19 years following surgery.⁶ Both patients were followed up and went on to develop in situ adenocarcinoma over periods of 1 and 3 years, respectively. This was managed with endoscopic mucosal resection. A detailed case report describes post-esophagectomy Barrett’s occurring 15 months after subtotal esophagectomy for a Barrett’s adenocarcinoma with progression to adenocarcinoma by 28 months.²³ The original proximal resection margin was free from metaplasia, effectively excluding the possibility of residual Barrett’s or carcinoma.

Of most concern to clinicians is the study by Wolfsen and colleagues that reports an exceptionally high incidence of dysplasia and adenocarcinoma in post-esophagectomy Barrett’s.¹³ This study included forty-five patients who had undergone esophagectomy for Barrett’s dysplasia or adenocarcinoma. The maximum follow-up period was 9 years. Three cases of postoperative Barrett’s with low-grade dysplasia (LGD) were identified, one with HGD, and two in association with invasive adenocarcinoma. The proximal resection margin is described as being composed of squamous epithelium in all cases. Mean time to the diagnosis of LGD was 44 months, to HGD was 88 months, and to adenocarcinoma was 13 months. There is clearly a marked discrepancy between the findings in this study and others that have reported outcomes following esophagectomy. No explanation for this is offered by the authors, and there is no obvious reason why this cohort should differ from the others reported in the literature. Surgical methods were similar to those in other studies, and PPIs are described as being routinely prescribed. Patients were drawn from a North American population, and the only marked difference between this and other series appears to be the inclusion solely of patients who underwent surgery for adenocarcinoma or dysplastic Barrett’s esophagus.

One case report of adenocarcinoma occurring after pediatric surgery was identified.³⁰ The report describes a 46-year-old female who developed adenocarcinoma on a background of extensive Barrett’s esophagus at the age of 46 following surgery for tracheo-esophageal fistula as an infant. Surgical details are not described, and it is not possible to determine if this represents a situation analogous to that occurring after subtotal esophagectomy. Gastric conduits may be used in the repair of tracheoesophageal fistula, particularly where this is associated with long-gap esophageal atresia, but in other cases, primary closure is the norm.³¹

SUMMARY AND IMPLICATIONS FOR CLINICAL PRACTICE

There is clear evidence that Barrett’s-type metaplasia occurs in a significant proportion of patients following subtotal esophagectomy and reconstruction with a gastric conduit. Such patients experience profound reflux, and this is likely to be the underlying etiology.
Columnar epithelium both with and without SIM occurs, and there is some suggestion that SIM represents a later stage of development. Postoperative metaplasia can occur in patients who have no previous history of Barrett’s esophagus or esophageal adenocarcinoma, and it can also occur in children and young adults who have undergone esophageal surgery and reconstruction with a gastric tube.

Data on the time to develop postoperative Barrett’s are inconsistent and tend to come from small studies that makes statistical analysis difficult. There are few reported cases occurring before 1 year.

Risk factors for postoperative Barrett’s esophagus are poorly defined. Most studies are too small for good-quality statistical analysis, and only one study has employed multivariate analysis. There appears to be a trend toward increased risk in patients with a preoperative diagnosis of Barrett’s esophagus, but statistical proof is lacking. Data from a single study suggest that a cervical anastomosis might be protective against reflux and Barrett’s esophagus. Evidence on the effect of pyloroplasty and postoperative PPI use is extremely limited.

Seven cases of adenocarcinoma arising within postoperative Barrett’s were identified by this literature search with a further four cases of dysplasia. One case of adenocarcinoma following surgery for tracheoesophageal fistula was also identified. There is low-grade evidence from case reports to show malignant progression that indicates that the metaplasia-dysplasia-adenocarcinoma sequence seen in sporadic Barrett’s esophagus can occur in the context of postoperative Barrett’s. The risk of malignant progression appears to be small, but given the lack of large scale, high-quality studies, it is not possible to accurately define this risk.

The high prevalence of Barrett’s metaplasia following esophageal reconstruction with a gastric conduit has led to some authors recommending routine endoscopic surveillance of such patients. Surgical or endoscopic resection of malignancy occurring within post-esophagectomy Barrett’s is possible and has been described by two groups. This provides some clinical justification for endoscopic follow up of patients, but it appears that the numbers of patients likely to benefit are extremely small. There is insufficient data to provide evidence-based recommendations for follow up.

Very few cases of malignant progression of post-esophagectomy Barrett’s are reported. Where these have occurred, there is frequently a delay of many years between surgery and malignancy. The vast majority of patients who undergo esophagectomy do so in later life for malignancy. There is little evidence to suggest that malignant progression of post-esophagectomy Barrett’s represents a significant clinical problem for these individuals. Specific patient groups including those undergoing surgery in childhood and early adulthood might be at higher risk because of their potential long-term survival, and it is these individuals who would be most likely to benefit from ongoing endoscopic surveillance.

Management of patients with post-esophagectomy Barrett’s

There are no national guidelines for the management of post-esophagectomy Barrett’s esophagus, and there is insufficient data to provide evidence-based recommendations. It seems sensible to suggest similar management to that for sporadic Barrett’s esophagus where surveillance intervals of 3–5 years are recommended. In our unit, we follow UK guidelines for surveillance every 2 years, although we consider a first repeat endoscopy 1 year after diagnosis where inflammation or food residue makes assessment difficult or where there is particular patient or clinician anxiety.

PPIs may reduce the risk of malignant progression in Barrett’s, but the evidence for this is largely indirect. These drugs do provide good control of reflux symptoms and promote healing of esophagitis, and it seems reasonable to support their routine use in patients with Barrett’s following esophagectomy. In the authors’ unit, all patients are prescribed PPI medication following esophagectomy.

The role of radiofrequency ablation in non-dysplastic Barrett’s is much debated, but it is not recommended outside of a research setting. There is no evidence to support radiofrequency ablation of non-dysplastic post-esophagectomy Barrett’s esophagus, and we are unaware of any units with experience of this. For the small numbers of patients who go on to develop HGD, this may be an option, and endoscopic mucosal resection has been successfully undertaken by one group.

Acknowledgments

The authors wish to thank Linda Errington, senior assistant librarian at the Walton Library, University of Newcastle upon Tyne for advice on the literature search strategy.

AUTHOR CONTRIBUTIONS

S M Griffin conceived the project. L J Dunn performed the literature search and wrote the manuscript. S M Griffin and J Shenfine were involved in revision of the manuscript.

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Practical approach to implementing dietary therapy in adults with eosinophilic esophagitis: the Chicago experience

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SUMMARY. Eosinophilic esophagitis (EoE) is a chronic immune/antigen-mediated esophageal disease characterized by esophageal dysfunction and esophageal mucosal eosinophilia. Diet therapy is effective in the treatment of EoE in both children and adults. The role of food allergens is well established in the pathogenesis and treatment of eosinophilic esophagitis. Empiric elimination with a six-food elimination diet (avoiding milk, wheat, egg, soy, peanuts/tree nuts, and fish/shellfish) demonstrates remission in over 70% of adults with this disease. Dietary therapy in adult EoE is becoming more accepted by both patients and clinicians. Dietary therapy can be effectively implemented in clinical practice with appropriate dietary education, patient resources, and close communication with physician and clinical staff.

The ability to identify specific food triggers to help tailor dietary therapy for long-term management allows for a return to consumption of most table foods. Furthermore, the diet approach avoids the need for chronic topical corticosteroid use and possible long-term side effects of these medications. The decision to proceed with dietary therapy should be decided by patient preference and available resources. A collaborative and multidisciplinary approach including gastroenterologists, allergists, nurses, and dietitians is essential in the success of this approach.

KEY WORDS: dietary therapy, dysphagia, eosinophilic esophagitis, nutrition assessment.

ABBREVIATIONS: EoE, eosinophilic esophagitis; GERD, gastroesophageal reflux disease; PPI, proton pump inhibitor; SFED, six-food elimination diet

INTRODUCTION

Eosinophilic esophagitis (EoE) is a common cause of food impaction and dysphagia in adults.1-3 Recent consensus guidelines define EoE as a chronic, immune/antigen-mediated esophageal disease characterized clinically by symptoms related to esophageal dysfunction and histologically by eosinophil-predominant inflammation.1,3 The concept of food allergens as the main antigenic trigger in EoE was introduced in a landmark study by Kelly and Sampson in pediatric patients with symptoms of gastroesophageal reflux disease (GERD) and histologic features of esophageal eosinophilia, both of which were unresponsive to acid suppression or fundoplication.

After treatment with an elemental or amino acid-based formula, both symptoms and histologic eosinophilia resolved.4,5 In this series, symptom recurrence occurred after open food challenges, further supporting a link between food allergens and EoE. Common food triggers in pediatric patients with EoE were found to be cow’s milk, soy, wheat, peanut, and egg.4,5 This experience of dietary therapy in EoE has been replicated in subsequent, larger pediatric series.6-10

Since these initial studies, three approaches to dietary elimination in EoE patients have evolved. The first is total elimination of all food allergens by placing patients on an elemental diet. Elemental diet (ED) has been shown to be the most effective at controlling histologic eosinophilia, there are many practical limitations to this approach.6 Children often require the use of feeding gastrostomy...
DIETS IN ADULTS
EFFECTIVENESS OF EMPIRIC ELIMINATION DIETS IN ADULTS

Although dietary therapy has long been utilized in pediatrics as first-line therapy for EoE, this approach has not been fully embraced in adults. Recently, empiric dietary elimination has been shown to be as effective in adults. Gonsalves et al. prospectively studied the efficacy of the SFED in 50 adults (25M/25F) with EoE. After 6 weeks of SFED, 70% of patients had histologic response of <10 eos/hpf, 94% had symptomatic improvement, and 74% had endoscopic improvement (see Table 1). In patients who responded, serial food reintroduction was undertaken and when the trigger food was identified, symptoms typically recurred within 5 days, and esophageal eosinophil counts returned to pretreatment values (P < 0.0001) on follow-up endoscopy. This reintroduction process identified the common food allergens as wheat (60%), milk (50%), soy (10%), nuts (10%), and egg (5%). Allergy testing with skin-prick testing was undertaken prior to the elimination diet but was predictive of food triggers in only 13% of cases. Another recent study from Spain demonstrated similar results in 67 adults with EoE after empiric elimination of wheat, rice, corn, legumes, peanuts, soy, egg, milk, fish, and shellfish resulting in histologic resolution of <15 eos/hpf in 73% of patients. Food reintroduction identified the common triggers as milk (61%), wheat (28%), eggs (26%), and legumes (23%). Results of allergy testing in this cohort of patients was also not predictive of their food trigger. This group also found that continued elimination of these food triggers was effective in maintaining remission.

ROLE OF PROTEIN REACTIVITY IN FOOD ALLERGY

From the field of food allergy, we now appreciate that food-triggered immune responses can occur through both immunoglobulin E (IgE)-mediated or non-IgE mediated processes, or even a combination of both. As a result, the mechanisms driving pathology in different patients may likely be quite different. However, the key trigger in both cases seems to be exposure to the food allergen. The questions of why

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some foods, as well as only specific proteins within these foods, are allergic triggers while so many others are not remains unclear. In the USA, studies have shown that the major food allergens are present in milk, egg, peanut, tree nuts, soy, wheat, fish, and shellfish. However, it is important to consider that more than 170 different foods have been described as causing IgE-associated food allergic reactions. In general, food allergens possess similarities in structure or functions that have allowed them to be grouped into families. For animal-derived food allergens, there are three: caseins (present in milk), tropomyosin (common in shellfish), and EF-hand proteins (common in fish). For plant allergens, there are four identified families: the Bet v 1 superfamily (common in fruits, vegetables, and soy), the Cupin superfamily (present in peanut, tree nuts, seeds, and other legumes), the Cysteine protease C1 family (seen in soy), the profilins (common in fruits, vegetables, and legumes), and the prolamin superfamily (present in cereals, fruits, vegetables, grains, peanuts, and tree nuts). Immune recognition of these proteins is likely due to lack of similarity to any human proteins, as it has been shown that foreign proteins with greater than 62% homology to self-proteins are unlikely to be allergenic. However, in addition to just protein sequence, differences in glycosylation and carbohydrate attachment to these proteins may also provide a driving influence on allergic sensitization.

Although allergic reactions are generally highly specific, due to the nature of antibody recognition of the triggering protein, in the case of some allergens, a phenomena called ‘cross-reactivity’ is sometimes observed. Cross-reactivity results when an antibody against one allergen is able to recognize sequences on a different allergen and trigger allergic responses. This is common for certain food allergens, particularly within the fish and tree nut categories. Importantly, these cross-reactivity responses can also straddle the lines between food and aeroallergens, and so an individual with an aero-allergy may be triggered by exposure to a cross-reactive food and vice versa. The role of aero-allergens in EoE deserves further investigation.

ADVANTAGES OF DIET THERAPY OVER MEDICAL THERAPY

Knowing that dietary therapy is also effective in adults provides the rationale to using this treatment approach as an alternative to swallowed topical corticosteroids. When discussing options for therapy with your patients, it is important to review the pros and cons of each treatment plan and try to identify goals of treatment with your patient. It is important to underscore that the goals of dietary therapy are not to stay on the ED or SFED indefinitely, but rather to undergo a process of food trigger identification to help tailor the diet for long-term maintenance therapy, if the diet were to work. Dietary elimination with food reintroduction has the ability to identify the actual triggers of the disease. This allows patients to have more control in their ability to alter their disease course, which in our experience has been important for many patients.

Dietary therapy in adult EoE has some practical advantages. As is commonly known, discontinuation of swallowed topical corticosteroids in the treatment of EoE can cause recurrence of patients’ histologic inflammation as well as symptoms, which often may necessitate chronic daily therapy. Avoidance of food allergens eliminates the need for chronic medication/corticosteroids to help control the disease. Rare side effects of topical corticosteroids include oral and esophageal candidiasis, osteopenia/osteoporosis, growth failure in children, cataracts, and adrenal suppression. Currently, these medications have not been FDA approved to be swallowed, which raises some concern about the knowledge of long-term safety with chronic use. These, as well as the cost of the long-term pharmacologic treatment, have been raised as concerns in young patients facing this chronic condition.

Early models explaining mechanisms involved in EoE pathogenesis support the presence of aeroallergens, food allergens, and skin sensitization in driving eotaxin-3-associated eosinophils into esophageal mucosa. Dietary therapy and topical glucocorticoids improve microscopic features of EoE but through different mechanisms. Dietary therapy, however, offers the advantage of getting to the root cause of the disease, i.e. food allergen avoidance rather than symptom and histologic control. As current guidelines suggest continued avoidance of food allergens in patients who are managed with diet therapy, targeted and individualized nutrition therapy is essential to success. Another key component to the successful use of dietary therapy is collaboration with a dietitian to assure proper patient education, nutritional assessment, and avoidance of cross-contamination. Our goal with this document is to help guide the clinician in implementing dietary therapy in an adult practice. The decision to use medical or dietary therapy should be individualized based on patient preference as well as available resources. Implementing diet therapy appropriately is essential and is discussed in the next section.

IMPLEMENTING SFED IN CLINICAL PRACTICE: THE CHICAGO EXPERIENCE

In patients who have met the diagnostic criteria for EoE as per current clinical guidelines, we offer dietary elimination as an alternative to topical
corticosteroids. A surprising number of adult patients choose dietary therapy as their first-line choice of treatment due to their desire to elucidate the relationship between food allergies and disease activity. It is important to note that during the initial endoscopic assessment of patients with suspected EoE, a strict biopsy protocol can be helpful in order to maximize sensitivity of achieving this diagnosis. For the initial endoscopic assessment as well as subsequent assessments, due to the patchy nature of EoE, it is important to obtain multiple biopsies throughout the esophagus at multiple levels.19 Our protocol has been to obtain at least four biopsies from each the distal and proximal esophagus while also trying to target areas of visual abnormalities in order to maximize sensitivity. Distal location

**Fig. 1** Suggested algorithm for management of eosinophilic esophagitis.

**Fig. 2** SFED dietary therapy: food reintroduction algorithm following successful response to SFED.

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has been defined as 5 cm above the squamocolumnar junction and proximal as 15 cm above the squamocolumnar junction.

Once a firm diagnosis of EoE has been established, discussions about available treatment options including dietary therapy are undertaken. Many factors are taken into account prior to embarking on dietary therapy including a candid discussion between patients and their physician regarding their goals of treatment and their assessment on being able to adhere to the diet. Patients previously diagnosed and treated with topical corticosteroids for EoE who are interested in transitioning to a dietary approach will have to discontinue the topical corticosteroids for at least 8 weeks prior to implementing SFED to assure that there is no residual effect from the prior therapy. Patients are usually continued on existing acid suppression, but recent characterization of PPI responsive esophageal eosinophilia needs to be considered. Patients may pursue an evaluation by an allergist to help control and identify other environmental allergens and assist with management of other atopic conditions. After the 6 weeks of the elimination diet, an upper endoscopy with biopsy is undertaken to assess treatment response, and the same biopsy protocol is used for diagnosis is completed. If treatment is successful, systematic food reintroduction is undertaken to help with food trigger reintroduction. See Figures 1 and 2 for disease treatment algorithm and dietary reintroduction timeline. The next section will serve as a guide for providing patients on SFED both nutritional balanced and allergen limited food options for meal planning. Recent labeling policies as well as improved consumer access to healthy, allergy-free foods allows for improved likelihood of success on SFED. Healthy, allergy-free substitutions can be found in appendix materials along with shopping guides, meal ideas, and answers to common diet questions.

WHEAT AVOIDANCE

Wheat was found to be a leading food trigger in two adult dietary studies. In EoE, wheat-free diets should be expanded to include gluten free where barley, rye, and conventionally produced oats would be excluded. Although it is unknown to what extent gluten is the offending protein, many gluten containing grains such as oats, barley, and rye are contaminated with wheat and could pose and unforeseen risk to adults with EoE. Areas of potential cross contamination of wheat can be found in Appendix materials.

As media popularity of gluten-free and wheat-free diets have grown among the general public, consumers have access to greater food options than in previous years. The market for products labeled as gluten and wheat free have experienced a compounded annual growth of 28% since year 2004. Despite their popularity, wheat-free products are largely refined, are not fortified, and can result in lower consumption and serum values of micronutrients including B vitamins, magnesium, and iron. Nutritional balance can be achieved however by careful addition of gluten-free grains including quinoa, buckwheat, gluten free oats, brown rice, and millet. These offer competitive levels of B vitamins, iron, zinc, and trace minerals. Quinoa is a pseudograin and falls into the seed category. Therefore, the inclusion of quinoa needs to be considered and individualized in patients with seed allergies.

MILK AVOIDANCE

Cow’s milk protein was found to be the most common trigger in children with EoE. Among adults, cow’s milk protein accounts for more than 50% of the food triggers patients with EoE. Milk and dairy products are rich in protein and are fortified with vitamins A and D as well as thiamin, riboflavin, B12, and calcium. Safe, allergy-free milk substitutes while on SFED include fortified rice milk, fortified hemp, and flax milk. In addition to obvious sources of dairy including milk, cheese, coffee cream, and ice cream, consumers need to know which foods can contain dairy derivatives in order to avoid while on SFED. Common hidden sources of contamination include milk solids in form of added butter, casein and whey, protein powder, chocolate, and marinades. Additionally, less obvious sources such as deli meats, sausages, hot dogs, chicken nuggets, and other processed meats can contain dairy solids or cheese flavorings, which can serve as a source of contamination.

EGG AVOIDANCE

Eggs are a highly bioavailable source of protein as well as B-vitamins. However, these nutrients can be easily covered through addition of legumes, seeds, quinoa, and other lean proteins. Egg and egg protein (albumin) are commonly found in mayonnaise, merengues, foams, marzipan, pastas, nougat, and baked goods. Egg and soy proteins are common emulsifiers (albumin and lecithin) and stabilizers and are often added to commercially prepared foods. Often patients will ask about whether they can receive flu shots while on SFED due to the use of egg albumin in the medium. However, concerns about egg in flu shots would not apply to patients on SFED as the inoculation is not oral. However, if abnormal allergy testing is present or patients have a known allergic reaction to egg, then egg free flu shots can be utilized.
Replacing eggs when cooking at home can easily be achieved by substituting with mashed fruit in baked goods or by using honey and mustard as emulsifiers in salad dressings and savory foods. Recent SFED diet results indicate the prevalence of egg allergies among adults being treated with diet therapy to be 5–26.2%.3,11

SOY AVOIDANCE

Soy beans belong to the legume family and are one of the few plant-based foods containing a complete amino acid profile similar to animal proteins. Soy beans and soy products can be rich in fiber, calcium, folic acid, iron, and essential fatty acids.29 Incorporating fortified allergy-free grains and milk substitutes, green leafy vegetables, and animal proteins allows for easy replacement of nutrients and a balanced diet.

The US Food and Drug Administration (FDA) exempts highly refined soybean oil from being labeled as an allergen. Unless a known IgE-mediated allergic response to soy is known, most individuals can safely consume soy lecithin and highly refined soy bean oils as the protein has been removed during processing. Patients should be instructed however to avoid cold pressed, expeller pressed, or extruded soybean oils as they still contain soy protein.30

Common sources of soy protein include soy sauce, Asian foods, snack products, veggie burgers, protein shakes, margarine, and nondairy creamer.

PEANUT AND TREE NUT AVOIDANCE

Peanuts and tree nuts are becoming more ubiquitous in the food supply as they confer significant cardiovascular benefit by reducing low-density lipoproteins. They are also a rich source of protein, monounsaturated fats, and antioxidants.31 The most common sources for peanut and tree nut contamination include baked goods, cereals, snack foods, and whole grain breads. Baked goods can often contain nuts or be contaminated with nuts during baking and need to be carefully considered in the diet. During the initial states of the SFED as wheat and dairy and eggs are limited, many baked goods are naturally excluded thus limiting exposure to nuts and tree nuts. When dining out, fried foods, breaded dishes, breads, deserts, condiments, salads, and the restaurant surfaces themselves serve as a potential medium for contamination.32,33 Healthy substitutions include seeds such as flax, hemp, or chia as well as wheat-free whole grains to provide complementary nutrition. Caution should be used when reading labels on seeds as they can often be produced in facilities and on equipment shared with tree nuts and peanuts.

AVOIDING FISH AND SHELLFISH

Among adults with EoE, fish and shellfish allergies are rare compared with other foods.3 In a recent prospective study conducted by these authors, there were no fish or shellfish allergens noted. Outside of direct consumption, hidden sources of fish and shellfish proteins can often include ethnic cuisine, Worcestershire sauce, fish oil, or omega-3-fortified products, soups containing fish stock, risotto, and stews. Although fish is an excellent source of lean protein and omega-3 fatty acids, dietary balance can be achieved through addition of lean poultry and seeds such as flax seeds and chia seeds.

LABEL READING AND AVOIDING CROSS-CONTAMINATION

In 2006, the US congress passed the Food Allergen Labeling and Consumer Protection Act,34 which mandated that companies list foods containing major allergens including milk, eggs, fish, shellfish, peanuts, tree nuts, wheat, and soy in plain language. This plain language declaration can be included in two ways. First, as a parenthetical statement embedded in the ingredients list or secondly by using the word ‘contains’ followed by the allergen. This declaration is required if allergens are present in any amount as in colorings, emulsifiers, starches, or flavorings.30

Successful avoidance depends on properly identifying both obvious and hidden sources of food allergens.35,36 Due to the global nature of the food supply, the presence of cross-contamination of food allergens is an issue for both healthcare providers and consumers.37 Unlike celiac disease and IgE-mediated food allergies, no clear agreement exists on the amount of allergens necessary to elicit an allergic reaction in EoE. Thus, patients following an SFED should be advised to reduce any possible exposure to allergens, as small amounts of allergens may be enough to illicit immune response.36–38

Concerns over food allergens in the diet and dining out rank as top factors impacting health-related quality of life in EoE.39,40 To ensure success, significant time is spent discussing common allergens inside and outside of the home as well as strategies that improve success while on SFED. Common sources of cross-contamination can include shared cooking equipment, shared sponges, condiments, and cookware surfaces. At home, reduction of cross-contamination can be achieved by encouraging segregated cook-top surfaces, sponges, toasters, and utensils for allergy-free food. Utilizing allergy-free stickers in
common areas such as shared refrigerators or office kitchens help identify allergy-free food to roommates, office mates, and family.41 Patients who are Christian and receive communion are encouraged to abstain from receiving communion wafers during SFED until the allergens are further elucidated. Restaurants serve as a significant source of cross-contamination and unintended allergens presented in form of desserts, fried foods, meat and cheese slicers, ethnic foods, and nutshell on establishment floor are all cause for concern.32,33 Food allergy training has been reported by less than 50% of restaurant personnel. Supporting the active role patients need to play in self-management of their disease.55 Additional strategies for dining out safely are addressed below.

MANAGING NUTRITIONAL LIMITATIONS OF SFED

Any restricted diet can pose significant nutritional risks and set the stage for both micronutrient and macronutrient deficiencies. Previous studies have not systematically evaluated the impact of the SFED and the EDs on nutritional status of adults with EoE. Limiting food groups such as milk, wheat, soy, eggs, fish, and nuts can significantly impact serum levels of zinc, B-vitamins, calcium, vitamin D, magnesium, selenium as well as dietary fiber and proteins if alternative sources of these vitamins are not incorporated. The 2010 Dietary Guidelines for adult Americans as well as dietary guidelines established by the Institute of Medicine and the US Department of Agriculture support a balance of fruits, vegetables, whole grains, lean proteins, and heart-healthy fats for a total diet approach.42,43 These nutritional goals can be met while on SFED with proper nutritional preplanning and the expertise of a registered dietitian skilled in food allergies and restricted diets. Recommendations for amounts and types of vitamins suggested while on SFED can be found in Appendix materials.

ADVICE FOR PROVIDERS: MAXIMIZING SUCCESS ON THE SFED

In addition to addressing nutrient balance, nutritional counseling needs to include guidance on minimizing the risk of cross-contamination. This includes education on proper label reading, awareness of high risk foods, and situations at risk for cross-contamination (i.e. dining out and social events such as holidays, parties, and family gatherings).44 Individualized dietary advice and options need to be customized based on local food availability and patient access. To help achieve increased success with the diet, our center has incorporated a dedicated 1-hour initial training session with our dietitian that includes nutrition assessment, education, and detailed diet materials. The SFED is individualized and fine-tuned in that session to include the following critical dietary modules:

- diet history to describe dietary pattern and typical day;
- assessment of food preferences, allergy symptoms, and oral allergy response;
- travel for pleasure and work during the diet intervention;
- dysphagia assessment (i.e. offering modified textures for proteins such as ground poultry and soft foods);
- cooking skills, label reading, and readiness to engage in meal planning;
- living situation (i.e. sharing kitchen with roommates or spouses and kids) and education on preventing cross-contamination at home;
- lifestyle coaching on dining out, socializing, and verbalizing needs in social settings; and
- adding in allergy-free multivitamin and calcium and vitamin D supplements given restricted nature of diet.

Patients are asked to keep a 3-day food diary regularly throughout the process to verify dietary adherence and nutrient adequacy, which can be faxed or emailed to the Registered Dietitian (RD) for evaluation. A food log should include what patients eat, when, how much, and brand names or restaurant names when available to guide dietary assessment.

Self-monitoring techniques such as food logs serve the dual purpose of providing data for the registered dietitian as well as a behavioral activation technique to keep patients engaged in dietary modification. Individuals who engage in self-monitoring techniques are more likely to adhere to a specialized diet compared with those who do not.45 Patients are encouraged to call or email team regularly regarding questions, symptoms, and questionable contaminant ingestion. With the use of encrypted email, phone or face-to-face visits, they are provided feedback on their food logs and dietary choices along with suggestions to improve nutrient density if necessary and continued avoidance of allergens. Food logs can often shed light on small details such as brand names or fast food items, salad bars, and snack foods, which may not be entirely safe on SFED. For example, plain baked potato chips are allowed on the SFED, but BBQ or sour cream and onion flavors contain dairy solids and need to be avoided. Additionally, salads prepared at salad bars are more likely to contain contaminants than salads prepared from home.

ADVICE FOR PATIENTS: MAXIMIZING SUCCESS ON THE SFED

Northwestern patients are encouraged to prepare most of their own meals to reduce risk of potential...
contaminants and improve success while on SFED. However, successful dining out can be achieved with a step-by-step guide and the use of food allergy cards to alert restaurants to consumer needs. A dining out checklist of common areas of cross-contamination (found in Appendix materials) can provide added safety to patients while on SFED. In addition to foods, alcoholic beverage such as beer and wine can serve as a source of allergens. Although gluten-free beers are available and labeled as such, many wines can contain unforeseen allergens. Wines are often ‘fined’ with animal proteins or clay to remove compounds including tannins, phenolic compounds, and processing residue. Fining improves the overall taste by reducing bitterness. Gluten, egg albumin, and dairy casein are all food additives currently utilized in the process of fining wines. Although the risk of having an allergic reaction to fined wines is small, asking restaurant and store members for vegan or dairy casein are all food additives currently utilized in the process of fining wines. Although the risk of having an allergic reaction to fined wines is small, asking restaurant and store members for vegan or biodynamic wines may reduce the likelihood of consumption can exacerbate GERD and may increase risk of allergen cross-reactivity. Dietary management of EoE in adults is a relatively new approach, and thus the extent to which alcohol promotes and maintains EoE is unknown.

Special variations on the SFED to include vegetarian or kosher dietary preferences can be accommodated through individualized planning on the part of the registered dietitian. For patients who wish to follow a vegetarian SFED, care is taken to provide adequate protein. During the diet history and food log review, the dietitian can assess if patients consume adequate amounts of dietary protein. If there are limited dietary options and the risk of inadequate protein is apparent, then supplemental protein powders can be integrated into the diet plan. There are several protein modular options that can be appropriate for patients depending on their level of sensitization including hydrolyzed brown rice, pea or seed protein powders, or specialized allergy-free amino acid supplements such as the Complete Amino Acid Mix supplement by Nutricia North America.

### ASSESSING RESPONSE TO DIETARY THERAPY AND IMPLEMENTING FOOD REINTRODUCTION PHASE

After completion of the 6-week elimination diet phase, an esophagogastroduodenoscopy (EGD) with biopsies are repeated to confirm histologic resolution and response to diet therapy. The same biopsy protocol used at baseline is pursued to maximize the sensitivity of catching a recurrence. Although there are clear guidelines about an eosinophil threshold to make the diagnosis of EoE, an absolute threshold to define response has not been established. As under normal physiologic conditions, the esophagus is devoid of eosinophils; in our study, we used a strict threshold level of <5 eosinophils/hpf to define response. Patients achieving histologic improvement (<5 eosinophils/high power field) begin systematic food reintroduction of food groups with each group being formally tested for 2 weeks with symptom monitoring before moving on to the next group. After two food groups are reintroduced, EGD with esophageal biopsy is repeated to monitor disease activity as there has been shown to be a poor correlation with symptoms and histology. Patients are instructed to call the gastroenterologist, nurse, or dietitian if they become symptomatic during food reintroduction phase so EGD can be repeated sooner if needed. In our study, most patients were symptomatic within 5 days of adding the trigger food. If a specific food group is implicated in disease reoccurrence based on histologic recurrence, then a 6-week wash out period of avoidance of that trigger food is recommended prior to initiating the next food group. This process is continued until all six-food groups are completed (Fig. 2). Typically, foods are reintroduced in order of least to most likely cause of triggering EoE. For instance, patients are advised to begin reintroduction with seafood for 2 weeks, followed by nuts for 2 weeks. If their follow-up endoscopy demonstrates continued remission, then egg is added for 2 weeks, followed by soy for 2 weeks. If their follow-up endoscopy demonstrates continued remission, then milk is added for 2 weeks, followed by wheat for 2 weeks. In some cases, due to the high likelihood of milk and wheat as triggers, patients have requested endoscopy to be performed after wheat introduction and before adding milk. This individualized approach can be entertained with consideration of other factors such as medical indication, risk/benefit discussion, insurance coverage, and patient preferences.

Although dietary elimination and food reintroduction has been considered first-line therapy in most pediatric patients, the process and timeline of food reintroduction is variable at many centers. In our pediatric group, during food reintroduction, one food is added back every 6 weeks and an endoscopy is performed after each food group. In order the expedite the process for our adults and to attempt to minimize the number of endoscopies needed during food reintroduction, we have streamlined this approach to what is described above.

Patients are counseled on the timeline of food reintroduction during the initial physician and dietitian visit as diet intervention is a minimum of 12 weeks and can be longer depending on the number of food triggers that are identified and number of relapses...
during reintroduction. Thankfully, in our experience, most patients are found to have just one food trigger. Regular medical and nutritional monitoring is essential to monitor changes in weight, dietary patterns, and adherence to supplements. When clinically indicated, nutritional blood work is suggested to safely monitor the restricted diet including complete metabolic profile, complete blood counts serum assessment of vitamin D, vitamins B12, and folic acid. If patients wish to transition to medical therapy during this process, they may do so at any time. In our experience, once dietary therapy is started, patients feel better within a few weeks and we have had a very low discontinuation rate. Once patients have completed the entire dietary protocol and food triggers are identified, they are advised to avoid their specific food trigger to help maintain remission. As long as patients remain stable on their diet, a follow-up endoscopy in 1 year may be performed to assure that histologic remission is maintained. Patients are advised that if there are times when food avoidance is not possible such as during increased travel for work or vacation or if they want to ingest the trigger food, use of swallowed topical corticosteroids may be used for a short time around times of contamination to help control symptoms as well as histologic eosinophilia. This approach allows for some flexibility while on the diet to help patients maintain adherence and also improve quality of life while on the diet.

HOW TO APPROACH PATIENTS WHO FAIL SFED?

Although the SFED offers a high success rate for patients, there are still around 30% of patients who experience either partial or no histologic improvement on the diet. In these cases, it is important to decipher whether or not the lack of response is from accidental contamination or from failure to exclude additional dietary allergens beyond the ‘Big Six’. Risk of contamination can be assessed through food logs and regular clinical visits with the physician and RD to review atypical dietary patterns, brand names included and frequency of dining out. If there is a high risk of contamination or known exposure to contaminants, then patients are given the option of continuing the diet for an extended period or altering their treatment plan and proceeding with medical therapy. If there is little chance of contamination based on the above assessment, then a clinical visit with the MD and the RD is appropriate to discuss next steps in clinical care. Options that are typically discussed include pursuing medical therapy at this time. If patients are still interested in pursuing dietary therapy, then it is essential to consider possible allergens other than only those in the SFED. Lucendo et al. recently reported a high prevalence of corn, legume, and rice allergies among their group of patients enrolled in empiric elimination.

Although this is likely due to regional differences in allergy triggers in the Spanish versus US cohorts, this raises the concern that other foods may be playing a role. In patients highly motivated to continue dietary therapy, additional food allergens may be eliminated including those with higher allergic potential including beef, corn, and legumes. Before embarking on this approach, patients are counseled that elimination of additional foods will likely lengthen the overall food reintroduction process and lead to additional endoscopies. In some patients where protein consumption is significantly limited with additional food elimination and weight loss is a concern, their calories are supplemented with elemental formula. In patients who continue to have histologic eosinophilia despite these additional eliminations are again reevaluated by the clinician and dietitian. These patients likely have a robust immunoreactivity to food allergens and may only respond to elemental formula. Patients are again counseled on switching to medical therapy at this point. Despite this, there are still some patients highly motivated to pursue dietary therapy who may be considered for treatment with ED. Before pursuing this approach, important discussion points with patients include quality of life, palatability of the formula, duration of overall dietary elimination process, and multiple endoscopic assessments needed during food reintroduction.

Thankfully, in our experience, most patients who fail SFED are due to contamination during the initial elimination period and repeating the process has lead to success. In the few who failed additional elimination and have gone on to elemental, 100% have resolved their EoE and were able to pursue reintroduction (unpublished data).

CONCLUSION

Dietary therapy significantly improves histopathologic features, symptoms, and endoscopic findings in EoE and should be offered to both children and adults with the disease. Dietary therapy can be effectively implemented in clinical practice with appropriate dietary education, patient resources, and close communication with physician and clinical staff. The ability to identify specific food triggers to help tailor dietary therapy for long term management allows for a return to consumption of most table foods. Furthermore, the diet approach avoids the need for chronic topical corticosteroid use and possible long-term side effects of these medications. The decision to proceed with dietary therapy should be decided by patient preference and available resources. A collaborative and multidisciplinary
approach including gastroenterologists, allergists, nurses, and dietitians is essential in the success of this approach.

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APPENDIX I

Dining out checklist

Look over menu ahead of time & call to ask questions about preparation methods

Review dietary modifications with wait staff

Educate yourself about cooking to better understand food ingredients

Send back food with allergen contamination i.e. croutons on salad

Ask about preparation methods including marinades, sauces and flavorings

Ask for clean cooking surface and clean utensils

Utilize restaurant card to aide kitchen staff in recognizing allergens

Be prepared with a plan B in case your needs are not met and the food is not safe

APPENDIX II

Healthy breakfast ideas: SAFE FOODS ON SFED

Breakfast is the most important meal of the day. Not only does it give you energy to start a new day, but breakfast is linked to many health benefits, including weight control and improved performance.

Studies show that eating a healthy breakfast can help give you:

-o A more nutritionally complete diet, higher in nutrients, vitamins and minerals
-o Improved concentration and performance in the classroom or the boardroom
-o More strength and endurance to engage in physical activity
-o Lower cholesterol levels

Whole grain + Lean Protein + Fruit/Vegetable = A well-balanced, high-energy breakfast with a healthful combination of fiber, vitamins, minerals and antioxidants. If you need additional heart healthy calories feel free to add in heart healthy fats such as olive, canola or safflower oils as well as avocado. You can also increase the portions of each to add healthy calories.

To make a healthy breakfast, choose one item from each column.
<table>
<thead>
<tr>
<th>Whole grain</th>
<th>Protein or milk alternative</th>
<th>Fruit/vegetable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enjoy Life Granola™ (1 cup) &amp; Hemp or Rice milk</td>
<td>Hormel Naturals™ Canadian bacon (2 slices)</td>
<td>Banana or berries (1 cup)</td>
</tr>
<tr>
<td>Gluten Free Oatmeal* (1 cup)</td>
<td>Fortified rice or hemp milk (1 cup)</td>
<td>Apple (1 sliced)</td>
</tr>
<tr>
<td>Cream of rice cereal (1 cup) cooked in rice or hemp milk</td>
<td>Enjoy Life™ trail mix (2 Tbsp)</td>
<td>Grapefruit (1/2) w/ Splenda</td>
</tr>
<tr>
<td>Ener-G™ Tapioca Rice bread (2 slices) with Jam</td>
<td>Hemp Milk</td>
<td>Banana (1 small)</td>
</tr>
<tr>
<td>Hot millet cereal*</td>
<td>Fortified rice or hemp milk (1 cup)</td>
<td>Melon (1 cup)</td>
</tr>
<tr>
<td>Corn tortilla (1–2)</td>
<td>Refried black beans (1/2 cup)</td>
<td>Salsa (1/4 cup)</td>
</tr>
<tr>
<td>Corn or Rice Chex™ (1 cup)</td>
<td>Fortified rice or hemp milk (1 cup)</td>
<td>Carrot Juice (1 cup)</td>
</tr>
<tr>
<td>Enjoy Life Granola Bar (1)</td>
<td>Jenny-O™ Turkey Sausage (2 links)</td>
<td>Pineapple (1 cup)</td>
</tr>
<tr>
<td>Note: * = Oats can be contaminated with wheat.</td>
<td><a href="http://www.glutenfree.oats.com">http://www.glutenfree.oats.com</a>.**</td>
<td>Orange (1)</td>
</tr>
</tbody>
</table>

**Healthy lunch/dinner ideas: SAFE FOODS ON SFED**

A balanced meal that is high in fiber and low in fat helps supply the body and mind with energy to avoid a mid-afternoon/late evening energy slump.

**Whole grain + Lean Protein + Fruit/Vegetable** = A well-balanced, high-energy meal. Round out the meal with a glass of fortified rice or hemp milk for healthy calories and extra protein and fats. If you need additional heart healthy calories, aim for healthy fats such as olive, canola or safflower oils as well as avocado. You can also increase the portions of each to add healthy calories.

To make a healthy choice each day, choose one item from each column.

<table>
<thead>
<tr>
<th>Whole grain</th>
<th>Protein</th>
<th>Fruit/vegetable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Tortilla (2)</td>
<td>Turkey breast (3 oz) *</td>
<td>Tomato and lettuce, baby carrots, avocado slices</td>
</tr>
<tr>
<td>Instant brown rice (1 cup cooked)</td>
<td>Black Beans (1 cup)</td>
<td>Broccoli and tomatoes</td>
</tr>
<tr>
<td>Ener-G™ Tapioca Rice bread (2 slices)</td>
<td>Lean Roast Beef (3 oz)* with mustard</td>
<td>Mixed greens with oil &amp; vinegar</td>
</tr>
<tr>
<td>Quinoa Pasta (1–2 cups cooked)</td>
<td>Skinless chicken breast slices (3 oz.)</td>
<td>Pear (1)</td>
</tr>
<tr>
<td>Baked Potato (1 medium)</td>
<td>Lean ground turkey or beef chili (1/2–1 cup)</td>
<td>Dairy free tomato sauce</td>
</tr>
<tr>
<td>Instant wild or brown rice (1 cup cooked)</td>
<td>Grilled turkey sausage</td>
<td>Apple</td>
</tr>
<tr>
<td>Quinoa (1 cup)</td>
<td>Homemade chili (1 cup)</td>
<td>Green salad with vinegar and olive oil</td>
</tr>
<tr>
<td>Baked Sweet Potato</td>
<td>Grilled Pork Tenderloin (olive oil, lemon juice marinade)</td>
<td>Strawberries (1 cup)</td>
</tr>
<tr>
<td>La Tortilla Factory™ Teff tortillas (1)</td>
<td>Hummus (1/2 cup)</td>
<td>Sautéed onions &amp; red or yellow bell pepper strips</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Banana (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mixed vegetables or garden salad</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kiwi (2)</td>
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<tr>
<td></td>
<td></td>
<td>Green beans</td>
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<tr>
<td></td>
<td></td>
<td>Applesauce (1/2–1 cup)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lettuce, tomato, cucumber &amp; black olives</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grapes (1 bunch)</td>
</tr>
</tbody>
</table>

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How to Read a Label for a Milk-Free Diet

All FDA-regulated manufactured food products that contain milk as an ingredient are required by U.S. law to list the word ‘milk’ on the product label.

Avoid foods that contain milk or any of these ingredients:
- butter, butter fat, butter oil, butter acid, butter ester(s)
- buttermilk
- casein
- casein hydrolysate
- caseinates (in all forms)
- cheese
- cottage cheese
- cream
- curds
- custard
- diacetyl
- ghee
- half-and-half
- lactalbumin, lactalbumin phosphate
- lactoferrin
- lactose
- lactulose
- milk (in all forms, including condensed, derivative, dry, evaporated, goat’s milk and milk from other animals, lowfat, malted, milkfat, nonfat, powder, protein, skimmed, solids, whole)
- milk protein hydrolysate
- pudding
- Recaldent®
- rennet casein
- sour cream, sour cream solids
- sour milk solids
- tagatose
- whey (in all forms)
- whey protein hydrolysate
- yogurt

Milk is sometimes found in the following:
- artificial butter flavor
- baked goods
- caramel candies
- chocolate
- lactic acid starter culture and other bacterial cultures

How to Read a Label for a Soy-Free Diet

All FDA-regulated manufactured food products that contain soy as an ingredient are required by U.S. law to list the word ‘soy’ on the product label.

Avoid foods that contain soy or any of these ingredients:
- edamame
- miso
- natto
- shoyu
- soy (soy albumin, soy cheese, soy fiber, soy flour, soy grits, soy ice cream, soy milk, soy nuts, soy sprouts, soy yogurt)
- soya

Soy is sometimes found in the following:
- Asian cuisine
- vegetable broth

Keep the following in mind:
- The FDA exempts highly refined soybean oil from being labeled as an allergen. Studies show most allergic individuals can safely eat soy oil that has been highly refined (not cold pressed, expeller pressed, or extruded soybean oil).
- Most individuals allergic to soy can safely eat soy lecithin.
- Follow your doctor’s advice regarding these ingredients.

How to Read a Label for a Peanut-Free Diet

All FDA-regulated manufactured food products that contain peanut as an ingredient are required by U.S. law to list the word ‘peanut’ on the product label.

Avoid foods that contain peanuts or any of these ingredients:
- artificial nuts
- beer nuts
- cold pressed, expeller pressed, or extruded peanut oil
- goobers
- ground nuts
- mixed nuts
- Peanut is sometimes found in the following:
- African, Asian (especially Chinese, Indian, Indonesian, Thai, and Vietnamese), and Mexican dishes
- baked goods (e.g. pastries, cookies)
- candy (including chocolate candy)
- chili

Keep the following in mind:
- Mandelsonas are peanuts soaked in almond flavoring.
- The FDA exempts highly refined peanut oil from being labeled as an allergen. Studies show that most allergic individuals can safely eat peanut oil that has been highly refined (not cold pressed, expeller pressed, or extruded peanut oil). Follow your doctor’s advice.

FARE

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info@foodallergy.org
http://www.foodallergy.org

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How to Read a Label for a Wheat-Free Diet

All FDA-regulated manufactured food products that contain wheat as an ingredient are required by U.S. law to list the word ‘wheat’ on the product label. The law defines any species in the genus *Triticum* as wheat.

**Avoid foods that contain wheat or any of these ingredients:**
- bread crumbs
- bulgur
- cereal extract
- club wheat
- couscous
- cracker meal
- durum
- einkorn
- emmer
- farina
- flour (all purpose, bread, cake, durum, enriched, graham, high gluten, high
- Wheat is sometimes found in the following:
  - glucose syrup
  - soy sauce

How to Read a Label for a Egg-Free Diet

All FDA-regulated manufactured food products that contain egg as an ingredient are required by U.S. law to list the word ‘egg’ on the product label.

**Avoid foods that contain eggs or any of these ingredients:**
- albumin (also spelled albumen)
- egg (dried, powdered, solids, white, yolk)
- eggnog
- lysozyme
- mayonnaise
- meringue (meringue powder)
- ovalbumin
- surimi

**Egg is sometimes found in the following:**
- baked goods
- egg substitutes
- marshmallows
- lecithin
- macaroni
- pasta

**Keep the following in mind:**
- Individuals with egg allergy should also avoid eggs from duck, turkey, goose, quail, etc., as these are known to be cross-reactive with chicken egg.

How to Read a Label for a Tree Nut-Free Diet

All FDA-regulated manufactured food products that contain a tree nut as an ingredient are required by U.S. law to list the specific tree nut on the product label.

**Avoid foods that contain tree nuts or any of these ingredients:**
- almond
- artificial nuts
- beechnut
- Brazil nut
- butternut
- cashew
- chestnut
- chinquapin nut
- coconut
- filbert/hazelnut
- gianduia *(a chocolate-nut mixture)*
- ginkgo nut
- Hickory nut
- litchi/lichee/lychee nut
- macadamia nut
- marzipan/almond paste
- Nangai nut

**Tree nuts are sometimes found in the following:**
- black walnut hull extract (flavoring)
- natural nut extract (e.g. walnut oil, almond oil)
- nut oils (e.g. walnut oil, almond oil)
- walnut hull extract (flavoring)
- nut distillates/ethanol extracts

**Keep the following in mind:**
- Mortadella may contain pistachios.
- There is no evidence that coconut oil and shea nut oil/butter are allergenic.
- Many experts advise patients allergic to tree nuts to avoid peanuts as well.
- Coconut, the seed of a drupaceous fruit, has typically not been restricted in the diets of people with tree nut allergy. However, in October of 2006, the FDA began identifying coconut as a tree nut. Medical literature documents a small number of allergic reactions to coconut; most occurred in people who were not allergic to other tree nuts. Ask your doctor if you need to avoid coconut.
- Talk to your doctor if you find other nuts not listed here.

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APPENDIX IV

Allergy free diet: getting started

What foods should I avoid?

Avoid the top 6 allergens: *wheat, soy, dairy, peanuts/tree nuts, fish/shellfish and eggs*

Where can I shop?

You can shop at any store however; many people find the best variety at local, specialty stores such as Whole Foods™, Trader Joe’s™, Fruitful Yield™ and Sunset Foods™. Additional specialty breads, products and baked goods can be purchased on-line through the following resources:

http://www.breadsfromanna.com
http://www.1-2-3glutenfree.com
http://www.glutenfreegrocery.com
http://www.glutino.com
http://www.glutenfremall.com

What should I look for on a food label?

All allergens will now be listed on a food label under ingredients. The Food Allergen Labeling and Consumer Protection Act (FALPCA) came into effect in 2006 and requires manufacturers to state the presence of allergens in their ingredient list.

What does the statement, ‘Good Manufacturing Practices used to segregate in a facility that also processes ALLERGEN’ mean?

This is to inform the consumer of what allergens are produced in the same facility as the product they are buying. These GMPs include, but are not limited to, thorough cleaning of machinery, line scheduling to segregate allergen and non-allergen ingredients, and line testing and would be appropriate to include on this diet. You should avoid foods that appear safe but state, ‘May contain traces of nuts, wheat, soy, dairy, eggs or fish’ as there may be cross contamination.

What is the timeline for following the elimination diet?

To start we eliminate all of the allergens for six weeks. After 6 weeks you will have another EGD (scope) to see if the Eosinophils have disappeared from your intestinal tract. Depending on the results you may be instructed to begin a food re-introduction phase where your medical team evaluates the relationship between food allergens and your GI symptoms. Your doctor or dietitian can create a specific timeline for you.

Can I dine out and travel while on the elimination diet?

Yes. Many restaurants offer allergen information to make dining out possible. However, it is difficult to control for cross contact with allergens and dining out should be limited during the elimination phase in order to achieve the best results possible. To limit contamination, you can speak with a manager and explain what modifications you need. You can also access restaurant menus on-line before hand to select best choices. There are several websites geared towards travel and dining for those with Celiac Disease (gluten allergy) as well as multiple food allergies:

http://www.bobandruths.com
http://www.glutenfreeonthego.com
http://www.eatingoutwithfoodallergies.com
http://www.allergyecats.com

It is important to tell restaurants what you need specifically asking for the following:

- Meats cooked in their own pan with only olive/vegetable oil, lemon juice, salt and pepper
- Kitchen staff to use fresh gloves and equipment when handling your food
- No marinades or sauces other than the allowed safe foods
- No broth, butter or soy sauce

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If food comes to you with an allergen accidentally added (croutons on salad) ask the kitchen to start over; it is not safe to remove and continue eating.

*Should I take vitamins while on the elimination diet?*

Yes. While you can achieve nutritional balance, many nutrients can be missing. A general multivitamin as well as calcium supplement with vitamin D are the key supplements recommended.

*How much calcium & vitamin D do i need?*

**Men:** Aim for 800–1000 mg/calcium and 600–800 IU vitamin D daily  
**Women:** Aim for 1200 mg calcium daily and 600–800 IU vitamin D

*Options for calcium & vitamin D supplements:*

- OsCal Chewable™ with D (2/day)  
- Caltrate™, Oscal™, Citrical plus D™ (2–4/day)

*Multi-vitamin (select one option):*

- Centrum™ (1/day) or  
- Nautre Made™ Allergy free multivitamin (1/day)  
- Little Critters Gummy Vites™ (2/day adults) or  
- Chewable Multivitamin available at http://www.kirkmanlabs.com

*Additional websites/resources for adults and kids with food allergies*

- **Allergy Eats**—website and mobile app devoted to finding safe dining out options when you have allergies. http://www.allergyeats.org  
- **Academy of Nutrition and Dietetics.** 1-800-877-1600; http://www.eatright.org: locate RD  
- **American Academy of Allergy, Asthma, & Immunology.** 1-800-822-2762; http://www.aaai.org  
- **American College of Gastroenterological Association (ACG).** Digestive Disease Specialists committed to quality in patient care. http://www.gi.org  
- **American Gastroenterological Association (AGA).** A research group devoted to advancing the science and practice of gastroenterology. http://www.gastro.org  
- **FARE: Food Allergy and Research Education.** http://www.foodallergy.org  
- **North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPghan).** 1-215-233-0808; http://www.naspghan.com  
- **Living Without magazine** for patients with multiple food allergies. PO Box 2126 Northbrook, IL 60065 http://www.livingwithout.com  
- **Mothers of Children Having Allergies** (MOCHA). A group of parents of children with food allergies, sharing information and supporting each other. www.mochallergies.org  
- **The Food Allergy Project.** A charitable organization, building a coalition of concerned parents, researchers, educators and experts. http://www.foodallergyproject.org

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# APPENDIX V

Shopping Guide: SAFE FOODS ON SFED

**AVOIDING**: fish/shellfish, nut/tree nuts, dairy, soy, eggs, wheat, seeds

<table>
<thead>
<tr>
<th>Whole grains</th>
<th>Proteins</th>
<th>Vegetables – <em>unlimited</em> (fresh or frozen)</th>
<th>Fruits – <em>unlimited</em> (fresh or frozen)</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRAINS</td>
<td></td>
<td>□ Artichoke hearts</td>
<td>□ Apricots</td>
<td>□ Avocado</td>
</tr>
<tr>
<td>□ Amaranth and amaranth flour</td>
<td>□ Azuki</td>
<td>□ Blackberries</td>
<td>□ Blackberries</td>
<td>□ Garlic</td>
</tr>
<tr>
<td>□ Brown or white rice</td>
<td>□ Garbanzo</td>
<td>□ Blueberries</td>
<td>□ Blueberries</td>
<td>□ Herbs and spices, fresh or dried</td>
</tr>
<tr>
<td>□ Uncle Ben’s™ 90-second rice</td>
<td>□ Kidney</td>
<td>□ Cantaloupe</td>
<td>□ Mandarins</td>
<td>□ Lemon/lime juice</td>
</tr>
<tr>
<td>□ Corn</td>
<td>□ Pinto</td>
<td>□ Cherries</td>
<td>□ Mango</td>
<td>□ Mustard</td>
</tr>
<tr>
<td>□ Cornmeal/poenta</td>
<td>□ White</td>
<td>□ Grapefruit</td>
<td>□ Nectarine</td>
<td>□ Fats/Oil:</td>
</tr>
<tr>
<td>□ Corn Tortilla</td>
<td>□ Lentils</td>
<td>□ Grapes</td>
<td>□ Orange</td>
<td>Canola</td>
</tr>
<tr>
<td>□ Corn Chips (baked or original)</td>
<td>□ Amy’s Organic™ Bean/lentil soups</td>
<td>□ Papaya</td>
<td>□ Persimmons</td>
<td>Grapeseed</td>
</tr>
<tr>
<td>□ Millet &amp; millet flour</td>
<td>□ Black Bean Chili</td>
<td>□ Peach</td>
<td>□ Peach</td>
<td>Olive</td>
</tr>
<tr>
<td>□ Tef &amp; Tef tortilla</td>
<td>□ Lentil soup</td>
<td>□ Pear</td>
<td>□ Pineapple</td>
<td>Safflower</td>
</tr>
<tr>
<td>□ Potatoes (w/skin)</td>
<td>□ Lentil vegetable</td>
<td>□ Refined grain products</td>
<td>□ Plum</td>
<td>Smart Balance</td>
</tr>
<tr>
<td>□ Sweet potatoes (w/skin)</td>
<td>□ Sausage (pork or turkey)</td>
<td>□ Oils:</td>
<td>□ Pomegranate</td>
<td>Light™</td>
</tr>
<tr>
<td>□ Pasta</td>
<td>□ Bratwurst</td>
<td>□ Fats:</td>
<td>□ Prunes</td>
<td>Earth Balance™ Soy</td>
</tr>
<tr>
<td>□ corn &amp; quinoa pasta</td>
<td>□ Italian Sausage</td>
<td>□ Vegetable oil</td>
<td>□ Raspberries</td>
<td>Free</td>
</tr>
<tr>
<td></td>
<td>□ Lentils</td>
<td>□ Fats:</td>
<td>□ Strawberries</td>
<td>Veganaise™ Soy free</td>
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<tr>
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<td>□ Rice noodles</td>
<td>□ Fats:</td>
<td>□ Plums</td>
<td>Vegan mayo</td>
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<td>□ Brown Rice Pasta</td>
<td>□ Fats:</td>
<td>□ Sweets/Treats:</td>
<td>□ Vinegars</td>
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<tr>
<td>□ Yucca</td>
<td>□ Poultry</td>
<td>□ Enjoy Life™ Chocolate chips, cookies, cereal bars</td>
<td>□ Beverages:</td>
<td>□ Beverages: La Croix™</td>
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<tr>
<td>□ Popcorn (air popped or stovetop)</td>
<td>□ Pork</td>
<td>□ Juice spritzers</td>
<td>□ Juice spritzers:</td>
<td>□ Juice spritzers: La Croix™</td>
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<tr>
<td>□ Fortified rice &amp; hemp milk</td>
<td>□ Lamb</td>
<td>□ Tea, hot/iced</td>
<td>□ Tea, hot/iced:</td>
<td>□ Tea, hot/iced: Juice spritzers</td>
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<tr>
<td>□ Cereals</td>
<td>□ Veal</td>
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<td></td>
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Dietary habits and esophageal cancer

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SUMMARY. Cancer of the esophagus is an underestimated, poorly understood, and changing disease. Its overall 5-year survival is less than 20%, even in the United States, which is largely a function of a delay in diagnosis until its more advanced stages. Additionally, the epidemiologic complexities of esophageal cancer are vast, rendering screening and prevention limited at best. First, the prevalence of esophageal cancer is unevenly distributed throughout the world. Second, the two histological forms (squamous cell and adenocarcinoma) vary in terms of their geographic prevalence and associated risk factors. Third, some populations appear at particular risk for esophageal cancer. And fourth, the incidence of esophageal cancer is in continuous flux among groups. Despite the varied prevalence and risks among populations, some factors have emerged as consistent associations while others are only now becoming more fully recognized. The most prominent, scientifically supported, and long-regarded risk factors for esophageal cancer are tobacco, alcohol, and reflux esophagitis. Inasmuch as the above are regarded as important risk factors for esophageal cancer, they are not the sole contributors. Dietary habits, nutrition, local customs, and the environment may be contributory. Along these lines, vitamins, minerals, fruits, vegetables, meats, fats, salted foods, nitrogen compounds, carcinogens, mycotoxins, and even the temperature of what we consume are increasingly regarded as potential etiologies for this deadly although potentially preventable disease. The goal of this review is to shed light on the less known role of nutrition and dietary habits in esophageal cancer.

KEY WORDS: carcinogen, esophageal cancer, mycotoxin, nitrogen compound, risk factor.

INTRODUCTION

Cancer of the esophagus is an underestimated, poorly understood, and changing disease. In 2000 there were approximately 412,000 new diagnoses of esophageal cancer worldwide, and combined with an associated 338,000 deaths each year, the disease ranks as the sixth most common cause of cancer-related death annually.¹ In the United States, there were 13,900 new cases and approximately 13,000 deaths from esophageal cancer in 2003.² As evidenced by the similarity of incidence and mortality rates, esophageal cancer is a particularly deadly disease. In fact, the overall 5-year survival is less than 20%, even in the United States, which is largely a function of a delay in diagnosis until its more advanced stages.³ Additionally, the epidemiologic complexities of esophageal cancer are vast, rendering screening and prevention limited at best. First, the prevalence of esophageal cancer is unevenly distributed throughout the world, whereby the disease is less common in the United States than in developing countries.⁴ Second, the two histological forms (squamous cell and adenocarcinoma) vary in terms of their geographic prevalence and associated risk factors. Indeed, contrary to the dominance of squamous cell cancer in the developing world, adenocarcinoma of the esophagus is more common in the United States than its squamous cell counterpart (58% vs. 36%, respectively, from 2003 to 2007).³ Third, some populations appear at particular risk for esophageal cancer, likely a combined result of genetic, environmental, socioeconomic, and/or dietary phenomena. And fourth, the incidence of esophageal cancer is in continuous flux among groups. For example, in the United States from 1974 to 1994, the rate of adenocarcinoma in white males increased an astounding 350%.⁴ Meanwhile, from the mid-1980s until 2006, esophageal cancer in blacks has dropped precipitously, although squamous cell...
carcinoma is still twice as common in blacks as compared with all other races (Fig. 1). Despite the varied prevalence and risks among populations, some factors have emerged as consistent associations while others are only now becoming more fully recognized.

The most prominent, scientifically supported, and long-regarded risk factors for esophageal cancer are tobacco, alcohol, and reflux esophagitis. These correlates by and large apply to gender, race, socioeconomic, and geography. Furthermore, cessation of tobacco and alcohol use is associated with a reduction in the risk for esophageal cancer. Inasmuch as the above are regarded as important risk factors for esophageal cancer, they are not the sole contributors. For example, many studies have highlighted genetic variations as an additional concern, primarily regarding populations at particularly high risk for esophageal cancer in China and elsewhere. Last, and as this paper aims to address, dietary habits, nutrition, local customs, and the environment may be contributory. Along these lines, vitamins, minerals, fruits, vegetables, meats, fats, salted foods, nitrogen compounds, carcinogens, mycotoxins, and even the temperature of what we consume are increasingly regarded as potential etiologies for this deadly although potentially preventable disease. Therefore, our goal was to perform a detailed review of all relevant and up-to-date articles pertaining to the role of nutrition and dietary habits on esophageal cancer in order to shed light on this relatively less known topic. This review was performed through an exhaustive PubMed search and cross-referencing of the citations of each relevant article.

VITAMINS AND MINERALS

The association between esophageal cancer and diet has been suspected since 1961 when Wynder and Bross noted that a diet rich in milk and vitamins reduced the risk of esophageal cancer. More recent epidemiologic studies have identified diet and nutrition as important factors in the risk for esophageal carcinoma. Some of the more prominent data regarding the impact of antioxidant vitamins and minerals stems from the Linxian and General Population Trials in China, whereby it was noted that those with poor nutritional intake had a higher risk of esophageal carcinoma and that diet supplementation with selenium, β-carotene, and vitamin E resulted in a 13% reduction in cancer mortality. Studies in this same population identified low levels of selenium and zinc as associated with esophageal cancer. Many of these findings carry over to Western civilizations, as Bollschweiler et al. have noted in Germany for instance, whereby low intake of vitamins C and E correlated with an increased risk of esophageal cancer. In a United States study, intake of fiber, β-carotene, folate, vitamin B₆, and vitamin C were inversely associated with both types of esophageal cancer, while cholesterol, animal protein, and vitamin B₁₂ were directly associated. Likewise, Steevens and colleagues demonstrated that consumption of citrus fruits high in vitamin C and raw vegetables correlated with decreased rates of esophageal adenocarcinoma. Vegetable consumption was also associated with reduced esophageal cancer risk in Canadian and Swedish studies, whereas phytoestrogens, a group of compounds naturally occurring in plants, were thought to play a protective role. Overall, the general reduction of esophageal cancer risk with increased intake of some vitamins and minerals is likely the effect of their antioxidant properties.

Retinol and β-carotene

In their study of the Linxian population in China, Abnet et al. identified that 12% of those aged 40–69 were vitamin A deficient and that the median β-carotene level was below the fifth percentile of those studied in the National Health and Nutrition Examination Survey III trial. These authors did not, however, find a definitive correlation between these
Squamous cell carcinoma of the esophagus increased intake of compounds in the prevention of esophageal cancer. Thus, it is noteworthy that the Chemopreventive Agent Development Research Group of the National Cancer Institute is focused on retinoids and vitamin A-like compounds and esophageal cancer. On the other hand, in studies of Western industrialized populations, Lagergren et al. and Bollschweiler et al. found a significant risk reduction for esophageal cancer with increased intake of β-carotene. Thus, the results regarding these compounds are mixed, although it is noteworthy that the Chemopreventive Agent Development Research Group of the National Cancer Institute is focused on retinoids and vitamin A-like compounds in the prevention of esophageal cancer.

**Vitamins C and E**

In 2002, for diets rich in vitamins C and E, respectively, Bollschweiler et al. published a report indicating a significant risk reduction in esophageal adenocarcinoma (0.33 and 0.13) as well as esophageal squamous cell carcinoma (0.31 and 0.17) (Table 1). Although only a moderately sized case-control trial of 149 people in Germany, their results concurred with earlier findings out of Sweden. However, in a more recent trial, no difference in the risk of esophageal cancer was identified with a higher supplemental vitamin E status. Therefore, more conclusive evidence is required regarding these particular vitamins.

### Vitamin D

Results regarding the study of vitamin D and esophageal cancer are mixed, and there appear to be two camps. Some reports indicate that low dietary intake of vitamin D is a risk factor for esophageal cancer. Yet, in 2007, Abnet et al. published a cross-sectional report of 720 people in Linxian, China, noting a significant correlation between elevated vitamin D levels and esophageal squamous dysplasia, which is a precursor to esophageal cancer. This work supported earlier findings of a positive association between elevated vitamin D and esophageal cancer. As these findings are varied, results regarding vitamin D and its link to esophageal cancer leave many questions that have yet to be explained.

### Zinc and selenium

Concurrent with earlier findings by Abnet et al. and Mark et al., Lu et al. identified a significant reduction in esophageal cancer risk with increased dietary intake of selenium and zinc. Specifically, they found a 70% decreased risk associated to selenium (odds ratio [OR] = 0.30; 95% confidence interval [CI] = 0.13–0.67) and a similar risk reduction with zinc (OR 0.28; 95% CI = 0.11–0.70). The Netherlands Cohort Study similarly demonstrated an inverse association between selenium intake and risk of esophageal squamous cell carcinoma. This relationship was also appreciated for esophageal adenocarcinoma, but only in select subgroups including women, never smokers, and low antioxidant consumers. Indeed, these studies correlated well with previous reports out of China for selenium intake and demonstrate a nearly ubiquitous trend for this mineral’s potential impact on esophageal cancer, despite non-concurrent ecological research in Iran.

### Riboflavin, folate, and B12

In 2005 Siassi and Ghaderian published a case-control study aimed at elucidating the effects of riboflavin on esophageal cancer. Their work followed that of others, who found that intake of riboflavin inversely correlated with the development of cancer of the esophagus. The work of Siassi and Ghaderian was interesting in that it involved inhabitants of the Gonbad District of Iran, who have the highest rate of esophageal cancer in the world. These authors, in their comparison with Iranians in a district 300

<table>
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<tr>
<th>Risk</th>
<th>95% Confidence interval</th>
<th>Significance</th>
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<tr>
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<tr>
<td>BMI1 0 = BMI &lt;25</td>
<td>5.42</td>
<td>1.29–15.92</td>
</tr>
<tr>
<td>1 = BMI 25.1–27.5</td>
<td>14.72</td>
<td>4.18–51.93</td>
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<tr>
<td>BMI2 0 = BMI &lt;25</td>
<td>2.25</td>
<td>0.58–8.60</td>
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<tr>
<td>1 = BMI &gt;27.5</td>
<td>8.69</td>
<td>2.62–28.81</td>
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<tr>
<td>Smoking 1 0 = 0 pack years</td>
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<td>1 = 1–20 pack years</td>
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<td>0.09–0.54</td>
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<td>Smoking 2 0 = 0 pack years</td>
<td>1.68</td>
<td>0.60–6.59</td>
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<td>1 = &gt; 20 pack years</td>
<td>5.08</td>
<td>1.41–18.31</td>
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<tr>
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<td>13.36</td>
<td>2.03–87.99</td>
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<tr>
<td>1 = 6–25 g/day</td>
<td>74.17</td>
<td>12.41–444.32</td>
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<tr>
<td>Alcohol 2 0 = &lt; 6 g/day</td>
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<td>1 = &gt; 25 g/day</td>
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<td>0.09–0.48</td>
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<tr>
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<td>2.62–28.81</td>
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<tr>
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<td>0.13</td>
<td>0.09–0.54</td>
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<tr>
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<td>0.60–6.59</td>
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<tr>
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<td>5.08</td>
<td>1.41–18.31</td>
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<td>Squamous cell carcinoma of the esophagus</td>
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<tr>
<td>Alcohol 1 0 = &lt; 6 g/day</td>
<td>13.36</td>
<td>2.03–87.99</td>
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<td>1 = 6–25 g/day</td>
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miles away (who have a 34–47-fold lower incidence than those in the Gonbad District), concluded that riboflavin deficiency may play a role in the high incidence of esophageal cancer in high-risk regions of Iran.

Low folate and vitamin B₁₂ have also been linked to esophageal cancer in a variety of studies, most of which emphasize high-risk populations in China and South Africa. More often than many of the nutrients previously discussed, folate and vitamin B₁₂ have been studied in the contexts of genomics. For example, Stolzenberg-Solomon et al. in their 2003 study of genetic polymorphisms in Linxian found that variations in the MTHFR and MTRR genes were positively associated with an increased risk of esophageal cancer in this region of China. This is important work because as the authors state, ‘deficiencies in folate and B₁₂ and alterations in MTHFR and MTRR functions may contribute to carcinogenesis through altered DNA methylation (e.g., DNA hypomethylation) and impeded thymidylate synthesis, resulting in nucleotide imbalances, increased uracil misincorporation in DNA, DNA strand breaks, and impaired excision repair, which may increase the susceptibility of DNA to mutations and damage’. Knowledge such as this forms tangible credibility that buttresses other information that may be at times regarded with less credibility because of numerous and uncontrollable confounders.

**NITROGENOUS COMPOUNDS**

Nitrites and nitrosamines are found in nearly every dietary source and habit that humans have, from pickled and preserved foods, to beer and whiskey, to tobacco smoking, and even to simple vegetables and water. Moreover, such compounds can even be formed by natural bodily processes, such as oral reduction of salivary nitrate. Although the role of nitrites and nitrosamines in esophageal cancer has not been fully elucidated, the evidence is quite suggestive. The Netherlands Cohort Study found a correlation between nitrite consumption and esophageal squamous cell carcinoma in men, with no such correlation in women given their significantly lower intake of nitrites. In their systematic review, Jakszyn and Gonzalez could not discern a certain association between these compounds and esophageal cancer, although they were hampered by the lack of controlled trials (Fig. 2). However, when they looked at foods known to be high in nitrogenous content, such as processed meats and preserved fish and vegetables, some associations were demonstrable (Fig. 3). Similarly, other studies have demonstrated a link with consumption of red meat and processed meat with esophageal squamous cell carcinoma. However, this link remains questionable, as other studies have shown no such association between red or processed meat consumption and esophageal cancer. Red meat contains high levels of iron, which can cause oxidative stress and DNA damage, along with heme iron, which can catalyze endogenous formation of nitrogenous compounds and may act as potent carcinogens. Other epidemiological studies echo the results linking nitrite consumption and esophageal cancer and cover numerous countries, although they are typically more associated with the orient than the occident. For instance, urinary excretion of N-nitrosamine acids and nitrate is pronounced in...
high-risk populations in China who are notorious for the consumption of pickled and preserved foods.\textsuperscript{57,58} Additionally, the effects of diets high in nitrites and nitrates may be compounded by a somewhat non-modifiable factor, that being local water. In a study focused on an endemic gastrointestinal area of Turkey, Turkdogan \textit{et al.} found that nitrite levels of drinking water were elevated as opposed to standard values and concluded that ‘a traditional diet rich in nitrate and nitrite is significant in the development of . . . esophageal and gastric cancers . . . in the Van region of Turkey’.\textsuperscript{59} In Nebraska, although Ward \textit{et al.} reached a similar conclusion that diets rich in nitrates and nitrates are a risk factor for esophageal cancer ($P = 0.015$), they also found that the effects of nitrates in water alone did not account for this increased risk.\textsuperscript{60} Likewise, Barrett \textit{et al.} concluded that nitrates in water, at least as they relate to northern England, are not an independent risk factor for esophageal cancer.\textsuperscript{61}

Another factor to consider is whether fat, which red meat contains high levels of, plays a role in esophageal cancer. A Swedish study recently showed that diets high in fat and low in carbohydrates were associated with elevated rates of esophageal adenocarcinoma.\textsuperscript{62} However, other studies have shown no such effect. In fact, O’Doherty and colleagues found that fat intake was associated with lower rates of esophageal adenocarcinoma in patients with normal body mass indices, although in obese patients there was no
such correlation.\textsuperscript{63} Nonetheless, further definition of the relation of nitrosamines, nitrites, and nitrates to esophageal cancer should be pursued, especially given findings reported by the American Institute for Cancer Research that concluded that both exogenous and endogenous exposure to these substances could increase the risk of esophageal cancer.\textsuperscript{64}

**MAIZE AND MYCOTOXINS**

Across the world, in countries including but not limited to China, South Africa, Iran, and Brazil, the association between mycotoxins and esophageal cancer has been pursued. This story originally began with a link between corn products (namely alcohol distilled from corn) and esophageal cancer in Africa, with one of the earlier reports coming from Paula Cook in 1971.\textsuperscript{65} However, the identification of the etiology for this manifestation would remain stagnant for some time, namely until the characterization of fumonisins in South Africa in 1988. Fumonisins, which are produced by fusarium fungi that grow preferentially on maize, were later found to have cancer-promoting activity.\textsuperscript{66,67} Since then, fumonisins have been noted to be particularly prevalent in many regions of the world with the highest incidence of esophageal cancer, such as Linxian (China), Transkei (South Africa), Mazandaran Province (Iran), and Santa Catarina (Brazil).\textsuperscript{67–70} Finally, and of tremendous interest, is that fumonisins are capable of reducing nitrates to nitrites, which brings full circle the discussion of the nitrosamine connection to carcinogenicity. Of course, however, one must keep in mind that multiple risk factors are often at play, especially when discussing alcohol distilled from maize and diets heavy in maize products, as not only is alcohol an independent risk factor for esophageal cancer, but maize has very little in the way of vitamins and minerals, which may be protective against developing cancer of the esophagus. Nonetheless, the story of fumonisins is compelling.

**COFFEE, TEA, AND THE TEMPERATURE OF DRINKS**

Unlike the stories of processed, pickled, and preserved foods as well and diets heavy in nitrates and nitrites, coffee and tea, in certain contexts, seem to offer a protective advantage in esophageal cancer. Stemming from earlier case–control studies suggesting an inverse correlation of coffee intake to esophageal cancer, Naganuma \textit{et al.} sought to investigate the potential protective effect via a more rigorous, population-based, prospective cohort study.\textsuperscript{71–73} The authors rationalized their approach by the fact that Japanese tend to have a fair amount of coffee intake, and the country itself has historically had a high prevalence of esophageal cancer. In a cohort comprising 494,935 person-years, Naganuma \textit{et al.} found that coffee did indeed appear protective of esophageal cancer. In comparing those who had one or greater cups per day versus those who did not drink coffee, the hazard ratio was 0.60 for esophageal cancer (95% CI: 0.37–0.97, \( P = 0.05 \)). Perhaps as important, the authors also found that the protective effects for oral, pharyngeal, and esophageal cancers seemed to persist for current drinkers or baseline smokers. Whether or not these results will be upheld in future studies is to be seen, although data exist demonstrating that components of coffee, such as caffeine, cafestol, and kahweol, have anticarcinogenic properties.\textsuperscript{74,75}

Like coffee, components of tea may have anticarcinogenic effects that result in the drink providing a protective advantage against esophageal cancer. However, studies are conflicting in whether or not tea offers some protection against esophageal cancer, which is likely the result of numerous confounders, which often may include the method in which it is served. For instance, Wu \textit{et al.} recently found in a study of a high-risk population in China that having a history of ever consuming tea in Ganyu was associated with an elevated odds ratio of esophageal cancer (1.9; 95% CI = 1.4–2.4).\textsuperscript{76} However, when an adjustment was made for the temperature of tea, this association did not persist, and the authors were able to conclude that drinking tea at high temperatures significantly increased the risk of esophageal cancer. Others have confirmed these same findings beyond the borders of China in countries such as Japan and Iran.\textsuperscript{77,78} Unfortunately, although there appears to be a trend implicating the temperature of tea with esophageal cancer, reports denying such an effect, like that of Ke \textit{et al.}, demand further research into the matter.\textsuperscript{79} and yet another confounder to deal with in studying the effects of tea on esophageal cancer are customs such as that in Kashmir, India, where salting of the tea is typical, and some purport a link to esophageal cancer.\textsuperscript{80} In this scenario, we are yet again forced to confront the ugly specter of nitrosation, as the preparation of salted tea may result in the formation of N-nitroso compounds. At present, it is probably best to stay within the confines of the conclusions of Boehm \textit{et al.} in their systematic review, whereby they state that ‘there is insufficient and conflicting evidence to give any firm recommendations regarding green tea consumption for cancer prevention’.\textsuperscript{81}

Last, it is likely worthwhile mentioning that the thermal effects of liquids as possible contributors to esophageal cancer are not relegated solely to tea, which may favor the argument of a thermal factor. One particular source that has garnished attention is maté (a tea-like drink), which is mainly popular in
Latin and South America. It may not be a coincidence that maté is common in areas with an elevated prevalence of esophageal cancer. The beverage is typically served hot and at times sipped through a straw, thereby placing the hot liquid directly into the oropharynx. Much like tea, reports have been mixed as to the association of hot maté and esophageal cancers, although some have found statistically significant correlations, even after adjusting for possible confounders.\(^{32,84}\)

**CONCLUSIONS**

Esophageal cancer is a relatively common disease worldwide, with a troublesome uptrend in incidence in the United States. Worse yet, the 5-year survival for those with esophageal cancer is quite poor, with little progress having been made in the last quarter century.\(^3\) A large, rate-limiting hurdle to making progress in the prevention and treatment of esophageal cancer is its numerous and poorly defined risk factors, aside from the better understood risks of tobacco and alcohol. Furthermore, underpowered studies or those of less applicability, although they note an association, are often insufficient to establish a large-scale public health intervention. Nonetheless, when taken together, some data are convincing, especially those involving diet and nutrient modification in populations at high risk for esophageal cancer. Fortunately, many of the basics remain applicable, such as cessation of tobacco and alcohol use as well as eating a diet rich in fruits and vegetables. By modifying simple risk factors (such as limiting tobacco and alcohol and supplementing basic nutrition) as well as through efforts to better understand less well-established aspects (such as nitrogens and thermal injury), perhaps strides can be made in preventing this disease, even on a population-wide basis.

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Adapted on May 2, 2010 from the National Cancer Institute's website, [Cited 5 Feb 2010.] Available from URL: http://prevention.cancer.gov/programs-resources/groups/cad/programs/agents


Radical lymphadenectomy in esophageal cancer: from the past to the present

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SUMMARY. Lymphadenectomy as an essential part of the surgical treatment has been one of the most controversial aspects in the management of esophageal cancers. The purpose of this article was to review the evolution, the current role, and the optimal extent of lymphadenectomy for the treatment of esophageal cancers. Studies discussing the outcome of esophagectomy with lymph nodes dissection and comparing among different extent of lymphadenectomy were used in the analysis. Several studies including recently published articles reveal that additional radical lymphadenectomy may be beneficial in some patients with non-extreme esophageal cancer undergoing esophagectomy, whereas two-field lymph node dissection is suitable for distal esophageal cancers regardless of the histology of the tumor. Minimally invasive surgery and neoadjuvant therapy combined with radical surgery seem to show more benefit in selected cases, but further studies should be required to clearly demonstrate their efficacy and safety. The expertise and experience of the surgeons should also be taken into account in determining the success of these radical procedures.

KEY WORDS: en bloc esophagectomy, esophageal cancer, radical lymphadenectomy.

INTRODUCTION

Lymph node dissection in esophageal cancer is still a controversial issue at present. It is known that esophageal cancer is associated with a high rate of nodal and distant metastasis even in its early course, and thus surgical treatment alone may not be able to cure the disease.1 On the other hand, some surgeons contend that more radical surgery can provide benefits of locoregional disease control and longer survival.2 Given the cost of morbidity and mortality, another debate concerns the proper extent of lymph node dissection. The aim of this article is to review the role of radical lymph node dissection including its advantages and disadvantages from the past to the current trend in the treatment of esophageal cancer.

MATERIALS AND METHOD

A literature search was conducted in Medline database using the combinations of terms esophageal cancer, esophagectomy, lymph node dissection, number of lymph nodes, minimally invasive esophagectomy (MIE), and neoadjuvant therapy. Only articles published in the English language between 1961 and 2012 were included. A manual search of relevant studies was also performed to complete this review. For the evidence of radical lymphadenectomy, data on perioperative and oncologic outcomes and numbers of lymph nodes retrieved extracted from comparative studies and relevant case series were demonstrated and used in the analysis.

Patterns of lymph node metastasis

Esophageal cancer has a high potential for metastasis even in the early stage. Deeper tumor invasion is also highly associated with increased frequency of lymph node involvement3–9 (Table 1). In squamous cell carcinoma, remarkable rates of positive nodes (8–12%) present when the tumor invades into muscularis mucosae (T1a-M3)3,4 whereas in adenocarcinoma, the prevalence of nodal metastasis in patients with T1a-M3 tumor, reported in recent large series seems to be lower (0–1.3%).10–12 A dramatic increase in the rate of lymph node metastasis presents in both cell types when the tumors invade into submucosal layer.
Another unique characteristic of esophageal cancer is the pattern of cancer cells spreading via the lymphatic system. Anatomically, the esophagus is different from other organs in which it is located through three body compartments. The lymph fluid from the esophagus is drained upwardly and downwardly by the richly submucosal lymphatic plexus before passing through the muscular coat into the lymph nodes. It is mainly drained in longitudinal, rather than in segmental direction. From this widespread pattern of lymphatic flow, metastasis can occur in any lymph nodes from the neck through the abdomen, which may be distant from the primary tumor. Some studies showed that the lymph originating from the esophagus at the level above the tracheal bifurcation was thought to drain into the thoracic duct, whereas the lymph formed under the bifurcation drains downwardly through the celiac and gastric lymph nodes. This result correlates with the findings from three-field lymph node dissection (3-FL) regarding the influence of the tumor location on the frequency of lymph node metastasis in each region. Chen et al reported the pattern of lymph node metastasis in thoracic esophageal cancer patients undergoing 3-FL. The frequency of cervical lymph node metastasis was at the highest rate of 49.5% in cases of upper thoracic tumor, whereas it was at the lowest rate of 17.2% in lower thoracic tumor. The contrary pattern was seen in the rate of intra-abdominal lymph node metastasis (12.2% vs. 51.7% in upper and lower tumor, respectively). However, in cases of cancers that cause lymphatic channel obstruction, the collateral pathway may be developed. Thus, the direction of lymphatic flow may be changed and unpredictable.

The background of lymph node dissection

History
Since the late 19th century with more accumulation of knowledge of the lymphatic systems of the internal organs and surgical experience, regional lymph nodes clearance has become a part of treatment of breast and other gastrointestinal cancers. In the 1960s, Nakayama and Logan proposed the role of celiac trunk and mediastinal node dissection, respectively, in the treatment of esophagogastric cancer. In the 1970s, Sannohe was the first to advocate the necessity of cervical lymphadenectomy on the basis of the incidence of cervical lymph node metastasis in his series. Then, after the 1980s, extensive lymph node dissection seemed to be more popular while the operative mortality rates were decreasing from the improvement of perioperative care and general anesthetic techniques.

The definition of lymph node dissection
Western and eastern surgeons differ in defining lymph node dissection because of the differences in their determination of the extent of the operation. A classification of lymphadenectomy from the International Society for Diseases of The Esophagus was conducted in 1994 in Munich; however, this seems not to be commonly used in literatures. With regard to the basics, three areas or fields involved in performing lymph node dissection are the upper abdomen, superior and inferior mediastinum, and neck. It is generally accepted that 3-FL is referred to a removal of nodal tissue through these three operative fields. However, some discord exists over the term ‘two-field lymph node dissection (2-FL)’, which is sometimes not clearly defined in the literatures. In Japan and some eastern countries where squamous cell carcinoma of the middle and lower esophagus is predominant, 2-FL as a standard surgical treatment is described as a removal of nodal tissue in the upper abdomen including lymph nodes around the branches of celiac artery and esophageal hiatus, and in the inferior and superior mediastinum including periesophageal nodal tissue and lymph nodes along both recurrent laryngeal nerves. On the other hand, adenocarcinoma of the lower esophagus and esophagogastric junction presents more frequently in western countries, and then dissection of lymph nodes in the superior mediastinum that seem distant from the primary tumor is not typically performed. Thus, 2-FL is usually referred to a removal of nodal tissue within the upper abdomen and inferior
mediastinum limited to the subcarinal level. Two- or three-field lymphadenectomy may be performed with en bloc esophagectomy (EBE), a more extensive removal of the lateral margin of the tumor as described later. Occasionally, the term ‘extended 2-FL’ or ‘total mediastinal lymphadenectomy’ is used in the western literatures when the lymph nodes in the superior mediastinum are removed.\textsuperscript{19,20}

Evidence of radical lymphadenectomy

At present, there are a number of related studies demonstrating the outcome of radical lymph node dissection in esophageal cancer. However, most of them are case series and retrospective studies that are not considered high-quality evidence. Therefore, it is necessary to review the related published data comparing associated operations regarding survival, tumor type, tumor recurrence, complication, and mortality after each operation, and related relevant factors to clarify whether and when this procedure should be adopted.

Transhiatal versus transthoracic esophagectomy

The extent of lymph nodes removal with transhiatal esophagectomy (THE) is usually limited whereas it can be more extensive in transthoracic esophagectomy (TTE). To examine the difference between these two approaches may help to elucidate the benefit of more radical surgery. Proponents of TTE claimed that its advantages included better exposure, ability to perform adequate lymph node dissection, and avoidance of an injury to the thoracic duct and tracheobronchial structures, whereas proponents of THE cited reduced pain, pulmonary complications, and operative time due to the lack of thoracotomy.\textsuperscript{1} Chang \textit{et al.}\textsuperscript{21} compared outcomes of THE and TTE in 868 esophageal cancer patients by using the Surveillance, Epidemiology, and End Results (SEER) database. The 5-year survival was not significantly different between groups after adjusting for a difference in tumor stages. Two largest prospective cohort studies by Rentz \textit{et al.}\textsuperscript{22} and Connors \textit{et al.}\textsuperscript{23} examined outcomes of 945 and 17 395 patients, respectively, who underwent esophagectomy. No difference in morbidity and mortality was seen, but the complication and mortality rates were significantly higher in TTE in a subgroup that underwent esophagectomy in low-volume hospitals or <10 cases/year. Currently, there are four randomized control trials (RCT)\textsuperscript{24–27} (Table 2), showing no definite survival benefit between groups. The long-term result of the largest study\textsuperscript{27} was reported, showing that there was still no significant difference in 5-year survival between groups.\textsuperscript{28} However, a subgroup analysis of patients with tumor that involved only the distal esophagus found that TTE resulted in a 14% overall 5-year survival advantage, although this was not statistically significant. Moreover, in patients who had lymph node metastases within 1–8 nodes in the resection specimen and underwent TTE significantly gained a 20% 5-year survival and 41% 5-year disease-free survival benefit when compared with THE group. In addition to these comparative studies, there are one systemic review and two meta-analyses demonstrating a similar end result. The first report was from Rindani \textit{et al.},\textsuperscript{29} who did a systemic review of the outcomes of 5483 esophageal cancer cases who underwent Ivor-Lewis or THE. No clear difference in overall morbidity, mortality, or 5-year survival was found. They also noted that, with a power of 80% and 5% confidence limits, it required 1180 and 3200 patients, respectively, in each arm of randomized control trials to demonstrate a significant difference in mortality and survival. Another meta-analysis by Hulscher \textit{et al.}\textsuperscript{30} examining 50 articles involving 7527 esophagectomy patients, transthoracic approach was associated with significantly lower anastomotic leakage, vocal cord paralysis, and cardiac complication but significantly higher pulmonary complication and overall mortality. However, there was no significant difference in 5-year survival. Boshier \textit{et al.}\textsuperscript{31} published the most recent meta-analysis reviewing data

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Histology (%)</th>
<th>n</th>
<th>Approach</th>
<th>Median survival (months)</th>
<th>5-year overall survival (%)</th>
<th>Hospital mortality (%)</th>
<th>Leak (%)</th>
<th>Pulmonary complication (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldminc \textit{et al.}\textsuperscript{24}</td>
<td>93</td>
<td>0 100</td>
<td>32</td>
<td>THE</td>
<td>12</td>
<td>NR</td>
<td>6.3</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>Chu \textit{et al.}\textsuperscript{25}</td>
<td>97</td>
<td>0 100</td>
<td>20</td>
<td>THE</td>
<td>16</td>
<td>NR</td>
<td>8.6</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>Jacobi \textit{et al.}\textsuperscript{26}</td>
<td>97</td>
<td>18 82</td>
<td>16</td>
<td>THE</td>
<td>NR</td>
<td>NR</td>
<td>6.3</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>Hulscher \textit{et al.}\textsuperscript{27}</td>
<td>02</td>
<td>96 2</td>
<td>95</td>
<td>THE</td>
<td>22</td>
<td>NR</td>
<td>6.3</td>
<td>12.5</td>
<td>50</td>
</tr>
<tr>
<td>Omloo \textit{et al.}\textsuperscript{28,29}</td>
<td>07</td>
<td>96 2</td>
<td>95</td>
<td>TTE</td>
<td>NR</td>
<td>34</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

\textsuperscript{†}This study is the 5-year follow-up data from the study of Hulscher \textit{et al.} \textsuperscript{‡}Significant. NR, not report; S, squamous cell carcinoma; THE, transhiatal esophagectomy; TTE, transthoracic esophagectomy.

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of 5905 patients from 52 articles, which included a recent randomized control trials literature in the 2000s. The main result of this study was similar to previous reports regarding the occurrence of postoperative complications and overall survival, which was not significantly different between groups.

**EBE**

First introduced by Logan\(^\text{16}\) for the treatment of cancer at the cardia, Skinner\(^\text{32}\) then extended the use of EBE to treat intrathoracic and abdominal esophageal cancer. After that, several case series have been reported, usually indicated for the treatment of lower esophageal cancer. This operation is described as the removal of the tumor-bearing esophagus within a wide envelop of surrounding tissue. Originally, all tissues between the esophagus and or vertebral bodies are removed in continuity with the esophagus including pleural surfaces, a necessary part of pericardium, segments of right intercostal arteries, segments of right and left intercostal veins, thoracic duct, and azygous vein.\(^\text{32}\) Resection of the azygous vein may help to increase the lateral margin of the primary tumor and enhance the nodal clearance. A study of lymph nodes adjoining the azygous vein in the specimens after EBE demonstrated that there were 65.2% of patients whose lymph nodes could be detected along the azygous vein, and the rate of metastasis in these lymph nodes was 7.6%.\(^\text{33}\) The original procedure has been modified in that the azygous vein and also intercostal vessels may be preserved.\(^\text{34}\) The data from a cadaveric study suggested that preservation of the azygous vein may not affect mediastinal lymphadenectomy because the average number of lymph nodes near the azygous vein was only 0.67 node per patient.\(^\text{35}\) Nevertheless, removal of the azygous and intercostal veins has still been practiced by many proponents performing EBE. Regional lymph nodes are also usually removed in the same fashion as 2-FL\(^\text{36}\) or occasionally dissected as 3-FL.\(^\text{37}\)

Improved overall survival and reduced mortality rate can be seen in reports in the last 20 years. These better outcomes may result from improved perioperative management and better patient selection.\(^\text{38}\) The morbidity rate is still high, ranging from 27.6% to 37.2% (Table 3). However, the proponents of EBE proposed that it provides benefits to the esophageal cancer patients regarding reduced recurrence and improved survival. The 31–52% rate of 5-year survival from recent series is higher than that from more limited surgery, ranging between 21% and 30%,\(^\text{1,41,42}\) The rate of recurrence in the surgical field is low, ranging between 1% and 11%,\(^\text{34,39,40}\) The overall recurrence rate is still high, and such recurrence is usually detected in the field outside the dissected area\(^\text{40,43}\) suggesting the effect in local disease control by surgery. A case–control study comparing between EBE and THE in esophageal adenocarcinoma patients who had R0 resection for transmural tumors (T3) and lymph node metastases (N1) with 20 or more nodes in the specimen was reported.\(^\text{34}\) The survival benefit of EBE could be demonstrated in patients with eight or fewer positive nodes. Nine or more involved lymph nodes may indicate a systemic disease, and the benefit of improving survival with this radical surgery is vanished.

**3-FL**

Many case series were reported by the proponents of radical surgery demonstrating the outcomes of 3-FL with overall 5-year survival rates ranging from 42% to 53%\(^\text{3,37,45–48}\) (Table 4), which seem to be better than those of non 3-FL that ranged at the rate between 20% and 30%.\(^\text{37}\) Mortality rates were in the range of 1.1–6.4%, comparable with major series describing standard esophagectomy. Overall morbidity including surgical and non-surgical-related complications varied widely between the rates of 31% and 80% (Table 5). Patient selection is important in order to reduce such morbidity. A retrospective study, by Nishimaki et al.,\(^\text{49}\) demonstrated that the potential benefit from 3-FL could not be detected in a subgroup of patients who had five or more metastatic nodes or simultaneous metastatic lymph nodes in all three fields.

Data of 3-FL in western countries seem to be scarce. 3-FL has lacked general acceptance among western surgeons because adenocarcinoma at the lower esophagus and esophagogastric junction occurs predominantly and the concern of increased complications especially recurrent laryngeal nerve injury.\(^\text{50}\)

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**Table 3** Outcomes of en bloc esophagectomy

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>n</th>
<th>Histology (%)</th>
<th>Perioperative death (%)</th>
<th>Positive lymph node (%)</th>
<th>5-year overall survival (%)</th>
<th>Major complication (%)</th>
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</thead>
<tbody>
<tr>
<td>Skinner(^\text{32})</td>
<td>83</td>
<td>80</td>
<td>A 49 S 54</td>
<td>11</td>
<td>63</td>
<td>18</td>
<td>NR</td>
</tr>
<tr>
<td>Lerut et al.(^\text{38})</td>
<td>92</td>
<td>54</td>
<td>35 65</td>
<td>7.4</td>
<td>NR</td>
<td>48.5</td>
<td>27.6</td>
</tr>
<tr>
<td>Dresnet et al.(^\text{39})</td>
<td>90</td>
<td>176</td>
<td>64 36</td>
<td>NR</td>
<td>NR</td>
<td>31</td>
<td>NR</td>
</tr>
<tr>
<td>Altorki and Skinner(^\text{34})</td>
<td>01</td>
<td>111</td>
<td>27 73</td>
<td>5.4</td>
<td>60</td>
<td>40</td>
<td>38.7</td>
</tr>
<tr>
<td>Hagen et al.(^\text{40})</td>
<td>01</td>
<td>100</td>
<td>100 0</td>
<td>6</td>
<td>63</td>
<td>52</td>
<td>NR</td>
</tr>
<tr>
<td>Siewert et al.(^\text{46})</td>
<td>01</td>
<td>527</td>
<td>0 100</td>
<td>7.9</td>
<td>NR</td>
<td>37.4</td>
<td>NR</td>
</tr>
</tbody>
</table>

A, adenocarcinoma; NR, not report; S, squamous cell carcinoma.

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However, results from series reported by Altorki et al.37 and Lerut et al.47 from North America and Europe were comparable with those from Japan, with good 5-year survival, low mortality, and average morbidity rates (Tables 4, 5).

Three-field versus lesser lymphadenectomy
Many non-randomized comparative studies were published demonstrating the oncologic advantages of 3-FL over non 3-FL groups. Isono et al.51 reported a group of retrospective data comparing results of 3-FL with 2-FL from major institutions in Japan. Five-year survival rates were 34.3% and 26.7% ($P < 0.001$), whereas 30-day operative mortality rates were 2.8% and 4.6% for 3-FL and 2-FL, respectively. The results from this report should be interpreted with caution because of a difference in patient selection and the superiority of the surgical outcome in 3-FL performing hospitals. Akiyama et al.2 compared esophageal cancer patients who had undergone 2-FL earlier in his career with those who underwent 3-FL. Significantly improved overall survival in patients undergoing extended cervical dissections was found with a 5-year survival of 53.3% versus 37.5% for those undergoing only 2-FL ($P = 0.0013$). However, survival benefit of 3-FL group was significantly better in only cases of upper and middle tumor. Other comparative studies also showed a similar trend of results in which more extensive lymph node dissection resulted in better overall survival52 or better 5-year survival in only cases of upper and middle tumor.18 There were two randomized studies trying to solve this controversy. Nishihiara et al.53 reported that 3-FL may be better although not significantly than 2-FL (5-year survival = 64.8% vs. 48.0; $P = 0.192$). This study had too small number of patients in each arm (32 vs. 30 cases, respectively) because of the strict criteria they followed. Another study by Kato et al.54 compared the outcome of 3-FL with 2-FL. Although the 5-year survival rate after 3-FL was significantly better than 2-FL (48.7% vs. 33.7%; $P < 0.01$), this study may not be validly accepted because of its discrepancy in randomization of the patients.

The impact of number of lymph nodes removed
The relationship between number of lymph nodes harvested and the survival is another clue to support the benefit of radical lymph node dissection in esophageal cancer. Several reports using a different size of database showed a similar trend. Altorki et al.37 studied retrospectively using single-institution database of 264 esophageal cancer patients who underwent esophagectomy. Higher numbers of lymph nodes removed were associated with better survival in either nodal or non-nodal metastatic cases. A study by Schwarz and Smith based on SEER database including 5620 esophageal cancer patients concluded that higher lymph node counts (>30) were associated with the best overall survival regardless the nodal status or histology.56 Currently, two multinational studies demonstrated this relationship. Peyre et al.57 examined the role of number of lymph nodes removed regarding whether it had an influence on the overall survival. Based on the data of 2303 patients with esophageal cancer undergoing esophagectomy, the number of lymph nodes removed was a good predictor of 5-year

Table 4 Oncologic outcomes after 3-FL

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>n</th>
<th>Positive lymph node (%)</th>
<th>A</th>
<th>S</th>
<th>5-year disease-free survival (%)</th>
<th>5-year overall survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akiyama et al.2</td>
<td>94</td>
<td>290</td>
<td>71.03</td>
<td>0</td>
<td>100</td>
<td>NR</td>
<td>53.5</td>
</tr>
<tr>
<td>Altorki et al.37</td>
<td>02</td>
<td>80</td>
<td>68.75</td>
<td>40</td>
<td>60</td>
<td>46</td>
<td>51</td>
</tr>
<tr>
<td>Osugi et al.45</td>
<td>02</td>
<td>247</td>
<td>63.50</td>
<td>0</td>
<td>100</td>
<td>NR</td>
<td>51</td>
</tr>
<tr>
<td>Fujita et al.18</td>
<td>03</td>
<td>176</td>
<td>67.04</td>
<td>0</td>
<td>100</td>
<td>NR</td>
<td>47</td>
</tr>
<tr>
<td>Lerut et al.47</td>
<td>04</td>
<td>174</td>
<td>66.70</td>
<td>55</td>
<td>45</td>
<td>46</td>
<td>41.9</td>
</tr>
<tr>
<td>Tachibana et al.48</td>
<td>05</td>
<td>141</td>
<td>50.00</td>
<td>0</td>
<td>100</td>
<td>NR</td>
<td>47.8</td>
</tr>
</tbody>
</table>

A, adenocarcinoma; NR, not report; S, squamous cell carcinoma.

Table 5 Morbidity and mortality after 3-FL

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>n</th>
<th>Hospital mortality (%)</th>
<th>Morbidity (%)</th>
<th>Leak (%)</th>
<th>Recurrent nerve injury (%)</th>
<th>Pulmonary infection (%)</th>
<th>Respiratory failure (%)</th>
<th>Cardiovascular complication (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akiyama et al.2</td>
<td>94</td>
<td>290</td>
<td>5.2</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Altorki et al.37</td>
<td>02</td>
<td>80</td>
<td>3.8</td>
<td>31</td>
<td>11.25</td>
<td>8.7</td>
<td>7.5</td>
<td>16.25</td>
<td>15</td>
</tr>
<tr>
<td>Fujita et al.18</td>
<td>03</td>
<td>176</td>
<td>2.0</td>
<td>NR</td>
<td>28</td>
<td>27</td>
<td>31</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Lerut et al.47</td>
<td>04</td>
<td>174</td>
<td>1.1</td>
<td>42</td>
<td>4.2</td>
<td>2.6</td>
<td>17.7</td>
<td>9.4</td>
<td>10.9</td>
</tr>
<tr>
<td>Tachibana et al.48</td>
<td>05</td>
<td>141</td>
<td>6.4</td>
<td>80</td>
<td>26</td>
<td>28</td>
<td>21</td>
<td>8</td>
<td>14</td>
</tr>
</tbody>
</table>

3-FL, three-field lymph node dissection; NR, not report.
overall survival, which could be maximized when 23 or more regional lymph nodes are removed. Another large international study was based on the Worldwide Esophageal Cancer Collaboration data including 4627 cancer patients who underwent esophagectomy. Improved survival with more extensive lymphadenectomy was seen in both node-negative and node-positive cancer patients except in the extreme group (High-grade dysplasia (Tis) and seven or more lymph nodes positive). For the optimal number of lymph nodes removed, it was recommended that a minimum of 10 nodes for T1, 20 nodes for T2, and 30 or more nodes for T3/T4 cancers should be obtained.

The questionable role of cervical lymph node dissection

A major concern in performing 3-FL is an increased risk of having complications especially recurrent laryngeal nerves injury. Some evidence suggests that cervical dissection may not be suitable for some patients with esophageal cancer. Several points should be taken into consideration for the determination of the most proper extent surgery for each individual.

Pattern of tumor recurrence and isolated recurrence at the neck

One of the potential benefits from neck dissection is to get rid of any unseen tumor cells that hide in the lymph node in order to reduce the chance of locoregional recurrences, which are often detected in the mediastinum, along recurrent laryngeal nerves and intra-abdominal lymph nodes ranging between the rates of 9.7% and 17.5%. These rates are not high when compared with those of distant metastasis ranging between the rates of 9.21% and 37.5%.

In fact, many local recurrences from those reports were synchronously detected with distant metastases indicating a systemic dissemination. When considering only cervical region, the incident of isolated cervical recurrences are quite rare even in non 3-FL studies. Law et al. reported the recurrent pattern in esophageal and gastric cardia cancer patients after undergoing esophagectomy without neck dissection. After 20 months of median follow up, the cervical recurrent rate was 11%. However, only 4% of those recurrences occurred as isolated cervical disease. Dresner et al. showed a very low rate of isolated cervical recurrences (1%) after undergoing esophagectomy with infracarina 2-FL in a group of middle and lower esophageal cancer patients. Mariette et al. published an only 3.6% of isolated cervical recurrences in 439 patients who underwent esophagectomy with 2-FL. The predominant histology of the tumor was squamous cell carcinoma that mostly located in the middle and lower part (68%). Thus, the benefit of cervical lymph node dissection is still in doubt particularly in the lower thoracic tumor.

Recurrent laryngeal node dissection by transthoracic route

The frequency of metastasis in lymph nodes along recurrent laryngeal nerves is high regardless of the location of the tumor in the thoracic esophagus. Clearing lymph nodes in this area is hoped to reduce local recurrence. Anatomically, both recurrent laryngeal nerves ascend from the superior mediastinum through the root of neck, and lymph nodes around the nerves in the mediastinum also continue to the deep cervical group as ‘cervicothoracic node’. In 2-FL, it is possible to remove such a group of nodes via transthoracic approach. Thus, additional neck dissection may add little benefit particularly in the lower esophageal tumor that has a lower frequency of cervical lymph node metastasis.

The impact of the location of lymph nodes removed

Udagawa et al. studied the impact of lymphadenectomy on the survival in 906 patients with thoracic esophageal cancer who underwent 3-FL. They analyzed the correlation between the frequency of lymph nodes metastasis, the location of tumors, and 5-year survival by using the efficacy index (EI), which was previously used in gastric cancer. It was interpreted to mean that a high number of EI in each group of lymph node was associated with a much better 5-year survival. For the tumor at the upper and middle thoracic esophagus, the high numbers of EI were seen in cervical lymph nodes, whereas for the lower thoracic tumor, the values of EI were very low. This suggests that cervical lymph node dissections may have minimal effects on the survival of patients with lower esophageal cancer.

Methods for a more targeted lymphadenectomy

A number of investigations of how to select the patients for being good candidates of 3-FL were proposed in order to diminish the surgical risk while the quality of the oncologic outcome is still maintained. Several investigators reported the efficacy of preoperative ultrasonography at the neck for detecting lymph node metastases in esophageal cancer cases with sensitivity, specificity, and accuracy of 74.5–82%, 94.1%, and 84.6–95% respectively. However, the local recurrence and survival in 2-FL group was not clearly defined in their study. The concept of sentinel node which is now a standard investigation in breast cancer and melanoma has also been examined in esophageal cancer. However, because of the widespread lymphatic drainage of the esophagus, it has been impossible to find such a method that is accurate enough. Some authors used a result of intraoperative pathological examination (IPE) of mediastinal recurrent laryngeal lymph nodes as a sentinel node for predicting metastasis in cervical lymph nodes. If the examination of recurrent nodes was negative
intraoperatively, cervical dissection would be omitted. Among 71 patients undergoing 3-FL, the rate of cervical lymph node metastasis was 40.9% in patients with recurrent nerve nodal positive compared with 10.2% in the negative group (P = 0.007). For another 31 patients who had negative IPE underwent only 2-FL. The rate of local recurrence at the neck was comparable between groups (6.7% after 3-FL vs. 2.6% after 2-FL).

Radical lymphadenectomy in the era of neoadjuvant therapy

Given the benefit of systemic control and downstaging of the tumor, neoadjuvant therapy has been increasingly used for the treatment of thoracic esophageal cancer. Although some studies were conducted with conflicting results, recent meta-analyses were reported supporting its utility. EBE and/or 2-FL are widely used in western countries with a radical intent. The combinations of these radical operations with neoadjuvant treatments were examined. A study by Rizzetto et al. showed the outcomes of 58 patients with esophageal adenocarcinoma undergoing either EBE or THE after receiving neoadjuvant therapy in which 90% of both groups received chemoradiotherapy. Overall 5-year survival and survival in patients with residual disease after neoadjuvant therapy was significantly higher in EBE group (overall survival: 51% vs. 22%; [P = .04]; survival with residual disease: 48% vs 9% [P = .02]). Locoregional recurrences were strikingly higher in THE than in EBE group (16.6% vs. 0%). Another study by Ozcecelik et al. demonstrated long-term outcomes of 114 esophageal cancer patients undergoing EBE. The majority of patients who underwent neoadjuvant treatment that was mostly chemoradiotherapy had clinical stage III cancers. The 5- and 10-year overall survival was 25% and 18%, respectively, and no difference was detected between neoadjuvant and surgery-alone group whose tumors were in the early stage (I and II). Recently, results from a multicenter randomized trial demonstrated that neoadjuvant chemoradiotherapy followed by esophagectomy with transthoracic 2-FL (with THE in cases of tumor at the esophagogastric junction) provided overall survival benefit and more R0 resection rate compared with surgery-alone for resectable esophageal cancer patients regardless of the histologic type of the tumor.

In eastern countries especially Japan, the major type of lymphadenectomy done is 2-FL with lymph node dissection along both recurrent laryngeal nerves in the mediastinum or 3-FL. Given a concern of perioperative complications regarding following the use of radiation, perioperative chemotherapy is the current standard treatment for resectable stage II/III esophageal squamous cell carcinoma, and radiation is used for locally advanced tumors or unresectable diseases. The important data supporting the application of adjuvant and neoadjuvant therapy was reported by the Japan clinical oncology group. The preceding data showed that postoperative adjuvant chemotherapy provided a significantly better 5-year disease-free survival than radical surgery alone to patients with node-positive cancers. Recently, the results of another trial comparing between using chemotherapy before and after esophagectomy with 2- or 3-FL in patients with stage II/III squamous cell carcinoma have been reported. Significantly improved 5-year survival was noted in the preoperative chemotherapy group (53% vs. 43%; P = 0.04). An investigation of the effect of preoperative chemoradiotherapy is preparing to undergo in the near future.

The impact of the number of lymph nodes and the ratio between metastatic lymph nodes and total lymph nodes removed was also investigated in the setting of neoadjuvant therapy. The data showed that either more numbers of lymph nodes retrieved or lower lymph node ratio were an independent predictor of improved survival in esophageal cancer patients who had neoadjuvant therapy followed by esophagectomy. This also suggests the role of lymphadenectomy in neoadjuvant treated patients.

The role of minimally invasive surgery in lymph node dissection

Since its first introduction in 1992, various types of MIE have been increasingly reported. With a minimally invasive method, it has been expected to provide reduced operative morbidity without worsening of the oncologic outcome. When lymph node dissection is concerned, it has to ensure that the quality of lymphadenectomy with MIE can be kept at least as equal as that with the open technique. Many cohort studies were reported with improved operative outcomes when more experience of surgeons was gained. Three meta-analyses were published comparing MIE with open esophagectomy. There was no significant difference in major morbidity and mortality between groups in all studies except in that of Nagpal et al. showing that reduced major pulmonary and anastomosis complications, lower operative blood loss, and shorter hospital stay were in favor of hybrid MIE. Regarding the oncologic outcome, the number of lymph nodes retrieved and 3-year survival was comparable between open and MIE groups in the study of Sgourakis et al. The most recent meta-analysis was reported by Dantoc et al. examining only the difference of oncologic outcomes of MIE versus open esophagectomy. Although no survival advantage was found between techniques, significantly higher numbers of lymph nodes removed were in favor of MIE group. It should be noted with regard to this study that lesser number of lymph nodes yield was apparent in low-experienced centers, and
significant number of lymph nodes could be seen in Japanese series where extended 2-FL and 3-FL were performed. Recently, the first randomized controlled trial comparing short-term outcomes of open esophagectomy and MIE demonstrated that pulmonary infection rate in the first 2 weeks postoperatively and intraoperative blood loss were significantly lower in MIE than in open-esophagectomy group, and no difference in the number of lymph nodes removed and mortality was noted between groups.87

**DISCUSSION AND CONCLUSION**

Regarding the evidence supporting radical lymph node dissection, most comparative studies and meta-analyses do not demonstrate the difference of the benefits of TTE versus THE. However, the survival advantage can be seen in a subgroup of distal esophageal cancers with limited lymph nodes metastasis in one randomized trial.27 A similar trend of results is noted in a cohort study44 comparing EBE with THE. Moreover, better survival outcomes of EBE and 3-FL than lesser extensive surgery can be seen in a number of cohort studies and case series. Studies of the impact of lymph nodes retrieved are also in favor of more lymph node dissection. These suggest that radical lymph node dissection either en bloc resection or at least 2-FL can provide an oncologic advantage especially in cases of distal esophageal tumors. However, this advantage may be limited to the patients who have limited extent of the disease (T1b–T3, N0–N1, M0). It is not justified to perform such aggressive operations in patients with early disease (T1a, N0, M0) because the risk of metastasis is very low, whereas in patients with large number of positive nodes (more than 5–8 nodes), radical surgery may not provide a survival advantage because the disease is probably systemic. Another issue is whether it is worthwhile to perform cervical dissection. Many case series demonstrated impressive oncologic outcomes but at the cost of high morbidity. However, data from cohort studies and outcomes researches regarding the impact of location of lymph nodes removed and pattern of tumor recurrence do not support performing neck dissection in cases of lower esophageal tumors. Therefore, cervical dissection is not recommended in patients with distal esophageal tumor. For proximal tumor, the role of neck dissection is still inconclusive because of limited evidence. Based on the data from some cohort studies, it may provide a potential survival and regional-controlled benefit when performing in rightly selected patients with limited extent of the disease in proximal esophageal cancers and only by a surgeon of vast experience in this field. More studies are required to define such a group of patients that benefits the most from this procedure.

Referring to the role of neoadjuvant therapy, the data suggest that it can enhance survival benefit of the patients with localized resectable esophageal cancer. This potential benefit also can be gained in the setting of radical lymphadenectomy, given results from the recent randomized controlled trials. However, there are some controversies that have to be defined including the optimal extent of lymphadenectomy, the type of neoadjuvant therapy, and the regimes of chemotherapeutic agents used.

Based on the data of MIE, it is suggested that MIE with lymph node dissection can be performed with oncologic outcomes comparable with those of the open technique in selected patients. This procedure should also be done in a center where it is highly capable of MIE for the best outcome. Well-designed comparative or randomized studies are required for more firm evidences.

In conclusion, radical lymph node dissection either at least 2-FL or en bloc resection is beneficial in providing better survival advantage in patients with non-extreme, resectable esophageal cancer. Multimodality treatment especially preoperative chemotherapy with or without radiotherapy should be considered in patients with locally advanced disease or clinically positive lymph node to enhance the oncologic outcome. MIE can be used, but the best outcome can be gained when performing in a highly capable center.

**Acknowledgment**

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**References**


Lymphadenectomy in esophageal cancer


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Exposure to an atomic bomb explosion is a risk factor for in-hospital death after esophagectomy to treat esophageal cancer


Department of Surgery, Hiroshima Red Cross Hospital and Atomic-bomb Survivors Hospital, Hiroshima, Japan

SUMMARY. Esophagectomy, one of the most invasive of all gastrointestinal operations, is associated with a high frequency of postoperative complications and in-hospital mortality. The purpose of the present study was to determine whether exposure to the atomic bomb explosion at Hiroshima in 1945 might be a preoperative risk factor for in-hospital mortality after esophagectomy in esophageal cancer patients. We thus reviewed the outcomes of esophagectomy in 31 atomic bomb survivors with esophageal cancer and 96 controls (also with cancer but without atomic bomb exposure). We compared the incidences of postoperative complications and in-hospital mortality. Of the clinicopathological features studied, mean patient age was significantly higher in atomic bomb survivors than in controls. Of the postoperative complications noted, atomic bomb survivors experienced a longer mean period of endotracheal intubation and higher incidences of severe pulmonary complications, severe anastomotic leakage, and surgical site infection. The factors associated with in-hospital mortality were exposure to the atomic bomb explosion, pulmonary comorbidities, and electrocardiographic abnormalities. Multivariate analysis revealed that exposure to the atomic bomb explosion was an independent significant preoperative risk factor for in-hospital mortality. Exposure to the atomic bomb explosion is thus a preoperative risk factor for in-hospital death after esophagectomy to treat esophageal cancer.

KEY WORDS: atomic bomb survivor, esophageal cancer, esophagectomy, postoperative complication.

INTRODUCTION

Despite advances in various therapeutic modalities, esophagectomy remains the mainstay of treatment for patients with esophageal cancer. Esophagectomy is associated with extremely high morbidity and mortality rates because a thoracoabdominal approach is frequently required. Despite recent advances in surgical, anesthetic, and intensive care techniques, in-hospital mortality after the procedure remains substantial, with rates reported to be up to 11%.1-4 As esophageal cancer usually occurs in the elderly and as many such patients have significant comorbidities, careful preoperative assessment of fitness for surgery and subsequent selection of appropriate candidates are important to improve the short-term outcomes of patients undergoing such surgery.

Interest in the effects of radiation on human health has increased in the time since the disaster that occurred at the Fukushima nuclear power plant secondary to the earthquake and tsunami of March 2011. Much has been learned from the first experience of large-scale exposure to ionizing radiation caused by the atomic bomb dropped on Hiroshima in August 1945. Epidemiological studies of the relationship between cancer incidence and radiation dose have been reported in atomic bomb survivors.5-8 Apart from the well-known increase in leukemia among such survivors, increases in the frequencies of solid cancers, including esophageal cancer, have been noted.5-8 No report has yet examined the postoperative complications experienced by esophageal cancer patients with a history of exposure to the atom bomb explosion. Therefore, in the present study, we reviewed the outcomes of esophagectomy, used to treat esophageal cancer, in 31 atomic bomb survivors and 96 controls, and compared the frequencies of postoperative complications and in-hospital mortality. Further, we
determined whether exposure to atomic bomb explosion could be considered to be a risk factor for in-hospital mortality of patients with esophageal cancer.

PATIENTS AND METHODS

Patients

One hundred twenty-seven Japanese patients with esophageal cancer were surgically treated at the Hiroshima Atomic Bomb Survivors Hospital from January 1993 to December 2012. Of these, 31 (24.4%) had been exposed to the atomic bomb explosion in Hiroshima in 1945. The remaining 96 patients (75.6%) had no history of exposure to the atomic bomb explosion but were also treated and served as controls. On the basis of distance from the hypocenter, the 31 esophageal cancer patients who were atomic bomb survivors were divided into two groups: a closely exposed group (9 cases, including 2 fetuses exposed in utero) that had been at sites within 2.5 km from the hypocenter; and a distantly exposed group (22 cases in all; 11 of whom had been at sites more than 2.6 km from the hypocenter and 11 of whom had been secondarily exposed).

Several clinicopathological factors, the surgical methods used, and postoperative complications were evaluated with reference to the AJCC TNM classifications of esophageal cancer. The criteria of preoperative co-morbidity were defined as previously described. The local ethics committee of Hiroshima Red Cross Hospital and Atomic-bomb Survivors Hospital approved the study. Consent was obtained from either the patient or the patient’s family.

Postoperative complications

Postoperative surgical complications were graded using the Clavien–Dindo classification. The grades were defined as follows. Grade I: any deviation from the normal postoperative course but without any need for pharmacological treatment or surgical, endoscopic, or radiological intervention; Grade II: complications requiring pharmacological treatment; Grade IIIa: complications requiring an intervention that was not performed under general anesthesia; Grade IIIb: complications requiring intervention under general anesthesia; Grade IV: life-threatening complications requiring ICU management; and Grade V: death. Pulmonary complications included pneumonia, acute respiratory distress syndrome, emphyema, atelectasis, pneumothorax, and pleural effusion. Surgical site infections (SSI) are classified as being either incisional or organ/space.

Statistical analysis

The clinicopathological features of and various postoperative complications in the two groups were compared with respect to exposure (or not) to atomic bomb explosion. Significant differences were established using Fisher’s exact test and the chi-squared test was used to examine distribution frequencies. Mann–Whitney’s U-test was used to analyze differences in continuous data between groups. Prognostic preoperative factors were examined using multivariate analysis (a logistic regression model of in-hospital mortality was constructed). A P-value of less than 0.05 was considered significant. Data were analyzed using JMP7 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Clinicopathological findings in esophageal cancer patients and operative features of esophagectomy used as treatment

The clinicopathological features of atomic bomb survivors and control patients are shown in Table 1. Of all factors, only age differed significantly in atomic bomb survivors compared with controls (P = 0.0390), being significantly higher in survivors (66.5 vs. 62.9 years).

In pathological features such as tumor location, histology, depth of tumor invasion, lymph node metastasis, and tumor stage, there were no significant differences between survivors and controls. Compared with distantly exposed survivors, the proportions of T1 patients and Stage I patients were significantly higher in closely exposed survivors (66.7 vs. 18.2%, 55.6 vs. 13.6%, Supporting Information Table S1).

The operative features of atomic bomb survivors and controls are shown in Table 2. We studied all details of operative procedures and reconstruction, operative time, and blood loss. Further, we also analyzed the times at which operations were performed. No significant between-group difference in any of these features was evident.

The number of patients having second primary cancer, before or after esophageal cancer, was 33 (34.4%) and 9 (29.0%) in the controls and atomic bomb survivors, respectively. The incidence of second primary cancer was not different significantly between those groups.

We examined the preoperative hematologic data of atomic bomb survivors and controls. There were no significant differences in hematologic data between those groups.

Postoperative complications in esophageal cancer patients

Details on postoperative courses, postoperative complications, and mortality are shown in Table 3. The duration prior to extubation of the endotracheal tube and the extents of severe pulmonary complications (Grade 4 or above), severe anastomotic leakage
Table 1  Clinicopathological features of control patients and atomic bomb survivors with esophageal cancers

<table>
<thead>
<tr>
<th>Feature</th>
<th>Control patients (n = 96)</th>
<th>Atomic bomb survivors (n = 31)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.9 ± 8.9</td>
<td>66.5 ± 7.6</td>
<td>0.0390</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>74 (77.1)</td>
<td>28 (90.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Female</td>
<td>22 (22.9)</td>
<td>3 (9.7)</td>
<td></td>
</tr>
<tr>
<td>Smoking (Brinkmann Index values)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>265 (28.3)</td>
<td>17 (54.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Yes</td>
<td>391 (41.7)</td>
<td>14 (45.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol (g ingested/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50</td>
<td>11 (17.1)</td>
<td>3 (18.8)</td>
<td>NS</td>
</tr>
<tr>
<td>50-100</td>
<td>36 (58.0)</td>
<td>12 (75.0)</td>
<td>NS</td>
</tr>
<tr>
<td>&gt; 100</td>
<td>36 (58.0)</td>
<td>6 (37.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac</td>
<td>31 (33.3)</td>
<td>8 (25.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>21 (23.6)</td>
<td>6 (19.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Liver</td>
<td>17 (17.9)</td>
<td>7 (22.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Renal</td>
<td>14 (15.0)</td>
<td>1 (3.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes</td>
<td>23 (25.0)</td>
<td>7 (22.6)</td>
<td>NS</td>
</tr>
<tr>
<td>ECG abnormalities</td>
<td>28 (29.5)</td>
<td>14 (45.2)</td>
<td>NS</td>
</tr>
<tr>
<td>FEV1%</td>
<td>50.3 ± 6.4</td>
<td>45.1 ± 3.9</td>
<td>NS</td>
</tr>
<tr>
<td>%VC</td>
<td>13 (13.5)</td>
<td>8 (25.8)</td>
<td>NS</td>
</tr>
<tr>
<td>ASA-PS status</td>
<td>13 (13.8)</td>
<td>8 (25.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Operation period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First decade (1993–2002)</td>
<td>54 (56.3)</td>
<td>15 (48.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Last decade (2003–2012)</td>
<td>43 (45.8)</td>
<td>16 (51.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Tumor location</td>
<td>5 (5.2)</td>
<td>1 (3.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Cervical</td>
<td>85 (89.5)</td>
<td>29 (93.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Thoracic</td>
<td>6 (6.3)</td>
<td>1 (3.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Histology</td>
<td>90 (94.7)</td>
<td>28 (93.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>1 (1.1)</td>
<td>1 (3.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>4 (4.2)</td>
<td>1 (3.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Depth of tumor invasion</td>
<td>32 (33.7)</td>
<td>10 (32.3)</td>
<td>NS</td>
</tr>
<tr>
<td>T1</td>
<td>12 (12.6)</td>
<td>4 (12.9)</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>31 (32.3)</td>
<td>10 (32.3)</td>
<td>NS</td>
</tr>
<tr>
<td>T3</td>
<td>40 (42.1)</td>
<td>11 (35.5)</td>
<td>NS</td>
</tr>
<tr>
<td>T4</td>
<td>43 (45.3)</td>
<td>11 (35.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Stage (UICC)</td>
<td>4 (4.2)</td>
<td>1 (3.2)</td>
<td>NS</td>
</tr>
<tr>
<td>0</td>
<td>33 (35.1)</td>
<td>11 (35.5)</td>
<td>NS</td>
</tr>
<tr>
<td>IA, IB</td>
<td>19 (20.0)</td>
<td>11 (35.5)</td>
<td>NS</td>
</tr>
<tr>
<td>IIIA, IIB, IIIC</td>
<td>10 (10.6)</td>
<td>8 (26.7)</td>
<td>0.0395</td>
</tr>
<tr>
<td>IV</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

%VC, percentage vital capacity; ASA-PS, American Society of Anesthesiologists Physical Status; ECG, electrocardiogram; FEV1%, forced expiratory volume in 1 s/forced vital capacity; NS, not significant.

Table 2  Operative details for control patients and atomic bomb survivors with esophageal cancers

<table>
<thead>
<tr>
<th>Feature</th>
<th>Control patients (n = 96)</th>
<th>Atomic bomb survivors (n = 31)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of esophagectomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6 (6.3)</td>
<td>0 (0.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Subtotal</td>
<td>70 (72.9)</td>
<td>27 (87.1)</td>
<td></td>
</tr>
<tr>
<td>Middle and lower</td>
<td>13 (13.5)</td>
<td>2 (6.5)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>7 (7.3)</td>
<td>2 (6.5)</td>
<td></td>
</tr>
<tr>
<td>Reconstructed organ</td>
<td>89 (95.7)</td>
<td>28 (93.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Stomach</td>
<td>3 (3.2)</td>
<td>1 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>8 (8.4)</td>
<td>2 (6.9)</td>
<td></td>
</tr>
<tr>
<td>Construction of anastomosis</td>
<td>23 (24.0)</td>
<td>10 (34.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Stapler</td>
<td>73 (76.0)</td>
<td>19 (65.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Location of anastomosis</td>
<td>81 (84.4)</td>
<td>27 (90.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Cervical</td>
<td>15 (15.6)</td>
<td>3 (10.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Thoracic</td>
<td>366 ± 121</td>
<td>401 ± 134</td>
<td>NS</td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>615 ± 441</td>
<td>781 ± 653</td>
<td>NS</td>
</tr>
<tr>
<td>Blood loss (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operation period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First decade (1993–2002)</td>
<td>54 (56.3)</td>
<td>15 (48.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Last decade (2003–2012)</td>
<td>42 (43.8)</td>
<td>16 (51.6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant.

Table 3  Postoperative complications in control patients and atomic bomb survivors with esophageal cancers

<table>
<thead>
<tr>
<th>Feature</th>
<th>Control patients (n = 96)</th>
<th>Atomic bomb survivors (n = 31)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of ICU stay (days)</td>
<td>4.0 ± 3.8</td>
<td>4.0 ± 8.8</td>
<td>NS</td>
</tr>
<tr>
<td>Extubation (POD)</td>
<td>1.0 ± 2.5</td>
<td>1.5 ± 8.4</td>
<td>0.0251</td>
</tr>
<tr>
<td>Oral intake (POD)</td>
<td>9.0 ± 21.2</td>
<td>9.0 ± 53.1</td>
<td>NS</td>
</tr>
<tr>
<td>Length of hospital stay (days)</td>
<td>30.0 ± 35.0</td>
<td>32.0 ± 73.8</td>
<td>NS</td>
</tr>
<tr>
<td>Pulmonary complications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I–V</td>
<td>33 (35.1)</td>
<td>11 (35.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Grade IIIa–V</td>
<td>19 (20.2)</td>
<td>10 (33.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Grade IV–V</td>
<td>10 (10.6)</td>
<td>8 (26.7)</td>
<td>0.0395</td>
</tr>
<tr>
<td>Anastomotic leakage</td>
<td>22 (23.4)</td>
<td>9 (30.0)</td>
<td></td>
</tr>
<tr>
<td>Grade I–V</td>
<td>2 (2.1)</td>
<td>6 (20.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Grade IIIa–V</td>
<td>12 (12.8)</td>
<td>8 (26.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Grade IV–V</td>
<td>4 (4.3)</td>
<td>7 (23.3)</td>
<td>0.0042</td>
</tr>
<tr>
<td>Surgical site infection</td>
<td>26 (27.7)</td>
<td>14 (48.3)</td>
<td>0.0422</td>
</tr>
<tr>
<td>30-day mortality</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>NS</td>
</tr>
<tr>
<td>In-hospital mortality</td>
<td>3 (3.1)</td>
<td>5 (16.1)</td>
<td>0.0208</td>
</tr>
</tbody>
</table>

ICU, intensive care unit; NS, not significant; POD, postoperative day.

Factors associated with in-hospital mortality from esophageal cancer

Of the 127 patients, in-hospital death occurred in 8 (6.3%). The associations between in-hospital (Grade 3b or above), surgical site infection, and in-hospital mortality, differed significantly between the two groups (P = 0.0251, 0.0395, 0.0042, 0.0422, and 0.0208, respectively). Atomic bomb survivors experienced a longer period of endotracheal intubation (1.5 postoperative days vs. 1.0), more severe pulmonary complications (26.7% vs. 10.6%), more severe anastomotic leakage (23.3% vs. 4.3%), a higher rate of SSI (48.3% vs. 27.7%), and higher in-hospital mortality (16.1% vs. 3.1%), than did controls.

In terms of distance from the hypocenter, significant differences were evident in the levels of pulmonary complications (all Grades), anastomotic leakage (Grades 3b-5), and in-hospital mortality (Table 4). The rate of pulmonary complications was significantly higher in the closely exposed group than in the distantly and secondarily exposed group (77.8% vs. 19.1%; P = 0.00085). The frequency of severe anastomotic leakage was significantly higher in the closely exposed group than in the other group (33.3% vs. 19.1%; P = 0.0046). In-hospital mortality was also significantly higher in the closely exposed group than in the other group (33.3% vs. 9.1%; p=0.0054).
mortality and candidate preoperative risk factors for such mortality are shown in Table 5. A history of exposure to the atomic bomb explosion, the presence of pulmonary comorbidities, and electrocardiographic abnormalities were all significantly associated with in-hospital mortality ($P = 0.0208, 0.0241,$ and $0.0016$, respectively). More atomic bomb survivors (62.5% vs. 21.9%), more of those with pulmonary comorbidities (62.5% vs. 22.7%), and more of those who were electrocardiographically abnormal (87.5% vs. 28.8%) suffered in-hospital death group than the other group. Greater mean age was associated with a tendency toward in-hospital death group compared with the other group (69.3 years vs. 63.4 years).

Table 4  Postoperative complications in control patients and atomic bomb survivors, with respect to distance from the hypocenter, with esophageal cancers

<table>
<thead>
<tr>
<th>Complications</th>
<th>Controls ($n = 96$)</th>
<th>Distance $&gt;2.6$ km† ($n = 22$)</th>
<th>Distance $&lt;2.5$ km† ($n = 9$)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of ICU stay (days)</td>
<td>4.0 ± 3.8</td>
<td>3.5 ± 5.4</td>
<td>8.0 ± 13.5</td>
<td>NS</td>
</tr>
<tr>
<td>Extubation (POD)</td>
<td>1.0 ± 2.5</td>
<td>1.0 ± 5.6</td>
<td>2.0 ± 13.0</td>
<td>NS</td>
</tr>
<tr>
<td>Oral intake (POD)</td>
<td>9.0 ± 21.2</td>
<td>9.0 ± 59.5</td>
<td>9.0 ± 21.0</td>
<td>NS</td>
</tr>
<tr>
<td>Length of hospital stay (days)</td>
<td>30.0 ± 35.0</td>
<td>31.0 ± 87.1</td>
<td>39.0 ± 35.3</td>
<td>NS</td>
</tr>
<tr>
<td>Pulmonary complications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I-V</td>
<td>33 (35.1)</td>
<td>4 (19.1)</td>
<td>7 (77.8)</td>
<td>0.0085</td>
</tr>
<tr>
<td>Grade IIIa-V</td>
<td>19 (20.2)</td>
<td>4 (19.1)</td>
<td>6 (66.7)</td>
<td>0.0141</td>
</tr>
<tr>
<td>Grade IV-V</td>
<td>10 (10.6)</td>
<td>3 (14.3)</td>
<td>5 (55.6)</td>
<td>0.0042</td>
</tr>
<tr>
<td>Anastomotic leakage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I-V</td>
<td>22 (23.4)</td>
<td>5 (23.8)</td>
<td>4 (44.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Grade IIIa-V</td>
<td>12 (12.8)</td>
<td>4 (19.1)</td>
<td>4 (44.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Grade IIIb-V</td>
<td>4 (4.3)</td>
<td>4 (19.1)</td>
<td>3 (33.3)</td>
<td>0.0046</td>
</tr>
<tr>
<td>Surgical site infection</td>
<td>26 (27.7)</td>
<td>10 (50.0)</td>
<td>4 (44.4)</td>
<td>NS</td>
</tr>
<tr>
<td>30-day mortality</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>NS</td>
</tr>
<tr>
<td>In-hospital mortality</td>
<td>3 (3.1)</td>
<td>2 (9.1)</td>
<td>3 (33.3)</td>
<td>0.0054</td>
</tr>
</tbody>
</table>

†On the basis of distance from the hypocenter, the 31 cases of esophageal cancer in atomic bomb survivors were divided into two groups: a closely exposed group of 9 (including 2 exposed in utero) who had been within 2.5 km from the hypocenter, and a distantly exposed group of 22 cases at sites who had been over 2.6 km from the hypocenter (11 cases) and secondarily exposed (11 cases). ICU, intensive care unit; NS, not significant; POD, postoperative day.

Table 5  Preoperative risk factors associated with in-hospital mortality after esophagectomy

<table>
<thead>
<tr>
<th>Factors</th>
<th>Hospital death group ($n = 8$)</th>
<th>The other group ($n = 119$)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>69.3 ± 8.4</td>
<td>63.4 ± 8.6</td>
<td>0.0522</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>8 (100.0)</td>
<td>94 (79.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (Brinkmann index values)</td>
<td>1038 ± 798</td>
<td>781 ± 637</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol (g ingested/day)</td>
<td>46.7 ± 46.3</td>
<td>51.4 ± 44.5</td>
<td>NS</td>
</tr>
<tr>
<td>Atomic bomb survivors</td>
<td>5 (62.5)</td>
<td>26 (21.9)</td>
<td>0.0208</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac</td>
<td>4 (50.0)</td>
<td>32 (26.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>5 (62.5)</td>
<td>27 (22.7)</td>
<td>0.0241</td>
</tr>
<tr>
<td>Liver</td>
<td>3 (37.5)</td>
<td>20 (16.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Renal</td>
<td>0 (0.0)</td>
<td>3 (2.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0 (0.0)</td>
<td>17 (14.3)</td>
<td>NS</td>
</tr>
<tr>
<td>ECG abnormalities</td>
<td>7 (87.5)</td>
<td>32 (28.8)</td>
<td>0.0016</td>
</tr>
<tr>
<td>Spirometry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1% &lt; 70</td>
<td>3 (37.5)</td>
<td>29 (25.7)</td>
<td>NS</td>
</tr>
<tr>
<td>%VC &lt; 80</td>
<td>0 (0.0)</td>
<td>11 (9.73)</td>
<td>NS</td>
</tr>
<tr>
<td>ASA-PS status</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0 (0.0)</td>
<td>14 (16.5)</td>
<td>NS</td>
</tr>
<tr>
<td>II</td>
<td>5 (83.3)</td>
<td>65 (56.5)</td>
<td>NS</td>
</tr>
<tr>
<td>III</td>
<td>1 (16.7)</td>
<td>6 (7.1)</td>
<td>NS</td>
</tr>
<tr>
<td>IV</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>NS</td>
</tr>
<tr>
<td>TNM classification (UICC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I, II</td>
<td>5 (62.5)</td>
<td>66 (55.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Stage III, IV</td>
<td>3 (37.5)</td>
<td>52 (44.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Residual tumor</td>
<td>1 (12.5)</td>
<td>12 (10.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Operation period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First decade (1993–2002)</td>
<td>5 (62.5)</td>
<td>64 (53.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Last decade (2003–2012)</td>
<td>3 (37.5)</td>
<td>55 (46.2)</td>
<td>NS</td>
</tr>
</tbody>
</table>

%VC, percentage vital capacity; ASA-PS, American Society of Anesthesiologists Physical Status; ECG, electrocardiogram; FEV1%, forced expiratory volume in 1 s/forced vital capacity; NS, not significant.

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Mortality. Multivariate analysis revealed that exposures were all significantly associated with in-hospital comorbidities, and electrocardiographic abnormalities (45.5%), progression of cancer (21.5%), and anastomotic leakage (8.9%). In the current study, we showed that exposure to the atomic bomb explosion, the presence of pulmonary comorbidities, and electrocardiographic abnormalities were all significantly associated with in-hospital mortality. Multivariate analysis revealed that exposure to the atomic bomb explosion and preoperative electrocardiographic abnormalities were independent significant preoperative risk factors for in-hospital mortality. Further, in-hospital mortality, and the frequencies of severe pulmonary complications and anastomotic leakage were significantly higher in the closely exposed group than in the other group. As atomic bomb survivors were significantly older than controls, we speculated that advanced age might contribute to the high mortality rate of survivors. However, the average age of the in-hospital death group was not significantly greater than that of the other group. Moreover, multivariate analysis showed that atomic bomb exposure and electrocardiogram abnormalities were independently associated with in-hospital mortality.

Analyses of mortality over the life spans of atomic bomb survivors have shown that mortality from malignant tumors exceeds control values. After averaging the data in terms of gender and age at exposure, the mortality increase was roughly 10% that of normal cancer rates in those exposed to 0.20 Sv, which was the mean dose delivered to survivors who were within 2.5 km of the Hiroshima hypocenter. Shimizu et al. found that atomic bomb survivors were at a significantly increased risk of mortality from esophageal cancer; the estimated relative risk at 1 Gy was 1.43 (the 90% confidence intervals were 0.08 and 0.67).

Several studies have shown that exposure to radiation due to the atomic bomb explosion causes long-term inflammatory effects, including reduced T-cell numbers in peripheral blood lymphocyte populations and increased plasma levels of proinflammatory cytokines. These effects were evident even over 50 years after radiation exposure. However, major stressful surgery, including esophagectomy, always triggers overproduction of proinflammatory cytokines. Moreover, surgical stress can trigger immunosuppression in response to overproduction of such cytokines. Prognostic relationships between development of complications and immunosuppression after esophagectomy, on the one hand, and survival, on the other hand, have previously been reported in esophageal cancer patients. Further, Deneve et al. reported that patients with HIV and AIDS who had lower CD4 counts were more likely to experience postoperative complications with increased mortality in abdominal operations. These data and references suggest that esophagectomy, a very invasive form of surgery, compromised the immune status of atomic bomb survivors, who were already mounting a persistent inflammatory response, and caused them to develop more severe postoperative complications and exhibit a higher level of in-hospital deaths. With respect to the long-term survival rate after esophagectomy, there were no significant differences between survivors and controls.

Table 6 Multivariate analysis of factors contributing to in-hospital mortality after esophagectomy

<table>
<thead>
<tr>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 65 years vs. over 65</td>
<td>2.858</td>
<td>0.480–25.228</td>
</tr>
<tr>
<td>Atomic bomb survivor</td>
<td>5.896</td>
<td>1.109–37.026</td>
</tr>
<tr>
<td>Respiratory comorbidity</td>
<td>4.910</td>
<td>0.909–31.575</td>
</tr>
<tr>
<td>ECG abnormalities</td>
<td>13.696</td>
<td>2.049–276.027</td>
</tr>
</tbody>
</table>

ECG, electrocardiogram.

DISCUSSION

Esophagectomy, used to treat esophageal cancer, is highly invasive and is frequently associated with in-hospital death secondary to development of postoperative complications. Jamieson et al. reviewed 312 papers containing data on 70 756 patients who underwent esophagectomies between 1990 and 2000 to treat esophageal cancer, and found that the in-hospital mortality rate was 8.8%. WHOoley et al. reported that the leading causes of in-hospital death after esophagectomy were pulmonary complications (45.5%), progression of cancer (21.5%), and anastomotic leakage (8.9%). In the current study, in-hospital death occurred in eight patients (6.3%), and the direct causes in four cases were anastomotic leakage (50.0%). Pulmonary complications occurred in three (37.5%) and cancer-progression in one (12.5%).

A number of studies have explored risk factors for in-hospital mortality following esophagectomy. Such work revealed that older age, the presence of pulmonary and cardiac comorbidities, and pulmonary status were independent risk factors. In the current study, we showed that exposure to the atomic bomb explosion, the presence of pulmonary comorbidities, and electrocardiographic abnormalities were all significantly associated with in-hospital mortality. Multivariate analysis revealed that exposure to the atomic bomb explosion and preoperative electrocardiographic abnormalities were independent significant preoperative risk factors for in-hospital mortality. Further, in-hospital mortality, and the frequencies of severe pulmonary complications and anastomotic leakage were significantly higher in the closely exposed group than in the other group. As atomic bomb survivors were significantly older than controls, we speculated that advanced age might contribute to the high mortality rate of survivors. However, the average age of the in-hospital death group was not significantly greater than that of the other group. Moreover, multivariate analysis showed that atomic bomb exposure and electrocardiogram abnormalities were independently associated with in-hospital mortality.

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In the present study of esophageal cancer patients treated with esophagectomy, we concluded that exposure to the atomic bomb explosion was associated with a longer period of endotracheal intubation, more severe pulmonary complications, more severe anastomotic leakage, and a higher level of in-hospital mortality, compared with controls. The severity of postoperative complications and the rate of in-hospital mortality were higher in survivors who had been at sites within 2.5 km of the hypocenter. A history of exposure to the atomic bomb explosion should thus be recognized as a preoperative risk factor for development of postoperative complications after esophagectomy.

AUTHORS’ CONTRIBUTIONS

Y. Nakashima: designing the study; collecting, analyzing, and interpreting the data; writing the report; making the decision to submit for publication


References


SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Table S1 Pathological features in control patients and atomic bomb survivors, with respect to distance from the hypocenter, with esophageal cancers.
Esophageal squamous cell carcinoma (ESCC): advance in genomics and molecular genetics

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SUMMARY. Esophageal cancer is aggressive and has poor prognosis. Esophageal squamous cell carcinoma (ESCC) is histologically the most prevalent type of esophageal cancer and ranked as the sixth leading cause of cancer death worldwide.1,2 In recent years, cancer has been widely regarded as genetic disease, as well as epigenetic abnormalities including DNA methylation, histone deacetylation, chromatin remodeling, gene imprinting and noncoding RNA regulation. In this review, we will provide a general overview of genes, proteins and microRNAs that are involved in the development of ESCC, which aims to enhance our understanding of molecular mechanisms implicated in ESCC development and progression.

KEY WORDS: esophageal cancer, MicroRNA, proteomics, transcriptomics.

Esophageal squamous cell carcinoma (ESCC) is histologically the most prevalent type of esophageal cancer and ranked as the sixth leading cause of cancer death worldwide.1,2 This neoplasm has a notable ethnic and geographic distribution being of high prevalence in China, Japan, Singapore and Puerto Rico.2,3 In some places such as China and Africa, nutritional deficiencies, intake of pickled vegetables or mycotoxin-contaminated foods and low status of socioeconomics are predispositions to ESCC.4–6 However, in other places like North America and Europe, heavy smoking and alcohol consumption are the main contributions to ESCC.3,9 In high-risk areas, family clustering has been observed in diverse populations, implying the risk factors are both environmental and genetic.10 In this review, we will discuss how the risk factors interact, and the genes, proteins, microRNAs that are involved in the development of ESCC, which aims to enhance our understanding of molecular mechanisms implicated in ESCC development and progression.

GENETIC FACTORS AND GWAS ANALYSIS

Genome-wide association studies (GWAS) is a cost-effective way to use a state-of-the-art research tool to apply a rapid method to analyze the genetic differences between people with a specific disease and healthy individuals.11 Recently, several groups have conducted GWAS studies for ESCC in Japanese and Chinese population.12,13 Three variants in high linkage disequilibrium on 12q24 confer their risks to Japanese ESCC in a gene–lifestyle interaction manner, with more pronounced risk enhancement seen in tobacco and alcohol users.12 Wang et al.13 conducted a GWAS study that also identifies the Chinese ESCC susceptibility loci at PLCE1 and C20orf54. Meanwhile, a shared susceptibility locus for both gastric adenocarcinoma and ESCC was found in PLCE1 at 10q23 (Table 1).14 Moreover, multiple independent associations’ susceptibility loci including 5q11, 6p21 and 21q22 were newly identified.15 Lack of PLCE1 would probably induce an effect on risk of squamous cell carcinoma of the head and neck associated with tobacco and alcohol exposure,16 and deficiency of riboflavin has been documented as a risk factor for ESCC and gastric cardia adenocarcinoma.13 These GWAS studies not only add to the known genetic factors that predispose individuals to
ESCC, but also highlight the importance of genetic factors and genetic heterogeneity in the development of these diseases, which could advance our understanding of the pathogenesis and carcinogenesis of ESCC. And, fine mapping and sequencing in these loci would be required to determine the optimal genetic variants to be studied in laboratory systems to explain these association signals in the future.

TRANSCRIPTOMICS AND SAGE STUDY

Transcriptomics, which can illustrate the molecular mechanisms and regulatory networks, refers to as gene expression profiling, and examines the variety of structure, function and regulation of all transcripts in a given cell.15 Our research group recently study ESCC by using this method, and identify a novel tumor suppressor gene (TSG), PTK6.18 The transcriptome obtained using SAGE technology not only conveys the identification of each expressed gene, but also quantifies its level of expression. In other words, compared with traditional microarray, the major advantage of the SAGE technology is that SAGE generates a library of thousands of expressed genes without any previous knowledge of the tissue’s repertoire. In order to get a better overview of biological events occurring in esophageal adenocarcinoma (EAC) and ESCC, recently, comparison of the SAGE-generated gene expression profiles have identified 1013 genes significantly differentially expressed transcripts,19 which indicates that ESCC is a highly active epithelium in several types of processes, including cell adhesion, signal transduction, cell death, immune response and cell growth.

PROTEOMICS

Proteomics is the entire set of proteins expressed by a transcriptome which can be seen as a precursor of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Alterations of gene, protein, microRNAs investigated in ESCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Alteration</td>
</tr>
<tr>
<td><strong>Gene</strong></td>
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</tr>
<tr>
<td>PLCE1</td>
<td>down</td>
</tr>
<tr>
<td>C20orf54</td>
<td>down</td>
</tr>
<tr>
<td>CHFR</td>
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</tr>
<tr>
<td>PI4</td>
<td>down</td>
</tr>
<tr>
<td>PI5</td>
<td>down</td>
</tr>
<tr>
<td>PI6</td>
<td>down</td>
</tr>
<tr>
<td>RASSF1A</td>
<td>down</td>
</tr>
<tr>
<td>DAPK</td>
<td>down</td>
</tr>
<tr>
<td>RUNX3</td>
<td>down</td>
</tr>
<tr>
<td>UCHL1</td>
<td>down</td>
</tr>
<tr>
<td>ZNF382</td>
<td>down</td>
</tr>
<tr>
<td>UPK1A</td>
<td>down</td>
</tr>
<tr>
<td>FHIT</td>
<td>down</td>
</tr>
<tr>
<td>MLH1</td>
<td>down</td>
</tr>
<tr>
<td>CRABP1</td>
<td>down</td>
</tr>
<tr>
<td>Protein</td>
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</tr>
<tr>
<td>TPM4</td>
<td>up</td>
</tr>
<tr>
<td>PRX1</td>
<td>up</td>
</tr>
<tr>
<td>MnSOD</td>
<td>up</td>
</tr>
<tr>
<td>SCCA1</td>
<td>up</td>
</tr>
<tr>
<td>HP19D1</td>
<td>up</td>
</tr>
<tr>
<td>Laminin receptor</td>
<td>up</td>
</tr>
<tr>
<td>Cathepsin D</td>
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</tr>
<tr>
<td>PK2</td>
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<td>Caretculin</td>
<td>up</td>
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<td>GRP78</td>
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<td>Galectin-7</td>
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<td>CD25B</td>
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<td>Prx VI</td>
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<tr>
<td>Micro RNA</td>
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<tr>
<td>hsa-miR-103/107</td>
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<td>MicroRNA-34b</td>
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<td>MicroRNA-92a</td>
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<td>MicroRNA518b</td>
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<td>MicroRNAlet-7</td>
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<tr>
<td>MicroRNA375</td>
<td>down</td>
</tr>
</tbody>
</table>

2-DE, two-dimensional electrophoresis; ESCC, esophageal squamous cell carcinoma; GWAS, genome-wide association studies.
the proteomics. There are two major strategies used for this method: gel-dependent and gel-independent strategies. In general, most studies on ESCC proteomics use the differences of tumor and its adjacent nontumor tissue to search for the biomarkers followed by immortalized cell lines. Comparative analysis showed that the overexpressions of tropomyosin isoform 4, prohibitin, peroxiredoxin 1 (PRX1) and manganese superoxide dismutase were upregulated in ESCC tissues and cancer cell lines while expressions of stratifin, prohibitin, squamous cell carcinoma antigen 1 were correlated inversely with dedifferentiation of ESCC. Later, five groups reported proteomic signatures associated with ESCC by using ESCC samples collected from different areas from China including low-risk region such as Beijing and Guangdong and some high-risk regions such as Linzhou, Anyang, Xinzhang. Subsequent multivariate analysis by Cox model recovered that upregulation of macrophage migration inhibitory factor and glucose-regulated protein 94 were independent poor prognostic factors, and several other reports revealed the clinical value of potential biomarkers such as HSP60, alpha-actin in 4 and 67 kDa laminin receptor, Prx VI, cathepsin D and PKM2, periplakin, calreticulin and GRP78, galectin-7 and anti-CDC25B antibody (Table 1).

Also, as a tool for determining the clinical utility of the identified proteins provides risk estimates based on multiple prospective studies. Light alcohol intake appears to be associated with alcohol drinking or the risk of ESCC in Asians, especially when coupled with high alcohol intake with risk of ESCC provides risk estimates based on multiple prospective studies. Light alcohol intake appears to be associated to ESCC mainly in studies in Asia, which suggests a possible role of genetic susceptibility factors. Both heterozygote Lys/Gln and (Lys/Gln + Gln/Gln) for XPD codon 751 genetic polymorphism were associated with an increased risk of developing esophageal cancer. Furthermore, heterozygote Lys/Gln and (Lys/Gln + Gln/Gln) for XPD codon 751 genetic polymorphism might have increased the risk of ESCC, but have no association with EAC. Also, Pro variant of TP53Arg72Pro is an important genetic hallmark contributing to ESCC risk.

Including DNA methylation, histone modification, chromatin remodeling and noncoding RNA regulation, epigenetics is an important component of functional genomics, which could be defined as the study of the interplay between the environment and genetics and the heritable changes which are not strictly dependent on the DNA sequence. Aberrant methylation of CpG islands on TSGs is a characteristic epigenetic feature of ESCC genomic DNA. Silencing the promoter of DNA of genes in ESCC could be summarized as cell cycle control genes CHFR, P14, P15, P16, RASSF1A, pro-apoptotic genes such as DAPK, RUNX3, UCHL1, ZNF382, metastasis-antagonizing genes like UPK1A, DNA repair genes FHIT, MLH1, growth factor response-related genes CRBP1, CRABP1 and others (Table 1). Detecting promoter methylation of TSGs has advantages compared with protein or RNA analysis because it is stable and highly sensitive. Thus, DNA methylation assays could be exploited as potent noninvasive diagnostic methods for clinical applications.

**MicroRNAs**

Recently, studies have demonstrated that microRNAs are intimately involved in the processes leading to ESCC. For the first time, Guo et al. have investigated expressed microRNAs in cryopreserved esophageal cancer tissues using advanced microRNA microarray techniques and identified seven microRNAs that...
could distinguish malignant esophageal cancer lesions from adjacent normal tissues, finding high expression of hsa-miR-103/107 correlated with poor survival by univariate analysis as well as by multivariate analysis. MicroRNA-21 targets PDCD4 at the posttranscriptional level and regulates cell proliferation and invasion in ESCC. Also, microRNA-34b-78 and microRNA-92a-79 have an oncogenic role in ESCC. Conversely, microRNA518b, microRNAlet-7p1 and microRNA37552 act as tumor suppressors (Table 1). Interestingly, MicroRNA-141 confers resistance to cisplatin-induced apoptosis by targeting YAP1. All these results indicate that microRNA expression profiles are important diagnostic and prognostic markers and also suggest that microRNAs might serve as biomarkers for response to chemotherapy.

GENE REGULATION NETWORKS IN ESCC

Our combined analyses of the activity spectra and proteomics data showed that the primary signaling pathways in ESCC were mitogen-activated protein kinase, WNT and AKT pathways, which lead the finding of regulatory networks cross-transcription factors, microRNAs and target genes. It is also vital to understand coactivation of ESCC-related TSGs as well as their relationship with microRNA and epigenetic regulation in the development of ESCC, which has prompted to the investigation of the microenvironment, immune surveillance and elimination, cancer cell transformation and invasion. With the increasing understanding of genetic regulation network, development of gene-based or microRNA-based therapy may lead to the eradication of ESCC and related diseases in the future.

CONCLUSION

Driven by both heritable and somatic alterations in DNA, cancer is genetic and epigenetic alterations which underpin not only carcinogenesis but also progression and eventual metastasis. Over the last decade, high-throughput technologies including cDNA microarray, proteomics, transcriptomics, GWAS and microRNA array have allowed us an unprecedented access to a host of cancer genomes, leading us to know more about their pathobiology and network. The challenge now is how to integrate such emerging information into clinical practice to achieve tangible benefits for cancer patients so that experience a shift from curative medicine to predictive and highly personalized medicine.

Acknowledgments

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References

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Translational research on esophageal adenocarcinoma: from cell line to clinic

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SUMMARY. Human esophageal adenocarcinoma (EAC) cell lines have made a substantial contribution to elucidating mechanisms of carcinogenesis and drug discovery. Model research on EAC relies almost entirely on a relatively small set of established tumor cell lines because appropriate animal models are lacking. Nowadays, more than 20% of all fundamental translational research studies regarding EAC are partially or entirely based on these cell lines. The ready availability of these cell lines to investigators worldwide have resulted in more than 250 publications, including many examples of important biomedical discoveries. The high genomic similarities (but certainly not completely identical) between the EAC cell lines and their original tumors provide rational for their use. Recently, in a collaborative effort all available EAC cell lines have been verified resulting in the establishment of a reliable panel of 10 EAC cell lines. It could be expected that the value of these cell lines increases as unlimited source of tumor material because new biomedical techniques require more tumor cells and the supply of viable tumor cells is diminishing because of neoadjuvant chemo(radio)therapy of patients with EAC. Here, we review the history of the EAC cell lines and their utility in translational research and biomedical discovery.

KEY WORDS: Barrett’s esophagus, cell line, esophageal adenocarcinoma, esophagus, in vitro model.

INTRODUCTION

Esophageal cancer is a significant and increasing health problem. In 2008, there were 450 000 new cases worldwide, which ranks this tumor type among the world’s most frequent malignancy.1 Although esophageal squamous cell carcinoma cases have steadily declined, the incidence of esophageal adenocarcinoma (EAC) has increased more rapidly than for any other cancer type. This has led to increased interest in fundamental research on molecular mechanisms underlying the origin of EAC. The current scale of biomedical cancer research on EAC requires an extensive source of tumor materials. It would appear that resection specimens of EAC could satisfy such needs. However, the extensive use of neoadjuvant chemo(radio)therapy, severely limits the availability of viable tumor cells. This has increased the importance of preoperative biopsies, only these biopsies contain a relatively small portion of tumor cells that can be used for research (as e.g. single nucleotide polymorphism arrays or genome-wide sequencing). Larger biopsies are optional but increase the risk of gastrointestinal bleeding and perforation. Taking more biopsies (for research purposes) is impaired by increasingly stringent requirements for protection of patients’ privacy rights by institutional review boards and by government legislation. These developments generate an increasing and ongoing demand for in vivo and in vitro models of EAC. In vivo models have the potential to unravel complex gene-environment interactions and to validate new therapeutic options. Unfortunately, rodents (in particular mice and rats) present several drawbacks to the engineering of a reliable model of EAC carcinogenesis.2,3 In contrast to the human esophagus, the rodent esophageal epithelium is keratinized and lacks submucosal glands. Furthermore, the gastroesophageal junction differs anatomically in that it has a more distal location and is in closer proximity to the pylorus. Also, the development of a genetically modified mouse model has been hindered so far by the absence of an identified gatekeeper mutation. However, recently it has
been shown that mutant mice lacking p63 develop Barrett’s metaplasia in a matter of days, with gene expression patterns nearly identical to those observed in the human condition. In the past, several rat models have been used yielding conflicting results. Some demonstrated a sequence similar to the human situation, whereas others failed to initiate true EAC or even Barrett’s metaplasia. Perhaps the most promising animal models of EAC are the rat esophagogastrroduodenal and esophagojjunostomy model, which generates 62% EACs 8 months after surgery and 26% EAC at 40 weeks after surgery, respectively. Because these surgical rat models have inherent problems, in vitro models have been widely used to attempt to understand the molecular basis of carcinogenesis. Thus far, only a small set of EAC cell lines is available for experimental cancer research. Recently, we verified the authenticity of all available EAC cell lines and confirmed the authenticity of 10 EAC cell lines. In this review, we focus on the utility of these cell lines that have been derived from human EAC for the study of this tumor type.

METHODS

Two searches were performed of the National Library of Medicine database (http://www.nlm.nih.gov/pubs/). The first search was done to determine the total number of publications (published in English) on EAC in the period between January 1, 2000 and December 31, 2009. Therefore, we used the following medical search heading terms (MESH terms): ‘esophageal neoplasms’ and ‘adenocarcinoma’. This search yielded 3229 publications. Eighty-three articles were excluded because the subject was not EAC. Then, we subdivided the publications (n = 3146) into three categories, namely (i) review articles (reviews, comments, editorials, letters and news); (ii) clinical research articles (clinical trials, clinical database research and epidemiological studies); and (iii) fundamental and fundamental translational articles (DNA, RNA, protein, in vitro and animal studies). Within this last category, we searched for studies in which EAC cell lines were used.

A second search was performed to identify the majority of publications that used EAC cell lines. The following MESH terms were used ‘esophageal neoplasms’ and ‘adenocarcinoma’ and ‘tumor cells, cultured’. On May 1, 2011, this search yielded 251 publications. Because we were involved in the establishment of the majority of EAC cell lines, we used our knowledge of literature to select relevant articles that illustrate the major purpose of this review, the utility of cell lines for the study of EAC. It should be noticed that this article is not a comprehensive review of all the literature. References and topics were selected to serve as representative examples.

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The use of EAC cell lines over the past decade

The increase of interest in research on EAC is illustrated by Figure 1. Over the past decade, the total number of studies has increased with 34%. Interestingly, the distribution of the three study types (reviews, clinical research or fundamental research) does not change. Also, the use of EAC cell lines in publications has increased (Fig. 2). In almost 20% of all fundamental research studies on adenocarcinoma of the esophagus, EAC cell lines have been used (Fig. 3). These figures reflect the extent of EAC cell line use in research on this tumor type.

History of EAC cell lines

Human EAC cells were first successfully cultured in Japan in 1984. The first cell line derived from a primary EAC was TE-7. Thereafter, only 16 EAC cell lines have been established namely: SK-GT-4, SK-GT-5 and BE-3; SEG-1, BIC-1 and FLO-1; JROECL9 (OE19), JROECL33 (OE33), JROECL47 and JROECL50; OACM5.1 and OACP4CE; KYAE-1; JH-EsoAd1; ESO26 and ESO51. There are several reasons for the limited number of available human EAC cell lines. At first, the establishment of cell lines is mainly based on trial and error, with relatively low success rates for EAC. The known
number of attempts to establish cell lines is most likely far below the total number of attempts because most failed efforts will not have appeared in literature. Why cell lines from EAC are so hard to establish is poorly understood. It depends on many factors like the number of viable tumor cells, and the ratio between these tumor cells and fibroblasts in the sample. Furthermore, a variety of nutritional and environmental conditions must be met for cells to thrive in culture that are often enigmatic to the establishers resulting in failure of in vitro growth of tumor cells. The major cause of failure appears to be the lack of tumor cell attachment to the plastic flasks and fibroblast overgrowth, as reported by several investigators.11,13,14 Secondly, before the era of multimodality treatment of EAC, the availability of resection specimens with large amounts of viable tumor cells was abundant. The need for an infinite source of material (one of the major advantages of cell lines) was limited, which resulted in a lack of drive among researchers to establish EAC cell lines. At third, several cases of cell line contamination among EAC cell lines have been identified, which reduced the number of reliable EAC cell lines.17,18 Ten cell lines, FLO-1, KYAE-1, SK-GT-4, OE19, OE33, JH-EsoAd1, OACP4C, OACM5.1, ESO26 and ESO51, were proven to derive from their original tissues.8 All these EAC cell lines, together with their genotyping information, have been deposited in publicly available cell line repositories to promote and facilitate future solid research on EAC. In the remaining part of this review, the pros and cons of EAC cell lines use will be discussed.

Advantages of EAC cell lines

Cell lines are populations of pure tumor cells without admixed stromal or inflammatory cells, which greatly aid in tumor cell characterization (Table 1). Furthermore, cell lines are capable of infinite replication, providing a limitless source of materials and permitting their dispersion to laboratories worldwide. This allows scientists to compare their results from identical materials. The relevance of human EAC cell lines depends on how representative these cell lines are as a model for all adenocarcinomas of the esophagus, and on how closely they resemble the tumors from which they were derived. EAC cell lines arise from subpopulations of the original tumor. One of the characteristics of EAC is intratumor heterogeneity, which is thought to be a result of clonal diversity. It has been suggested that duodenogastroesophageal reflux-induced ulceration and inflammation can induce tumor suppressor gene mutations that give rise to multiple distinct clones of metaplastic tissue. Clonal expansion of populations with greater selective advantage leads to dominant and widespread clones.19 To establish an EAC cell line, a (random) sample is taken from a certain area of the tumor, which implies that there is selection of a set of subclones due to sampling. Then, during the cell culturing process, selection of the most robust, fast-growing subpopulation occurs.
Furthermore, it is not surprising that most EAC are derived from poorly differentiated tumors (except cell line JH-EsoAD1 that is derived from a moderately differentiated adenocarcinoma) that already accumulated the mutations required for indefinite growth in vitro. Thus, from an already poorly differentiated EAC, the most undifferentiated subpopulation grows out to a cell culture. Although the selection process that occurs, EAC cell lines (some) retain properties of their corresponding original tumor. Previously, we have demonstrated that the EAC cell lines retain the TP53 mutations that were detected in the primary tumors. Furthermore, a study that genetically characterized four EAC cell lines (OE19, OE33, OACP4CE and OACM5.1) by 24-color fluorescent in situ hybridization and array comparative genomic hybridization showed multiple chromosomal regions of gains and losses. These findings are consistent with data obtained by array comparative genomic hybridization and single nucleotide polymorphism arrays in studies using primary tumor tissue. However, also differences between primary tumors and the corresponding cell lines have been observed. As for example, expression of surface marker HLA-DR has been observed in the primary tumor, of which cell line OE33 has been derived; however, the cell line does not express this marker. These results emphasize the need for more investigations regarding the question how representative these EAC cell lines are for their primary tumor. Moreover, this kind of research has become feasible because the patient’s derived tumor materials have been traced.

Almost all studies using EAC cell lines studies have been performed on adherent cultures of tumor cells. Under these conditions, cells are grown flat, as monocultures on plastic tissue culture plates. This model has several advantages in that cells are easy to grow and maintain, the cultures are pure and free from contaminating cells, and the methods of protein/RNA/DNA extraction are relatively simple. To address the lack of complexity in this adherent culture model, more complex coculture systems have been developed. In these models, epithelial cells are cultured on top of a layer of collagen and fibroblasts, with media being fed into the system form below. Recently, an organotypic culture system with a collagen matrix and a fibroblast feeder layer has been used to describe growth of Barrett’s esophagus cell lines. Only for one EAC cell line (OE19) such an organotypic model has been reported. Also, recently spheroids of EAC cell lines OE19, OE33 and JH-EsoAd1 have been established. These EAC spheroids were maintained in a serum-free medium that is thought to enrich for stem and progenitor cells. Limiting dilution analysis for OE33 and JH-EsoAd1 spheroid cultures showed significantly enhanced clonogenic potential as compared with their parental cell line. In vivo assays are currently performed by transplantation of serially diluted cells into immunocompromised mice to establish the presence and frequency of tumor-initiating cells. Xenograft transplants can be generated from all EAC cell lines (Boonstra et al.; xenografts from cell line FLO-1 were recently obtained by us in mouse strain NOD/SCID/IL2-Rγ(null)). The most widely used translational application of these EAC cell line xenografts has been the in vivo testing of different chemotherapeutic agents such as trastuzumab or temozolomide. More recently, an orthotopic mouse model has been generated by transplanting esophageal tumor fragments (derived from a previous subcutaneously injection of OE19 cells into the flanks of a female NMRI nude mice) into the abdominal esophagus of the mice. Orthotopic transplantation models are thought to represent a more clinically relevant tumor model with respect to tumor site and metastasis; however, its limitations include technical skill, time and cost. Also the therapeutic efficacy is more difficult to assess in contrast to the relative ease of subcutaneous tumor measurements.

As reviewed by Sharma et al., the rapidly expanding use of cancer cell lines to predict the clinical efficacy of new agents is already affecting the course of drug development and is now becoming an important tool for the biotechnology and pharmaceutical industries, in which efforts that are focused on molecularly targeted cancer therapies are accelerating. Genome-wide profiling of copy number alterations of EAC samples and cell lines have identified regions that harbor potential targets for therapy, such as amplifications on chromosomes 7q21-22 and 17q21, harboring the e-MET and

Table 1 Advantages and disadvantages of esophageal adenocarcinoma cell lines

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Pure population of tumor cells</td>
<td>Possible selection of minor tumor subpopulations not characteristic of the original population</td>
</tr>
<tr>
<td>Possibility of wide distribution to investigators worldwide</td>
<td>Possible acceleration of genomic instability</td>
</tr>
<tr>
<td>Limitless replicative ability</td>
<td>Absence of stromal, immune and inflammatory cells</td>
</tr>
<tr>
<td>Availability of in vivo and in vitro tests for the evaluation of invasiveness and tumorigenicity</td>
<td>Absence of vascularization</td>
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<tr>
<td>Ability to utilize a single passage repeatedly</td>
<td>Difficulty of evaluating metastatic potential</td>
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<tr>
<td>Identification of specific genetic, epigenetic and cytogenetic changes and confirmation of their importance to the origin or maintenance of the malignant state</td>
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<tr>
<td>Ability for phenotypic or genotypic selection or manipulation</td>
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<tr>
<td>Growth as substrate dependent or substrate independent cells</td>
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<tr>
<td>Determination of specific environmental conditions or growth factor requirements for optimal growth</td>
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<td>Identification and testing of conventional and novel therapeutic approaches</td>
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<td>Development of models to study multistage pathogenesis</td>
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This table is adapted from Gazdar et al.24
ERBB2 genes, respectively. In vitro testing of a monoclonal antibody against the c-ERBB2 receptor and c-MET inhibitor PHA665752 showed marked growth inhibition in EAC cell lines. These results led to the design of a phase I-II clinical trial among patients with EAC. EAC cell lines do also play a role in studying mechanisms of chemo- and/or radioresistance, which remains a significant clinical problem, with only ~25% of patients achieving a complete pathological response after neoadjuvant chemoradiation therapy.

In Figure 4, we present preliminary data on the chemosensitivity of six EAC cell lines to paclitaxel and carboplatin. In concordance with other literature findings, cell line OE33 is relatively more sensitive for platin than cell line OE19. By continuously exposing drug and/or radiosensitive EAC cells to treatment over a period of time, it is often possible to eliminate the majority of cells while selecting for the expansion of relatively rare drug-resistant clones. Recently, a radio-resistant subline of cell line OE33 has been established. In addition, a recent patent claimed the establishment of 5-fluorouracil-resistant sublines of cell lines OE19 and OE33 (http://www.freepatentsonline.com/; United States Patent Application 20110014303). Variants that are better adapted to the new environment may result in an increased frequency of mutational changes during in vitro growth. However, it has been demonstrated that the mutation rate during prolonged cell culture is limited. Cancer cell lines constantly generate variants with phenotypic and/or genotypic differences from the predominant population caused by exposure to different conditions (such as media, sera, trypsin, carbon dioxide levels, humidity and temperature). Similar to tumor cells in vivo, cells in vitro adapt their phenotype, by epigenetic (potentially reversible) or genetic mechanisms (irreversible), to the conditions to which they are exposed. Variants that are better adapted to the new environment may result in an increased frequency of mutational changes during in vitro growth. However, it has been demonstrated that the mutation rate during prolonged cell culture is limited.
conditions are likely to be selected. In general, the passage number of the EAC cell lines is high (generally above the 50), except for the relatively new established cell lines ESO26, ESO51 and JH-EsoAd1. Cell lines at high passage numbers experience alterations in morphology, response to stimuli, growth rates, protein expression and transfection efficiency compared with low passage cells (http://www.atcc.org/CulturesandProducts/TechnicalSupport/TechnicalLiterature/tabid/580/Default.aspx). Therefore, it can be expected that these differences also occur in EAC cell lines; however, data on this issue is lacking. Despite our efforts to authenticate all available EAC cell lines, cross-contamination or misidentification of EAC cell lines is a continuous threat. To overcome these problems, it is important to standardize the culture methods of the cell lines and to minimize the passage number levels (especially for new EAC cell lines).

CONCLUSIONS

Human EAC cell lines, while not ideal model systems, offer a model to study pathogenesis and that can serve as a filter to fast track the most promising compounds and enables high-throughput science over short periods (such as c-MET and c-ERBB2 inhibitors). It could be expected that the value of these cell lines increases as unlimited source of tumor material becomes available EAC cell lines, cross-contamination or misidentification of EAC cell lines is a continuous threat. To overcome these problems, it is important to standardize the culture methods of the cell lines and to minimize the passage number levels (especially for new EAC cell lines).

References

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Homozygosity in the ApoE 4 polymorphism is associated with dysphagic symptoms in older adults

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SUMMARY. Apolipoprotein E (ApoE) is the most well-described genetic risk factor for Alzheimer’s disease and nonpathological cognitive decline. While possession of the E2 allele may have protective properties, substantial research evidence suggests the E4 allele increases the risk of cognitive degeneration. As neurodegenerative processes are implicated in swallowing dysfunction, we hypothesized that the presence of ApoE 4 would be predictive of dysphagia symptoms in older adults. Eight hundred members of a genetically well characterized community dwelling elderly cohort received the Sydney oropharyngeal dysphagia questionnaire via mail. Cognitive function was also measured using the modified Telephone Interview of Cognitive Status (TiCS-m) and depression with the Geriatric Depression Score (GDS). ApoE allele was genotyped on blood samples from all subjects and data analyzed using standard statistical software (SPSS version 16). Completed questionnaire response rate was 79% (23.5% men, 76.5% women; mean age 81 ± 5 years; range 69–98 years). Possession of one or more of the ApoE 4 and 2 alleles was found in 23.5% and 16%, respectively. Swallowing score was significantly related to GDS (rho 0.133, P < 0.001**) and age (rho 0.107, P < 0.007**) but not general cognitive function as measured by TiCS-m. Self-reported swallowing function was not significantly associated with heterozygosity of any allele or homozygosity for E2 or E3 alleles. Although infrequent (1.1% of all subjects) ApoE E4 homozygosity was significantly associated with higher swallowing scores compared to all other allele combinations (P = 0.033) and while attenuated, was still predicted in multivariate regression modeling (B = 0.812; SE = 0.323; P = 0.012). We report the association between ApoE 4 homozygous genotype and self-reported oropharyngeal dysphagia symptoms in community-dwelling older adults. As this association is weakened by the multivariate analysis and the population frequency of ApoE 4 allele homozygosity is low, this finding while intriguing requires replication in larger independent cohorts.

KEY WORDS: Apolipoprotein E, dysphagia, genetic polymorphism, older adult, swallow questionnaire.

INTRODUCTION

Apolipoprotein E (ApoE), the most abundant brain lipoprotein, has been reported to be the most common genetic risk factor for Alzheimer’s disease.1,2 Additionally, the ApoE gene polymorphism is thought to be a risk factor in vascular dementia,3,4 the second commonest cause of dementia; further Fritze et al.5 showed that the presence of ApoE 4 allele was significantly associated with depression. Recent literature supports the current theory that ApoE 4 could also contribute to cognitive decline in the healthy elderly;6–8 hence there is mounting evidence for ApoE playing a major role in neurodegeneration and functional neurologic performance.

Dysfunction in swallowing is a common complaint among older individuals especially where there may be underlying neurological disease (e.g. stroke). But the factors that predict age-related dysphagia are barely understood. Studies have shown that half of the patients may develop swallowing disorders after stroke.9 These complications include aspiration pneumonia,10,11 malnutrition, and/or a prolonged stay in the hospital.12 Furthermore, neurodegenerative
disorders such as Alzheimer’s and Parkinson’s disease are associated with worsening swallowing performance. Despite this and the growing literature on genetic predisposition to neurologic illness, there are few research reports examining the genes associated with human swallowing in health or disease. Further research in this domain is crucial as genetic polymorphisms enable useful insights at the molecular level in a number of domains of stroke and neurodegenerative research, like for example pathogenesis\textsuperscript{13} and therapy.\textsuperscript{14} Although the genetics of common disorders generally supports polygenic associations of small effect size, single loci with sufficient association can now be detected.\textsuperscript{15} Given the interest in ApoE, and its links to neurological function, we hypothesized that the possession of ApoE 4 may predict dysphagia symptoms in older adults. We therefore conducted a study to examine ApoE genotype interactions with data collected via the a symptom profiling swallowing questionnaire\textsuperscript{16} in a large cohort of healthy elderly subjects.

**METHODS**

**Study population**

Represented the surviving members of the University of Manchester Longitudinal Study of ‘Cognition in Normal Healthy Old Age’ and consisted of 800 individuals living in the Manchester and Newcastle area.\textsuperscript{17} This population was compiled in 1983 to carry out a longitudinal study examining the etiology, nature, extent, time course, and changes in cognitive function of over 6000 normal healthy individuals aged 50 years and over. At initial participation, the cohort had no evidence of cognitive impairment. Since 2003, the surviving actively engaged volunteers in this study have repeated cognitive assessment using the Telephone Interview for Cognitive Status – modified (TICS-m), which is now in its fourth wave in 2011–2012. From this, cognitive performance can be assessed and validated thresholds for impairment are published.\textsuperscript{18}

**Genotyping**

The DNA used in genotyping experiments was extracted from venesected whole blood samples. The genotyping was done performed at the Centre for Integrated Genetic Medical Research at University of Manchester as part of previous study on cognitive ageing in the same cohort. Sequenom (Sequenom Inc, San Diego, USA) was committed using the iPLEX method. This method has been described previously by Ghebranious et al.\textsuperscript{19}

**Swallow questionnaire**

The questionnaire itself contained 17 questions concerning swallowing function. Individuals were asked to enter their responses onto a 105-mm visual analog scale.\textsuperscript{20} The score of 16 questions ranged between 0–100. On the left-hand side of the line, a statement such as ‘Never occurs’ or ‘No difficulty at all’ was noted. The opposing statement could be found on the right-hand side of the scale such as ‘Occurs every time I swallow’ or ‘Unable to swallow at all’. Subjects were asked to mark the point which they felt best represented using a single ‘X’. Consequently, a score of 0–100 for each question could be assessed. The score corresponded to the distance in millimeters from the origin of the scale. Placing a mark within the first 5 mm of the line was considered as zero for that question. On one question, it was feasible to score 0, 20, 40, 60, 80, or 100 depending upon which category the subject placed his mark. The maximum possible total score was 1700. A higher score indicated greater swallowing dysfunction. A score of ≥180 was arbitrarily considered to indicate swallowing difficulty, based on the findings of Wallace et al.\textsuperscript{16} in neurogenic dysphagic patients but recognizing that the study population were not a diseased cohort, such that any dysphagia symptoms might be considered abnormal. We therefore chose to apply cut-offs of 100 and 180 to include subjects with both subtle and more significant dysphagia symptoms.

**Cognitive and mood tests**

The Telephone Interview Cognitive Screen – modified (TICS-m) was performed on the study population (see study population). This is a short screening tool for measuring cognitive performance. The version used in this study was composed of 13 items. The maximum score one could achieve was 39 points.\textsuperscript{21} The TICS-m aims to test orientation, concentration, immediate and delayed memory, naming, calculation, reasoning, and comprehension. A TICS-m score below 21 was set to define ‘cognitive impairment’.\textsuperscript{21} To quantify low mood and depression, the validated Geriatric Depression Scale (GDS) questionnaire (15-item version) was used.\textsuperscript{22} The maximum possible score on the questionnaire to obtain was 15. A score above 5 can be used to indicate case-ness for mild clinical depression.

**Statistical analysis**

All data were analyzed using SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). After visual inspection of the data, nonparametric univariate analyses were performed using Mann–Whitney U’s nonparametric test and Spearman’s nonparametric as appropriate. For multivariate approaches, linear regression models were then calculated with different dependants as the total swallowing score distribution would not support this type of analysis (presence of left skewedness). We therefore split data into tertiles and then used these groups (low, medium, and
high-swallowing score) as the dependant for the regression models. A \( P \)-value of <0.05 was taken to indicate biological statistical significance.

**RESULTS**

**Symptom profiles**

Eight hundred members of the study cohort received the Sydney oro-pharyngeal dysphagia questionnaire via mail. The survey resulted in a final number of 634 completed questionnaires response rate over 79% (23.5% men, 76.5% women; mean age 81 ± 5 years; range 69–98 years). Details are shown in Table 1. A breakdown of the swallowing scores according to age, based across decades, is given in Table 2.

The three most common symptoms found in the participants with scores suggestive of dysphagia (scores >180) were the following: Difficulty swallowing hard foods (question 5), a feeling of food getting stuck in the throat (question 9), a sensation of choking/coughing on swallowing (question 10).

ApoE with the three alleles (E 2, 3, and 4) led to six genotypes with the frequencies as in Table 1. For heterozygotes, the ApoE 2 allele was found in 14.3% and the ApoE 4 allele in 22.3%. These would be in keeping with published genotype frequencies for Caucasians.

In initial correlation analysis, age (\( \rho = 0.107 \), **\( P < 0.007 \)) and GDS score (\( \rho = 0.133 \), **\( P < 0.001 \)) were significantly related to the total swallowing score. In other words, advanced age and high-depression score were accompanied by higher total swallowing score. Using the cut-off 100 and 180, significant relations with age (\( U = 35271, Z = -2.522, *P = 0.012 \); \( U = 23041.5, Z = -3.218, *P = 0.016 \)) and GDS score (\( U = 32910.5, Z = -2.413, *P = 0.001 \); \( U = 21413, Z = -3.126, *P = 0.002 \)) were found. By contrast, there was no association between swallowing score and TICS-m score (\( U = 36958.5, Z = -0.818, P = 0.413 \); \( U = 25455.5, Z = -0.280, P = 0.780 \)). There was also no relationship between gender and the total swallowing scores (\( P = 0.987 \)) or with either cut-off (\( P = 0.536, P = 0.801 \)).

As seen in Figure 1, while ApoE 4 appeared graphically to show higher swallowing scores, given the sample size (\( n = 7 \)) and wide inter-quartile ranges for the more populated genotypes, this was not significant.

Total swallowing score was also not significantly associated with possession of E4 (\( U = 31357, Z = -0.728, P = 0.467 \) or E2 (\( U = 22609, Z = -0.287, P = 0.774 \)) alleles. Moreover, no categorical relationship could be revealed between the six different genotypes and the two predefined swallow score cut-offs (100 and 180). Looking specifically at ApoE 4 homozygosity, there was no association between the cut-off 100 (\( P = 0.593, P = 0.899 \) and 180 (\( P = 0.895, P = 0.875 \)).

As seen in Figure 1, while ApoE 4 appeared graphically to show higher swallowing scores, given the sample size (\( n = 7 \)) and wide inter-quartile ranges for the more populated genotypes, this was not significant.

Table 1: Description of study cohort characteristics

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Mean (Percentage) Range</th>
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<tbody>
<tr>
<td>Male (( n = 149 ))</td>
<td>81 ± 5(years) 23.5% 69–98 (years)</td>
</tr>
<tr>
<td>Female (( n = 485 ))</td>
<td>81 ± 5(years) 76.5% 69–98 (years)</td>
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</tbody>
</table>

Table 2: Analysis of Sydney oro-pharyngeal dysphagia questionnaire according to age by decade

<table>
<thead>
<tr>
<th>Decade (Yrs)</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
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<tr>
<td>60–69</td>
<td>4</td>
<td>96.1850</td>
<td>137.5509</td>
<td>35.2333</td>
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<tr>
<td>70–79</td>
<td>245</td>
<td>78.6452</td>
<td>114.8981</td>
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<td>80–89</td>
<td>350</td>
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<tr>
<td>All</td>
<td>634</td>
<td>96.1850</td>
<td>120.0212</td>
<td>40.0000</td>
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</table>

GDS, Geriatric Depression Scale; TICS-m, Telephone Interview Cognitive Screen-modified.
ApoE 4 homozygotes showing greater swallowing scores ($U = 1179.5$, $Z = -2.130$, *$P = 0.033$).

The examination of the distribution of swallowing questionnaire scores showed a skewed distribution (Fig. 2).

To permit multivariate analysis, the total swallowing score were split into three tertiles (swallowing total score tertiles: lower = 20.0; upper = 73.34) as dependent variables and potential related variables as predictors (listed in Table 3). This showed that the linear regression model significantly predicted tertiles of swallowing score ($r = 0.173$; $r^2 = -0.030$; $P = 0.002$). The significant predictors of higher swallowing questionnaire scores in this model were: older age ($B = 0.014$; SE = 0.007; $P = 0.04$); higher GDS ($B = 0.044$; SE = 0.017; $P = 0.012$); the presence of ApoE 4 homozygosity ($B = 0.812$; SE 0.323; $P = 0.012$).

DISCUSSION

We have reported a novel association between ApoE 4 homozygosity and dysphagia symptoms in older adults. Although the frequency of this genotype is low, no other allele combination showed any effect with swallowing symptoms in this carefully studied population.

In the elderly, swallowing disorders are considered as a substantial problem. They are associated with significant morbidity.$^{24,25}$ Recent studies suggest that dysphagia may be found in up to 16% of the frail elderly.$^{26,27}$ It is also recognized that the older population is at increased risk of dysphagia because of the growing incidence of conditions such as stroke along with age-related impairment of swallowing function.$^{28-30}$ One issue that arises in studies of dysphagia in ‘normal’ populations is how is this defined. In our study, we chose the cut-off of >180 in the Sydney swallowing questionnaire to reflect the
fact that in contrast to diseased populations, any major symptom of dysphagia would be ‘abnormal’. As there is no consensus on this, we recognize that that this figure can only be arbitrary, but our previous studies have supported its use.

In this study, we focused on plausible genetic associations with dysphagia. There was a reassuring lack of interaction between age and genotype when we chose to study ApoE for the reasons given above. This suggests that differences in white matter structure between ApoE 4-carriers and non-carriers are not accompanied by significant differential brain changes with age. Additionally, Filippini et al. studied structural and functional effects of the ApoE polymorphism in young healthy ApoE 4-carriers and matched non-carriers. Their brain activity was investigated both at rest and during an encoding memory paradigm using blood oxygen level-dependent functional magnetic resonance imaging. The encoding task led to greater hippocampal activation in E4-carriers compared to non-carriers. The authors concluded that the E4 allele modulates brain function decades before any clinical or neurophysiological changes with age. The relationship between self-reported dysphagic symptoms and ApoE genotype in our study population is likely to be complex and involve a number of other variables including psychological, environmental, and dietary factors. Moreover, one of the limitations of our data was that we did not capture specific information about functional status and frailty. It is possible that these factors will have influenced the dysphagia scores, although the cohort was considered to be in good health and home dwelling, so would be at the high end of functional performance. ApoE is associated with anatomical and functional brain changes in middle-aged and elderly healthy subjects. It is implicated in brain mechanisms related to white matter development and repair. Generally, there are marked ApoE–isoform specific differences in neuronal protection, repair, and remodeling. The most important mechanisms in each disorder associated with ApoE still have to be clarified. But it can be assumed that the isoform specific differences underlie a genetically determined susceptibility to the outcome from AD and to acute brain injury based on ApoE 4 conferring relative vulnerability. In the future, the examination of ApoE transgenic mice could lead to a greater understanding of the pathophysiological role of ApoE in the central nervous system.

The ApoE genotype is known to be related to cognition and depression. Indeed, Holland et al. previously revealed an association between depression and dysphagic symptoms. These findings are keeping with results reported by Kim et al. The researchers showed that in East Asian population the ApoE 4 allele is a stronger predictor of incident dementia in the presence of depressive syndrome. It should be emphasized that our study found no effect linking cognitive performance to swallow score or ApoE status although this could be a reflection of the small sample size. Nevertheless, it might be speculated that ApoE status, depression, and cognitive impairment are overlapping factors in the presence or absence of swallowing dysfunction, which may become more pronounce during the ageing process. Consequently, cognition, dementia, and swallowing may be closely associated. Cortical regions which are involved in normal swallowing are affected by AD. This includes the anterior cingulate cortex and insula (inferior frontal gyrus pars operculum). Other studies have shown activity in the anteromedial temporal lobe during unimpaired swallowing. This cortical region shows significant atrophy.

Table 3  Results linear regression of age, gender, cognitive performance, mood and homozygosity for Apolipoprotein E4 allele and tertiles of swallowing symptoms

<table>
<thead>
<tr>
<th>Model enter</th>
<th>Unstandardized beta (B)</th>
<th>Standard error</th>
<th>t</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>1.428</td>
<td>0.767</td>
<td>1.862</td>
<td>0.063</td>
</tr>
<tr>
<td>Gender</td>
<td>0.017</td>
<td>0.081</td>
<td>0.209</td>
<td>0.834</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.014</td>
<td>0.007</td>
<td>2.057</td>
<td>0.04</td>
</tr>
<tr>
<td>TICS-m total score</td>
<td>0.002</td>
<td>0.009</td>
<td>0.198</td>
<td>0.843</td>
</tr>
<tr>
<td>GDS total score</td>
<td>0.044</td>
<td>0.017</td>
<td>2.531</td>
<td>0.012</td>
</tr>
<tr>
<td>Apolipoprotein E 4 (homozygous versus other genotype)</td>
<td>0.812</td>
<td>0.323</td>
<td>2.514</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Linear regression with enter model. Dependent: tertiles of Sydney oro-pharyngeal dysphagia questionnaire total score; independents: age (in years), gender (male, female), Telephone Interview Cognitive Screen-modified (TICS-m total score); Geriatric Depression Screen (GDS total score); Apolipoprotein E 4 status (homozygous or other genotype).
in AD.\textsuperscript{40} As a result, cortical regions that are both activated in unimpaired and degenerative swallowing due to AD are overlapping. However, Humbert \textit{et al.}\textsuperscript{24} provided evidence that the neurophysiology of swallowing in early AD involved increased blood oxygen level dependent responses in both unaffected swallowing cortical areas and in regions usually involved in AD. Although memory dysfunction is among the first obvious symptoms of AD, these results imply that the brain areas underlying swallowing function also show signs of compensation before a clinical swallowing examination was carried out. It would be interesting to speculate that AD patients with ApoE 4 alleles and swallowing dysfunction may have differential swallow activation patterns (or reduced compensation) to those without the ApoE 4 allele. Future work may be best directed at looking at the genotype–functional interaction at the brain and swallowing level.

In agreement with the univariate analysis, multivariate regression of the effect of ApoE 4 and total swallowing score accounting for age, mood, and cognitive performance showed the effect of the E4 allele still significant. The current study does not permit further analysis to examine for interactions between E4 allele and these multivariate significant predictors. This again supports the assumption that analyses in larger cohorts with similar phenotype and genotype data are needed to answer this question. It has to be emphasized that the findings while novel, require further validation in a larger cohort as the study population consisted only of 800 individuals living in the United Kingdom.\textsuperscript{17} It has also be kept in mind that the response rate was good but may produce bias compared those who did not respond.

In summary, we have found a novel relationship between ApoE 4 homozygosity and swallowing dysfunction in an otherwise healthy older population. While this finding has physiologic plausibility with respect to changes in brain function, cognitive impairment, and mood, this finding requires replication of the association between dysphagic symptoms and the presence of the ApoE 4 allele in independent larger cohorts.

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DISCLOSURE STATEMENT

There are no actual or potential conflicts of interest.

References