American Gastroenterological Association Institute Technical

Review on The Role of Upper Gastrointestinal Biopsy to Evaluate Dyspepsia in the Absence of Mucosal Lesions

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*QUESTION:* If endoscopic biopsies are sent from any of the aforementioned areas, are specific biopsy techniques recommended and are special stains needed to supplement hematoxylin and eosin (H&E) histologic examination?

**Summary and Discussion**
**Introduction**

Physicians performed more than 7 million esophagogastroduodenoscopy (EGD) procedures in the United States in 2009, a number that is likely higher currently. (1) Mucosal tissue acquisition and analysis are fundamental components of an accurate diagnostic strategy for many upper gastrointestinal (GI) disorders. The techniques of endoscopic tissue acquisition and indications for biopsies have been published previously in review papers and a guideline from the American Society of Gastrointestinal Endoscopy (ASGE). (2-6). The importance of close communication and a cooperative interaction between the endoscopist and pathologist in determining appropriate biopsy techniques, tissue identification, tissue transport and histological preparation including the use of special stains has been emphasized previously. (7)

This Technical Review summarizes existing literature that focuses directly on the question of whether endoscopic tissue sampling obtained during an EGD performed in an adult with dyspepsia and normal appearing mucosa would alter patient health outcomes compared to not obtaining a biopsy. Secondly, if biopsies are obtained in this situation, is there a role of special staining techniques and preparation beyond standard hematoxylin and eosin (H/E) stain and analysis. Third, is there evidence to support specific biopsy practice patterns, such as the use of multiple sample jars for a single organ (esophagus, stomach and duodenum). Finally, when biopsies are obtained from normal appearing mucosa do the results affect important health outcomes.
In the last decade, image-enhanced endoscopy (IEE) has come into widespread use by many endoscopists. IEE is accomplished through the use of either dyes applied during the exam (chromoendoscopy) or is instrument based and is discussed below (see section on the esophagus). These techniques are reviewed in detail elsewhere. (8) IEE is most useful to identify neoplastic lesions of both the upper and lower GI tract and usually follows careful inspection with white light imaging. In addition, such techniques can help identify intestinal metaplasia and even subtle changes of celiac sprue. IEE may obviate the need for tissue sampling and these techniques will be briefly discussed within appropriate sections of this article.

Payers determine rates of biopsy during EGD by analyzing administrative (coding) data submitted using the Healthcare Common Procedure Coding System (HCPCS) and its Level One Common Procedural Terminology (CPT) codes for EGD and anatomic pathology. The CPT code for diagnostic EGD is 43235 and for EGD with biopsy it is 43239. The most common anatomic pathology codes used for endoscopic biopsies are 88305 (Level 4 pathology analysis) with CPT codes 88312, 88313 and 88342 used for most special stains applied to endoscopy biopsies. (9)

Recently, the use and potential overuse of endoscopy biopsies has been a focus of the United States General Accounting Office (GAO) (10). The GAO analyzed biopsy rates for procedures performed by gastroenterologists, dermatologists and urologists and found that approximately 66 percent of the $1.94 billion in expenditures for anatomic pathology in 2010 were for CPT code 88305 with most of these codes linked to procedures performed within these three specialties. The GAO
also noted that when a physician practice owned an anatomic pathology laboratory (codes for the procedure and anatomic pathology originated in the same site of service or Tax Identification Number), the rates of biopsy were substantially higher compared with practices that did not own a pathology laboratory. Finally, they found that when practices acquired pathology laboratories, the rates of biopsy rose by approximately 25% compared to the practice’s own biopsy rates prior to acquisition of a lab. This has prompted some members of Congress to request a review of Section 1877 of the Social Security Act, commonly known as the “Stark Law” that covers exceptions to the Center for Medicare and Medicaid’s (CMS) ban on physician self-referral. (11) Currently, pathology services that are an integral part of a physician practice are exempt under Stark Laws. These practices may suggest to some that the decision to biopsy during EGD is not solely based on clinical judgment.

With this and other factors in mind, the American Gastroenterological Association Institute (AGA) Governing Board determined that there was need for critical analysis of current literature (Technical Review) and a Medical Position Statement (MPS or guideline) concerning the appropriateness of endoscopic biopsies during EGD, especially when no visually apparent lesions are noted. The decision to biopsy mucosa is straightforward when lesions are visible, but the decision is less clear when the examination of the esophagus, stomach and duodenum all are normal in appearance. Therefore, this review summarizes current literature on the health impact of endoscopic biopsies obtained during an EGD performed in an adult with dyspepsia but a normal appearance. Specifically, we
sought to determine whether such biopsies would reveal disorders or diseases that would have not been found without a biopsy. Furthermore, we wondered whether disorders found in this manner changed clinical decisions, improved the value of care or added to patients’ quality of life.

**Methods**

**Overview**

This review collects and evaluates pertinent literature concerning endoscopic biopsies of the upper GI tract for the AGA’s Medical Position Panel, which in turn will produce the final Medical Position Statement (MPS) as described previously. (12) Methods for deriving focused clinical questions, systematically reviewing and rating the quality of evidence for each outcome, and rating the overall quality of evidence were based on the *Grading of Recommendations Assessment, Development and Evaluation* (GRADE) framework, which have been described in detail. (13-27) The PICO format frames a clinical question by defining a specific patient population (P), intervention (I), comparator (C), and outcome(s). We divided the upper GI tract into three anatomic sections (esophagus, stomach and duodenum) and asked two fundamental questions for each section as follows:

1. In the defined population, do biopsies from the normal appearing anatomic segment lead to a diagnosis that would have been missed had biopsies not been taken, and does this change clinical outcomes?

2. If endoscopic biopsies are sent from any of the aforementioned areas, are specific biopsy techniques recommended and are special stains
needed to supplement hematoxylin and eosin (H&E) histologic examination?

Within each anatomic section, we defined critical disorders or diseases that would be found during an endoscopic examination and discussed the role of biopsies in the face of a normal appearing mucosa identified at endoscopy using white light high definition imaging. Finally, we determined if the presence of an immune competent or immune compromised state led to different criteria and thresholds for obtaining biopsies.

**Types of Participants, Interventions and Comparators**

Population: This TR is focused on patients greater than 18 years of age who present for endoscopy with dyspepsia and no alarm symptoms (dysphagia, anemia, bleeding, weight loss) combined with normal appearing mucosa on EGD. Erythema was classified as normal appearance of the mucosa. In studies that reported outcomes for patients with erythema separately from those with normal appearance, we reclassified erythema as “normal” and recalculated outcomes accordingly.

Intervention: The intervention for each question is routine tissue acquisition and analysis. A secondary intervention (Question # 2) would be the use of any special stain beyond routine H&E staining.

Comparator: The intervention (biopsy) is compared to no biopsy. The use of special stains is compared to restricting histologic examination to H&E staining only.
Outcome: If biopsies are sent how accurately is the disease of interest identified in the absence of gross endoscopic findings? What is the likelihood of changing health outcomes of the patient? What is the accuracy of the biopsies for identifying disorders, and are there additional aids (special stains for example) that would add to biopsy accuracy? We assumed no prior treatment for Helicobacter pylori (HP). Finally, do biopsies obtained in a single organ but at different locations necessitate individual processing or may they be grouped together as the latter may also provide significant cost savings.

Outcomes of Interest

We defined disorders or diseases of interest that would be identified through the use of biopsy and attempted to prioritize outcomes by patient importance, ability to treat and whether treatment would improve patients’ health. The relative importance of each outcome was discussed during conference calls and consensus opinion was used in ranking when no firm published data existed. Only critical and important diseases and their outcomes were included in the results and discussion sections. If decisions were made based on a population studied, we favored studies focused on Western populations.

Harmful outcomes, due to adverse events derived from treatment of disorders found on biopsy or the negative effects of not diagnosing a disorder in the absence of a biopsy were reviewed as they may factor in final MPP recommendations. We assumed a very low rate of harm due to the biopsy itself. The minimal clinically important difference (often referred to as the smallest difference that clinicians and patients care about) is useful for decision making, as it
represents the threshold for a clinically meaningful improvement for an individual patient. The difficulty in setting this threshold lies in assigning an objective threshold to a subjective metric especially since much of the literature reviewed for this guideline is twenty to thirty years old and based on subjective analysis or observational studies.

**Information Sources and Study Selection**

A GRADE methodologist (GL), with input from the authors and a medical librarian (Kellee Kaulback), developed and conducted several serial literature searches. The following bibliographic databases were searched through the OVID interface: MEDLINE, MEDLINE In-Process & Other Non-Indexed Citations, EMBASE and Cochrane Central Register of Controlled Trials (CENTRAL) on November 18, 2013. The main search strategy comprised of controlled vocabulary, including the National Library of Medicine’s Medical Subject Headings (MeSH) that were exploded, as well as free text words. The main terms included and combined were terms related to “biopsy”, “histology”, “microscopy”, “immunohistochemistry” or “staining” combined with terms related to “dyspepsia”. The exact synthesis of the main search strategy is provided in Appendix 1.

The results were limited to English language and humans. We applied filters to exclude conference abstracts, editorials, letters to the editor and case reports. No limitations were applied on the year of publication. The criterion for selecting studies for this technical review was compatibility with the PICO frame (population, interventions, comparator and outcomes) as defined above. Each title and abstract
of the references identified by the search was independently checked by at least two authors, who removed obviously irrelevant reports. The full text of all potentially relevant studies was obtained and assessed for relevance to this project, again by at least two authors. The final decisions on inclusion were based on consensus. We included primary studies and systematic reviews. In selecting studies, we followed the umbrella systematic review approach in which we identified published systematic reviews that fit predetermined eligibility criteria and ranked articles based on publication type and methodological rigor. (28)

A systematic review was eligible for inclusion if it evaluated the aforementioned outcomes of interest (outcomes important to patients). We supplemented this by trying to identify any additional randomized controlled trials (RCTs) not included in the systematic reviews as well as references of relevant articles from the systematic reviews. When the systematic reviews did not provide sufficient information for us to fully apply the GRADE approach (such as rigorous assessment of risk of bias, assessment of heterogeneity, adequate description of studies to judge directness) we attempted to retrieve and assess the individual primary studies. When systematic reviews were not up to date or were incomplete, we performed additional focused literature searches in MEDLINE and attempted to perform our own systematic reviews and meta-analyses using the Cochrane Collaboration’s RevMan 5.3 software. (29)

Evaluating the Evidence: Risk of Bias and Study Quality Appraisal

Within the GRADE framework, RCTs start as high-quality evidence but can be rated down for 5 possible reasons. Using GRADE, the quality of evidence for each
outcome was evaluated for the following domains: risk of bias, inconsistency, indirectness, imprecision, and publication bias (see Glossary of Terms in Supplementary Methods). When the systematic reviews did not provide sufficient information to judge the quality of the evidence, individual studies were retrieved. Evidence ratings and qualitative judgments were determined via video conference discussion and consensus. For each question, an overall judgment of quality of evidence was made for a body of evidence that encompassed all critical outcomes.

When available, quantitative estimates of effect were applied from existing systematic reviews. Quality of evidence was presented in the form of answers to the two major questions for each anatomic region as discussed above. Answers to the question are organized around major conditions of interest for each organ.

**Results**

**Esophagus**

**QUESTION:** In the defined population, do biopsies from the esophagus lead to diagnoses that would have been missed had biopsies not been taken, and does this change clinical outcomes?

**RESULTS FOR IMMUNE COMPETENT PATIENTS:**

**Gastroesophageal Reflux Disease (GERD)**

In the presence of endoscopically normal appearing mucosa, the clinical question would be whether “microscopic” GERD is an important diagnosis to
establish and does it change management in a way that adds value to a patient’s health or quality of life.

Multiple histologic variations from normal have been described in patients with GERD in the absence of endoscopically evident ulceration, erosions or other abnormalities. These findings include: (a) increased thickness of basal cell layer; (b) increased length of epithelial papillae; (c) increased number of intraepithelial cells; (d) presence of neutrophils, eosinophils and lymphocytes; (e) ballooning of epithelial cells; (f) dilation of intercellular spaces; and, (g) increase in ectasia and number of intrapapillary capillaries (30). The most common variations used to diagnose reflux-induced changes include basal cell hyperplasia (BCH), papillae elongation (PE), and dilatation of intercellular spaces (DIS) (30-32). These variations from normal have been documented in both erosive and non-erosive disease found in patients with established GERD and with experimental induction of gastroesophageal reflux. Earlier studies using electron microscopy in non-erosive reflux disease (NERD) patients suggest that DIS may be the earliest form of injury in GERD. (33-34) Furthermore, these histologic findings have been shown to improve with acid suppressing therapy (35-38). Unfortunately, resolution of these findings is not consistently complete.

When these abnormalities are combined to form a histologic score, they have been found to be valuable in distinguishing NERD and hypersensitive esophagus from functional heartburn (FH) when groups are defined by a pH/impedance study and symptom index. (31) Specifically, the presence of microscopic esophagitis was able to differentiate patients with NERD from those with FH and healthy volunteers
with an accuracy of 79% (95% CI 70–88%), a sensitivity of 74%, a specificity of 86%, with positive and negative predictive values of 86% and 73%, respectively. DIS and PE were found to be the most sensitive markers of NERD. Another recent study also documented good sensitivity (85%) and specificity (65%) in distinguishing hypersensitive esophagus from GERD. (39)

There are several limitations of these studies. As pH/impedance was used as the gold standard in these studies, it is difficult to know if microscopic esophagitis represents a better gold standard. To establish such a gold standard, one would require, randomized prospective trials comparing microscopic changes and pH monitoring to another standard, for example response to therapy. Furthermore, important questions concerning standard definition of findings and number of esophageal biopsies needed for microscopic GERD diagnosis have not been published. (32) A recent meta-analysis suggests that it is inadequate to use a single histologic abnormality to define GERD. (40) Location of esophageal biopsies may also influence the sensitivity of histologic findings in GERD. (41)

Finally, the lack of specificity in reference to other esophageal disease is another limitation to the use of histologic markers of gastroesophageal reflux. For example, all of the findings listed above have been documented in patients with eosinophilic esophagitis, particularly DIS. (42)

Thus, there is no recognized health benefit to justify performance of endoscopic esophageal mucosal tissue sampling to diagnosis GERD in patients presenting with dyspepsia and having a normal appearing esophagus at endoscopy, although the lack of any RCT, or even observational studies specifically addressing
this question would relegate the quality of evidence to very low. This conclusion also has been endorsed by a group of clinical GERD experts during a recent consensus conference on NERD where there was a 95% agreement that biopsy-based abnormalities are insufficiently validated to be used as a measure of health outcomes in clinical trials. (43). The evidence on the role of esophageal biopsies for the diagnosis of GERD is summarized in Table 1.

Table # 1. Summary of evidence table for the question “In the defined patient population, do biopsies from the esophagus lead to diagnosis of reflux esophagitis that would have been missed if no biopsies had been taken, and does this change clinical outcomes?”

<table>
<thead>
<tr>
<th>All patients (immune competent and immune compromised)</th>
<th>Quality assessment</th>
<th>Summary of Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (studies)</td>
<td>Risk of bias</td>
<td>Inconsistency</td>
</tr>
<tr>
<td>Diagnosis of histological reflux esophagitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(IMPORTANCE OF OUTCOME: Not important for decision making)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43 participants ¹ (1 cross-sectional study)</td>
<td>Boyd 1996</td>
<td>serious ²</td>
</tr>
<tr>
<td>Effect on clinical outcomes ¹ by treating histological reflux esophagitis that was diagnosed by biopsying normal-looking mucosa (IMPORTANCE OF OUTCOME: important but not critical for decision making)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No studies identified</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Eosinophilic Esophagitis (EoE)

EoE is now a common esophageal disease with an estimated prevalence of 0.4% in a Swedish population. (44-46) In adults, dysphagia is the most common presenting symptom. (47) In one series from a U.S. nationwide database of patients

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¹ 43 out of 66 patients had normal endoscopy
² Histological criteria not validated; only 2 esophageal biopsies were taken; unclear if the pathologist was blinded (most likely not)
³ 23% of the participants had GERD symptoms in addition to dyspeptic symptoms; study population (United Arab Emirates) with high prevalence of \(H. pylori\) infection (50% by histology)
⁴ Small sample size
⁵ By definition, the disorder wouldn’t have been diagnosed if biopsies hadn’t been obtained, because on endoscopy the mucosa looked normal
⁶ The relative effect is not applicable. It will be infinite, because the diagnostic rate without biopsies is zero.
⁷ Resolution or improvement of dyspeptic symptoms, changes in quality of life, effect on survival/mortality after treating the disorder (histological reflux esophagitis) that was diagnosed by biopsying normal-looking mucosa
with esophageal eosinophilia, 71% of patients had dysphagia, 27% had heartburn and 14% had abdominal pain or dyspepsia. Notably, patients in this study were not comprehensively evaluated for EoE. In a study in which EoE was more rigorously defined, the most frequent symptoms were dysphagia (70.1%), heartburn (47%), chest pain (29%) and epigastric pain (29%). (48) If patients have heartburn like dyspepsia as the predominant symptom that prompts evaluation, the prevalence of EoE is low. For example, when 66 patients with refractory symptomatic heartburn were evaluated for EoE, there was a prevalence of 8.8%. (49) The majority of these patients also had dysphagia, a symptom that would have initiated endoscopic evaluation with biopsy (an alarm symptom), and all had an atopic history. In a study of patients with PPI-refractory heartburn alone, only 1 of 105 patients had EoE. (50) No studies specifically address dyspepsia as a presenting symptom of EoE in adults. Thus, dyspepsia as a presenting symptom in adults with EoE is not well characterized and should be considered uncommon. Symptoms more likely to suggest EoE include dysphagia and/or a history of atopic disease. Additionally, it is uncommon for adult patients with EoE to have a normal appearing esophagus on endoscopy. Whereas earlier studies suggested normal appearance in up to 10-20% of patients, (51) more recent studies suggest that a normal appearing esophagus even using white-light imaging, is found in less than 10% of patients. (52-53) In a recent meta-analysis, a finding of at least one endoscopic abnormality was found in 93% of patients with EoE. (54) Thus, in addition to noting that dyspepsia alone is an uncommon presenting symptom of EoE, a normal appearing esophagus in the
presence of EoE also is uncommon, making the combination of these two characteristics in an adult patient with EoE even more unlikely.

Therefore, as was the case with GERD, there is no clear evidence that obtaining biopsies from an esophagus with an endoscopically normal appearance in an adult patient with dyspepsia without alarm symptoms for the purpose of diagnosing EoE can change clinical outcomes. The quality of evidence for this statement is moderate based on multiple studies suggesting that EoE is found almost always in the face of endoscopic abnormalities. It should be noted that when biopsies for EoE are sent (because of symptoms or other reasons to suspect EoE), most experienced pathologists prefer biopsies obtained from both the mid and distal esophagus and placed in separate (labeled) jars, a practice that vastly improves the ability to distinguish EoE from GERD.

The evidence on the role of esophageal biopsies for the diagnosis of EoE is summarized in Table 2.

While not the subject of this TR, it should be noted that in children with EoE, the above arguments do not hold true since dyspepsia and abdominal pain are more common presenting symptoms. (47, 55-57) Thus, in contrast to adults, performance of esophageal biopsies in children with dyspepsia and a normal appearing esophagus is warranted to search for EoE.

Table #2. Summary of evidence table for the question "In the defined patient population, do biopsies from the esophagus lead to diagnosis of esophageal eosinophilia that would have been missed if no biopsies had been taken, and does this change clinical outcomes?"

<table>
<thead>
<tr>
<th>All patients (immune competent and immune compromised)</th>
<th>Quality assessment</th>
<th>Summary of Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk of bias</td>
<td>Inconsistency</td>
<td>Indirectness</td>
</tr>
<tr>
<td>--------------------------------------------------------</td>
<td>--------------------</td>
<td>---------------------</td>
</tr>
</tbody>
</table>
Participants (studies)

<table>
<thead>
<tr>
<th>Diagnosis of esophageal eosinophilia (IMPORTANCE OF OUTCOME: Important but not critical for decision making)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 participants (1 cross-sectional study)</td>
</tr>
<tr>
<td>• Ronkainen 2007</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No biopsies taken</th>
<th>Biopsies taken</th>
<th>[95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not serious</td>
<td>Serious</td>
<td>NA</td>
</tr>
<tr>
<td>Biopsies taken</td>
<td>Very Low</td>
<td>0.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Effect on clinical outcomes by treating esophageal eosinophilia that was diagnosed by biopsying normal-looking mucosa (IMPORTANCE OF OUTCOME: Important but not critical for decision making)

<table>
<thead>
<tr>
<th>No studies identified</th>
</tr>
</thead>
</table>

Intestinal Metaplasia

Esophageal adenocarcinoma (EAC) is a lethal disease and there is evidence that diagnosing and appropriate management of its precursor, Barrett’s esophagus (BE), may reduce risk of disease progression and mortality in some selected patients. There is also evidence that intestinal metaplasia of the cardia is a metaplastic phenomenon (58-59) not present in early childhood, and develops as a result of acid reflux. (60) Finally, there is substantial evidence that the precursor cell for BE originates in the gastric cardia. (61) Although these data and others ostensibly make a compelling argument to screen for intestinal metaplasia confined to the cardia (cardia intestinal metaplasia or CIM) in a patient with dyspepsia and a normal appearing GE junction, there are no data that demonstrate finding CIM in the absence of overt endoscopic changes of BE leads to any health benefit related to GERD or EAC. Only one published study provided information about long-term consequences of CIM. (62) In this population-based cohort study set in Olmsted

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* The results for individuals with ≥ 15 eosinophils were not reported separately for those who had dyspepsia without heartburn or dyspepsia with only mild heartburn

* By definition, the disorder wouldn’t have been diagnosed if biopsies hadn’t been obtained, because on endoscopy the mucosa looked normal

* Comment to technical review authors, not to be published: I have only counted the definite EoE cases. Please advise if I should add the probable as well.

* The relative effect is not applicable. It will be infinite, because the diagnostic rate without biopsies is zero.
County, Minnesota, 401 patients with Barrett’s esophagus (columnar segment >1 cm with intestinal metaplasia) and 86 patients with intestinal metaplasia in biopsies from the gastroesophageal junction (IMGEJ) diagnosed from 1976 to 2006 were followed for a median interval of 7-8 years. No patient with IMGEJ progressed to EAC in contrast to BE subjects with visible BE who had a cumulative risk of progression of 7% at 10 years and increased risk of death from EAC (standardized mortality ratio 9.62). The overall survival of subjects with BE and IMGEJ did not differ from that expected in similar age- and sex-distributed white Minnesota populations. Whether these data suggest that biologically, CIM is a different lesion compared to intestinal metaplasia of the esophagus or that mechanistic transformation to dysplasia and cancer is similar but less likely or far lower for the former is unclear. These findings also need to be taken in context with the commonality of CIM. In the first study of CIM, 18% of patients undergoing endoscopy for any reason had IMGEJ. In a similar study, in an unselected group of patients undergoing endoscopy in a Veteran’s Affairs Hospital, Morales and colleagues found CIM in 24 (23%) of 104 patients, a prevalence similar to another study reported by Dias Pereira and colleagues finding IMGEJ in 25% of patients. Although other studies have demonstrated a prevalence as low as 5%, these data still emphasize the ubiquity of this pathologic finding and the implications for a diagnosis for which further medical interventions have not been proven to be of any health benefit. Furthermore, the finding of IMGEJ on initial endoscopy may not be confirmed on repeat endoscopy. In one study of 85 patients with IMGEJ initially identified, only 10 patients had this pathologic finding.
reconfirmed with repeat biopsies approximately 1 year later. (67) As a result, the frequent finding of IMGEJ, its unreliability in follow up biopsies, the lack of clear data suggesting that dysplasia is a common outcome and the substantial cost of a potential surveillance program mitigate against a cardia biopsy in patients with dyspepsia and no endoscopic evidence suggesting BE.

An important limitation of this statement rests, however, in defining the cardia endoscopically and characterizing what is a normal squamocolumnar junction. These recommendations apply to what the endoscopist views as a normal squamocolumnar junction and variability among endoscopists in distinguishing a normal squamocolumnar junction from endoscopically appearing Barrett’s has been demonstrated. (68) Whether these guidelines will apply to short lengths of columnar appearing mucosa in the esophagus that might not fulfill criteria for Barrett’s metaplasia by some guidelines is unclear.

We conclude that there is no evidence that obtaining an endoscopic biopsy from a normal appearing squamo-columnar junction in a patient with dyspepsia for the purpose of finding intestinal metaplasia affects patient health outcomes. The quality of evidence for this statement is very low due to the presence of only a single long-term observational study (and no RCT) that approaches this question.

The evidence on the role of endoscopic biopsies for the diagnosis of CIM is summarized in Table 3.

Table #3. Summary of evidence table for the question “In the defined patient population, do biopsies from the esophagus lead to diagnosis of intestinal metaplasia at the GEJ that would have been missed if no biopsies had been taken, and does this change clinical outcomes?”
<table>
<thead>
<tr>
<th>Participants (studies)</th>
<th>No biopsies taken</th>
<th>Biopsies taken (95% CI)</th>
<th>No biopsies taken</th>
<th>Biopsies taken (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis of intestinal metaplasia at the gastroesophageal junction</td>
<td>Importance of outcome: important but not critical for decision making</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No studies identified</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Effect on clinical outcomes by treating IM at the GEJ that was diagnosed by biopsying normal-looking mucosa</th>
<th>Importance of outcome: important but not critical for decision making</th>
</tr>
</thead>
<tbody>
<tr>
<td>86 participants (1 cohort study)</td>
<td>Not serious</td>
</tr>
<tr>
<td>Jung et al. AJG 2011</td>
<td>Not serious</td>
</tr>
<tr>
<td></td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

### Lymphocytic Esophagitis

Lymphocytic esophagitis is a histologic diagnosis defined by an increase in intraepithelial lymphocytes without a concordant increase in granulocytes. (69-70)

The etiology is unclear but possible associations include GERD, inflammatory bowel disease, EoE (71) and HP-related gastritis. (72)

In the largest series of 81 patients identified with lymphocytic esophagitis, other noted associations included achalasia, asthma and allergies. Common symptoms included dysphagia in 54 (%), chest/abdominal pain in 36 (%), heartburn in 38 (%), nausea in 24 (%) and odynophagia in 10 (%) patients.

Notably, this group of patients was identified over an 11-year period at a tertiary referral center and some data suggested an increasing incidence. The most common esophageal endoscopic appearance was normal in 30%, but other findings included strictures, rings, esophagitis, erythema and nodularity. The most commonly prescribed and effective therapy was use of proton pump inhibitors. Given the plethora of potential etiologies and symptoms associated with this entity, lymphocytic esophagitis at this time appears to be a non-specific histologic reaction potentially to a variety of causes without specific characteristics or treatments.

Although it may be increasing in incidence, it still appears to be uncommon. Given
the caveats on making this "diagnosis", routine screening for this entity in patients with dyspepsia is not warranted at this time.

There is no clear evidence that obtaining an endoscopic biopsy from a normal appearing esophagus in a patient with dyspepsia for the purpose of finding lymphocytic esophagitis affects clinical outcomes. The quality of evidence for this statement is very low due to no RCT or long-term observational studies that approach this question.

Neoplasia

Most esophageal malignancies are recognizable through the appearance of a mucosal or submucosal mass lesion. This includes not only common cancers of the esophagus such as esophageal adenocarcinoma (EAC) or squamous cell cancer, but also unusual cancers such as lymphoma and benign neoplasms such as leiomyomas and granular cell tumors. Furthermore, almost all symptomatic neoplastic esophageal lesions present with dysphagia and/or odynophagia. Dyspepsia is a rare presenting symptom unless the malignancy extends to involve the gastric side in which case it is difficult to distinguish whether the tumor is of esophageal or gastric primary.

The only data that address the possibility of esophageal cancer detection in a normal appearing esophagus is from high-risk populations. Unfortunately, the sensitivity of white light endoscopy for detecting visible dysplasia or carcinoma in situ in an otherwise asymptomatic individual is poor. This has been shown through numerous studies comparing the sensitivity of standard endoscopy to
chromoendoscopy using staining techniques. For example, in a study on 225 healthy adults with positive balloon cytology for squamous cell cancer from a high risk area in China, only 62% were detected with white light endoscopy whereas 96% were found with Lugol’s iodine staining. (73)

Noting that the sensitivity for balloon cytology is generally low, it further emphasizes the lack of reliability of endoscopy, let alone, esophageal biopsies for detecting microscopic dysplasia or cancer. Given the length of the esophagus, a random biopsy protocol of squamous epithelium, such as used in Barrett’s esophagus has not been applied either.

A modern version of chromoendoscopy not requiring application of stains is termed “Image-enhanced endoscopy” or IEE as mentioned previously and includes Narrow Band Imaging (NBI – Olympus), iScan (Pentax) and FICE (Fujinon). In a recent study using NBI, detection of EAC was significantly improved in a high risk Japanese population versus white light alone (p value <0.001) (74)

Other enhanced optical imaging techniques including trimodal imaging, confocal microscopy and autofluorescence have been applied to early squamous cancer detection but at present, there is no evidence for enhanced health benefits and many of these imaging techniques are outside the expertise of general gastroenterology. (75) As a result, the lack of sensitivity of white light endoscopy for detecting early squamous cell cancer, the difficulty of applying enhancing techniques by the general gastroenterologist and the relative rarity of this microscopic cancer in a normal esophagus but for the patients with a history of head and neck cancer or high risk geographic non-Western populations, make routine
endoscopic screening and biopsy for esophageal cancer impractical in general practice.

There is no clear evidence that obtaining an endoscopic biopsy from a normal appearing esophagus in a patient with dyspepsia for the purpose of finding cancer would change clinical outcomes. The quality of evidence for this statement is low since there are no RCT’s or long-term observational trials that approach this question, but it is acknowledged that this condition is rare in the Western world.

**Conclusion:** In the defined population (and in the absence of alarm symptoms), there is no clear evidence that obtaining biopsies from an esophagus with an endoscopically normal appearance in an immune competent adult patient will reveal any unsuspected esophageal condition that would affect patient health outcomes. The overall quality of evidence for all critical and important outcomes is very low.

**IMMUNE COMPROMISED PATIENTS**

There is no evidence from the literature that disorders described above in immune competent patients differ in presentation and endoscopic appearance in patients who are immune compromised. Therefore conclusions about the lack of added benefit for tissue sampling of normal appearing mucosa should be similar for immune compromised patients for most disorders. Graft versus host disease and infections are disorders that might act differently in patients with a compromised immune system and will be discussed separately.
Acute and Chronic Graft Versus Host Disease (GVHD)

Clinically diagnosed GVHD of the esophagus, usually presenting with dysphagia, is rare with few cases reported. (76) On the other hand, when random biopsies are taken of the esophagus in patients suspected for GVHD, esophageal involvement can be found. For example, in a study of 197 patients undergoing 338 endoscopies for suspected GVHD, 19 patients underwent esophageal biopsies with positive findings in 33.3% (77) Of greater importance were the findings of 89% and 80% sensitivity and specificity, respectively, when gastric and duodenal biopsies were taken. Notably, in a prospective series of twenty-seven patients evaluating upper versus lower endoscopy in the diagnosis of GVHD, (78) the esophageal biopsies were positive in 11 patients but negative in 7 where other sites of the upper and lower GI tract were positive. Only one patient in the series had dysphagia. No patient had esophageal involvement without positive biopsies elsewhere in the gastrointestinal tract. Thus, as dysphagia (and certainly dyspepsia) is an uncommon presentation of GVHD and as other luminal sites are more likely to yield positive biopsies for GVHD when suspected, obtaining biopsies of the normal appearing esophagus in a patient with suspected GVHD with dyspepsia alone is not indicated.

We conclude that there is no clear evidence that obtaining an endoscopic biopsy from a normal appearing esophagus in a patient who has undergone tissue transplant for the purpose of finding graft-vs-host disease can change clinical outcomes and the quality of evidence is low.
**Infection**

There is scant if any data on the value of performing endoscopic esophageal biopsies in an immune compromised patient without esophageal symptoms or endoscopic abnormalities for detection of an opportunistic infection. Indeed, in most studies, esophageal biopsies are performed at targeted lesions such as ulcers or plaques. (79) For example, in a series of 141 HIV patients diagnosed with Candida esophagitis, all had endoscopically visible plaques of variable degree ranging from a patchy distribution to uniform coalescence with stricture and/or ulcer formation. (80) In bone marrow transplant candidates, Candida esophagitis is uncommonly discussed in the literature. In an older study of 56 patients randomized to ketoconazole or placebo prior to induction chemotherapy and transplantation for acute leukemia, two patients were strongly suspected of developing Candida esophagitis. (81) In organ transplant patients, Candida esophagitis is uncommon overall but one of the more common infections. For example in a study of 547 organ transplant patients, only 12 patients developed Candida esophagitis. (82) Also, numerous other opportunistic infections were reported in this series, none of which involved the esophagus.

A similar pattern follows with cytomegalovirus (CMV) in the immune compromised host. For example, in 33 HIV patients with CMV esophagitis, 141 esophageal ulcers were seen endoscopically but with patterns from giant single deep ulcers to multiple superficial shallow ulcerations. Odynophagia was uniformly present in all patients. (83) Different from Candida infection, the most common site
of CMV infection in the gastrointestinal tract is not esophagus in the immune compromised patient. In one study of 190 GI tract biopsies HIV positive patients, the majority of those patients were CMV positive in the colon or rectum, followed by stomach, small intestine and perianal area. (84) These findings are similar to other immunosuppressed groups such as transplant patients. For example, in patients who have undergone small bowel transplantation, almost all CMV infections are in the small intestine and not the esophagus. (85) Similarly, Candida infections are most likely in the blood and abdominal cavity. In the previously mentioned series of organ transplant patients, 16 of 547 patients developed CMV infection, none of which were in the esophagus. (82) This is important when “screening” for CMV is to be considered. In contrast, herpes simplex virus (HSV) is a squamous epithelium disease that more commonly occurs in the esophagus compared to CMV in immune compromised patients. However, patients with HIV diagnosed with esophageal HSV infection all had esophageal symptoms severe enough to warrant endoscopy with obvious ulceration seen in most series, and were not found incidentally. (86-87)

It is also unclear whether the discovery of a microorganism in an endoscopically normal appearing esophagus is clinically relevant or is an incidental finding. In a study of biopsies taken from 79 HIV infected patients analyzed by polymerase chain reaction, 22 biopsies had more than one pathogen identified. In this study, biopsies appeared to be more discriminating in demonstrating a single infection. In a randomly selected population of 175 Danish subjects undergoing endoscopy, 164 had mucosal brushings for Candida albicans of which 12.3% were positive in patients with and in 25.1% without COPD. (88) There was no significant
correlation between endoscopic gross findings, such as plaques, and the presence of Candida on esophageal brushing. Similarly, in a study of matched groups of asthma patients with or without inhaled steroid use, esophageal colonization with Candida occurred in 31% of the 40 patients in the control group and was no different than the group on steroids. (89) Finally, in a classic study from Laine and colleagues, esophageal brushings taken from 110 HIV patients revealed Candida in 57 patients, CMV in 31 and HSV in 10. (90) Notably, 86 of the 110 patients had abnormal endoscopic findings. Only five patients with a normal appearing esophagus on endoscopy had a positive brushing further supporting the likelihood that identification of an opportunistic infection in the esophagus is typically associated with an abnormal esophagus.

Thus, since Candida and viral infections occur in the esophagus almost always associated with symptoms and endoscopic findings, and since there is no clear understanding of prevalence or the natural course of an incidentally found esophageal infection in a normal appearing esophagus, obtaining endoscopic biopsies of a normal appearing esophagus in an immune compromised patient is unlikely to affect clinical outcomes. The quality of evidence for this statement is judged to be very low, as shown in Table 4.

Table #4. Summary of evidence table for the question “In the defined patient population, do biopsies from the esophagus lead to diagnosis of opportunistic infections that would have been missed if no biopsies had been taken, and does this change clinical outcomes?”

<table>
<thead>
<tr>
<th>Immune compromised patients</th>
<th>Quality assessment</th>
<th>Summary of Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Risk of bias</td>
<td>Inconsistency</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusion: In the defined population (and the absence of alarm symptoms), there is no clear evidence that obtaining biopsies from an esophagus with an endoscopically normal appearance in an immune compromised adult patient with dyspepsia will reveal any unsuspected esophageal condition that would affect patient health outcomes. The overall quality of evidence for all critical and important outcomes is very low.

Question: If endoscopic biopsies are sent from any of the aforementioned areas, are specific biopsy techniques recommended and are special stains needed to supplement hematoxylin and eosin (H&E) histologic examination?

12 Unclear if the pathologist was blinded (most likely not)
13 At the time that the study was conducted, routine prophylaxis against CMV was not given. Nowadays, prophylaxis and preemptive therapy for CMV is standard practice. Also, the immunosuppressant treatment has changed
14 Small sample size
15 By definition, the disorder wouldn't have been diagnosed if biopsies hadn't been obtained, because on endoscopy the mucosa looked normal
16 The relative effect is not applicable. It will be infinite, because the diagnostic rate without biopsies is zero.
17 All patients received prophylactic treatment with oral nystatin. Also, the immunosuppressant treatment has changed
Although biopsies from a normal appearing esophagus are not recommended, if for some reason biopsies are obtained, there is no evidence that special stains (such as Alcian blue or Periodic acid Schiff) are necessary for a pathologist to diagnose intestinal metaplasia or other conditions in the immune competent host. Goblet cells can be recognized on routine H&E stains and this holds true for both the gastroesophageal junction and the tubular esophagus. (91-92) Similarly, there is no need for special stains to be used to identify microorganisms in esophageal biopsies in most situations, and no indication for performing special stains prior to review of H&E stains. On the contrary, one study of HIV infected patients found that most organisms including Candida, CMV and HSV can be recognized on H&E staining. (93) However, special stains to diagnose specific organisms may be necessary in certain clinical situations or based on specific microscopic changes (i.e., severe inflammation obscuring cellular detail, rarity of or atypical appearance of putative organisms), but these should be ordered only after examination of H&E stained samples. The quality of evidence for this statement is moderate.
Conclusion: In the defined adult population (both immune competent and immune compromised), there is no clear evidence that routine use of special stains on biopsies obtained from an esophagus with a normal appearance on EGD can increase diagnostic accuracy or affect clinical outcomes. Use of special stains after initial H/E examination may improve diagnostic accuracy in some circumstances (severe inflammation for example). The overall quality of evidence for all critical and important outcomes is moderate.
Stomach

QUESTION: In the defined population, do biopsies from the stomach lead to diagnoses that would have been missed had biopsies not been taken, and does this change clinical outcomes?

Clinically significant disorders found in the stomach during EGD include Helicobacter pylori (HP) infection, reactive gastropathy, chronic atrophic gastritis (autoimmune-mediated gastritis and other rare forms of non-HP chronic gastritis), intestinal metaplasia, gastric dysplasia, cancers (various types), lymphocytic gastritis, collagenous gastritis and eosinophilic gastritis or gastroenteritis.

Outcomes of successful treatment included resolution or improvement of dyspeptic symptoms (for more than 6 months), a change in quality of life, incidence of metaplasia or dysplasia, reduction in the incidence of and mortality from gastric cancer. Secondary outcomes include reduction of resource utilization. Complications include the very low risk of gastric biopsies and problems related to the treatment of these conditions.

RESULTS FOR IMMUNE COMPETENT PATIENTS

Helicobacter pylori (HP) Infection: General Considerations

Diagnosis of HP infection is important in and of itself, since finding HP almost always prompts treatment. HP is the strongest known risk factor for gastric cancer, which is the second leading cause of cancer-related deaths worldwide. (94) However, whether biopsies from a normal appearing stomach would lead to a
diagnosis of HP infection that would have been missed if no biopsies were taken is less meaningful compared to other disorders because non-invasive diagnostic tests (serology, breath tests and stool antigen tests) are now widely available and less costly when compared to endoscopy. Thus, past literature can be difficult to apply to current clinical practice. In our review, we assumed that patients present for open access endoscopy and no prior HP evaluation is available. Hence, should biopsies be sent in the face of a normal EGD but when a patient has dyspepsia?

In 1999, Jaakkomainen published a report that included two meta-analyses of 23 observational studies. (95) One analysis compared the prevalence of HP in patients with functional dyspepsia (FD) and the other assessed the effect of eradication on patient outcomes. There were 8291 participants included with a 55.2% prevalence of HP infection in patients with FD. Despite these findings, it would be misleading to claim that biopsies alone would lead to 552 more diagnoses of HP infection per 1000 patients during the current era because of alternative diagnostic methods. The evidence derived from this analysis is rated as very low quality because all studies were observational with statistically significant heterogeneity (p< 0.001) among the studies, variability in the definition of FD, tests used to diagnose HP and variability in the baseline prevalence of infection among the studies included. More importantly, the design of the studies does not allow conclusions to be drawn about how many diagnoses would be missed without biopsies.

When clinical outcomes are considered, some evidence suggests a positive effect. A 2006 Cochrane Review (96) involved 3566 participants in 17 RCT's. In 360
per 1000 HP infected patients, dyspepsia symptoms resolved after HP eradication compared to 290 per 1000 patients whose symptoms resolved without treatment. Relative risk for resolution of dyspepsia was 0.90 (0.86 to 0.94) with a number needed to treat to cure one case of dyspepsia equal to 14 (95% confidence interval of 10-25). Evidence was rated moderate due to a moderate risk of bias (13 out of 17 RCT’s were at unclear risk of bias for allocation concealment) and moderate indirectness because most studies reported outcomes without defining HP status at the end of the follow up period. Two additional RCT’s were published based on Chinese literature and appeared after the afore-mentioned meta-analysis. (97-98) Both showed a statistically significant effect of HP eradication on FD but both had limited (12 month) follow up and one had methodological and reporting flaws. Neither altered the effect estimate or the level of evidence. (97-98) Additional RCT’s published after the 2006 Cochrane meta-analysis both showed a trend towards improvement of FD with HP eradication but did not change the conclusions of the Cochrane paper. (99-100)

In 2011, Moayyedi published an updated review (101) as a comment on a large Brazilian RCT on HP eradication. (102) Adding that current study to the Cochrane Review changed the Number Needed to Treat from 14 to 13, although the NNT in the Brazilian study was 9. As Moayyedi points out, there is strong evidence to suggest eradication of HP relieves FD in some patients and can reduce gastric inflammation and motility problems.

Adverse events were reported in two of the Cochrane reported RCT’s among 410 participants. The most common adverse events were diarrhea and taste
disorder related to treatment. The evidence was graded as low due to a high risk of selection bias (only 2 out of 17 trials reported adverse events) and a small number of events.

Finally, Ford et al reported the effect on the subsequent risk of gastric cancer in 2014. (103) Six RCT’s were pooled for meta-analysis of 6497 participants although only three studies were at low risk of bias. When pooled separately the results remained robust. Populations were different from the one considered in this guideline with 5 of 6 studies performed in populations with a high risk of gastric cancer (4 studies in China and 1 in Japan) and many participants had visible lesions at endoscopy. Overall quality of evidence was low due to serious indirectness and imprecision but there were 24 patients (per 1000) with gastric cancer developing in patients not treated for HP compared to 16 in the treatment group (Relative risk 0.66 (0.46 to 0.95). The number needed to treat was 124 (from 78 to 843).

An analysis of guidelines published between 2007 and 2012 was published in 2013, (104) and showed that eradication of HP has modest effect on FD but there appears to be a subset of patients that derive long-term symptom resolution after HP eradication.

Two major United States guidelines concerning HP and dyspepsia were published in 2005 (AGA) and 2007 (American College of Gastroenterology - ACG). (105-106) The AGA guideline stated “Biopsy specimens should be obtained for H. pylori at the time of endoscopy and eradication therapy offered to those who are infected because this may reduce the risk of subsequent peptic ulcer disease and gastric malignancy.” Eradication therapy was superior to placebo (RR, 0.91; 95% CI,
0.87–0.96), with an NNT of 17 (95% CI, 11–33), a conclusion similar to the Cochrane Review of Moayyedi. (96) The ACG guideline stated that treatment of HP infection with the goal of symptom resolution in FD was “controversial”.

Despite almost two decades of work including multiple RCT’s, systematic reviews, meta-analyses, numerous observational studies and at least nine major guidelines, we continue to recommend that biopsies should be performed during EGD for dyspepsia and HP eradication should be offered when found, realizing that there will be only a modest overall effect on symptoms of dyspepsia with a subgroup of complete responders.

In summary, using the GRADE assessment, biopsies for HP performed during EGD for dyspepsia in the presence of a normal gastric endoscopic appearance if HP has not been diagnosed by other (non-invasive means) and subsequent eradication therapy for proven HP infection leads to improved clinical outcomes (quality of evidence is very low for biopsy and moderate for offering eradication therapy). Eradication should lead to resolution of dyspeptic symptoms by 3-12 months in a higher proportion of patients compared to placebo (quality of evidence is moderate). Treatment may not have a clinically important effect on quality of life compared to placebo (quality of evidence is low) and treatment causes adverse events in a higher proportion of patients compared to placebo (quality of evidence is low). The evidence is summarized in Tables 5 and 6. It should be noted that non-invasive testing and not repeat EGD should be performed to assure HP eradication whenever possible due to cost and safety considerations.

Table #5. Summary of evidence table for the question “In the defined patient population, do biopsies from the stomach lead to diagnosis of H. pylori that would have been missed if no biopsies had been taken?”
**Participants (studies)**

<table>
<thead>
<tr>
<th></th>
<th>Limitation</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Imprecision</th>
<th>Publication bias</th>
<th>Overall quality of evidence</th>
<th>Study event rates (%)</th>
<th>Relative effect (95% CI)</th>
<th>Anticipated absolute effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (studies)</td>
<td>None</td>
<td>Serious</td>
<td>Serious</td>
<td>None</td>
<td>Not reported</td>
<td>⊕⊕⊕⊝</td>
<td>8291 participants</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

**Diagnosis of H. pylori infection** *(IMPORTANCE OF OUTCOME: Critical for decision making)*

<table>
<thead>
<tr>
<th></th>
<th>Biopsies taken</th>
<th>No biopsies taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (studies)</td>
<td>23 observational studies pooled by meta-analysis</td>
<td>23 observational studies pooled by meta-analysis</td>
</tr>
<tr>
<td>Limitation</td>
<td>None</td>
<td>Serious</td>
</tr>
<tr>
<td>Inconsistency</td>
<td>Serious</td>
<td>Serious</td>
</tr>
<tr>
<td>Indirectness</td>
<td>None</td>
<td>Not reported</td>
</tr>
<tr>
<td>Imprecision</td>
<td>None</td>
<td>Very low</td>
</tr>
<tr>
<td>Publication bias</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Overall quality of evidence</td>
<td>⊕⊕⊕⊝</td>
<td>MODERATE</td>
</tr>
<tr>
<td>Study event rates (%)</td>
<td>55.2%</td>
<td>552 more disorders diagnosed per 1000</td>
</tr>
<tr>
<td>Relative effect (95% CI)</td>
<td>Not applicable</td>
<td>(range: from 94 more to 875 more)</td>
</tr>
</tbody>
</table>

**Table#6. Effect on clinical outcomes by treating H. pylori infection that was diagnosed by biopsying normal-looking mucosa**

<table>
<thead>
<tr>
<th></th>
<th>Risk of bias</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Imprecision</th>
<th>Publication bias</th>
<th>Overall quality of evidence</th>
<th>Study event rates (%)</th>
<th>Relative effect (95% CI)</th>
<th>Absolute effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution of symptoms of functional dyspepsia</td>
<td>Mode rate</td>
<td>None</td>
<td>Moderate</td>
<td>None</td>
<td>None</td>
<td>⊕⊕⊕⊝</td>
<td>290 cured per 1000 patients</td>
<td>Relative risk 0.90 (0.86 to 0.94)</td>
<td>Number needed to treat to cure one case of dyspepsia: 14 (95% CI 10 to 25)</td>
</tr>
<tr>
<td>Quality of life</td>
<td>None</td>
<td>Serious</td>
<td>25</td>
<td>None</td>
<td>Could not be</td>
<td>⊕⊕⊝</td>
<td>Not reported</td>
<td>Standardized</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

18 Jaakkimainen BMJ 1999
19 Statistically significant heterogeneity (p< 0.001) among studies
20 There was variability among studies with regards to the definitions of functional dyspepsia, the tests used to diagnose H. pylori infection, and the baseline prevalence of H. pylori infection in the general population. Most importantly, the design of these studies does not allow us to draw conclusions on how many diagnoses of Hp infection would have been missed if no biopsies were taken.
21 The quality of evidence was low (because it was derived from observational studies) and was further downgraded to very low due to inconsistency and indirectness
22 Biopsies were obtained from all patients
23 Moayyedi Cochrane Dat Syst Rev 2006
24 Those two RCTs do not substantially alter the effect estimate of the 2006 meta-analysis and do not alter the level of evidence.
25 13 out of 17 RCTs were at unclear risk of bias for allocation concealment.
26 There was moderate indirectness because most studies reported outcomes according to whether the patients received eradication regimen or not, but not according to Hp status at the end of the follow up period
27 The results shown refer to the pooled analysis of 17 RCTs that assessed outcomes between 3 and 12 months. This did not affect the quality of evidence: the result was robust when the analysis was restricted to RCTs that assessed outcomes at 12 months (the relative effect was not reported; the relative risk reduction was 10%, 95% CI 6% to 14%).
28 The effect size is derived from the Cochrane meta-analysis (Moayyedi et al. Cochrane Dat Syst Rev 2006)
29 High risk of bias due to selective reporting for this outcome. Only 3 out of 17 RCTs reported outcomes related to quality of life.
30 Two different scales were used to assess outcomes. Two RCTs reported outcomes as Psychological General Well Being and one RCT as Short Form 36. In both scales lower scores are poorer
31 Although the 95%CI includes no effect (zero), neither the upper nor the lower limits of the 95%CI are suggestive of a large effect. This result is suggestive of evidence that there is no difference of clinical importance, therefore the results can be considered precise.
### RCTs pooled by meta-analysis

<table>
<thead>
<tr>
<th>Assessed (only 3 studies)</th>
<th>Mean difference (-0.12 to 0.15)</th>
</tr>
</thead>
</table>

### Adverse events

<table>
<thead>
<tr>
<th>418 participants (2 RCTs not pooled by meta-analysis)</th>
<th>Serious</th>
<th>None</th>
<th>Serious</th>
<th>Could not be assessed (only 3 studies)</th>
<th>⊕⊕⊝⊝ LOW</th>
<th>Pooled results were not reported</th>
</tr>
</thead>
</table>

### Effect on risk of gastric cancer

<table>
<thead>
<tr>
<th>6497 participants (6 RCTs pooled by meta-analysis)</th>
<th>None</th>
<th>None</th>
<th>Serious</th>
<th>Serious</th>
<th>None detected</th>
<th>Relative risk (0.46 to 0.95)</th>
<th>NNT 124 (from 78 to 843)</th>
</tr>
</thead>
</table>

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**HP Infection: Clinical Utility of Routine Testing for cagA and vacA Variants**

In general, patients infected with cagA and/or vacA genotype have a much greater risk of developing peptic ulcer, atrophic gastritis and gastric cancer. (109)

Several studies assessing the prevalence of cagA and vacA (109-112), and a European guideline from 2012 all conclude that routine testing for these variants is unlikely to be justified and this practice cannot be justified for clinical practice (quality of evidence high). (113)

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**HP Infection: Eradication and Gastric Adenocarcinoma**

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32 High risk of bias due to selective reporting for this outcome. Only 2 out of 17 RCTs reported adverse events per treatment group.

33 Small number of events.

34 Ford et al. BMJ 2014

35 Only 3 of the studies were at low risk of bias, but when these studies were pooled separately, the results remain robust. Therefore, the QoE was not downgraded for this criterion

36 The population was different from the population of interest for our guideline. They included asymptomatic individuals (the majority of which did not have dyspepsia); participants without malignancy at study entry were included regardless of whether they had peptic ulcer or normal findings at baseline endoscopy. Furthermore, 5 out of 6 studies were performed populations at high risk for gastric cancer (4 studies in China and 1 in Japan) and the sixth study was performed in Colombia

37 Small number of events (127 in total)
It is beyond the scope of this TR to discuss in detail the relationship between HP and gastric cancers. A well-done systematic review and meta-analysis were published in 2014. (103) The meta-analysis assessed whether “searching for *Helicobacter pylori* and treating with eradication therapy leads to a reduction in incidence of gastric cancer among healthy asymptomatic infected individuals”. The duration of follow up ranged from >4 years to 14.7 years and summarized data from China, Japan, the United States and the United Kingdom. The lifetime risk of gastric cancer in men ranges from 1.8% in the US (3.1% in the UK) to 19.5% in China (19.2% in Japan) (women tend to be at slightly less risk in all countries compared to men). The NNT to prevent one case of cancer in men is 163.4 (102.9 to 1111.1) in the US (245.1 in women), 94.9 (163.4 in women) in the UK, 15.1 (9.5 to 102.6) in China (23.7 in women) and 15.3 (9.6 – 104.2) in Japan (23.0 in women). Evidence of the beneficial effect of mass HP eradication in Asia was published in 2004 (114), but does not apply to Western populations. Other reports also demonstrate increased risk of gastric cancer in HP infected patients (115-116). Quality of evidence was judged to be moderate to low in these studies due to their observational nature and the fact that normal appearing mucosa on EGD was not the predominant finding.

In summary, for high-risk countries (Asia and others), screening with EGD and biopsy for HP and gastric cancers and mass eradication paradigms are widely practiced and make sense because of the low NNT data. In Western countries with a low prevalence of HP, population-based EGD screening for gastric cancer or HP infection is not cost effective (high quality of evidence).
HP Infection: Eradication and MALT lymphoma

A systematic review, published in 2010, found that 42% of patients with primary gastric mucosa-associated lymphoid tissue (MALT) lymphoma did not have alarm symptoms and 8.4% had normal or “hyperemic” mucosa. (117). Of the 136 patients with normal or hyperemic mucosa, 135 had low-grade lymphoma and only one had high-grade lymphoma. One other publication was found describing 93 patients with dyspepsia as a primary symptom and two had B cell monoclonality, but both had a prior diagnosis of MALT lymphoma. (118) The evidence concerning the importance of EGD biopsies to discover gastric lymphoma is considered to be of very low quality.

Premalignant Gastric Conditions (Gastric Atrophy, Intestinal Metaplasia and Dysplasia)

In the defined patient population, biopsies from the stomach can lead to diagnosis of HP-related premalignant gastric conditions that would have been missed if no biopsies had been taken. Nevertheless, the true incidence of these histological findings in the absence of visible endoscopic lesions differs so widely in different populations and age cohorts even within the same country, that it is impossible to provide a single estimate for their prevalence (quality of evidence is low). Identification of these management changing lesions can be made through image-enhanced endoscopy (8) but only indirect evidence was found so the quality of evidence for this assertion is low (116). This topic has been addressed in detail
by a recent guideline from Europe using rigorous methodology (119) but there was no concrete evidence for use of random biopsies to detect these lesions, intestinal metaplasia or atrophy suspected at endoscopy. Using IEE, the need for routine biopsies in this situation could be even less than using white-light endoscopy alone.

**HP Infection: Eradication and Effect on Well-being and Quality of Life**

Quality of life was assessed in 839 participants in a pooled meta-analysis from three RCT's in the same Cochrane Review. (96) There was a high risk of bias in these studies due to selective reporting as only 3 of the 17 trials reported quality of life scores. The evidence was graded as low. Several “before and after” studies have been published concerning the effect of HP eradication on quality of life. (120-121). Overall the quality of evidence supporting biopsy is very low due to the observational nature of each of these studies, but all concluded that EGD or HP eradication did not affect psychological well-being.

**Summary Conclusions**

HP infection can be present in the stomach even with a normal appearance on EGD. There is clear evidence (high quality) that at least some patients with dyspepsia can benefit from diagnosis and eradication of HP infection. Eradication of HP, once found, can lead to symptom improvement and prevention of associated diseases or conditions, including peptic ulcer disease, chronic gastritis, precancerous lesions (chronic atrophic gastritis, intestinal metaplasia and dysplasia), MALT lymphoma and gastric adenocarcinoma. Decisions about routine
biopsy of asymptomatic patients is not directly relevant to this review and would depend on the age, ethnicity, country of origin and other factors that often are not apparent in an open access system. As a result, this decision must be individualized and left to the best judgment of the endoscopist in a shared decision with the patient.

**Conclusion # 1: In the defined population, biopsy of normal appearing gastric mucosa can detect HP infection that would be missed on the exam if biopsies were not obtained. The quality of evidence is very low. One must note that there are non-invasive methods to detect HP infection.**

**Conclusion # 2: Detection of HP infection with tissue biopsy and its eradication in patients with dyspepsia is associated with symptom improvement and reduction of risk for HP-related comorbidities including gastric cancer compared to no biopsy (or no eradication). The quality of evidence is moderate based on multiple RCT’s that show a modest effect with a NNT of about 13-14. The effect on symptom resolution is not universal and it does not appear to improve wellbeing. Quality of evidence for this statement is low.**
Non-HP Infections in Immune Competent Patients

Infections other than HP do exist in adult stomachs. Giardia lamblia can co-exist with HP but always in the presence of chronic atrophic gastritis (122). Fungal density in material from the stomach of patients with diabetes or those treated with steroids, antibiotics or cytostatic drugs are increased, but this finding does not correlate with symptoms or serum anti-fungal antibodies. This suggests that this colonization is secondary and of little clinical significance (123). In general the quality of evidence in these case series is of very low quality and its applicability to U.S. populations is unknown. Both are observational studies with significant limitations.

IMMUNE COMPROMISED PATIENTS

Infections found in the stomach of patients with a compromised immune system include CMV, HHV-6, cryptosporidiosis and perhaps fungi. A study of 219 consecutive transplant patients (kidney or kidney/pancreas) who developed upper GI symptoms (dyspepsia (n=31), dysphagia, or bleeding) underwent EGD. (124) Biopsies were taken from areas of mucosal abnormality; in normal EGDs (n=9) “multiple random biopsies of duodenal, gastric and esophageal mucosa” were taken (the number and exact location of biopsies was not stated). At this time, prophylaxis and aggressive therapy for CMV were not standard practice (compared to the current time). CMV was detected in 2 out of 9 patients with normal EGD and both patients had dyspepsia. This study provides very low quality of evidence but it appears reasonable to conclude that if biopsies were not performed, CMV would
have been missed. Several studies of both CMV and fungal infection emphasize the fact that most infections are associated with changes on endoscopic appearance. (124-126)

There are no available data that allow an answer to the question of whether routine gastric biopsies in a transplant (or otherwise immune compromised) patient should be performed in the presence of an endoscopically appearing normal stomach. One reason to explain this absence of data is that most immunocompromised patients are routinely screened and treated for CMV and HP. Our only conclusion from the scant literature available is that, if in 1991-1993 we were unlikely to detect CMV in biopsies from esophagus and duodenum (and by inference the stomach) in symptomatic patients with normal EGD, then presently it will be even more unlikely to detect CMV in such biopsies (argument against routine biopsies in such patients). The quality of evidence for this statement is very low.

We can also conclude, based on data from two studies that in patients infected with HIV on highly active antiretroviral therapy (HAART), gastric CMV infection occurs only among patients with low CD4 counts (less than 300) and always in with the presence of gastric erosions or ulcers. (127-128). Studies looking for cryptosporidiosis and fungal infections in the stomach of AIDS patients with diarrhea or upper GI symptoms did not report endoscopic findings but did find that gastric biopsies revealed both types of infections in a high number of such patients.

The quality of evidence from all of the above studies is very low (as shown in Table 7), but it is clear that important diagnoses are found in some severely immune compromised patients, albeit usually in the presence of endoscopic abnormalities.
### Immune compromised patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants (studies)</th>
<th>Risk of bias</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Imprecision</th>
<th>Publication bias</th>
<th>Overall quality of evidence</th>
<th>Study event rates (%)</th>
<th>Relate effect (95% CI)</th>
<th>Anticipated absolute effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>moderate</td>
<td>serious 46</td>
<td>serious 41</td>
<td>NA</td>
<td>☒☒☒.hasClass=&quot;evidence&quot;</td>
<td>VERY LOW</td>
<td>0% 45</td>
<td>2/9 (22%: 95% CI 7% to 76%) 43</td>
<td>NA 44</td>
</tr>
<tr>
<td></td>
<td>1 participant</td>
<td>moderate 12</td>
<td>none</td>
<td>Very serious 45</td>
<td>NA</td>
<td>☒☒☒.hasClass=&quot;evidence&quot;</td>
<td>VERY LOW</td>
<td>0% 46</td>
<td>0%</td>
<td>NA 47</td>
</tr>
<tr>
<td></td>
<td>(1 cross-sectional study)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

#### Diagnosis of CMV infection (IMPORTANCE OF OUTCOME: critical for decision making)

- **57 participants** (1 cross-sectional study)
  - Graham 1995

- **1 participant** (1 cross-sectional study)
  - Kaplan 1997

#### Effect on clinical outcomes 48 by treating CMV infection that was diagnosed by biopsying normal-looking mucosa (IMPORTANCE OF OUTCOME: critical for decision making)

- No studies identified

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Table #7. Summary of evidence table for the question “In the defined patient population, do biopsies from the stomach lead to diagnosis of opportunistic infections that would have been missed if no biopsies had been taken, and does this change clinical outcomes?”

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38 Unclear if the pathologist was blinded (most likely not)
39 Inconsistent results among the two studies
40 At the time that the study was conducted, routine prophylaxis against CMV was not given. Nowadays, prophylaxis and preemptive therapy for CMV is standard practice. Also, the immunosuppressant treatment has changed
41 Small sample size
42 By definition, the disorder wouldn’t have been diagnosed if biopsies hadn’t been obtained, because on endoscopy the mucosa looked normal
43 Among those with normal endoscopic findings. Both of these patients with CMV had dyspepsia.
44 The relative effect is not applicable. It will be infinite, because the diagnostic rate without biopsies is zero.
45 Very small size (only 1 patient had EGD without erosions or ulcers)
46 By definition, the disorder wouldn’t have been diagnosed if biopsies hadn’t been obtained, because on endoscopy the mucosa looked normal
47 The relative effect is not applicable. It will be infinite, because the diagnostic rate without biopsies is zero.
48 Resolution or improvement of dyspeptic symptoms, changes in quality of life, effect on survival/mortality after treating the disorder that was diagnosed by biopsying normal-looking mucosa
Conclusion: In patients with a compromised immune system and symptoms of dyspepsia, serious infections can be present in the absence of mucosal abnormalities (albeit in low prevalence) and finding infections would change management and potentially alter health outcomes. Quality of evidence is very low.

Question: If endoscopic biopsies are sent from any of the aforementioned areas, are specific biopsy techniques recommended and are special stains needed to supplement hematoxylin and eosin (H&E) histologic examination?

Evaluation of the technique for obtaining endoscopic biopsies of the stomach is essential given the variability in the number of biopsies obtained by endoscopists and the addition of special stains used by pathologists (either routinely or reflexively) in conjunction with H&E staining. The topic of special stains to diagnose H. pylori infection has significant economic consequences and has been the subject of a recent guideline from the Rodger C. Haggitt Gastrointestinal Pathology Society. (129) The conclusion of this group of GI pathologists is stated as follows: “We suggest that use of ancillary stains is appropriate when biopsies show chronic, or chronic active gastritis without detectable H pylori in hematoxylin and eosin-stained sections, but performing them “up front” on all gastric biopsies is generally unnecessary.” (129)
Conclusions are based on the fact that HP infection is almost always found in the context of chronic gastric inflammation. (130-131). There are instances where HP infection can be detected with little or no inflammation. Unfortunately, in our analysis, only three studies could be found that used methods appropriate to calculate rates of true positive, true negatives, false positives and false negatives for HP infection and chronic inflammation. There are no published studies from which one could calculate the prevalence of HP infection that could not be detected on routine H/E staining in the absence of chronic inflammation.

With regards to the number and location of biopsies taken to detect HP infection, the literature contains descriptions of biopsy protocols from 2 tissue samples to 12 in various parts of the stomach. The evidence is summarized in Tables 8 and 9.

Table #8. What is the diagnostic accuracy of various biopsy protocols for obtaining biopsies from stomach (in the absence of mucosal lesions) for diagnosis of \textit{H. pylori}?

<table>
<thead>
<tr>
<th>Biopsy Protocol</th>
<th>Participants (studies)</th>
<th>Study design</th>
<th>Factors that may decrease quality of evidence</th>
<th>Overall quality of evidence</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 biopsies (1 body, 1 antrum)</td>
<td>164 (1 study) [49]</td>
<td>Observation al</td>
<td>Very serious [50]</td>
<td>Not applicable</td>
<td>No</td>
</tr>
<tr>
<td>3 biopsies (1 body, 1 antrum, 1 incisura)</td>
<td>46 (1 study) [51]</td>
<td>Observation al</td>
<td>Not applicable</td>
<td>No</td>
<td>serious</td>
</tr>
<tr>
<td>4 biopsy protocol (2 body, 2 antrum)</td>
<td>76 (1 study) [52]</td>
<td>Observation al</td>
<td>Serious [53]</td>
<td>Not applicable</td>
<td>No</td>
</tr>
</tbody>
</table>

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[50] Incorporation bias for the reference standard
[51] el-Zimaity. Hum Path 1999
[52] The study population was enriched with patients with known intestinal metaplasia, but this would tend to decrease the sensitivity of histology. Therefore, if any selection bias was introduced, it would tend to weaken the results.
[54] Reference standard not adequately sensitive
Table #9. What is the diagnostic accuracy of various biopsy protocols for obtaining biopsies from stomach (in the absence of mucosal lesions) for diagnosis of intestinal metaplasia?

<table>
<thead>
<tr>
<th>Biopsy Protocol</th>
<th>Participants (studies)</th>
<th>Study design</th>
<th>Factors that may decrease quality of evidence</th>
<th>Overall quality of evidence</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 biopsies</td>
<td>No studies</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td>3 biopsies</td>
<td>46 (1 study)</td>
<td>Observation al</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>No serious</td>
</tr>
<tr>
<td>4-biopsy protocol (2 body, 2 antrum)</td>
<td>348 (2 studies)</td>
<td>Observation al</td>
<td>Serious</td>
<td>none</td>
<td>No serious</td>
</tr>
<tr>
<td>5-biopsy protocol (Updated Sydney System: 2 body, 2 antrum, 1 incisura)</td>
<td>46 (1 study)</td>
<td>Observation al</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>No serious</td>
</tr>
<tr>
<td>7-biopsy protocol (3 antrum, 1 angulus, 1 greater curvature corpus, 2 lesser curvature corpus)</td>
<td>112 (1 study)</td>
<td>Observation al</td>
<td>moderate</td>
<td>Not applicable</td>
<td>No serious</td>
</tr>
</tbody>
</table>

General conclusions from ten publications can be summarized as follows: all protocols accurately diagnosed HP with close to 100% sensitivity (quality of evidence ranged from very low to moderate) but all, including a 12-biopsy protocol had low sensitivity for premalignant gastric conditions and could not be used to

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55 el-Zimaity. Hum Path 1999
56 The study population was enriched with patients with known intestinal metaplasia, but this would tend to decrease the sensitivity of histology. Therefore, if any selection bias was introduced, it would tend to weaken the results.
57 el-Zimaity. Hum Path 1999
58 The study population was enriched with patients with known intestinal metaplasia
60 Reference standard not adequately sensitive
61 el-Zimaity. Hum Path 1999
62 The study population was enriched with patients with known intestinal metaplasia
63 de Vries. Helicobacter 2010
64 spectrum bias (all patients had known intestinal metaplasia and/or dysplasia)
provide adequate surveillance in an at risk population (chronic atrophic gastritis for example).

The Updated Sydney System is commonly used in clinical practice and involves a 5-biopsy protocol with specimens from the lesser and greater curve of the antrum within 2-3 cm of the pylorus, from the lesser curvature of the corpus (4 cm proximal to the angularis), from the middle portion of the greater curvature of the corpus (8 cm from the cardia and one from the incisura angularis) (137) El-Zimaity (133) compared the diagnostic yield of eleven different protocols from 3 to 12 biopsies with the gold standard of 20 biopsies in a research protocol that was enriched with known cases of intestinal metaplasia. The updated Sydney System (5 biopsies) identified 100% of HP infections, but the 5-biopsy protocol as well as all other biopsy protocols missed a significant proportion of diagnoses of intestinal metaplasia (diagnostic yield no better than 85%). They noted that a 3-biopsy approach (1 each from body greater curve, antrum greater curve and incisura) identified 100% of HP.

Experienced GI pathologists can determine the anatomic location of biopsy specimens sent from the stomach, which obviates the need for separating specimens into multiple jars. All gastric biopsy specimens sent for HP diagnosis should be submitted in a single jar as a means to limit costs without compromising accuracy or patient health outcomes.
Conclusion #1: The updated Sydney System (5 biopsies) (137) identified 100% of HP infections and balances diagnostic accuracy with resource and time expenditure although a 3-biopsy protocol (same areas of the stomach biopsied) also identified 100% of HP infections and has been endorsed in other society guidelines. (2,140) Quality of evidence is moderate due to the observational nature of all studies.

Conclusion #2: If tissue sampling includes biopsies from the antrum, incisura and body, with some exceptions, cases of HP infection can be identified by experienced pathologists using standard H&E staining. Occasionally, specimens demonstrate chronic gastritis with no identifiable HP on H/E or suspected cases of HP infection demonstrate little or no chronic gastric inflammation. In these circumstances, immunohistochemical staining is a cost effective means to determine whether HP infection is present. There is no evidence published that supports use of other special stains (in addition to immunohistochemical staining) to increase accuracy of this diagnosis. Use of immunohistochemistry or other special staining techniques prior to initial reviewed of H/E slides by the pathologist is not recommended. This practice does not substantially improve accuracy of diagnosis or patients’ clinical outcomes although if practiced routinely, this will increase overall procedural costs. Quality of evidence for this statement is moderate.
Conclusion # 3: There is no published evidence that submitting biopsies samples from different anatomic locations in the stomach in separate jars increases accuracy of diagnosing HP infection and this practice will increase overall health care costs substantially.
Duodenum

QUESTION: In the defined population, do biopsies from the duodenum lead to diagnoses that would have been missed had biopsies not been taken, and does this change clinical outcomes?

Clinically significant disorders found in the duodenum on endoscopic biopsy include celiac sprue, Giardia lamblia, duodenal eosinophilia, disaccharidase deficiency, gastric metaplasia, Whipple’s disease and opportunistic infections. Outcomes of successful treatment include resolution or improvement of symptoms, a change in quality of life and a reduction in long-term complications of disorders (such as with celiac sprue for example). Complications include the low risk of obtaining duodenal biopsies and problems related to treatment of disorders found on biopsy. As with other sections, this section will consider two subgroups of patients; those with intact immune systems and those with immune systems that are compromised.

RESULTS FOR IMMUNE COMPETENT PATIENTS

Celiac disease

Celiac disease (CD) is an autoimmune disease that has a genetic basis but is triggered by ingestion of gluten in predisposed individuals. Its prevalence is estimated to be 0.8% in the United States though many people remain undiagnosed. (141) There is an increased risk of malignancy, osteoporosis, autoimmune manifestations and other comorbidities that are reduced in incidence with initiation of a gluten free diet after careful evaluation and diagnosis. While there are serologic
tests that aid in the diagnosis, endoscopic biopsy of the duodenum remains an essential step in disease diagnosis and management. (141)

Established indications for obtaining a duodenal biopsy to evaluate for the presence of celiac disease include the presence of one or more of a variety of signs, symptoms, predisposing diseases, positive family history and/or characteristic endoscopic findings at upper endoscopy, including the symptom of dyspepsia. (142) Some guidelines suggest that dyspepsia can be considered a symptom of celiac disease (143-144), and that duodenal biopsies should be considered in specific patients with isolated dyspepsia (persistent symptoms, age > 55, alarm symptoms). While dyspepsia is not listed as a characteristic symptom of celiac disease in most consensus and guideline documents, this is a noted association in clinical practice (143-145) The number of studies examining the utility of obtaining screening duodenal biopsies in patients with dyspepsia and normal endoscopic duodenal appearance in order to detect celiac disease are few.

Ford et al published a systematic review and meta-analysis of 15 case series and case-control studies in 2009 that applied serological tests and/or distal duodenal biopsy for CD to unselected adults with dyspepsia. (146) Five were case-control studies with controls consisting of healthy volunteers in three studies, blood donors in one study, and individuals with other GI symptoms who did not meet criteria for either dyspepsia or functional gastrointestinal disorders in the fifth study. Prevalence of biopsy-proven CD following positive serology was higher in patients with dyspepsia (3.2% in cases vs. 1.3% in controls), but without statistical significance (OR 2.85; 95% CI 0.60–13.38). Prevalence of biopsy-proven CD was 1%
(95%CI 0.76-1.46%) in ten studies performing duodenal biopsy as the initial test for CD; only one of these studies was a case-control study.

Use of endoscopic markers of villous atrophy are not accurate for detection of CD in patients with dyspeptic symptoms since there was a prevalence of biopsy-proven celiac disease of 1.3% in cases compared to 0% in controls (146). Therefore, the estimate for the prevalence of CD in patients with dyspepsia has a tight 95% confidence interval, but there is uncertainty (imprecision) as to whether this prevalence is statistically different than the prevalence of CD in the general population. The prevalence of CD in patients with functional dyspepsia (normal endoscopy) was not assessed.

We therefore conclude that the prevalence of biopsy-proven CD in subjects with dyspepsia is 1% and is higher than in controls, although this difference is not statistically significant. This suggests that subjects with dyspepsia are no more likely to harbor undiagnosed CD than controls.

In other publications, Collin et al. assessed if there is an association between GERD symptoms and CD in primary practice in Finland. (148-149) The first was a large well-conducted and well-reported study. The second study population largely overlaps with his initial publication and includes three studies that were contained in the Ford meta-analysis. (147) The prevalence of CD (diagnosed by duodenal histology) was 0.9% among patients with esophagitis, 0.6% among patients with GERD symptoms, 1.0% among patients with dyspepsia and 12% among patients with a clinical suspicion of CD. In CD patients, reflux symptoms were mild prior to initiation of gluten-free diet (GFD) but nevertheless were significantly improved.
after 1 year on GFD. There is no mention whether any of these patients were started on PPI treatment during that year. The authors concluded that the study did not support the concept that patients with GERD symptoms should be screened for CD. The association between these two conditions is, at most, weak, but a GFD may still bring symptomatic relief for reflux symptoms in CD.

The question as to whether CD is characterized by endoscopic abnormalities has been approached. Dickey et al concluded that endoscopic markers of CD were insensitive as 30 of 129 patients studied (23%) lacked endoscopic findings using standard white light imaging. (150) The most commonly seen findings were a mosaic pattern mucosa (68 patients, 53%) and scalloping of duodenal folds (74 patients, 57%). The prevalence of visible lesions was significantly lower for partial villous atrophy (15 of 26 patients, 58%) than for subtotal or total VA (84 of 103 patients, 82%) (p = 0.02). The conclusion was that CD, even in a high-risk population, could indeed be present with a normal appearing duodenum and thus, biopsy would needed for endoscopy-related diagnosis. Their performance is likely poorer in an unselected dyspeptic population. IEE has been reported as useful in this situation and may emerge as an alternative to tissue sampling. At this time, however, the skill to diagnose CD using IEE in the context of normal white-light imaging is limited.

Finally, one has to also consider the impact of false positive biopsy diagnoses when assessing pathologic samples for celiac disease. This is particularly true in early grade celiac lesions, such as Marsh 1-2, where expensive testing and
worrisome follow up may be needed for what was originally a non-specific inflammatory response to HP or non steroidal anti-inflammatory drugs.

In a prior AGA guideline, (143) there is no specific recommendation for or against routine biopsy in patients with a normal endoscopy but based on the following statement it is implied that such a practice is not encouraged.

“It is the position of the American Gastroenterological Association (AGA) Institute that testing for celiac disease should be considered in symptomatic individuals who are at particularly high risk. These include those with unexplained IDA, a premature onset of osteoporosis, Down’s syndrome, unexplained elevations in liver transaminase levels, primary biliary cirrhosis, and autoimmune hepatitis. Situations in which testing for celiac disease should be selectively considered during the medical evaluation, especially if symptoms that could be the result of celiac disease are present, include type 1 diabetes mellitus, autoimmune thyroid disease, Sjögren’s syndrome, unexplained recurrent fetal loss, unexplained delayed puberty, selective IgA deficiency, irritable bowel syndrome, Turner’s syndrome, peripheral neuropathy, cerebellar ataxia, and recurrent migraine, as well as children with short stature and first- and second-degree relatives of patients with celiac disease.”

This statement in the AGA guideline would be consistent with the British Society of Gastroenterology guideline from 2014 that also implied that routine biopsies should not be taken in all patients and listed a group of disorders that should prompt biopsy. They did not specifically mention dyspepsia. (142)

The 2013 ACG guideline on celiac disease states that there is no consensus regarding which symptoms or laboratory abnormalities require CD evaluation but
goes on to state that mucosal biopsies of the duodenum should be considered in patients with dyspepsia who undergo EGD. (143). The 2005 AGA TR states “the exclusion of celiac disease should be considered” in patients with dyspepsia. (144)

There is evidence that quality of life is improved with early diagnosis of CD, even in those who are “asymptomatic” but these data come from observational studies and no systematic review has been done to answer this question. (151) The quality of evidenced for this statement is very low (table 10).

**Conclusion:** In the defined population (dyspepsia and no alarm symptoms) and a normal appearing duodenum there is no clear evidence that mucosal biopsies will detect CD more than would be expected in a control population (approximately 1%). If there are conditions present that suggest a higher than average risk for CD (defined risk factors are discussed above) then biopsies would be more likely than control to detect CD and early treatment might be beneficial. The evidence for this statement is very low.
Table # 10. Summary of evidence table for the question “In the defined patient population, do biopsies from the duodenum lead to diagnosis of celiac disease that would have been missed if no biopsies had been taken?”

<table>
<thead>
<tr>
<th>Study event rates (%)</th>
<th>Relative effect (95% CI)</th>
<th>Anticipated absolute effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>1% (95% CI 0.76% - 1.46%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Giardia lamblia

Giardia lamblia is the most common protozoan parasitic infection worldwide, caused by the ingestion of contaminated water or food. Methods for detecting G. lamblia include stool examination by immunoassay, direct search in unfixed wet mounts of duodenal biopsies or aspirates, and examination of formalin fixed duodenal biopsies processed for histology using routine H&E stains. Additional stains and immunofluorescence may also be employed. Since symptoms of giardiasis vary, and some patients may be asymptomatic or complain only of abdominal pain, it is reasonable to consider whether biopsies to detect G. lamblia are indicated in dyspepsia. In a prospective study of 137 consecutive patients with dyspepsia or IBS, excluding patients with alarm symptoms and symptoms lasting more than 1 year, the presence of G. lamblia was evaluated by three different techniques. (152) Stool sample examinations and direct search of wet mounts of duodenal biopsies were equally sensitive in detecting infection in 9/137 patients.

65 Ford APT 2009
66 Serious risk of bias mainly spectrum bias as noted by Ford et al.
67 Significant heterogeneity
Histologic examination detected only 2/9 patients diagnosed by the other two techniques (sensitivity 22.2%). Eight of the 9 patients had diarrhea.

Since stool examination was as sensitive as techniques requiring tissue biopsies, these findings suggest that duodenal biopsy is not necessary to diagnose G. lamblia infection. Two additional studies from Pakistan report Giardiasis in 9 to 44% of patients with dyspepsia in duodenal biopsies. (153-154) However, both studies suffer from methodological limitations, making the results difficult to interpret.

Based on the limited literature examining the association of Giardia infection to patients with dyspepsia and no alarm symptoms and the availability and accuracy of stool testing, duodenal biopsy is unlikely to be the best approach for the diagnosis of Giardia infection in this group.

**Duodenal Eosinophilia**

Recently, several studies and reviews from a single research center have suggested a role for duodenal eosinophilia in the pathophysiology of non-ulcer dyspepsia. In a Swedish adult population based, case-controlled study of 51 non-ulcer dyspepsia (NUD) patients and 48 controls, duodenal mucosal eosinophils were counted in routine H&E stained sections by two blinded, independent observers. (155) Mean eosinophil counts were significantly increased in the first and second portion of the duodenum of patients with NUD compared to controls (33 vs. 18 per high power field), with an odds ratio of 11.7 (95% confidence interval, 3.9-34.9). Gastric eosinophil counts were not increased in the NUD group. The authors
postulate a role for eosinophils in the symptoms of NUD, possibly via eosinophil mediated histamine release from tissue mast cells. In a second study from the same group (156), mean eosinophil counts and prevalence of duodenal eosinophilia (20.2/5 high power fields; 47.3%) were significantly higher in patients with postprandial distress syndrome than in controls (15/5 high power fields; 22.5%). No difference between controls and patients with nausea and vomiting, reflux type pain or other types of abdominal pain were found. Increased eosinophil counts were also found in patients with a history of allergies.

While these findings may suggest a role for mucosal eosinophils in upper GI symptoms, including dyspepsia, the evidence is limited at present. In addition, there is no evidence that knowledge of eosinophil counts in duodenal biopsies can improve or dictate different management, patient outcome or our understanding of the prognosis in FD. Therefore, outside of research protocols, there is no evidence that clinical outcomes will be affected by obtaining duodenal biopsies in patients with dyspepsia, no alarm symptoms and a normal duodenal appearance for the purposes of diagnosing duodenal eosinophilia at this time.

**Gastric Metaplasia**

Gastric metaplasia of the duodenum is commonly found during routine endoscopy. The prevalence of gastric metaplasia is high in patients with current or past history of duodenal ulcer and in patients with HP. (157) Gastric metaplasia was reported in 33% to 70% of patients with FD, with an increase in FD compared to healthy controls (33.3% vs 12%) (158) However, there is no evidence that the
diagnosis of gastric metaplasia changes the management or outcome in patients
with dyspepsia nor clear evidence that it is causative of dyspepsia. Therefore,
obtaining biopsies of the endoscopically normal duodenum in a patient with
dyspepsia and no alarm symptoms is unlikely to change clinical outcomes.

**Whipple’s Disease**

Maiwald, et al (159) studied three groups of patients for evidence of
Tropheryma. whippelii in small intestinal biopsies using histology and the
polymerase chain reaction (PCR) to detect bacterial DNA. One group consisted of
173 patients who underwent upper gastrointestinal endoscopy for the evaluation of
dyspepsia, abdominal pain, or possible gastroesophageal reflux or peptic ulcer
disease, without signs of malabsorption or consideration of Whipple disease. All
patients in this group had negative histology and PCR for T. whippelii. Therefore,
obtaining biopsies of the endoscopically normal duodenum in a patient with
dyspepsia and no alarm symptoms is unlikely to change clinical outcomes.

**IMMUNE COMPROMISED PATIENTS**

No specific data exist reporting the prevalence of graft versus host disease in
the defined patient population. Therefore, a clear conclusion on the clinical utility of
obtaining duodenal biopsies in this setting cannot be reached. However, upper and
lower endoscopy is frequently performed in patients at risk for graft versus host
disease, as the inclusion or exclusion of that diagnosis has important therapeutic
implications. In addition, the endoscopic appearance found in GVHD varies
depending on the severity of the process. Therefore, the decision to biopsy the
duodenum or any other site of the gastrointestinal tract in the susceptible patient
should be based on clinical suspicion.

There is little data regarding the prevalence of opportunistic infections
diagnosed by obtaining biopsies from normal duodenal mucosa in immune
compromised patients with dyspepsia, since most reported diagnoses are from
patients with additional symptoms and with visible lesions. In two studies of at
least 500 HIV positive patients on HAART therapy with dyspepsia and CD4 counts <
300, 2 diagnoses of cryptosporidium, 2 of G. lamblia, and one of Mycobacterium
were made on biopsies from endoscopically normal duodenum. (160-161) No CMV
infections were detected in endoscopically normal duodenal mucosa in either study.

In summary, there is very limited evidence suggesting that routine biopsies
of the endoscopically normal duodenum in patients with dyspepsia can increase the
diagnostic yield of opportunistic infections slightly (compared to obtaining only
gastric and esophageal biopsies), but the incremental yield is low. However, the
decision to biopsy immune compromised patients will take into account the degree
of clinical concern and the benefits of early diagnosis and treatment.

**Question:** If endoscopic biopsies are sent from any of the aforementioned areas, are specific
biopsy techniques recommended and are special stains needed to supplement hematoxylin
and eosin (H&E) histologic examination?
The available data regarding the diagnostic accuracy of methods of duodenal biopsy in the setting of celiac disease are insufficient for constructing evidence profiles. However, studies provide indirect evidence to support obtaining at least 4-6 biopsies that include the duodenal bulb and distal duodenum (162-167). A retrospective cohort study of 132,352 patients without known celiac disease compared the prevalence of newly diagnosed celiac disease in biopsies with ≥4 and <4 specimens. The probability of a new diagnosis of celiac disease increased from 0.7 to 1.8% when 4 or more specimens were obtained (p< 0.001). (162)

Regarding the issue of biopsy site (bulb vs distal duodenum), a prospective study of 461 patients, including 126 with newly diagnosed celiac disease, 85 with established celiac disease and 250 controls was performed. Five biopsies were taken, one from the duodenal bulb and four from quadrants of the second part of the duodenum. Nine percent of newly diagnosed celiac disease and 14% of established celiac disease patients had villous atrophy in the bulb only. Variable severity between the bulb and distal duodenum was noted in both patient groups (17% in newly diagnosed; 36% in established celiac disease) with the majority of those showing more severe histology in the bulb. (163) This study cites three additional studies in adults in which 1.8 to 9.5 % of patients had villous atrophy only in duodenal bulb biopsies. (164-166)

In a later prospective study of 77 patients undergoing clinically indicated EGD with duodenal biopsies, 4 biopsies were taken from the second portion of the duodenum and 4 from the bulb at the 3-, 6-, 9-, and 12-o’clock positions. In 28/77 patients with newly diagnosed celiac disease, the most severe villous atrophy was
noted in distal duodenal biopsies and in the bulb from either the 9- or 12-o’clock position (96.4% sensitivity). (167) The difference between the 12-o’clock and 3-o’clock biopsy position in detecting the most severe villous atrophy was 92% (24/26) versus 65% (17/26) (p=0.02). Five of 28 (18%) of patients had abnormalities confined to the bulb only.

Practice guidelines emphasize the fact that mucosal changes in celiac disease may be patchy, and therefore recommend multiple biopsies, variably specified as 4-6 in number, with more recent guidelines including a specific recommendation to include biopsies from the duodenal bulb (2 biopsies) as well as the second duodenum (4 biopsies) (142-145).

The use of special stains in diagnosing duodenal disorders is usually not necessary. Because Marsh 1 and 2 lesions have normal villous architecture, the recognition of a possible abnormality that could represent celiac disease rests on the pathologist’s perception and counting of an increase in intraepithelial lymphocytes (IELs) in an otherwise normal duodenal mucosa. CD3 (T-cell marker) immunohistochemistry stains have been utilized in a number of studies to highlight intraepithelial T-cells for the purposes of counting IEL/enterocyte ratios (168). However, studies using H&E counting methods (villous tip or villous side and tip methods) show similar results as those obtained using CD3 stains. (169-172) If CD3 stains are employed to report IEL counts in the setting of normal villous architecture, it is suggested that pathologists run parallel stains on control biopsies from normal and known celiac patients to gain experience with counting, in order to
avoid the possibility of artificial increases that may be detected simply from utilizing a more sensitive technique of identification.

Based on available data, the performance of CD3 stains in suspected celiac disease is not specifically recommended, but may be performed at the discretion of the pathologist in the setting of normal villous architecture, or for study purposes.

**Conclusion:** In the defined population, if biopsies are obtained for the purposes of diagnosing celiac disease, 6 biopsies obtained from the duodenal bulb (2) and from the distal duodenum (4) in four-quadrant fashion will yield maximal diagnostic accuracy. Performance of special stains, including CD3 stains in suspected celiac disease does not increase diagnostic accuracy in the hands of expert GI pathologists. There are no data about increased diagnostic accuracy with special stains in any of the other conditions discussed above. Quality of evidence is very low due to the observational nature of all studies with methodological limitations.

**Summary and Discussion**

This review has focused on the need for biopsies to diagnose clinically important conditions in the absence of endoscopic mucosal abnormalities. We reviewed literature dating back to the beginning of the modern endoscopic era to
find any evidence for routine biopsy in adult patients with mild upper GI symptoms (dyspepsia) and a normal EGD.

We could find no clear evidence that esophageal biopsies added value to patient care in this situation. In the case of gastric biopsies, there was evidence that biopsy of the normal appearing stomach could yield a diagnosis of HP and that treatment of HP infected patients would improve dyspepsia in some patients and additionally reduce risk of subsequent peptic ulcer disease, gastritis and gastric cancer. Finally, there is no clear evidence that obtaining duodenal biopsies in patients who are at low risk of CD (no concurrent risk factors) and have a normal EGD may change clinical outcomes.

This review will support development of the MPS concerning endoscopic biopsies in an adult with dyspepsia and who has a normal appearing upper GI tract on EGD. Conclusions emanating from this MPS will have significant implications on practice patterns in the United States (both for the endoscopist and the pathologist) and concurrently, implications on resource use related to upper GI endoscopy.
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APPENDIX 1
Search strategy for the main search

The following databases were searched:

- EBM Reviews - Cochrane Central Register of Controlled Trials October 2013
- Embase 1980 to 2013 Week 46
- Ovid MEDLINE(R) 1946 to November Week 1 2013
- Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations November 18, 2013

The search string was as follows:

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<tr>
<td>2 (dyspepsia or dyspeptic).ti,ab.</td>
<td>26631</td>
</tr>
<tr>
<td>3 1 or 2</td>
<td>42484</td>
</tr>
<tr>
<td>4 exp gastrointestinal biopsy/ use emez</td>
<td>26141</td>
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<td>5 *Biopsy/ use mesz,cctr</td>
<td>13832</td>
</tr>
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<td>6 (biopsy or biopsies or bioptic).ti,ab.</td>
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</tr>
<tr>
<td>7 exp immunohistochemistry/</td>
<td>911342</td>
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<tr>
<td>8 exp Histology/ or exp microscopy/</td>
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<td>(stain or stains or staining or alcian or H&amp;E or &quot;H/E&quot; or PAS or Hematoxylin or eosin or (ihc adj4 tryptase) or (tryptase adj4 immunohistochemistry) or gms or immunofluorescence or Tzanck or Giemsa or Warthin-Starry or Genta or Steiner or (McMullen* adj2 stain*) or (mcmullen* adj2 modif*) or (H pylori adj2 silver) or (h pylori adj2 antibod*) or HpSS or gastrin or chromogranin or mucicarmine).ti,ab.</td>
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(aspirate adj10 (duoden* or small bowel or bile or small intestine or small intestinal)).ti,ab.

or/4-15

3 and 16

limit 17 to english abstract [Limit not valid in CCTR, Embase; records were retained]

limit 17 to english [Limit not valid in CCTR; records were retained]

limit 18 to (animal/ or animals/ or animal studies/) [Limit not valid in CCTR, Ovid MEDLINE(R), Ovid MEDLINE(R) In-Process; records were retained]

limit 20 to (animal/ or animals/ or animal studies/) [Limit not valid in CCTR, Ovid MEDLINE(R), Ovid MEDLINE(R) In-Process; records were retained]

Human/ or Humans/

animal/ or animals/ or animal studies/

21 not (22 and 23)

20 not 24

limit 25 to "all child (0 to 18 years)" [Limit not valid in CCTR, Embase; records were retained]

exp Child/

adult/ or aged/

26 not (27 and 28)

25 20 not 24

limit 30 to (conference abstract or conference proceeding or editorial or letter or note or case reports or comment or consensus development conference) [Limit not valid in CCTR, Embase, Ovid MEDLINE(R), Ovid MEDLINE(R) In-Process; records were retained]

limit 30 to (case report or comment or conference or congresses or editorial or letter) [Limit not valid in Embase, Ovid MEDLINE(R), Ovid MEDLINE(R) In-Process; records were retained]

30 not (31 or 32)

remove duplicates from 33