Hepatocyte Paraffin 1 Antigen as a Biomarker for Early Diagnosis of Barrett Esophagus

Jennifer A. Jeung, MD,1 Justin J. Coran, MA,2 Chen Liu, MD, PhD,1* and Diana M. Cardona, MD3*

Key Words: Barrett esophagus; Carbamoyl phosphate synthetase I; Hepatocyte paraffin 1; HepPar1

DOI: 10.1309/AJCPYOBVGS4CGA8Y

Abstract

We evaluated hepatocyte paraffin 1 (HepPar1) antigen expression, a sensitive marker of small intestinal differentiation, in combination with morphologic features to demonstrate intestinal differentiation in cases equivocal for Barrett esophagus (BE). Clinicopathologic features and HepPar1 expression were recorded for 54 BE cases, 45 consistent with reflux esophagitis (RE) cases, and 65 “suspicious” for BE (SBE) cases. The SBE category included RE cases with 2 or more morphologic changes associated with BE or metaplastic reaction to injury (eg, multilayered epithelium, squamous islands, goblet cell mimickers, pancreatic metaplasia). HepPar1 was expressed in all 54 BE cases, 4 of 45 RE cases, and 24 of 65 SBE cases. In SBE cases, 2 or more morphologic changes were associated with HepPar1 expression in 37% of cases (24/65), 3 or more features in 59% (13/22), and 4 or more features in 100% (4/4) (P ≤ .004). The combination of certain morphologic changes and HepPar1 expression in clinically suspicious distal esophageal biopsy cases without goblet cells supports the presence of evolving intestinal metaplasia.

Barrett esophagus (BE), which develops secondary to chronic gastroesophageal reflux disease, is the major risk factor for the development of esophageal dysplasia and adenocarcinoma.1-7 Studies suggest that the histologic changes seen in BE are a continuum from an initial metaplastic change of the squamous esophageal mucosa to an intermediate columnar epithelium followed by goblet cell metaplasia.2,8,9 This columnar epithelium with goblet cells, defined as “intestinal metaplasia” (IM), is widely accepted as having potential for malignant progression.5,7 The American College of Gastroenterology requires an endoscopically identifiable columnar-type mucosal change in the distal esophagus combined with histologic confirmation of goblet cells for the diagnosis of BE.7 The American Gastroenterological Association additionally supports the requirement of goblet cells on biopsy after endoscopic identification of metaplastic columnar epithelium replacing the normal stratified squamous epithelium of the distal esophagus on endoscopy, stating that “intestinal metaplasia is the only type of esophageal columnar epithelium that predisposes to malignancy.”10 However, other studies have suggested that goblet cells alone may not be the earliest histologic indicator of intestinal differentiation.2,11 This metaplastic columnar epithelium of the esophagus without goblet cells demonstrates DNA content abnormalities similar to columnar epithelium with goblet cells,12 and immunohistochemical reactivity against Cdx-2, villin, and Das-1 highlights the presence of intestinal differentiation.11,13-15 In fact, this non–goblet cell metaplastic columnar epithelium may have a similar or possibly greater risk of neoplastic progression than that of columnar epithelium with goblet cells.2,3,12,16,17

Upon completion of this activity you will be able to:

• define the American College of Gastroenterology (ACG) requirements for a diagnosis of Barrett esophagus and compare and contrast these with the British Society of Gastroenterology (BSG) requirements.
• define the antigen target of hepatocyte paraffin 1 (HepPar1) antibody and describe the pattern of immunohistochemical staining.
• discuss the different morphologic features that can be identified in distal esophageal biopsies and how these correlate with clinical impression and potential markers of intestinal differentiation.

The ASCP is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians. The ASCP designates this journal-based CME activity for a maximum of 1 AMA PRA Category 1 Credit™ per article. Physicians should claim only the credit commensurate with the extent of their participation in the activity. This activity qualifies as an American Board of Pathology Maintenance of Certification Part II Self-Assessment Module.

The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.

Questions appear on p 158. Exam is located at www.ascp.org/ajpccme.
Hepatocyte paraffin 1 (HepPar1) antigen, recently identified as carbamoyl phosphate synthetase I (CPS1), the rate-limiting enzyme in the urea cycle,18 is a marker of intestinal differentiation. Specifically in small intestinal mucosa, antibody to HepPar1 antigen demonstrates granular cytoplasmic reactivity within absorptive cells without reactivity in goblet or Paneth cells. Several studies have identified HepPar1 antibody as a sensitive and specific marker of the IM found in BE.1,19 An advantage of assessing CPS1 expression at the gastroesophageal junction (GEJ) is its presence in the absorptive cells of IM but absence within the columnar cells of cardiac-type mucosa.19

Although in the United States the presence of goblet cells in distal esophageal biopsy specimens is necessary for a diagnosis of BE, several other morphologic characteristics have been shown to be highly associated with BE. For example, Shields et al20 identified multilayered epithelium and basal squamous-appearing cells with superficial columnar cells as a transitional state in squamous-to-columnar metaplasia that may be nearly 100% specific for cases of distal esophageal IM.9,14,21 Srivastava et al22 described crypt atrophy, branching, and disarray, in addition to multilayered epithelium, as also being specific for BE. In addition, goblet cell mimickers, such as “pseudogoblet cells” and “columnar blues,” pancreatic metaplasia, Paneth cells, and reepithelialized squamous mucosa are also thought to represent metaplastic change within the distal esophagus.11,23-26

As the incidence of esophageal adenocarcinoma continues to increase,27 identifying BE through endoscopic screening with biopsies remains increasingly important.7,28,29 Defining the earliest changes associated with intestinal differentiation and identifying at-risk patients is crucial for care given the survival advantage of early cancer detection vs symptomatic presentation.7 By using HepPar1 antigen expression as a sensitive and specific marker of intestinal differentiation, our purpose in this study was to assess the presence of BE-associated features as a way to predict the presence of IM within distal esophageal biopsy specimens without goblet cells. In clinically suspected cases, the presence of these BE-associated morphologic features and expression of HepPar1 antigen may indicate the need for increased surveillance or a shorter interval to repeated biopsy in an attempt to diagnose BE according to the current American College of Gastroenterology guidelines.

Materials and Methods

Case Selection and Morphologic Analysis

This study was approved by the institutional review board at the University of Florida College of Medicine, Gainesville. Any case representing a patient younger than 18 years was excluded from this study per institutional review board permission requirements.

By using the diagnostic term “consistent with reflux esophagitis” (RE), we retrospectively searched the Department of Pathology files for cases that had a final diagnosis of RE between January 1, 2000, and April 1, 2009, and initial case selection consisted of 587 total cases. All available clinical records were reviewed to determine the sex and age of patients, endoscopic appearance of the distal esophagus/GEJ region, the presence or absence of a previous diagnosis of BE or IM, and available clinical follow-up. In addition, cases with the final diagnosis of “Barrett esophagus” were searched from January 1, 2006, to December 31, 2006, and the first 100 cases were selected.

Based on the procedure reports, endoscopic findings were recorded as positive or negative for features suggestive of or consistent with BE, including presence of an irregular Z-line, “salmon-colored mucosa,” and/or displacement of the squamocolumnar junction proximal to the GEJ. Per endoscopic reports, distal esophageal biopsy specimens were obtained from the neosquamocolumnar junction in cases with endoscopic abnormalities or the actual squamocolumnar junction/GEJ in endoscopically normal-appearing cases. In addition, distal esophageal biopsy specimens obtained from patients older than 18 years with reportedly normal endoscopic findings and no significant histologic changes were used as control samples.

Category selection was determined after the cases were reviewed at a multiheaded scope by 2 pathologists (J.A.J. and D.M.C.), and inclusion required histologic identification of both squamous and glandular mucosa (squamocolumnar mucosa) for each case. Cases originally diagnosed as consistent with RE (defined by the presence of intraepithelial eosinophils associated with basal cell hyperplasia and elongation of the lamina propria rete pegs in the squamous component) were reviewed, and the columnar component was assessed for the presence of “BE-associated” histologic features. The cases that had 2 or more BE-associated morphologic features in the columnar component—crypt disarray and atrophy, multilayered epithelium, Paneth cells, squamous mucosal islands, pancreatic metaplasia, or columnar cells with basophilic mucin on H&E stain—were categorized as “suspicious” for BE (SBE). A total of 65 cases met these criteria and were included in the SBE category. Of the remaining cases, in which the features consistent with RE were confirmed and fewer than 2 BE-associated features were identified, the first 45 cases were kept for inclusion in the study.

Cases involving patients who had a previous diagnosis of BE were excluded from the RE and SBE categories. For the BE cases, the diagnosis was confirmed by the reviewing pathologists, and the first 54 cases were included in the study. The presence of any BE-associated morphologic
feature and/or additional features associated with IM (incomplete IM, epithelium with gastric foveolar mucosa intermixed with goblet cells; complete IM, epithelium with goblet cells and absorptive enterocytes with a brush border; squamous epithelium overlying columnar crypts with IM; and hybrid glands) were recorded for those cases. In addition, the presence of low-grade dysplasia, high-grade dysplasia, and/or esophageal adenocarcinoma as previously diagnosed by consensus among gastrointestinal pathologists was recorded.

Immunohistochemical Staining

Immunohistochemical staining was performed by routine methods on 4-μm-thick, formalin-fixed, paraffin-embedded tissue sections. Briefly, after the slides were dried for 2 hours in a 60°C oven, they were placed on a Ventana BenchMark automated immunostainer (Ventana Medical Systems, Tucson, AZ) and dewaxed, and heat-induced epitope retrieval was performed with the Ventana CC1 retrieval solution for 30 minutes at 95°C to 100°C. Primary mouse monoclonal antibody, antihuman HepPar1 (clone OCH1E5, DAKO North America, Carpinteria, CA) at a 1:50 dilution, was applied to the sections at 37°C for 32 minutes. Presence of the antigen was visualized using the UltraView DAB detection kit (Ventana Medical Systems). Slides were counterstained with Ventana Hematoxylin, taken off the stainer, and then dehydrated, cleared, and mounted with permanent mounting media. Immunoactivity was considered positive if cells demonstrated discrete granular cytoplasmic staining.

Statistical Methods

The presence of HepPar1 antibody immunoactivity was correlated with the recorded morphologic findings in RE, BE, and SBE cases and clinical features using $\chi^2$ analysis.

Results

The clinical features of the patient groups are summarized in Table 1. Table 2 summarizes the morphologic findings of patient groups.
findings and HepPar1 immunohistochemical results for all cases. Within the RE category, 19 cases demonstrated 1 BE-associated morphologic feature (multilayered epithelium, squamous mucosal islands, pancreatic metaplasia, or nongoblet columnar cells with basophilic mucin). Only 4 (9%) of 45 cases demonstrated focal HepPar1 antibody immunoreactivity. 2 of the 4 had 1 of the aforementioned BE-associated morphologic features and endoscopic features suspicious for BE. The 54 BE cases demonstrated the following morphologic features: 46 (85%) had incomplete IM; 8 (15%), complete IM; 27 (50%), squamous epithelium overlying crypts with IM; 7 (13%), hybrid glands; 19 (35%), crypt disarray and atrophy; 9 (17%), multilayered epithelium; 13 (24%), Paneth cells; 24 (44%), squamous mucosal islands; and 3 (6%), pancreatic metaplasia. In addition, 7 of the 54 BE cases contained low-grade dysplasia, 3 had high-grade dysplasia, and 1 had esophageal adenocarcinoma. HepPar1 antigen was expressed in all 54 of the BE cases (100%). The expression of HepPar1 antigen was lost in areas of low-grade dysplasia, high-grade dysplasia, and esophageal adenocarcinoma. None of the 5 control cases demonstrated HepPar1 antigen expression (data not shown).

**Image 1A** and **Image 1B**: A distal esophageal mucosa with evidence of reflux esophagitis demonstrated by basal cell hyperplasia and intraepithelial eosinophils (H&E, ×200). Immunohistochemical stain using hepatocyte paraffin 1 (HepPar1) antibody demonstrates no expression in biopsy consistent with reflux esophagitis (×200).

**Image 1C** and **Image 1D**: Distal esophageal biopsy demonstrating columnar mucosa with goblet cells (intestinal metaplasia [IM]) histologically diagnostic for Barrett esophagus (H&E, ×200). HepPar1 antigen expression is identified in areas of IM (×200).
The SBE group (n = 65) demonstrated the following BE-associated morphologic features: 54 (83%) had nongoblet columnar cells with basophilic mucin, 39 (60%) had multilayered epithelium, 26 (40%) had pancreatic metaplasia, 31 (48%) had squamous mucosal islands, 3 (5%) had Paneth cells, and 3 (5%) had crypt disarray and atrophy. HepPar1 antigen was expressed in 24 (37%) of 65 cases, with 4 additional cases demonstrating focal expression. HepPar1 antigen expression was identified as discrete granular cytoplasmic immunoreactivity in the vicinity of the described BE-associated features, most commonly within columnar cells adjacent to or within pancreatic metaplasia, multilayered epithelium, or columnar cells with blue mucin. The presence of 2 or more BE-associated features was associated with HepPar1 antigen expression in 24 (37%) of 65 cases, 3 or more features with CPS1 expression in 13 (59%) of 22 cases, and 4 or more features with CPS1 expression in 4 (100%) of 4 cases (P ≤ .004). Specifically, multilayered epithelium, pancreatic metaplasia, and columnar cells with basophilic mucin were significantly associated with HepPar1 antibody immunoreactivity (P ≤ .01). The association between HepPar1 antigen expression and the presence of squamous mucosal islands, Paneth cells, and crypt disarray/atrophy was not statistically significant. The clinical and endoscopic features of the 24 SBE cases with HepPar1 antigen expression are summarized in Table 3.

*Image 2*: A, Barrett esophagus with low-grade dysplasia (H&E, ×200). B, Hepatocyte paraffin 1 (HepPar1) antigen expression is lost in areas of low-grade dysplasia (×200). C, Barrett esophagus with focal high-grade dysplasia (H&E, ×200). D, HepPar1 antigen expression is lost in areas of high-grade dysplasia (×200).
Identification of at-risk patients who could benefit from early follow-up is made through the histologic diagnosis of BE, the precursor lesion of dysplasia and esophageal adenocarcinoma. Currently, the histologic diagnosis of BE requires the demonstration of unequivocal goblet cells by H&E staining within distal esophageal biopsy specimens. However, goblet cells are not always apparent, and perhaps more important, this morphologic feature may not be the earliest sign of intestinal differentiation. Thus, recognition of suspicious morphologic features and the judicious use of biomarkers that can detect this metaplastic state may be important for clinical practice.

For decades, the use of different histochemical stains and immunohistochemical markers for the evaluation of IM in esophageal biopsy specimens has been studied. Periodic acid–Schiff/alcian blue, pH 2.5, stains neutral and acidic cytoplasmic mucins of goblet and nongoblet columnar epithelium. However, several authors have noted nonspecific staining...
with periodic acid–Schiff/alcian blue, including staining of foveolar and glandular epithelium of the cardia and distal esophagus, which can potentially lead to a false-positive BE diagnosis.13,14,24,26,31-34

Several immunohistochemical markers have been used as potential indicators of intestinal differentiation in esophageal columnar epithelium with and without goblet cells, including MUC2, villin, Cdx-2, and Das-1.2,9,11,13,14,24 The MUC2 antibody reacts with the mucin in goblet cells; therefore, the staining intensity depends on the density of goblet cells, indicating that this marker may not be a reliable marker of early intestinal differentiation.1,2,35

The antibody to villin (a cytoskeleton protein in the microvillus core of the brush border) is a less specific marker for IM, as it can also be reactive within fundic-type mucosa.11 However, in a study by Shi et al,11 cytoplasmic expression of villin in columnar-lined mucosa without goblet cells was proposed as a sign of early intestinal differentiation.

Phillips et al13 demonstrated that goblet and non–goblet cells in 100% of BE cases studied exhibited nuclear immunoreactivity for antibody to Cdx-2 (a transcription factor involved in intestinal epithelial differentiation and maintenance). That study also demonstrated that 30% of cases with

<table>
<thead>
<tr>
<th>Feature</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (range) y</td>
<td>51 (21-79)</td>
</tr>
<tr>
<td>Male</td>
<td>13 (54)</td>
</tr>
<tr>
<td>Female</td>
<td>11 (46)</td>
</tr>
<tr>
<td>Endoscopy findings suspicious for Barrett esophagus†</td>
<td>13 (54)</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage) unless otherwise indicated.
† Suspicious findings included a proximally displaced squamocolumnar junction, an irregular Z-line, and salmon-colored mucosa.
jejunal-type esophageal epithelium with no suspicion of IM were focally Cdx-2+, which was postulated to represent evidence of early intestinal differentiation as opposed to lack of specificity.13

In a study by Glickman et al,15 Das-1 (a monoclonal antibody developed against a colonic epithelial protein) was detected in 88% or more of BE cases with reactivity in 35% of cases of junctional-type epithelium without IM. While some of these markers proved highly sensitive and/or specific for BE, none of these studies specifically evaluated expression in cases that lacked goblet cells but were morphologically suspicious owing to the presence of BE-associated histologic features. Ours is the first study to systematically evaluate HepPar1 antigen expression as a marker of intestinal differentiation in the nongoblet columnar epithelium of distal esophageal biopsy specimens with BE-associated morphologic features.

Several studies have revealed that HepPar1 antigen (CPS1) is expressed in small intestinal mucosa but not in normal esophageal, gastric, or colonic mucosa, which varies from other markers (ie, MUC2, villin, Cdx-2, and Das-1) previously used in the evaluation of BE.1,18 Unlike MUC2 antibody, for which the staining intensity depends on the density of goblet cells, HepPar1 antigen is expressed in the absorptive cells and may be detected in samples with IM even if goblet cells are rare or not present in a particular section.1 Unlike villin, which may be expressed in fundic-type mucosa, HepPar1 was not found to be expressed in gastric mucosa.1,18 HepPar1 antigen is expressed in the complete and incomplete forms of IM and in focal and diffuse IM, which potentially makes HepPar1 antibody a useful tool in small biopsy specimens.1 Cdx-2 and Das-1 have been found to be expressed in 30% and 35%, respectively, of esophageal biopsy specimens demonstrating junctional epithelium without goblet cells or suspicion of IM.1,13,15 These findings may support evidence of early intestinal differentiation, but could support lack of specificity.

In our study, all cases diagnosed as BE (with endoscopic features and histologically unequivocal goblet cells) demonstrated HepPar1 antibody immunoreactivity. Of the cases that were designated as SBE on histologic grounds, HepPar1 antigen expression was present in 37% with 2 or more described BE-associated morphologic features, 59% with 3 or more BE-associated morphologic features, and 100% with 4 or more BE-associated morphologic features. These results indicate that identification of increasing numbers of BE-associated morphologic features correlates with a greater likelihood of HepPar1 antigen expression, supporting the presence of IM. Granular cytoplasmic immunoreactivity in columnar cells was typically present in the vicinity of the described BE-associated features, implying that these histologic features do not necessarily equal IM, but rather indicate that the tissue displays intestinal differentiation, despite the absence of identifiable goblet cells. Only 4 of the RE cases had focal HepPar1 antibody immunoreactivity, of which 2 demonstrated 1 BE-associated morphologic feature and endoscopic findings suspicious for BE. Of the 110 non-BE cases, only 2 (1.8%) expressed HepPar1 antigen without the presence of at least 1 BE-associated feature. HepPar1 antigen expression is uncommonly identified in cases without morphologic changes that are associated with intestinal differentiation. It is interesting that, similar to findings by Chu et al,1 HepPar1 antigen expression was lost in areas of adjacent low-grade dysplasia, high-grade dysplasia, and adenocarcinoma. The loss of HepPar1 antigen expression with development of dysplasia or malignant expression is not an event unique to the esophagus. Loss of antigen detection has been described in poorly differentiated hepatocellular carcinomas, IM-associated gastric adenocarcinomas, and small intestinal adenomas with high-grade dysplasia and/or invasive adenocarcinoma.1,18,36

There is ongoing debate about the clinical significance of detecting non–goblet cell forms of IM. Do they carry the same risk of progression to dysplasia and/or adenocarcinoma? According to the British Society of Gastroenterology, an endoscopically abnormal area suggestive of BE proximal to the GEJ with biopsy findings of columnar-lined mucosa is enough to diagnose BE regardless of the presence of goblet cells.37 Several studies supporting the British Society of Gastroenterology guidelines have concluded that nongoblet columnar epithelium may have neoplastic potential, and people who have specialized columnar mucosa without goblet cells have a similar risk of neoplastic progression as do people with goblet cells.12,16,38 Bright et al39 found a 2.5% incidence of significant findings (high-grade dysplasia or adenocarcinoma) in a 3-year endoscopic surveillance study of patients who had endoscopic abnormalities suspicious for BE with columnar-lined mucosa with or without goblet cells.

Currently, the American Society for Gastrointestinal Endoscopy guidelines state that screening endoscopy for BE may be appropriate in patients with gastroesophageal reflux disease symptoms, but no further screenings are necessary after a negative examination.40 It is interesting that Gatenby et al38 reported that up to 40% of cases of BE in their study would have been missed based on initial biopsy using the current American Society for Gastrointestinal Endoscopy criteria. They further reported that 54.8% and 90.8% of patients with non–goblet cell columnar metaplasia demonstrated IM at 5 and 10 years of follow-up, respectively.38 A limitation of our study is the lack of long-term follow-up and adequate rebiopsy samples, given the initial diagnosis of RE. The median follow-up period for the 65 patients in our SBE category was 3 years (range, 0-9 years). Although 14 patients underwent rebiopsy, only 2 had adequate squamocolumnar junction samples, 1 of which contained diagnostic goblet cells of BE.

There is great importance in making a diagnosis of BE for an individual patient, as patients with BE have reportedly
higher psychological stress and financial burden than the general population. However, BE remains the major risk factor for the development of esophageal dysplasia and adenocarcinoma. Mortality from esophageal cancer is high, and there is a significant survival advantage with early cancer detection vs symptomatic presentation.

Currently in the United States, the identification of goblet cells in distal esophageal biopsy specimens is the only histologic criterion used to diagnose BE and initiate follow-up screening. At times, the diagnosis of BE can be difficult to make on H&E-stained slides because of inconspicuous or rare goblet cells, sampling error, or goblet cell mimickers. Based on our results, recognition of the well-described BE-associated morphologic features predicts the presence of HepPar1 antigen expression, a sensitive and specific marker of IM. The presence of certain morphologic features (specifically multilayered epithelium, pancreatic metaplasia, and columnar cells with blue mucin) and overall 2 or more of the proposed BE-associated morphologic features were significantly associated with HepPar1 antigen expression. No long-term follow-up studies or surveillance guidelines of patients with clinicopathologic features suspicious for BE but without diagnostic goblet cells exist. However, one could argue that given the evidence of metaplastic change, reported increased neoplastic risk, and the need for early cancer detection, recognition of these earlier/non–goblet cell lesions is important, as opposed to implying a completely negative screening. Use of the clinical impression, the presence of these suspicious morphologic changes, and possibly phenotypic markers of intestinal differentiation may prove to be an additional method of diagnosing IM of the distal esophagus as opposed to the use of goblet cells alone.

**References**


