Pyloric and foveolar type metaplasia are important diagnostic features in Crohn’s disease that are frequently missed in routine pathology

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DOI: 10.14670/HH-18-167
Article type: ORIGINAL ARTICLE
Accepted: 2019-09-20
Epub ahead of print: 2019-09-20
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SHORT RUNNING TITLE: Pyloric Metaplasia and Crohn’s.

KEY WORDS: Pyloric metaplasia, foveolar metaplasia, Ulcer Associated Cell Lineage (UACL), Crohn’s, ileum

Word Count: 3946

Conflict of Interest
No conflicts of interest were included in this article.
ABSTRACT

Pyloric type metaplasia (PYME) as evidence of chronic mucosal damage, is one of the main histopathological findings for diagnosing Crohn’s Disease (CD) in terminal ileum biopsies, according to the latest guidelines but still frequently underdiagnosed in routine pathology. Foveolar metaplasia (FOME) changes in mucosa, another aspect of the chronic post-inflammatory Ulcer Associated Cell Lineage (UACL), have only been reported in a few cases. However, their clinical significance has not been investigated in depth except in pouchitis. The aim of this study was to investigate the importance of meticulous study of terminal ileum biopsies for the recognition of PYME/FOME as an adjunct finding helpful for the diagnosis of CD. In the present study, two experienced gastrointestinal pathologists, have reviewed 105 terminal ileum biopsies from 105 patients with CD, using a protocol of 15 sections on average per biopsy. In 21% (22/105) of cases PYME was recognized and in 4% (4/105) FOME was also present. PYME/FOME had not been detected in 83% of these cases in the original reports. FOME was also identified in terminal ileum biopsies, a feature not reported previously in CD. Conclusively, PYME/FOME can be easily missed in terminal ileum biopsies from patients with suspected or known CD unless a meticulous study of the histologic material is carried out combined with awareness of the pathologist about its importance.

INTRODUCTION

Terminal ileum biopsy is of major importance in establishing histological diagnosis of Crohn’s disease (CD) in conjunction with clinical, laboratory, imaging and endoscopic data. Granulomas, mucosal architectural alterations, discontinuous chronic or active chronic inflammation comprise pathognomonic findings for CD, in the proper clinical setting (Magro et al., 2013; Langner et al., 2014; Gionchetti et al., 2017). However, granulomas are detected in only 15-36% of intestinal biopsies and there is an urgent need for other morphological findings consistent with CD to establish the correct diagnosis (Jenkins et al, 1997; Feakins, 2013; Gionchetti et al., 2017).

Pyloric gland metaplasia (PYME) is a feature indicative of chronic mucosal damage, commonly related to mucosal ulceration and repair (Ulcer Associated Cell Lineage -UACL). UACL is activated by various stimuli, including infections with

Although, pyloric metaplasia can be detected generally in chronic small bowel inflammation, its diagnostic value is highlighted in CD. PYME presence in ulcerative colitis (UC) is considered extremely rare as opposed to the presence of Paneth metaplasia in UC (Goldenring, 2018). There are few studies with small number of cases mentioning a very low prevalence of PYME detection of 1% in UC with back wash ileitis (Haskell et al, 2005, Yantiss and Odze, 2007) and 15% in NSAID’s associated ileitis (Lengeling et al., 2003; Yantiss and Odze, 2007). However, metaplastic changes were found in up to 40% of pouch biopsies of patients with UC and restorative proctocolectomy with ileal pouch-anal anastomosis. In this context, PYME appears to be a marker for chronic antibiotic-refractory pouchitis or CD of the pouch (Kariv et al., 2010, Agarwal et al., 2013, Langner et al., 2014; Ramai et al., 2017). Biopsies from ileal pouch also present colonic type metaplasia with villous atrophy and crypt hyperplasia (Langner et al., 2014; Ascolani et al., 2014).

PYME in CD has been well described in ileectomies as well as in 2–27% of ileal biopsies, and the significance of its detection has been described in ECCO/ESP histopathology guidelines, ESP histology paper for inflammatory bowel disease and a few other reports (Geboes et al, 1998; Koukoulis et al., 2002; Magro et al., 2013; Langner et al., 2014; Ramai et al., 2017).

There is evidence of bidirectional (pyloric type and foveolar) “mucous cell metaplasia” in CD. Metaplastic lesions are composed of cells which resemble gastric gland mucous cells and/or gastric surface mucous cells (Kushima et al, 1997; Buisine et al., 1999; Longman et al., 2000; Buisine et al., 2001; Kaneko et al., 2008). Foveolar metaplasia (FOME) has not been reported in endoscopic ileal biopsies so far. It seems that the incidence of detection of these metaplastic changes is highly variable and dependent on the meticulous study of the histological specimen (Surawicz, 1982; Langner et al., 2014). Metaplastic glands are usually few and can be easily missed, unless a careful examination of multiple sections is carried out.

The purpose of this study was to evaluate the incidence of PYME and/or FOME in terminal ileum biopsies taken from patients with known or suspected CD and to correlate these results with the typical histological findings of the disease, as
well as the extent of the histopathological assessment as reflected in the number of the examined sections.

**MATERIALS AND METHODS**

For the purposes of this study, we retrieved, from archives of our private Histopathology Laboratory “Istodagnostiki” in Thessaloniki, Greece, 105 consecutive terminal ileum biopsies taken during ileocolonoscopy from 105 patients with suspected or known CD, studied for diagnostic purposes from March 2008 to December 2010; both histological reports and slides were reviewed anonymously. All patients had clinical, radiological or endoscopic findings compatible with suspected or known CD. The samples were fixed in 10% neutral formalin for 12 to 24 hours and were then routinely processed. The study included the hematoxylin-eosin original slides used for the initial routine diagnosis. Histochemical PAS/Alcian blue stain was performed for the initial diagnosis in 3 cases with questionable PYME or suspected FOME. No additional sections were cut.

The slides were reviewed for the presence of PYME and/or FOME by two pathologists experienced in gastrointestinal pathology (AG, SM), according to the previously referred morphological criteria for metaplastic lesions (Kushima et al., 1997; Jenkins et al, 1997; Langner et al., 2014; Gionchetti et al., 2017). The biopsies in which metaplastic changes were recognized were further analyzed regarding the following parameters: 1) the clinical information obtained from the histological report, with regard to whether CD was already known to be present or if it was diagnosed for the first time, 2) the disease location 3) the number of tissue fragments in the biopsy, 4) the number of sections containing PYME and/or FOME in relation to the total number of sections in the biopsy, 5) the presence of granulomas, not related to crypt injury, 6) the presence of active chronic or acute active inflammation (intraepithelial neutrophils, cryptitis, crypt abscesses, surface epithelial neutrophils, erosion, ulceration), 7) crypt distortion 8) the description of metaplastic changes in the original report.
RESULTS
Pyloric and/or foveolar metaplasia were identified in 22/105 terminal ileum biopsies (21%), of whom 6 (27%) had known CD, while 16 (73%) were diagnosed for the first time with CD. The analysis of these 22 biopsies including demographic data (age and gender), clinical information and histologic evaluation are shown in Table 1. Patient age ranged from 17 to 64 years old and regarding their gender, 14 of them were males and 8 females.

The cases with known CD had received treatment, but the medication used was not available in the initial referral notes, except for one patient (No 6 in table 1) treated with mesalazine and azathioprine. One patient (no 21 in table1) underwent colectomy 20 years ago and was in clinical remission at the time of biopsy. None of the patients had a history of regular NSAID intake.

In 11 cases endoscopic findings were not available. In 9 of 22 patients with PYME/FOME CD extended to the colon, with histological detected lesions. In one of them there was upper gastrointestinal tract involvement in stomach and duodenum. Eleven of cases with PYME/FOME had endoscopically aphthous ulcers, nodularity or redness in terminal ileum, findings compatible with active disease; for the remaining cases endoscopic findings were not available in the initial referrals.

In 18/22 biopsies (82%) only pyloric metaplasia was found, whereas in 4/22 cases (18%), FOME was present additionally with PYME. Histochemical PAS/Alcian blue stain confirmed the presence of metaplastic lesions in 3 cases with questionable PYME or suspected FOME. The pyloric glands formed small aggregates near ulcers and were usually located in the deep lamina propria (Fig.1). FOME was found in the surface epithelium, always above the underlying metaplastic pyloric type glands and was characterized by tall columnar mucous secreting cells, resembling the foveolar gastric epithelium (Fig. 2A, 2B).

Regarding the number of fragments, the biopsies consisted of 3-12 tissue fragments (with a mean of 6 tissue fragments per biopsy) each. Concerning the number of serial paraffin sections present on the slides in each biopsy, this varied from 10 to 24 (with a mean of 15 sections per biopsy). In 8 out of 22 biopsies (36%) pyloric metaplasia was present in all sections. In 14/22 (64%) biopsies metaplasia was present in 28.5-80% (mean 51.7%) of the sections.

Granulomas were detected in only 3/22(14%) biopsies. Discontinuous chronic inflammation and crypt abnormalities were present in all cases. In most cases,
(21/22), active inflammation was noted. In only one case, pyloric metaplastic glands were recognized without active inflammation. This case concerned a 38-year-old woman with known CD in clinical remission after colectomy 20 years ago. The metaplastic changes were described in the original report in only 6/22 cases (27% or 6% overall). FOME was originally reported in one case along with PYME. Four of the cases with metaplastic changes in the initial report had metaplasia in all serial sections and only one of them was a known CD. In the remaining 16 cases, PYME was identified during the review of the slides for the present study. Among cases with PYME and FOME, one involved the case of the 38-year-old woman in clinical remission after surgical treatment and it was the only one without active inflammation but with discontinuous chronic inflammation and crypt distortion at the time of biopsy.

**DISCUSSION**

The diagnosis of CD in terminal ileum biopsies remains a challenge for the pathologist. The final diagnosis is usually based on a constellation of clinical, laboratory, endoscopic and histopathological features. Although active inflammation and granulomas are the cardinal morphological findings of CD, the former is not pathognomonic for the disease and the latter are only found in a small percentage of cases (Theodossi et al., 1994; Yantiss and Odze 2007; Feakins, 2013; Langner et al., 2014). PYME/FOME is a metaplastic response of the intestinal mucosa to the ulceration and active inflammation caused by CD. The role of PYME/FOME in CD has been investigated and discussed by several authors. There is evidence that these metaplastic changes constitute an adaptive response of the intestinal mucosa to the chronic injury caused by the active inflammation and ulceration in CD (Kushima et al., 1997; Geboes et al, 1998; Longman et al, 2000, Buisine et al., 2001; Koukoulis et al., 2002; Kaneko et al., 2008; Feakins, 2013; Goldenring, 2018). Although metaplasia is indicative of chronic tissue injury, it is emphasized that chronicity, as evidenced by mucosal structural alterations, is already present in the first diagnosis of inflammatory bowel disease (Longman et al., 2000).

Pathogenetically, the presence of PYME/FOME is related to the activation of the so called UACL which is the ubiquitous reparative machinery throughout the GI tract. As we already have mentioned PYME/FOME can be detected in ileal injury of
variable etiology, including drugs (NSAIDs), infections (H. pylori, tuberculosis), chronic radiation injury, diverticulosis, cryptogenic multifocal ulcerous stenosis enteritis, intussusception, Behcet’s disease even in Meckel’s diverticulum (coexisting with CD or ectopic gastric mucosa). However, its diagnostic value is highlighted in CD (Lee FD, 1964; Yokohama et al, 1977; Lee RG., 1989; Prabhu et al, 1994; Chi and Hanauer, 2003; Yantiss and Odze, 2007; Hamilton et al., 2015; Setaffy et al, 2015; Goulart et al, 2016; Goldenring, 2018). Consequently, its presence as an indication of CD should be evaluated with caution and always in conjunction with a detailed clinical history. Evidence of chronicity, such as mucosal architectural damage orients diagnosis towards CD in terminal ileum biopsies.

PYME was historically reported in 1964 (Lee FD, 1964). In our series PYME was found in 21% of the cases on review, an incidence close to the highest percentage reported by other authors (Geboes et al., 1998; Koukoulis et al., 2002.). In the British Gastroenterology Society guidelines, European Society of Pathology and European Crohn’s and Colitis Organization (ECCO) guidelines, PYME is highlighted as a histological finding in CD, identifying its diagnostic value in the case of pouchitis (Jenkins et al, 1997, Feakins, 2013, Magro et al., 2013, Langner et al, 2014, Gionchetti et al., 2017).

In our study, granulomas, the most characteristic finding in CD, were identified in only 3/22 biopsies (14%) with PYME/FOME. Active inflammation was present in almost all cases with PYME/FOME (21 out of 22), except for one patient with known CD in clinical and endoscopic remission. The presence of active inflammation could be explained by the fact that the sections with PYME/FOME in their majority (73%), were taken from patients with suspected CD as 1st diagnosis with probably active and symptomatic disease. Furthermore, in 50% of cases with PYME/FOME there were endoscopic findings compatible with active disease. Unfortunately, endoscopic data as previously mentioned were not available in the initial notes for the remaining cases.

Another interesting finding in our study was the detection of FOME of the intestinal mucosa in 4/105 (4%) CD biopsies. FOME has been described in a few studies, but to date there is no reference regarding its presence in terminal ileum biopsies (Kushima et al, 1997). In our cases, FOME was found in the superficial epithelium and was always accompanied by PYME in the lamina propria.
Pyloric metaplastic glands in our study were found in the lamina propria and were present in all sections of 8/22 biopsies, whereas in the remaining, they were identified in 28-80% of the sections. In this study the average number of sections examined per biopsy was 15, which is the usual average number of sections examined per GI biopsy in our laboratory. There are no specific criteria regarding the required number of sections to be examined, but in various reports the number of serial sections from a single biopsy varies between 2 and 6, and for identifying even a small granuloma, reaches 90 (Surawicz, 1982; Theodossi et al., 1994; Langner et al., 2014). Consequently, a well oriented embedding of tissue fragments and multiple sections are required to enhance the possibility to detect PYME/FOME.

We should also mention the limitations of our study, which were the small number of cases, missing clinical and lab data, information about treatment, exact endoscopic findings, which can be explained by the fact that the biopsies were received from several private endoscopic centers. Clinical information was obtained either from the referral note or after personal communication with the attending clinicians. PAS/Alcian blue stain was performed for the initial diagnosis only in three cases with questionable PYME or suspected FOME. Although it is well known that metaplastic pyloric glands are MUC6 positive, immunohistochemistry was not performed routinely, as it would be an additional cost for the patients. Classic morphology, in combination with PAS/Alcian blue histochemistry in cases of doubt, are sufficient for the diagnosis in most cases, as mentioned by Langner C et al, in the ESP IBD histopathological practice guide (Langner et al., 2014).

A substantial number of cases with PYME/FOME (73%) were missed in the original examination of the slides and were only detected during review for the present study. In these missed cases, the diagnosis of CD was based on other known histopathological features, as well as on clinical and endoscopic evidence. It is usual in routine practice when dealing with terminal ileum biopsies from a patient with suspected or known CD to focus on active inflammation and on granulomas (Langner et al, 2014).

Conclusively, PYME/FOME, though non-specific is a useful adjunct evidence of chronic mucosal damage in CD in the proper clinical setting. PYME/FOME identification can be easily missed during routine histological examination. However, the pathologist should be aware of the whole spectrum of histopathologic features of the CD and search for them. A meticulous study of terminal ileal biopsy based on
multiple well oriented sections, is necessary for the identification of PYME/FOME in suspected or known CD.

ACKNOWLEDGEMENTS
The authors thank Dr Dina Tiniakos for reviewing the manuscript.

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**Legends of Figures**

Fig. 1: PYME in ileum biopsy in CD (arrow). Hematoxylin+ Eosin stain.

Fig. 2: PYME (arrow) and FOME (*) in ileum biopsy in CD. 2A: Hematoxylin+ Eosin stain. 2B: Periodic Acid -Schiff (PAS) / Alcian Blue stain.
<table>
<thead>
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<th></th>
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<th>Age</th>
<th>Clinical data</th>
<th>Endoscopy</th>
<th>CD extent Histologically confirmed</th>
<th>Tissue fragments/biopsy</th>
<th>Crypt distortion</th>
<th>Active inflammation</th>
<th>Granulomas</th>
<th>Sections with PYME/FOME n(%)</th>
<th>PYME/FOME in original report</th>
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<tbody>
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<tr>
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