Human Pathology (2016) **54**, 31–36

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

In this issue

Intraepithelial lymphocytosis is a frequent finding in biopsies from ileal pouch–anal anastomoses☆,☆☆

David F. Schaeffer MD[a,b,*], Joanna C. Walsh MB, BCh[a], Andrea D. Tyler PhD[c,d], Ofer Ben-Bassat MD[c,d], Mark S. Silverberg MD[c,d], Robert H. Riddell MBBS[a,d], Richard Kirsch MBChB, PhD[a]

[a]*Department of Laboratory Medicine and Pathology, Mount Sinai Hospital, Toronto, ON, Canada M5G 1X5*
[b]*Department of Pathology and Laboratory Medicine, The University of British Columbia, Vancouver, BC V6T 2B5*
[c]*Division of Gastroenterology, Department of Medicine, Mount Sinai Hospital, Toronto, ON, Canada M5G 1X5*
[d]*Zane Cohen Centre for Digestive Diseases, Toronto, ON M5T 3H7*

Received 4 January 2016; revised 26 February 2016; accepted 3 March 2016

Keywords:
Intraepithelial lymphocytosis;
Ileal pouch–anal anastomoses;
IPAA;
Celiac disease;
Ulcerative colitis;
Familial adenomatous polyposis

Summary Following restorative proctocolectomy with an ileal pouch–anal anastomosis, the small bowel mucosa undergoes several specific histologic adaptions, which may be unrelated to the underlying disease or symptoms of pouchitis. An increase in intraepithelial lymphocytes (IELs) has not been described as part of this spectrum. Mucosal biopsies of the ileal pouch and afferent limb of 230 patients (mean age: 45.7y [18.3–74.7], gender [female/male]: 117/113) with a functioning ileal pouch–anal anastomosis (mean time since ileostomy closure: 10.8 months) and associated clinically annotated outcome data were assessed for IELs/100 enterocytes. Forty-two patients (18.3%) showed an increase in IELs (≥ 20 IELs/100 enterocytes [range 20-39]), in pouch and/or afferent limb biopsies. Intraepithelial lymphocytosis was more commonly observed in afferent limb compared to pouch biopsies (18.8% *vs* 8.3%; $P = .42$) and in familial adenomatous polyposis compared to ulcerative colitis patients (16% *vs* 8%; $P = 0.36$), but neither difference reached statistical significance. No cases with increased IELs displayed severe villous blunting. Increased IELs were not significantly associated with age, sex, ethnicity, smoking history, time since ileostomy, use of antibiotics, biologic agents, anti-diarrheal agents or probiotics, C-reactive protein levels or differential white cell count. None of the 42 patients with increased IELs had positive celiac serology (anti-human tissue transglutaminase IgA [ELISA] with corresponding total serum IgA). Intraepithelial lymphocytosis in pouch biopsies may represent a subclinical response to an altered bacterial microenvironment. Pathologists should be aware that intraepithelial lymphocytosis is part of the spectrum of changes in pouch biopsies, and only rarely is due to celiac disease.

☆ Statement of author contributions: D.F.S. conceived of the study with R.K., R.H.R., and M.S. and wrote the first draft of the manuscript. J.C.W. helped in the histology analysis and contributed to the writing of the manuscript. A.T. and O.B.B. contributed to the writing and review of the manuscript and assisted in the interpretation of the statistical analysis. M.S. conceived the study with R.K., R.H.R., and D.F.S. and helped with the preparation of the manuscript. R.H.R. conceived of the study with R.K., M.S., and D.F.S. and helped with the preparation of the manuscript. R.K. conceived the study with R.H.R., M.S., and D.F.S., helped with the preparation of the manuscript, and functioned as senior author.

☆☆ Disclosures: None.

* Corresponding author at: Department of Pathology, Vancouver General Hospital, 910 W. 10th Ave., Vancouver, British Columbia, Canada V5Z 1M9.
E-mail address: david.schaeffer@vch.ca (D. F. Schaeffer).

http://dx.doi.org/10.1016/j.humpath.2016.03.007
0046-8177/

1. Introduction

Despite the increased use of biological agents in the setting of inflammatory bowel disease, restorative proctocolectomy with ileal pouch–anal anastomosis (IPAA) continues to be the surgical treatment of choice for ulcerative colitis (UC) patients with medically refractory disease and/or dysplasia, and for the majority of patients with familial adenomatous polyposis (FAP) [1,2]. Although this procedure improves patients' health-related quality of life and substantially reduces the risk for UC-associated neoplasia, mechanical/surgical, inflammatory and functional complications are common. Some of these complications can lead to pouch failure with pouch excision or permanent diversion. A meta-analysis of 43 studies including over 9000 patients estimated the frequency of pouch failure to be 7% with a median follow-up of 37 months; at 60 months follow-up the frequency increased to 9% [3].

The most common long-term complication of IPAA is pouchitis – active idiopathic inflammation of the ileal reservoir [4–6]. The prevalence of pouchitis has been reported to be as high as 30% to 60% within the first postoperative year [7] and 23% to 46% at 10 to 11 years after IPAA surgery [1,8]. However, the definition of pouchitis remains controversial, which may in part explain the wide variations in its reported prevalence. The clinical definition of pouchitis is based on patient symptoms and response to antibiotics; however, symptoms of pouchitis are non-specific and may be seen in other conditions [9,10]. The Pouchitis Disease Activity Index (PDAI) applies quantitative scores to clinical symptoms, endoscopic inflammatory changes and histologic inflammation [11]. Heuschen et al, devised the Pouchitis Activity Score (PAS), which incorporates similar elements to the PDAI, but in addition includes features of chronic inflammation as part of the histology score [12]. The overall score distinguishes between three grades of pouch inflammation, namely mild "adaptive" inflammation, moderate pouchitis and severe pouchitis. Depending on the severity, acute changes may include neutrophils in the lamina propria or epithelium, crypt abscess formation, or mucosal ulcerations [13].

Well-documented histological changes accompanying mucosal adaption to the pouch environment include architectural distortion, villous blunting, crypt hyperplasia and a chronic inflammatory infiltrate within the lamina propria. More recently we have observed a higher than expected prevalence of intraepithelial lymphocytosis in pouch biopsies. While an intraepithelial lymphocytosis has been reported in pouch biopsies in the context of celiac disease [14], it has not been described in the spectrum of adaptive changes in neorectal ileal pouch mucosa. In this study we sought to determine the prevalence of intraepithelial lymphocytosis in ileal pouch biopsies, and its relationship to pouchitis (clinical, endoscopic and histologic), the underlying disease (FAP versus UC), celiac serology and potentially related clinicopathologic variables.

2. Materials and methods

2.1. Patient selection

This study was part of a larger ongoing study examining the etiology of pouchitis and was approved by the hospital's Research Ethics Board. As part of the Mount Sinai Hospital Pelvic Pouch database, patients were recruited during regular pouch follow-up at Mount Sinai Hospital in Toronto, Canada. All patients with confirmed UC, inflammatory bowel disease of the colon–unclassified or FAP before colectomy and who had undergone IPAA at least 6 months before recruitment were included in the study for a total cohort of 230 patients. Biopsies were taken from within the pouch itself (1 biopsy) and 5 to 10 cm into the afferent limb (1 biopsy) and fixed in 10% neutral formalin, processed and sections stained with hematoxylin and eosin. Of these 230,158 patients had both a pouch and afferent limb biopsy, 39 patients had a pouch biopsy only and 33 patients had an afferent limb biopsy only. During the pouchoscopy, physicians documented the appearance of the pouch and afferent limb using previously described criteria for pouch inflammation (PDAI and PAS scores). Peripheral blood was also collected for clinical evaluation of celiac serology.

2.2. Histological evaluation

Histologic evaluation of hematoxylin and eosin–stained sections was undertaken by 2 independent observers (D.F.S. and J.C.W.) who were blinded to all clinical information. In each specimen, intraepithelial lymphocyte (IEL) counts were obtained by assessing 5 adjacent, well-oriented villi. In each of the 5 villi the number of IELs per 20 enterocytes in the tip of the villus was counted. Twenty lymphocytes per 100 enterocytes was considered increased [15,16]. Immunohistochemical stains for lymphocytes (ie, CD3, CD4, and CD8) were not performed on a routine basis to quantify the number of IELs.

2.3. Serum analyses

Serum samples were analyzed for IgA antibodies against human recombinant tTg using a commercial kit (Prometheus Therapeutics, San Diego, CA) according to the manufacturers' instructions. A reading of above 7 AU/mL was considered positive. Total serum IgA levels were determined by a routine turbidimetric method.

2.4. Statistical analysis

Descriptive statistics, Fischer exact test (categorical variables) and Student t test (continuous variables) were performed as appropriate. Two-tailed statistical significance was declared if P was $\leq .05$. All analyses were computed with JMP v11.2.1 (SAS Institute, Cary, NC).