Collagenous sprue is not always associated with dismal outcomes: a clinicopathological study of 19 patients

Efsevia Vakiani[1], Carolina Arguelles-Grande[2], Mahesh M Mansukhani[3], Suzanne K Lewis[2], Heidrun Rotterdam[3], Peter H Green[2] and Govind Bhagat[3]

[1]Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA; [2]Department of Medicine, Columbia University, New York, NY, USA and [3]Department of Pathology, Columbia University, New York, NY, USA

Collagenous sprue is associated with high morbidity; however, the etiology of this disorder is unclear. Data regarding the pathological and clinical manifestations of patients with collagenous sprue are also limited. We, thus, undertook this study to gain insight into the etiology, disease manifestations and outcomes of collagenous sprue. We searched our departmental database (1999–2008) to identify cases of collagenous sprue and to obtain clinical and laboratory data. Small bowel histology, including thickness of subepithelial collagen, intra-epithelial lymphocyte phenotype and results of T-cell clonality assays were evaluated. Nineteen patients (15 women, 4 men, age 22–80 years, mean 57 years) were identified. Seventeen (89%) had celiac disease and two had unclassified sprue; 9 of 17 (53%) celiac disease patients had refractory disease; 5 of 15 (33%) lacked diarrhea (atypical presentation), including 2 of 6 (33%) with active (untreated) celiac disease and 3 of 9 (33%) with refractory celiac disease. Autoimmune disorders were seen in 12 of 19 (63%) patients and microscopic colitis (n = 7), lymphocytic gastritis (n = 2) or collagenous gastritis (n = 2) were seen in nine patients. Subepithelial collagen thickness was mildly (n = 6), moderately (n = 10), or markedly (n = 3) increased and villous atrophy was total (n = 13) or subtotal (n = 6). Phenotypically aberrant intraepithelial lymphocytes were not detected in any case. Polymerase chain reaction analysis showed a dominant T-cell clone in the only patient with refractory celiac disease type II. Histological improvement occurred in 7 of 11 (64%) patients. Overall, 8 of 19 (42%) responded to gluten-free diet, including 2 of 9 (22%) with refractory celiac disease and 10 responded to immunomodulatory therapy, including 6 of 9 (67%) with refractory celiac disease. Only one patient died from complications of refractory celiac disease. No patient developed lymphoma. The vast majority of our patients with collagenous sprue had celiac disease. Although, many patients required immunomodulatory therapy for symptom control, a subset responded to gluten-free diet alone. In our experience, collagenous sprue patients had relatively good clinical outcomes.

Collagenous sprue is a clinicopathological entity characterized by diarrhea and malabsorption accompanied by the histological findings of subepithelial collagen deposition and severe villous atrophy of

small bowel mucosa. The term collagenous sprue was first introduced by Weinstein *et al*[2] in 1970, although descriptions of subepithelial deposits of eosinophilic hyaline material, consistent with collagen, date back to 1947.[1,2] Only isolated case reports and small series have been published since then.[3–16]

The occurrence of collagenous sprue has been reported in individuals with celiac disease, tropical sprue, milk intolerance, myotonic dystrophy, malignancy-related paraneoplastic syndrome, and common variable immunodeficiency.[4,5,10,11,14,17,18] These

studies have suggested that subepithelial collagen deposition might be a non-specific feature.

Regardless of the etiology, collagenous sprue is thought to portend a poor prognosis with severe morbidity and mortality[2,4,6,11,13] and has been described as a manifestation of refractory sprue.[5,11] A few studies, however, have reported a good response to treatment and favorable outcomes.[4,8–10] The discrepant findings have been attributed to inclusion of cases with mild alterations of the subepithelial collagen table in some studies, but currently there are no well-defined histological criteria for diagnosing collagenous sprue.

The aim of our study was to describe the etiology of collagenous sprue and provide objective histological criteria for diagnosis. We also assessed the relationship between the extent of subepithelial collagen deposition in small bowel biopsies and other pathologic findings, clinical presentation, and disease outcomes of individuals with collagenous sprue seen at our institution.

Materials and methods

Case Selection

We searched our departmental database to identify patients with increased subepithelial collagen documented on a review of their small bowel biopsies by HR, GB, and PHG, over a 10-year period (January 1999–December 2008). In the absence of established criteria regarding the thickness of small bowel subepithelial collagen required for diagnosing collagenous sprue, small bowel biopsies from two groups of control patients (individuals lacking small bowel pathology and untreated celiac disease patients), evaluated at our center during the study interval, were also assessed in order to establish the threshold (or 'cutoff' value) for 'normal' and celiac disease-related changes in subepithelial collagen thickness.

Clinical Data

Clinical data, including results of serology, HLA–DQ type, presence of other immune or autoimmune conditions, symptoms at presentation, endoscopic findings, type of treatment, response to treatment, and outcome were collected from the treating physicians. Symptoms were classified as classical (diarrhea predominant) or atypical (anemia, bone loss or screening).[19] The clinical presentations of celiac disease were classified as (1) active celiac disease (patients with untreated celiac disease), (2) patients on gluten-free diet, and (3) refractory celiac disease (disease refractory to gluten-free diet for at least 6 months). The latter was further subclassified as primary or secondary, based on the absence or presence of previous response to gluten-free diet, and as type I or type II, based on the presence of a

polyclonal or clonal population of intraepithelial lymphocytes.[20–22] Cases where the underlying etiology could not be ascertained were designated as unclassified sprue. This study was approved by our Institutional Review Board.

Histologic Evaluation

Hematoxylin- and eosin-stained sections of all diagnostic and follow-up small bowel biopsies, and gastric and colonic biopsies, were evaluated where available. The degree of villous atrophy was classified as partial, subtotal and total corresponding to Marsh stages 3a, 3b and 3c, respectively, as described previously.[23] Presence of a thickened subepithelial collagen layer, intraepithelial lymphocytosis, epithelial damage (eg, cytoplasmic vacuolization, loss of columnar shape), detachment of surface epithelium, and severity of lamina propria chronic inflammation (mild—inflammatory cells occupying 1/3 of the lamina propria; moderate—inflammatory cells occupying 2/3 of the lamina propria; marked—entire lamina propria occupied and distended by inflammatory cells) were recorded. Presence and distribution of neutrophils was noted. The number of eosinophils was counted in 10 random high-power fields and the mean per high power field was calculated.

Masson trichrome-stained sections were used to evaluate and measure the subepithelial collagen thickness in all small bowel biopsies using an ocular micrometer (magnification $\times 600$) and an Olympus BX41 microscope (Olympus America, Center Valley, PA, USA). Three different fields were measured in well-oriented biopsies and mean thickness was calculated, tendrils of collagen extending into the lamina propria were not included in the measurement. Distribution of subepithelial collagen was classified as patchy if only a subset of biopsy pieces showed increased collagen or if only portions of the biopsy pieces showed increased collagen and diffuse if all biopsies showed increased subepithelial collagen along their entire length.

Phenotypic Analysis

Immunohistochemical stains were performed with antibodies against CD3, CD8 and smooth muscle antigen (DAKO, Carpinteria, CA, USA) on paraffin sections after heat-induced antigen retrieval using an autostainer (Universal staining system, DAKO) according to standard methods. The Envision Plus kit (DAKO) was used for detection. CD3+ and CD8+ intraepithelial lymphocytes were counted per 500 enterocytes. Intraepithelial lymphocytosis was graded as mild (30–50 intraepithelial lymphocytes/100 enterocytes), moderate (51–75 intraepithelial lymphocytes/100 enterocytes) or marked (>75 intraepithelial lymphocytes/100 enterocytes). Cases were deemed to have a significant expansion of

CD8– intraepithelial lymphocytes, if the percentage of CD3 + lymphocytes expressing CD8 was ⩽50%.

Four-color flow cytometric analysis (FACScan; BD Biosciences, San Diego, CA, USA) was performed on

Figure 1 Trichrome-stained sections show thin BM in normal controls (**a**, ×400) and some active celiac disease patients (**b**, ×400). Minimal thickening of the BM was seen in 60% of the biopsies from active celiac disease patients (**c**, ×400).

small bowel biopsies using a panel of antibodies against T- and NK-cell antigens, and CD103 as described.[24] Data were analyzed using the Cell Quest software (BD Biosciences).
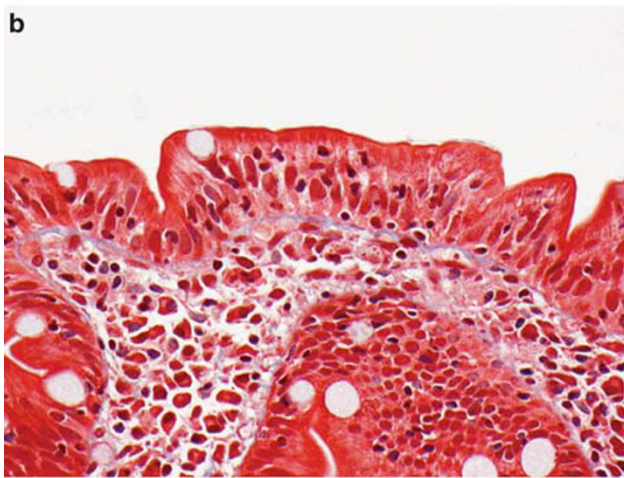
T-Cell Receptor Gene Rearrangement Analysis

Polymerase chain reaction to detect T-cell receptor-β gene rearrangement was performed using DNA extracted from fresh or paraffin-embedded biopsies and the 'Biomed-2' primers and protocol (InVivo-Scribe Technologies, San Diego, CA, USA).[25]

Statistical Analysis

Fisher's exact test, Student's *t*-test and one-way ANOVA were performed to evaluate differences between datasets and a *P*-value <0.05 was considered significant.

Results

Evaluation of Subepithelial Collagen

Ten small bowel biopsies from consecutive, unselected adults with normal villous architecture (7 women, 3 men, mean age 63 years, range 23–87 years) who had reflux esophagitis ($n = 3$), reactive gastropathy ($n = 5$) and *Helicobacter pylori*-associated gastritis ($n = 2$), served as one set of controls. Trichrome-stained sections highlighted a thin, often discontinuous, basement membrane <1.5 μm in thickness (Figure 1a). As it has been proposed that active celiac disease patients frequently have subepithelial basement membrane thickening or fibrosis,[4,11] trichrome-stained biopsies from 20 individuals with active celiac disease, 10 each with subtotal and total villous atrophy (15 women, 5 men, mean age 43 years, range 19–73 years) were evaluated. Subepithelial basement membrane thickness was similar to normal controls (< 1.5 μm) in 8 (40%) (Figure 1b) and 12 (60%) biopsies showed minimal fibrosis (mean $3 \pm 0.7 \mu$m, range 1–5 μm) (Figure 1c). Thus, the 'minimal' inclusion (and diagnostic) criterion of collagenous sprue for this study was any case with a mean subepithelial collagen thickness exceeding 5 μm (>mean + 2 s.d. of active celiac disease controls).

The study cases were divided into three groups: (1) mild fibrosis (>5 μm–10 μm, $n = 6$)—splayed and multilayered collagen strands with entrapped capillaries (Figures 2a and b); (2) moderate fibrosis (>10 μm–20 μm, $n = 10$)—collagen deposits of variable thickness with stellate extensions into the superficial lamina propria and along lateral portions of villi and crypts (Figures 2c and d); (3) marked fibrosis (>20 μm, $n = 3$)—dense, hyalinized subepithelial collagen bands with an amorphous appearance occupying 1/3 to 1/2 of the lamina propria (Figures 2e and f). The majority of cases (12/19,
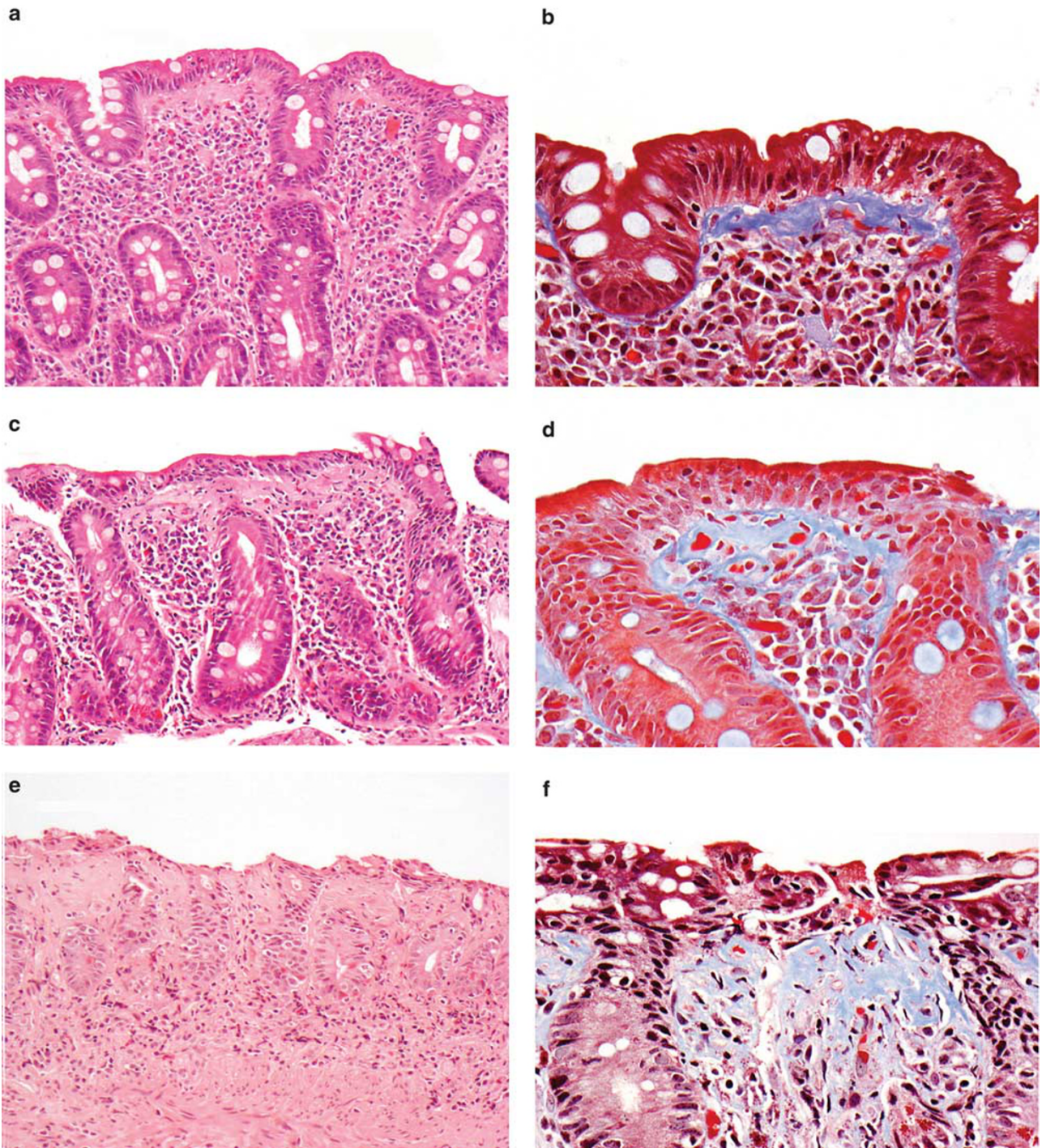
Figure 2 Representative examples of collagenous sprue cases with mild subepithelial fibrosis (case no. 5; (**a**), H&E, ×200; (**b**), Trichrome, ×400), moderate fibrosis (case no. 16; (**c**), H&E, ×200; (**d**), Trichrome, ×400) and marked fibrosis (case no. 19; (**e**), H&E, ×200; (**f**), Trichrome, ×400).

63%) across all grades of fibrosis had a patchy distribution of collagen (Table 2) and showed significant variability in the extent of fibrosis; nine (75%) had at least one biopsy piece with diffuse fibrosis (and at least one piece that lacked appreciable fibrosis), two (17%) showed fibrosis in all pieces, although some biopsy pieces only showed partial fibrosis, and one (8%) case had an admixture of partially fibrotic and normal appearing pieces. All pieces with partial fibrosis showed fibrosis of at least 1/3 of the length of the biopsy.

Table 1 Clinical features of patients with collagenous sprue

Patient	Age (years)	Sex	Serologies	HLA–DQ	Etiology and clinical presentation at dx of CS	Childhood Dx	Duration of symptoms before dx (years)	Interval between etiologic dx and CS (years)	Type of symptoms	Response to GFD	IMRx	Response to IMRx	Outcome (follow-up duration)	Other autoimmune or immune disorders
1	54	F	IgA tTG+	ND	Primary RCD (type I)	No	10	3	Classical (diarrhea)	No	Prednisone	Yes	A (4.3 years)	None
2	66	F	IgA tTG+	DQ2/DQ8	ACD	No	10	1	Classical (diarrhea)	No	Budesonide	Yes	A[b] (0.8 years)	IgA nephropathy
3	22	F	IgGgliadin+, IgA tTG+	ND	ACD	Yes	0	16	Atypical (screening)	Yes	None	NA	A (7.5 years)	DM type I, peripheral neuropathy
4	74	F	IgA tTG+	ND	ACD	No	Not known	0	Atypical (anemia)	Yes	None	NA	A (2 years)	None
5	80	F	IgA and IgG gliadin+, IgA tTG+	ND	Secondary RCD (type I)	No	2	4.4	Classical (diarrhea)	Yes	None	NA	A (6.3 years)	Dermatitis herpetiformis
6	65	M	IgA gliadin+, IgA tTG+	ND	ACD	No	10	0	Classical (diarrhea)	Yes	None	NA	A (1.4 years)	None
7	34	F	IgA EMA+	ND	ACD	No	0.5	0	Classical (diarrhea)	Yes	None	NA	A (6.8 years)	ANA 1:1280
8	74	F	ND	ND	Secondary RCD (type I)	No	8	12	Classical (diarrhea)	No	Prednisone Azathioprine	Yes	LTF (0.3 year)	Autoimmune hepatitis
9	46	F	IgA and IgG gliadin+	ND	Secondary RCD (type I)	Yes	1	45	Classical (diarrhea)	No	Prednisone	Yes	A (3.6 years)	Autoimmune hepatitis
10	82	F	ND	ND	Secondary RCD (type I)	No	32	39	Classical (diarrhea)	No	Azathioprine	Yes	LTF (0.4 year)	None
11	59	F	IgA and IgG gliadin+, IgA EMA+	ND	Secondary RCD (type I)	No	20	5.3	Atypical (anemia, intermittent diarrhea)	Yes	None	NA	A (5 years)	None
12	58	F	IgA and IgG gliadin−, IgA tTG−, IgA EMA−, anti-enterocyte−	DQ2	Unclassified sprue	No	30	0	NA[a] (diarrhea)	No	Prednisone Budesonide	Yes	A (2 years)	Hypogamma-globulinemia
13	30	F	IgA and IgG gliadin+, IgA tTG+	ND	CD on GFD	Yes	30	29	Classical (diarrhea)	Yes	None	NA	A (3 years)	Psoriasis
14	63	F	IgA gliadin+, IgA tTG− anti-enterocyte−	ND	Unclassified sprue	No	0.8	0	NA[a] (diarrhea)	No	Prednisone	Yes	A (0.9 years)	Autoimmune arthritis
15	65	M	IgA tTG+	ND	CD on GFD	Yes	5	28	Classical (diarrhea)	Yes	None	NA	A (0.4 years)	None
16	63	M	IgA tTG+	DQ2	ACD	No	0.5	1	Classical (diarrhea)	No	Budesonide	Yes	A[b] (1.3 years)	IgA deficiency
17	69	F	ND	DQ2	Primary RCD (type I)	No	0.5	1.2	Atypical (osteoporosis)	No	Prednisone Azathioprine Cyclosporine	Yes	A[b] (9.9 years)	Peripheral neuropathy and dermatitis herpetiformis

Table 1 Continued

Patient	Age (years)	Sex	Serologies	HLA-DQ	Etiology and clinical presentation at dx of CS	Childhood Dx	Duration of symptoms before dx (years)	Interval between etiologic dx and CS (years)	Type of symptoms	Response to GFD	IMRx	Response to IMRx	Outcome (follow-up duration)	Other autoimmune or immune disorders
18	25	F	IgA gliadin+, IgA tTG+	DQ2	Secondary RCD (type I)	No	2	8	Atypical (anemia)	No	None	NA	A^c (3.9 years)	Pernicious anemia
19	63	M	IgA and IgG gliadin+	DQ2	Primary RCD (type II)	No	0.8	0.5	Classical (diarrhea)	No	Prednisone Azathioprine	Yes (transient)	D (1.7 years)	None

tTG, transglutaminase; EMA, endomysial; NA, non-applicable; ND, not done; CD, celiac disease; RCD, refractory celiac disease; ACD, active celiac disease; ANA, anti-nuclear antibodies; GFD, gluten-free diet; IMRx, immunomodulatory therapy; DM, diabetes mellitus; LTF, lost to follow-up.

aDesignation of classical vs atypical presentation applicable only to CD patients.

bCurrently doing well on GFD.

cContinues to be symptomatic.

Clinical Characteristics

The clinical features of the 19 study patients (15 women, 4 men, mean age 57 years, range 22–80 years) are summarized in Table 1. Seventeen of 19 (89%) individuals had celiac disease, comprising 3% (17/554) of all celiac disease patients who had biopsies reviewed at our center during the study period; 14 had positive serologies for celiac disease and three were diagnosed before serologies were routinely performed, based on histological findings and a response to gluten-free diet. Of these patients, nine (53%) had refractory celiac disease at the time collagenous sprue was diagnosed (three primary, and six secondary refractory celiac disease, all except one had refractory celiac disease type I), six (35%) had active celiac disease and two (12%) were well controlled on gluten-free diet (endoscopy was performed for abdominal pain and dysphagia—biopsies revealed reactive gastropathy and esophageal ulceration, respectively). Of the 15 patients with refractory celiac disease or active celiac disease, 67% had classical and 33% had an atypical presentation (Table 1). Mean duration of celiac disease was 10.2 years (range 0–45 years). Four patients had an initial childhood diagnosis of celiac disease. Two of these patients (cases nos. 13, 15) had increased subepithelial collagen despite being well controlled on gluten-free diet at presentation, but they had a history of long periods of gluten ingestion before re-diagnosis of celiac disease as adults. Two patients (cases nos. 9, 15) showed severe villous atrophy, but no fibrosis on biopsy 5 years before their diagnostic biopsy.

In two (11%) patients, designated as having unclassified sprue, an immune-mediated etiology of enteropathy was considered likely based on the atypical histological features (see section 'Histologic Findings') and presence of hypogammaglobulinemia in one and autoimmune arthritis in the other patient; celiac disease was excluded based on a combination of negative serologies and non response to gluten-free diet. Anti-enterocyte antibodies were not detected in these two patients.

All patients with available information ($n = 6$) were HLA–DQ2 + , including one who did not have celiac disease. Overall, 12 (63%) patients had coexistent autoimmune or immune disorders (Table 1), 7/9 (78%) patients had microscopic colitis, whereas lymphocytic gastritis ($n = 2$) and collagenous gastritis ($n = 2$) were detected in 4/16 (25%) (Table 2). A diffuse process affecting the entire gastrointestinal tract (either intraepithelial lymphocytosis or subepithelial collagen deposition) was seen in two patients (case nos. 15, 18, Table 2). None of the patients had evidence of inflammatory bowel disease.

Histologic Findings

Total and subtotal villous atrophy was seen in 68 and 32% of cases, respectively (Table 2). Surface epithelial damage was seen in all cases and detach-

Table 2 Collagenous sprue: endoscopic and pathologic findings

Patient	Endoscopic findings (duodenum)	Degree of villous atrophy	SE collagen thickness			Distribution of collagen			LP inflammation	Neutrophils		No. of eos per HPF	No. of IELs/ 100 enterocytes (degree)	Percentage of CD8+IELs	PCR results	Other GI pathology
			Category	Range (μm)	Avg (μm)	Overall	Affected no. of bx pcs/ total no. of bx pcs	Distribution of fibrosis in affected pcs		Present	Location					
1 dx bx	Not known	Total	Mild	7.6–11	8.7	Patchy	3/4	2 D, 1 P	Moderate	Yes	LP, SE	4	35 (mild)	78	Polyclonal	Collagenous colitis
f/u (1 year)	Not known	Subtotal	Mild	8.5–10.2	9.1	Patchy	2/4	2 D	Moderate	Yes	LP, SE	5	38 (mild)	63	Polyclonal	
f/u (3 years)	Not known	Total	Mild	8.5–10.2	9.1	Patchy	3/5	1 D, 2 P	Mild	Yes	LP, SE	7	37 (mild)	54	Polyclonal	
2 dx bx	NOD, SC	Subtotal	Mild	7.6–9.3	8.7	Patchy	4/7	4 P	Moderate	Yes	LP, SE, CE	11	92 (marked)	82	Polyclonal	Lymphocytic colitis
3 dx bx	MP, AF, SC	Subtotal	Mild	8.5–10.2	9.3	Diffuse	3/3	3 D	Moderate	No	NA	13	32 (mild)	60	Polyclonal	
f/u (1 year)	MP, RF, SC	Partial	Wnl	1.7–3.4	2.3	NA	NA	NA	Moderate	No	NA	20	62 (moderate)	65	Polyclonal	
4 dx bx	Not known	Subtotal	Mild	8.5–11	9.6	Patchy	3/3	1 D, 2 P	Marked	Yes	LP, SE	17	69 (moderate)	81	Polyclonal	
5 dx bx	MP, AF	Total	Mild	9–10.2	9.8	Diffuse	6/6	6 D	Marked	Yes	LP	30	32 (mild)	72	Minor clone	
f/u (4 year)	NOD, AF	Total	Mild	7.8–11	9.6	Diffuse	9/9	9 D	Moderate	Yes	LP, SE	32	43 (mild)	75	Polyclonal	
6 dx bx	SC	Total	Mild	9–10.2	9.8	Patchy	3/4	2 D, 1 P	Moderate	Yes	LP	20	82 (marked)	48	Polyclonal	
f/u (1 year)	SC	Partial	Mild	5.4–8.5	7.5	Patchy	3/8	3 P	Mild	Yes	LP	32	73 (moderate)	45	Polyclonal	
7 dx bx	SC	Total	Moderate	10.2–11.7	11.2	Patchy	2/4	2 D	Moderate	Yes	LP	35	32 (mild)	40	Polyclonal	Lymphocytic colitis
f/u (1 year)	RF	Partial	Wnl	1.7–3.4	2.3	NA	NA	NA	Moderate	No	NA	8	12 (absent)	33	Polyclonal	
8 dx bx	MP	Total	Moderate	9.6–12.7	11.4	Diffuse	5/5	5 D	Moderate	No	NA	40	72 (moderate)	28	Minor clone	Lymphocytic colitis
f/u (3 months)	Not known	Subtotal	Wnl	3.4–7.6	4.3	NA	NA	NA	Moderate	No	NA	9	52 (moderate)	47	Polyclonal	
9 dx bx[a]	MP, NOD	Total	Moderate	11–12.7	12.1	Diffuse	10/10	10 D	Marked	Yes	LP, SE, CE	38	38 (mild)	40	Polyclonal	
10 dx bx	AF, MP	Subtotal	Moderate	9–15	12.3	Patchy	3/5	1 D, 2 P	Moderate	No	NA	7	55 (moderate)	50	Polyclonal	Collagenous colitis
f/u (5 months)	AF, MP, SC	Partial	Wnl	1.7–7.6	4.1	NA	NA	NA	Moderate	Yes	LP	25	43 (mild)	55	Polyclonal	
11 dx bx	AF	Subtotal	Moderate	10.2–13.7	12.6	Patchy	4/6	2 D, 2 P	Marked	No	NA	40	58 (moderate)	40	Polyclonal	
12 dx bx	NOD	Total	Moderate	13.6–17	15.8	Patchy	5/7	4 D, 1 P	Moderate	Yes	LP, SE, CE	15	15 (absent)	80	Polyclonal	Collagenous gastritis
f/u (4 months)	Not known	Subtotal	Mild	3.4–11.9	6.2	Patchy	1/3	1 P	Moderate	Yes	LP	7	8 (absent)	ND	ND	
f/u (1 year)	Not known	Partial	Wnl	1.0–5.1	3.3	NA	NA	NA	Mild	Yes	LP	8	10 (absent)	ND	ND	
f/u (1.5 year)	Not known	Normal	Normal	1.0–1.5	<1.5	NA	NA	NA	Mild	No	NA	7	6 (absent)	ND	ND	
13 dx bx	SC	Total	Moderate	14.3–19.2	16.7	Diffuse	3/3	3 D	Moderate	Yes	LP	50	20 (absent)	90	Polyclonal	
14 dx bx	AF	Total	Moderate	13.6–15.3	14.1	Diffuse	3/3	3 D	Marked	Yes	LP, SE, CE	19	8 (absent)	80	ND	
15 dx bx[a]	AF	Total	Moderate	12–15.6	13.7	Patchy	3/4	2 D, 1 F	Marked	No	NA	52	25 (absent)	88	Polyclonal	Lymphocytic gastritis Lymphocytic colitis
16 dx bx	NOD, AF	Subtotal	Moderate	16.2–19.4	18	Diffuse	9/9	9 D	Moderate	Yes	LP	29	70 (moderate)	87	Polyclonal	
f/u (1 year)	AF	Partial	Wnl	1.0–3.4	2.2	NA	NA	NA	Moderate	No	NA	15	20 (moderate)	80	ND	
17 dx bx	MP, NOD	Total	Marked	20–24	21.3	Patchy	2/3	2 D	Marked	Yes	LP, SE, CE	23	42 (mild)	76	Polyclonal	
f/u (2 year)	MP, RF	Subtotal	Mild	8.5–10.2	9.6	Patchy	1/5	1 P	Moderate	Yes	LP, SE	56	8 (absent)	62	ND	
18 dx bx	NOD	Total	Marked	22–28	24	Patchy	4/5	4 D	Marked	No	NA	61	62 (moderate)	92	Polyclonal	Collagenous gastritis Collagenous colitis

Table 2 Continued

Patient	Endoscopic findings (duodenum)	Degree of villous atrophy	SE collagen thickness			Distribution of collagen			LP inflammation	Neutrophils		No. of eos per HPF	No. of IELs/ 100 enterocytes (degree)	Percentage of CD8+IELs	PCR results	Other GI pathology
			Category	Range (μm)	Avg (μm)	Overall	Affected no. of bx pcs/ total no. of bx pcs	Distribution of fibrosis in affected pcs		Present	Location					
f/u (3 year)	NOD	Total	Marked	18–34	25	Diffuse	4/4	4 D	Marked	No	NA	56	32 (mild)	75	Polyclonal	
19 dx bx	AF, SC	Total	Marked	22–60	38	Patchy	6/6	4 D, 2 P	Moderate	Yes	LP, SE, CE	50	85 (marked)	70	Clonal	Lymphocytic gastritis

NOD, nodular mucosa; SC, scalloping; AF, absent folds; RF, reduced folds; MP, mosaic pattern; bx, biopsy; dx, diagnostic; f/u, follow-up; wnl, within normal limits; ND, not done; NA, not applicable; D, diffuse; P, partial; LP, lamina propria; SE, surface epithelium; CE, crypt epithelium.
[a]These patients had a biopsy 5 years before the diagnostic biopsy that showed severe villous atrophy but no fibrosis.

ment of the surface epithelium was observed in the majority (89%). Neutrophils were observed in 68% of cases, but no association between the presence or location of neutrophils and the degree of lamina propria inflammation or fibrosis was noted. The average number of eosinophils per high power field was significantly higher in cases with moderate and marked fibrosis compared to those with mild fibrosis (32.5 and 44.7 *vs* 15.8, $P = 0.04$), although no association with the extent of lamina propria inflammation was observed. An immunohistochemical stain for smooth muscle antigen did not show any increase in the number of myofibroblasts in the subepithelial zone in any group (data not shown).

One case of unclassified sprue (case no. 12, Figures 3a and b) showed total villous atrophy, surface epithelial damage, moderate chronic inflammation of the lamina propria and distension by prominent lymphoid follicles with reactive germinal centers (nodular lymphoid hyperplasia), neutrophilic infiltrate, and cryptitis. The second case of unclassified sprue (case no. 14, Figures 3c–f)) showed total villous atrophy with crypt atrophy and loss, extensive pericryptal fibrosis, and cryptitis, scattered crypts had apoptotic bodies. The inflammatory infiltrate consisted of lymphocytes, plasma cells, neutrophils, eosinophils, and histiocytes. Granulomas were seen in association with degenerating crypts and mucin extravasation, which was especially prominent in Brunner's glands. Stains for bacterial, fungal, and viral organisms were negative in both cases.

Evaluation of Intraepithelial Lymphocytes

Fifteen of 17 (88%) celiac disease cases showed increased intraepithelial lymphocytes, but the two cases of unclassified sprue did not. The degree of intraepithelial lymphocytosis and the percentage of CD8 + intraepithelial lymphocytes in all cases evaluated are described in Table 2. The mean number of intraepithelial lymphocytes did not differ significantly between cases with mild, moderate and marked fibrosis (57, 38, 63, $P = 0.2$). A significant expansion of CD3 + CD8– intraepithelial lymphocytes (50%) was restricted to cases with mild ($n = 1$) or moderate ($n = 5$) fibrosis (Figure 4).

Flow cytometry was performed in four cases, including three of six with significant CD3 + CD8– intraepithelial expansions. In all cases, the CD8– intraepithelial lymphocytes corresponded to expansions of T-cell receptor-$\gamma\delta$ + lymphocytes (range 22–43% of all gated cells). Intraepithelial lymphocytes with aberrant phenotype, namely concurrent CD8 loss and surface CD3 and/or T-cell receptor downregulation, were not detected.

Polymerase chain reaction for T-cell receptor-β gene rearrangement showed polyclonal products in 15/18 (83%) cases tested (Table 2). Minor clones, in a polyclonal background, seen in two cases were not
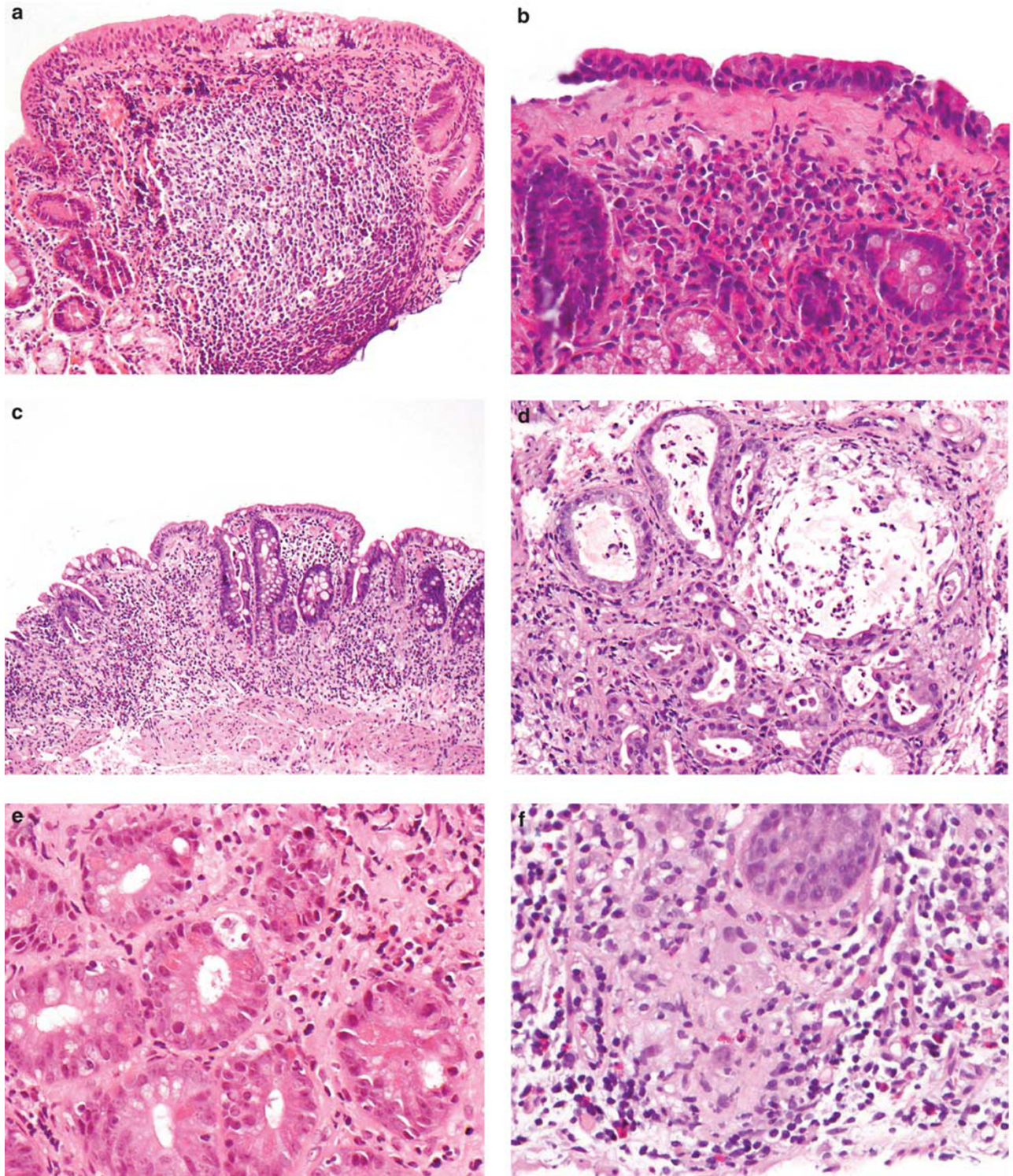
Figure 3 Histological features of unclassified sprue (H&E). Prominent lymphoid follicles indicating nodular lymphoid hyperplasia (**a**, ×100) and subepithelial fibrosis, but an absence of intraepithelial lymphocytosis (**b**, ×200) in case no. 12. Crypt atrophy and loss (**c**, ×100), degenerating Brunner's glands and mucin extravasation (**d**, ×200), crypt with apoptotic cells (**e**, ×400) and pericryptal granulomas (**f**, ×400), in addition to subepithelial fibrosis, were present in case no. 14.

detected in follow-up biopsies, suggesting the presence of transient T-cell expansions with skewed T-cell receptor repertoires either in the epithelial compartment or the lamina propria. These cases did

not meet the criteria for refractory celiac disease type II.[5] A dominant clone was detected in the small bowel biopsy in only one case. The intraepithelial lymphocytes (CD3+ CD8+) lacked an aberrant
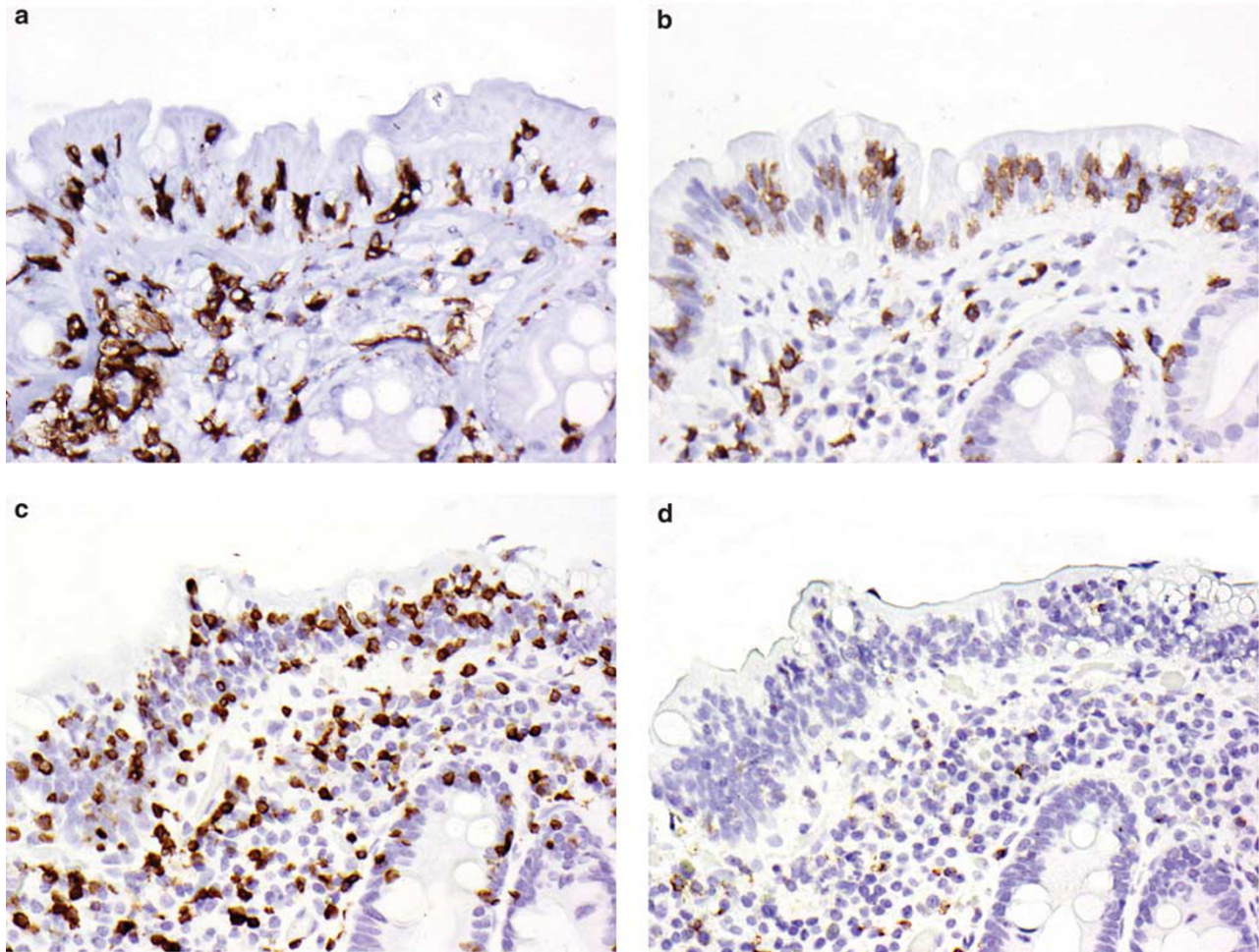
Figure 4 Representative cases of collagenous sprue showing expansions of CD3 + CD8 + IELs (case no. 4; (**a**) CD3, (**b**) CD8; × 400) and CD3 + CD8– IELs (case no. 8, (**c**) CD3, (**d**) CD8; × 400).

phenotype by immunohistochemical staining; however, as flow cytometry was not performed in this case, surface CD3 expression could not be evaluated. This case was classified as refractory celiac disease type II, as rare cases of refractory celiac disease type II with similar phenotype have been described.[24,26] A clonal product of identical size was also detected in the lung of this patient, but not in peripheral blood.

Association of Subepithelial Fibrosis with Disease Presentation, Response to Treatment and Outcome

The clinical presentation of patients with mild fibrosis was refractory ($n = 2$) and active ($n = 4$) celiac disease, whereas moderate thickening was observed in individuals with refractory ($n = 4$) and active celiac disease ($n = 2$) and those on gluten-free diet ($n = 2$) or with unclassified sprue ($n = 2$). All three individuals with marked thickening had refractory celiac disease. Atypical presentation of celiac disease was seen in patients across all grades of fibrosis. There was no association between a

diarrhea predominant presentation and the degree of fibrosis (4/6 with mild fibrosis *vs* 10/13 with moderate/marked fibrosis, $P = 0.4$). Similarly, frequencies of coexistent autoimmune/immune disorders or other gastrointestinal pathology did not differ significantly between patients with mild fibrosis and those with moderate/marked fibrosis (3/6 *vs* 9/13, $P = 0.6$ and 2/6 *vs* 7/13, $P = 0.6$, respectively).

Eight of 17 (47%) celiac disease patients did well on gluten-free diet; four with mild and four with moderate fibrosis. Two of the latter had secondary refractory celiac disease type I. Response to gluten-free diet was seen more frequently in patients with mild compared to those with moderate/marked fibrosis, although the difference was not statistically significant (4/6 *vs* 4/13, $P = 0.3$). Similarly, though not statistically significant, patients who responded to gluten-free diet had a lower frequency of autoimmune/immune conditions and other gastrointestinal pathology compared with those who did not (4/8 *vs* 8/11, $P = 0.4$, 2/8 *vs* 7/11, $P = 0.2$).

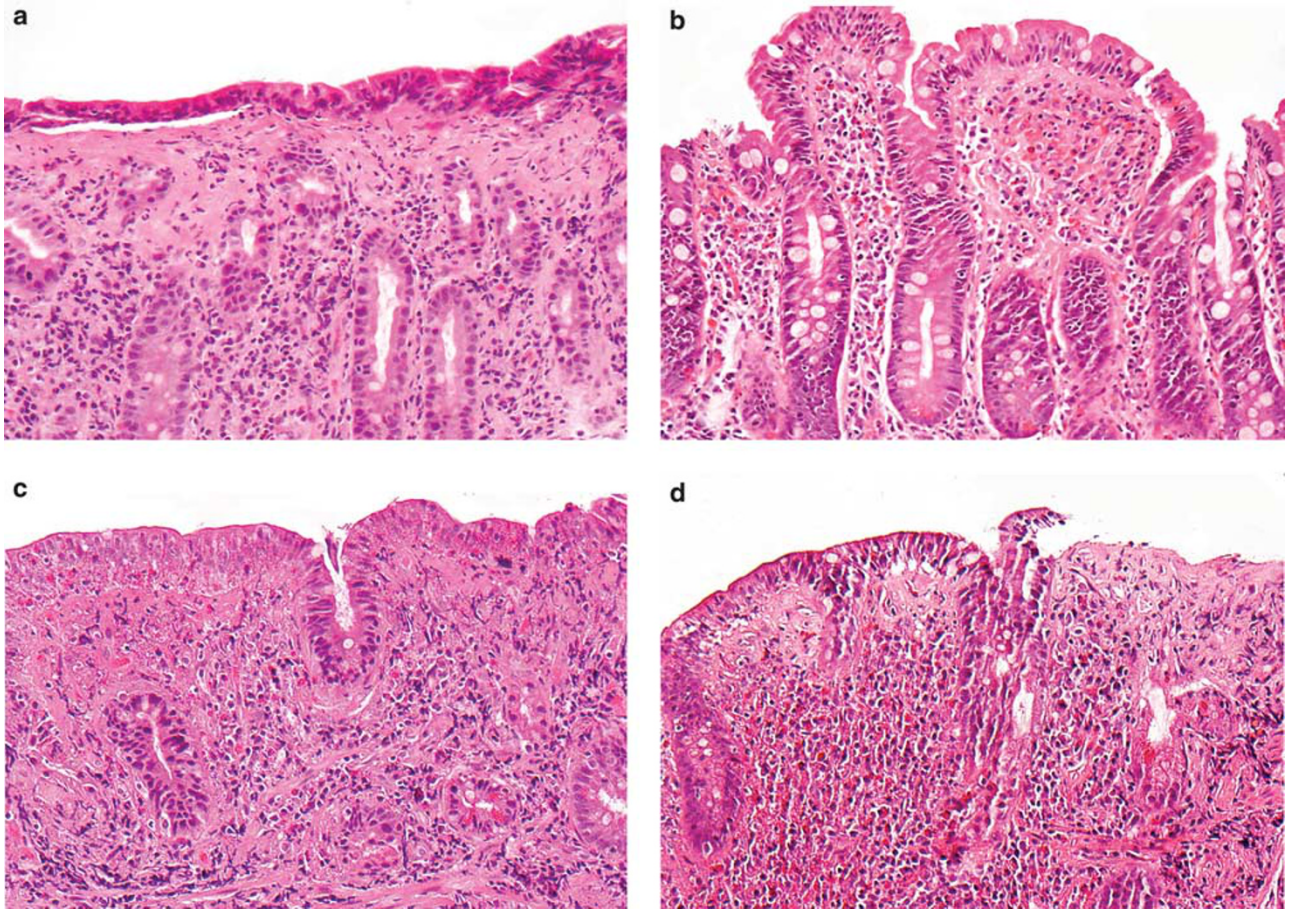Three patients required parenteral nutrition. Ten patients, across all grades of fibrosis, received

Figure 5 Diagnostic and follow-up small bowel biopsies (H&E): case no. 17 shows initial total villous atrophy and marked subepithelial fibrosis (**a**, ×200), subsequently showing subtotal villous atrophy and mild fibrosis (**b**, ×200); case no. 18 shows total villous atrophy and marked fibrosis (**c**, ×200) without improvement 3 years later (**d**, ×200).

immunomodulatory therapy. A response to immu-nomodulatory therapy was seen in all patients across different clinical presentations (Table 1). Response was transient in the patient with refrac-tory celiac diseased type II. One patient (case no. 17) with refractory celiac disease type I initially re-quired immunomodulatory therapy, but is currently doing well on a gluten-free diet, whereas one patient (case no. 18) with refractory celiac disease type I refused immmunomodulatory therapy and has per-sistent iron-deficiency anemia on a gluten-free diet.

Sixteen of 19 (84%) patients are currently alive (mean follow-up of 3.7 years, range 0.4–9.9 years); two were lost to follow-up after 3 and 5 months. The patient with refractory celiac disease type II died from complications of malnutrition 1.7 years after diagnosis of collagenous sprue. No patient devel-oped lymphoma.

Endoscopy and Colonoscopy Findings

The endoscopic appearance of the duodenal mucosa was abnormal at diagnosis of collagenous sprue in 17 patients with available information (Table 2). Mucosal abnormalities persisted in all eight patients who had follow-up endoscopy, with qualitative changes seen in 6/8 (75%) cases. Despite persistent endoscopic abnormalities, 6/8 (75%) and 5/8 (63%) cases showed improvement of villous architecture and reduction in fibrosis, respectively (see section 'Follow-Up Biopsies'). These findings are indicative of slow and possible incomplete (patchy) recovery, which is not detectable on endoscopy and parallel observations in uncomplicated celiac disease, where endoscopy has been shown to have low sensitivity in predicting histological alterations (50–80%).[27] None of the patients had ulcerations or erosions at diagnosis or follow-up. The colon had an unremarkable appearance in all nine cases evaluated.

Follow-Up Biopsies

A reduction in subepithelial collagen was seen in 7 of 11 (64%) patients with follow-up small bowel biopsies (0.3–3 years post diagnosis of collagenous sprue) across all grades of fibrosis (Table 2, Figures 5a and b), the thickness reaching active celiac

disease control range in 5, with 1 case each showing showing mild or no fibrosis. Improvement was seen in two patients on gluten-free diet and five treated with immunomodulatory therapy. Decreased fibrosis was accompanied by decreased degrees of villous atrophy in all cases. Persistent fibrosis was seen in four patients (Table 2, Figures 5c and d). One patient received immunomodulatory therapy, whereas three were on gluten-free diet. The degree of villous atrophy improved in two and no change was observed in two cases. Intraepithelial lymphocyte numbers were quite variable in both situations and did not appear to correlate with histological improvement (Table 2).

Discussion

Collagenous sprue has historically been considered a syndrome of recalcitrant malabsorption associated with high morbidity and mortality.[2,11] This view has been challenged by a few.[4,8] However, the clinical and pathological manifestations of this disorder have not been investigated adequately, as data regarding its prevalence and etiology are limited and definitional criteria are suboptimal. Our study represents the first large comprehensive single institution study describing the histopathological and phenotypical features, clinical characteristics, therapeutic strategies, and outcomes of individuals with a thickened small bowel subepithelial collagen table.

Qualitative descriptions of increased subepithelial fibrosis have been reported in 36–42% of small bowel biopsies from celiac disease patients,[4,28] which have been interpreted as common and non-specific findings lacking any clinical significance.[4] It has been argued that a diagnosis of collagenous sprue requires the presence of 'a clear-cut layer of subepithelial collagen that extends into the lamina propria.'[11] Still others have based the diagnosis of collagenous sprue on the presence of $>10\,\mu m$ thickness of the subepithelial collagen band, using criteria proposed for the diagnosis of collagenous colitis.[5] Morphometric analysis of the subepithelial collagen layer in small bowel biopsies from patients with active celiac disease, or for that matter other small intestinal diseases, has not been described. Hence, there is a lack of consensus as to what represents a 'significant' increase in subepithelial collagen, hampering the promulgation of histological criteria required for diagnosing collagenous sprue.

Our morphometric analysis was conservative avoiding areas with tangential orientation, which can lead to an erroneous impression of increased subepithelial collagen.[29] Mild thickening of the basement membrane, not exceeding 5 μm, was seen in 60% of active celiac disease control patients who had severe villous atrophy, confirming previous reports.[4,28] Hence, we suggest that only cases with small bowel subepithelial collagen thickness $>5\,\mu m$ (approximately the diameter of a lymphocyte) should be diagnosed as collagenous sprue and recommend that a note indicating this finding (and if possible the degree) be provided in the histopathology report. This would alert the physician to the possibility of an atypical disease course.

Histological improvement was seen in the majority of our patients with available follow-up biopsies, irrespective of disease presentation and symptoms and across all grades of fibrosis, though without any identifiable predictors of response. Whether these findings represent true histological improvement or sampling artifacts can be debated. The concurrent improvement in mucosal architecture with a decrease in the extent of fibrosis and amelioration of clinical symptoms of our patients, supports the view of previous investigators, who documented the disappearance of collagen deposits on follow-up biopsies and suggested that collagenous sprue might be reversible in certain instances.[4,8–10] In this context, it is worth reiterating that subepithelial collagen fibrosis was patchy in the majority of our cases, both at diagnosis and at follow up, with the extent and severity varying from piece to piece, as well as within a single biopsy piece. This observation is in keeping with the original description of collagenous sprue[2] and mirrors the variability of mucosal architectural abnormalities in uncomplicated celiac disease,[30] We, thus, recommend obtaining multiple biopsies from different regions of the proximal small bowel (altogether 4–6 pieces) to ensure adequate sampling, as has been suggested for celiac disease.[31]

Our study and previous reports indicate that most patients with collagenous sprue are middle-aged to elderly females.[5,11] In our series, 21% of the individuals were younger than 35 years, highlighting the fact that young adults may occasionally be affected.[32] However, in contrast to collagenous gastritis,[33,34] collagenous sprue is rare in children, with only two cases described in the literature.[1,13] The most common etiology of collagenous sprue in our patients was celiac disease, accounting for 89% of all collagenous sprue cases. The prevalence of collagenous sprue in this patient population was quite low (3%), despite the inherent referral bias in our study. Thus, it is likely that the true prevalence of collagenous sprue among celiac disease patients in the community is lower. The relationship between celiac disease and collagenous sprue has remained controversial over the past several decades as many of the reported patients failed to respond to a gluten-free diet,[3,8,10,11,32] and most cases lacked serologic or other clinical data that could have suggested celiac disease as the etiology.[3,8,10,11] Nonetheless, Bossart *et al*[4] observed 'moderate to marked' subepithelial collagen in 8% of their celiac disease patients. In two recent studies of refractory sprue, collagenous sprue accounted for 50 and 34% of all cases, the underlying etiology of collagenous sprue

being refractory celiac disease in 40 and 86% of the cases, respectively.[5,11]

Collagenous sprue can also be a manifestation of other disorders. Eleven percent of our patients did not have celiac disease. Although the precise etiology could not be determined conclusively, immune-mediated mechanisms appeared likely. These patients did not respond to a gluten-free diet, but are currently symptom free on maintenance steroids. Small bowel biopsies from these patients had atypical histological features and lacked intraepithelial lymphocytosis. The latter may help distinguish cases of collagenous sprue unrelated to celiac disease. However, this feature should be interpreted in the context of other clinical, serological and histological variables, as patients on gluten-free diet often lack increased intraepithelial lymphocytes.[35] Conversely, before attributing increased intraepithelial lymphocytes to underlying celiac disease, other mimics of celiac disease, which have also been implicated as etiologic agents of collagenous sprue, such as tropical sprue, common variable immunodeficiency and autoimmune enteropathy, should be excluded.[17,18,36] Unusual histological features observed in our patients with unclassified sprue, included a peculiar pattern of crypt destruction, reminiscent of graft-versus-host disease, accompanied by a granulomatous reaction and nodular lymphoid hyperplasia, which can also be seen in small bowel biopsies from individuals with common variable immunodeficiency.[17]

An increased prevalence (63%) of coexisting autoimmune conditions was noted in our patients, which is higher than the prevalence reported in celiac disease patients (5–30%).[37,38] This might explain the more pronounced skew in gender distribution, that is, female predominance observed in our series. An association with microscopic colitis, lymphocytic gastritis and/or collagenous gastritis was also seen, the former being more common and occurring at a higher frequency compared with celiac disease patients in general.[39]

A notable finding of our study, from a clinical perspective, was the significant frequency (33%) of atypical or silent presentation of celiac disease patients who had collagenous sprue, which contrasts with the prevailing view. Atypical presentations are being increasingly recognized in celiac disease patients,[40] likely because of a combination of more widespread use of serological testing and the changing nature of celiac disease. Furthermore, although the majority of our patients had refractory celiac disease, collagenous sprue was seen in patients with active celiac disease, confirming previous case reports,[9,10,15] and interestingly even in asymptomatic individuals on gluten-free diet, which has not been previously reported. A history of celiac disease since childhood with long periods of gluten ingestion and the documentation of subepithelial fibrosis developing in follow up biopsies raise the possibility that duration of disease could be a factor in disease pathogenesis.

Our patient profile also differed from a previous study of refractory celiac disease type II, which described collagenous sprue in 86% of patients[5] and the few individuals with collagenous sprue in the study of Daum *et al.*[26] All but one of our patients with refractory celiac disease had type I disease, and in all cases evaluated, increased CD8- intraepithelial T-cells represented expansions of $\gamma\delta +$ T-cell receptor-bearing lymphocytes. The only patient with refractory celiac disease type II had CD8 + intraepithelial lymphocytes, clonal T-cell receptor-β rearrangement and evidence of extraintestinal dissemination of the T-cell clone.[26,41] Refractory celiac disease type II patients with CD8 + intraepithelial lymphocytes were not observed by Cellier *et al*,[5] but rare cases of clonal CD8 + intraepithelial lymphocyte expansions have been reported.[24,26] Our patient with refractory celiac disease type II was the only one documented to have died. Further studies are needed to ascertain whether the incidence of refractory celiac disease type II is higher in collagenous sprue patients with fatal outcomes.

Patients who did not respond to gluten-free diet were more likely to have moderate or marked fibrosis, but only 62% of patients in both groups combined had refractory celiac disease. Of interest, 38% of these patients had refractory 'atypical' symptoms rather than diarrhea. It is important to note that patients with severe fibrosis also had a higher prevalence of autoimmune conditions and colonic or gastric pathology. These observations suggest that refractory symptoms and the need for immunomodulatory therapy might reflect a heavier or more complex disease burden or generalized immune deregulation and that collagen deposition might represent an epiphenomenon, as has been alluded to previously.[32] Future studies evaluating the genetic and immune function profiles of such patients should help clarify these assumptions. Of patients treated with immunomodulatory therapy, 90% are currently alive with good control of their symptoms. Corticosteroids, either in the form of prednisone or budesonide, were the most commonly used immunomodulatory therapy. Steroids are accepted as a therapy for severe, poorly responsive celiac disease and refractory celiac disease.[5] Budesonide is an attractive drug to treat patients with collagenous sprue, as it acts locally on the mucosa and has few systemic side effects. Used widely in Crohn's disease, it has been shown to benefit patients with poorly responsive celiac disease.[42]

The etiology of collagenous sprue is not known. Increases in procollagen I and tissue inhibitor of metalloproteinase-1 mRNAs, unopposed by increases in mRNA levels of matrix metalloproteinases 1 and 3 (key enzymes of extracellular matrix degradation), have been reported in collagenous sprue.[32] Elevations of matrix metalloproteinases 1, 3, and 9 and tissue inhibitor of metalloproteinase -1 proteins have been described in active celiac disease, indicating continual matrix remodeling in

celiac disease.[43] An imbalance between collagen synthesis and degradation might underlie increased subepithelial fibrosis, especially in celiac disease-associated collagenous sprue. No increase in smooth muscle antigen-positive myofibroblasts in our case corroborates a previous report that suggested increased synthesis and/or secretion of collagen, rather than an increase in cells producing it, in collagenous sprue patients.[32] The increased number of eosinophils in biopsies displaying more severe grades of fibrosis suggests a role for these cells in the pathogenesis of collagenous sprue, as has been hypothesized in collagenous colitis and collagenous gastritis,[44,45] as eosinophils are known to have profibrogenic effects.[46] Contributions of, and interactions between, epithelial, inflammatory and stromal cell-derived inflammatory mediators, cytokines, and extracellular matrix components, implicated in inducing fibrosis in other inflammatory intestinal diseases, also need to be defined for collagenous sprue.[47] Elucidating polymorphisms in genes leading to increased production of profibrogenic factors or increased sensitivity to them might also be worthwhile endeavors.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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