CME/SAM

The Significance of Duodenal Mucosal Atrophy in Patients With Common Variable Immunodeficiency

A Clinical and Histopathologic Study

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Upon completion of this activity you will be able to:
- list histologic features commonly observed in small intestinal biopsies from patients with common variable immunodeficiency (CVID).
- describe histologic features that can be seen in both CVID and in celiac disease.
- discuss the role of celiac antibodies and of response to a gluten-free diet in diagnosing celiac disease in patients with CVID.

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Questions appear on p 305. Exam is located at www.ascp.org/ajcpcme.

Abstract

Gastrointestinal manifestations and villous atrophy can be seen in patients with common variable immunodeficiency (CVID). In some patients, infectious agents may be responsible, whereas in others, celiac disease (CD) may be the cause. In this study, we investigate the causes and the histopathologic features seen in patients with CVID. Eleven patients with CVID and villous atrophy underwent duodenal biopsies, human leukocyte antigen (HLA) typing, and testing for all celiac antibodies. Fifteen patients with CVID and normal villi and 6 patients with CD but without CVID served as controls. Histologic response to a gluten-free diet (GFD) allowed a diagnosis of CD in 3 of 11 patients. In the remaining 8, the lack of a histologic response to a GFD or HLA typing excluded CD. Celiac antibodies gave conflicting results and were of no help. Polymorphonuclear infiltrates and lesions like graft-versus-host disease are seen more often in flat mucosa unresponsive to a GFD. However, the specificity of these findings remains to be determined and response to a GFD remains the only diagnostic criteria for CD in these patients. Villous atrophy was gluten-sensitive in 3 of 11 patients with CVID. It was not related to gluten-responsive CD in most patients.

Common variable immunodeficiency (CVID) is the most frequent symptomatic form of primary humoral immunodeficiency. Its onset occurs more frequently in the second or third decade of life, with no appreciable differences between the sexes; its prevalence in the general population is between 1 in 10,000 and 1 in 250,000. Although most cases are sporadic, in almost 10% of cases the inheritance is familial.[1]

It is known that in patients with this condition the gastrointestinal tract is frequently involved, manifesting either as symptoms or histopathologic lesions.[2-10] In 30% of cases, gastrointestinal involvement was demonstrated to be the result of infestations of *Giardia lamblia*, a protozoan parasite whose presence in the small intestine of these patients is facilitated by the absence of secretory immunoglobulin (Ig) A, a hallmark in most patients with CVID.[2,4]

In a significant percentage of patients, however, intestinal lesions are not caused by infectious/parasitic agents.[4,5] In particular, Crohn disease–like lesions[4-6,10] and lymphoid nodular hyperplasia[2,3,7,8,10] have been described, as well as a condition of atrophy of the duodenal mucosa characterized by villous atrophy, crypt hypertrophy, and an increase of T-lymphocyte infiltrate.[2-5,9,10] This finding is similar to the histopathologic alterations found in untreated celiac disease (CD), a gluten-sensitive condition characterized by a variable degree of villous atrophy, a significant association with the human leukocyte antigen (HLA) DQ2 and DQ8 alleles, and highly specific and sensitive serum antibodies, such as gliadin, endomysial, and tissue transglutaminase antibodies.[11]

It is therefore understandable why patients with CVID and an alteration of duodenal mucosa pose a difficult differential diagnostic problem that includes CD. This problem is not only because the specific antibodies for CD have no diagnostic role in patients with CVID[2,5] but also because the 2 conditions can be associated in the same patient.[12,13] The histologic response of a flat mucosa to a gluten-free diet (GFD) therefore represents the only diagnostic criterion for establishing whether these patients with CVID also have CD. But even this is not without problems, because the histologic response must be evaluated after a long period (at least 12-15 months), and if a histologic recovery does not occur, the differential diagnosis with refractory CD cannot be made.[11]

Some authors have indicated a series of histopathologic aspects characterizing an altered mucosa in CVID but not in untreated CD.[5,8,14-16] This occurs in the absence of plasma cells and the presence of a polymorphonuclear infiltrate (PMNI) and graft-versus-host disease–like lesions (GVHDL). Although these histopathologic aspects are of help in differentiating between a flat mucosa in CVID and a flat mucosa in CD, but with no CVID, it is not yet clear whether the finding of these lesions in the flat duodenal mucosa of patients with CVID can confirm or exclude the concomitant presence of CD.

Our study had 2 aims. The first one (clinical study) was to evaluate whether the patients with CVID and an altered duodenal mucosa are affected by CD. The second one (histopathologic study) was to evaluate whether absence of plasma cells, PMNI, and GVHDL are of help in confirming or excluding the presence of CD in patients with CVID and an altered duodenal mucosa, without having to wait for the 12 to 15 months needed to evaluate the histologic response to a GFD.

Materials and Methods

Patients and control subjects were regularly followed up at the Coeliac Centre/First Dept of Internal Medicine, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy, and at the Pediatrics Clinic, Spedali Civili di Brescia, University of Brescia, Brescia, Italy. The study was approved by the local ethics committee at the Fondazione IRCCS Policlinico San Matteo.

Clinical Study

Eleven adult patients (3 women; mean age, 43 ± 10 years) with duodenal mucosa atrophy (Marsh stage ≥3; Corazza-Villanacci classification B2; intraepithelial lymphocyte count >25/100 enterocytes) and CVID diagnosis according to European Society for Immunodeficiencies (ESID) criteria were included in our retrospective clinical study.[1,17] The ESID criteria are as follows: male or female patient who has a marked decrease of immunoglobulin (Ig) G (at least 2 SD below the mean for age) and a marked decrease in at least 1 of the isotypes IgM or IgA, and fulfils all of the following criteria: (1) onset of immunodeficiency after age 2 years; (2) absence of isohemagglutinins and/or poor response to vaccines; and (3) exclusion of other defined causes of hypogammaglobulinemia. All the patients had been referred to our units because of symptoms of frank malabsorption (diarrhea, steatorrhea, and weight loss). All these patients were reassessed by means of gastroscopy with multiple duodenal biopsies performed before and after the start of a GFD and by searching for both IgA and IgG class CD-specific antibodies. More specifically, a search was conducted for endomysial antibodies (EMA) using indirect immunofluorescence on monkey esophagus and jejunum (The Binding Site, Birmingham, England), and for deaminated gliadin and tissue transglutaminase with an enzyme-linked immunoassay (Eurospital, Trieste, Italy).[18] Finally, all these patients underwent HLA typing, stool parasitology, and culture tests.

On the basis of our reassessment, the patients were classified into 3 groups: patients in whom CD was confirmed, patients in whom CD was excluded, patients in whom CD could neither be confirmed nor excluded.

Histopathologic Study

The presence or absence of plasma cells, GVHDL, and PMNI was evaluated in hematoxylin-stained duodenal biopsy specimens from the same 11 patients with CVID and villous atrophy included in the clinical study **Image 1**, **Image 2**, and **Image 3**. Duodenal biopsy slides from 21 control subjects were also evaluated; 15 of them were patients with CD but without CVID. More precisely, 7 of them were on a gluten-containing diet and the duodenal biopsies showed villous atrophy (4 women; mean age, 35 ± 13 years), while the other 8 were on a GFD with a good histologic response (5 women; mean age, 34 ± 10 years). Finally, we reassessed the biopsy specimens of 6 patients with CVID and no intestinal lesions (3 women; mean age, 41 ± 13 years).

Results

Clinical Study

Table 1, **Table 2**, and **Table 3** show the results of our clinical reassessment. CD was diagnosed in 3 of 11 patients (Table 1). The cornerstone for this diagnosis was the clinical and histologic response to a GFD (Marsh stage ≤1 in all of them). These patients were all DQ2 or DQ8 positive.

Table 2 shows the 3 patients in whom it was possible to exclude the diagnosis of CD with certainty, based on the absence of HLA DQ2 and DQ8. Moreover, symptoms of malabsorption did not improve and duodenal biopsy showed persistence of a Marsh stage ≥3 lesion in all of them in spite

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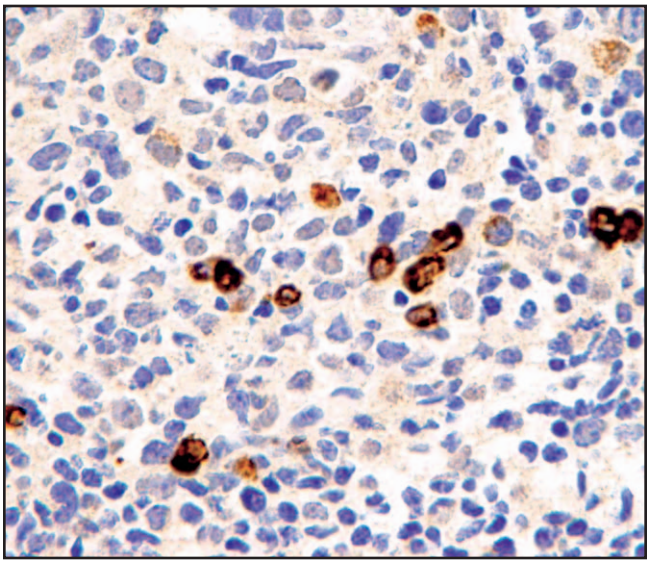

Image 1 Scattered plasma cells in duodenal mucosa (λ chain antibody stain, ×100).

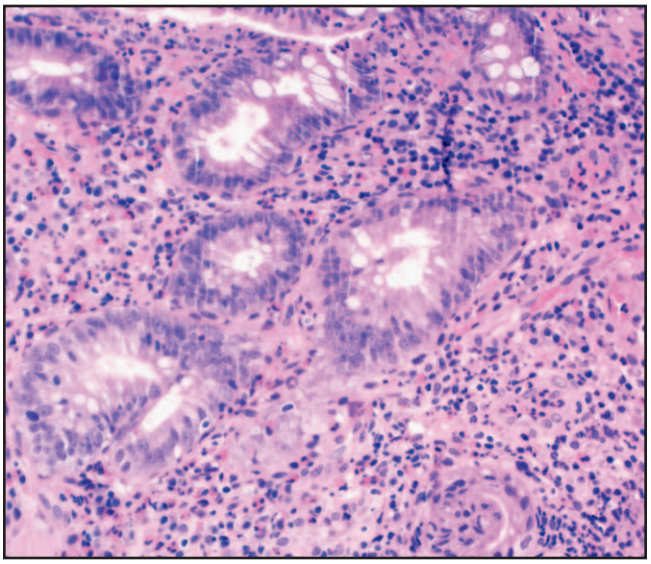

Image 2 Duodenal mucosa showing crypt with a heavy hyperplastic appearance demonstrated by mucous reduction without dysplasia and intense inflammatory infiltrate mainly consisting of eosinophils infiltrating into the crypt epithelium (H&E, ×40).

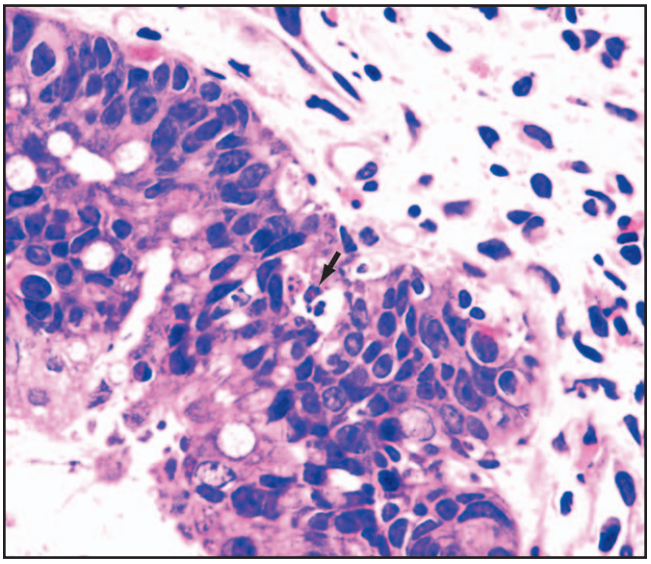

Image 3 Duodenal mucosa showing graft-vs-host-disease–like appearance demonstrated by atrophy and marked hyperplasia of the crypt with lymphoid inflammatory elements infiltrating into the crypt epithelium and presence of an apoptotic body (arrow) (H&E, ×40).

of a GFD; however, 1 patient stopped the GFD after 2 months once the HLA typing was performed.

Finally, Table 3 shows the 5 patients in whom CD could neither be confirmed nor excluded, because of the lack of a clinical and histologic response to GFD only (Marsh stage ≥3) as well as the presence of HLA alleles compatible with CD.[19] Moreover, the clinical evaluation of these patients was made even more difficult by the fact that 2 of them (patients 8 and 10) experienced a mucosal response once they started taking corticosteroid therapy and the diet had returned to one containing gluten. One of them (patient 8) developed B-cell small-bowel lymphoma and died at age 46 years.

All these 11 patients had been on a GFD for 24 ± 22 months, and compliance, assessed by means of a dietary interview performed by expert personnel, was considered to be very strict in all of them. Ten of these 11 patients were receiving intravenous immunoglobulins replacement therapy. The only 1 who was not receiving this therapy was a 52-year-old woman who was shown to be negative to all celiac antibodies (patient 11, Table 3). Finally, stool tests excluded *G lamblia* and other gastrointestinal infections in all of them.

Histopathologic Study

Absence of plasma cells was found in 10 of 11 patients who had both CVID and a flat duodenal mucosa (patients 1-4, 6-11 in Tables 1 through 3). Absence of plasma cells was seen in 5 of 6 control subjects with CVID but without flat duodenal mucosa. However, absence of plasma cells was not detected in any of the celiac controls without CVID. This is, therefore, a highly sensitive and specific aspect for CVID, but it is of no help in understanding whether villous atrophy in a patient with CVID is the result of concomitant CD or not.

PMNI and GVHDL were seen in a few patients with CVID and flat mucosa. In particular, PMNI was detected in 5 of the 10 patients with absence of plasma cells and villous atrophy (patients 4 and 8-11 in Tables 2 and 3). Finally, GVHDL was detected in 3 of the 5 patients with PMNI (patients 8, 10, and 11 in Table 3). To find these lesions only in patients in whom CD was not confirmed is very interesting.

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Finally, GFD had no effect on absence of plasma cells, PMNI, and GVHDL. Similar to other authors, we found an increased intraepithelial lymphocyte count and follicular hyperplasia in the biopsy samples we studied[5,16]; however, they did not differ and they were of no help in distinguishing between patients with CVID with and without CD (data not shown).

Discussion

Gastrointestinal symptoms or intestinal lesions are frequently seen in patients with CVID. It is well known that *G lamblia* is the main cause in these cases.[1-10,14] In the last few years we found only 1 case with this organism among patients with CVID and villous atrophy referred to our unit (a patient not included in the current series). This is because of the preselection process that these patients go through before being referred to our centers.

On the basis of our reassessment, CD was present in 27% of the cases (3/11) and could be excluded in the same percentage. In the remaining cases (5/11, 46%), the diagnosis of CD could neither be confirmed nor excluded with any certainty. However, it seems likely that in these doubtful cases the diagnosis of CD can be excluded. Alternatively, we would have to hypothesize that these cases showed a form of CD refractory to GFD. This would mean that in patients with CVID, the prevalence of refractory CD would be greater than the prevalence of uncomplicated CD. To us, that seems unlikely.

We confirm that absence of plasma cells in the duodenal mucosa is a highly specific aspect of CVID.[15,16] We found

Table 1
Clinical Data of Patients With Common Variable Immunodeficiency and a Flat Duodenal Mucosa With Confirmed Celiac Disease

Patient	Sex	Age (y)	HLA	Months on a GFD	Histological Response to a GFD	Positive Antibodies
1*	M	47	DQ2+	45	Yes	EMA IgG
2	F	27	DQA1*0501,*0505 DQB1*0201,*0301	13	Yes	EMA IgA EMA IgG
3	M	42	DQA1*0104,*0505 DQB1*0303,*0503	6	Yes	EMA IgG

EMA, epithelial membrane antigen; GFD, gluten-free diet; HLA, human leukocyte antigen; Ig, immunoglobulin.
* Human leukocyte antigen genomic typing was not available for patient 1.

Table 2
Clinical Data of Patients With Common Variable Immunodeficiency and a Flat Duodenal Mucosa Without Celiac Disease

Patients	Sex	Age (y)	HLA	Months on a GFD	Histologic Response to a GFD	Positive Antibodies
4	M	28	DQA1*0102,*0302 DQB1*0602,*0303	12	No	EMA IgG
5	M	46	DQA1*0201,*05 DQB1*0302,*0302	2	No	None
6	M	35	DQA1*0101,*0505 DQB1*0301,*0501	31	No	None

EMA, epithelial membrane antigen; GFD, gluten-free diet; HLA, human leukocyte antigen; Ig, immunoglobulin.

Table 3
Clinical Data of Patients Affected by Common Variable Immunodeficiency and a Flat Duodenal Mucosa in Whom Celiac Disease Could Neither Be Excluded nor Confirmed

Patient	Sex	Age (y)	HLA	Months on a GFD	Histologic Response to a GFD	Positive Antibodies
7	M	53	DQA1*0201,*030101 DQB1*0202,*0302	18	No	None
8	M	46	DQA1*0102,*0501 DQB1*050201,*0201	6	No	EMA IgG
9	M	44	DQA1*0501,*0501 DQB1*0201,*0201	29	No	EMA IgG
10	F	59	DQA1*0102,*030101 DQB1*0502,*0302	76	No	None
11	F	52	DQA1*0201,*0505 DQB1*0202,*0302	29	No	None

EMA, epithelial membrane antigen; GFD, gluten-free diet; HLA, human leukocyte antigen; Ig, immunoglobulin.

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it in the great majority of patients and control subjects with CVID (15/17). This was, however, regardless of the presence or absence of villous atrophy and whether a response to a GFD was seen. It does not, therefore, help in understanding whether these patients have CD. PMNI and GVHDL were, instead, found in fewer patients with CVID. Although our sample was small, we found these lesions only in patients in whom CD was not confirmed. Because of our small sample, we cannot confirm the hypothesis that CD could be excluded in these patients and, therefore, that starting a GFD is of no use; it suggests a cue for future studies.

Our results demonstrate that, in most cases, the search for CD-specific antibodies is of no use. Only 1 of the 3 patients with confirmed CD tested positive to epithelial membrane antigen (EMA) IgA (Table 1). The detection of positive EMA IgG in these patients should not surprise us. Tables 2 and 3 show that EMA IgG was also present in patients in whom CD could be excluded, and thus EMA IgG has no specificity for CD. On the other hand, CD-specific antibodies are useful in identifying selective IgA deficiency.[20]

The lack of serologic findings in these patients with CVID has been described.[5] These patients may have false-negative test results because they cannot mount an appropriate antibody response. On the other hand, they may have a false-positive result from passive transfer of antibodies in the pooled human immunoglobulin used for replacement therapy. To verify this hypothesis we tested a sample of therapeutic immunoglobulins (Ig vena 50 g/L, Kedrion Biopharmaceuticals, Castelvecchio Pascoli, Italy) for IgA and IgG EMA. We first diluted the sample to obtain the immunoglobulin physiologic concentration of 5 g/L. We then tested it at different titers (1:5, 1:10, 1:20, 1:40). Although IgA EMA was clearly negative, IgG EMA was positive.

Based on our results, we therefore conclude that the only criterion that makes it possible to confirm the diagnosis of CD in patients with CVID and a flat mucosa is still a histologic response to a GFD; HLA typing can be very useful in excluding a diagnosis of CD; the search for CD-specific antibodies, certainly of use in patients with a selective IgA deficiency,[20] has no role in the diagnosis of patients with CVID.

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