The Morphologic Features of Primary Epstein-Barr Virus Infection in the Gastrointestinal Tract

An Approach to Correct Diagnosis

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Abstract: Epstein-Barr virus (EBV) is a herpes virus that has been shown to contribute to the development of multiple tumor types. There are isolated reports on EBV infection in the gastrointestinal tract, but, notably, there are none with detailed descriptions of the morphologic features. It is believed to be difficult to generalize the histologic features, as EBV does not cause viral inclusions in infected cells. This study was aimed at describing the pathologic changes of nonlymphomatous gastrointestinal EBV lymphoproliferative diseases in both immunocompetent and immunocompromised settings; the former was focused on chronic active EBV infection, and the latter was about the infection in posttransplantation patients. A retrospective search identified a total of 26 cases of EBV lymphoproliferative disease in the gastrointestinal tract—8 in immunocompetent patients and 18 in immunocompromised patients. We found that there was no difference in pathologic findings in immunocompetent and immunocompromised patients, which ranged from essentially subtle to severe mucosal architectural distortion with prominent lymphoid aggregates. Most cases showed either focal or diffuse lymphocytic infiltration comprised of small to intermediate-sized lymphocytes with a round shape and increased cytoplasm when compared with mature small lymphocytes. Focal lymphoepithelial lesions and karyorrhexis can be seen. Although gastrointestinal primary EBV lymphoproliferative diseases are not common, the mortality is high. Awareness of the potential histologic features combined with suspicion of EBV infection from clinical presentation, radiographic findings, and/or EBV serologies can aid in the diagnosis of primary EBV infection in the gastrointestinal tract.

Key Words: Epstein-Barr virus, gastrointestinal tract, chronic active EBV infection, immunocompetent, immunocompromise

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The Epstein-Barr virus (EBV) is a double-stranded DNA virus that belongs to the Herpes family. EBV infection has an overall prevalence of over 90% worldwide.[1] EBV can infect multiple cell types, including epithelial cells, B, T, and natural killer (NK) lymphocytes, and mesenchymal cells such as smooth muscle cells. It has been well established that there is an association between EBV infection and tumorigenesis such as Burkitt and NK/T lymphomas, lymphoepithelial carcinoma, and some sarcomas. More recently, it was demonstrated that some mature small B-cell lymphomas might be accompanied by EBV infection (eg, follicular lymphoma).[2]

Clinically, primary EBV infection can have a variety of manifestations. The most common presentation is infectious mononucleosis (IM), which is an acute clinical syndrome characterized by fever, sore throat, and generalized lymphadenopathy. Chronic active Epstein-Barr virus infection (CAEBV) presents with similar symptoms; however, the symptoms and detection of EBV DNA persist for at least 3 months.[3–5] For reasons that are not fully understood, immunocompetent Asian individuals have a strong predisposition for the development of CAEBV.[3,6]

Historically, the terms CAEBV and Epstein-Barr virus–associated lymphoproliferative disease (EBV-LPD) have both been used to describe the same entity, leading to discrepancies with regard to the definitions among previous studies. Some authors believe that these 2 terms are nearly equivalent, with CAEBV being the favored clinical term, whereas EBV-LPD is the preferred pathologic classification. Others suggest EBV-LPD to be simply a severe form of CAEBV.[6,7] The 2017 World Health Organization (WHO) classification of lymphohematopoietic

tumors has updated the EBV-associated lymphoid diseases, and the updated classification indicates that CAEBV is one of the many subtypes of EBV-LPD and comprises a spectrum of lymphoid tissue diseases including hyperplastic, borderline, and neoplastic diseases.[3]

CAEBV has 2 types—CAEBV–T/NK-cell type and CAEBV–B-cell type, which account for 98% and 2% of cases, respectively. The grading system of systemic CAEBV–T/NK cell type was also clarified by the 2017 WHO: A1 cases are polymorphic and polyclonal, A2 cases are polymorphic and monoclonal, A3 cases are monomorphic and monoclonal, and B cases are monomorphic and monoclonal and show a fulminant clinical course. The A1 to A3 categories are thought to represent a continuous spectrum of CAEBV from lymphoproliferative disorder (A1 to A2) to overt lymphoma (A3), whereas the B category is equivalent to systemic EBV-positive T-cell lymphoma of childhood.[3]

In a recent review article by Rezk et al,[8] the authors divided EBV-associated lymphoproliferative lymphoid and histiocytic/dendritic proliferations into several groups: reactive, B cell, T/NK cell, immunodeficiency, and histiocytic/dendritic cell proliferations. Reactive proliferations were subcategorized into reactive lesions with no malignant potential and varied malignant potential; interestingly, they included acute IM in the former and CAEBV in the latter.[8]

Primary EBV infection has a lytic stage and 3 types of latent infection.[1] Detection of EBV-related molecules including nuclear antigens (EBNA), latent membrane proteins, and short noncoding RNAs (EBER1 and EBER2) in blood and tissue can be used to differentiate between lytic infection and latent infection. Previous studies suggest that EBV viral capsid antigen (VCA) immunoglobulin (Ig) M, VCA IgG, and EBNA-1 IgG can distinguish acute from past infection—specifically, the presence of VCA IgM without EBNA-1 IgG indicates an acute infection.[9] Detection of EBV load (DNA) in the affected tissue and/or peripheral blood is necessary for diagnosing CAEBV, which is highly associated with lytic infection[4]; hence, in this study, serologic studies were used to verify active EBV infection.

It is well known that many viral infections form characteristic inclusions, providing clues for their diagnosis. Unfortunately, no inclusion bodies are formed in EBV-infected cells, resulting in a rarity of systematic histologic studies published with regard to EBV-associated gastrointestinal lymphoproliferative diseases (LPDs). In the few isolated case reports, some cases were previously misdiagnosed as inflammatory bowel disease (IBD) or EBV-related lymphoma among other diseases.[10–14] In the present study, the histologic features of nonlymphomatous primary gastrointestinal EBV infection are described, including changes from EBV gastroenteritis in patients with CAEBV gastrointestinal infection (A1/A2), and EBV gastrointestinal infection in immunocompromised posttransplantation individuals. Cases of EBV-associated lymphomas such as Burkitt lymphoma, EBV-positive large B-cell lymphoma, or CAEBV infection with atypia and neoplastic features (A3) are not included in the current study. The top differentials are also discussed briefly.

Although gastrointestinal primary EBV-LPD is not common, the mortality is high regardless of the extent or grade.[7,15] Patients with CAEBV die of hemorrhage, perforation, multiorgan failure, disseminated intravascular coagulation, or other severe complications. Making the correct diagnosis of EBV infection is a pivotal step for appropriate clinical management, as misdiagnosis potentially leads to improper treatments, delaying appropriate therapies and/or compromising the already damaged immune system.

MATERIALS AND METHODS

All cases were from the Department of Pathology at Zhongnan Hospital of Wuhan University in China and the University of California at Los Angeles in the United States. This project has been approved by the Ethic Consensus Committee in China and the Internal Review Board of the University of California at Los Angeles, respectively. The electronic medical records were retrospectively searched to identify cases of EBV-positive gastrointestinal infection from 2013 to 2018. Cases diagnosed with primary EBV infection in the gastrointestinal tract (GI) tract were retrieved. Some identified cases with initial onset of disease before 2013 were also included in this series.

The following requirements were met for case selection:

(1) Patients had to have an established diagnosis of acute EBV infection via a positive monospot test for heterophile antibody and positive serum VCA IgM but had to be negative for VCA IgG and EBNA-1 IgG.
(2) In CAEBV patients, cases had to meet the revised WHO diagnostic criteria including IM–like symptoms (in our cases, including but not limited to abdominal pain, diarrhea, and hematochezia) and increased EBV DNA ($>10^{2.5}$ copies/mg) in the peripheral blood persisting for >3 months, histologic evidence of organ disease, and demonstration of EBV RNA or viral protein in affected tissues in patients without known immunodeficiency, malignancy, or autoimmune disorders.
(3) For the posttransplantation group, patients had to have undergone a solid organ transplant—most were either multivisceral transplant cases or small intestinal transplant cases.
(4) The patients must not have had a diagnosis of lymphoma or monomorphous posttransplant lymphoproliferative disease (PTLD).

Cases of EBV+ gastrointestinal infection initially identified included 16 cases of CAEBV patients and 18 cases of posttransplantation patients. Only 8 of the 16 cases of CAEBV gastrointestinal infection had follow-up and were included here (Table 1). Characteristics of all included cases are listed in Tables 1 and 2. Also included were control cases in which patients had EBV viremia but had negative staining for EBV early RNA (EBER) in tissue samples (not shown in the tables). In situ hybridization (ISH) for EBER was

TABLE 1. Clinical, Laboratory, and Pathologic Findings in Immunocompetent Patients

Case No.	Age (y)	Sex	Organ Involvement	Sample	EBV DNA	Duration	Subtype and Grade	Summary of Pathologic Findings, EBER-positive Cells/HPF	Follow-Up
1	50	Male	Small bowel	Resection	4.5E5	1 y	CAEBV–T-cell, grade 2	Ulceration, mild crypt distortion in the margin of the ulcer, aggregation of atypical lymphoid cells, and also infiltration of mixed inflammatory cells in submucosa, > 50	PR
2	27	Female	Colon	Resection	4.8E5	3 y	CAEBV–T-cell, grade 1	Ulceration and infiltration of small lymphocytes with little atypia in the mucosa. Lymphoid aggregates and vascular hyperplasia are prominent in the submucosa, > 100 only in mucosa	PR
3	26	Male	Duodenum, lymph node	Biopsy	2.52E5	1 y	CAEBV–T-cell, grade 2	Fragmented tissue with surface necrosis and diffuse lymphoid infiltrates in the lamina propria and submucosa. Atypical rounded, medium-sized lymphocytes infiltrate the epithelium, and karyorrhectic debris is noted, > 100	Recovered
4	22	Male	Colon, spleen	Biopsy	1.02E6	3 mo	CAEBV–T/NK, grade 2	Multiple biopsies show that the mucosa between ulcerations seems normal, with lymphoid hyperplasia mimicking follicles. The infiltrating lymphocytes exhibit a round shape and increased cytoplasm compared with mature small lymphocytes, > 100	Died
5	15	Male	Ileum, skin	Resection	7.2E5	1 y	CAEBV–T/NK, grade 2	Diffuse infiltration of atypical lymphocytes in lamina propria, and karyorrhexis and focal necrosis are noted, > 200	Died
6	26	Female	Colon	Resection	4.06E4	1.5 y	CAEBV–T-cell, grade 2	Ischemic change and ulceration in mucosa without obvious crypt abnormality. Scattered polymorphic lymphocytes infiltrates the intestinal wall (monoclonal TCR rearrangement), > 100	Recovered
7	28	Male	Colon	Biopsy	2.0E5	6 mo	CAEBV–T/NK, grade 2	No crypt abnormality, lymphoid infiltrates in mucosa and submucosa with mild atypia (monoclonal TCR rearrangement), > 100	PR
8	51	Male	Colon, mouth	Biopsy	3.76E5	16 y	CAEBV–T-cell, grade 1	Ulceration of the mucosa with scattered small lymphocytes in the lamina propria and submucosa, > 200	PR

E indicates 10^n; PR, partially resolved; TCR, T-cell receptor.

performed on the tissue block in all potential cases via an automated ISH instrument (either Leica Bond Max or BenchMark XT). The cutoff for positivity used was > 30 and > 50 EBER-positive lymphocytes per high-power field (HPF, 0.238 mm^2) on average for biopsies and resection samples, respectively, in the immunocompetent group.[15,16] Immunohistochemical stains such as B, T, and NK cell markers were performed accordingly. Two pathologists including 1 hematopathologist reviewed the cases. Primary EBV gastroenteritis may be part of the multiorgan systemic disease or the primary organ of involvement. There is no established grading system available, as there were no other studies focusing on its pathologic features previously published. As mentioned above, CAEBV has a well-established WHO grading scheme; hence, we applied the same criteria and simplified A1 to A3 to grades 1 to 3 (G1 to G3) only in our CAEBV group. Because posttransplantation is in a different setting from the CAEBV group, we just

TABLE 2. Clinical and Laboratory Findings in Immunocompromised (Posttransplant) Patients

Case No.	Age (y)	Sex	EBV DNA (Copies/mg)	Summary of Pathologic Findings, and EBER-positive Cells/HPF	Onset of EBV Viremia After Transplant (y)
1	15	Male	78	Mildly increased lymphocytes in lamina propria with karyorrhexis, 15	5
2	8	Female	334	Lymphoid hyperplasia is noted with no increase in crypt apoptotic activity, 30	0
3	3	Female	1049	Moderate villous blunting and crypt hyperplasia with surface erosion, atypical lymphoid infiltrate with lymphoepithelial lesions, >100	3
4	5	Male	84	Focal erosions and lymphoid hyperplasia, 15	2
5	13	Female	500	Minimally increased crypt apoptotic activity, >100	0
6	5	Male	114	Prominent lymphoid aggregates present, 20	NA
7	28	Female	82	A prominent benign-appearing lymphoid aggregate present, 10	1
8	57	Female	186	Focal minimally increased crypt apoptotic activity, 30	NA
9	13	Male	669	Mildly increased crypt apoptotic activity, 20	0
10	13	Male	230	Benign-appearing lymphoid aggregates, 30	0
11	13	Female	704	No histopathologic abnormality, 10	NA
12	28	Male	2565	No histopathologic abnormality and a few benign-appearing lymphoid aggregates are noted, 30	5
13	39	Male	10	Minimally increased crypt apoptotic activity, 10	NA
14	8	Female	188	Benign-appearing lymphoid aggregates present, 10	NA
15	34	Male	186	Large aggregate comprised of lymphocytes with round shape and increased cytoplasm, >100	NA
16	12	Female	54	Atypical lymphoid proliferation with lymphoepithelial lesions, >100	NA
17	10	Male	752	Focal dense lymphoplasmacytic infiltrates, 20	0
18	20	Male	116	Mucosa with lymphoid hyperplasia mimicking lymphoid follicle and medium-sized lymphocytes, 20	0

0 indicates the same year as transplantation; NA, not available.

checked the status of EBV infection rather than grading them in this study.

RESULTS

CAEBV Infection in the GI

This group (Table 1, cases 1 to 8) was comprised of predominantly young adults with an average age of 30 years. The duration of illness ranged from 3 months to 16 years. A longer clinical course was seen in G1 cases, such as in cases 2 and 8, with durations of 3 and 16 years, respectively. Common symptoms and signs were abdominal pain, fever, lymphadenopathy and splenomegaly, reflux gastritis, diarrhea, hematochezia, and weight loss. EBV DNA copies in the peripheral blood were all over 10^3 copies/mg, and some cases reached 10^6 copies/mg. Some patients presented with predominantly gastrointestinal involvement (cases 1, 2, 6, and 7), whereas others showed multiorgan involvement (cases 3, 4, 5, and 8). Occasionally, imaging studies showed a thickened gastric or intestinal wall and enlargement of retroperitoneal lymph nodes. Endoscopically, diffuse granular, erythematous mucosa and multiple ulcers were common findings within the stomach or intestinal tract, even mimicking skip lesions seen in Crohn disease (case 4, Fig. 1).

In general, the pathologic findings varied according to grade. In G1 cases, the morphologic changes are subtle and easy to overlook, such as those seen in cases 2 (Fig. 2) and 8 (not photographed). Ischemic changes or mucosal erosions were noted in some cases, but no cases showed obvious acute enterocolitis–like changes such as cryptitis or crypt abscesses, or chronic enterocolitis–like changes such as crypt architectural distortion. A population of small to medium-sized lymphocytes showing little or no atypia infiltrated the mucosa. There was no loss of T-cell markers, but ISH showed numerous EBER-positive lymphocytes in the mucosa, which were not present in the deeper tissue, and a relatively low Ki-67 proliferation index (case 2, not shown in Fig. 2).

As for G2 cases, all cases showed a focal or diffuse inflammatory cell infiltrate within the lamina propria and submucosa composed of small to intermediate-sized lymphocytes with mild to moderate atypia (Fig. 3), with crypt architecture either preserved or slightly distorted. Interestingly, some cases showed lymphoid aggregates with obvious follicle formation resembling the outline of a normal lymphoid follicle in the submucosa; however, the lymphocytes within the aggregates were atypical, showing a round shape with increased cytoplasm when compared with mature small lymphocytes (Figs. 3D, E). Infiltration of the epithelium by individual lymphocytes was noted. Focal necrosis and karyorrhexis were prominent in some cases (Figs. 3, 4). The biopsy fragments in some cases showed extensive crush artifact. There were mature plasma cells, lymphocytes, and histiocytes in the background with few eosinophils and neutrophils. Immunophenotypically, the atypical lymphocytes were strongly positive for T-cell markers CD3 and CD8 and were negative for CD4. Immunohistochemical stains were used to categorize all cases in this cohort as either T-cell or T/NK-cell type CAEBV, with the latter group showing CD56 positivity in addition to T-cell markers. Some cases showed weak to strong staining with CD30. All cases were

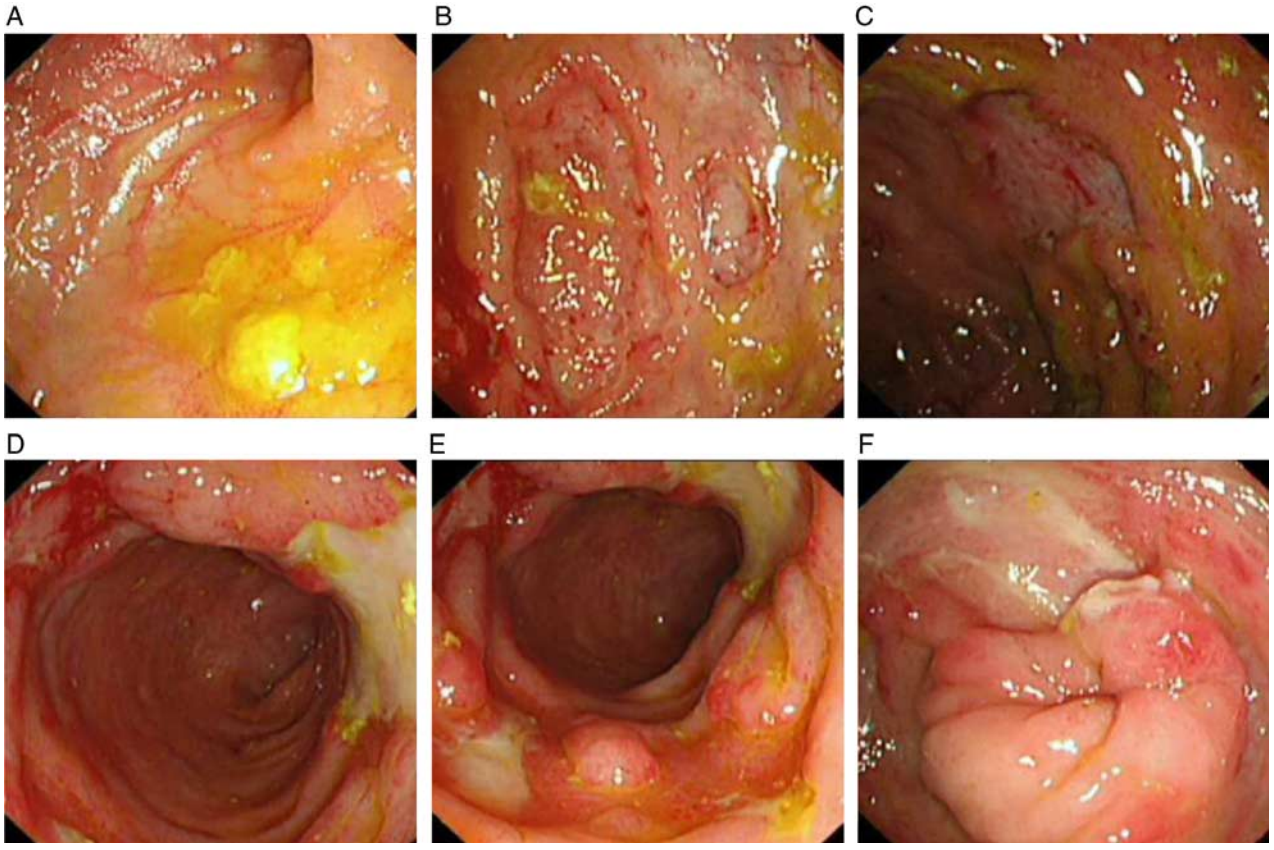

FIGURE 1. Colonoscopic images from case 4 show sporadically distributed ulcerations in different parts of the GI tract, including the terminal ileum (A), ileocecal region (B, C), descending colon (D, E), and sigmoid colon (F). The clinical impression from the colonoscopy was Crohn disease.

positive for EBER by ISH, as stated in the inclusion criteria. There was evidence of a clonal T-cell receptor in all cases in which T-cell gene rearrangement studies were performed. All cases met the diagnostic criteria for grade A2: polymorphic histologic changes and monoclonal immunophenotype.

Follow-up showed that 6 patients partially or fully recovered after treatment, and 2 patients died of complications of EBV infection (bowel perforation, untreatable massive intestinal hemorrhage, and disseminated intravascular coagulation) instead of overt lymphoma.

EBV Infection in the GI After Transplantation

All 18 patients were status after small bowel or multivisceral transplantation for a variety of indications including tufting enteropathy, microvillus inclusion disease, intestinal failure secondary to chronic intestinal pseudo-obstruction, or jejunal atresia. The majority did not have any clinical symptoms and were undergoing routine follow-up endoscopic surveillance at the time of biopsy. All patients had increased EBV DNA copies in the blood, ranging from 10 to over 2000 copies/mg. They represented EBV latent infection rather than acute infection, even though the EBV load in some cases was not low. The time when EBV DNA became detectable in the peripheral blood following transplantation varied from the same year to 7 years.

Microscopic findings were similar to those seen in immunocompetent patients, ranging from subtle changes to overt mucosal alterations. Some cases showed only subtle mucosal changes including expansion of the lamina propria by nonspecific inflammatory cells with no remarkable atypical lymphoid infiltrate. Other cases showed erosion and enlarged submucosal lymphoid follicles containing small to medium-sized lymphocytes with round to oval nuclei and abundant cytoplasm (Figs. 5A, C). Infiltration of the epithelium by individual lymphocytes and nuclear fragments was easily identified (Figs. 5E, G, and H).

The number of EBER-positive cells within the tissue detected by ISH showed no correlation with the number of EBV DNA copies in the peripheral blood or the histologic findings. For instance, some unincluded patients had a high EBV DNA level but only rare to no positive cells in the tissue (similar to our control cases). In contrast, some cases showed very low DNA copies, whereas the biopsy tissue exhibited numerous positive cells. In addition, cases 15 and 18 in Table 2 shared similar histologic changes; however, the number of EBER-positive cells was very different (Figs. 5B, D).

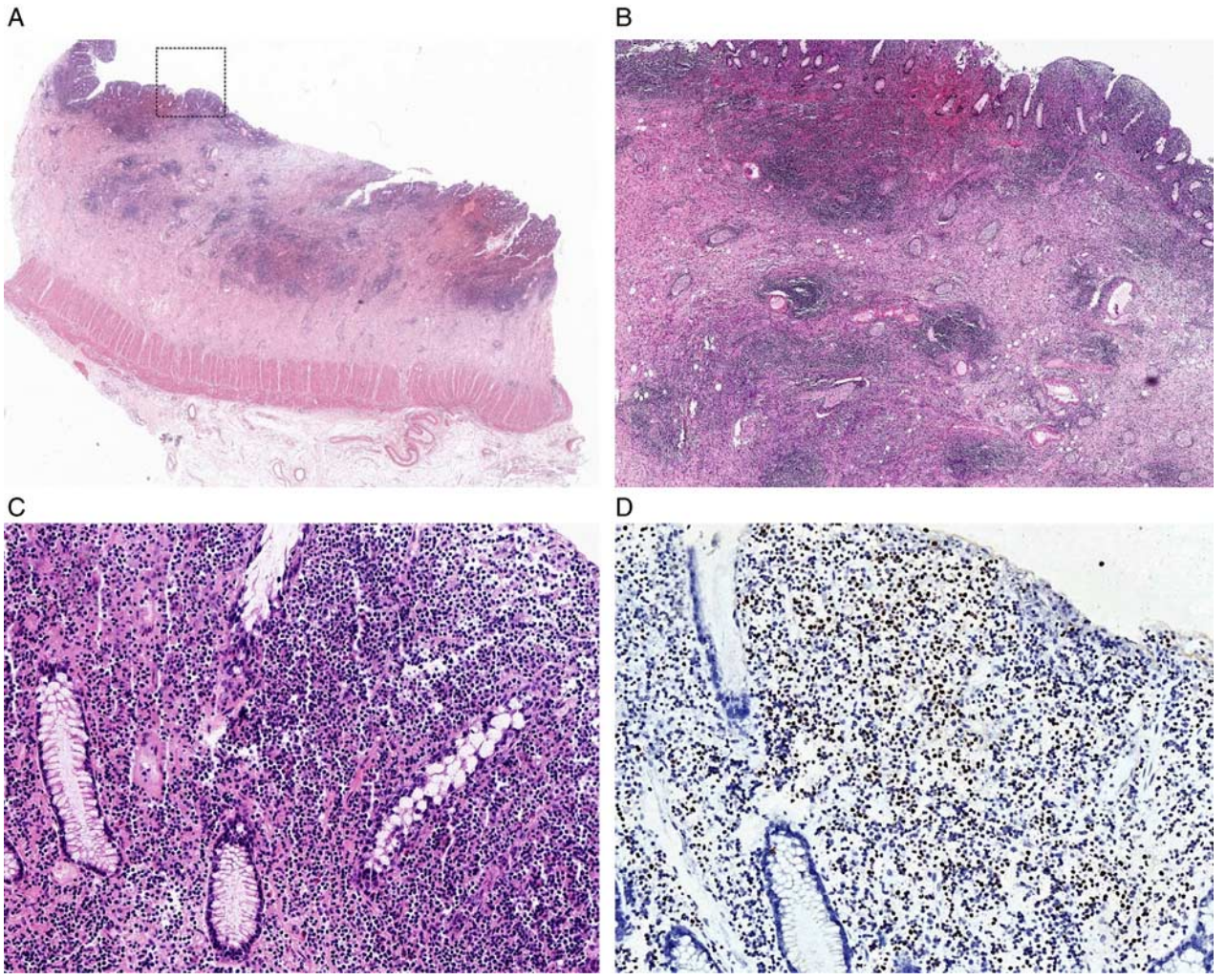

FIGURE 2. From case 2, sections of the resected colon from a 27-year-old woman with gastrointestinal symptoms for 3 years. A low-power view shows ulceration of the mucosa and an expanded submucosal layer (A, the box indicating the area shown in B and C at higher power view). Lymphoid aggregates and vascular hyperplasia are prominent in the submucosa as well (B). A high-power view shows small lymphocytes with minimal atypia infiltrating the mucosa (C), and ISH for EBER shows numerous positive cells present in the mucosa (D) but not in the deeper tissue.

DISCUSSION

In the past decade, progress has been made in EBV-related research.

It is already known that IM and CAEBV are highly associated with EBV lytic infection. Usually, both the quantity and quality of the $CD8^+$ T-cell response are critical to control EBV infection. $CD8^+$ T cells isolated from acute IM individuals recognize a wide variety of EBV epitopes.[17] In contrast, $CD8^+$ T cells from persons with persistent IM (CAEBV) recognize only a few EBV epitopes.[6,8,18] CAEBV likely involves a cellular immune defect that fails to control the proliferation of infected lymphocytes and leads to restricted latency with activation of viral lytic proteins.[8,19] Lytic genes play important roles in cell proliferation of EBV-positive cells; however, many lytic gene products have not been identified yet nor studied thoroughly.[8,19]

In the GI tract, EBV gastroenteritis and CAEBV infective enteritis have been commonly accepted terms, although EBV-positive LPD involving the GI tract might be more accurate, which would encompass all EBV-positive entities. Primary EBV gastrointestinal infection including both acute IM–like lesions and CAEBV are prone to be overlooked or misdiagnosed without much experience.[10–13]

Patients in this study were predominantly young adults, consistent with other reports.[7,15] They frequently presented with acute or intermittent fever and gastrointestinal symptoms such as abdominal pain, diarrhea, and hematochezia with or without extragastrointestinal manifestations. Some patients also had lymphadenopathy, hepatosplenomegaly, or other symptoms. Endoscopic examination showed nonspecific findings and may not be useful to the diagnosis. Radiologic images may show a

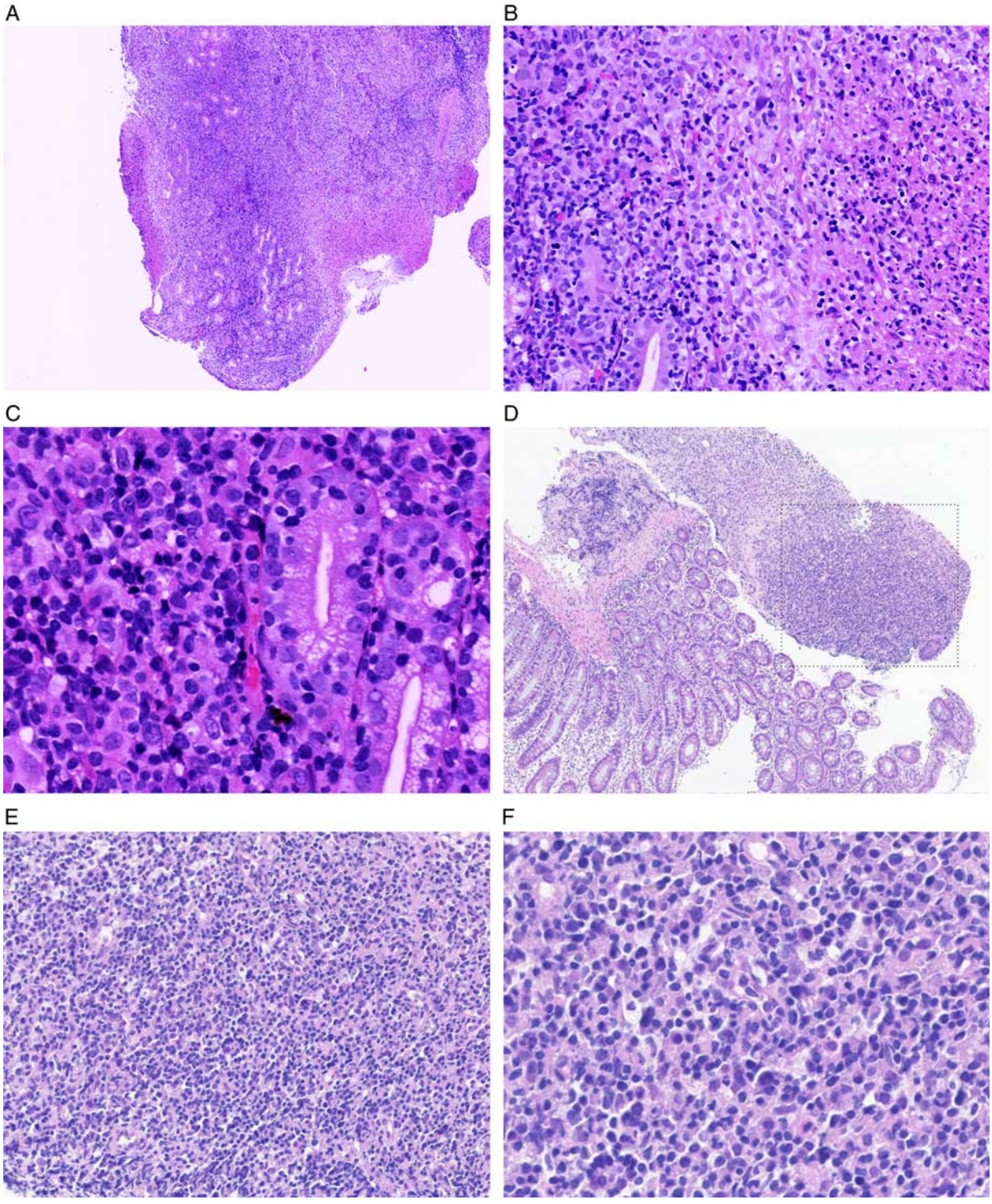

FIGURE 3. From case 3 (A–C), a duodenal biopsy shows fragmented tissue with surface necrosis and diffuse lymphoid infiltrates in the lamina propria and submucosa. There are no lymphoid follicles (A). Karyorrhectic debris is noted (B). Atypical rounded, medium-sized lymphocytes infiltrate the epithelium (C). From case 4 (D–F), a 22-year-old man with fever of unknown origin, intermittent abdominal pain, and hematochezia for 3 months. His blood EBV DNA copy number was 1.02E6. Colon biopsies show that the mucosa between ulcerations appears normal, with lymphoid hyperplasia mimicking follicles (the box in D indicating the area shown in E and F at higer power view). The infiltrating lymphocytes exhibit a round shape and increased cytoplasm compared with mature small lymphocytes (E, F). He was ultimately diagnosed with EBV-associated T/NK LPD, grade 2.

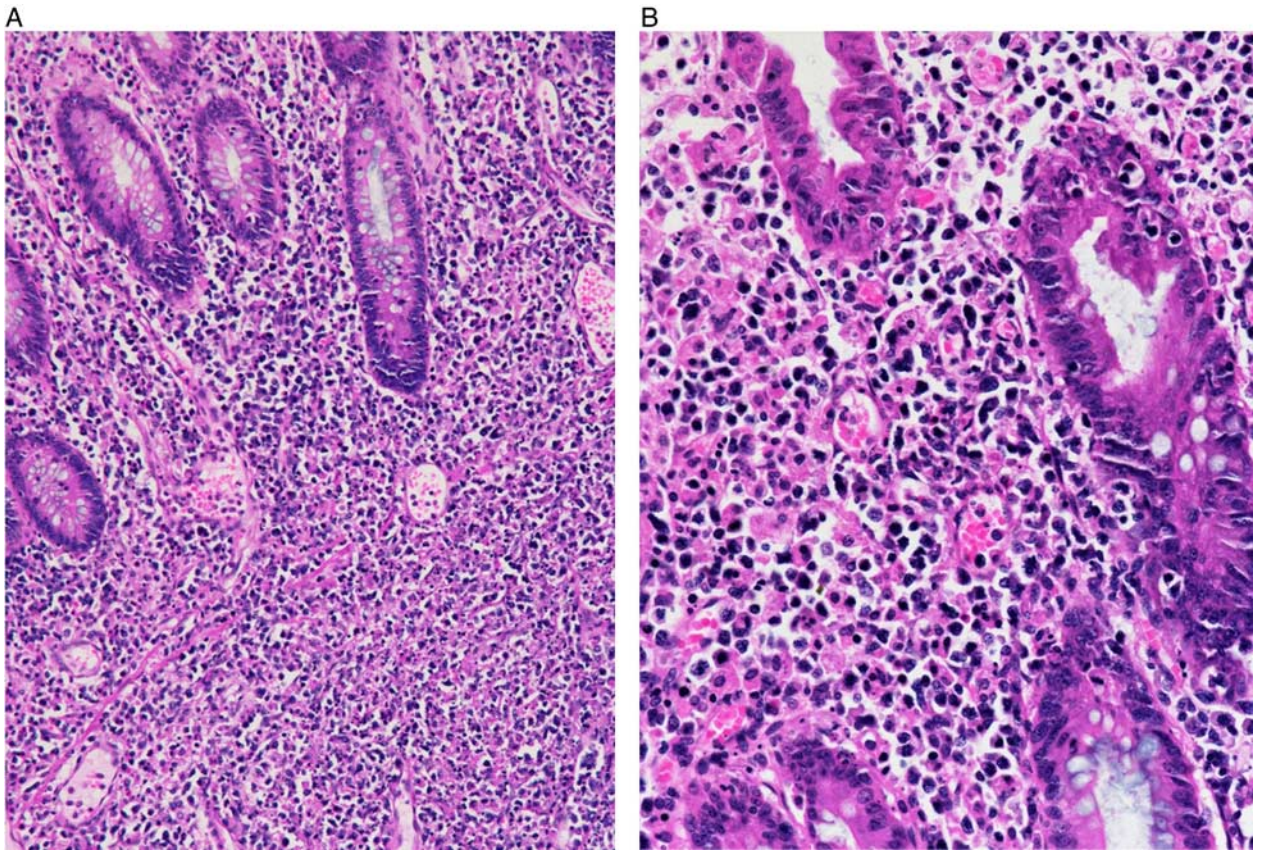

FIGURE 4. From case 5, ileal resection due to perforation in a 15-year-old boy. The sections show diffuse infiltrates of atypical lymphocytes and prominent karyorrhexis (A, B). Similar findings were also seen in biopsies from other organs including the skin. He was diagnosed with EBV-positive T/NK-cell LPD and died after the operation.

thickened gastric or intestinal wall and/or enlarged mesenteric lymph nodes.

A combination of histologic findings, clinical history, and laboratory correlation are crucial for the accurate diagnosis of acute IM–like lesion and CAEBV of the GI tract. For example, acute IM–like infection is a self-limited disease, but the possibility of misdiagnosis as lymphoma is high because of the presence of large atypical immunoblastic cells with abnormal immunophenotype. We have previously reported a rare case of acute IM–like gastritis.[20] The patient was previously healthy and presented with acute fever and epigastric pain. His radiologic images and microscopic examination resembled lymphoma to some extent, but he was found to have laboratory test abnormalities including a positive monospot test for heterophile antibody and positive serum VCA IgM, which was crucial to the final diagnosis.

All diagnostic criteria must be met to a make a diagnosis of CAEBV of the GI tract. First, the disease duration for CAEBV should be at least 3 months besides the laboratory abnormality. Second, the number of EBV-positive cells must meet the suggested cutoff (at least 30/HPF). If the number of EBER-positive cells is below the recommended cutoff, it suggests a likely latent infection or superimposed infection rather than primary infection.[15,16] For G1 CAEBV, it is very important to perform EBER ISH, as the morphologic changes are subtle, and a high index of suspicion should be rendered if the medical history and manifestation are consistent with primary EBV infection. In G2 CAEBV cases, lymphocytes within the aggregates are atypical, showing a rounded shape with increased cytoplasm, when compared with mature small lymphocytes. We speculate that these cytotoxic ($CD8^+$) lymphocytes are reactive lymphocytes or variant lymphocytes, which become large in size as a result of EBV stimulation, just like a "Downey cell" cytologically. Third, the exclusion of other potential etiologies is of great importance. There is a wide differential for gastrointestinal EBV infection including, but not limited to, herpes simplex virus infection, IBD with or without superimposed EBV infection, NK/T-cell lymphoma, EBV-positive peripheral T-cell lymphoma, and EBV mucosal cutaneous ulceration.

Differentiating gastrointestinal EBV infection from IBD can be challenging. In addition, there are cases of IBD superimposed with EBV infection, and cases that develop EBV infection after treatment for IBD, which can occasionally be fatal.[21,22] A recent study compared the clinicopathologic features between GI CAEBV infection and IBD.[15] The authors suggested that CAEBV infective

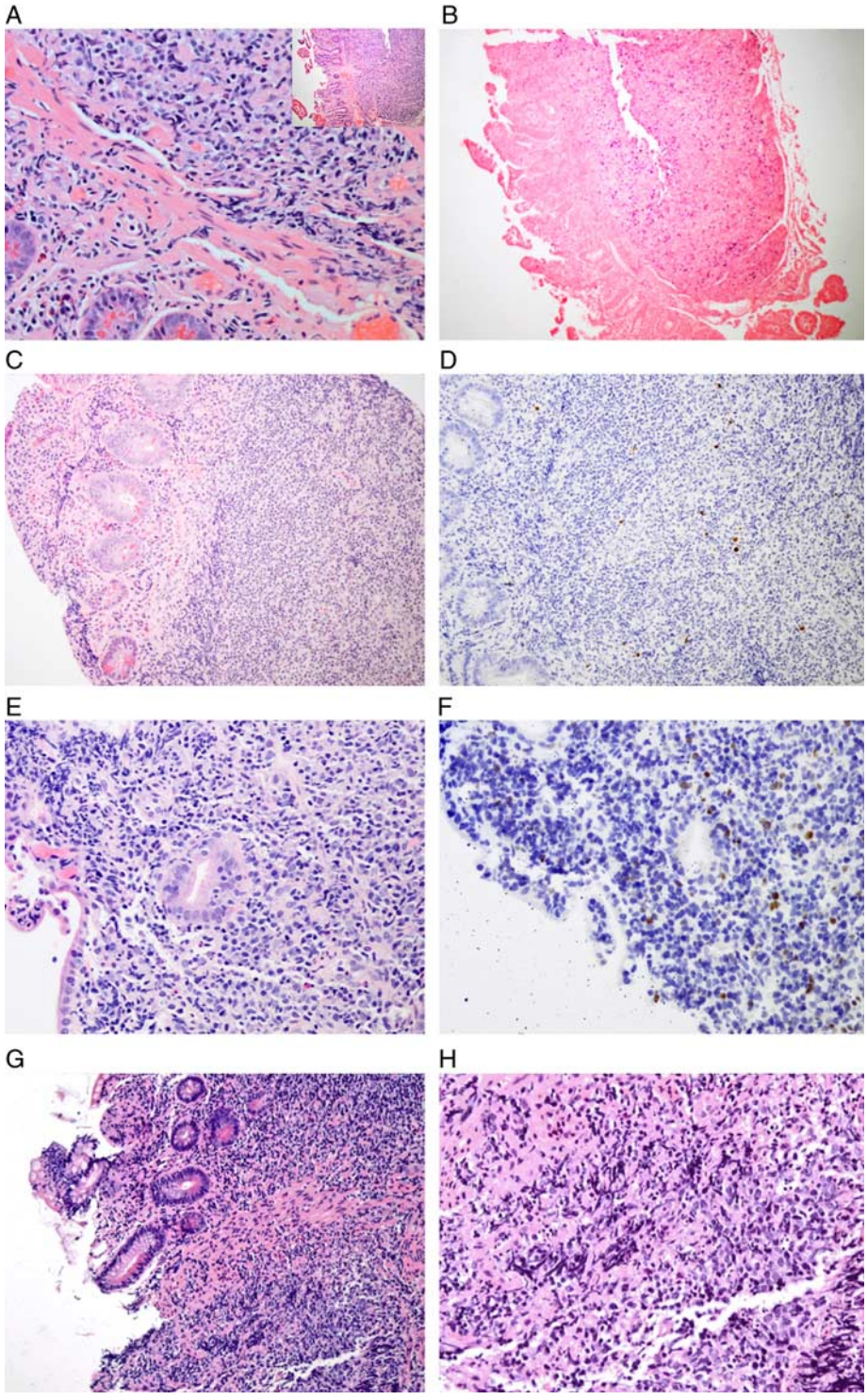

FIGURE 5. These biopsies are from patients in cases 1, 3, 15, and 18 (Table 2) following organ transplant. Some morphologic changes are similar to those seen in immunocompetent patients. Lymphocytes with a round shape and rich cytoplasm constitute lymphoid follicle–like structures that are seen in biopsies from case 15 (A and low-power inset) and case 18 (C), which show a variable number of EBER-positive cells from numerous (B) to few (D), respectively. Moreover, lymphoepithelial lesions are easily identified in case 3 (E), which is positive for EBV on EBER staining (F). Karyorrhexis is found in case 1 (G, H).

enteritis should be suspected for patients who exhibited IBD-like symptoms but with intermittent high fever and showing irregular, small, and shallow ulcers in both large and small intestines. ISH for EBV should be performed in suspicious cases, which is critical to establishing the diagnosis. In our study group, there were a couple of cases that looked like IBD by endoscopic findings. Again, the overall findings, including clinical history, laboratory results including the status of EBV infection, along with histologic examination for the patterns of ulceration and the presence or absence of chronic changes in the mucosa, are crucial in distinguishing IBD from EBV infection. Findings that favor EBV infection include acute or intermittent fever, hepatosplenomegaly, generalized lymphadenopathy, and increased EBV DNA load. It seems that the risk of EBV infection may be higher in inflamed mucosa than in normal mucosa. It had been reported that EBER1 was detected in ~60% of Crohn disease and ulcerative colitis cases, but not in noninflammatory controls.[22] In a recent study by Pezhouh et al,[23] the authors found that EBV infection is closely related to refractory IBD. For young IBD patients, the diagnosis of EBV infection requires a high index of suspicion, particularly when clinical symptoms are atypical, there is no symptomatic improvement on IBD-related treatment, there is hepatosplenomegaly, and there is seroconversion on EBV serological screening. There is a proposed algorithm for treatment management of IBD patients who are either EBV seropositive or negative.[24]

Both extranodal NK/T-cell lymphoma and CAEBV may show similar histologic features and positive stains for CD56 and EBER. Clinically, CAEBV patients usually present with systemic symptoms and/or abnormal laboratory results from the onset of the disease and suffer a recurrent disease course for months or even years, gradually worsening; however, NK/T-cell lymphoma often starts with local lesions, and then progressively spreads throughout the whole body.

EBV-positive mucocutaneous ulcer is a B-cell lymphoproliferative disorder characterized by discrete ulcerations in the oropharynx, skin, and GI tract. It differs from CAEBV, however, in pathogenesis and clinical course, and in gross, microscopic, and immunohistochemical findings. For example, mucocutaneous ulcer is closely related to iatrogenic immunosuppression or is common in aged people, and presents characteristically with isolated, sharply circumscribed ulcers.[3] The tumor cells are B cells. Importantly, it has a self-limited clinical course and responds well to the reduction of immunosuppression and conservative management. Clearly, these are not features in our cases, as our patients are predominantly young adults with no known immunodeficiency.

The GI tract is a common site of involvement by PTLD, seen in ~20% of PTLD cases.[25] Previous studies have shown that around one third of pediatric patients who receive liver transplantation present with GI bleeding or diarrhea at the time of EBV infection in the first year.[26] In our posttransplant group, most patients had asymptomatic infection but, similarly, at least one third of EBV infections occurred within the first year of transplantation (notably, the date of onset of EBV infection was not documented in some cases). The cases included in this study did not meet the diagnostic criteria for monomorphic PTLD and showed morphologic features similar to those of immunocompetent cases. Surveillance endoscopy with biopsy remains important for early diagnosis of GI PTLD.

Although some progress has been made in the understanding of CAEBV, the prognosis of this disease is not promising. Some patients with CAEBV die within several years. Those patients with late onset of disease, thrombocytopenia, and T-cell infection have significantly poorer outcomes.[27] The treatment depends on the infected cell type and the general health condition of the patients. For example, rituximab can be used to treat B-cell LPD. If it is a T/NK-cell LPD, antivirus therapy does not offer much help. At Zhongnan Hospital, some patients had symptoms partially resolved with supportive treatment such as infusion of serum γ-globulin in combination with glucocorticoids, cyclosporin, or mild chemotherapy, whereas others did not benefit from these treatments, and their prognosis was always dismal. It seems that the only cure is allogeneic hematopoietic stem cell transplantation. It is essential for early identification of potential severe complications, such as disseminated intravascular coagulation, multiorgan failure, gastrointestinal bleeding/perforation, myocarditis, coronary artery aneurysms, and sepsis, and to treat the patients timely to save lives when the complications occurs.

In summary, the approach to prompt and correct diagnosis of EBV infection in the GI tract starts with recognition of morphologic clues. The histology of CAEBV G1 is subtle, which always shows dense small mature lymphocytes in the lamina propria but without mucosal architecture distortion. Some unique morphologic features have been observed for G2 cases, such as atypical round shape lymphoid cells with increased cytoplasm, lymphoid follicle–like aggregates, lymphoepithelial lesions, focal necrosis, and karyorrhexis. It is vital to review clinical history and laboratory tests related to EBV, especially anti-VCA-IgG and EBV DNA, and to perform EBER on tissue if suspicion for primary EBV infection in the GI is raised, particularly in young patients. It is also important to keep in mind that CAEBV is a spectrum composed of benign, borderline, and malignant diseases. The accurate diagnosis requires comprehensive assessment of clinical history, laboratory results, histologic findings, immunohistochemical and ISH results, and sometimes the results of molecular testing.

REFERENCES

1. Williams H, Crawford DH. Epstein-Barr virus: the impact of scientific advances on clinical practice. *Blood*. 2006;107:862–869.
2. Kurt H, Medeiros LJ, Khoury JD, et al. Epstein-Barr virus-positive follicular lymphoma. *Br J Haematol*. 2018;182:757.
3. Steven H, Swerdlow EC, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: International Agency for Research on Cancer (IARC); 2017.
4. Okano M, Kawa K, Kimura H, et al. Proposed guidelines for diagnosing chronic active Epstein-Barr virus infection. *Am J Hematol*. 2005;80:64–69.
5. Kimura H, Ito Y, Kawabe S, et al. EBV-associated T/NK-cell lymphoproliferative diseases in nonimmunocompromised hosts: prospective analysis of 108 cases. *Blood*. 2012;119:673–686.

6. Cohen JI, Kimura H, Nakamura S, et al. Epstein-Barr virus-associated lymphoproliferative disease in non-immunocompromised hosts: a status report and summary of an international meeting, 8–9 September 2008. *Ann Oncol*. 2009;20:1472–1482.
7. Ohshima K, Kimura H, Yoshino T, et al. Proposed categorization of pathological states of EBV-associated T/natural killer-cell lymphoproliferative disorder (LPD) in children and young adults: overlap with chronic active EBV infection and infantile fulminant EBV T-LPD. *Pathol Int*. 2008;58:209–217.
8. Rezk SA, Zhao X, Weiss LM. Epstein-Barr virus (EBV)-associated lymphoid proliferations, a 2018 update. *Hum Pathol*. 2018;79:18–41.
9. De Paschale M, Clerici P. Serological diagnosis of Epstein-Barr virus infection: problems and solutions. *World J Virol*. 2012;1:31–43.
10. Jeong JE, Kim KM, Jung HL, et al. Acute gastritis and splenic infarction caused by Epstein-Barr virus. *Pediatr Gastroenterol Hepatol Nutr*. 2018;21:147–153.
11. Hisamatsu A, Nagai T, Okawara H, et al. Gastritis associated with Epstein-Barr virus infection. *Intern Med*. 2010;49:2101–2105.
12. Wang Z, Zhang W, Luo C, et al. Primary intestinal Epstein-Barr virus-associated natural killer/T-cell lymphoproliferative disorder: a disease mimicking inflammatory bowel disease. *J Crohns Colitis*. 2018;12:896–904.
13. Zheng X, Xie J, Zhou X. Epstein-Barr virus associated T-cell lymphoproliferative disease misdiagnosed as ulcerative colitis: a case report. *Int J Clin Exp Pathol*. 2015;8:8598–8602.
14. Karlitz JJ, Li ST, Holman RP, et al. EBV-associated colitis mimicking IBD in an immunocompetent individual. *Nat Rev Gastroenterol Hepatol*. 2011;8:50–54.
15. Liu R, Wang M, Zhang L, et al. The clinicopathologic features of chronic active Epstein-Barr virus infective enteritis. *Mod Pathol*. 2019;32:387–395.
16. Jianlan X, Yuhua H, Yuanyuan Z, et al. Acute Epstein-Barr virus-positive cytotoxic T cell lymphoid hyperplasia in the upper aerodigestive tract, mimicking extranodal natural killer/T cell lymphoma, nasal type. *Virchows Arch*. 2019;474:219–226.
17. Bharadwaj M, Burrows SR, Burrows JM, et al. Longitudinal dynamics of antigen-specific CD8+ cytotoxic T lymphocytes following primary Epstein-Barr virus infection. *Blood*. 2001;98:2588–2589.
18. Cohen JI, Jaffe ES, Dale JK, et al. Characterization and treatment of chronic active Epstein-Barr virus disease: a 28-year experience in the United States. *Blood*. 2011;117:5835–5849.
19. Murata T. Encyclopedia of EBV-encoded lytic genes: an update. *Adv Exp Med Biol*. 2018;1045:395–412.
20. Chen ZM, Shah R, Zuckerman GR, et al. Epstein-Barr virus gastritis: an underrecognized form of severe gastritis simulating gastric lymphoma. *Am J Surg Pathol*. 2007;31:1446–1451.
21. Makino Y, Tani C, Miyokawa N, et al. Multiple intestinal ulcers associated with primary Epstein-Barr virus infection in a patient with rheumatoid arthritis undergoing methotrexate therapy. *Intern Med*. 2015;54:2851–2855.
22. Yanai H, Shimizu N, Nagasaki S, et al. Epstein-Barr virus infection of the colon with inflammatory bowel disease. *Am J Gastroenterol*. 1999;94:1582–1586.
23. Pezhouh MK, Miller JA, Sharma R, et al. Refractory inflammatory bowel disease: is there a role for Epstein-Barr virus? A case-controlled study using highly sensitive Epstein-Barr virus-encoded small RNA1 in situ hybridization. *Hum Pathol*. 2018;82:187–192.
24. Lam GY, Halloran BP, Peters AC, et al. Lymphoproliferative disorders in inflammatory bowel disease patients on immunosuppression: lessons from other inflammatory disorders. *World J Gastrointest Pathophysiol*. 2015;6:181–192.
25. Cruz RJ Jr, Ramachandra S, Sasatomi E, et al. Surgical management of gastrointestinal posttransplant lymphoproliferative disorders in liver transplant recipients. *Transplantation*. 2012;94:417–423.
26. Cao S, Cox K, Esquivel CO, et al. Posttransplant lymphoproliferative disorders and gastrointestinal manifestations of Epstein-Barr virus infection in children following liver transplantation. *Transplantation*. 1998;66:851–856.
27. Kimura H, Morishima T, Kanegane H, et al. Prognostic factors for chronic active Epstein-Barr virus infection. *J Infect Dis*. 2003;187:527–533.