Title

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Immune checkpoint inhibitor-induced gastrointestinal injury: prevalence of cytomegalovirus, adenovirus and Epstein-Barr virus

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ABSTRACT

Aims Widespread use of immune checkpoint inhibitors (ICIs) for treatment of advanced malignancies led to an increase in number of immune-related adverse events such as ICI gastrointestinal (GI) injury (ICIGI). The resulting immune dysregulation of the GI mucosa is believed to predispose patients to viral infections. We characterised the histopathological features of ICIGI and the frequency of viral infections such as cytomegalovirus (CMV), adenovirus, and Epstein-Barr virus (EBV).

Methods Single-centre retrospective study (2011–2020).

Results 81 GI biopsies from 31 patients with ICIGI (65% male (20/31), 35% female (11/31)) with advanced malignancies were reviewed. Most patients received ipilimumab and nivolumab (14/31, 45%), followed by pembrolizumab (9/31, 29%), ipilimumab (4/31, 13%), nivolumab (2/31, 6%) and combination of all three medications (2/31, 6%). Average regimen prior to incidence of diarrhea was three cycles. Evidence of colitis or erythema by endoscopy was present in 77% of cases, while 23% showed normal endoscopy. Histologically, the predominant ICIGI findings were active inflammation (84%), including cryptitis (77%), crypt abscesses (65%), lymphocytic colitis-like (LCL) pattern (61%), increase in epithelial apoptosis (74%) and/or surface injury (81%). Only one case showed diffuse CMV positivity (3%) with characteristic CMV viral cytopathic effects present on H&E stain and four cases were positive for rare EBV (13%). Adenovirus infection was not identified.

Conclusion While our cohort is small, ICIGI generally demonstrates active inflammation including cryptitis and crypt abscesses in the colon, LCL pattern, and an increase in epithelial apoptosis. Upfront immunohistochemistry for viral infection without high-degree of clinical and histologic suspicion is not recommended.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Immune checkpoint inhibitors (ICIs) are associated with immune-related adverse effects, that include gastrointestinal (GI) injury. The prevalence and impact of viral infections such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV) in the context of ICI-induced GI injury (ICIGI) is uncertain, necessitating further investigation.

WHAT THIS STUDY ADDS

⇒ This study demonstrates that: (1) ICIGI predominantly presents with active inflammation and cryptitis and/or a lymphocytic colitis-like pattern; (2) viral infections (CMV and EBV) are rare in ICIGI.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The study presents common histological findings seen in ICIGI and emphasises the need to correlate histology with clinical history. Routine viral testing can be avoided in most cases, but can be considered in therapy refractory cases or those with high clinical suspicion of viral disease. Thus, reducing unnecessary diagnostic studies.

INTRODUCTION

Immune checkpoint inhibitors (ICIs) are effective therapies for advanced malignancies.[1] They work by blocking immune checkpoints, such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed death 1 (PD-1) and PD-1 ligand (PD-L1) receptors.[1] CTLA-4 is a homologue of CD28 regulating T-cells activation through competitive binding.[1] Similarly, PD-1 is an inhibitory receptor suppressing T-cell activation by binding to its ligands, PD-L1 and PD-L2.[2] By blocking suppression of the antitumour response, ICIs allow the immune system to recognise and destroy tumour cells.[3] Commonly used ICIs approved for advanced malignancies include nivolumab (anti-PD-1), pembrolizumab (anti-PD-1), atezolizumab (anti-PD-L1), avelumab (anti-PD-1) and ipilimumab (CTLA-4 inhibitor). These medications are associated with immune-related adverse effects (irAEs) involving the skin, endocrine system, lungs, liver and gastrointestinal (GI) tract.[3–5] One of the most common irAE is ICI-gastrointestinal-injury (ICIGI), involving the colon.[3] ICIGI often presents with diarrhoea and a median onset of 6 weeks after starting therapy.[6]

The exact pathogenesis of ICIGI is unknown but is suspected to occur due to diffuse inflammation secondary to the loss of regulation of GI mucosal immunity.[5 7] Endoscopic features of ICIGI often include erythema, erosion, ulceration and granulation resembling ulcerative colitis (UC).[5 6] Histological evaluation of the inflammation pattern may provide clues to the medication type being used. Previous studies demonstrate that anti-PD-1 and anti-CTLA-4-associated-colitis can present with increased epithelial cell apoptosis and active colitis

(cryptitis and crypt abscesses).[8] A lymphocytic colitis-like (LCL)-pattern of injury has also been observed, along with surface epithelial injury (goblet cell loss). As such histologically ICIGI can mimic inflammatory bowel disease (IBD).[9]

Corticosteroids are the first-line therapy for ICIGI to prevent progression to fatal complications such as perforation.[8] Refractory cases can be treated with infliximab, a tumour necrosis factor-α antagonist, or vedolizumab, an antibody against α4β7 integrin.[8 9] However, immunosuppressive therapy increases risk for adenovirus, Epstein-Barr virus (EBV) and cytomegalovirus (CMV) infections.[8–11] The rates of viral infections in IBD cases are well established by PCR, with reported rates as high as 81% for CMV and 76% EBV in UC compared with 66% for CMV and 55% for EBV in Crohn's disease (CD).[12]

The infection/reactivation rates of CMV and EBV in patients on ICIs are less documented. Interestingly, CMV has been reported to occur disproportionately more among patients with severe ICI-associated pneumonitis and colitis refractory to standard immunosuppressive treatments.[10 13] Franklin *et al* found that 5 of 41 patients with ipilimumab ICI colitis were therapy refractory and CMV positive.[10] CMV induced hepatitis in a patient receiving ipilimumab has also been reported.[14] EBV positive lymphoproliferations have been described by Pugh *et al* in 4 of 13 patients on anti-CTLA-4 therapy (alone or in combination with anti-PD-1). These four patients developed EBV positive lymphoproliferations manifesting as florid ulcers.[15] Although data on CMV and EBV infections in ICI colitis exist, many aspects remain unclear. Our study characterises the endoscopic and histological features of ICIGI caused by ipilimumab, pembrolizumab, nivolumab and their combinations. We also evaluate the prevalence of viral infections (adenovirus, CMV and EBV) across all biopsies.

METHODS

Study design and patient characteristics

Reviewed cases include 81 GI biopsies (31 patients) with advanced malignancies receiving ICI that developed ICIGI between 2011 and 2021. Patients with a history of IBD or any prior colitis were excluded. Demographics, medical history, ICI regimen(s) (ipilimumab, nivolumab, pembrolizumab or combination), endoscopy reports and histology slides were reviewed.

Endoscopic and histological features

The endoscopic reports were reviewed for any abnormalities such as ulceration, erosion, erythema, edema, friability and/or granularity. Two pathologists reviewed all biopsies. A scoring system (0=absence; 1=presence) was developed for each feature. 10 features were evaluated: (1) active inflammation or activity defined as presence of neutrophils; (2) intraepithelial neutrophils (specifically denoting neutrophils infiltrating squamous mucosa or GI crypt/pits; (3) crypt abscesses (clusters of inflammatory cells in a crypt); (4) LCL pattern of injury (presence of more than 20 lymphocytes per 100 enterocytes); (5) lamina propria expansion (LPE) (either lymphoplasmacytic or lymphohistiocytic); (6) architectural distortion (irregular mucosal surface, crypt atrophy, loss of crypt parallelism, crypt irregularity, tortuosity, dilatation, variation in shape/size and crypt branching); (7) increased epithelial cell apoptosis (presence of more than two apoptotic bodies); (8) surface epithelial damage (erosion/loss of goblet cells); (9) basal plasmacytosis; (10) granulomas.

Two main categories of injury patterns were generated: active colitis or LCL pattern of injury. The H&E slides were also reviewed for evidence of viral cytopathic effects (CMV and adenovirus).

Immunohistochemistry and EBV-encoded small RNA1 in situ hybridisation

Immunohistochemistry (IHC) was performed on all biopsies with available tissue as previously described.[16] Three biopsies for one patient were insufficient for evaluation. Antibodies included, adenovirus (ready-to-use, cat#212M-16-ASR, clone 20/11&2/6, Cell Marque) and CMV (1:200, Dako, cat#M085401-1, clone CCH2+DDG9). IHC was performed on FFPE tissue using automated Leica Bond-Max (Leica-Microsystem IHC platform). EBV-encoded small RNA in situ hybridisation (ISH) was performed as previously described an automated instrument (BenchMark XT; Ventana-Medical-Systems, Tucson, AZ). In this system, the ISH iVIEW Blue Plus Detection Kit consisted of a primary rabbit anti-DNP antibody and biotin-streptavidin system for detecting EBV probes in either epithelial cells or lymphocytes (Ventana catalogue no. 760-097760-1209).[11]

Statistics

GraphPad Prism V.9.2.0 was used. Due to the small number of cases the findings were reported by: (1) descriptively comparing the most common/predominant findings of each ICIGI pattern (majority defined as ≥50% of the cases); (2) comparing categorical ICIGI features via Fisher's exact test (online supplemental table 3).

RESULTS

Patient clinical characteristics

A total of 31 patients with 81 GI biopsies fit the study criteria and 20 (65%) were males and 11 (35%) were females (table 1). Age ranged from 28 to 78 years (median age of 64). Malignancies includes: metastatic melanoma (17/31, 55%), lung carcinoma (4/31, 13%), ovarian carcinoma (3/31, 10%), renal cell carcinoma (1/31, 3%), urothelial carcinoma (1/31, 3%), cholangiocarcinoma (1/31, 3%), oligodendroglioma (1/31, 3%), Hodgkin's lymphoma (1/31, 3%), B cell lymphoma (1/31, 3%) and metastatic squamous cell carcinoma (1/31, 3%). The treatment regimens for individual patients: nivolumab (2/31, 6%), ipilimumab (4/31, 13%), pembrolizumab (9/31, 29%), ipilimumab and nivolumab combination (14/31, 45%), ipilimumab, nivolumab and pembrolizumab combination (2/31, 6%).

Endoscopy

Endoscopic findings correlated with the predominant histology injury pattern (table 1). Most common endoscopic findings includes inflammation, colitis, or erythema in 77% of cases (24/31). Normal endoscopy was observed in 23% (7/31) with pathology revealing LCL pattern (5/7, 71%), active colitis with LCL pattern (1/7, 14%) and colonic mucosa with focal crypt architectural distortion (1/7, 14%). Cases with predominant histological findings of active colitis/ileitis (23/31, 74%) demonstrated endoscopic findings of pancolitis/gastritis with ulceration (6/31, 19%), pancolitis without ulceration (11/31, 35%), erythema (5/31, 16%), edema (2/31, 6%), erosion (1/31, 3%) or localised inflammation (ie, duodenitis) (1/31, 3%). One case of LCL pattern presented as focal colonic erythema endoscopically.

Table 1 Cohort characteristics, pathological and endoscopic features of immune checkpoint inhibitor-induced gastrointestinal injury

No.	Age*	Sex	ICI	Cy	Malignancy	Predominant histology injury pattern	Predominant endoscopic findings	V
1	65–70	M	I	3	Melanoma	Active colitis	Pancolitis without ulceration	
2	50–55	M	I	2	Melanoma	Active colitis	Pancolitis without ulceration	
3	25–30	F	I	2	Melanoma	Active ileitis Active chronic colitis	Pancolitis with focal ulcerations	
4	50–55	F	I	3	Melanoma	Active chronic colitis with lymphocytic colitis-like	Pancolitis without ulceration	E, C
5	45–50	F	P	9	High-grade papillary serous carcinoma	Lymphocytic colitis-like	Normal	
6	70–75	M	P	1	Lung squamous cell carcinoma	Active chronic duodenitis	Duodenitis	
7	65–70	F	P	4	Lung adenocarcinoma	Colon, focal crypt damage	Normal	E
8	65–70	M	P	1	Urothelial carcinoma	Active chronic colitis	Pancolitis without ulceration	
9	65–70	M	P	1	Lung adenocarcinoma	Active gastritis	Gastritis with ulceration	
10	60–65	F	P	1	High-grade serous carcinoma	Lymphocytic colitis-like	Focal colon erythema	
11	45–50	M	P	1	Oligodendroglioma	Mild active esophagitis with lymphocytic injury and apoptosis, reactive gastropathy, mild acute ileitis, active chronic colitis lymphocytic-like injury	Diffuse stomach and colon erythema without ulceration	E
12	60–65	M	P	1	Melanoma	Lymphocytic colitis-like	Normal	
13	50–55	F	P	1	Serous carcinoma	Lymphocytic colitis-like	Normal	
14	30–35	F	N	4	Hodgkin's lymphoma	Lymphocytic colitis-like	Normal	
15	70–75	M	IN	2	Melanoma	Active chronic colitis	Pancolitis without ulceration	
16	45–50	M	INP	1	Melanoma	Active chronic colitis	Pancolitis, discontinuous, with ulceration	
17	55–60	F	INP	1	Melanoma	Active chronic colitis	Pancolitis without ulceration	
18	45–50	F	IN	2	Melanoma	Active chronic colitis with granulomas	Pancolitis with ulceration	E
19	25–30	F	IN	1	Melanoma	Active chronic gastritis, active duodenitis	Diffuse erythematous mucosa without ulceration	
20	65–70	M	IN	1	Melanoma	Active chronic colitis	Pancolitis without ulceration	
21	65–70	M	IN	3	Melanoma	Active chronic colitis	Mild erythematous mucosa, focal ulceration, edema	
22	60–65	M	IN	1	Metastatic SCC	Active chronic colitis	Pancolitis without ulceration	
23	75–80	M	IN	9	Renal cell carcinoma	Active chronic colitis	Mild edema and erythema	
24	65–70	M	IN	1	Melanoma	Active chronic colitis	Pancolitis with erosion	
25	65–70	M	IN	1	Lung adenocarcinoma	Active chronic colitis	Diffuse moderate erythema from anus to cecum	
26	65–70	M	IN	3	Melanoma	Active chronic colitis	Pancolitis without ulceration	
27	60–65	M	IN	3	Melanoma	Active colitis	Pancolitis with ulceration	
28	60–65	M	IN	1	Melanoma	Active colitis with lymphocytic colitis-like	Normal	
29	60–65	M	IN	1	Melanoma	Active colitis	Mild diffuse colon erythema	
30	65–70	F	IN	4	Cholangiocarcinoma	Active colitis	Left colitis without ulceration	
31	75–80	M	N	1	B-cell lymphoma	Lymphocytic colitis-like	Normal	

*As age is considered a direct patient identifier, exact age was replaced by an age range.
C, CMV; Cy, cycles; E, EBV; I, ipilimumab; ICI, immune checkpoint inhibitor; N, nivolumab; P, pembrolizumab; SCC, small cell carcinoma; V, xvirus.

Characterising histology patterns of ICIGI

Predominant histological features of ICIGI were active inflammation/activity (particularly cryptitis), increase in epithelial apoptosis and surface injury

From most to least common ICIGI features seen on biopsies: active inflammation (60/81, 74%), cryptitis (54/81, 67%), surface injury (particularly goblet cell loss) (50/81, 62%) and increase in epithelial apoptosis (45/81, 56%) (figures 1 and 2, online supplemental table 1). Less frequent features included LCL pattern (36/81, 44%), crypt abscesses (34/81, 42%), LPE by inflammatory cells (14/14/81, 17%) and architectural distortion (14/81, 17%). Granulomas (2/81, 3%) and basal lymphoplasmacytosis (3/81, 4%) were the least common.

Majority of ICIGI histological events were in the colon

The majority of the ICIGI histological findings occurred in the colon vs other regions of the GI tract (6/10 features; activity, cryptitis, crypt abscesses, LCL pattern, increase in epithelial apoptosis and surface injury) (figure 1, online supplemental table 1). All esophageal biopsies contained mild increase in epithelial apoptosis (2/2, 100%). Only two granulomas were found; one in the ileum (1/11, 9%) and one in the colon (1/57, 2%). Crypt abscesses, and architectural distortion, were only present on colonic biopsies. Stomach was infrequently affected, but in 33% (2/6) of the cases contained active inflammation, LPE, epithelial apoptosis and surface injury. In the small intestine inflammation (10/16, ~60% of cases) and intraepithelial neutrophils (7/16, ~40% of cases) was predominant.

Histological differences between pembrolizumab, Ipilimumab and nivolumab associated colitis

Active inflammation

Active inflammation (intraepithelial neutrophils and crypt abscesses) was more prevalent in ipilimumab colitis. Active inflammation in the colon occurred in all ipilimumab associated colitis cases (5/5), versus 36% (8/22) of pembrolizumab and 33% (1/3) of nivolumab cases (figure 3, online supplemental table 2). Colonic active inflammation was significantly increased with

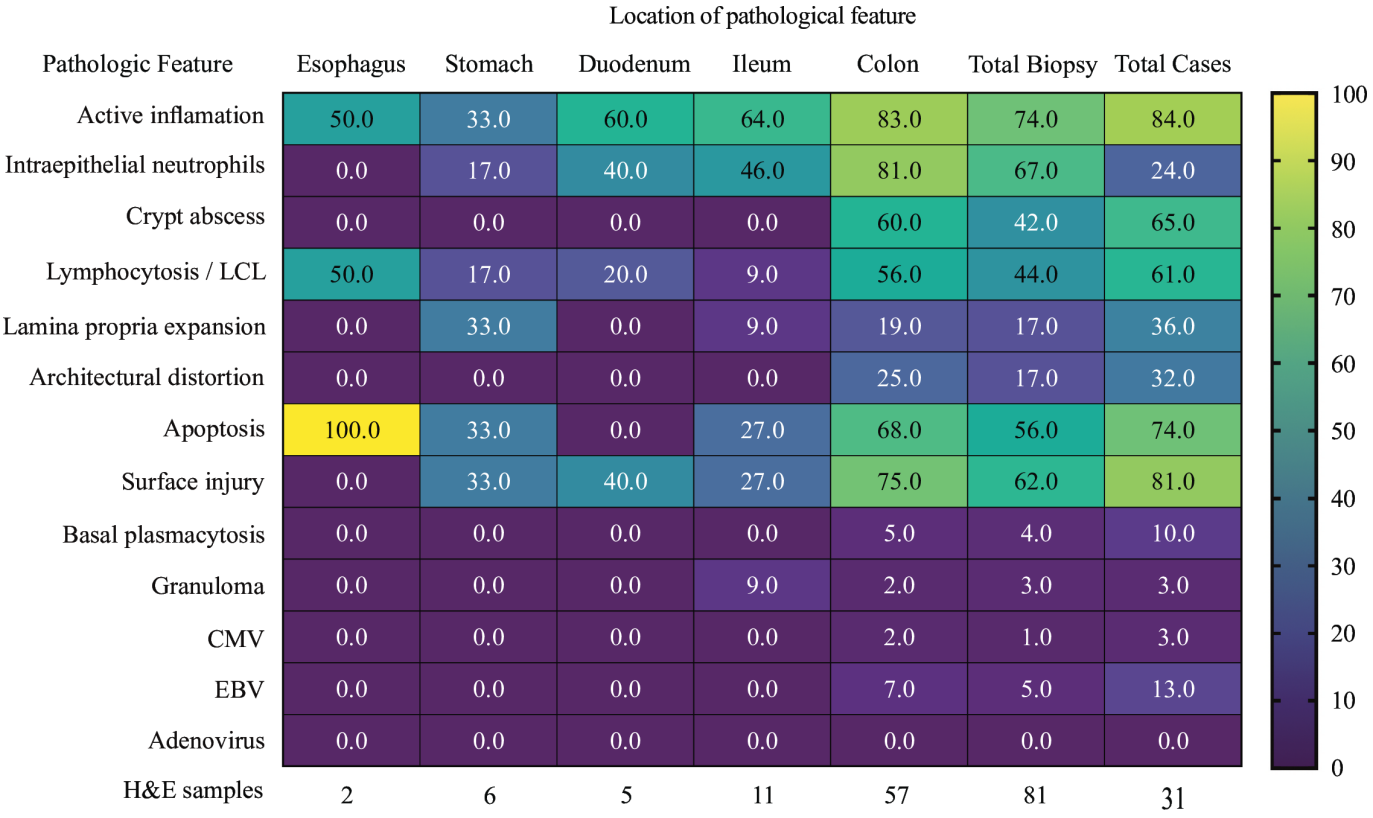

Figure 1 Combined histological findings seen in immune checkpoint inhibitor gastrointestinal injury. A heat-map demonstrating the predominant histological findings of immune checkpoint inhibitor gastrointestinal injury when all inhibitors are combined and viewed together as a group. CMV, cytomegalovirus; EBV, Epstein-Barr virus; LCL, lymphocytic colitis-like pattern.

ipilimumab than pembrolizumab (100% (4/4) vs 8/22 (36%); p<0.05) (online supplemental table 3).

Lymphocytic colitis-like pattern

The predominant injury pattern in nivolumab and pembrolizumab associated colitis (100% (2/2) and 78% (7/9) of cases, respectively) was LCL (figure 3, online supplemental table 2). In contrast, LCL pattern was present in only 25% (1/4) of ipilimumab colitis cases. More cases in the colon showed LCL pattern with pembrolizumab versus ipilimumab (91%, 10/11, vs 60%, 3/5; p<0.05) (online supplemental tables 1 and 2).

Increased epithelial apoptosis

Apoptotic epithelial injury was a characteristic feature of pembrolizumab colitis (78%, 7/9 cases). In contrast, increased epithelial apoptosis was seen in 50% (1/2) of ipilimumab and 50% (2/4) nivolumab colitis cases (figure 3, online supplemental table 2). The proportion of colonic biopsies with increased crypt epithelial apoptosis was significantly higher with pembrolizumab than ipilimumab (91%, 10/11 vs 20%, 2/5; p<0.05) (online supplemental tables 2 and 3).

Combination ICI

Patients on combination therapy had an overlap between active inflammation and LCL pattern in the colon. All patients on ipilimumab and nivolumab showed active inflammation (14/14), with 57% (8/14) having LCL pattern (figure 3, online supplemental table 2). An additional finding included increase in crypt epithelial apoptosis (11/14, 79%).

Adenovirus, CMV IHC and EBV-ISH

Adenovirus IHC (78 biopsies, 30 patients) was negative. However, three biopsies in two different patients contained diffuse non-specific staining and both patients were treated with combination of ipilimumab and nivolumab (not shown) (online supplemental table 1). Of the 78 biopsies in 31 patients stained for EBV, 4 biopsies (4/48, 5%) in 4 different patients (4/30, 13%) contained mild positivity (figure 4A,B, online supplemental table 1). Of the four positive EBV cases, one patient was treated with ipilimumab, two were treated with pembrolizumab and one was treated with ipilimumab and nivolumab. Of the 81 biopsies from 31 patients stained for CMV, one case (1/81, 1%) was positive (figure 4C,D, online supplemental table 1), which also tested positive for focal EBV. This patient was treated with ipilimumab. The four EBV positive cases had activity, cryptitis, crypt abscesses and crypt architectural distortion in 75% (3/4) of cases. While LCL pattern, increase in crypt epithelial apoptosis, surface injury and basal plasmacytosis was present in 50% (2/4) of cases.

DISCUSSION

This is a detailed study of the endoscopic and histopathological features of ICIGI secondary to the common ICIs: nivolumab, ipilimumab, pembrolizumab and their combinations. Our study uniquely characterises overall and independent injury patterns of ICI and assesses the prevalence of viral infections. The endoscopic findings generally correlated with the expected histopathological pattern of injury. Normal endoscopy findings were observed in most cases of LCL pattern. Endoscopic findings of pancolitis or pangastritis usually demonstrated active

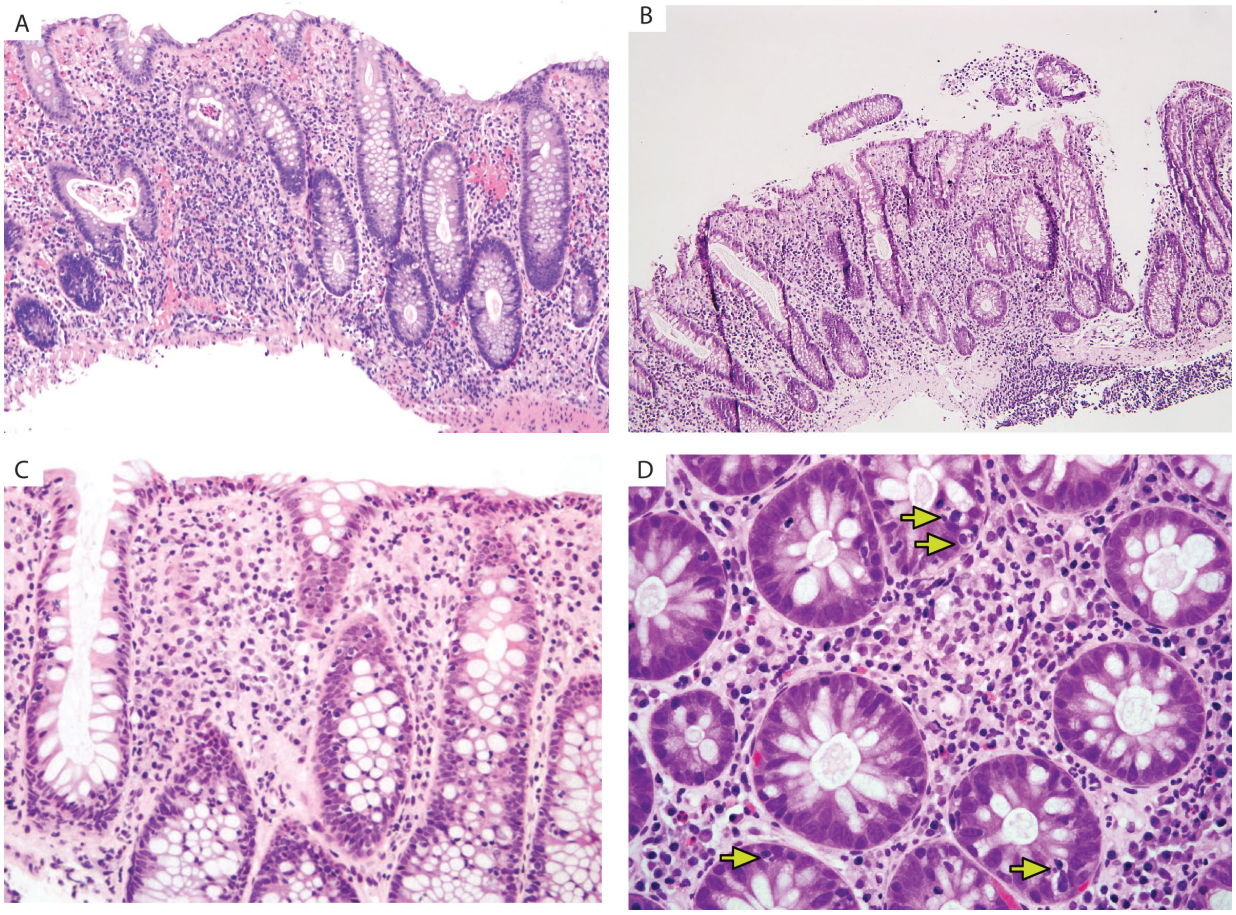

Figure 2 Predominant histological findings found in immune checkpoint inhibitor gastrointestinal injury. (A, B) Active inflammation, cryptitis, crypt-abscesses; (C) lymphocytic-like pattern of injury; (D) representative demonstration of apoptosis (arrows).

inflammation by histology. Our endoscopic and histological findings are similar to prior studies.[9 17 18]

Four general colonic injury patterns secondary to ICI have been described: (1) acute self-limiting colitis, (2) lymphocytic colitis, (3) collagenous colitis and (4) apoptotic colopathy.[19] The updated list now includes: active colitis, chronic active colitis, microscopic-like colitis and graft-vs-host-disease-like, among others.[20] In our cohort across all ICIs, major findings on colonic biopsies were active colitis (mainly cryptitis and crypt abscesses), LCL pattern and apoptotic injury. Similar findings were described with individual ICI.[3 9 17 18] In our cohort, no single feature was specific for an individual ICI. However, LCL pattern, increase in crypt epithelial apoptosis, and surface injury was the predominant ICIGI pattern secondary to PD-1 inhibitors (pembrolizumab and nivolumab). The presence of active colitis, was a minor finding in some cases. Our findings are in line with prior published work.[9] A study by Nielsen *et al*, demonstrated that incidence of mild and severe colitis was low with PD-1 and PD-L1 inhibitors (1.2%–0.2%, respectively).[21] This is likely because PD-1 and PD-L1 inhibition targets a downstream signalling pathway compared with CTLA-4, making it more specific and less prone to irARs.[20] Supporting studies indeed show that active colitis pattern with increased crypt epithelial apoptosis is an infrequently encountered pattern in PD-1 ICIGI.[3 9 17 18]

Prior studies evaluated the correlation between ICI type and specific histological changes related to ICI colitis. Two studies found no correlation.[22 23] Others demonstrated that anti-CTLA-4 therapy was more likely to cause active and less likely to cause chronic and microscopic colitis.[24] All biopsies in our cohort of ipilimumab patients contained active inflammation (cryptitis and/or crypt abscesses). This is in line with the majority of literature.[3 9 17 18 21] However, adverse events secondary to ipilimumab may sometimes include increased crypt epithelial apoptosis, intraepithelial lymphocytes or crypt elongation.[3]

Most patients taking pembrolizumab or nivolumab demonstrated LCL pattern and an increase in crypt epithelial apoptosis. This finding is in line with prior reports.[3 5 19] Relative to ipilimumab associated colitis, patients with ICIGI due pembrolizumab contained more LCL pattern and an increase in crypt epithelial apoptosis and less active inflammation.[10 13]

In our cohort, the prevalence of viral infections (CMV, adenovirus and EBV) was low. Only four patients (13%, 4/31 patients; 5%, 4/81 biopsies) contained mild EBV positivity, with one having a concurrent CMV infection (3%, 1/31 patients; 1%, 1/81 biopsies), and CMV viral cytopathic effects on H&E. This patient's CMV PCR was positive. Our findings suggest that upfront routine testing for EBV and CMV in ICIGI is not necessary when there is low suspicion based on histological and clinical context.

The reported incidence of CMV infection/reactivation in ICI-treated patients is low (0.3%–7.7%).[25 26] Due to these low rates, routine IHC testing is not recommended without specific clinical or histological indication, especially in non-refractory cases. However, CMV IHC may be indicated if ICI colitis is refractory to standard treatment. Tay *et al* found that 3.8% of patients with therapy-refractory ICI colitis had CMV colitis.[25] Franklin

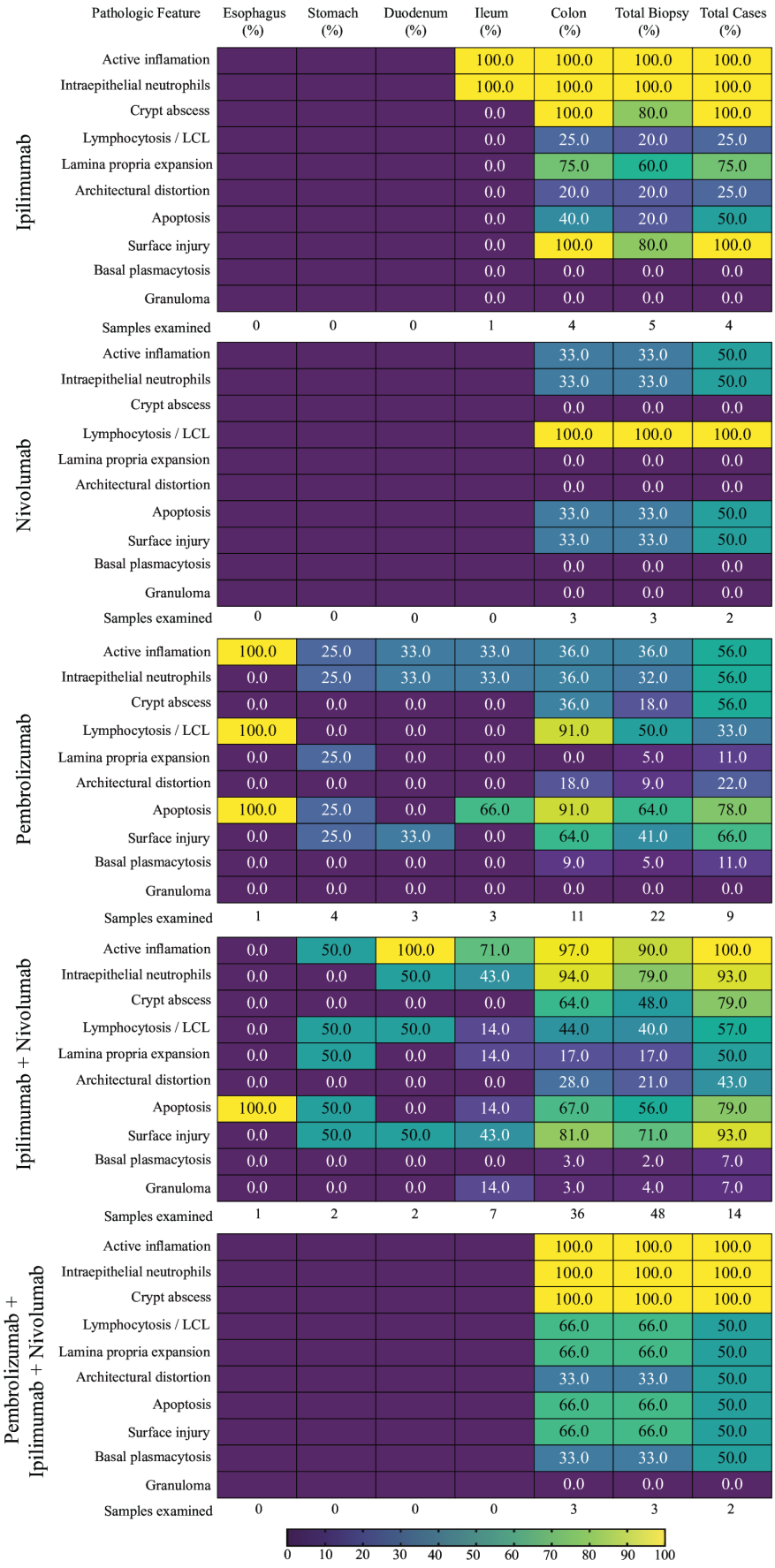

Figure 3 Histological findings observed with each immune checkpoint inhibitor regimen. A heat-map representation comparing the histological findings of immune checkpoint inhibitor gastrointestinal injury features secondary to nivolumab, ipilimumab, pembrolizumab, ipilimumab+nivolumab and pembrolizumab+ipilimumab+nivolumab regimens. LCL, lymphocytic colitis-like pattern; LP, lamina propria.

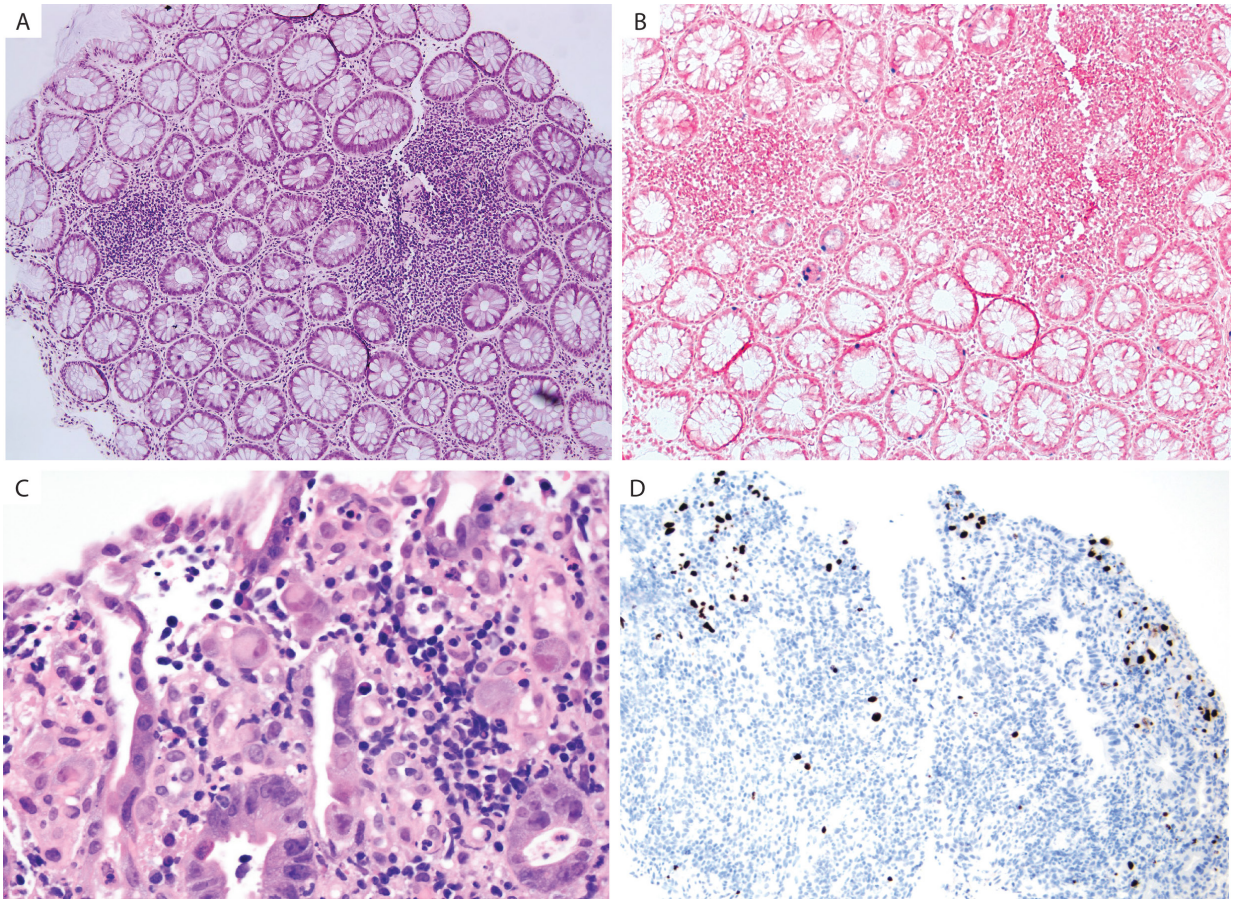

Figure 4 Viropathic findings detected in immune checkpoint inhibitor gastrointestinal injury. (A) H&E of relatively unremarkable colon mucosa; (B) colon mucosa with EBV virus particles by EBER ISH highlighting positive epithelial cells and intraepithelial lymphocytes; (C) H&E of viropathic cellular findings in colon mucosa confirmed; (D) confirmation of viropathic findings by CMV immunohistochemistry. CMV, cytomegalovirus; EBER, Epstein-Barr virus-encoded small RNAs; EBV, Epstein-Barr virus; ISH, in-situ hybridisation.

et al reported a 12.2% CMV positivity rate among patients with therapy-refractory ICI colitis related to ipilimumab.[10] Additionally, Lankes *et al* described a case where CMV reactivation was detected in a patient with anti-TNF-refractory colitis after ICI therapy, and antiviral treatment resulted in clinical improvement.[27] As such, CMV IHC can be indicated in refractory ICI colitis cases and is clinically actionable even when the staining is focal. Additionally, the histopathological presentation of CMV infection in the GI tract varies widely. From a severe inflammatory response with frank ulceration to scattered crypt apoptotic bodies associated with viral inclusions and a minimal inflammatory response characterised by mild acute neutrophilic inflammation.[28] As such, viral infection can show overlapping histological features with ICIGI, such as apoptosis. Therefore, it may be warranted to include a note in the pathology report indicating that infection should be clinically excluded.

The clinical significance of CMV is underscored by the observation that CMV reactivation is linked to more severe disease and poorer outcomes in UC patients undergoing immunosuppressive therapy. Kuwabara *et al* found that dense CMV infection correlated with severe UC in 22% of patients and was associated with steroid resistance.[29] Kuwabara *et al* also noted that scattered CMV infection was found in 75% of patients with moderate UC. Domènech *et al*, also confirm that CMV infection is associated with therapy refractory UC.[30] Of note, in their cohort two patients with active UC were considered to have false-positive CMV IHC because of a single positive cell. One patient had positive CMV IgG serology, and no CMV disease present on the surgical specimen. The other patient had negative CMV serology. Neither patient received ganciclovir.

The exact incidence of EBV infection and reactivation in patients treated with ICI is unknown and this is an active area of investigation. In our study, EBV positivity was mild and focal. The clinical significance of focal EBV labelling is not clear. EBV is commonly found in inflamed GI mucosa and is not always pathogenic. Ryan *et al* found that EBV DNA is detected in inflamed GI tissues including those affected by conditions like gastritis, CD and UC.[31] EBV therefore may be localising preferentially to inflamed lesions, and acting like an innocent bystander rather than being a primary pathological driver. In the context of ICI therapy, this changes. Pugh *et al* reported the presence of EBV positive lymphoproliferations in 4 of 13 (30%) patients receiving anti-CTLA-4 (alone or in combination with anti-PD-1).[15] These four patients developed florid EBV positive ulcers and had worse outcomes (steroid refractory colitis and colon perforation). Additionally, Pugh *et al* also reported one case of an EBV ulcer that was coinfected with CMV.[15] As such, focal EBV labelling, without lymphoproliferative lesions or ulceration can be a possible innocent bystander effect. However, in certain contexts particularly with severe immune dysregulation, EBV may contribute to the pathology and is clinically actionable. This is supported by the fact that both focal and diffuse EBV were detected with refractory IBD.[11] Furthermore, EBV colitis does not contain specific histological features, but the presence

of increased infiltrating B-cell lymphocytes in inflamed gastric and colonic mucosa can raise the suspicion for EBV in the appropriate context.[32]

Our small cohort is the major limitation of our study. Larger cohorts are needed to better dissect the finer details. Despite this, our findings support and contribute to prior work on this topic. In our cohort, anti-PD-1 associated colitis most often presented with an LCL pattern with a background of active inflammation with increased crypt epithelial apoptosis. While, anti-CTLA-4 associated colitis often presented with an active colitis pattern of injury, however these features can overlap. We also demonstrated that while EBV and CMV can be a contributing factor to the pathology, the prevalence of viral infections in our cohort was low. Carefully evaluating the H&E-stained slides for viral cytopathic effect appears sufficient to rule out viral infections. Thus, routine viral IHC is not recommended in ICIGI, unless the patient is refractory to therapy.

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REFERENCES

1 Johnson DB, Nebhan CA, Moslehi JJ, *et al*. Immune-checkpoint inhibitors: long-term implications of toxicity. *Nat Rev Clin Oncol* 2022;19:254–67.
2 Dong Y, Sun Q, Zhang X. PD-1 and its ligands are important immune checkpoints in cancer. *Oncotarget* 2017;8:2171–86.
3 Assarzadegan N, Montgomery E, Anders RA. Immune checkpoint inhibitor colitis: the flip side of the wonder drugs. *Virchows Arch* 2018;472:125–33.
4 Khoja L, Day D, Wei-Wu Chen T, *et al*. Tumour- and class-specific patterns of immune-related adverse events of immune checkpoint inhibitors: a systematic review. *Ann Oncol* 2017;28:2377–85.
5 Zhang ML, Deshpande V. Histopathology of Gastrointestinal Immune-related Adverse Events: A Practical Review for the Practicing Pathologist. *Am J Surg Pathol* 2022;46:e15–26.
6 Prieux-Klotz C, Dior M, Damotte D, *et al*. Immune Checkpoint Inhibitor-Induced Colitis: Diagnosis and Management. *Target Oncol* 2017;12:301–8.
7 Pauken KE, Dougan M, Rose NR, *et al*. Adverse Events Following Cancer Immunotherapy: Obstacles and Opportunities. *Trends Immunol* 2019;40:511–23.
8 Nishida T, Iijima H, Adachi S. Immune checkpoint inhibitor-induced diarrhea/colitis: Endoscopic and pathologic findings. *World J Gastrointest Pathophysiol* 2019;10:17–28.
9 Chen JH, Pezhouh MK, Lauwers GY, *et al*. Histopathologic Features of Colitis Due to Immunotherapy With Anti-PD-1 Antibodies. *Am J Surg Pathol* 2017;41:643–54.
10 Franklin C, Rooms I, Fiedler M, *et al*. Cytomegalovirus reactivation in patients with refractory checkpoint inhibitor-induced colitis. *Eur J Cancer* 2017;86:248–56.
11 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;82:187–92.
12 Wakefield AJ, Fox JD, Sawyerr AM, *et al*. Detection of herpesvirus DNA in the large intestine of patients with ulcerative colitis and Crohn's disease using the nested polymerase chain reaction. *J Med Virol* 1992;38:183–90.
13 Morelli T, Fujita K, Redelman-Sidi G, *et al*. Infections due to dysregulated immunity: an emerging complication of cancer immunotherapy. *Thorax* 2022;77:304–11.
14 Uslu U, Agaimy A, Hundorfean G, *et al*. Autoimmune Colitis and Subsequent CMV-induced Hepatitis After Treatment With Ipilimumab. *J Immunother* 2015;38:212–5.
15 Pugh MR, Leopold GD, Morgan M, *et al*. Epstein-Barr Virus-Positive Mucocutaneous Ulcers Complicate Colitis Caused by Immune Checkpoint Regulator Therapy and Associate With Colon Perforation. *Clin Gastroenterol Hepatol* 2020;18:1785–95.
16 Chornenkyy Y, Mejia-Bautista M, Brucal M, *et al*. Liver Pathology and SARS-CoV-2 Detection in Formalin-Fixed Tissue of Patients With COVID-19. *Am J Clin Pathol* 2021;155:802–14.
17 Verschuren EC, van den Eertwegh AJ, Wonders J, *et al*. Clinical, Endoscopic, and Histologic Characteristics of Ipilimumab-Associated Colitis. *Clin Gastroenterol Hepatol* 2016;14:836–42.
18 Adler BL, Pezhouh MK, Kim A, *et al*. Histopathological and immunophenotypic features of ipilimumab-associated colitis compared to ulcerative colitis. *J Intern Med* 2018;283:568–77.
19 Zhang ML, Neyaz A, Patil D, *et al*. Immune-related adverse events in the gastrointestinal tract: diagnostic utility of upper gastrointestinal biopsies. *Histopathology* 2020;76:233–43.
20 Losurdo G, Angelillo D, Favia N, *et al*. Checkpoint inhibitor-induced colitis: An update. *Biomedicines* 2023;11:1496.
21 Nielsen DL, Juhl CB, Chen IM, *et al*. Immune checkpoint Inhibitor-Induced diarrhea and Colitis: Incidence and Management. A systematic review and Meta-analysis. *Cancer Treat Rev* 2022;109:102440.
22 Pai RK, Pai RK, Brown I, *et al*. The significance of histological activity measurements in immune checkpoint inhibitor colitis. *Aliment Pharmacol Ther* 2021;53:150–9.
23 Geukes Foppen MH, Rozeman EA, van Wilpe S, *et al*. Immune checkpoint inhibition-related colitis: symptoms, endoscopic features, histology and response to management. *ESMO Open* 2018;3:e000278.
24 Isidro RA, Ruan AB, Gannarapu S, *et al*. Medication-specific variations in morphological patterns of injury in immune check-point inhibitor-associated colitis. *Histopathology* 2021;78:532–41.
25 Tay KH, Slavin MA, Thursky KA, *et al*. Cytomegalovirus DNAemia and disease: current-era epidemiology, clinical characteristics and outcomes in cancer patients other than allogeneic haemopoietic transplantation. *Intern Med J* 2022;52:1759–67.
26 Panneerselvam K, Szafron D, Amin RN, *et al*. Cytomegalovirus infection among patients with cancer receiving immune checkpoint inhibitors. *Ann Gastroenterol* 2022;35:522–31.
27 Lankes K, Hundorfean G, Harrer T, *et al*. Anti-TNF-refractory colitis after checkpoint inhibitor therapy: Possible role of CMV-mediated immunopathogenesis. *Oncoimmunology* 2016;5:e1128611.
28 Karamchandani DM, Chetty R. Apoptotic colopathy: a pragmatic approach to diagnosis. *J Clin Pathol* 2018;71:1033–40.
29 Kuwabara A, Okamoto H, Suda T, *et al*. Clinicopathologic characteristics of clinically relevant cytomegalovirus infection in inflammatory bowel disease. *J Gastroenterol* 2007;42:823–9.
30 Domènech E, Vega R, Ojanguren I, *et al*. Cytomegalovirus infection in ulcerative colitis: a prospective, comparative study on prevalence and diagnostic strategy. *Inflamm Bowel Dis* 2008;14:1373–9.
31 Ryan JL, Shen Y-J, Morgan DR, *et al*. Epstein-Barr virus infection is common in inflamed gastrointestinal mucosa. *Dig Dis Sci* 2012;57:1887–98.
32 Choi C, Yu Q, Deb PQ, *et al*. Rare case of EBV-induced colitis in an immunocompetent individual. *BMJ Open Gastroenterol* 2020;7:e000360.