



Review Article

Ulcerative Colitis and Cytomegalovirus Infection: From A to Z

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Abstract

Despite multiple studies, the role of cytomegalovirus [CMV] infection in exacerbating the severity of inflammation in ulcerative colitis [UC], and its response to treatment, remain debatable. Additionally, the optimal diagnostic tests for CMV infection in the setting of UC relapse, and timing of antiviral treatment initiation, remain unclear. The challenge faced by gastroenterologists is to differentiate between an acute UC flare and true CMV colitis. It seems that the presence of CMV colitis, as defined by the presence of intranuclear or intracellular inclusion bodies on haematoxylin and eosin [H&E] staining and/or positive immunohistochemistry [IHC] assay on histology, is associated with more severe colitis. Patients with CMV infection and acute severe colitis are more resistant to treatment with corticosteroids than non-infected patients. This refractoriness to steroids is related to colonic tissue CMV viral load and number of inclusion bodies [high-grade CMV infection] which may have a pronounced effect on clinical outcomes and colectomy rates. Whereas many studies showed no effect for antiviral treatment on colectomy rates in CMV-infected UC patients, there was a significant difference in colectomy rates of patients with high-grade infection who received anti-viral therapy compared with those who did not receive treatment. It was therefore proposed that high-grade CMV disease indicates that the virus is acting as a pathogen, whereas in those with low-grade CMV disease, the severity of IBD itself is more likely to influence outcome. The different algorithms that have been put forward for the management of patients with UC and concomitant CMV infection are discussed.

Key Words: Cytomegalovirus; ulcerative colitis

1. Introduction

Cytomegalovirus [CMV] is a ubiquitous virus belonging to the herpes virus family, with human species being the only natural host.¹ Infection with CMV is very common and leads to a lifelong dormant phase.^{2,3} The virus may be reactivated when the immune system is compromised, leading to CMV disease. The first case report on CMV infection in ulcerative colitis [UC] was described by Powell *et al.* in 1961.⁴ Since then, a multitude of studies on the association between CMV infection and UC have been published. Yet, no causative relationship has been demonstrated between CMV and development

of UC, as the prevalence of latent infection is not different between UC patients and healthy controls.⁵ However, the debate continues about the role of CMV in inducing a flare of UC or worsening its severity. In addition, whether CMV alters the resistance of UC to treatment and in turn affects disease outcomes such as length of hospitalisation, need for colectomy, or even mortality rates, remain unclear. It is debatable whether CMV is responsible for the severity of colitis inflammation or if it is just an innocent bystander or a marker of underlying disease severity.⁶ Many questions are raised by gastroenterologists when faced with a patient with severe UC and

suspected CMV infection. Questions such as the appropriate diagnostic test for CMV, the optimal time to start antiviral therapy, the duration of antiviral therapy, and how to alter immunosuppressant medications, remain unanswered. Our aim is to review the available data on the association between UC and CMV, in order to answer some of these unanswered questions and to propose an approach for the management of concomitant CMV infection in UC patients.

2. Epidemiology of CMV Infection

2.1. Do patients with UC have an increased prevalence of CMV infection?

The epidemiology of CMV infection and colitis depends on the definition used to diagnose the infection, on the severity of colitis, on the population studied including immune status, and on geographical distribution. Unfortunately, there is a lack of a gold standard definition of clinically relevant CMV infection and intestinal disease. In a systematic review of the literature, Romkens *et al.*⁷ identified 21 different definitions for CMV infection, eight definitions for CMV intestinal disease, and three definitions for CMV reactivation. In the published literature, the term CMV infection, CMV intestinal disease, or CMV colitis are used interchangeably. The highest prevalence of CMV disease is found in studies that used positive serum polymerase chain reaction [PCR], followed by studies using antigenaemia, in their case definition for CMV infection [both defined as serum CMV replication]. In case of diagnosing CMV intestinal disease, the highest prevalence of CMV infection was seen in studies that used tissue PCR [>10 copies/mg tissue].

The prevalence of latent CMV infection defined by positive IgG serology ranges from 45% to 100%,^{2,3} and tends to be highest in South America, Africa, and Asia and lowest in Western Europe and the USA.⁸ In a recent meta-analysis, no difference in latent CMV infection was found between patients with inflammatory bowel disease [IBD] and controls (69.6% vs 51.8%, odds ratio [OR] = 1.36, 95% confidence interval [CI] = 0.45–4.14, $p = 0.59$),⁵ suggesting that there is no difference in the exposure to CMV and susceptibility for the latency phase of the virus between these groups. However, there was a significant difference between the two groups in terms of detection of CMV IgM [5.5% vs 0.59%, OR 7.14, 95% CI = 1.58–32.25, $p = 0.01$], with higher rates among IBD patients. Similarly, there was a significant increased risk for serum CMV replication as evidenced by CMV antigenaemia [40.4% vs 6.6%, OR 7.4] and presence of blood CMV DNA [42.5% vs 26.4%, OR 4.99] in patients with UC compared with healthy controls.

Lopes *et al.*⁹ prospectively followed 95 patients with endoscopically active IBD. The frequency of positive tissue CMV DNA [colonic CMV replication] was 12.1% in IBD patients compared with 0% in 50 healthy volunteers [$p = 0.017$]. Only one patient with IBD had positive serum CMV replication. However, in acute severe colitis, the reported presence of colonic CMV replication is much higher at 21–34% of patients.^{2,10,11} Shimada *et al.*¹² examined colonic CMV replication from patients presenting with ulcerations in the colon, and found that the prevalence rate in IBD patients is higher than in patients who had ulcerations from causes other than IBD [24.4% vs 7.7%] although the viral load was very low. A similar prevalence was found in UC patients in the paediatric age group (Box 1).¹³

Factors that have been associated with an increased risk of CMV reactivation in IBD include female sex, pancolitis, advanced age,^{14–16} immunosuppressive therapy [steroids and azathioprine]^{14,15} [OR 6.7], disease duration less than 60 months [OR 7.7], and blood leukocytes count less than 11/nl [OR 4.49].¹⁴ Although chronic infliximab use

Box 1. Evidence for the association of CMV infection with UC exacerbation, severity, and outcome.

Higher rates of CMV IgM, CMV antigenaemia, and presence of blood or colonic CMV DNA in patients with UC compared with healthy controls^{5,9}

Higher prevalence of positive colonic tissue CMV PCR in patients who have ulcerations secondary to IBD compared with patients presenting with ulcerations from causes other than IBD¹²

In acute severe colitis, the reported presence of tissue CMV DNA, histological examination, and IHC are much higher than in patients with endoscopically active UC^{2,10,11,13}

The severity of colitis with punched-out ulcers, irregular ulcers, or cobblestone-like appearance is associated with the presence of intranuclear or intracellular inclusion bodies on H&E and/or positive IHC assay on histology^{24–26}

Patients with acute severe colitis and CMV infection are more resistant to treatment with corticosteroids than non-infected patients^{5,13}

High tissue CMV DNA [more than 250 copies/mg tissue] predict resistance to steroids and immunosuppressive medications^{21,32,36}

Patients with positive tissue CMV PCR or high-grade CMV colitis [defined by the presence of inclusion bodies and/or positive IHC in colonic biopsies] have increased risk of colectomy^{13,22,27,28,37,65}

Patients with high-grade CMV colitis may respond to antiviral treatment and have decreased colectomy rates^{16,21,35,65,67,73}

Patients with high tissue CMV viral load may respond to antiviral treatment without additional UC therapy, whereas patients with low viral load [<5500 copies/ μ g DNA] would benefit from intensifying UC therapy¹⁶

CMV, cytomegalovirus; UC, ulcerative colitis; PCR, polymerase chain reaction; IBD, inflammatory bowel disease; IHC, immunohistochemistry; H&E, haematoxylin and eosin.

was not found to be associated with increased risk of CMV infection in two studies,^{17,18} there was a strong association in another study.¹⁹

3. Association Between Severity of UC Flare and CMV Infection

3.1. Does CMV infection lead to a worse UC flare?

The role that CMV infection plays in exacerbating the severity of UC remains unclear. Whereas some studies showed a higher prevalence of endoscopically severe UC in patients with CMV antigenaemia compared with non-infected patients,²⁰ others did not find any relationship between positive blood CMV DNA or CMV tissue IHC [colonic CMV replication] and Mayo endoscopic score.^{21,22} Histologically, a more severe colitis was reported in patients with CMV infection who had punched-out ulcers and wide mucosal defects on colonoscopy,^{15,16,22,23} especially with positive tissue CMV PCR. It seems that the presence of CMV colitis, as defined by the presence of intranuclear or intracellular inclusion bodies on haematoxylin and

eosin [H&E] staining and/or positive IHC assay on histology, is associated with more severe endoscopic colitis with punched-out ulcers, irregular ulcers, or cobblestone-like appearance.^{24,25} Endoscopic deep ulcerations were found in 97.2% of CMV colitis [diagnosed by H&E and IHC] compared with 57.7% in controls [$p < 0.05$].²⁶ The number of inclusion bodies per high power field was significantly higher in those with punched-out ulcers [25.7% vs 60%, $p < 0.05$].²⁴ In two recent studies that define true CMV colitis as presence of inclusion bodies or positive IHC on colonic biopsies, patients with CMV colitis had higher disease activity on presentation as evidenced by the total Mayo score^{25,26} and Mayo endoscopic subscore²⁶ compared with CMV-negative patients.

4. CMV Infection and Outcome of UC Flare

4.1. Does CMV infection alter the outcome of UC flare?

Whether CMV infection would lead to a relapse in UC or alter its natural history, outcome, and response to therapy, is also controversial. There is now convincing evidence that patients with CMV infection and acute severe colitis are more resistant to treatment with corticosteroids than non-infected patients. In a recent meta-analysis using data from 1306 patients, 52.9% of patients in the CMV-positive group [as defined by detection of CMV by any method] were observed to have steroid resistance, compared with 30.2% of patients in the CMV-negative group. CMV infection might cause a nearly 4-fold risk of steroid resistance in IBD patients [OR 3.63, 95% CI = 1.99–6.62, $p < 0.0001$].⁵ In the stratified analysis by CMV detection method, latent CMV infection was not found to be a risk factor for the occurrence of steroid-resistant IBD.

Similar results were obtained in the paediatric age group. In a recent retrospective case-control study, a total of 56 children with acute UC were included. Of these, 15 patients were diagnosed with CMV based on colonic biopsy histological assessment by H&E and/or by IHC. More CMV-positive patients with acute UC were resistant to intravenous corticosteroids as compared with CMV-negative patients [93% and 56%, respectively, $p = 0.009$].¹³ However, whether this is related to the infection itself or whether CMV infection is a surrogate marker for severity of inflammation is unclear.

A study with a small number of patients with steroid-resistant colitis reported a 50% [three out of six patients] risk of colectomy when CMV was positive [defined by the presence of inclusion bodies and/or positive IHC in colonic biopsies] as compared with 15% [two out of 13 patients] when it was negative.²⁷ The presence of CMV inclusion bodies, indicated by H&E and positive IHC staining on colonic biopsies, was also found to be a poor prognostic factor for future hospitalisations in 257 adult patients who presented with UC flare [38.97% in patients with CMV colitis compared with 16.3% in controls]. However, there was no difference in colectomy rates [5.6% vs 2.3%]. In multivariable analysis, the hazard ratio for poor outcome [hospitalisation or colectomy] was found to be 2.27.²⁶

Similar results were obtained in the study by Cohen *et al.* in paediatric patients.¹³ There was no difference in colectomy rates during hospitalisation between patients with and without colonic CMV replication. However, by 12 months, the difference in the rate of colectomy between the two groups became more obvious [33% vs 13%, $p = 0.049$]; the statistical significance was not retained on multivariate analysis.

Similar findings were reported by Schenk *et al.*,²⁸ where eight out of 24 [33.3%] adult UC patients with positive CMV PCR [qualitative] underwent proctocolectomy, compared with 10 out of

84 [11.9%] with tissue-negative CMV PCR during 52 months of follow-up [$p = 0.026$]. On multivariate analysis, colonic CMV replication was an independent risk factor for colectomy (hazard ratio [HR]) 3.287, = CI 1.293–8.354, $p = 0.012$). The fact that CMV positivity in these studies showed impact only during follow-up favours the possibility that CMV is probably a surrogate marker of severe colitis.¹³

In contrast, in a retrospective case-controlled study involving 26 patients with UC relapse and serum CMV replication diagnosed by positive blood CMV PCR, there were no differences in length of hospital stay or colectomy rates compared with patients with negative CMV infection [15.4% vs 23.1%].²⁹ Similarly, in a prospective study of 69 patients with moderate-to-severe UC, the outcome regarding surgery and relapse was not altered by serum CMV replication [found in 48 patients].³⁰ A recent national survey from the USA found a very low prevalence of CMV infection in UC patients admitted to the hospital and discharged with the diagnosis of CMV infection [0.45%].³¹ Although the tests used to diagnose CMV infection were not mentioned, the survey showed no increased risk of colorectal surgery or mortality associated with CMV infection. Yet, an increased hospital stay and total charges were reported amongst those concomitantly infected with CMV.[£]

4.2. Is CMV viral load important in determining UC flare outcome?

The next question is whether the severity of CMV infection, as demonstrated by the viral load in blood, the level of antigenaemia, the tissue CMV DNA, or the presence of inclusion bodies on histological examination, determines the outcome of a UC flare.

In a prospective observational study on 16 patients with a moderate-to-severe flare of UC and concomitant CMV infection, Roblin *et al.*²¹ found no relationship between colonic tissue viral load and severity of colonic inflammation, but tissue CMV DNA of more than 250 copies/mg tissue predicted resistance to steroids and immunosuppressive medications with a sensitivity of 100%, a specificity of 66.6%, a likelihood ratio of 4.3, and a receiver operating characteristic [ROC] of 0.85.

Studying CMV DNA in colonic tissue of 32 patients with UC, Paul *et al.*³² came to a similar conclusion that a cutoff value of $\geq 2.5 \log_{10}$ copies/mg of tissue could predict steroid refractoriness [$p < 0.001$] with a sensitivity of 79.2% and a specificity of 84.3%.

Although CMV expression in colonic tissue by PCR was not predictive of poor steroid responsiveness in patients with moderate to severe colitis, Jung *et al.*³³ found that the mean colonic viral load in patients with CMV colitis was higher in poor responders [1140 copies/mg tissue] compared with responders [492 copies/mL, $p < 0.017$]. In addition, serum CMV viral load was significantly higher in steroid-refractory UC compared with controls. A study on a prospectively evaluated IBD patient cohort suggested that tissue CMV DNA of $\geq 10^3$ copies/ 10^5 cells by reverse transcription PCR [RT-PCR] can differentiate between superimposed viral colitis and latent infection.³⁴

In addition, Nguyen *et al.*³⁵ presented evidence from 43 IBD patients, showing that the severity of CMV involvement on histological examination correlates well with both clinical outcomes and colectomy rates. Patients stratified with a high-grade colitis [defined by presence of viral inclusion bodies on both routine H&E and special IHC stains] had a higher colectomy rate [57%] than patients with a low-grade colitis [30%] [defined by absence of viral inclusion bodies on routine H&E stains but presence of positive CMV IHC stains against CMV monoclonal antigens]. Similarly, among the

IHC-positive patients with colitis, the presence of >5 IHC-positive cells per 2 mm tissue was significantly higher in the poor steroid responders [50%] than in the responders [6.6%, $p < 0.009$].³³

Furthermore, Kuwahara *et al.*³⁶ demonstrated that patients with high CMV IHC-positive cells (>10 cells per section) require higher doses of steroids and have higher rates of surgery compared with those who have low CMV IHC-positive cells [one to nine cells per section]. One recent study showed that the colectomy risk is higher in UC patients who have more than or equal to five IHC-positive cells per biopsy section as opposed to those who have up to five IHC-positive cells [$p = 0.014$],²² whereas another study found, on multivariate analysis, that colectomy was associated with more than two positive cells per biopsy [$p = 0.048$].³⁷

It seems that in ‘true CMV colitis’ with high tissue viral load and inclusion bodies [i.e. high-grade CMV colitis], the virus may be the culprit for gut inflammation while UC-induced inflammation plays a minor role (Box 2). In these patients, antiviral therapy may

be effective in treating the inflammatory process and may alter the clinical course, including the need for colectomy. On the other hand, in patients with low-grade CMV colitis, the virus is most likely an innocent bystander, and the underlying IBD colitis is the main driver for gut inflammation. In these cases, aggressive optimisation of treatment with immunosuppression rather than antiviral therapy may control the inflammation and improve clinical outcome.^{38,39}

5. Pathophysiology of CMV Infection and Steroid Resistance in UC

5.1. How to explain the increased risk of CMV infection in patients with UC and steroid resistance?

After a primary, mostly subclinical infection, CMV enters a latency phase in fibroblasts, endothelial cells, monocytes, or granulocyte stem cells.¹ The virus may lie latent for life in colonic mucosa under continuous surveillance of the immune system, particularly by natural killer [NK] cells. Patients with UC are more susceptible to CMV reactivation for many reasons. First, they have inherent impaired NK activity and mucosal immunity.^{40,41} Second, immunosuppression impairs T lymphocytes, resulting in reactivation of the virus. Third, corticosteroids that are frequently used in UC were found to induce viral replication in vitro.⁴² Finally, the increased production of cytokines, especially TNF- α , was shown to promote CMV reactivation, as infected monocytes differentiate into macrophages following stimulation by inflammatory cytokines. During the differentiation and stimulation process, CMV can be reactivated and can infect epithelial, vascular endothelial, and interstitial cells,⁴³ resulting in an increase in inflammatory mediators such as IL-6⁴⁴ which may further exacerbate the severity of colitis. Therefore, the use of corticosteroids in the setting of inflammation from UC and immunosuppressive therapy may reactivate the virus, causing more inflammation with increased migration of CMV-infected monocytes and macrophages into the inflamed tissue, propagating more virus replication in the presence of TNF- α and thus creating a vicious cycle with worsening of intestinal inflammation^{1,6,43} [Figure 1]. Conversely, in Crohn’s disease [CD], CD4+ T cells produce interferon alpha that inhibits CMV reactivation, which may explain the difference in the rates of CMV infection between UC and CD.^{45,46}

Box 2. Different definitions of high-grade CMV infection or ‘true CMV colitis’ which may affect severity of colitis, resistance to steroids treatment, response to antivirals, or colectomy rate.

- Intracellular or intranuclear inclusion bodies on H&E and/or positive IHC on histology^{24,35,67,73}
- Tissue CMV DNA more than 250 copies/mg tissue²¹
- Tissue CMV DNA more than 2.5 log₁₀ copies/mg of tissue³²
- Tissue CMV DNA more than or equal 10³ copies/10⁵ cells by reverse transcription PCR [RT-PCR]³⁴
- Tissue CMV viral load more than 5500 copies/ μ g DNA¹⁶
- Tissue CMV IHC-positive cells more than 10 cells per section³⁶
- Tissue CMV IHC-positive cells more than or equal to five cells per biopsy section²²
- Tissue CMV IHC-positive cells more than two cells per biopsy section³⁷

CMV, cytomegalovirus; PCR, polymerase chain reaction; IHC, immunohistochemistry; H&E, haematoxylin and eosin.

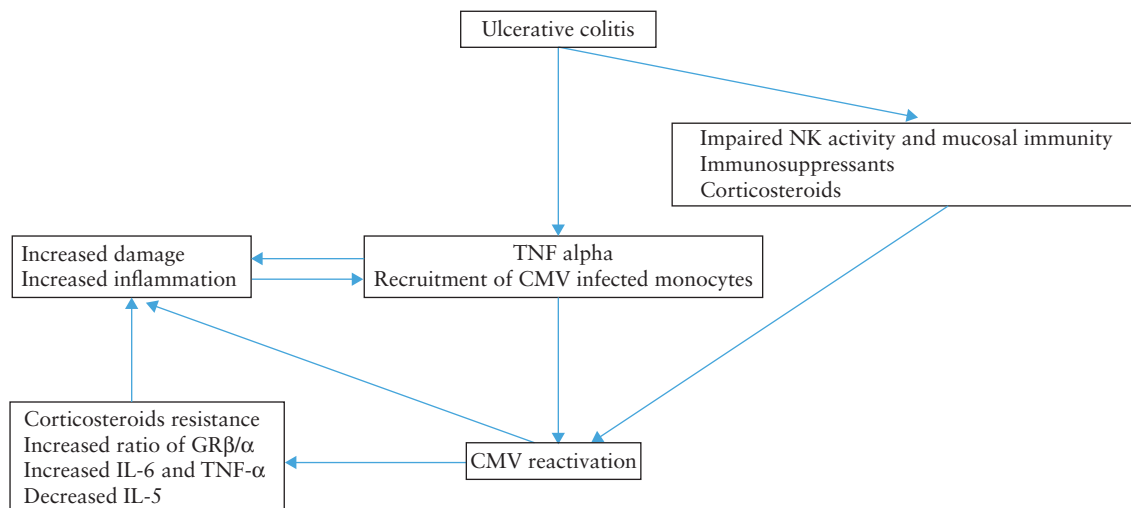


Figure 1. Pathophysiology of cytomegalovirus [CMV] reactivation and colitis.

A recent study tried to unveil the pathophysiology of steroid resistance in patients with CMV infection.⁴⁷ In general, glucocorticoids exert potent immunosuppressive and anti-inflammatory effects after binding to glucocorticoid receptors α [GR α]. However, glucocorticoids bound to glucocorticoid receptors β [GR β] exert no effect. In active UC, monocytes and macrophages infiltrate intestinal lesions.⁴⁸ Using a human mononuclear cell line, Wang *et al.*⁴⁷ demonstrated a change in glucocorticoid receptors [GRs] during the lytic phase of CMV reactivation. Specifically, there was a significant increased ratio of GR β / α in the CMV lytic infection group compared with that in the control group. These changes may explain refractory response to steroid treatment. Increased GR β and the ratio of GR β to GR α have been shown to be associated with glucocorticoid resistance in general and in patients with UC.^{49,50} In addition, the CMV lytic infection group had increased pro-inflammatory cytokines IL-6 and TNF- α and a decrease in the anti-inflammatory cytokine IL-5, which may explain the exacerbation of inflammation in UC [Figure 1].

6. Diagnosis of CMV Infection

6.1. What is the best way to diagnose CMV infection and CMV colitis?

The available methods to diagnose intestinal CMV infection include histology, serology, polymerase chain reaction [PCR] for CMV DNA in the blood or intestinal tissue, and CMV pp65 antigen.¹⁵ The problem faced by gastroenterologists is to differentiate between acute UC flare and CMV colitis. Both share similar clinical features including fever, malaise, diarrhoea, haematochezia, abdominal pain, and weight loss. Although some endoscopic findings are more common in patients with CMV colitis, such as punched-out ulcers, there are no reliable pathognomonic features on endoscopy that can distinguish between the two conditions.² In addition, it is important to differentiate between CMV infection and CMV disease. CMV infection or exposure which can be detected by CMV serology, serum antigenaemia or even positive CMV PCR does not necessarily cause CMV disease. These above named tests have been shown to correlate poorly with active CMV disease.^{2,51} The latter should be associated with symptoms or CMV-related tissue damage.⁶

The sensitivity of IgM serology in detecting CMV disease is around 15–60%.¹⁵ CMV antigenaemia assays that detect the CMV pp65 antigen in circulating leukocytes have shown sensitivities of 60–100% and specificities of 83–100% for CMV infection.⁵² Although the presence of antigenaemia does not correlate with detection of CMV in colonic tissue in patients with UC,^{27,30} in one recent study from Korea a good correlation between CMV antigenaemia and CMV colitis, defined as the presence of inclusion bodies and/or positive IHC in the colonic mucosa, was found.⁵³ Blood CMV PCR can reliably detect and quantify CMV viraemia with good sensitivity and specificity [65–100% and 40–92%, respectively].^{52,54} Some studies have reported a correlation between identification of CMV by PCR in blood and CMV colitis by H&E or IHC among UC patients.^{10,30,51,55} The prevalence of CMV viraemia [viral load >137 IU/mL] in patients with a tissue diagnosis of CMV is only 30%.⁵⁶ The sensitivity of CMV viraemia in other studies ranged from 18% to 47%.^{10,27,57–59} A high cut-off value of 1150 copies/mL in one study was found to increase the specificity of blood CMV PCR to 78.9% in predicting CMV colitis.²⁴

In UC therefore, H&E, IHC, and tissue PCR [colonic CMV replication] are the methods of choice, in decreasing order, to determine the presence of CMV in colonic tissue as virus presence in

the colon, rather than serology, antigen or virus detection in blood [serum CMV replication] which is more likely to be related to the colonic inflammation. H&E staining typically shows enlarged cells with thickened nuclear membrane and large intranuclear inclusion bodies [owl's eyes] [Figure 2] representing active CMV replication indicating CMV colitis.² H&E staining has a high specificity [92–100%], but its sensitivity is in the range of 10–87%⁶ and the diagnosis is observer-dependent. Special IHC staining [Figure 3], specific to one of immediate early [IE] antigens, increases the diagnostic sensitivity [78–93%] and specificity [92–100%] of the histological examination for colonic CMV.² IHC is important in the routine evaluation of IBD patients with severe disease before proceeding with step-up medical treatment or even surgery.⁶⁰ Although a weak recommendation with low quality evidence, the British Society of Gastroenterology [BSG] guidelines recommend checking for CMV disease using H&E staining which would identify typical CMV inclusions, and preferably also IHC and/or quantitative tissue PCR.⁶¹ The most recent ECCO guidelines concur with the BSG guidelines on the use of H&E and IHC, but emphasise the fact that multiple, rather than occasional, intranuclear inclusions are usually clinically significant.⁶² In most studies, no correlation was found between histology [H&E, IHC] and tissue viral DNA.⁶ The clinical significance of a positive PCR of colonic tissue without other histological signs of infection remains unclear. The presence of tissue viral DNA without histological features of viral infection is likely to represent low-level

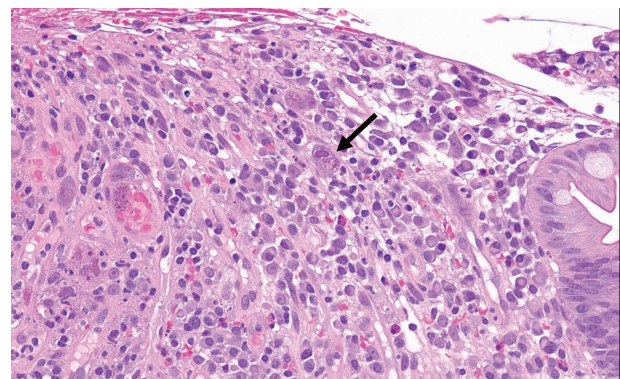


Figure 2. Haematoxylin and eosin [H&E] staining of colonic mucosal biopsy showing enlarged cells with thickened nuclear membrane and intranuclear inclusion bodies [arrow] representing active cytomegalovirus [CMV] replication [magnification 200x].

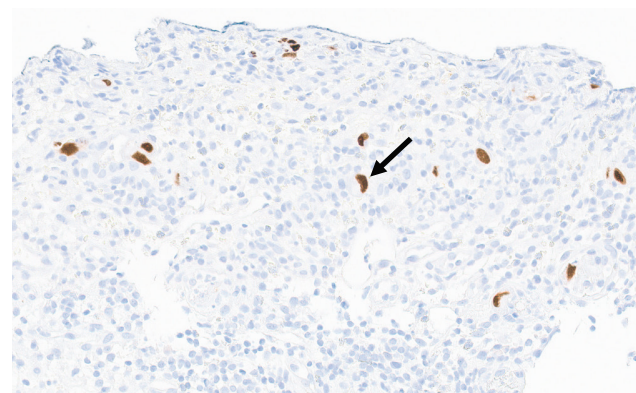


Figure 3. Special immunohistochemical [IHC] staining specific to one of immediate early [IE] antigens showing positive cells [arrow] [magnification 100x].

reactivation or latent CMV infection.⁶ Therefore, it has been suggested that quantitative rather than qualitative determination of tissue CMV PCR should be performed, as the viral load has been associated with CMV colitis and response to antiviral treatment.³² Short of quantitative tissue CMV PCR, and in an attempt to increase the sensitivity of H&E, it was recently suggested that the number of biopsies to be taken from the sigmoid colon of patients with UC should be 11 to achieve an 80% probability of a positive biopsy.⁵⁶ Whether a smaller number of biopsies will be sufficient if targeted biopsies are taken from the ulcer base, where the virus can be better detected, needs further investigation.^{56,63}

As CMV infection in UC depends not only on viral factors and viral load but also on the host immune reaction, obtaining information on both factors may differentiate between CMV disease and bystander viral activation. Theoretically, combining observations on host factors, such as CMV-specific T cell responses and viral factors such as data on CMV replication, should help in this difficult clinical situation.

In a proof of concept study, Kim *et al.*⁶⁴ used interferon γ -releasing assays [IGRA] for CMV and CMV PCR to diagnose active gastrointestinal CMV disease, defined as histological detection of intranuclear inclusion bodies or positive IHC and clinical improvement on ganciclovir treatment. The sensitivity and specificity of CMV replication in biopsy tissue [positive IHC staining] and low CMV IGRA result, for predicting gastrointestinal CMV disease in suspected patients, were quite high at 92% [95% CI = 62–100] and 100% [95% CI = 74–100], respectively. More studies are needed to clarify the importance of the IGRA test in UC patients with CMV infection.[£]

7. Effect of Anti-viral Treatment

7.1. Does antiviral therapy in UC flare with CMV infection improve outcome?

Although some studies showed that anti-viral treatment with ganciclovir or foscarnet improved outcomes,^{10,20,21,65–69} others showed no benefit.^{29,70} It is now becoming clearer that response rates depend on CMV viral load in colonic tissue.

Several studies analysed the importance of tissue CMV viral load in predicting clinical response to antiviral therapy and subsequently colectomy rates. Whereas many studies showed no effect for antiviral treatment on the rate of colectomy in CMV-infected UC patients in general,^{21,35,65,67,71} different results were obtained and different conclusions were reached on subgroup analysis. There was a significant difference in the colectomy rate between high-grade [≥ 5 inclusions in any single fragment on histology IHC] and low-grade CMV colitis [< 5 inclusions on IHC]. Patients with low-grade CMV colitis were significantly more likely to undergo surgery than those with high-grade colitis [HR 2.13, 95% CI = 0.85–5.33].⁶⁵ Patients with low-grade CMV colitis who did not receive antiviral therapy were nearly five times more likely to have surgery within 1 year than patients with high-grade colitis who were treated [HR 4.81, 95% CI = 1.60–14.48].

Nguyen *et al.*³⁵ classified CMV disease into low-grade [CMV detected by IHC only] and high-grade [CMV detected on H&E] categories, and compared colectomy rates in treated and untreated patients between the two groups. Antiviral treatment did not change the colectomy rates for those with low-grade disease [31% if treated and 29% if not treated], but did change outcomes for those with high-grade disease [44% if treated and 83% if untreated]. The authors proposed that high-grade CMV disease indicates that the

virus is acting as a pathogen, whereas in those with low-grade CMV disease the severity of the IBD itself is more likely to influence outcome.³⁵

Roblin *et al.*²¹ demonstrated that the majority of patients [seven out of eight] with a high tissue viral load [> 250 copies per mg of colonic tissue] responded to antiviral therapy despite failing three consecutive lines of immunosuppressive therapy. One meta-analysis on nine studies, with a total of 176 patients, concluded that the 30-day colectomy risk in patients who received antiviral therapy was significantly higher than in patients who did not receive antiviral therapy [OR 2.4, 95% CI = 1.05–5.5].⁷⁰ However, this review was criticised for the inappropriate inclusion of four studies in the analysis.⁷²

In contrast, a recent meta-analysis on 15 studies describing antiviral therapy in UC patients presenting with CMV infection reached a different conclusion.⁶⁷ Out of 333 patients, 189 [43.2%] were treated with antiviral therapy and 189 [56.8%] were not. The diagnosis of CMV was made primarily by H&E and/or IHC in seven studies and by tissue PCR in five studies. There was no difference in the risk of colectomy between patients treated with antiviral therapy and those not treated [OR 0.92, 95% CI = 0.31–2.76]. The results did not change when analysing only the studies in which the diagnosis was made on tissue biopsies. However, in corticosteroid refractory patients, the risk of surgery was lower in those treated with antivirals [eight studies; OR 0.2, 95% CI = 0.08–0.49]. In subgroup analysis, there was a difference in colectomy rate with anti-CMV viral treatment when the diagnosis was based on histological criteria [H&E and/or IHC] [three studies; OR 0.06, 95% CI = 0.01–0.34] but not on tissue PCR [four studies; OR 0.31, 95% CI = 0.09–1.11; in three out of the four studies the diagnosis was based on qualitative PCR]. As previously mentioned, the latter finding may be due to the fact that PCR is a very sensitive test in detecting low viral loads and even detecting latent virus.

Following this meta-analysis, two further studies were published providing more evidence on the importance of antiviral treatment in improving outcome of patients with UC and high CMV tissue viral load.^{16,73} In a retrospective case-control study, Wang *et al.*⁷³ identified 41 UC patients who had positive CMV on colonic biopsy, defined as one or more CMV virocytes diagnosed by H&E and/or IHC; 24 patients [58.5%] were treated for the infection. Antiviral therapy significantly improved surgery-free survival within 30 days and the difference between the two groups was sustained for 70 months [$p < 0.01$]. However, hospitalisation, rehospitalisation, and mortality rates were similar between the two groups. Okahura *et al.*¹⁶ found that patients with high tissue CMV viral load may respond to antiviral treatment without additional UC therapy, whereas patients with low viral load [< 5500 copies/ μ g DNA] would benefit from intensifying UC therapy. The cut-off point for virus load to start antiviral treatment is not well established or standardised. Further studies are needed before coming up with sound recommendations.

8. Use of Immunosuppressive and Biologic Therapies

8.1. Do immunosuppressive and biologic therapies affect CMV infection? Can they be used during UC flare?

It is now well established that steroids [even a short course] and immunomodulators are associated with increased risk of CMV reactivation.^{14,15,17,45,74,75} In a prospective study of 61 UC patients, treatment with azathioprine in addition to steroids was found to be a significant risk factor associated with CMV infection, defined as

positive IgM antibody, or detection of CMV DNA by PCR on colonic biopsy, or positive findings on histological assessment of H&E-stained colonic biopsy.¹⁵ A recent meta-analysis by Shukla *et al.*¹⁷ showed that exposure to corticosteroids [12 studies, 1180 patients; OR 2.05, 95% CI = 1.40–2.99] and thiopurines [14 studies, 1273 patients, 24.1% exposed; OR 1.56, 95% CI = 1.01–2.39] was associated with increased risk of CMV reactivation. However, this risk could not be demonstrated when stratifying the studies for the use of tissue CMV PCR for diagnosis.

The effect of anti-TNF alpha agents on the outcome of patients with CMV colitis has also been studied. As compared with patients not exposed to TNF alpha antagonists, exposure to this drug was not associated with an increased risk of CMV reactivation [seven studies, 818 patients; OR 1.44, 95% CI = 0.93–2.24].¹⁷ In addition, treatment with anti-TNF alpha did not adversely affect the outcome of patients with CMV reactivation.^{76,77} Although no studies have specifically looked at the effect of anti-TNF alpha antagonists on viral replication in patients with UC and CMV, these agents might theoretically lead to a reduction in macrophage differentiation and CMV reactivation.⁷⁸

Cyclosporine causes immunosuppression through inhibition of T cell proliferation, and therefore may lead to an increased risk of CMV reactivation. A few small non-comparative studies reported the development of CMV infection in approximately 80% of severe UC patients [18 out of 23 and five out of six] treated with cyclosporine.^{27,79} CMV reactivation occurred 7–10 days after starting intravenous [IV] cyclosporine and resulted in exacerbation of colitis. Therefore, it was recommended not to start cyclosporine in patients with severe colitis and CMV infection. However, a recent retrospective multicentre study, on 110 patients with acute severe UC and CMV infection, showed no difference in the colectomy rate at 1, 3, and 12 months between patients who received ganciclovir alone, or ganciclovir + infliximab, or ganciclovir + cyclosporine.⁸⁰ Therefore, it seems that infliximab or cyclosporine can be given in patients with CMV infection as long as antiviral therapy is administered.

By depleting the intestine of circulating lymphocytes, vedolizumab may also favour CMV reactivation due to diminished constant immune surveillance, and therefore it may theoretically increase the risk of CMV infection. The available data are scarce. A review on the safety data from six published trials showed no risk for increased CMV infection with the use of vedolizumab,⁸¹ and even resolution of colonic CMV infection in a patient with UC following vedolizumab treatment was reported.⁸² However, a recent study published in abstract form showed an increased risk of CMV reactivation with vedolizumab as compared with infliximab, in patients who had positive CMV IgG serology before starting treatment.⁸³ Using a Cox model and correcting for disease severity, the hazard ratio was 2.3 [95% CI = 0.5–9.3] compared with infliximab and 5.1 [0.6–41] in case of concomitant steroid use. During CMV reactivation, clinical, endoscopic, and biological severity were higher in the vedolizumab group.

In the UNIFI study, there were two reported cases of CMV colitis encountered among the UC patients receiving ustekinumab in the every-12 week maintenance arm.⁸⁴ There has been a single reported case of serious CMV colitis in a Crohn's disease patient who was receiving ustekinumab.⁸⁵ After treatment with ganciclovir, the patient did well and her infection resolved. Regarding the risk of CMV colitis in patients receiving tofacitinib, only a single UC patient in the OCTAVE Induction 2, receiving tofacitinib 10 mg twice daily,

had developed CMV colitis.^{86,87} There have been no other reports of CMV colitis from tofacitinib reported in the literature. Studies have shown high rates of recurrent CMV infections among UC patients, with rates ranging between 13.5% and 57%.^{26,80} Despite these high rates, to date there are no recommendations for the use of secondary CMV prophylaxis in non-transplant patients.

9. Approach to Patients With Acute Colitis and CMV

9.1. What to do for UC patients who have a concomitant CMV infection?

It is important to keep a high clinical suspicion for CMV infection/reactivation among UC patients who are presenting with worsening of their gastrointestinal symptoms, regardless of their immunosuppression status. A delay in the diagnosis and subsequent management may be associated with poor outcomes, including increased colectomy rates. There are several ways to test for CMV disease including serological markers [CMV serology, antigen testing, and DNA PCR] which, as previously mentioned, are not very accurate in identifying an acute active infection. The gold standard testing for CMV gastrointestinal disease remains histological, based on H&E stains and special IHC testing +/- IE antigen testing, followed by tissue PCR testing. This is thought to be related to the local effect of CMV on colonic tissue.

There have been several published algorithms regarding the approach and management of CMV infection in UC patients, all of which have minor differences between them. Most algorithms propose checking for CMV colitis in patients with severe colitis who are refractory to steroids. Once the colonoscopy is performed, based on endoscopic evaluation [i.e., large punched-out ulcers] and based on histological testing, be it H&E stains, IHC stains, or tissue CMV PCR, the decision is whether to start antiviral therapy, add anti-TNF therapy, increase immunosuppression, and/or stop the corticosteroids.^{32,70}

Beswick *et al.*⁸⁸ recently published an algorithm taking into account serum CMV IgG testing in those patients with symptoms of severe colitis. In patients whose serum CMV IgG returns positive, and who are refractory to steroids and/or other immunosuppressive agents, serum CMV PCR and a colonoscopy for tissue testing are performed. Management then depends on the degree of serum CMV PCR viral load as well as the H&E and IHC tissue testing. Tissue CMV PCR is performed only in cases of high CMV suspicion in those patients who have a negative IHC stain.

Pillet *et al.*¹¹ proposed a simpler algorithm, in which patients with steroid-refractory or steroid-dependent UC would undergo a colonoscopy for CMV detection, and then based on the results, they would be classified to have high-grade CMV density, low-grade CMV density, or no CMV. Further management would be dictated based on quantification of CMV density on the colonic tissue. Patients with no CMV would require intensification of their immunosuppression to treat the underlying UC, and patients with high-grade CMV [tissue CMV DNA >250 copies/mg or >four inclusions on IHC] would require antiviral therapy and anti-TNF if deemed necessary. Patients with low-grade CMV density [tissue CMV DNA 10–250 copies/mg or ≤four inclusions on IHC], would require antiviral therapy only in cases where endoscopically they had large colonic ulcers or if they were ill and admitted to the hospital.¹¹

This protocol is slightly similar to that proposed by Shukla *et al.*,⁶⁷ which categorises UC patients with CMV disease into

low-grade CMV or high-grade CMV, the latter of which requires treatment with antiviral therapy. As for the low-grade CMV patients, those need to be assessed for response to corticosteroids and then, accordingly, the decision to give antiviral therapy or escalate immunosuppression would be made.

An earlier study by Pillet *et al.* proposes an algorithm which starts by checking serum IgG status and then, depending on response of clinical symptoms to corticosteroids, decides on need for colonoscopy for tissue testing of CMV tissue PCR.⁸⁹ Similar to what was published previously, patients with low CMV burden on tissue [<10 copies/mg of tissue] would need escalation of their immunosuppressive therapy, and those with high CMV burden on tissue [>250 copies/mg of tissue] would need antiviral therapy with or without anti-TNF therapy. As for the patients who have a fair CMV burden [i.e., those falling between the low and high burden groups—between 10 and 250 copies/mg], the recommendation is to initiate anti-TNF therapy.

We propose to proceed with a colonoscopy in patients with severe colitis, especially if they are steroid-refractory, after common infectious pathogens have been excluded. During the colonoscopy, biopsies are obtained for H&E staining to check for inclusion bodies, in addition to IHC stains and a quantitative CMV tissue PCR. If patients have multiple inclusion bodies, we propose antiviral treatment regardless of CMV tissue PCR or positive IHC stains. If the inclusion bodies are negative and patients have high CMV tissue PCR [>250 copies/mg of tissue] or high IHC staining [$>four$ cells/section], then we would also propose initiation of anti-viral therapy.

The preferred antiviral agent is intravenous ganciclovir at a dose of 5–7.5 mg/kg twice daily, for 2 weeks.^{6,39,75,78} Although based on very low-quality evidence, the BSG makes strong recommendations on using intravenous ganciclovir 5 mg/kg twice daily while continuing conventional therapy with corticosteroids or rescue medications with infliximab or cyclosporine.⁶¹ ECCO guidelines, on the other hand, recommend intravenous ganciclovir 5 mg/kg twice daily for 3–5 days, then oral valganciclovir at 900 mg orally twice daily for 2–3 weeks. The advice of virology/microbiology should be taken into account regarding the route and duration of therapy. In cases of ganciclovir resistance or intolerance [e.g., myelotoxicity], foscarnet [for 2–3 weeks] is an alternative.⁶⁰ It remains unclear what best follow-up method should be used to confirm resolution of the CMV colitis after completion of treatment.

Also, it is unclear what to do with a patient's immunosuppression in the event of initiation of antiviral therapy. The ECCO guidelines recommend that in patients with severe steroid-resistant colitis who have CMV detected in colonic tissue, in addition to starting antiviral therapy, immunomodulators could be discontinued. Immunomodulators, however, must be discontinued in cases of systemic CMV disease.⁶⁰ Although the recently published American College of Gastroenterology guidelines recommend checking for CMV infection in patients with acute severe UC, there are no recommendations on how to manage the immunosuppressant medications.⁹⁰ CMV infection exacerbates the underlying UC, so continuation of the immunomodulators and initiation/continuation of anti-TNF therapy have been described as acceptable.^{38,39} The main controversial medications are the corticosteroids and, to date, it is unclear if these should be discontinued or not in patients with CMV colitis who are receiving antiviral therapy. It is the authors' preference to induce remission with anti-TNF therapy and quickly taper off the steroids while continuing intravenous ganciclovir.

10. Future Research

There have been multiple reviews, meta-analyses, and algorithms proposed for the management of CMV colitis in patients with UC. Despite that, there remain multiple unanswered questions related to the optimal diagnosis of CMV colitis, what criteria to use, and what to do with corticosteroids, immunomodulators, and anti-TNF agents. After initiation of antiviral therapy, it remains uncertain how long to continue treatment to ensure complete resolution of the CMV colonic infection. A prospective randomised controlled trial, targeting patients who are admitted to the hospital with acute severe colitis and who are found to have CMV infection on tissue biopsies, should be examined.

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Conflict of Interest

VK has received grants from Ferring, Abbvie, and Takeda, and consultancy fees from Shire and Janssen. RL is on the advisory boards of Aspen, AbbVie, Celgene, Ferring, Hospira, Janssen, Pfizer, MSD, and Takeda, and has received research funds from Janssen, Shire, and the Gastroenterological Society of Australia. The other authors have no conflict of interest to disclose.

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FM and JH contributed to the references search and writing-up of the manuscript. VK contributed to the review design and manuscript revision. RL contributed to manuscript revision and intellectual content.

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