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Article type: Review article

Received: March 21, 2019.

Accepted: May 15, 2019.

Published online: May 21, 2019.

ISSN: 1897-9483

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Perspectives of cytomegalovirus infection in ulcerative colitis

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Short title: CMV infection in UC
Key words: cytomegalovirus, herpes virus, inflammatory bowel disease, ulcerative colitis, immunosuppression

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Conflict of interest: none declared.

Abstract
Herpes virus infection causes severe and fatal disease in individuals having suppressed immunity. In inflammatory bowel disease (IBD) patients, particularly those with ulcerative colitis (UC) patients undergoing immunosuppressive therapy or unresponsive to medical therapy, cytomegalovirus (CMV) has been found to be associated with significant clinical morbidity. In addition, other herpes viruses particularly human herpes virus 6 (HHV6) and Epstein-Barr virus (EBV) have been identified recently in colonic mucosa of IBD individuals
although the relationship between herpes virus infection other than CMV and IBD exacerbation remains unknown to date. Hence, this review highlights herpes virus infection in UC patients emphasizing on prevalence and diagnostic strategy of CMV infection, prevalence of single or combined infection by herpes virus (HHV6 and EBV) in addition to CMV. Furthermore, significance of genotyping of CMV in UC is discussed.

**Introduction**

Clinically and pathologically distinct two medical conditions such as, ulcerative colitis (UC) and Crohn’s diseases (CD) together are termed as inflammatory bowel disease (IBD). UC is characterized by continuous inflammation limited to colonic mucosa whereas CD is characterized by transmural inflammation and skip lesions limited to mucosal and submucosal layer and involve mouth to perianal region [1]. Therapeutic options include use of glucocorticoids, thiopurines, or tumor necrosis factor inhibitors (TNFi) as a monotherapy or combined use of glucocorticoids with immunosuppressive drugs, such as, azathioprine, cyclosporine A, and/or biologics. Currently, anti α-4/β-7 integrin antibody such as, vedalizumab, and Janus kinase inhibitors (JAKi) such as, tofacitinib have been approved as novel immunosuppressant therapy in UC [2]. Use of different biologics and/or immunosuppressive agents as a monotherapy or in combination with each other have been reported as a risk factor for serious infections in patients with UC. Very recent systemic review and meta-analysis compared precisely regarding the risk of infections with TNFi, vedalizumab, and immunosuppressing agents in IBD patients. This meta-analysis showed that risk of serious infections is higher in combination therapies of TNFi and corticosteroids than TNFi monotherapy. On the other hand, higher association of infections with TNFi monotherapy than combined therapies of TNFi and immunosuppressing agents other than corticosteroids was observed in IBD patients. Furthermore, thiopurines are primarily
responsible for viral infections, which sometimes becomes serious and in some cases, may need hospitalization. Moreover, very little data is available regarding the effect of vedolizumab and tofacitinib on the risk of serious infection. Several clinical trials of vedolizumab haven’t reported any significant risk of serious infections, particularly in gut [3]. However, tofacitinib treated UC patients had higher prevalence of infections by herpes zoster virus and cytomegalovirus (CMV) than placebo [4]. Further, IBD patients have been reported as a high-risk group for serious infection due to poor nutritional status, inflamed mucosa, most importantly due to use of immunosuppressive drugs as an effective therapeutic option. Mostly reported infection in IBD is occurred by CMV [5].

CMV is a double stranded DNA virus included in β-herpesvirinae group. CMV commonly infects people of all ages and undergoes life-long latency like other herpes viruses. Endothelial cells as well as blood mononuclear cells have been reported as reservoirs for CMV following primary infection. In a quiescent stage, CMV usually doesn’t show meaningful clinical manifestations except mononucleosis. However, latent CMV becomes reactivated due to immunodeficiency disease [6] and prescribed drugs, such as corticosteroids, which cause profound immunosuppression. Diagnosed cases of IBD are often placed on long-term prednisolone and/or other immunosuppressive drugs and may develop iatrogenic immunosuppression. That’s the reason why immunosuppressive drugs have been reported as one of the most important stimuli to reactivate CMV from inactive state in IBD [7, 8]. Previous epidemiological data regarding IBD coexistent with CMV infection revealed that ulcerative colitis patients had increased risk of being infected by CMV rather than CD (<5%) [9]. CMV infection in UC may present with two conditions. One is UC co-exist with CMV colitis where CMV itself causes colitis, another is UC with CMV infection [10]. In this review, we will particularly focus on CMV infection in UC emphasizing on diagnostic strategy by reviewing published data from our laboratory and other literatures. We make a
sketch to distinguish between CMV induced colitis and CMV infection in UC patients (Fig. 1). Furthermore, colonic infection by herpes virus other than CMV such as, infection by human herpes virus 6 (HHV6) and Epstein-Barr virus (EBV) will be discussed briefly in this review.

**Prevalence of CMV infection in UC**

By definition, positive serology or positive reaction for CMV DNA in relevant clinical samples for example, blood, stool, intestinal fluid by polymerase chain reaction (PCR) is infection by CMV. Analysis of feces by PCR seems to be more specific for elucidating colonic infection by CMV in UC. The report on exact prevalence of CMV in UC is scarce. After a careful review of published data, variations in the prevalence of CMV infection in UC have been observed due to use of different diagnostic techniques by different laboratory. Therefore, the actual rate of CMV infection in UC is not clear. Association between UC and CMV infection was first reported in 1961 [11]. Since then, numerous studies have been conducted considering the role of CMV in UC. Recently, prospective study reported that significantly higher CMV DNA was detected in 47% (8/17) IBD refractory to conventional therapies compared to non-refractory IBD patients (21.7%; 5/23) and controls (5%; 2/40) [12]. Studies suggested that CMV infection rate appears to be higher in UC particularly in steroid refractory cases. One study analyzed the prevalence of CMV infection in corticosteroid free, thiopurine-free 105 UC patients and corticosteroid refractory 82 UC patients by serology, CMV antigen testing method, CMV DNA testing by PCR, and by histology. In this study, prevalence was 75.2% and 69.5% in corticosteroid free UC patients serologically and by CMV antigenemia respectively. In case of refractory group, prevalence was 81.7%, 32.9%, 77.6% and 25.9% in order of serology, CMV antigenemia, PCR and histological examination [13]. Another study reported that 75% steroid refractory UC patients
were positive for CMV by CMV antigenemia [14]. Prevalence of CMV in active UC was 10% (12/122) by the immunohistochemical method [15]. The prevalence of CMV was 29.4% in active UC patients who didn’t undergo any immunosuppressive therapies [16] and 56.7% in active UC patients who were refractory to immunosuppressive therapies [17] through testing of CMV DNA by PCR in colonic mucosa.

**Endoscopic evaluation**

Studies suggested that colonoscopic findings to diagnose CMV infection in UC were complicated. The authors considered two aspects of colonic changes; one is mucosal defects, another is ulcerative changes after analyzing colonoscopic images retrospectively. Mucosal defects include loss of visible blood vessel under mucosa, erythematous change, fragile mucosa which bleeds easily on slight contact of endoscope, mucosal edema, exudates composed of blood and pus. Wide spread mucosal defect, clearly demarcated round ulcer, ulcer along the colon, irregular ulceration, and cobblestone-like appearance were assessed to reveal ulcerative changes (Fig. 2). Punched-out, longitudinal, and irregular ulceration has been recommended as characteristic colonoscopic findings in UC patients complicated by CMV infection [18, 19]. However, most of the studies have reported that in case of active UC patients, who were seemed to be susceptible to CMV infection, endoscopic features of UC and CMV infection/colitis overlap with each other [20-23]. Thus, endoscopic evaluation might have less significant role in making accurate diagnosis of CMV in UC patients.

**Diagnostic strategy**

CMV infection produces adverse effect on clinical course of UC. Therefore, appropriate diagnosis of CMV infection in UC is necessary. Many methods are available to define colonic infection by examining CMV-specific antibodies, presence of CMV and CMV DNA in
peripheral blood and colonic specimen such as, tissue, colonic fluid or feces in order to
determine relationship with concomitant clinical symptoms. Following techniques are
currently used to investigate colonic infection by CMV.

**Detection of IgM and IgG antibodies to cytomegalovirus**

Serology helps us to determine previous exposer to virus. Serological analysis is usually
performed by enzyme linked immunosorbent assay (ELISA) using serum sample. Both IgM
and IgG antibodies titer are necessary to diagnose CMV infection. Increased IgM antibody
titer indicates primary infection, takes 2 years to disappear from individual’s serum, and is
increased rarely during reactivation of CMV (0.2-1%). CMV specific IgG antibody titer is
analyzed in two alternate serum samples collected at an interval of 2-4 week. Four-fold
increase of IgG antibody is used as one criterion to diagnose CMV. However, serological
analysis is considered as a non-specific test to diagnose colonic CMV infection [24].

**PP65 antigenemia**

Detection of PP65 antigen in peripheral blood mononuclear leucocytes through
immunofluorescence generally indicates active infection or reactivation of CMV with
sensitivity of 60-100% and a specificity of 83-100% [25, 26]. However, similar to serology,
positive result in blood samples doesn’t reflect the concurrent CMV infection in colon [27]. If
there is neutropenia, CMV antigenemia test reveals false negative result [8]. Positive results
of CMV antigenemia reflect systemic infection; however, they do not always correlate with
colic infection in UC [28].

**Histological detection of CMV in colonic tissue**
Primarily, histological analysis of colonic biopsy specimen is performed by hematoxylin and eosin (H&E) staining followed by microscopy. CMV generates inclusion bodies in nucleus and cytoplasm. Large-sized cells approximately 25-50 μm in diameter containing intranuclear and cytoplasmic inclusion bodies visible under microscope as an owl’s-like eye are defined as cytomegalic cell, which are typical features of CMV infection in tissue. Cytomegalic inclusion bodies are found very rarely for which histological analysis is considered as a low sensitive technique for detecting CMV in intestinal tissue. It is important to note that collection of biopsy accessing deep mucosal layer is very difficult for endoscopists due to inflamed mucosa in UC. Superficial mucosal layer is collected as biopsy specimen to prevent risk of bleeding and mucosal damage. Therefore, epithelial cells, rarely infected by CMV, can be visible under microscope following H&E staining. That’s the reason why histological analysis provides false negative result [29-32]. Area of collecting tissue along the colon and quantity of tissue specimen for revealing CMV infection is not clear. One study showed that density of CMV positive cells were higher in base and edge of the ulcer whereas CMV positive cells were not found in the uninvolved portion of colon through IHC [32]. One study recommended collecting 11 biopsies to assess CMV in UC [33]. Although CMV is heterogeneously distributed along the colon, rectum part was shown to be affected predominantly by CMV infection rather than other area of colon. As rectal part is more prone for CMV infection and rectum is usually the most inflamed area in UC, hence, CMV might has more affinity to be accumulated in inflamed zone [34]. Ulcer bed is seemed to be a reservoir of CMV [35, 36], which area can’t be accessed due to risk of bleeding and perforation of intestine. Furthermore, the immunohistochemical study has been employed to improve the sensitivity of histology. Histology combined with immunohistochemistry (IHC) is considered as golden standard for CMV [37, 38]. Very recently published one article showed that patients with ≥ 2 positive CMV-IHC-positive cells/biopsy had higher risk for
colectomy [39]. Another article strictly recommended to consider 5 positive cells/biopsy as CMV positive IHC. In addition, very recent publication analyzed the diagnostic accuracy of immunohistochemistry and they have considered IHC positive when only one positive cells were present [40]. But this study performed immunohistochemistry in those patients who were suspected case of CMV colitis in UC clinically and endoscopically [41]. However, histological analysis is time-consuming technique and required to ensure great effort by pathologists.

**Diagnosis of intestinal CMV infection by PCR**

Amplification of CMV DNA by qualitative and quantitative PCR assay are used to detect CMV in blood, urine, colonic tissue, and feces. Colonic samples such as, colonic mucosa and feces, are more specific to identify colonic infection by CMV than PCR in blood and urine. Similar to antigenemia test, positive CMV DNA in blood is not related to colonic infection [42-45]. Recently, one study analyzed mucosal tissue for detecting CMV infection by PCR. In that study, among the UC patients with positive CMV DNA by mucosal PCR, 56% were negative by CMV antigenemia testing [46]. It is noted that employment of non-invasive testing method such as, stool analysis by PCR, is beneficial for UC patients. Qualitative or quantitative analysis by PCR has been introduced to detect CMV DNA in feces [47-50]. In UC, fecal analysis carries some advantages. Physician faces some difficulties during collecting tissue through endoscope in UC particularly in those patients who have flare-up sometimes. There is possibility of bleeding on touch. Sometimes, severe patients deny going through endoscopic examination. Thus, stool analysis is easy, non-invasive, and convenient for patients. However, the European Crohn’s and Colitis Organisation has recommended to use fresh stool samples for performing PCR [51]. In our previously published article, qualitative multiplex PCR method using fresh stool samples has been suggested as a rapid,
feasible screening tool to detect CMV DNA in UC patients. Qualitative tests only can detect CMV infection; however, it is not useful for diagnosing CMV induced colitis. Tissue histology or colonic tissue PCR is recommended for diagnosing CMV induced colitis. Positive finding of CMV on PCR were defined as reactivation by previous study [17]. PCR indicates the presence of colonic infection by CMV rather than CMV colitis. Furthermore, positive finding of colonic PCR for CMV DNA is not false positive but a warning for treating UC patients who are refractory to immunosuppressive therapies [52].

**Risk factors in the occurrence of CMV infection in UC**

Three major criteria have been identified by experimental studies: (1) inflamed colonic tissue with ulcer which acts as a reservoir of CMV, (2) impaired immunity of UC, (3) use of immunosuppressive drugs such as, corticosteroids, cyclosporine or their combination. Several lines of evidence have indicated that corticosteroids itself or combined use of corticosteroids and immunosuppressive drugs worked as fundamental risk factor for CMV infection in UC [53-55]. Recently, in one retrospective study, old age, high endoscopic score and higher dose of corticosteroids has been shown as predisposing factors for CMV infection in UC [56]. Another study diagnosed CMV infection by CMV antigenemia and reported that all CMV-positive patients received corticosteroid therapy in comparison to CMV-negative patients [19]. Other authors demonstrated that after prescribing ganciclovir therapy and after discontinuing steroid, steroid resistant symptoms of UC were improved [21]. Our experimental data have revealed that immunosuppressive drugs increased the risk of CMV infection in UC patients besides disease activity [56]. Likewise, A large retrospective observational study demonstrated CMV-positive all UC patients had history of corticosteroid therapy. It has been stated that CMV induced colitis symptoms are developed due to immunosuppressed condition particularly in organ transplants, human immunodeficiency
virus-infected patients, and in immunosuppressive drug users [57]. In a systemic review of case series, it has been reported that all UC patients were infected by CMV after receiving corticosteroids for a prolonged period. UC patients under the treatment of both azathioprine and steroids were found to be increased risk of CMV infection in a prospective study [58]. Recently, meta-analysis data revealed that use of corticosteroids and thiopurines are associated with CMV reactivation in UC [59].

**Combined infection by herpes viruses in UC**

Evidence of combined infection of CMV, EBV, and/or HHV-6 in UC remains very little. In our laboratory, multiplex PCR analysis of herpes virus DNA using stool samples demonstrated the prevalence of CMV (36.6%), EBV (36.6%), and HHV6 (11.3%) in UC patients and the simultaneous presence of CMV and EBV, and/or HHV6 were significantly higher in UC active patients (24.1%). Our study also suggested the possible synergistic role of these three herpes viruses in the pathogenesis of UC [56]. Consistent with these findings, one study examined herpes virus DNA in colonic tissue and peripheral blood samples collected from UC. That study demonstrated the higher prevalence of CMV (81%), EBV (76%), and HHV6 (76%) in UC than the control. Authors also showed that simultaneous presence of HHV6 and CMV and/or EBV were significantly higher in colonic tissue of UC patients (76%) than the control (29%) ($P < 0.05$), which further suggested the synergistic role of these three viruses in the pathogenesis of UC [60]. In solid organ transplant recipients, it has been reported that HHV-6 play a role in developing CMV infection [61, 62]. Similarly, EBV reactivation was also demonstrated in large scale of primarily infected patients by CMV [63]. Several studies have been reported on combined infection by herpes virus DNA in UC. In one study, 66 UC patients were enrolled to analyze herpes virus DNA using colonic tissue through multiplex PCR assay and combined prevalence of herpes virus (CMV, EBV, and/or HHV6)
was elucidated in 10.6% patients with UC. Further analysis demonstrated the association of combined infection (CMV with concurrent EBV or HHV6) with clinical course in UC. This research revealed that EBV or HHV-6 could synergistically exacerbate the intestinal inflammation or increase the risk of CMV reactivation, which further increased the risk of surgical approach [64]. Another study investigated the rate of EBV, CMV, and HHV6 infection as 53.7%, 24.4%, and 39% respectively in UC by mucosal PCR and mixed infection was not clarified in that study [65]. Based on these previous findings, it is highly likely that mucosal infection rates of EBV and HHV6 are higher in UC besides CMV infection, which may imply the role of combined herpes virus (CMV, EBV, and HHV6) infection in the pathogenesis of UC. CMV is frequently reactivated in UC particularly in individuals taking corticosteroids, thiopurines, TNFi [66, 67]. There is also significant association of CMV infection with clinical morbidity such as, toxic megacolon, risk of colectomy, high mortality [68]. However, there has been no clear evidence of risk regarding other herpes viruses or combined presence of human herpes viruses with CMV in UC. On the other hand, there are a few cases of herpes simplex virus associated colitis in UC [69-71]. UC patients taking immunosuppressive medications are at high risk for varicella zoster virus (VZV) reactivation [72]. However, herpes simplex virus-1/2 or VZV infection is not common in colonic tissue [73].

**Genotyping of CMV in UC**

Despite the presence of extensive results on distribution of pathogenic strains of CMV in congenital infection, solid organ transplant recipients, hematopoietic stem cell transplant groups, and acquired immunodeficiency syndrome, very little data are available in UC regarding genotypic distribution of CMV. Our previous study revealed that glycoprotein B1, N3, and H2 were the most likely frequent genotypes in UC. Clinical correlation demonstrated
that gB1 and gH2 gene might have a role in producing symptoms in UC [74]. However, due to lack of sufficient data, it was not feasible to make a firm conclusion. Likewise, gB1 was displayed as the most prevalent genotype of CMV in UC [75]. Genotypic analysis in a large scale could make demarcation between pathogenic and non-pathogenic strain of CMV to figure out pathogenic role of CMV in UC [76].

**Conclusion**

As there is growing evidence in herpes virus particularly CMV infection in UC, future research should be undertaken necessarily to resolve several crucial issues such as, clear prevalence rate of CMV infection, exact role in exacerbation of colitis symptoms, the best diagnostic and preventive strategy, accurate timing for starting antiviral therapy, and exact mechanism in causing reactivation or in enhancing the pathogenicity of CMV infection as well as particular role of individual herpes virus infection or combined infection in UC.

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Figure 1. Schematic representation of distinguishing cytomegalovirus (CMV) colitis from CMV infection in ulcerative colitis (UC) patients by reviewing literatures. Exacerbation of clinical symptoms (excessive watery/bloody diarrhea, fever, fatigue, abdominal pain), presence of endoscopic features of CMV colitis, non-responsive to conventional therapy of UC, positive immunohistochemistry (IHC) and/or positive reaction of colonic tissue PCR reveal CMV colitis, whereas presence/absence of exacerbating symptoms, negative IHC but positive blood/ stool PCR indicate CMV infection. CMV infection may not require anti-viral treatment or discontinuation of therapy of UC but the warning for monitoring patients to prevent the risk of CMV colitis.
Figure 2. Colonoscopic picture of cytomegalovirus-associated punched-out round ulcer in the rectum of active ulcerative colitis.