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Clinical utility of cytomegalovirus antigenemia assay and blood cytomegalovirus DNA PCR for cytomegaloviral colitis patients with moderate to severe ulcerative colitis

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KEYWORDS
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Abstract

Background and aims: Clinical usefulness of cytomegalovirus (CMV) antigenemia assay and blood CMV polymerase chain reaction (PCR) in patients with ulcerative colitis (UC) needs to be evaluated.
Methods: Medical records of moderate to severe UC patients between January 2001 and December 2012 were reviewed retrospectively. Diagnostic performances of CMV antigenemia assay and blood PCR to predict CMV colitis, and clinical outcome according to the results were analyzed. CMV colitis was diagnosed by H&E staining and/or CMV immunohistochemistry.
Results: Of the 229 study subjects, 83 patients (36.2%) had CMV colitis. The sensitivity and specificity of CMV antigenemia assay were 47.0% and 81.7%, and those of blood CMV DNA PCR were 44.3% and 87.9%, respectively. If either CMV antigenemia or PCR was positive in the presence of significant ulcers, the sensitivity and specificity of having CMV colitis were 67.3% and 75.7%, respectively, with the area under the receiver operating characteristic curve value

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; IQR, interquartile range; LR+, positive likelihood ratio; LR−, negative likelihood ratio; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value; UC, ulcerative colitis.

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of 0.717. Among patients with significant ulcers, positive CMV antigenemia (33/50 [66.0%] *vs.* 31/102 [30.4%]; $p < 0.001$) and positive blood CMV PCR (25/37 [67.6%] *vs.* 24/86 [27.9%]; $p < 0.001$) showed significantly higher probability of CMV colitis than blood test-negative patients. UC-CMV colitis patients with positive CMV antigenemia showed significantly higher rate of colectomy than those with negative antigenemia (13/39 [33.3%] *vs.* 5/44 [11.4%]; $p = 0.015$). *Conclusions:* Although CMV antigenemia and blood CMV PCR showed low sensitivity for diagnosing CMV colitis, the specificity values were high. Among UC-CMV colitis patients, CMV antigenemia showed significant association with subsequent colectomy.

1. Introduction

Cytomegalovirus (CMV) is an important pathogen causing a wide spectrum of disorders, ranging from asymptomatic infection in immunocompetent hosts[1] to disseminated disease in immunocompromised patients.[2,3] Although gastrointestinal involvement of CMV is rare in immunocompetent individuals, clinically significant CMV gastrointestinal disease may occur in immunocompromised patients such as transplant recipients or in immunocompetent hosts of advanced age.[3,4] In addition, CMV can lead to worsening of colitis in patients with moderate to severe ulcerative colitis (UC), mainly in those with corticosteroid-refractory disease.[5–8] Therefore, a reliable and rapid diagnostic method for detecting superimposed CMV colitis is essential in patients with moderate to severe UC.[9]

In the 2009 European Crohn's & Colitis Organization (ECCO) guidelines, histopathology combined with either tissue polymerase chain reaction (PCR) or immunohistochemistry (IHC, using monoclonal antibodies against CMV immediate early antigen) was suggested for diagnosing CMV colitis.[10] Using CMV DNA detected in colonic tissue with PCR to diagnose CMV colitis is a matter of debate, since it can represent either false positivity[11] or CMV reactivation.[12] Although the best approach involves confirming the presence of CMV with histological analysis using IHC staining,[13–15] tissue biopsy could possibly lead to hemorrhage or perforation related with endoscopic examination, especially if significant, large, and bleeding ulcerative lesions are present.[16–18] In addition, during the time to get results of IHC staining and/or colonic tissue CMV DNA PCR, the clinical deterioration may occur in UC patients. Therefore, blood tests for CMV antigen or CMV DNA could be considered as a possible alternative or complement for endoscopic biopsy.

Most studies have focused on the role of CMV antigenemia and blood CMV DNA PCR for identifying CMV infection, especially among transplant patients.[3,18–20] The sensitivity of the CMV antigenemia assay was reported to be 69.4% in detecting CMV pneumonitis among lung transplant patients.[20] The overall performance characteristics of the antigenemia assay in predicting CMV disease among solid organ transplant patients have been reported to include a sensitivity of 64% and a specificity of 81%.[21] In a study of hematopoietic stem cell transplant patients, plasma CMV DNA PCR showed higher sensitivity for detecting CMV in the blood than CMV antigenemia assay (98.8% *vs.* 45.2%, respectively), and the PCR assay detected more episodes of active CMV infection than did the antigenemia assay,[22] although the clinical utility of the PCR assay is disputed due to its low specificity. For diagnosing CMV gastrointestinal disease among immunocompromised patients, the sensitivity and specificity of the antigenemia assay were 54.4% to 65.4% and 87.5% to 93.6%, respectively.[18,23] Among patients with UC, however, few studies have been performed on the clinical utility of CMV antigenemia and blood CMV DNA PCR for diagnosing CMV colitis, and the diagnostic role of these tests in patients with UC is uncertain.

Therefore, we evaluated the clinical utility of CMV antigenemia assay and blood CMV DNA PCR for diagnosing CMV colitis in patients with moderate to severe UC.

2. Patients and methods

2.1. Patients

Medical records of patients with moderate to severe UC who had undergone biopsy of colonic tissue on suspicion of superimposed CMV colitis from January 2001 to December 2012 at Asan Medical Center (Seoul, Republic of Korea) were enrolled in the study. Patients who were tested for either CMV antigenemia assay or blood CMV DNA PCR were included. The patients' disease severity was graded by the Mayo score,[24] and patients with a total Mayo score of 6 or higher were included. Patients were endoscopically suspected to have superimposed CMV colitis if significant ulcers were present, which was defined by a Blackstone score of 8 or more, with more than 10 large ulcers (>5 mm) per 10 cm segment.[25,26] Two endoscopists, who had colonoscopic experience of more than 1000 cases each, determined whether the ulcers were significant in a blinded manner by reviewing endoscopic images retrospectively. Examples of significant and non-significant ulcers are shown in Fig. 1. For diagnosis of CMV colitis, colonic tissues were obtained from ulcers and were immunohistochemically stained for CMV. Patients were excluded if neither CMV antigenemia assay nor blood CMV DNA PCR was performed. The attending physician decided whether it was necessary to treat the CMV disease. In general, patients who either failed to show response to corticosteroids initially or who were aggravated despite initial response were treated with ganciclovir. The clinical data of 229 patients were collected from the medical records and reviewed retrospectively. Regarding the medication history, previous use of corticosteroids was classified into four groups as follows: (1) high-dose (intravenous or oral corticosteroids 40 mg/day or more); (2) moderate-dose (20 mg/day or more for >2 months); (3) low-dose (oral <20 mg/day or oral >20 mg/day for <2 months); and (4) no use of corticosteroids.[27] Recent use of thiopurines within the past one month[27] and anti-tumor necrosis factor agent use within the past eight weeks were also

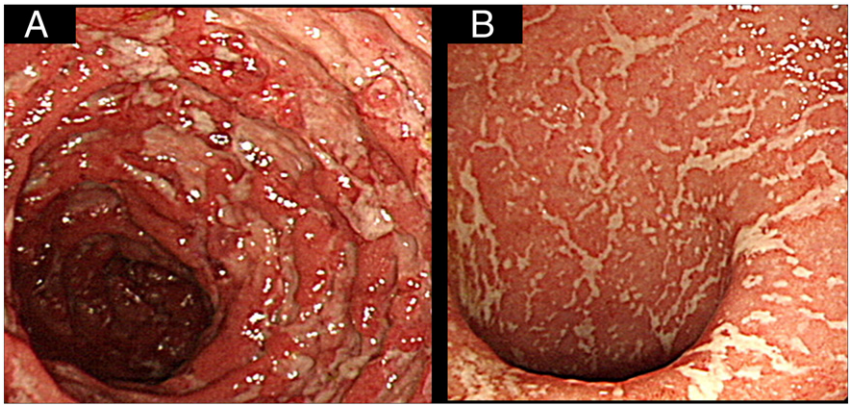

Figure 1 Significant *vs*. non-significant ulcers. A, Large deep, and punched-out "significant" ulcers. B, Multiple shallow ulcers with hyperemic mucosal change ("non-significant" ulcers).

investigated. We compared the clinical characteristics on the day of admission or endoscopy (whichever was earlier) between UC patients with CMV colitis and those without CMV colitis. Further data after evaluation of CMV status including ganciclovir and infliximab therapy and colectomy on the same admission were also collected.

2.2. Definition of CMV colitis

CMV colitis was defined as cases showing one or more inclusion bodies on hematoxylin and eosin (H&E) staining and/or positive CMV IHC staining in colonic biopsies.[18,23,28,29] Patients with negative results in both H&E staining and CMV IHC staining were classified as not having CMV colitis. Accordingly, the study subjects were classified as UC-CMV colitis group or UC only group.

2.3. CMV antigenemia assay and blood CMV DNA PCR test

The CMV antigenemia assay was carried out using the Light Diagnostics™ CMV-pp65 Antigenemia Immunofluorescence Assay (Chemicon International, Temecula, CA) for identifying the lower matrix protein pp65 of CMV in isolated peripheral blood leukocytes. The assay result was expressed as the number of CMV antigen-positive cells per 200,000 leukocytes, and a positive result for the CMV antigenemia assay was defined as one or more CMV-positive cells per 200,000 leukocytes applied.

The blood CMV DNA PCR was quantified using a commercially available real-time PCR test using a QIAamp Blood Mini Kit (QIAGEN, Valencia, CA).[30] A positive result for blood CMV PCR was defined as more than 250 copies/mL.

2.4. Microscopic examination and immunohistochemistry

One to six 1 to 3 mm tissues were obtained with colonoscopic biopsy. The tissues were immediately immerged in 10% neutral-buffered formalin and then, embedded in paraffin. For the microscopic examination, 4 μm thick sections were transferred to glass slides and stained with hematoxylin and eosin. For immunohistochemical staining, sections of 5 μm thickness were obtained with microtome from the paraffin block, transferred onto adhesive slides, and dried at 62°C for 30 min. Immunohistochemical staining with antibody against CMV (CCH2 + DDG9, 1:100, Dako, Glostrup, Denmark) was performed using a Benchmark automatic immunostaining device (Ventana Medical System, Tucson, AZ) according to the manufacturer's protocol. Slides were counterstained with hematoxylin. Positive results on immunohistochemical staining were classified into "rare positive staining cells (one or two positive cells)" and "multiple positive cells".[29]

2.5. Statistical analysis

Chi-squared or Fisher's exact tests were used to compare categorical variables, and Student's *t*-test or Mann–Whitney's *U* test for continuous variables, where appropriate. We used Cohen's κ coefficient index to evaluate the interobserver agreement on the presence of significant ulcers on endoscopy. Diagnostic performances of the CMV antigenemia test and the blood CMV PCR were expressed in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR−) with 95% confidence intervals (CIs). To identify the number of positive findings among the CMV antigenemia, blood CMV PCR, and significant ulcers on endoscopy that best predicted CMV colitis, the receiver operating characteristic (ROC) curves were plotted, and compared using a nonparametric method reported previously.[31]

Stata ver. 12.1 was used for the analysis (StataCorp, College Station, TX) and *p*-values less than 0.05 were considered statistically significant.

2.6. Ethical considerations

This study was approved by the institutional review board at Asan Medical Center (IRB no. 2012-0458).

3. Results

3.1. Demographic characteristics of the study subjects

There were 324 patients who were suspected of having CMV colitis. Among these, 72 patients had not been tested for either CMV antigenemia assay or blood CMV PCR, and were excluded. Also, patients with Mayo score of <6 were excluded (n = 23). The remaining 229 patients were included for analysis. There were 125 male patients (54.6%), and the median age at presentation was 42 years (interquartile range [IQR], 31–55). There were 83 patients who were diagnosed as having CMV colitis (36.2%, Table 1). The patients in the UC-CMV colitis group were older (median age, 48 *vs*. 41 years; $p = 0.005$), and a greater proportion of the UC-CMV colitis group was on either corticosteroids (56/83 [67.5%] *vs*. 67/146 [45.9%], $p = 0.002$) or thiopurines (23/83 [27.7%] *vs*. 20/146 [13.7%], $p = 0.009$) than in the UC only group. The Mayo score was significantly higher in the UC-CMV colitis patients (median 10 *vs*. 9; $p = 0.011$).

3.2. Endoscopic findings of study subjects

Comparing the agreement on the presence of significant ulcers among endoscopists, complete agreement was obtained in 83.8% of cases (n = 192, 37 cases of discrepancy). The Cohen's κ coefficient was 0.56. Among the agreed cases, 156 cases (81.3%) were classified as significant ulcers and 36 (18.7%) as non-significant. In cases where the endoscopists had agreed upon the presence/absence of significant ulcers, there was a significantly higher number of CMV colitis cases in the significant ulcer group compared with the non-significant ulcer group (65/156 [41.7%] *vs*. 8/36 [22.2%], $p = 0.036$).

3.3. Diagnostic accuracy indices of CMV antigenemia assay and blood CMV DNA PCR test

CMV antigenemia assay was performed in 225 patients (98.3%), and blood CMV PCR test in 177 patients (77.3%). In 173 patients (75.5%), both blood tests (*i.e*. CMV antigenemia and blood CMV PCR) were performed. The sensitivities of CMV antigenemia assay and blood CMV PCR test for CMV colitis were 47.0% and 44.3%, respectively (Table 2). The specificities of CMV antigenemia assay and blood CMV PCR test were 81.7% and 87.9%, respectively. The positive predictive values of these tests were 60.0% and 65.9%, respectively, and the negative predictive values were 72.5% and 75.0%, respectively. The sensitivity of significant ulcers on endoscopy (among agreed cases, n = 192) for CMV colitis was higher (88.9%) than the blood tests, but the

Table 1 Demographic characteristics of patients with moderate to severe UC (N = 229) who were tested for superimposed CMV colitis with endoscopic biopsy.

Variables	UC-CMV colitis (n = 83, 36.2%)	UC only (n = 146, 63.8%)	*p*-Value
Male gender	47 (56.6%)	78 (53.4%)	0.640
Age, yrs, median (IQR)	48 (36–59)	41 (28–51)	0.005
Duration of disease, months, median (IQR)	10 (2–59)	21 (4–64)	0.301
Disease extent in the last examination			0.755
Proctitis	11 (13.3%)	14 (9.6%)	
Left-sided	28 (33.7%)	45 (30.8%)	
Extensive	36 (43.4%)	71 (48.6%)	
Unknown	8 (9.6%)	16 (10.9%)	
Body mass index (kg/m^2), median (IQR)	20.2 (18.3–23.1)	20.7 (18.6–22.9)	0.609
Mayo score, median (IQR)	10 (9–11)	9 (8–10)	0.011
Corticosteroid use			<0.001 [a]
No	27 (32.5%)	79 (54.1%)	
Low-dose	6 (7.2%)	21 (14.4%)	
Moderate-dose	16 (19.3%)	23 (15.8%)	
High-dose	34 (41.0%)	23 (15.8%)	
Thiopurine use [b]	23 (27.7%)	20 (13.7%)	0.009
Anti-TNF agent use [c]	2 (2.4%)	4 (2.7%)	>0.999
Significant ulcers on endoscopy [d]	64/72 (88.9%)	92/120 (76.7%)	0.036
CMV antigenemia positive [e]	39/83 (47.0%)	26/142 (18.3%)	<0.001
Blood CMV DNA PCR positive [f]	27/61 (44.3%)	14/116 (12.1%)	<0.001

CMV, cytomegalovirus; PCR, polymerase chain reaction; TNF, tumor necrosis factor; UC, ulcerative colitis.
Data are number (%) of patients or median (interquartile range [IQR]), unless otherwise specified.

[a] *p*-trend.
[b] Within the last one month.
[c] Within the last 8 weeks.
[d] Among patients for whom the endoscopists had agreed upon the presence/absence of significant ulcers (n = 192).
[e] Among patients who were tested for CMV antigenemia (n = 225).
[f] Among patients who were tested for blood CMV PCR (n = 177).

Table 2 Diagnostic accuracy indices of CMV antigenemia, blood CMV DNA PCR, and significant ulcers on endoscopy to predict CMV colitis.

Findings	Sensitivity	Specificity	PPV	NPV	LR+	LR−
CMV antigenemia [a]	47.0% (35.9–58.3)	81.7% (74.3–87.7)	60.0% (47.1–72.0)	72.5% (64.9–79.3)	2.6 (1.7–3.9)	0.7 (0.5–0.8)
Blood CMV PCR [b]	44.3% (31.5–57.6)	87.9% (80.6–93.2)	65.9% (49.4–79.9)	75.0% (66.9–82.0)	3.7 (2.1–6.5)	0.6 (0.5–0.8)
Both blood tests positive [c]	44.2% (29.1–60.1)	90.6% (82.9–95.6)	67.9% (47.6–84.1)	78.4% (69.6–85.6)	4.7 (2.3–9.6)	0.6 (0.5–0.8)
Significant ulcers on endoscopy [d]	88.9% (79.3–95.1)	23.3% (16.1–31.9)	41.% (33.2–49.2)	77.8% (60.8–89.9)	1.2 (1.0–1.3)	0.5 (0.2–1.0)
Combinations of positive findings [e]						
One or more	96.3% (87.3–99.5)	21.3% (13.5–30.9)	41.3% (32.6–50.4)	90.9% (70.8–98.9)	1.2 (1.1–1.4)	0.2 (0.0–0.7)
Two or more	63.0% (48.7–75.7)	80.9% (71.4–88.2)	65.4% (50.9–78)	79.2% (69.7–86.8)	3.3 (2.1–5.2)	0.5 (0.3–0.7)
All findings	31.5% (19.5–45.6)	91.5% (83.9–96.3)	68.0% (46.5–85.1)	69.9% (61–77.9)	3.7 (1.7–8)	0.7 (0.6–0.9)
Significant ulcers with either CMV antigenemia or blood CMV PCR positivity [f]	67.3% (52.5–80.1)	75.7% (64–85.2)	66.0% (51.2–78.8)	76.8% (65.1–86.1)	2.8 (1.8–4.4)	0.4 (0.3–0.7)

CMV, cytomegalovirus; LR+, positive likelihood ratio; LR−, negative likelihood ratio; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value.
Values are expressed with their 95% confidence intervals based on binomial distribution.
[a] Among patients who were tested for CMV antigenemia assay (n = 225).
[b] Among patients who were tested for blood CMV PCR test (n = 177).
[c] Both CMV antigenemia assay and blood CMV PCR tests positive *vs.* both tests negative (n = 139).
[d] Among cases on which the endoscopists had agreed on the presence/absence of significant ulcers (n = 192).
[e] Findings, meaning CMV antigenemia, blood CMV PCR and significant ulcers, among patients who were tested for both CMV antigenemia assay and blood CMV PCR test, and among cases on which the endoscopists had agreed on the presence/absence of significant ulcers (n = 148).
[f] Among patients with agreed significant ulcers and both blood tests available (n = 119).

specificity was lower (23.3%). Combining these tests, if any blood test was positive (*i.e.*, CMV antigenemia assay or blood CMV DNA PCR) in the presence of significant ulcers, the sensitivity and specificity in predicting CMV colitis were 67.3% and 75.7%, respectively, with the area under the ROC curve value of 0.717 (Fig. 2). Among patients who showed significant ulcers on endoscopy and were tested for CMV antigenemia (152 out of 192 agreed cases), those who were positive for CMV antigenemia assay showed significantly higher probability of CMV colitis (33/50 [66.0%]) than the CMV antigenemia-negative patients (31/102 [30.4%]; $p < 0.001$). The results were similar for the blood CMV PCR test among patients with significant ulcers (25/37 [67.6%] *vs.* 24/86 [27.9%], respectively; $p < 0.001$). However, among patients with non-significant ulcers, only the CMV antigenemia assay showed a significant impact on the diagnosis of CMV colitis (CMV colitis among antigenemia-positive cases, 4/8 [50.0%]; CMV colitis among antigenemia-negative cases, 4/28 [14.3%]; $p = 0.032$), whereas the blood CMV PCR test did not (CMV colitis among PCR-positive cases, 1/2 [50.0%]; CMV colitis among PCR-negative cases, 4/27 [14.8%]; $p = 0.320$). In a stratified analysis (Table 3), the PPV of the blood tests was high in the presence of significant ulcers (66.0% and 67.6% for CMV antigenemia and blood CMV PCR, respectively), whereas the NPV was high with non-significant ulcers (85.7% and 85.2%, respectively).

3.4. Clinical course of UC-CMV colitis patients

Among UC-CMV colitis patients, there were 61 (73.5%) who subsequently underwent ganciclovir treatment. Having positive results on both CMV antigenemia assay and blood CMV PCR had significant association with subsequent administration of ganciclovir (Table 4, ganciclovir administration with both tests positive, 18/19 [94.7%]; both tests negative, 15/24 [62.5%]; $p = 0.026$).

In total, there were 40 patients (17.5%) who underwent colectomy during the same admission period. Among the UC-CMV colitis patients, there were 18/83 cases (21.7%) who underwent colectomy, and having positive CMV antigenemia assay showed significantly higher rate of colectomy (13/39 [33.3%] *vs.* 5/44 [11.4%]; $p = 0.015$) (Table 4).

Although ganciclovir administration and colectomy rates were higher with multiple positively staining cells on immunohistochemistry (56/75 [74.7%] and 17/75 [22.7%], respectively), they were not significantly different from those of patients showing rare positive cells (3/6 [50.0%] and 0/6 [0%]; with $p = 0.337$ and 0.334, respectively) (Table 4).

4. Discussion

In this study, we evaluated the diagnostic performances of CMV antigenemia assay and blood CMV PCR for CMV colitis

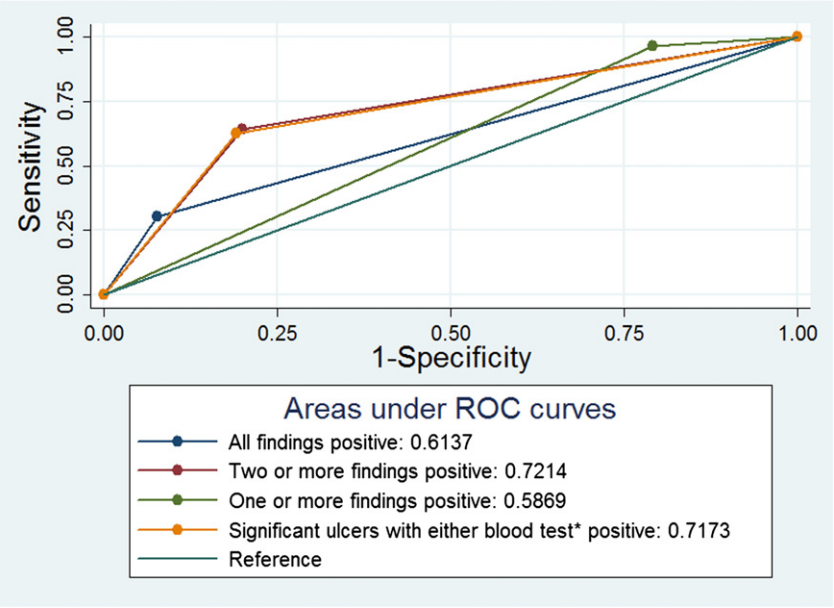

Figure 2 Receiver operating characteristic (ROC) curves of the number of positivity among CMV antigenemia assay, blood CMV PCR, and presence of significant ulcers on endoscopy in diagnosing CMV colitis. CMV, cytomegalovirus; PCR, polymerase chain reaction. *Blood test includes CMV antigenemia and blood CMV PCR. Analysis was performed among patients who were tested with both CMV antigenemia assay and blood CMV DNA PCR, and among cases where the endoscopists agreed on the presence/absence of significant ulcers (n = 148).

in moderate to severe UC patients. Our data showed that the sensitivities of CMV antigenemia assay and blood CMV PCR test were low (47.0% and 44.3%, respectively), whereas the specificities were relatively high (81.7% and 87.9%, respectively). The sensitivity values are lower than those of a previous study[18] among immunocompromised patients (64.9% by CMV antigenemia assay and 73.0% by blood CMV PCR), but higher than a small sized study[32] among UC patients (17.6% with antigenemia). The colectomy rate among UC-CMV colitis patients was significantly higher with positive antigenemia assay (33.3% *vs.* 11.4%; $p = 0.015$), whereas the degree of positively staining cells on immunohistochemistry showed no significant association with further colectomy.

Having used a stricter diagnostic criterion, the frequency of CMV colitis was lower in our study (36.7%) than those of previous studies (52.5%–56.7%).[18,32] We believe that the lower frequency by strict criteria could have led to lower sensitivity values of the blood tests. Also, non-real-time PCR method for blood CMV PCR test in our study could have contributed to a lower sensitivity. Since the presence of significant ulcers on endoscopy showed higher sensitivity and lower specificity than blood tests, we hypothesized that

Table 3 Diagnostic accuracy indices of CMV antigenemia, blood CMV DNA PCR according to presence of significant ulcers on endoscopy to predict CMV colitis.

Findings	Sensitivity	Specificity	PPV	NPV	LR+	LR−
Significant ulcers						
CMV antigenemia [a]	51.6% (38.7–64.2)	80.7% (70.9–88.3)	66.0% (51.2–78.8)	69.6% (59.7–78.3)	2.7 (1.6–4.4)	0.6 (0.5–0.8)
Blood CMV PCR [b]	51.0% (36.3–65.6)	83.8% (73.4–91.3)	67.6% (50.2–82.0)	72.1% (61.4–81.2)	3.2 (1.8–5.7)	0.6 (0.4–0.8)
Both [c]	51.5% (33.5–69.2)	86.9% (75.8–94.2)	68% (46.5–85.1)	76.8% (65.1–86.1)	3.9 (1.9–8.1)	0.6 (0.4–0.8)
Non-significant ulcers						
CMV antigenemia [a]	50.0% (15.7–84.3)	85.7% (67.3–96.0)	50.0% (15.7–84.3)	85.7% (67.3–96.0)	3.5 (1.1–11.0)	0.6 (0.3–1.2)
Blood CMV PCR [b]	20.0% (0.5–71.6)	95.8% (78.9–99.9)	50.0% (1.3–98.7)	85.2% (66.3–95.8)	4.8 (0.4–64.6)	0.8 (0.5–1.3)
Both [c]	33.3% (0.8–90.6)	95.2% (76.2–99.9)	50% (1.3–98.7)	90.9% (70.8–98.9)	7.0 (0.6–84.8)	0.7 (0.3–1.6)

CMV, cytomegalovirus; LR+, positive likelihood ratio; LR−, negative likelihood ratio; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value.
Values are expressed with their 95% confidence intervals based on binomial distribution.
Analysis performed on cases for which the endoscopists had agreed on the presence/absence of significant ulcers (n = 192).
[a] Among patients who were tested for CMV antigenemia assay (n = 188).
[b] Among patients who were tested for blood CMV PCR test (n = 152).
[c] Both CMV antigenemia assay and blood CMV PCR tests positive *vs.* both negative, among significant ulcer-agreed patients tested for both blood tests (n = 118).

Table 4 Clinical course of UC-CMV colitis patients according to the blood tests and IHC staining results.

CMV colitis patients (n = 83)	Ganciclovir treatment	*p*-value	Infliximab treatment	*p*-value	Colectomy	*p*-value
CMV antigenemia [a]		0.096		>0.999		0.015
Positive (n = 39)	32 (82.1%)		1 (2.6%)		13 (33.3%)	
Negative (n = 44)	29 (65.9%)		1 (2.3%)		5 (11.4%)	
Blood CMV PCR [b]		0.086		>0.999		0.930
Positive (n = 27)	23 (85.2%)		1 (3.7%)		5 (18.5%)	
Negative (n = 34)	22 (64.7%)		1 (2.9%)		6 (17.7%)	
CMV antigenemia and blood PCR [c]		0.026		>0.999		0.680
Both positive (n = 19)	18 (94.7%)		1 (5.3%)		4 (21.1%)	
Both negative (n = 24)	15 (62.5%)		1 (4.2%)		3 (12.5%)	
Positive IHC staining cells [d]		0.337		0.144		0.334
Multiple positive cells (n = 75)	56 (74.7%)		1 (1.3%)		17 (22.7%)	
Rare positive cells (n = 6) [e]	3 (50.0%)		1 (16.7%)		0 (0%)	

CMV, cytomegalovirus; IHC, immunohistochemical; PCR, polymerase chain reaction.
[a] Among CMV colitis patients who were tested for CMV antigenemia assay (n = 83).
[b] Among CMV colitis patients who were tested for blood CMV PCR test (n = 61).
[c] Among CMV colitis patients who were tested for both CMV antigenemia assay and blood CMV PCR test (n = 43).
[d] Among 83 UC-CMV colitis patients, there were 81 patients who were diagnosed by IHC staining, and two by H&E staining.
[e] One or two positive cells.

combination of these tests could provide compensatory clinical information. Still, the combination of these findings yielded sensitivity of 67.3%, with the area under the ROC curve value of 0.717. Although the sensitivity and PPV of this combination approach were not high, the blood tests may aid in clinical decision making in differential diagnosis of CMV colitis with their high specificity (75.7%) and negative predictive value (76.8%).

CMV colitis is a major cause of aggravation and clinical deterioration among patients with UC, and diagnosis at an early stage is essential.[14,33,34] However, clinical diagnosis of CMV colitis can be difficult, as it may merely be a surrogate marker for severe disease, not real aggravating role of CMV.[11] Considering the high false positive rate of colonic tissue PCR, some authors suggested only tissue IHC, and not tissue CMV PCR, to be used to diagnose CMV colitis.[11,35] In several previous studies, tissue CMV PCR-positive cases were classified as a separate diagnostic category,[23] or were given clinical significance.[32] However, the significance of a positive tissue CMV PCR in the absence of other histological signs of infection remains unclear.[11] We therefore used a stricter diagnostic criterion for CMV colitis using H&E and IHC staining only, and cases with tissue CMV PCR-positivity only were categorized into UC only group. The role of CMV in the exacerbations of UC is the topic of continuing debate.[11] Matsuoka et al.[36] and several others[37] reported that CMV reactivation disappears without antiviral treatment in UC patients. However, Roblin et al. reported that antiviral therapy has allowed some tissue CMV-positive patients with severe colitis who were resistant to corticosteroids or anti-TNFs to achieve clinical remission and avoid colectomy.[38] Also, Yoshino et al. reported that among 12 CMV DNA-positive patients with UC treated with ganciclovir, 10 patients (83.3%) went into remission.[32] Other studies showed 60.0% to 83.3% response to antiviral therapy among patients with steroid-refractory disease and CMV reactivation.[5,6,8] Both the American College of Gastroenterology Practice Guidelines and the European Crohn's and Colitis Organization Consensus also recommend treatment with antiviral agents when CMV is detected in colonic tissue in refractory UC patients.[10,39] Whether or not to treat CMV colitis is beyond the scope of this study, but since CMV antigenemia showed significant association with colectomy rate in our data, the presence of CMV antigenemia may provide clinically meaningful information among UC-CMV patients.

In our patients with CMV colitis, the median age was older and more patients were on corticosteroids or thiopurines than those with non-CMV colitis, which is in accordance with previous studies.[14,33] Also, more patients had significant ulcers in the CMV colitis group. In a recent study by Suzuki et al.,[40] punched-out ulceration, longitudinal configuration, irregular ulceration, and cobblestone-like appearance were more frequently observed with CMV colitis. Complicated CMV infection in UC is considered to cause significant colonic ulcers.[40] However, judging the ulcers as significant or not could be inconsistent among endoscopists. Still, there was a relatively high degree of agreement between two endoscopists in our study (83.7% of cases). By using cases where two blinded endoscopists had agreed upon the presence of significant ulcers, we intended to include only cases with certainty in "significant" ulcers. According to our results, CMV antigenemia assay and blood CMV PCR test should have diagnostic utility only in conjunction with significant ulcers on endoscopy, since these tests showed no meaningful predictability in diagnosing CMV colitis for cases with non-significant ulcers.

This study has several limitations. First, the intensity and the number of positively staining cells per high power field on tissue IHC staining have not been taken into account, which could have provided additional information. Second, this was a retrospective study, conducted in a single center, which could cause biased results. Third, tissue real-time PCR was not used in our study. In a recent report,[38] cut-off values of CMV copies by real-time PCR were associated with resistance to treatment. Therefore, real-time PCR could have provided additional information regarding diagnosis of CMV colitis in our study.

In conclusion, CMV antigenemia assay and blood CMV DNA PCR seem to have low sensitivities for diagnosing CMV colitis in patients with moderate to severe UC, and these tests should be interpreted in conjunction with endoscopic findings. Considering the low sensitivity values, CMV antigenemia and blood DNA PCR could not substitute for endoscopic biopsies. However, since the specificity and negative predictive values of CMV antigenemia and blood CMV PCR are relatively high, these tests could aid in early differential diagnosis of CMV colitis. Also, CMV antigenemia assay may predict clinical course of UC-CMV colitis patients.

Conflict of interest statement

Suk-Kyun Yang has received a research grant from Janssen Korea Ltd. For the rest of the authors, there is no conflict of interest in this study.

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Statement of authorship: JWK, SJB and BDY conceived the study; JWK, SJB, and BDY collected and interpreted the data; CLK collected data; JK and SAK gave advice in pathological interpretation; SHP, SKP, DHY, KWJ, KJK, JSB, SJM and JHK cared patients and critically reviewed the manuscript; JWK and SJB drafted the manuscript; SKY and BDY cared the patients and critically reviewed and revised the manuscript; BDY is the guarantor of the article and approved the final manuscript. All authors read and approved the final manuscript.

References

1. Colomba C, Lalicata F, Siracusa L, Di Bona D, Giammanco G, De Grazia S, et al. Cytomegalovirus infection in immunocompetent patients. Clinical and immunological considerations. *Infez Med* 2012;**20**:12–5.
2. Gallant JE, Moore RD, Richman DD, Keruly J, Chaisson RE. Incidence and natural history of cytomegalovirus disease in patients with advanced human immunodeficiency virus disease treated with zidovudine. The Zidovudine Epidemiology Study Group. *J Infect Dis* 1992;**166**:1223–7.
3. Mori T, Mori S, Kanda Y, Yakushiji K, Mineishi S, Takaue Y, et al. Clinical significance of cytomegalovirus (CMV) antigenemia in the prediction and diagnosis of CMV gastrointestinal disease after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2004;**33**:431–4.
4. Kim CH, Bahng S, Kang KJ, Ku BH, Jo YC, Kim JY, et al. Cytomegalovirus colitis in patients without inflammatory bowel disease: a single center study. *Scand J Gastroenterol* 2010;**45**:1295–301.
5. Cottone M, Pietrosi G, Martorana G, Casa A, Pecoraro G, Oliva L, et al. Prevalence of cytomegalovirus infection in severe refractory ulcerative and Crohn's colitis. *Am J Gastroenterol* 2001;**96**:773–5.
6. Domenech E, Vega R, Ojanguren I, Hernandez A, Garcia-Planella E, Bernal I, et al. Cytomegalovirus infection in ulcerative colitis: a prospective, comparative study on prevalence and diagnostic strategy. *Inflamm Bowel Dis* 2008;**14**:1373–9.
7. Maher MM, Nassar MI. Acute cytomegalovirus infection is a risk factor in refractory and complicated inflammatory bowel disease. *Dig Dis Sci* 2009;**54**:2456–62.
8. Papadakis KA, Tung JK, Binder SW, Kam LY, Abreu MT, Targan SR, et al. Outcome of cytomegalovirus infections in patients with inflammatory bowel disease. *Am J Gastroenterol* 2001;**96**:2137–42.
9. Kaufman HS, Kahn AC, Iacobuzio-Donahue C, Talamini MA, Lillemoe KD, Hamilton SR. Cytomegaloviral enterocolitis: clinical associations and outcome. *Dis Colon Rectum* 1999;**42**:24–30.
10. Rahier JF, Ben-Horin S, Chowers Y, Conlon C, De Munter P, D'Haens G, et al. European evidence-based consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. *J Crohns Colitis* 2009;**3**:47–91.
11. Lawlor G, Moss AC. Cytomegalovirus in inflammatory bowel disease: pathogen or innocent bystander? *Inflamm Bowel Dis* 2010;**16**:1620–7.
12. Nakase H, Yoshino T, Matumura K, Honzawa Y, Yamamoto S, Matsuura M, et al. Positive finding of colonic polymerase chain reaction for cytomegalovirus DNA is not false positive but a warning for treating patients with ulcerative colitis refractory to immunosuppressive therapies. *Inflamm Bowel Dis* 2011;**17**:E13–4.
13. Drew WL. Diagnosis of cytomegalovirus infection. *Rev Infect Dis* 1988;**10**(Suppl 3):S468–76.
14. Goodgame RW. Gastrointestinal cytomegalovirus disease. *Ann Intern Med* 1993;**119**:924–35.
15. Kambham N, Vij R, Cartwright CA, Longacre T. Cytomegalovirus infection in steroid-refractory ulcerative colitis: a case–control study. *Am J Surg Pathol* 2004;**28**:365–73.
16. Almeida N, Romaozinho JM, Amaro P, Ferreira M, Cipriano MA, Leitao MC. Fatal mid-gastrointestinal bleeding by cytomegalovirus enteritis in an immunocompetent patient. *Acta Gastroenterol Belg* 2009;**72**:245–8.
17. Frank D, Raicht RF. Intestinal perforation associated with cytomegalovirus infection in patients with acquired immune deficiency syndrome. *Am J Gastroenterol* 1984;**79**:201–5.
18. Nagata N, Kobayakawa M, Shimbo T, Hoshimoto K, Yada T, Gotoda T, et al. Diagnostic value of antigenemia assay for cytomegalovirus gastrointestinal disease in immunocompromised patients. *World J Gastroenterol* 2011;**17**:1185–91.
19. Michaelides A, Liolios L, Glare EM, Spelman DW, Bailey MJ, Walters EH, et al. Increased human cytomegalovirus (HCMV) DNA load in peripheral blood leukocytes after lung transplantation correlates with HCMV pneumonitis. *Transplantation* 2001;**72**:141–7.
20. Moon SM, Sung H, Kim MN, Lee SO, Choi SH, Kim YS, et al. Diagnostic yield of the cytomegalovirus (CMV) antigenemia assay and clinical features in solid organ transplant recipients and hematopoietic stem cell transplant recipients with CMV pneumonia. *Transpl Infect Dis* 2012;**14**:192–7.
21. Greanya ED, Partovi N, Yoshida EM, Shapiro RJ, Levy RD, Sherlock CH, et al. The role of the cytomegalovirus antigenemia assay in the detection and prevention of cytomegalovirus syndrome and disease in solid organ transplant recipients: a review of the British Columbia experience. *Can J Infect Dis Med Microbiol* 2005;**16**:335–41.
22. Gimeno C, Solano C, Latorre JC, Hernandez-Boluda JC, Clari MA, Remigia MJ, et al. Quantification of DNA in plasma by an automated real-time PCR assay (cytomegalovirus PCR kit) for surveillance of active cytomegalovirus infection and guidance of preemptive therapy for allogeneic hematopoietic stem cell transplant recipients. *J Clin Microbiol* 2008;**46**:3311–8.
23. Jang EY, Park SY, Lee EJ, Song EH, Chong YP, Lee SO, et al. Diagnostic performance of the cytomegalovirus (CMV) antigenemia

assay in patients with CMV gastrointestinal disease. *Clin Infect Dis* 2009;**48**:e121–4.
24. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N Engl J Med* 1987;**317**:1625–9.
25. Omiya M, Matsushita M, Tanaka T, Kawamata S, Okazaki K. The absence of large ulcer predicts latent cytomegalovirus infection in ulcerative colitis with positive mucosal viral assay. *Intern Med* 2010;**49**:2277–82.
26. Blackstone MO. Differentiation of ulcerative colitis from Crohn's colitis. Endoscopic interpretation: normal and pathologic appearance of the gastrointestinal tract. New York: Raven Press; 1984. p. 464–96.
27. Colombel JF, Loftus Jr EV, Tremaine WJ, Pemberton JH, Wolff BG, Young-Fadok T, et al. Early postoperative complications are not increased in patients with Crohn's disease treated perioperatively with infliximab or immunosuppressive therapy. *Am J Gastroenterol* 2004;**99**:878–83.
28. Preiksaitis JK, Brennan DC, Fishman J, Allen U. Canadian Society of Transplantation consensus workshop on cytomegalovirus management in solid organ transplantation final report. *Am J Transplant* 2005;**5**:218–27.
29. Mills AM, Guo FP, Copland AP, Pai RK, Pinsky BA. A comparison of CMV detection in gastrointestinal mucosal biopsies using immunohistochemistry and PCR performed on formalin-fixed, paraffin-embedded tissue. *Am J Surg Pathol* 2013;**37**:995–1000.
30. Mendez J, Espy M, Smith TF, Wilson J, Wiesner R, Paya CV. Clinical significance of viral load in the diagnosis of cytomegalovirus disease after liver transplantation. *Transplantation* 1998;**65**:1477–81.
31. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988;**44**:837–45.
32. Yoshino T, Nakase H, Ueno S, Uza N, Inoue S, Mikami S, et al. Usefulness of quantitative real-time PCR assay for early detection of cytomegalovirus infection in patients with ulcerative colitis refractory to immunosuppressive therapies. *Inflamm Bowel Dis* 2007;**13**:1516–21.
33. Baroco AL, Oldfield EC. Gastrointestinal cytomegalovirus disease in the immunocompromised patient. *Curr Gastroenterol Rep* 2008;**10**:409–16.
34. Galiatsatos P, Shrier I, Lamoureux E, Szilagyi A. Meta-analysis of outcome of cytomegalovirus colitis in immunocompetent hosts. *Dig Dis Sci* 2005;**50**:609–16.
35. Kandiel A, Lashner B. Cytomegalovirus colitis complicating inflammatory bowel disease. *Am J Gastroenterol* 2006;**101**:2857–65.
36. Matsuoka K, Iwao Y, Mori T, Sakuraba A, Yajima T, Hisamatsu T, et al. Cytomegalovirus is frequently reactivated and disappears without antiviral agents in ulcerative colitis patients. *Am J Gastroenterol* 2007;**102**:331–7.
37. Leveque N, Brixi-Benmansour H, Reig T, Renois F, Talmud D, Brodard V, et al. Low frequency of cytomegalovirus infection during exacerbations of inflammatory bowel diseases. *J Med Virol* 2010;**82**:1694–700.
38. Roblin X, Pillet S, Oussalah A, Berthelot P, Del Tedesco E, Phelip JM, et al. Cytomegalovirus load in inflamed intestinal tissue is predictive of resistance to immunosuppressive therapy in ulcerative colitis. *Am J Gastroenterol* 2011;**106**:2001–8.
39. Kornbluth A, Sachar DB. Ulcerative colitis practice guidelines in adults (update): American College of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 2004;**99**:1371–85.
40. Suzuki H, Kato J, Kuriyama M, Hiraoka S, Kuwaki K, Yamamoto K. Specific endoscopic features of ulcerative colitis complicated by cytomegalovirus infection. *World J Gastroenterol* 2010;**16**:1245–51.