Performance of Cytomegalovirus Real-Time Polymerase Chain Reaction Assays of Fecal and Plasma Specimens for Diagnosing Cytomegalovirus Colitis

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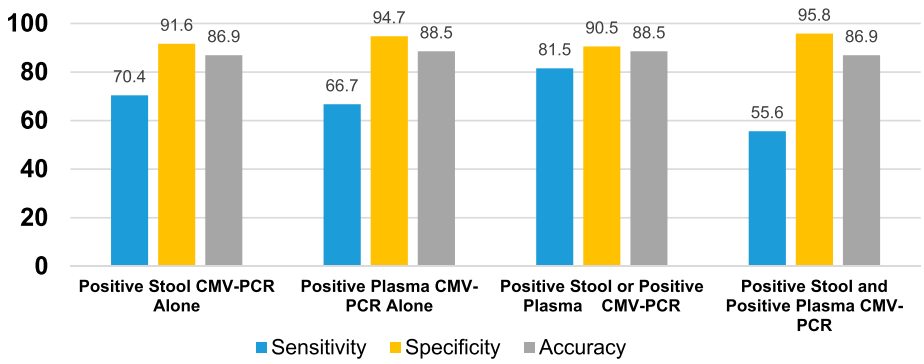
INTRODUCTION: **Cytomegalovirus (CMV) viral load detected by real-time polymerase chain reaction (PCR) in plasma or stool may facilitate detection of CMV colitis.**

METHODS: **This prospective study enrolled 117 patients with clinically suspected CMV colitis. Patients presenting with gastrointestinal symptoms and having increased risk of CMV infection were eligible. All participants underwent colonoscopy with tissue biopsy. Five patients underwent colonoscopy twice because of clinical recurrence, resulting in a total of 122 colonoscopies. Stool CMV-PCR and plasma CMV-PCR were performed within 7 days before/after colonoscopy. Twenty asymptomatic volunteers also underwent the same protocol.**

Stool and Plasma CMV-PCR in Diagnosis of CMV Colitis

- 117 patients with clinically suspected CMV colitis underwent colonoscopy
- Stool and plasma specimens were obtained within 7 days of colonoscopy



Both positive tests can be used to rule in,
while both negative tests can be used to rule out CMV colitis

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RESULTS: **Twenty-seven (23.1%) of 122 colonoscopies yielded positive for CMV colitis. The sensitivity and specificity was 70.4% and 91.6% for stool CMV-PCR and 66.7% and 94.7% for plasma CMV-PCR, respectively. The sensitivity of either positive plasma or positive stool CMV-PCR was 81.5%, which is significantly higher than that of plasma CMV-PCR alone ($P = 0.045$). However, positive results from both tests yielded a specificity of 95.8%, which is significantly higher than that of stool CMV-PCR alone ($P = 0.045$). There was a good and significant correlation between stool CMV-PCR and plasma CMV-PCR ($r = 0.71$, $P < 0.01$), and both tests significantly correlated with the cytomegalic cell count ($r = 0.62$, $P < 0.01$ for stool and $r = 0.64$, $P < 0.01$ for plasma). There were no positive stool or plasma CMV-PCR assays among volunteers.**

DISCUSSION: **The results of this study strongly suggest that the combination of stool CMV-PCR and plasma CMV-PCR can be used to confidently rule in (both positive) or rule out (both negative) a diagnosis of CMV colitis.**

KEYWORDS: performance; real-time polymerase chain reaction assays; fecal and plasma specimens; diagnosing; cytomegalovirus colitis; CMV colitis

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A917.

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INTRODUCTION

Cytomegalovirus (CMV)-related gastrointestinal (GI) diseases are common and can involve various organs, including the oral cavity, esophagus, stomach, small intestine, and large intestine—the last of which is the most common site of CMV-GI infection (1–4). Two-thirds of patients with CMV-GI disease are immunocompromised (5–7). The risk factors for CMV-GI infection include HIV infection with low CD4 count (2,8,9), post–organ transplantation (10–14), and taking immunosuppressive agents, especially corticosteroids (6,7,15,16). Interestingly, CMV-GI disease is being increasingly reported in immunocompetent hosts. The important risk factors for CMV-GI disease in immunocompetent patients were reported to be old age and being critically ill with multiple comorbid illnesses (6,7,16–19). Inflammatory bowel disease (IBD) also increases the risk of developing CMV-GI disease, despite not receiving immunosuppressive agents (20–22).

Diagnosis of CMV colitis requires colonoscopy with tissue biopsy. The presence of viral inclusion on histologic specimens stained with hematoxylin and eosin (H&E) has high specificity, but low sensitivity for diagnosing CMV colitis, with reported sensitivities and specificities of 10%–87% and 92%–100%, respectively. Immunohistochemistry (IHC) stain increases the diagnostic sensitivity to 78%–93%. Therefore, the combination of H&E staining and IHC has been recommended as the gold standard for diagnosing CMV colitis (23–25). Tissue polymerase chain reaction (PCR) assay, which amplifies CMV DNA, has been reported to increase the diagnostic sensitivity to 92%–96.7% (23,26). However, because of its high sensitivity, colonic tissue PCR might detect latent CMV replication in colonic tissue in patients who do not have a clinically significant infection (27–29). Quantitative tissue PCR, which is able to select different cutoffs of tissue viral load, has been reported to increase the specificity while maintaining sensitivity (30). Furthermore, there is a good correlation between tissue CMV viral load and the density of CMV-infected cells (31). The European Crohn's and Colitis Organization guideline recommends that colonic tissue PCR can be used to diagnose CMV colitis in patients with IBD (32). However, the data to define the optimal cutoff of tissue viral load for diagnosis of CMV colitis are currently limited to a few studies (30,33).

Although colonoscopy with tissue sampling remains the mainstay method for diagnosing CMV colitis, colonoscopy may not be feasible in some situations because of the high risk of procedural complications, such as in patients with profound neutropenia, thrombocytopenia, or severe illnesses. Plasma CMV-PCR is a noninvasive test that detects CMV viremia, which could be associated with CMV-GI disease. However, the results of previous studies that investigated the use of plasma CMV-PCR for diagnosing CMV colitis are inconsistent (34,35). Moreover, there has been increasing interest in stool CMV-PCR in diagnosing CMV colitis. Stool CMV-PCR is a noninvasive test that can be either qualitative or quantitative. In a pilot study, Herfarth et al (36) reported a sensitivity and specificity of stool CMV-PCR for detecting CMV DNA of 83% and 93%, respectively, in 19 patients with IBD. Since then, only a few studies have reported the performance of stool CMV-PCR for diagnosing CMV colitis, and the results of those studies are inconsistent. The reported sensitivity ranges from 16.7% to 85%, and the specificity ranges from 71% to 96% (37–40). Furthermore, and importantly, the diagnostic performance of combining plasma and stool CMV-PCR, which may improve the diagnostic performance, has not been studied.

Accordingly, the aim of this study was to investigate the diagnostic performance of stool CMV-PCR, plasma CMV-PCR, and the combination of stool CMV-PCR and plasma CMV-PCR for diagnosing CMV colitis using tissue histopathology (H&E stain and IHC) as a standard reference in patients with clinical suspicion of CMV colitis. We also evaluated the correlation between the number of CMV-infected cells in colonic tissue and the CMV viral load in stool, plasma, and colonic tissue.

METHODS

Study design and participants

This prospective cohort study was conducted at Siriraj Hospital, Mahidol University, Bangkok, Thailand, from October 2020 to October 2021. Patients older than 18 years with clinical suspicion of CMV colitis were enrolled into the study group. Twenty asymptomatic volunteers were also enrolled—all of whom underwent the same study protocol. Clinical suspicion for CMV colitis was established by the presence of at least one risk factor from the list in section 1 below and presenting with at least one GI tract symptom from the list in section 2 below.

1. Risk factors for CMV colitis:
   a. Immunocompromised status, including at least one of the following: HIV infection, solid organ or hematologic stem

cell transplantation, active hematologic malignancy, or receiving immunosuppressive agents or chemotherapy at the time of enrollment.
   b. Patients without obvious immunocompromised status, but having some risk factors for CMV colitis, including being critically ill (defined by the presence of organ failure or requiring inotropic agents); age older than 60 years; or having multiple comorbid illnesses, including diabetes mellitus (DM), atherosclerosis, or chronic kidney disease (6,7,16–19).
   c. IBD (20–22).
2. Presenting GI tract symptoms, including diarrhea, lower GI bleeding, bowel ileus, or pseudointestinal obstruction.

All participants underwent colonoscopy with tissue biopsy. Histopathologic analysis of the tissue biopsy was performed using both H&E and IHC stain. An experienced GI pathologist (N.A.) counted the number of infected cells in biopsy specimens with IHC. Two pieces of colonic tissue were sent for quantitative CMV-PCR (CMV R-GENE kit, limit of detection 450 copies/mL; bioMérieux SA, Marcy-l'Étoile, France). Patients who could not be proceeded to colonoscopy with tissue biopsy were excluded. Quantitative stool CMV-PCR (CMV R-GENE kit, limit of detection 450 copies/mL; bioMérieux SA) and quantitative plasma CMV-PCR (cobas CMV, limit of detection 150 copies/mL; Roche Diagnostics, Basel, Switzerland) were performed within 7 days before or after colonoscopy.

Patients diagnosed with CMV colitis based on detection of either cytomegalic cells on H&E stain or IHC-CMV cells were treated with ganciclovir for at least 2 weeks and then underwent follow-up colonoscopy with tissue biopsy to confirm complete treatment response. Stool specimens were also recollected and sent for CMV-PCR. The patients diagnosed with non-CMV colitis received treatment appropriate to their diagnosis. Twenty asymptomatic volunteers were enrolled into our asymptomatic control group, and all 20 of those subjects followed the same study protocol. A flow diagram describing subject enrollment, the study protocol, and the diagnostic outcome for both study patients and asymptomatic volunteers is shown in Figure 1.

CMV-infected cell morphologic diagnosis and cell count methods

The H&E and CMV IHC study (clone CCH2+DDG9, DAKO, Denmark, 1:150 dilution) slides were retrieved from our archive and digitalized by whole slide scanner (Pannoramic 1,000, 3DHISTECH Ltd., Hungary) with ×40 objective lens (numerical aperture 0.95, pixel resolution 0.12 μm).

The digital H&E and CMV-IHC slides were reviewed (CaseViewer software, 3Dhistech) by pathologists. A CMV-positive cell is defined by the presence of IHC-positive intranuclear and/or intracytoplasmic inclusion. The CMV-positive cells and area of the biopsied tissue were counted and calculated by digital image analysis software (Quantcenter, 3DHISTECH) and recorded as the number of CMV-positive cells per mm^2 (Figures 2a,b).

Stool, plasma, and colonic tissue PCR technique

For stool CMV-PCR, CMV DNA from stool was extracted using a MagLEAD 12gC automated extraction platform (Precision System Science, Chiba, Japan). Briefly, the stool specimen was resuspended with phosphate-buffered saline at a concentration of 20% (g/mL) and then subjected to a freeze/thaw cycle 3 times. The supernatant was then collected and used for nucleic acid extraction. Quantitative CMV-PCR was performed using a CMV R-GENE kit (bioMérieux SA) according to the manufacturer's instructions.

For plasma CMV-PCR, 500 mL of plasma was placed into a Cobas AmpliPrep/Cobas TaqMan platform for nucleic acid extraction, amplification, and quantitation. Testing of plasma was performed using a Cobas AmpliPrep/Cobas TaqMan CMV assay (Roche Diagnostics) following the manufacturer's instructions.

For tissue CMV-PCR, the biopsied tissue was cut with scissors into small pieces and placed into a lysis buffer containing guanidine isothiocyanate. The tissue and buffer mixture was mixed thoroughly and incubated at room temperature for 20 minutes. The MagLEAD 12gC automated extraction platform (Precision System Science) was used to extract CMV DNA from 200 mL of lysed tissue. Extraction was performed according to the manufacturer's recommended protocol. Viral DNA was eluted with 100-mL buffer and used for real-time PCR assay. Quantitative CMV-PCR was performed using a CMV R-GENE kit (bioMérieux SA) according to the manufacturer's instructions. Ten milliliters of extracted DNA was added into the amplification mix, and the PCR was performed using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA).

Study outcomes

Primary outcomes. The performance of stool CMV-PCR, plasma CMV-PCR, and their combinations was calculated using the detection of either cytomegalic cells on H&E stain or IHC-CMV cells as the reference standard for diagnosis of CMV colitis among 117 patients with clinically suspected CMV colitis. Correlation between

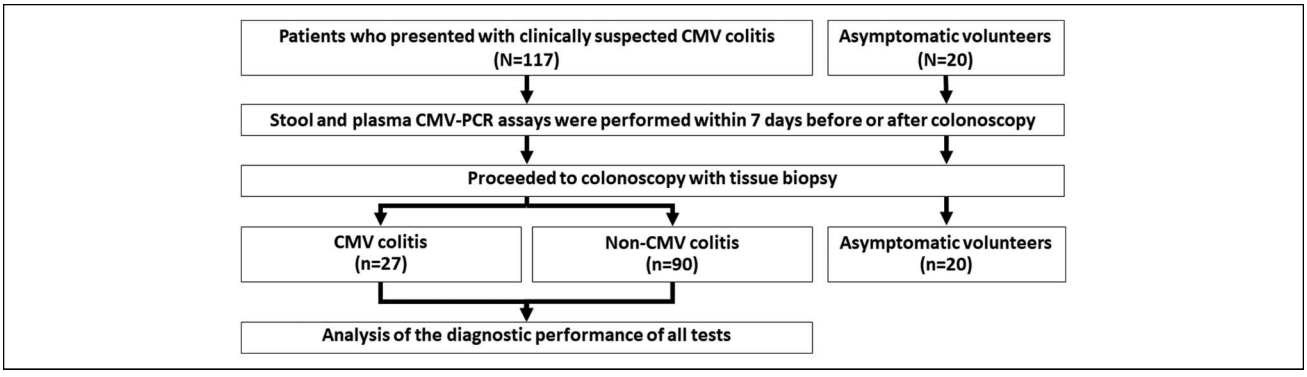

Figure 1. Flow diagram describing subject enrollment, the study protocol, and the diagnostic outcome for both study patients and asymptomatic volunteers. CMV, cytomegalovirus; CMV-PCR, cytomegalovirus polymerase chain reaction.

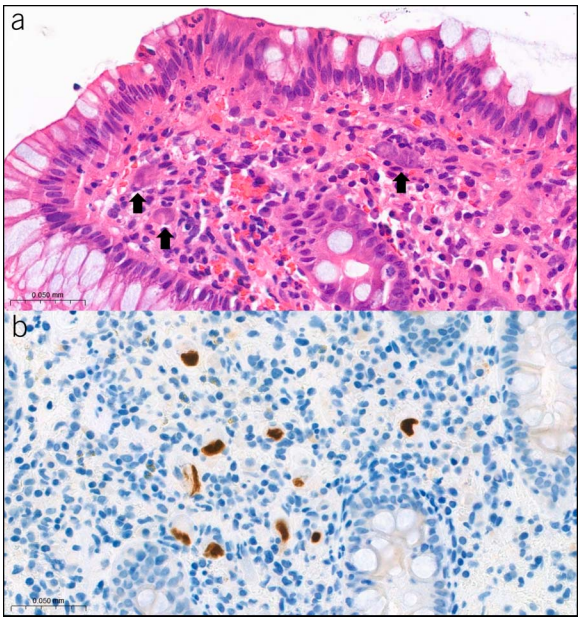

Figure 2. Images of H&E (×1,000) CMV-infected cells (arrow) reveal cytomegalic change with intranuclear and/or intracytoplasmic inclusions (**a**) and CMV IHC (×1,000) CMV-positive cells (**b**). CMV, cytomegalovirus; H&E, hematoxylin-eosin; IHC, immunohistochemistry.

cytomegalic cell count and quantitative tissue CMV-PCR, stool CMV-PCR, and plasma CMV-PCR was calculated. We also evaluated the performance of stool CMV-PCR in following up patients after treatment.

Secondary outcomes. The performance of the diagnostic tests using tissue CMV-PCR as the reference standard was assessed. Subgroup analysis assessed the performance of the diagnostic tests in patients with IBD, patients with immunocompromised status, and patients without obvious immunocompromised status but with some risk factors for CMV colitis.

Statistical analysis

Descriptive statistics were used to summarize patient characteristics. Continuous variables are expressed as median and interquartile range (IQR) or mean ± SD, and categorical variables are presented as number of subjects and percentage. Standard 2-group comparison methods were used, including independent *t* test (for normally distributed data) or Wilcoxon rank-sum test (for non-normally distributed data) for continuous data, and χ^2 test or Fisher exact test (depending on the size of the sample) for categorical data.

The diagnostic performance of stool CMV-PCR and plasma CMV-PCR is reported as the area under the receiver operating characteristic curve (AUC). The DeLong test was used to compare the AUCs of the stool and plasma CMV-PCR tests. After that, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using the optimal cutoff value obtained from the Youden Index. The McNemar test was used to determine the statistical significance of differences among stool CMV-PCR, plasma CMV-PCR, and their combination. Spearman correlation coefficients (*r*) were used to determine the correlation between the number of infected cells in colonic tissue and stool CMV-PCR, plasma CMV-PCR, and colonic tissue CMV-PCR. All statistical analyses were performed using SAS version OnDemand for Academics (SAS Institute, Cary, NC). A 2-tailed *P* value of <0.05 was regarded as being statistically significant for all tests.

The protocol for this study was approved by the Siriraj Institutional Review Board on October 1, 2020 (COA no. Si 810/2020), and was in accordance with both the 1964 Declaration of Helsinki (and all of its subsequent amendments) and Good Clinical Practice guidelines. The study was registered at http://clinicaltrials.gov (NCT05522283). All study and volunteer participants provided signed informed consent before participation in the study.

RESULTS

Patient characteristics

A total of 117 patients with clinical suspicion of CMV colitis were included, and of those, 27 (23.1%) patients were definitively diagnosed with CMV colitis. The most common diagnoses in the non-CMV colitis group were ulcerative colitis (n = 21), Crohn's disease (n = 11), drug-induced colitis (n = 9), and acute hemorrhagic rectal ulcer syndrome or stercoral ulcers (n = 8). A comprehensive list of non-CMV colitis diagnoses is shown in Supplementary Digital Content (see Supplementary Table S1, http://links.lww.com/CTG/A917). Patient and asymptomatic volunteer baseline characteristics are shown in Table 1. The mean age of study patients was 53 years, and 48% were men. Sixty-seven of 117 (57.3%) patients were immunocompromised, 11 (9.4%) had HIV infection, and 43 (36.7%) were treated with corticosteroids. Seventy patients (59.8%) were hospitalized. Of those, 37 patients had GI symptoms at admission, and the other 33 patients were admitted because of other indications and developed GI symptoms during hospitalization.

Study patient age and sex were not significantly different between the CMV and non-CMV groups. The proportion of patients with HIV infection tended to be higher in the CMV group, but the difference was not statistically significant. Furthermore, the dose of corticosteroids was significantly higher in the CMV colitis group ($P = 0.003$). The patients with CMV colitis were more seriously ill based on their higher rates of hospitalization, admission to the intensive care unit, acute kidney injury, and lower mean albumin levels. The common presentations in both groups were not different, including diarrhea and lower GI bleeding, with a median duration from symptom onset to hospital presentation of 14 days. Endoscopic findings revealed more ulcerative lesions in the CMV colitis group than in the non-CMV colitis group (88.9% vs 57.8%, respectively; $P = 0.002$). Among the 20 asymptomatic volunteers, the mean age was 52 years, and 45% were men. The mean age and sex distribution in the control group were not significantly different from the mean age and sex distribution in both of the study groups. All 20 volunteer subjects had normal colonoscopic findings.

Diagnostic performance of stool CMV-PCR, plasma CMV-PCR, and their combination using histopathologic diagnosis as the reference standard

Of the 117 study patients enrolled, 5 underwent colonoscopy twice because of recurrent symptoms, resulting in a total of 122 stool CMV-PCR tests, and 122 plasma CMV-PCR tests. Of those 122 colonoscopies, 27 colonoscopies yielded positive for CMV on histopathology. Table 2 shows the minimum (0th percentile), 25th percentile, median (50th percentile), 75th percentile, and maximum (100th percentile) values of each diagnostic test. As shown in Table 2, the median stool CMV viral load was 2,357 copies/mL (IQR: 0–6,626) in the CMV group, which was

Table 1. Patient baseline characteristics of all study patients, compared between study patients with and without CMV colitis, and of asymptomatic volunteers[a]

Characteristics	All study patients (N = 117)	CMV colitis (n = 27)	Non-CMV colitis (n = 90)	*P*	Asymptomatic volunteers (n = 20)
Age (yr)	53.3 ± 18.4	55.8 ± 15.5	52.5 ± 19.2	0.41	52.10 ± 14.91
Male sex	56 (47.9%)	13 (48.2%)	43 (47.8%)	0.97	9 (45.0%)
Underlying conditions					
Diabetes mellitus	18 (15.4%)	5 (18.5%)	13 (14.4%)	0.61	3 (15.0%)
Coronary artery disease	10 (8.6%)	5 (18.5%)	5 (5.6%)	0.03	3 (15.0%)
Cerebrovascular disease	14 (12.0%)	4 (14.8%)	10 (11.1%)	0.60	1 (5.0%)
Chronic kidney disease	19 (16.2%)	5 (18.5%)	14 (15.6%)	0.71	0 (0.0%)
HIV	11 (9.4%)	5 (18.5%)	6 (6.7%)	0.06	0 (0.0%)
CD4 count (cells/mm^3)	33.5 (18–293)	33.5 (27–156)	155.5 (8–495)	1.00	
Organ transplantation	12 (10.3%)	2 (7.4%)	10 (11.1%)	0.58	0 (0.0%)
Malignancy	18 (15.4%)	5 (18.5%)	13 (14.4%)	0.61	2 (10.0%)
Autoimmune disease	10 (8.6%)	2 (7.4%)	8 (8.9%)	0.81	1 (5.0%)
Inflammatory bowel disease	39 (33.3%)	6 (22.2%)	33 (36.7%)	0.16	0 (0.0%)
Active IBD	27/39 (69.2%)	4/6 (66.7%)	23/33 (69.7%)	1.00	
Immunosuppressive drugs	54 (46.1%)				
Corticosteroid	43 (36.7%)	7 (25.9%)	36 (40.0%)	0.18	0 (0.0%)
Prednisolone dose (mg/d)	7.5 (5–15)	30 (15–40)	5 (5–15)	**0.003**	0 (0.0%)
Other immunosuppressants	44 (37.6%)	10 (37.0%)	34 (37.8%)	0.94	0 (0.0%)
Chemotherapy	11 (9.4%)	4 (14.8%)	7 (17.8%)	0.27	1 (5.0%)
Immunocompromised status	67 (57.3%)	16 (59.3%)	51 (56.7%)	0.81	1 (5.0%)
Status					
Inpatient	70 (59.8%)	21 (77.8%)	49 (54.4%)	**0.03**	0 (0.0%)
GI symptoms at admission	37/70 (52.8%)	12/21 (57.1%)	25/49 (51.0%)	0.64	
ICU	15 (12.8%)	7 (25.9%)	8 (8.9%)	**0.02**	0 (0.0%)
On ventilator	19 (16.2%)	6 (22.2%)	13 (14.4%)	0.33	0 (0.0%)
On inotropic drugs	17 (14.5%)	7 (25.9%)	10 (11.1%)	0.055	0 (0.0%)
Acute kidney injury	24 (20.5%)	11 (40.7%)	13 (14.4%)	**0.003**	0 (0.0%)
Clinical presentations					
Symptom duration (d)	14 (2–60)	15 (2–42)	14 (2–60)	0.54	
Diarrhea	81 (52.1%)	14 (51.8%)	47 (52.2%)	0.97	
Lower GI hemorrhage	45 (38.5%)	13 (48.1%)	32 (35.6%)	0.23	
Abdominal pain	19 (16.2%)	1 (3.7%)	18 (20.0%)	0.07	
Fever	17 (14.5%)	6 (22.2%)	11 (12.2%)	0.19	
Laboratory findings					
Hemoglobin (mg/dL)	9.99 ± 2.39	9.41 ± 2.31	10.16 ± 2.40	0.15	
WBC (cells/uL)	7,878 ± 4,450	8,456 ± 6,846	7,705 ± 3,462	0.59	
Albumin (mg/dL)	3.21 ± 0.87	2.77 ± 0.78	3.35 ± 0.86	**0.002**	
Endoscopic findings					
Ulceration	76 (65.0%)	24 (88.9%)	52 (57.8%)	**0.002**	
Irregular/geographic	46 (39.7%)	16 (59.3%)	30 (33.7%)	**0.017**	
Deep ulcer	18 (15.5%)	7 (25.9%)	11 (12.4%)	0.09	
Punch out lesion	4 (3.5%)	2 (7.4%)	2 (2.2%)	0.23	

Table 1. (*continued*)

Characteristics	All study patients (N = 117)	CMV colitis (n = 27)	Non-CMV colitis (n = 90)	*P*	Asymptomatic volunteers (n = 20)
Inflamed mucosa	65 (55.6%)	18 (66.7%)	47 (52.2%)	0.18	
Mucosal hemorrhage	26 (22.2%)	9 (33.3%)	17 (18.9%)	0.11	
Location					
Ileocecum	42 (36.2%)	11 (40.7%)	31 (34.8%)	0.57	
Ascending and/or transverse	34 (29.3%)	11 (40.7%)	23 (25.8%)	0.13	
Descending and/or sigmoid	50 (42.7%)	15 (55.7%)	35 (38.9%)	0.12	
Rectum	60 (51.3%)	18 (66.7%)	42 (46.7%)	0.06	

Data presented as number and percentage, mean plus/minus SD, or median and interquartile range.
A *P* value < 0.05 indicates statistical significance which are highlighted in bold.
CD4, cluster of differentiation 4; CMV, cytomegalovirus; GI, gastrointestinal; IBD, inflammatory bowel disease; ICU, intensive care unit; WBC, white blood cells.
[a]Asymptomatic volunteers are the subjects who undergo colonoscopy for colorectal cancer screening without any gastrointestinal symptoms.

significantly higher than the viral load value of 0 copies/ml (IQR: 0–0) in the non-CMV group ($P < 0.001$). Similarly, the median plasma CMV viral load was significantly higher in the CMV group than in the non-CMV group (345 copies/mL [IQR: 0–25,900] vs 0 copies/mL (IQR: 0–0), respectively; $P \leq 0.001$). As shown in Figure 3, the AUC for diagnosing CMV colitis was 0.818 (95% confidence interval [CI]: 0.724–0.911) for the stool CMV-PCR assay and 0.809 (95% CI: 0.715–0.902) for the plasma CMV-PCR assay. There was no significant difference between the stool CMV-PCR AUC and the plasma CMV-PCR AUC ($P = 0.854$). The identified optimal cutoff values to define a positive CMV finding was 450 copies/mL for the stool CMV-PCR assay and 161 copies/mL for the plasma CMV-PCR assay.

Using the immediately aforementioned cutoff values, 27 stool tests and 23 plasma tests were positive. As shown in Table 3 and Figure 4, stool CMV-PCR alone had a sensitivity and specificity of 70.4% and 91.6%, respectively, whereas the corresponding values for plasma CMV-PCR were 66.7% and 94.7%, respectively. When both tests were combined, positive results for both tests yielded a specificity of 95.8%, which is significantly higher than the 91.6% specificity of stool CMV-PCR alone ($P = 0.045$), and resulted in a PPV of 78.9% (95% CI: 60.6–97.3). Furthermore, the sensitivity of either positive plasma CMV-PCR or positive stool CMV-PCR was 81.5%, which was significantly higher than the 66.7% sensitivity of positive plasma CMV-PCR alone ($P = 0.045$), and resulted in a NPV of 94.5% (95% CI: 89.8–99.2). There were 12 inconsistent results between stool CMV-PCR and plasma CMV-PCR. Four of 8 (50%) positive stool CMV-PCR and negative plasma CMV-PCR tests were implicated in a final diagnosis of CMV colitis, and CMV colitis was diagnosed in 1 of 4 (25%) negative stool CMV-PCR and positive plasma CMV-PCR tests. There were no positive results for any stool CMV-PCR or plasma CMV-PCR tests among any of the 20 asymptomatic volunteer subjects.

Table 2. Range of quantitative data specific to cytomegalic cell counts per area of colonic tissue biopsy(cell/mm^2) and copies per milliliter from tissue, stool, and plasma CMV-PCR compared among patients with CMV colitis, patients with non-CMV colitis, and asymptomatic volunteers

Study group	Variables	Minimum (0th percentile)	25th percentile	Median (50th percentile)	75th percentile	Maximum (100th percentile)
CMV colitis	Cytomegalic cells, cells/mm^2	1.46	4.57	6.26	7.97	11.82
	Tissue, copies/mL	0	3,040	56,199	979,107	29,463,461
	Stool, copies/mL	0	0	2,357	6,626	425,317
	Plasma, copies/mL	0	0	345	25,900	283,000
Non-CMV colitis	Cytomegalic cells, cells/mm^2	0	0	0	0	0
	Tissue, copies/mL	0	0	0	0	1,992,358
	Stool, copies/mL	0	0	0	0	43,793
	Plasma, copies/mL	0	0	0	0	13,400
Asymptomatic volunteers	Cytomegalic cells, cells/mm^2	0	0	0	0	0
	Tissue, copies/mL	0	0	0	0	0
	Stool, copies/mL	0	0	0	0	0
	Plasma, copies/mL	0	0	0	0	0

CMV, cytomegalovirus; PCR, polymerase chain reaction.

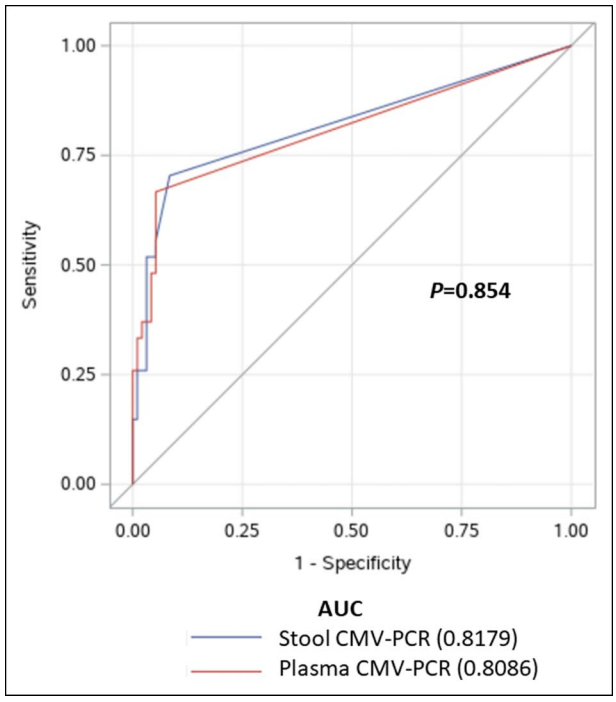

Figure 3. Receiver operating characteristic (ROC) curves to evaluate the sensitivity and specificity of the stool cytomegalovirus polymerase chain reaction (CMV-PCR) assay and the plasma CMV-PCR assay for diagnosing CMV colitis. AUC, area under the curve.

Correlation between cytomegalic cell count and quantitative tissue CMV-PCR, stool CMV-PCR, and plasma CMV-PCR

The correlations between cytomegalic cell count and quantitative data from tissue CMV-PCR, stool CMV-PCR, and plasma CMV-PCR are shown in Supplementary Digital Content (see Supplementary Table S2, http://links.lww.com/CTG/A917). The median cytomegalic cell count was 6.3 cells per mm^2 of colonic tissue biopsy from patients diagnosed with CMV colitis. The median viral load from tissue, stool, and plasma CMV-PCR was 56,199 copies/mL, 2,357 copies/mL, and 345 copies/mL, respectively. There was a good and significant correlation between stool CMV-PCR and plasma CMV-PCR ($r = 0.71$, $P < 0.0001$). Furthermore, both of those tests significantly correlated with cytomegalic cell count ($r = 0.62$, $P < 0.0001$ for stool CMV-PCR, and $r = 0.64$, $P < 0.01$ for plasma CMV-PCR) and with tissue CMV-PCR ($r = 0.68$, $P < 0.0001$ for stool CMV-PCR and $r = 0.68$, $P < 0.0001$ for plasma CMV-PCR), as shown in Supplementary Digital Content (see Supplementary Table S2, http://links.lww.com/CTG/A917).

Follow-up stool CMV-PCR to evaluate for CMV colitis after treatment

Twenty-six of the 27 patients diagnosed with CMV colitis were treated with intravenous ganciclovir or oral valganciclovir. The median duration of treatment was 21 days. After treatment, 13 patients underwent follow-up colonoscopy, and stool CMV-PCR was performed in 8 of 13 patients. Among the 4 patients in that group who were initially diagnosed with CMV colitis and who had a positive stool CMV-PCR, all 4 of the follow-up stool tests were negative for CMV after treatment. The follow-up stool CMV-PCR remained negative in the other 4 patients with CMV colitis that had a negative stool CMV-PCR before treatment.

Diagnostic performance of stool CMV-PCR, plasma CMV-PCR, and their combination using tissue CMV-PCR as the reference standard

When using the tissue CMV-PCR as the reference standard, 40 patients had CMV colitis. The sensitivity and specificity of stool CMV-PCR were 60.0% and 96.3%, respectively, whereas the corresponding values of plasma CMV-PCR were 52.5% and 97.6%, respectively. The sensitivity and specificity of pathological diagnosis were 57.5% and 95.1%, respectively. No difference was observed among each diagnostic modality ($P = 0.77$ for stool CMV-PCR and plasma CMV-PCR, $P = 0.80$ for stool CMV-PCR and pathological diagnosis, $P = 1.00$ for serum CMV-PCR and pathological diagnosis).

Subgroup analysis in patients with IBD, patients with immunocompromised status, and patients without obvious immunocompromised status but with some risk factors for CMV colitis

Among 122 colonoscopies, 42 were performed in patients with IBD (6 CMV and 36 non-CMV), 36 were performed in immunocompromised patients (10 CMV and 26 non-CMV), and 44 were performed in immunocompetent patients (11 CMV and 33 non-CMV). Stool CMV-PCR had a sensitivity and specificity of 33.3% and 94.4% in patients with IBD, 70.0% and 84.6% in immunocompromised patients, and 90.9% and 93.9% in immunocompetent patients, respectively. In comparison, plasma CMV-PCR had a sensitivity of 0% for patients with IBD, a sensitivity and

Table 3. Diagnostic performance of the 5 evaluated diagnostic modalities

Testing modalities	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
Stool CMV-PCR alone	70.4% (53.1–87.6)	91.6% (86.0–97.2)	70.4% (53.1–87.6)	91.6% (86.0–97.2)
Plasma CMV-PCR alone	66.7% (48.9–84.4)	94.7% (90.2–99.2)	78.3% (61.4–95.1)	90.9% (85.2–96.6)
Positive stool CMV-PCR and positive plasma CMV-PCR	55.6% (36.8–74.3)	95.8% (91.7-99.8)[a]	78.9% (60.6-97.3)[a]	88.3% (82.1–94.5)
Positive stool CMV-PCR or positive plasma CMV-PCR	81.5% (66.8-96.1)[a]	90.5% (84.6–96.4)	71.0% (55.0–86.9)	94.5% (89.8-99.2)[a]
Tissue PCR	85.2% (71.8–98.6)	83.2% (75.6–90.7)	59.0% (43.5–74.4)	95.2% (90.6–99.8)

[a]The highest value for that diagnostic performance parameter.
CI, confidence interval; CMV, cytomegalovirus; PCR, polymerase chain reaction; PPV, positive predictive value; NPV, negative predictive value.

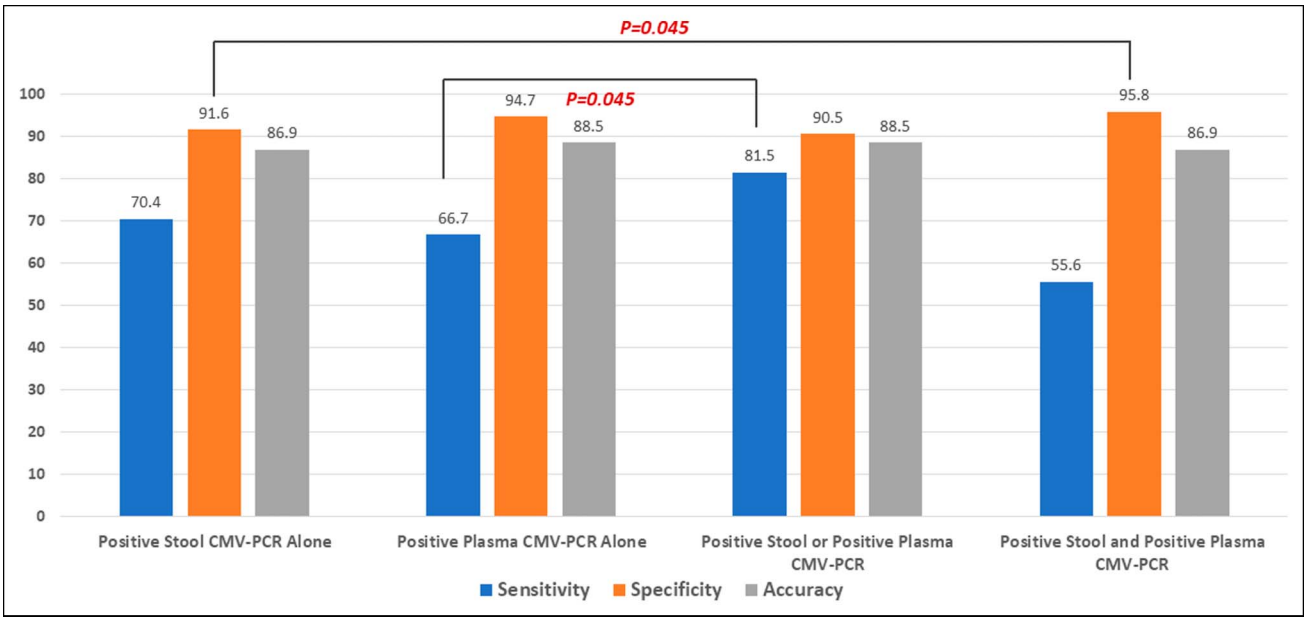

Figure 4. Diagnostic performance of a positive stool cytomegalovirus polymerase chain reaction (CMV-PCR) alone, a positive plasma CMV-PCR alone, a positive stool CMV-PCR or positive plasma CMV-PCR, and the combination of a positive stool CMV-PCR and a positive plasma CMV-PCR.

specificity of 90.0% and 84.6% for immunocompromised patients, and 81.8% and 96.9% for immunocompetent patients, respectively.

DISCUSSION

This prospective study reported the performance of stool CMV-PCR, plasma CMV-PCR, and their combinations in diagnosis of CMV colitis, the correlations among each diagnostic test, and the performance of stool CMV-PCR in following up after antiviral treatment. There have been few studies evaluating the performance of stool CMV-PCR in diagnosis of CMV colitis; however, the participants, the methods for definite diagnosis of CMV colitis, the PCR methods, and the results are varied as we summarized in Table 4 (36–43).

To best of our knowledge, our study is the first prospective study comprehensively collect and evaluate stool CMV-PCR, plasma CMV-PCR, tissue CMV-PCR, and CMV-infected cell count on histopathology from the same group of patients. All samples were collected within 7 days before or after colonoscopy. We included a broad variety of patients with risk factors for developing CMV colitis, including both immunocompromised and immunocompetent patients. We found an overall sensitivity and specificity of stool CMV-PCR for diagnosing CMV colitis of 70% and 91%, respectively, when using histopathologic findings as the reference standard, and our results are comparable with those from several previous reports (36–39). Most previous studies were conducted in immunocompromised patients. Micheal et al, Ganzenmeuller et al, and Prachasitthisak et al included various types of immunocompromised patients and reported sensitivity of 66%–71%, and specificity of 85%–96% (38,39,41). Two studies specifically included only patients with IBD. The reported sensitivity and specificity were 83%–84.7% and 71.4%–93%, respectively (36,37). Two studies specifically included only stem cell transplantation patients, and one of those studies required enrolled patients to have graft-vs-host disease. They reported the sensitivity and specificity of stool CMV-PCR to be 16.7%–28.6% and 76.2%–86.3%, respectively (40,43).

The diagnostic performance of plasma CMV-PCR for diagnosing CMV colitis in our study was 67% sensitivity and 95% specificity. A previous prospective study by Prachasitthisak et al (39) that included various types of immunocompromised patients reported the sensitivity and specificity of plasma CMV-PCR to be 100% and 85%, respectively. A prospective study by Harfarth et al (36) that included only patients with IBD reported a sensitivity of 80%, and a specificity of 100%, but plasma CMV-PCR was collected in only some patients because it was not in the study protocol. A retrospective study by Zavrelova et al (40) that included only stem cell transplant patients reported a sensitivity of 66.7%, and a specificity of 71.4%. Finally, a study by Chan et al (42) that included only intensive care unit patients reported the sensitivity of plasma CMV-PCR to be 100%.

Despite stool CMV-PCR alone and plasma CMV-PCR alone having been reported to have good performance for diagnosing CMV colitis, the reported sensitivity ranges from 60% to 70%, and the reported specificity ranges from 80% to 90%, as discussed above. The use of these 2 CMV-PCR tests in combination may improve the diagnosis of CMV colitis; however, combination use of these 2 tests in this clinical setting has not been previously studied. In this study, the combination of positive stool CMV-PCR and positive plasma CMV-PCR increased the specificity to 96% with a 79% PPV. Accordingly, when both tests are positive and colonoscopy is not feasible, the start of treatment for CMV colitis should be considered. When we evaluated this combination of CMV-PCR tests and only one or the other test was positive, we found the sensitivity to be 81% with a 94% NPV. As such, a negative result on both tests is strongly suggestive of no presence of CMV colitis.

To strengthen the reliability of the stool and plasma CMV-PCR tests, we analyzed their correlation to other parameters, particularly cytomegalic cell count from tissue histopathology. We found a significantly strong correlation among stool CMR-PCR, plasma CMV-PCR, cytomegalic cell count, and tissue CMV-PCR. By contrast, previous studies reported conflicting

Table 4. **Summary of previous studies that investigated the diagnostic performance of stool CMV-PCR for diagnosing CMV colitis**

Study	Study design	Population, n	CMV colitis incidence	Reference standard	PCR method	Stool collection	Stool PCR Sn/Sp	Plasma PCR Sn/Sp	Tissue PCR Sn/Sp	Cytomegalic cells
Michel et al, 1995	Prospective	36 ICM (17 AIDS and 19 post–stem cell transplant); 15 colonoscopies were performed in symptomatic patients	6/15 (40%)	H&E or IHC	Taq DNA polymerase LOD 2 × 10^4 CMV genome equivalents per ml (In house analysis)	Fresh stool 5 mL within 4 wk of colonoscopy	Positive 4/6 (66.7%)		Positive 6/15 (40%)	
Harfarth et al, 2010	Prospective pilot	19 IBD (11 UC and 8 CD); (steroid 16/19, thiopurine/MTX 7/19, anti-TNF 4/19)	6/19 (32%)	Tissue PCR	Primers and TaqMan probe LOD = 500 cp/mL (In house analysis)	1.5 g of fresh stool	✓ Sn 83% Sp 93%	✓ 15 cases Sn 80% Sp 100%	✓ Gold standard	
Chan et al, 2014	Retrospective	18 ICU patients with CMV colitis (excluded: post-transplant, HIV)	Biopsy-proven CMV colitis = 8 Probable CMV colitis = 10	H&E or IHC Probable CMV: Symptomatic + blood CMV-PCR pos + colonic ulcer or stool CMV-PCR pos	Primers and TaqMan probe (In house analysis)	Not mentioned (retrospective)	Positive 8/18 (44.4%), 2/3 biopsy-proven, 5/7 probable CMV	All positive		
Ganzenmueller et al, 2014	Retrospective	66 ICM (42% SCT, 26% SOT, 5% HIV, and 14% IBD)	12/66 (18%)	H&E or IHC tissue PCR >0.14 cp/cell	AB 7500 Cycler LOD = 500 cp/mL (In house analysis)	Not mentioned (retrospective)	✓ Sn 67% Sp 96%	CMV Antigen Sn 83%	✓ Gold standard	Significant correlation between stool and tissue CMV-PCR
Sun et al, 2015	Prospective	56 GVHD biopsy-proven in post-HSCT	7/58 (12%)	H&E or IHC	AB 7300 Cycler LOD = 1,000 cp/mL (QIAamp stool Mini Kit, Qiagen GmbH, German)	Stool and blood PCR within 24 hr of colonoscopy	✓ Sn 28.6% Sp 86.3%			
Prachasitthisak et al, 2017	Prospective	29 ICM (12 SOT, 4 SCT, 3 CMT, and 5 corticosteroid use)	7/27 (26%)	H&E	LOD = 20 cp/mL (Abbott RealTime CMV assay, USA)	Not mentioned (prospective)	✓ Sn 71% Sp 85%	✓ Sn 100% Sp 85%		

Table 4. (*continued*)

Study	Study design	Population, n	CMV colitis incidence	Reference standard	PCR method	Stool collection	Stool PCR Sn/Sp	Plasma PCR Sn/Sp	Tissue PCR Sn/Sp	Cytomegalic cells
Zavrelova et al, 2018	Retrospective	45 SCT	6/69 (8.7%)	H&E or IHC	LOD = 100 cp/mL (QIAamp DNA Mini Kit, Qiagen)	Not mentioned (retrospective)	✓ Sn 16.7% Sp 76.2%	✓ Sn 66.7% Sp 71.4%		
Magdziak et al, 2020	Prospective	75 active UC (89 tests) (steroid 72%, immunosuppressant 40%, and biologic 10%)	19/89 (21%)	H&E or IHC	Rotor Gene 6000/ Rotor Gene Q apparatus LOD = 42.5 cp/mL (QIAamp Stool Mini Kit, Qiagen of Hilden, German)	200 μL of supernatant from 215 to 220 mg of stool	✓ Sn 84.7% Sp 71.4%			✓ No correlation with stool PCR
This study, 2022	Prospective	117 patients (67 ICM and 50 ICP) (12 SOT, 11 HIV, 54 immunosuppressant, and 39 IBD) (122 tests) 20 asymptomatic volunteers	27/122 (22%)	H&E or IHC	LOD 450 copies/mL (CMV R-gene kit, bioMérieux, France)	Stool and blood PCR within 7 d of colonoscopy	✓ Sn 70.4% Sp 91.6%	✓ Sn 66.7% Sp 94.7%	✓ Sn 85.2% Sp 83.2%	✓ Strong correlation with colonic biopsy, stool, and blood PCR

AB, Applied Biosystems; CD, Crohn's disease; CMT, chemotherapy; CMV, cytomegalovirus; DNA, deoxyribonucleic acid; GVHD, graft-vs-host disease; H&E, hematoxylin and eosin stain; HSCT, hematopoietic stem cell transplantation; IBD, inflammatory bowel disease; ICM, immunocompromised; ICP, immunocompetent; ICU, intensive care unit; IHC, immunohistochemistry; LOD, limit of detection; MTX, methotrexate; PCR, polymerase chain reaction; SCT, stem cell transplant; Sn, sensitivity; SOT, solid organ transplant; Sp, specificity; TNF, tumor necrosis factor; UC, ulcerative colitis.

results. Ganzenmueller et al reported a significant association between stool CMV-PCR and tissue CMV-PCR, but Magdziac et al found no significant association between cytomegalic cell count and stool CMV-PCR (37,38). Furthermore, we performed follow-up stool CMV-PCR after treatment. We found that all previous positive results became negative after the treatment, which was consistent with the results of follow-up tissue histopathology.

Because tissue CMV-PCR was used as the standard diagnostic tool in some studies (36,38), we did another analysis using tissue CMV-PCR as the reference standard. The sensitivity of both stool and plasma CMV-PCR slightly decreased to 60% and 52.5%, respectively, whereas the specificities remained high, 96.3% for stool and 97.6% for plasma CMV-PCR.

We also did a subgroup analysis categorizing the patients according to the inclusion criteria. We found that the sensitivity of both stool and plasma CMV-PCR in patients with IBD was lower than previously reported (36,37). The difference in the PCR method might attribute to this variation (Table 4). Interestingly, we found that the sensitivity of stool CMV-PCR was higher than plasma CMV-PCR (33.3% vs 0%). This could be because CMV infection in patients with IBD was localized rather than systemic reactivation. The study by Ganzenmueller et al (30), which found that many patients with CMV intestinal disease showed no concomitant CMV antigenemia, suggesting a localized intestinal CMV replication, supported this hypothesis. By contrast, among immunocompromised patients, plasma CMV-PCR was more sensitive to stool CMV-PCR (90% vs 70%) in detecting CMV colitis. This result was consistent with the previous studies including only immunocompromised patients (39,40).

Strengths

This study has several important strengths. First, it is the largest study to investigate the performance of stool CMV-PCR for diagnosing CMV colitis. Second, our study is the first to report the results of stool CMV-PCR, plasma CMV-PCR, tissue CMV-PCR, and cytomegalic cell count from histopathology from the same cohort of patients. Having both stool CMV-PCR data and plasma CMV-PCR data allowed us to assess the performance of stool CMV-PCR, plasma CMV-PCR, and their combination, which our results strongly suggest will confer benefit in clinical practice. Furthermore, because all parameters were evaluated quantitatively, we are able to show strong correlations among stool CMV-PCR, plasma CMV-PCR, tissue CMV-PCR, and cytomegalic cell count from histology. Third, we repeated stool CMV-PCR after completing treatment to assess the correlation between stool CMV-PCR and histologic findings in the same patients at different time points. Fourth and last, we also enrolled 20 asymptomatic volunteers who were subjected to the same study protocol. Analysis of those 20 volunteers showed no presence of CMV in any volunteer for either the stool CMV-PCR or the plasma CMV-PCR. All these strengths support the value of the combined use of stool CMV-PCR and plasma CMV-PCR as a reliable alternative for diagnosing CMV colitis in clinical practice in scenarios where colonoscopy is, for some reason, contraindicated.

Limitations

This study has some mentionable limitations. First, this study was conducted in a single national tertiary care center that is routinely referred complex cases believed to be unmanageable at other levels of care. As such, the characteristics of patients in this study may not necessarily reflect the characteristics of patients in other care settings. Furthermore, the prevalence of CMV colitis in this study may be higher than in other settings. Second, the colonic tissue specimens were not obtained from the same anatomical location, and the number of biopsies was not equal among patients. However, the GI pathologist counted all cytomegalic cells in all tissue specimens, and we calculated the number of cytomegalic cells per mm^2 of the biopsy piece. Third and last, we did not enroll a separate group of asymptomatic volunteers who were immunocompromised. This is potentially notable because a previous study reported the possible presence of CMV viremia in asymptomatic immunocompromised patients (44).

Conclusion

The results of this study strongly suggest that the combination of stool CMV-PCR and plasma CMV-PCR can be used to rule in (both tests positive) or rule out (both tests negative) a diagnosis of CMV colitis with a high degree of confidence. In addition to the advantage of these PCR tests being noninvasive, they can be used in hospitalized patients with critical illness, with multiple comorbidities, or with immunocompromised status—all of which render colonoscopy an unfeasible or less favorable diagnostic alternative. In patients with contradictory results between the 2 tests, colonoscopy with tissue biopsy should be performed to establish the diagnosis. Moreover, follow-up stool CMV-PCR may be performed after treatment to ensure treatment success.

CONFLICTS OF INTEREST

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Study Highlights

WHAT IS KNOWN

- ✓ Diagnosis of cytomegalovirus colitis requires colonoscopy with tissue biopsy.
- ✓ Colonoscopy can cause serious complications, particularly in patients with severe illnesses.

WHAT IS NEW HERE

- ✓ The combined use of stool cytomegalovirus (CMV) polymerase chain reaction and plasma CMV polymerase chain reaction can be used to rule in (both tests positive) or rule out (both tests negative) a diagnosis of CMV colitis with a high degree of confidence.

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