

Diagnostic Performance of the Cytomegalovirus (CMV) Antigenemia Assay in Patients with CMV Gastrointestinal Disease

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Of 149 patients with suspected cytomegalovirus (CMV) gastrointestinal disease, 51 (36%) confirmed cases, 6 (4%) probable cases, and 64 (45%) instances of non-CMV gastrointestinal disease were analyzed using the CMV antigenemia assay; 22 patients (5%) with indeterminate gastrointestinal disease were excluded. The sensitivity and specificity of the CMV antigenemia assay (defined as detection of ≥ 1 positive cells per 200,000 leukocytes) for diagnosis of CMV gastrointestinal disease were 54% (95% confidence interval, 41%–68%) and 88% (95% confidence interval, 77%–94%), respectively.

Cytomegalovirus (CMV) gastrointestinal disease is a major cause of morbidity and mortality in immunocompromised patients, especially transplant recipients [1] and human immunodeficiency virus–infected patients [2]. Occasionally, reactivation of CMV infection in the gastrointestinal tract can lead to severe complications in immunocompetent hosts, especially in those of advanced age [3]. Recently, the value of noninvasive diagnostic methods, such as the CMV blood antigenemia assay, has been demonstrated for preemptive therapy to prevent the development of CMV pneumonitis after hematopoietic stem cell transplantation (HSCT) [4–6]. However, only a few studies on the diagnostic value of the CMV antigenemia assay for CMV-related diseases other than pneumonitis (including gastrointestinal disease, retinitis, and hepatitis) have appeared, and

only small numbers of patients were examined in these studies [1, 7–9]. Therefore, the clinical utility of the CMV antigenemia assay for diagnosis of CMV gastrointestinal disease in immunocompromised or elderly patients and in transplant recipients remains unknown. Thus, we evaluated the clinical utility of the CMV antigenemia assay for diagnosis of suspected CMV gastrointestinal disease.

Methods. We reviewed the medical records of patients with suspected CMV gastrointestinal disease for whom endoscopy and the CMV antigenemia assay (≤ 48 h after endoscopy) were performed at the Asan Medical Center (Seoul, Republic of Korea) during the period from January 2005 through July 2008. The CMV antigenemia assay used the monoclonal antibodies C10/C11 (Biotest) and was performed as described elsewhere [5]. Counts are expressed as the number of CMV-positive cells per 200,000 leukocytes.

CMV gastrointestinal disease was categorized in a manner modified from a previous report [10]. Confirmed CMV gastrointestinal disease was defined as symptoms or signs of upper or lower gastrointestinal disease plus the detection of CMV by histologic testing or immunohistochemical examination of biopsy specimens from macroscopic lesions found by endoscopy, with no evidence of other pathogens. Patients were classified as having probable CMV gastrointestinal disease if (1) they presented with symptoms or signs of upper or lower gastrointestinal disease in the absence of any other documented cause, (2) if macroscopic mucosal lesions were noted by endoscopy, (3) if CMV polymerase chain reaction (PCR) of biopsy specimens yielded a positive result, and (4) if they experienced clinical improvement while receiving anti-CMV therapy. Patients were classified as having non-CMV gastrointestinal disease if they experienced clinical improvement in the absence of any evidence of CMV infection or of any other definitive cause of gastrointestinal disease, without receipt of antiviral therapy. Patients were classified with indeterminate gastrointestinal disease if CMV infection could not be excluded but the above criteria were not satisfied. Diagnostic performance was expressed in terms of sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio.

Results. A total of 149 patients with suspected CMV gastrointestinal disease were identified. Of these patients, we excluded 6 who had multiple-organ CMV diseases. Thus, 143 patients were enrolled in the study. Of these patients, 55 (38%) had undergone transplantation, 49 (34%) had received immunosuppressive agents, and 3 (2%) had human immuno-

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Table 1. Clinical characteristics of 121 patients with suspected cytomegalovirus (CMV) gastrointestinal disease.

Characteristic	Final diagnosis	
	CMV gastrointestinal disease ^a (n = 57)	Non-CMV gastrointestinal disease (n = 64)
Age, mean years \pm SD	52.2 \pm 16.2	45.9 \pm 16.0
Male sex	36 (63)	30 (47)
Underlying condition or illness		
Transplantation		
All	21 (37)	23 (36)
Kidney	9 (16)	6 (9)
Hematopoietic stem cell	7 (12)	12 (19)
Heart	3 (5)	0
Liver	1 (2)	4 (6)
Pancreas	1 (2)	1 (2)
Inflammatory bowel disease	8 (14)	11 (17)
Rheumatologic disease	3 (5)	5 (8)
Hematologic malignancy	6 (11)	4 (6)
Solid tumor	6 (11)	6 (9)
HIV infection	1 (2)	2 (3)
Age \geq 60 years	10 (18)	8 (13)
None	2 (4)	5 (8)
Suspected infection site		
Esophagus	3 (5)	11 (17)
Stomach	11 (19)	2 (3)
Small intestine	4 (7)	3 (5)
Colon	36 (63)	45 (70)
\geq 2 sites	3 (5) ^b	3 (5) ^c

NOTE. Data are no. (%) of patients, unless otherwise indicated. HIV, human immunodeficiency virus; SD, standard deviation.

^a Patients with CMV gastrointestinal disease included those with confirmed cases (n = 51) and those with probable cases (n = 6). Twenty-two patients with indeterminate gastrointestinal disease were excluded from analysis.

^b Multiple sites included stomach and colon, stomach and small intestine, and esophagus and colon for 1 patient each.

^c Multiple sites included stomach and small intestine, esophagus and stomach, and small intestine and colon for 1 patient each.

deficiency virus infection. The clinical categories were confirmed CMV gastrointestinal disease (51 [36%] of 143 patients), probable CMV gastrointestinal disease (6 patients [4%]), indeterminate gastrointestinal disease (22 patients [15%]), and non-CMV gastrointestinal disease (64 patients [45%]). Patients who were classified as having indeterminate gastrointestinal disease were excluded from final analysis. The baseline clinical characteristics of patients with CMV gastrointestinal disease and non-CMV gastrointestinal disease are presented in table 1.

When the cutoff value for a positive CMV antigenemia assay was \geq 1 CMV-positive cell per 200,000 leukocytes, the sensitivity of the assay was 54% (95% confidence interval [CI], 41%–68%), the specificity was 88% (95% CI, 77%–94%), the positive predictive value was 79% (95% CI, 64%–91%), the negative predictive value was 68% (95% CI, 57%–78%), the positive likeli-

hood ratio was 4.35 (95% CI, 2.18–8.68), and the negative likelihood ratio was 0.52 (95% CI, 0.39–0.70) for diagnosis of CMV gastrointestinal disease. In a subgroup analysis that included transplant recipients (n = 44), the sensitivity was 76% (95% CI, 53%–92%), the specificity was 87% (95% CI, 66%–97%), the positive predictive value was 84% (95% CI, 60%–97%), the negative predictive value was 80% (95% CI, 59%–93%), the positive likelihood ratio was 5.84 (95% CI, 1.97–17.23), and the negative likelihood ratio was 0.27 (95% CI, 0.13–0.59) for diagnosis of gastrointestinal CMV disease. The diagnostic accuracy of diagnostic tests for CMV gastrointestinal disease for the 121 patients with suspected CMV gastrointestinal disease, by underlying disease category, is shown in table 2.

Discussion. The CMV antigenemia assay is one of the most widely used methods for detection of reactivation of CMV infection in a variety of clinical settings [1]. The introduction of the CMV antigenemia assay has contributed to the successful use of preemptive therapy for CMV disease in allogeneic HSCT recipients [4–6]. Despite the high sensitivity for detection of viral reactivation before the onset of CMV pneumonitis, CMV antigenemia does not necessarily precede the onset of other CMV diseases [5]. However, only a few studies have addressed this issue. Mori et al. [5] reported that CMV antigenemia preceded disease onset in only 4 (21%) of 19 HSCT recipients with CMV gastrointestinal disease. However, all 19 patients subsequently tested positive for CMV antigenemia after the diagnosis of CMV gastrointestinal disease [5]. Fica et al. [7] also showed that the CMV antigenemia test result was positive for 18 (58%) of 31 solid-organ transplant recipients with CMV end-organ disease in whom CMV gastrointestinal disease (22 [71%] of 31) was the most frequent form of CMV end-organ disease. However, it is unclear how many patients with CMV gastrointestinal diseases tested positive for CMV antigenemia. In this retrospective study, we assessed the clinical utility of the CMV antigenemia assay in other immunocompromised or elderly patients with suspected CMV gastrointestinal disease, as well as in transplant recipients. Our study demonstrated that the CMV antigenemia assay has limited sensitivity (54%; 95% CI, 41%–68%) for diagnosis of CMV gastrointestinal disease. To our knowledge, this is the largest study to systemically assess the diagnostic performance of the CMV antigenemia assay in patients with suspected CMV gastrointestinal disease.

Visualization of intranuclear inclusions in stained tissue and immunohistochemical staining of tissue where the presence of CMV is suspected have been used as standard reference methods to confirm CMV gastrointestinal disease [7]. However, the sensitivity of this combined methodology was 75%, compared with that of PCR [11]. Thus, we included patients with confirmed CMV gastrointestinal disease (i.e., intranuclear inclusions and CMV-positive immunohistochemical stains) and with probable CMV gastrointestinal disease (i.e., positive results of

Table 2. Accuracy of diagnosis of cytomegalovirus (CMV) gastrointestinal disease in 121 patients with suspected CMV gastrointestinal disease

Group, test	Sensitivity		Specificity		PPV, % (95% CI)	NPV, % (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	
	<i>n</i> / <i>N</i> ^a	Percentage (95% CI)	<i>n</i> / <i>N</i> ^b	Percentage (95% CI)					
All patients (<i>n</i> = 121)									
Endoscopy									
Light microscopy plus IHC examination	51/57	89 (78–96)	64/64	100 (94–100)	100 (93–100)	91 (82–97)	Not applicable	0.11 (0.05–0.22)	
PCR of tissue specimens	24/29	83 (64–94)	32/39	82 (66–92)	77 (59–90)	86 (71–95)	4.61 (2.31–9.20)	0.21 (0.09–0.47)	
CMV antigenemia									
≥1 CMV-positive cell/200,000 leukocytes	26/57	54 (41–68)	56/64	88 (77–94)	79 (64–91)	68 (57–78)	4.35 (2.18–8.68)	0.52 (0.39–0.70)	
≥5 CMV-positive cells/200,000 leukocytes	24/57	42 (29–56)	64/64	100 (94–100)	100 (86–100)	66 (56–75)	Not applicable	0.58 (0.46–0.72)	
Transplant recipients (<i>n</i> = 44)									
Endoscopy									
Light microscopy plus IHC examination	20/21	95 (77–99)	23/23	100 (85–100)	100 (83–100)	96 (79–99)	Not applicable	0.11 (0.02–0.70)	
PCR of tissue specimens	9/10	90 (56–99)	11/12	92 (62–99)	90 (56–99)	92 (62–99)	10.80 (1.64–71.34)	0.11 (0.02–0.70)	
CMV antigenemia									
≥1 CMV-positive cell/200,000 leukocytes	16/21	76 (53–92) ^c	20/23	87 (66–97)	84 (60–97)	80 (59–93)	5.84 (1.97–17.23)	0.27 (0.13–0.59)	
≥5 CMV-positive cells/200,000 leukocytes	15/21	71 (48–89) ^d	23/23	100 (85–100)	100 (78–100)	79 (60–92)	Not applicable	0.29 (0.14–0.56)	
Immunocompromised patients who did not receive a transplant (<i>n</i> = 52)									
Endoscopy									
Light microscopy plus IHC examination	19/24	79 (58–93)	28/28	100 (88–100)	100 (82–100)	85 (68–95)	Not applicable	0.21 (0.09–0.45)	
PCR of tissue specimens	10/11	91 (59–99)	15/19	79 (54–94)	71 (42–92)	94 (70–99)	4.32 (1.77–10.52)	0.12 (0.02–0.76)	
CMV antigenemia									
≥1 CMV-positive cell/200,000 leukocytes	10/24	42 (22–63) ^c	26/28	93 (77–99)	83 (52–98)	65 (48–79)	5.83 (1.41–24.06)	0.63 (0.44–0.89)	
≥5 CMV-positive cells/200,000 leukocytes	7/24	29 (13–51) ^d	28/28	100 (88–100)	100 (59–100)	62 (47–76)	Not applicable	0.70 (0.55–0.92)	

NOTE. Twenty-two patients with indeterminate gastrointestinal disease were excluded from analysis. CI, confidence interval; IHC, immunohistochemical; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value.

^a No. of patients with a positive test result/no. of patients tested.

^b No. of patients with a negative test result/no. of patients tested.

^c *P* = .02 for transplant recipients versus immunocompromised patients who did not receive a transplant.

^d *P* = .005 for transplant recipients versus immunocompromised patients who did not receive a transplant

CMV PCR of biopsy specimens with clinical improvement during antiviral therapy) as the reference standard for CMV gastrointestinal disease.

Quantitative real-time PCR is considered more sensitive than the CMV antigenemia assay [12]. However, Mori et al. [5] reported that the PCR assay yielded positive results for only 50% of HSCT recipients with CMV gastrointestinal disease. In our study, quantitative real-time PCR and the CMV antigenemia assay were simultaneously performed for 29 patients with CMV gastrointestinal disease. Of these patients, 14 (48%) had positive results with quantitative PCR; all of these patients also had positive results for the CMV antigenemia assay. Thus, we assume that noninvasive diagnostic methods, such as the CMV antigenemia assay or quantitative real-time blood PCR, may be not useful to rule out gastrointestinal CMV disease. We did not address the kinetics of the CMV antigenemia assay during antiviral therapy. Therefore, we do not know how many patients had delayed detectable CMV antigenemia after receipt of antiviral therapy. Additional studies of this topic are needed. In

addition, inter- and intra-assay variability for the CMV antigenemia assay limit the comparability of our findings [12].

In conclusion, the outcome of this study suggests that the CMV antigenemia assay is of limited value in the diagnosis of CMV gastrointestinal disease. When the low assay sensitivity is considered, the CMV antigenemia assay is not useful to exclude CMV gastrointestinal disease, and the results need to be interpreted in the context of clinical presentation and other diagnostic test results.

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