International Journal of Medical Sociology and Anthropology Vol. 9 (8), pp. 001-011, August, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research paper

Histopathologically documented gastrointestinal cytomegalovirus infection in immunosuppressed patients: Clinicopathologic analysis with serum quantitative PCR correlation

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Accepted 12 February, 2020

Cytomegalovirus (CMV) infection of the gastrointestinal (GI) tract is associated with high mortality in immunosuppressed patients. Few studies have correlated serum viral load with histopathological findings in this setting. Gastrointestinal biopsies from 28 immunosuppressed patients showing CMV cytopathic effect were studied by immunohistochemistry (IHC) and *IN SITU* hybridization (ISH). Quantitative evaluation of CMV-infected cells was correlated with clinicopathologic data, including plasma PCR. 85.7% (24/28) of our patients had detectable viremia. Viral load did not correlate with concentration of CMV-infected cells in tissue, type of infected cell (endothelial, epithelial, smooth muscle, or stromal), microscopic mucosal ulceration, CMV inclusions in multiple sites within the GI tract, endoscopic findings, or presence of systemic symptoms. All PCR-negative cases (4/28) occurred in late onset CMV infection or disease. Most cases of biopsy-proven GI CMV infection and disease are associated with positive viremia by plasma PCR. We found no correlation between viral load and any of the clinicopathologic parameters analyzed in our study. Some cases of late-onset CMV infection and disease may have no detectable CMV viremia by PCR.

Key words: Cytomegalovirus (CMV), immunohistochemistry, *in situ* hybridization, gastrointestinal.

INTRODUCTION

Cytomegalovirus (CMV) is a double-stranded DNA virus belonging to the Herpesviridae family (Weller, 1971), first described in 1956 (Smith, 1956). Latent CMV infection is extremely common worldwide, with reported prevalence in the adult population being as high as 100% (De Jong et al., 1998). Like other Herpesviruses, CMV may persist in a latent state after acute infection (Sinclair et al., 1996). Reactivation of CMV occurs most often in immunosuppressed patients and can present as gastroenteritis, hepatitis, pneumonitis, marrow sup-pression, retinitis and encephalitis, frequently repre-senting a severe disease associated with high morbidity and mortality (van Burik et al., 2001; Sarkio et al., 2005; Osarogiagbon et al., 2000; Abbott et al., 2002).

Although GI CMV infection can usually be suspected clinically, especially in high-risk patient populations, the definitive diagnosis of GI CMV disease requires a combination of clinical symptoms, histopathological findings (CMV inclusions), and presence of mucosal lesion(s) on endoscopic examination in case of luminal disease. Much emphasis has been given recently to molecular techniques as early markers and predictors of CMV disease in immunocompromised patients. CMV

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antigenemia and circulating CMV DNA levels seem to correlate, to a certain extent, with development of visceral disease (Saltzman et al., 1992; Gourlain et al., 2003; Dpdt et al., 1997; Mori, et al., 2004; Boeckh et al., 2004; Boeckh et al., 1998), and serial testing of high-risk patients is now routine in many centers. Nonetheless, a very small number of studies to date have documented the association between cases of histopathologically-proven CMV disease and circulating CMV DNA, as assessed by plasma PCR (van Burik et al., 2001; Dodt et al., 1997; Fica et al., 2007), a technique that has repeatedly been shown to be the most useful for follow-up of high-risk patients (Dodt et al., 1997; Boeckh et al., 1998). In addition, little is known about the correlation between histopathologic findings and CMV viral load.

In this study, we present one of the largest series of biopsy-proven GI CMV cases in immunocompromised hosts with corresponding plasma CMV quantitative PCR. Histologic, immunohistochemical and *in-situ* hybridization findings are correlated with clinical data.

MATERIALS AND METHODS

Case selection

All cases of GI CMV infection in the immunosuppressed population diagnosed on histopathologic examination of biopsy or surgical specimens over a period of six years were collected, after Institutional Review Board (IRB) approval. Our inclusion criteria included: (1) Clinically known immunosuppression (human immune-deficiency virus (HIV) infection, bone marrow transplantation (BMT), solid organ transplantation (SOT), or therapeutic immunosuppresion for other reasons); (2) positive histopathology for CMV on gastrointestinal biopsy or surgical specimen; (3) available CMV plasma viral load, as assessed by CMV quantitative PCR testing (COBAS Amplicor CMV Monitor Test, Roche Diagnostics, Branchburg, NJ, USA), performed within 10 days of tissue sampling; (4) sufficient and adequate tissue for immunohisto-chemical and *in-situ* hybridization studies. Patients receiving therapeutic doses of antiviral medication at the time of biopsy or PCR testing were excluded. The diagnosis of GI CMV was based on the presence of typical CMV cytopathic effect as seen on hematoxylin-eosin stained sections, including nuclear and cellular enlargement with presence of characteristic nuclear and/or cytoplasmic inclusions.

Study population and clinical data

A total of 28 patients (36 specimens, including 35 CMV-positive biopsies and one CMV-positive resection specimen) met our criteria and were included in the study. Fourteen patients were males and fourteen were females, with a mean age of 49.4 years (range: 23 – 71). Twenty four of 28 patients included in this study met criteria for GI CMV disease, defined in this study as: (1) gastrointestinal symptoms; (2) endoscopically abnormal GI mucosa and (3) presence of CMV-infected cells (CMV cytopathic effect) identified histolopathologically. In cases of hepatic CMV infection, abnormal transaminase or bilirubin levels, rather than abnormal endoscopic findings, were required for diagnosis of CMV disease. Four patients were classified as having “CMV infection” due to the absence of any abnormal findings on upper endoscopy or colonoscopy. Six patients were excluded from the study due to unavailable PCR testing (n = 2), PCR testing done over 10 days from biopsy procedure (n = 1) and PCR testing done after initiation of CMV antiviral therapy (n = 3). Demographic information and symptoms associated with CMV disease were retrieved from electronic medical records. All patients had detailed records from outpatient clinic visits or hospital admission/discharge summaries within one week of biopsy. Endoscopic and/or colonoscopic reports were available for all GI CMV patients with luminal disease, except for one patient with CMV colitis diagnosed after surgery.

Histopathology, immunohistochemistry, and *IN-SITU* hybridization

In order to assess the number of infected cells in each biopsy sample, IHC and ISH studies were performed on each biopsy, using the Bond-max autostainer (Leica Microsystems, Bannockburn, IL, USA), with prediluted CMV probe and NCL-CMV pp65 monoclonal antibody (1:500 dilution) (Novocastra, Newcastle upon Tyne, UK), respectively. The concentration of CMV infected cells per ten high-power field (HPF) was assessed by counting the total number of cells showing definite CMV staining in each biopsy fragment or representative section of surgical specimen and dividing this number by the total number of HPFs present in the slide. Different magnifications were used to estimate the area of each biopsy sample (mm^2), depending on the fragment’s size. The area of each biopsy sample was then converted to number of HPFs, defined in this study as 400X magnification, or 0.21 mm^2.

Statistics

Statistical difference in viral loads among different groups was calculated using the Mann-Whitney U test, Kruskal-Wallis test, or Fisher’s exact test. Correlation between CMV viral loads and number of infected cells by IHC and ISH was assessed using the Spearman’s correlation coefficient.

RESULTS

Serum quantitative PCR

We included a total of 36 CMV-positive samples (28 patients) from various sites in the GI tract for which CMV quantitative PCR results were available and collected in accordance with the time limits stipulated in our study. Overall, 24 of 28 patients (85.7%) had detectable viremia and the mean viral load was 20,172 copies/µL (range: 0 to 121,000). In the group of patients meeting criteria for GI CMV disease, a positive PCR was seen in 21 of 24 patients (87.5%), with a mean viral load of 19,235 (range: 0 -121,000). In patients who did not meet criteria for CMV disease, PCR was positive in 3 of 4 cases (75%), with a mean viral load of 25,800 (range 0 - 50,000) (Table 1). Among the four cases with negative CMV serum PCR, two were SOT and two were BMT patients, who were diagnosed with gastric (2 patients), duodenal (1 patient), and both gastric and esophageal CMV disease (1 patient). Two of these patients had multiple symptoms, including constitutional symptoms, and 3 of 4 had an abnormal endoscopic examination. One patient with normal endoscopic examination was classified as ‘CMV infection’ and the other three as ‘CMV disease’. The three

Table 1. Characteristics of especial patient subgroups compared to all patients.

	Focal CMV-positive cells (n=3)*	Normal endoscopic examination (n=5)†	Negative PCR (n=4)	Synchronous extra-GI CMV infection(n=2)	All patients (n=28)
Mean age	55.6(range: 53-57)	47.4 (range: 41-71)	50.2 (range: 23-71)	41.5 (range: 24-59)	49.4 (range 23-71)
Gender	1 males, 2 females	2 males, 3 females	3 males,1 female	1 male, 1 female	14 males, 14 females
Condition					
SOT	33% (1/3)	40% (2/5)	50%(2/4)	66% (2/3)	53% (15/28)
BMT	66% (2/3)	20% (1/5)	50%(2/4)	–	39% (11/28)
HIV	–	40% (2/5)	–	–	7% (2/28)
Mean post-transpant Day (range)	SOT: n/a BMT: 112 (75-150)	SOT: 587‡ BMT: 124	SOT: 1110(587-1634) BMT: 580 (234 –927)	SOT: 57 (48-66) BMT: –	SOT: 182 (30-574) BMT: 268 (41-1634)
CMV site					
	Colon (n=2) Duodenum (n=1)	Colon (n=4) Duodenum (n=1)	Stomach (n=2) Stomach + esophagus (n=1) Duodenum (n=1) Ileum (n=2)	Stomach + duodenum (n=1) Liver (n=1) Lung (n=2)	Esophagus (n=3) Stomach (n=13) Duodenum (n=7) Colorectal (n=9) Liver (n=2)
Endoscopic finding:					
Ulceration	0% (0/4)	–	25%(1/4)	100% (1/1)§	44% (12/27)
Erythema	25% (1/4)	–	0% (0/4)	–	22% (6/27)
Nodularity	25% (1/4)	–	50%(2/4)	–	15% (4/27)
Normal	25% (1/4)	100% (5/5)	25%(1/4)	–	18% (5/27)
Mean viral load [copies/∝L] (range)	2,330 (990-3,200)	21,660 (0-50,000)	0	75,000 (50,000-100,000)	20,172 (0-121,000)
Symptom(s)					
Abdominal pain	–	40% (2/5)	25%(1/4)	50% (1/2)	28% (8/28)
Nausea/vomiting	33% (1/3)	20% (1/5	50%(2/4)	–	35% (10/28)
Diarrhea	66% (2/3)	60% (3/5)	100% (4/4)	–	50% (14/28)
Constitutional	–	–	50%(2/4)	50% (1/2)	42% (12/2

Table 1. Continued.

Number of CMV+ cell [cells/10 hpf] (range)					
IHC	0.73 (0.1-1.8)	11.6 (0.1-50)	39.4 (3.4-98)	11.86 (2-22.3)	21 (0.1-98.7)
ISH	0.24 (0.1-0.31)	8.56 (0.1-40)	24.8 (5.7-40)	22.6 (6.6-40)	12.8 (0.1-99)
Type of CMV+ cells					
Epithelial	0% (0/4)	0% (0/5)	20% (1/4)	50% (1/2)	30% (11/36)
Endothelial	0% (0/4)	20% (1/5)	50% (2/4)	100% (2/2)	25% (9/36)
Stromal	100% (4/4)	100% (5/5)	100% (4/4)	100% (2/2)	100% (36/36)

*Cases of liver CMV were not included. † One patient had CMV infection with endoscopic abnormalities at a separate site. ‡ Data not available for 3 patients. § Endoscopic examination not performed (hepatic CMV). CMV, cytomegalovirus; PCR, polymerase chain reaction; BMT, bone marrow transplantation; HIV, human immunodeficiency virus; SOT, solid organ transplantation; IHC, immunohistochemistry; ISH, in-situ hybridization; hpf, high-power field.

PCR-negative patients who met criteria for CMV disease (two BMT and one SOT patients) had late onset CMV disease (mean 931 days; range 234-1634). The forth CMV PCR-negative patient, who did not fulfill criteria for CMV disease, also had late CMV infection (post-transplant day 587). The CMV infection in PCR-negative patients was similar to that of PCR-positive patients in regards to clinical (presence of systemic symptoms and endoscopic appearance of affected organ) and histopathologic (presence of mucosal ulceration, number of CMV infected cells by IHC and ISH) parameters (Table 1). Serum PCR was performed within two days of tissue sampling in all four patients, and none received therapeutic or prophylactic doses of antiviral agents within several weeks prior to testing. None of the patients with negative PCR had histopathologic evidence of CMV infection simultaneously in the upper and lower GI tract.

Underlying disease

All patients included in this study were immunocompromised due to various underlying etiologies (Table 6). A significant proportion of the patients included in our study were either solid organ transplant (SOT) (n = 13), or bone marrow transplant (BMT) (n = 11) patients. Only two of 28 patients (7%) were HIV positive, reflecting the relatively low prevalence of HIV infection within the patient population seen at our institution. Subgroup analysis showed that the average CMV viral load in SOT, HIV and BMT and patients was not significantly different ($P \geq 0.32$). None of the patients were being treated for CMV at the time of viral load determination.

Information regarding transplantation date was available for 24/26 transplant patients. The average time from transplantation to histopathologic diagnosis of CMV infection was 254 days (range, 30 - 1634) for all transplant recipients, 268 days (range, 41 - 1634 days) for SOT patients, and 182 days (range, 30 - 574 days) for BMT patients ($P \geq 0.45$) (Table 6). 12 of 24 patients (50%) for whom clinical information regarding date of transplantation was available had late onset CMV infection or disease (defined as > 3 months after transplantation for SOT patients and > 100 days for BMT patients).

Site(s) of CMV involvement

The most common site of involvement in the GI tract was the stomach (13 cases/36.1%), followed by the colon (8 cases/22.2%), and the duodenum (7 cases/19.4%). A smaller number of cases were also diagnosed in the esophagus (3 cases/8.3%), liver (2 cases/5.5%), rectum (2 cases/5.5%) and ileum (1 case/2.7%). No statistically significant difference in viral load was observed in patients with CMV in the esophagus, stomach, duodenum, ileum, colon, and rectum (Table 3). Patients with CMV of the esophagus and ileum had lower viral counts compared to other sites, but the number of affected patients was insufficient to establish the significance of this finding.

Correlation between CMV viremia and concentration of infected cells by IHC and ISH

No correlation was found between viral load and

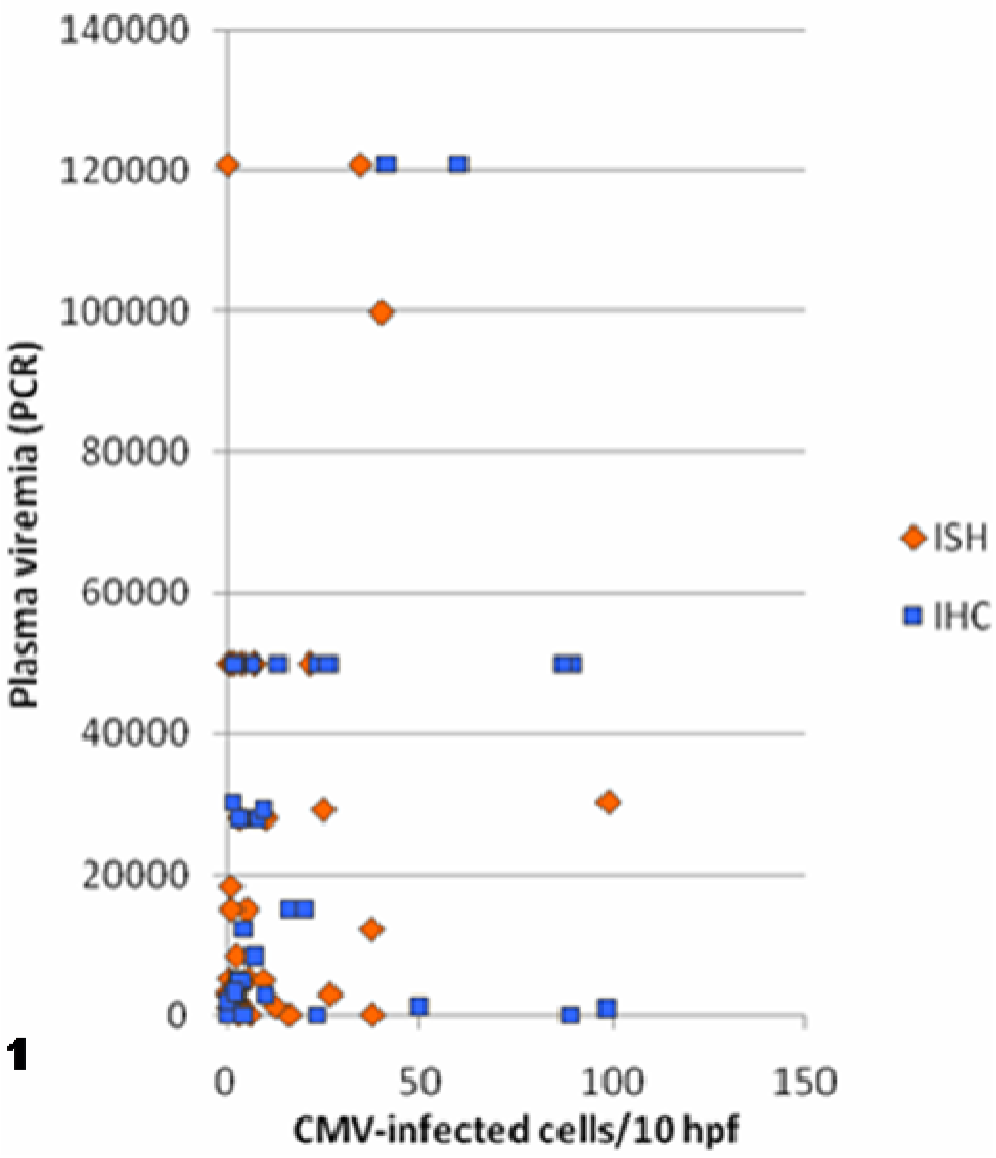

Figure 1. No statistical correlation is seen between the level of plasma viremia by quantitative serum polymerase chain reaction (PCR) and the number of cytomegalovirus (CMV)-infected cells/10 high power fields (hpf) by either immunohistochemical (IHC) or *in-situ* hybridization (ISH) analysis (r = -0.01 and -0.02, respectively; Spearman correlation coefficient).

of CMV-infected cells, either by IHC or ISH (r = -0.01, and -0.02, respectively) (Figures 1, 2A, and B). Patients whose biopsies showed cytopathic changes in endothelial cells (mean viral load 16,203 copies/µL) had similar viral loads compared to patients with cytopathic changes seen in epithelial cells (17,211 copies/µL) or stromal cells (20,172 copies/µL)($P \geq 0.18$) (Table 2). In 3 of 36 biopsies (3 patients), only a small number of CMV-positive cells were present in each individual biopsy sample (defined as less than 5 cells total by IHC and ISH, and less than 2 cells/10hpf). A low viral load was seen in Number all three patients (mean 2,330; range: 990 - 3,200). None of these patients had CMV infection in more than one GI site, extra-GI infection, or constitutional symptoms. No ulceration was seen on endoscopic examination in any of these patients. The clinical and pathologic findings in this group are summarized in Table 1.

Histologic findings

No significant difference in mean viral load was observed in patients with mucosal ulceration on microscopic examination in at least one biopsy with CMV inclusions (17,537 copies/µL) compared to patients with no

Table 2. Patient characteristics according to type of CMV infected cell on tissue biopsy.

	Epithelial cells (n=9/36 biopsies)	Endothelial cells (n=10/36 biopsies)	All biopsies (n=36)
Mean age	47.3 (range: 23-62)	48.4 (range:23-71)	49.4 (range 23-71)
Gender	4 males, 5 females	6 males, 4 females	14 males, 14 females
Condition			
SOT	44% (4/9)	50% (5/10)	47% (17/36)*
BMT	55% (5/9)	40% (410)	41% (15/36)*
HIV	–	10% (1/10)	11% (4/36)*
CMV site			
	Stomach (n=5) Duodenum (n=2) Colorectal (n=2)	Stomach (n=2) Stomach + esophagus (n=1) Duodenum (n=4) Colorectal (n=2) Liver (n=1)	Esophagus (n=3) Stomach (n=13) Duodenum (n=7) Ileum (n=2) Colorectal (n=9) Liver (n=2)
Viral load [copies/∝L)	17,211 (range: 0-50.000)	16,203 (range: 0-100,000)	20,172 (0-121,000)
Symptom(s)			
Abdominal pain	22% (2/9)	40% (4/10)	22% (8/36)
Diarrhea	44% (4/9)	60% (6/10)	27% (10/36)
Nausea/vomiting	55% (5/9)	30% (3/10)	38% (14/36)
Flu-like symptoms	11% (1/9)	30% (3/10)	8% (3/36)
Constitutional symptoms	22% (2/9)	50% (5/10)	33% (12/36)
Number of CMV+ cell			
[cells/10 hpf[(range)			
IHC:	23.9 (1-90)	36 (2-87.5)	21 (0.1-98.7)
ISH:	16.4 (1-99)	17.8 (3.1-37.5)	12.8 (0.1-99)
Type of CMV+ cells			
Endothelial	22% (2/9)	–	27% (10/36)
Epithelial	–	20% (2/10)	25% (9/36)
Stromal	100% (9/9)	100% (10 /10)	100% (36/36)
Additional histologic			
Findings			
Mucosal ulceration	55% (5/9)	80% (8/9)†	58% (20/34)**

* Eight patients had biopsies from more than one site showing cytomegalovirus inclusions. † Only mucosal biopsies included. CMV, cytomegalovirus; IHC, immunohistochemistry; ISH, in-situ hybridization; hpf, high-power field.

no evidence of mucosal ulceration (26,411 copies/µL) (*P* = 0.26). Among patients with microscopic evidence of mucosal ulceration, CMV infected endothelial cells were seen in 41% (7/17) of patients, while CMV-positive epithelial cells were present in 35% (6/17). Of patients with CMV-positive endothelial cells, 88.9% (8/9) had microscopic evidence of mucosal ulceration, compared to 48% (12/25) of patients without endothelial cell CMV infection (P = 0.08).

Overall, 20 of 28 patients included in this study had additional tissue biopsies from at least one separate site within the GI tract showing no evidence of CMV infection. Of patients with upper GI CMV infection, 12 had biopsies from a different segment of the upper GI tract (either esophagus, stomach, or duodenum) showing no viral cytopathic effect. These biopsies showed normal mucosa (8 cases), possible GVHD (2 cases), chronic gastritis (1 case), and chronic active gastritis (1 case). Five of these

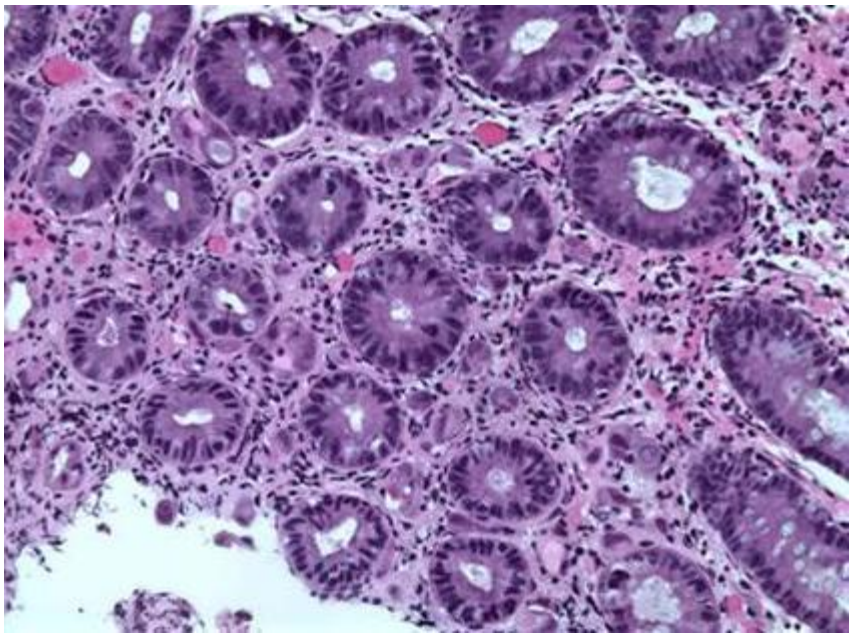

Figure 2A. Colonic mucosa with numerous stromal and endothelial cells showing cytomegalovirus cytopathic effect (H and E, 200x magnification).

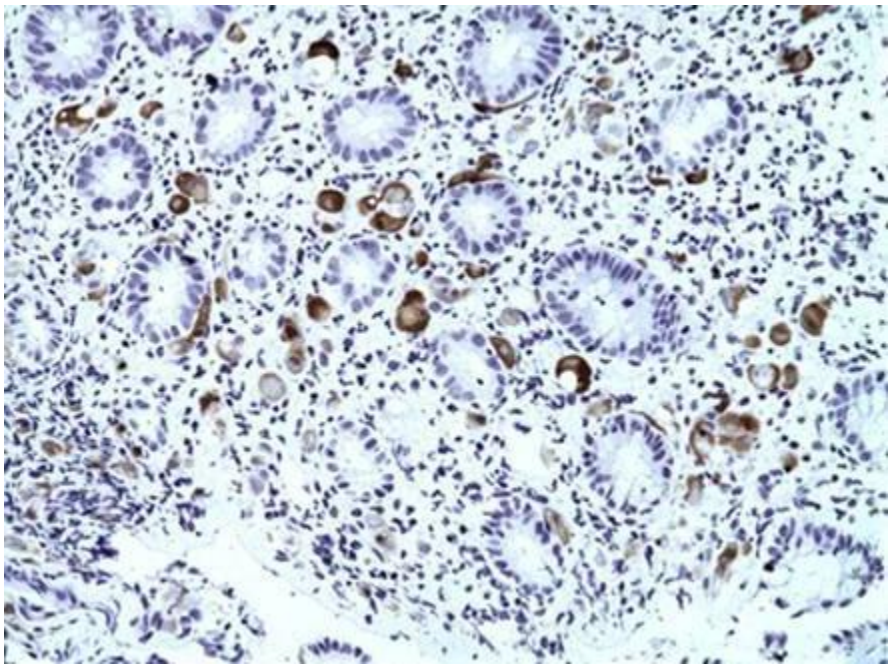

Figure 2B: Immunohistochemistry for cytomegalovirus (pp65) demonstrating nuclear and cytoplasmic staining of cytomegalovirus-infected cells (200x magnification).

patients also had colonic mucosal biopsies showing normal mucosa (4 cases) and nonspecific ulceration (one case). Of patients with colonic CMV, 3 had ileal biopsies showing normal mucosa, and one had biopsies from an upper endoscopy showing no abnormalities. Two patients had concomitant CMV pneumonitis diagnosed histopathologically between 6 and 20 days after the diagnosis of GI CMV. Demographic data for this subgroup is

Table 3. Biopsy site and viral load by PCR.

Biopsy site	Mean CMV viral load [copies/µL] (range)
Esophagus (n = 3)	516 (0 - 1,200)
Stomach (n = 13)	20,854 (0 - 121,000)
Duodenum (n = 7)	21.328 (0 - 49,800)
Ileum (n = 2)	27.550 (5,100 – 50,000)
Colorectal (n = 9)	16,571 (990 - 50,000)
Liver (n = 2)	50,000 (48,000 - 52,000) *P* = 0.3

PCR, polymerase chain reaction.

Table 4. Viral load (PCR) according to symptom(s) at presentation.

Symptom	Viral load (copies/µL) (range)	
Gastrointestinal		
Abdominal pain (n = 8)	24,562 (0-121,000)	
Nausea/vomiting (n = 10)	12,270 (0-50,000)	
GI bleeding (n = 1)	350 (350-350)	
Diarrhea (n = 14)	11,849 (0-50,000)	*P*=.54
Constitutional		
Fever (n=9)	30,477 (0-121,000)	
Weight loss (n = 3)	6,100 (0-18,300)	
Fatigue (n = 3)	33,333 (0-50,000)	
Flu-like symptoms (n = 3)	30,066 (12,200-50,000)	*P* = 0.29
Constitutional symptoms absent: n = 15	14,682 (0-50,000)	
Constitutional symptoms present: n = 12	28,550 (0-121,000)	***P* = 0.09**

summarized in Table 1.

Clinical and endoscopic findings

All patients with GI CMV included in our study were symptomatic at the time of endoscopic examination with the exception of one patient for whom no clinical information was available. All patients had at least one GI tract-related symptom (abdominal pain, nausea, vomiting, diarrhea, or GI bleeding). A tendency towards a higher viral load was seen in patients presenting with constitutional symptoms (mean 28,550 copies/µL) as compared to patients presenting exclusively with GI tract-related symptoms (14,682 copies/µL) (*P* = 0.09) (Table 4).

Endoscopic findings were documented in 27 of 28 patients. The endoscopic appearance of the biopsied areas was classified as: (a) ulceration/erosion; (b) inflammation/erythema/mucosal friability; (c) polyp(s)/ mucosal nodularity and (d) normal mucosa. One patient presented with upper GI bleeding but specific endoscopic abnormalities were not documented. No statistically significant difference as seen in mean viral loads of patients presenting with endoscopic lesions classified as ulceration/erosion compared to those showing other types of lesion on endoscopy (27,104 versus 15,857 copies/µL, *P* = 0.3) (Table 5). Interestingly, a low to undetectable viremia was seen in all four patients with lesions classified as mucosal nodularity (0 - 2,800 copies/µL). On colonoscopy, even a normal appearance of the colonic mucosa was not predictive of a low viral load. In fact, 2 of 3 cases in which colonoscopic examination showed a normal colonic mucosa were associated with high viral loads (> 50.000 copies/ µL).

Four patients had normal colonoscopies or upper endoscopies, and for this reason, did not meet criteria for CMV disease. These patients did not differ significantly from CMV disease patients with regards to viral load (25,800 vs. 19,235 copies/µL, *P* = 0.42) or other clinicopathologic parameters (Table 1).

DISCUSSION

GI CMV disease occurs primarily in immunosuppressed patients and accounts for significant morbidity and mortality in this population. It is thought to represent, in the majority of cases, reactivation of latent infection with subsequent systemic dissemination. In fact, immunosuppressed patients will often have very high

Table 5. Endoscopic findings and viral load by PCR.

Endoscopic finding	Viral load (copies/µL) (range)	
Upper endoscopy (n = 19):	**17,575 (0-121,000)**	
Ulceration/erosion (n = 9)	**25,394 (0-121,000)**	
Other findings (n = 10)	**10,539 (0-50,000)**	***P* = 0.13**
Erythema/inflammation (n = 5)	20,278 (990-50,000)	
Nodularity/polyp (n = 4)	1000 (0-2,800)	
Normal mucosa (n = 1)	**0 (0-0)**	
Colonoscopy (n = 8):	**22,725 (0-50,000)**	
Ulceration/erosion (n = 3)	**23,533 (0-50,000)**	
Other findings (n = 5)	22,240 (2,900-50,000)	***P* = 0.65**
Erythema/inflammation (n = 1)	2,900 (2,900-2,900)	
Nodularity/polyp (n = 0)	–	
Normal mucosa (n = 4)	27,075 (3,200-50,000)	
All endoscopic procedures (n = 27):	**20,842 (0-121,000)**	
Ulceration (n = 12)	**27,104 (0-121,000)**	
Other findings (n = 15)	**15,857 (0-50,000)**	***P*=0.3**

PCR, polymerase chain reaction.

Table 6. Underlying etiology of immunosuppresion, viral load (PCR), and time from transplantation in the different patient groups.

Underlying disease	CMV viral load [copies/µL] (range)		Post-transplantation day (range)
Solid organ transplant (SOT):			
All SOT (n=15)	**30,833 (1,200-121,000)**		268 (41-1634)
Liver transplant (n=8)	32,588 (0-121,000)		130 (34-587)
Kidney transplant (n=3)	20.733 (0-50,000)		771 (314-1634)
Lung transplant (n=2)	29,200 (8,400-50,000)		66 (66-66)*
Heart transplant (n=2)	15,600 (3,200-28,000)	*P* = 0.78	71 (71-71)*
Bone marrow transplant (n=11)	**8,840 (0-50,000)**		182 (30-574)
HIV positive (n=2)	**27,550 (5,100-50,000)**	***P* = 0.32**	N/A

* Data not available for one of two patients; PCR, polymerase chain reaction; CMV, cytomegalovirus; HIV, human immunodeficiency virus.

plasma viral loads, and present with systemic symptoms and evidence of infection in multiple organs. In such cases, the GI tract is probably part of a multi-organ process secondary to hematogenous dissemination from a primary reactivation site. However, in some cases, no evidence of disseminated infection is found, even when highly sensitive molecular techniques are employed (Husain, 2009). Therefore, essential aspects of the pathophysiology of this disease are yet to be elucidated. Our data supports the concept that, in the majority of cases in the immunosuppressed population, GI CMV is part of a systemic disease rather than of a localized process.

Quantitative PCR is a highly sensitive method for detection of CMV and is generally regarded as the preferred method for diagnosis of CMV infection in clinical practice. Reported sensitivity has ranged from 72 to 100% in various studies (Mori et al., 2004; Boeckh et al., 2004; Fica et al., 2007; Kulkarni et al., 2001; Sia et al., 2000), possibly reflecting the lack of uniformity in the definition of CMV disease and technical differences between PCR methods utilized by different institutions. In our study, we defined GI CMV disease in accordance to current consensus criteria (Ljungman et al., 2002) and found 85.7% and 87.5% of immunosuppressed patients with GI CMV infection and GI CMV disease, respectively, diagnosed by histopathologic examination, to have detectable CMV viremia by plasma PCR. Viremic patients did not differ significantly from plasma PCR-negative patients in regards to age, underlying condition, site of positive biopsy or microscopic findings (number of infected cells/10HPF's, type of infected cell, and presence of ulceration on microscopic examination). Six patients were excluded from the study due to either

unavailable or delayed PCR testing (n = 3) or antiviral treatment at the time of PCR testing or endoscopic biopsy (n = 3). This may have introduced some degree of bias into our results. Information regarding the exact reason why CMV PCR was not obtained in a few of our cases was unclear. It could be speculated, however, that lack of PCR testing may have been due to clinically mild disease or nonspecific symptoms which did not raise suspicion for an infectious process, even in the setting of immunosuppression. On the contrary, empiric initiation of antiviral therapy may have been prompted by a more ominous presentation. Therefore, it is plausible to raise the question of whether the prevalence of viremia in the six excluded patients may differ from that observed in our study group.

Several studies have shown that high viral loads are highly predictive of visceral disease (Saltzman et al., 1992; Gourlain et al., 2003; Dodt et al., 1997; Mori et al., 2004; Boeckh et al., 2004; Boeckh et al., 1998). Our data suggest, however, that viral loads can be quite variable in PCR-positive patients and do not correlate well with the histopathologic parameters evaluated in this study (Table 1).

Interestingly, all four PCR-negative patients had late onset CMV disease (3 patients) or infection (1 patient). Atypical clinical presentation has been reported in cases of late onset CMV infection, including absence of clinical symptoms, fever or hematologic abnormalities, usually seen cases diagnosed within the first 3 months following transplantation (Husain et al., 2009). Our study indicates that a negative serum quantitative PCR may represent another atypical feature seen in late onset CMV infection and CMV disease. One-third (4/12) of the late onset CMV cases had a negative PCR in our study, while all early CMV cases were PCR-positive. Our data, therefore, suggest that serum PCR-negative cases of GI CMV may be significantly more common in late onset disease and that a negative PCR does not reliably exclude CMV infection in this setting.

From a pathophysiologic standpoint, the existence of patients with GI CMV disease with no detectable viremia and no evidence of extra-GI infection is intriguing. The wide variation of viral loads observed in PCR-positive patients, often at very low levels, raises the possibility that systemic infections with viral loads which are below the lower limit of detection of currently employed molecular methods may exist. Some authors suggest, however, that different CMV glycoprotein B genotypes may be associated with viral tropism for specific organs and may also influence CMV dissemination potential (Tarrago et al., 2003; Meyer-Konig et al., 1998; Meyer-Konig et al., 1998; Sinzger et al., 1999; He et al., 2006) , providing an alternative explanation for the occurrence of serum PCR-negative GI CMV cases.

Microscopically, none of the features analyzed in our study has shown any predictive value in regards to viral load, including number of infected cells per 10 HPF (by IHC and ISH) (Figure 1), type of infected cell (endothelial, epithelial, smooth muscle, or stromal) and presence of mucosal ulceration (Tables 1 and 2). We specifically addressed the question of whether endothelial cell infection would correlate with a higher prevalence of widespread infection, which has been hypothesized by other authors (Roberts et al., 1989; Hinnant et al., 1986). No correlation was found between the presence of endothelial cell infection recognized on H and E, IHC, or ISH sections and viral load, histopathologic evidence of infection in multiple sites within the GI tract, and systemic symptoms. Eighty percent of cases in which endothelial cells with CMV inclusions were identified showed histological evidence of mucosal ulceration, reflecting the tendency of CMV-induced vascular/endothelial damage to cause ischemic type injury leading to mucosal ulceration (Golden et al., 1994) (Table 2).

Several authors have questioned the significance of the presence of CMV-infected cells in certain clinical scenarios (Goodgame et al., 1993; Hinnant et al., 1986; Eyre-Brook et al., 1986; Berk et al., 1985; Cooper et al., 1977). In some series of GI CMV disease patients, a significant proportion of the biopsies were found to have microscopic evidence of a second disease process, such as opportunistic infections, ischemia, or inflammatory bowel disease, raising questions about the role of CMV infection in these cases (van Burik et al., 2001; Roberts et al., 1989; Hinnant et al., 1986). In addition, significant evidence exists that CMV infection outside the GI tract (e.g. lungs) may not be clinically significant in certain circumstances, especially when other disease processes are concomitantly identified (Boeckh et al., 1998; Tamm et al., 2001; Millar et al., 1990; de Maar et al., 2003). Therefore, it is hypothesized that, in some situations, CMV may also be present in the GI tract as a bystander rather than as a primary pathogenic agent. In our study, only 3 of 28 patients (10.7%) showed evidence of a second disease process (all three cases were BMT patients, including two cases of possible GVHD and one case of esophageal candida infection). In both cases in which GVHD was suggested, increased apoptotic activity was seen at a different site than the CMV infection, in which CMV infection was excluded by immunohisto-chemistry. It is not clear, however, that those changes truly reflect GVHD or are secondary to undetected CMV infection in the nearby mucosa. One of the two patients was also on mycophenolate mofetil, which may cause changes indistinguishable from those seen in GVHD. All these patients had positive CMV PCR, and two patients had evidence of CMV infection in multiple GI sites. In our population of immunosuppressed patients, therefore, CMV infection was the only pathogenic process identified on GI biopsies in the majority of cases and was frequently associated with clinical evidence of systemic infection (positive serum PCR and constitutional symptoms), suggesting that CMV is, in most instances, a primary pathogenic agent in this clinical scenario.

In summary, most cases of biopsy-proven GI CMV infection and disease in immunosuppressed patients are associated with detectable viremia by plasma PCR. The presence of CMV infected cells identified on H and E slides usually reflects clinically significant disease regardless of plasma viremia, location within the GI tract, number and type of infected cells, or associated histologic findings. We found no correlation between histologic parameters and viral load. Among transplant recipients, PCR-negative GI CMV cases may occur more commonly in late-onset disease. GI CMV infection is a complex disease and further research is needed to elucidate the mechanisms of viral dissemination and tropism for specific organ systems.

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