Detection of Cytomegalovirus DNA in Plasma as an Adjunct Diagnostic for Gastrointestinal Tract Disease in Kidney and Liver Transplant Recipients

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(See the Editorial Commentary by Ison on pages 1560–1.)

Background. Cytomegalovirus (CMV) disease is the most common infectious complication after solid organ transplantation, frequently affecting the gastrointestinal (GI) tract. There are limited data on quantitative polymerase chain reaction (qPCR) for plasma CMV DNA as an adjunct diagnostic method for GI tract disease in kidney and liver transplant recipients.

Methods. We reviewed all records of adult kidney and liver transplant recipients with a GI tract biopsy and plasma CMV qPCR result within 15 days of biopsy during a 6.5-year period at our center. CMV GI tract disease was defined as histopathologic evidence of CMV on biopsy by immunohistochemistry or visualization of inclusion bodies.

Results. GI tract biopsy and qPCR results were available for 81 kidney and liver transplant recipients; 20 cases of confirmed CMV GI tract disease were identified. Overall, the sensitivity of qPCR for diagnosing CMV GI tract disease was 85% (95% confidence interval [CI], 61%–96%), and the specificity was 95% (95% CI, 85%–99%). For CMV-seronegative recipients (R⁻) with CMV-seropositive donors (D⁺), the sensitivity of qPCR was 100% (95% CI, 59%–99%), and the specificity was 80% (95% CI, 30%–99%). The lowest sensitivity was observed in CMV D⁺/R⁺ cases (72.7%; 95% CI, 39%–93%). The mean plasma CMV copy number in patients with GI tract disease was 3.84 log₁₀ (38 334 copies/mL).

Conclusions. Plasma CMV qPCR had good sensitivity and excellent specificity for CMV GI tract disease in kidney and liver transplant recipients. Its sensitivity was 100% in CMV D⁺/R⁻ cases but 72.7% in CMV D⁺/R⁺ cases. This variation in assay performance according to host serostatus may reflect differences in disease pathogenesis.

Keywords. CMV; viremia; colitis; kidney and liver transplant recipients.

Cytomegalovirus (CMV) infection and disease are common in solid organ transplant (SOT) recipients [1], even with antiviral prophylaxis [2–4]. *CMV infection* is

defined as viral replication without symptoms. *CMV disease* refers to viral replication with symptomatic illness [5, 6] and is categorized as (1) CMV syndrome with fever, malaise, and abnormal findings, such as leukopenia or thrombocytopenia, or (2) tissue-invasive disease with end-organ damage from the virus [6]. Tissue-invasive disease most commonly affects the gastrointestinal (GI) tract, resulting in esophagitis, gastritis, enteritis, or colitis [7]. Proven CMV GI tract disease requires a biopsy obtained by esophagogastroduodenoscopy and/or colonoscopy with histologic or culture-based evidence of CMV [5].

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Noninvasive molecular tests have advanced our understanding of this clinical entity and affected outcomes [8]. Detection and quantification of CMV pp65 antigen or CMV DNA in blood with quantitative polymerase chain reaction assays (qPCR) are used. However, the utility of qPCR for CMV DNA in plasma as an adjunct diagnostic for CMV GI tract disease in SOT recipients has not been well described. Findings of recent studies suggest that plasma CMV DNA is frequently not detected in cases of CMV GI tract disease. In one small study (11 SOT recipients with tissue-invasive CMV disease), qPCR sensitivity was 73%, though the number of cases of GI tract disease was not specified [9]. In a larger retrospective study of a mixed cohort (immunocompetent patients, SOT recipients, and stem cell transplant recipients), the sensitivity of plasma qPCR for CMV GI tract disease was 48% based on results from 29 patients; the sensitivity in SOT recipients was not specified [10]. Finally, Grim et al [11] reported on 12 cases of CMV GI tract disease in SOT recipients; 4 (50%) of 8 patients had detectable plasma CMV DNA. Given the limited data in SOT recipients, we reviewed a large cohort of kidney and liver transplant recipients at our institution to determine the sensitivity and specificity of qPCR for plasma CMV DNA as an adjunct in the diagnosis of CMV GI tract disease.

METHODS

Study Population

This single-center study was approved by the Institutional Review Board of Johns Hopkins Hospital (JHH). The database of the Comprehensive Transplant Center of the JHH was reviewed to identify all adult patients (aged >18 years) who received a kidney or liver transplant from 1 June 2003 (when plasma CMV DNA qPCR testing was introduced) to 31 December 2009. These patients were cross-matched to the pathology database to identify those who underwent GI tract biopsies (esophagus, stomach, small bowel, or colon). The final study patient population included all abdominal SOT recipients during the study period with (1) gastrointestinal symptoms, (2) a GI tract biopsy, and (3) a plasma CMV DNA qPCR result within 15 days of the biopsy. For patients in whom >1 GI tract biopsy was performed, the first biopsy was considered.

Immunosuppression and CMV Prophylactic Regimens

Kidney transplant recipients receive anti-thymocyte globulin (Thymoglobulin; Genzyme) as induction of immunosuppression at JHH. HLA-incompatible (positive cross-match) kidney transplant recipients receive alternate-day plasmapheresis and 100 mg/kg of intravenous CMV immune globulin (Cytogam; CSL Behring) to remove donor-specific anti-HLA antibody and quadruple-drug immunosuppression (daclizumab or thymoglobulin, tacrolimus or sirolimus, mycophenolate mofetil, and

steroids) [12]. Patients with persistent donor-specific anti-HLA antibody may also receive anti-CD20 antibody therapy or/and splenectomy. Blood group-incompatible live donor kidney transplant recipients receive anti-IL2 receptor antibody therapy. Liver transplant recipients receive basiliximab or intravenous corticosteroids for induction; maintenance immunosuppression includes prednisone, tacrolimus, and mycophenolate mofetil. Based on clinical indications, sirolimus rather than tacrolimus may be used.

The institutional protocol for CMV prophylaxis was unchanged during the study period. Ganciclovir or valganciclovir was initiated after transplantation, based on the CMV serostatus of the donor (D) and recipient (R). After transplantation, CMV-seropositive recipients (R⁺) receive valganciclovir (450 mg/d) for 3 months; CMV-seronegative recipients with CMV-seropositive donors (D⁺/R⁻) receive valganciclovir (900 mg) for 6 months, and CMV-seronegative recipients with CMV-seronegative donors (D⁻/R⁻) receive acyclovir or valacyclovir for 3 months. In patients treated for graft rejection, prophylaxis is restarted for 3 months.

Clinical Variables

The following data were reviewed and collected: demographics, organ transplants (type and date), immunosuppressive regimen, rejection episodes within 30 days of biopsy, laboratory data (creatinine levels and white blood cell and platelet counts within 3 days of biopsy), CMV D/R serostatus, CMV prophylaxis within 30 days before biopsy, plasma CMV qPCR results, symptoms that prompted endoscopy, and macroscopic and histopathologic findings from endoscopy. For kidney transplants, donor characteristics were also collected.

Definitions

The following definitions were used: *proven CMV GI tract disease*, evidence of tissue invasion by CMV in biopsies, demonstrated by immunohistochemical stain (Cell Marque; CMV monoclonal antibody; clone, DDG9/CCH2) or viral cytopathic effect [5]; *upper GI tract disease*, disease of the esophagus, stomach, duodenum, jejunum, and/or ileum; *lower GI tract disease*, disease of the sigmoid and/or colon; *no CMV GI tract disease*, absence of CMV on biopsy, irrespective of the plasma CMV DNA qPCR result.

qPCR Assay

During the study period, 3 CMV qPCR assays were used at JHH, with varying limits of detection (LODs): (1) June 2003 through August 2008, the COBAS Amplicor CMV Monitor assay (Roche Diagnostics; LOD, 600 DNA copies/mL plasma); (2) September 2008 through September 2009, an in-house protocol using the Qiagen M48 DNA extraction robot and Qiagen qPCR analyte-specific reagents (LOD, 300 copies/mL); and (3)

October through December 2009, an in-house protocol with Qiasymphony extraction (Qiagen analyte-specific reagents; LOD, 50 copies/mL).

Statistical Analysis

The incidence of invasive CMV GI tract disease was calculated by dividing the number of biopsy-proven CMV GI tract disease cases by the number of transplants performed during the study period. The sensitivity, specificity, and 95% confidence intervals (CIs) were calculated for the study population and for subgroups including upper or lower GI tract disease, transplant type, and CMV serostatus. Estimated positive predictive values (PPVs) and negative predictive values (NPVs) were determined as a function of specificity. Categorical variables were compared with Pearson χ^2 tests; Fisher exact tests were used with expected cell values <5 . Continuous variables were compared with Mann-Whitney U tests. Differences were considered significant at $P \leq .05$. Predictors of CMV GI tract disease were identified using a multivariable logistic regression model built in a step-wise fashion using independent variables from the univariable analyses whose P values were $<.05$. Statistical analyses were performed using Stata software, version 11.1 (StataCorp; 2010).

RESULTS

Eighty-one transplant recipients (49 kidney, 25 liver, 4 kidney-liver, 2 kidney-pancreas, and 1 kidney-heart transplant recipients) with GI tract symptoms, GI tract biopsies, and plasma CMV DNA qPCR results were identified during the study period. One case of upper GI tract CMV disease in a kidney transplant recipient was excluded because no qPCR results were available. Demographics and clinical characteristics are presented in Table 1. Invasive CMV GI tract disease was found in 20 of 81 patients (24.7%; 11 kidney, 7 liver, 1 kidney-pancreas, and 1 kidney-liver transplant recipient); there was no biopsy evidence of CMV GI tract disease in 61/81 (75.3%). The incidence of invasive CMV GI tract disease was 1.1% (11 of 978 recipients), 2.2% (7 of 322), and 1.7% (1 of 60) for kidney, liver, and kidney-pancreas transplant recipients, respectively.

Proven CMV GI tract disease occurred a median of 206.5 days (range, 48–1133 days) after transplantation. Fifteen (75%) of 20 cases occurred within the first 12 months, and 13 (87%) of these cases occurred 3–9 months after transplantation (Figure 1). The earliest episode occurred 48 days after transplantation in a CMV D⁺/R[−] patient with primary CMV infection. Valganciclovir prophylaxis had been discontinued in all 8 patients with CMV GI tract disease that occurred between 3–6 months: 5 were CMV D⁺/R⁺ patients off valganciclovir per institutional practice and 3 were CMV D⁺/R[−] patients in whom therapy was discontinued for other reasons (leukopenia in 2 and unknown

Table 1. Clinical Characteristics of 81 Kidney and Liver Transplant Recipients With Suspected CMV GI Tract Disease

Characteristic	No. (%) ^a		P Value
	CMV GI Tract Disease (n = 20)	No CMV GI Tract Disease (n = 61)	
Age, median (range), y	54.5 (33–79)	51 (21–77)	.20
Female sex	11 (55)	35 (57.4)	.85
White race	15 (75)	44 (72.1)	.80
Transplant type			.65
Kidney	11 (55)	38 (62.3)	
Liver	7 (35)	18 (29.5)	
Kidney-liver	1 (5)	3 (4.9)	
Kidney-pancreas	1 (5)	1 (1.6)	
Kidney-heart	0	1 (1.6)	
Prior transplant	5 (25)	13 (21.3)	.73
Kidney transplant information ^b			
Donor deceased	5 (45.4)	13 (34.2)	.49
Donor unrelated	6 (54.5)	21 (55.3)	.97
HLA incompatible	6 (54.5)	19 (50.0)	.79
Immunosuppression induction ^b			
Thymoglobulin	11 (100)	28 (73.7)	.06
Plasmapheresis	4 (36.4)	15 (39.5)	.85
Rituximab	3 (27.3)	16 (42.1)	.38
Daclizumab	2 (18.2)	3 (7.9)	.33
Immunosuppression maintenance ^b			.18
Tacrolimus based	8 (72.7)	34 (89.5)	
Sirolimus based	3 (27.3)	4 (10.5)	
Rejection ^c	5 (25)	5 (8.2)	.06
CMV serostatus			.004
D ⁺ /R ⁺	11 (55)	36 (59.0)	
D ⁺ /R [−]	7 (35)	5 (8.2)	
D [−] /R [−]	2 (10)	20 (32.8)	
CMV prophylaxis ^c	4 (20)	32 (52.5)	.004

Abbreviations: CMV, cytomegalovirus; D⁺, donor CMV seropositive; D[−], donor CMV seronegative; GI, gastrointestinal; R⁺, recipient CMV seropositive; R[−], recipient CMV seronegative.

^a Data represent No. (%) of transplant recipients, except where otherwise indicated (for age).

^b Data provided only for patients who received a kidney transplant (11 with and 38 without CMV GI tract disease).

^c Within 30 days before biopsy.

reasons in 1). Late CMV disease (>12 months after transplantation) developed in 5 patients.

One-year follow-up data were available for 19 of 20 patients with CMV GI tract disease. Four patients (21%) died within the first year; in 2, death occurred within 2 weeks of CMV GI tract disease, and CMV was considered a contributing cause of death. The other 15 patients (79%) recovered with treatment of CMV GI tract disease. One-year follow-up data were available

Figure 1. Timing of cytomegalovirus (CMV) gastrointestinal (GI) tract disease diagnosis in transplant recipients relative to transplantation. Abbreviations: D⁺, donor CMV seropositive; D⁻, donor seronegative; R⁺, recipient seropositive; R⁻, recipient seronegative.

in 57 of 61 patients without CMV GI tract disease; 47 (82%) were alive at 1 year, and 10 (17.5%) had died.

CMV qPCR Assay Performance

Seventeen of the 20 patients (85%) with documented CMV GI tract disease had CMV DNA detected in plasma by qPCR, with a mean DNA copy number of $3.84 \log_{10}$ (38 334 copies/mL; range, 1.69 – $5.65 \log_{10}$) (Figure 2). The mean DNA copy numbers were $4.68 \log_{10}$ (47 710 copies/mL) in CMV D⁺/R⁺ and $4.25 \log_{10}$ (27 210 copies/mL) in D⁺/R⁻ cases ($P = .59$). PCR assays in use during the study period had varying LODs, with 55 (68%), 21 (26%), and 5 (6%) of the tests performed using assays with LODs of 600, 300, or 50 copies/mL plasma, respectively.

Plasma CMV DNA was undetectable in 3 patients with biopsy-proven CMV GI tract disease (15%), designated as patients 1–3 (Table 2). These 3 patients were all kidney transplant recipients, CMV D⁺/R⁺, no longer receiving CMV prophylaxis, with disease diagnosed at 5, 9, and 12 months after transplantation, respectively. Two of the 3 cases occurred when the assay with an LOD of 600 copies/mL was in use.

The majority (58 of 61; 95.1%) of transplant recipients without CMV GI tract disease by biopsy had undetectable plasma CMV DNA. Three of 61 (4.9%) had detectable CMV DNA in plasma without evidence of CMV GI tract disease at biopsy, designated as patients 4–6 (Table 2). Patient 4 had a CMV plasma DNA level of $2.49 \log_{10}$ (307 copies/mL). The clinical diagnosis was thought to be mycophenolate mofetil–associated GI tract toxicity, and CMV-specific treatment was not administered. Patient 5 was a kidney-heart transplant recipient (CMV D⁺/R⁻) in whom induction-dose intravenous ganciclovir was started 11 days after the first detectable CMV DNA qPCR result. Esophagogastroduodenoscopy was performed 8 days after treatment initiation and revealed chemical gastritis. This patient was treated for probable CMV GI tract disease despite no evidence of CMV on biopsy. Finally, patient 6 was a kidney transplant recipient (CMV D⁻/R⁻) with a plasma CMV viral load of $5.54 \log_{10}$ (346 736 copies/mL) in whom intravenous ganciclovir was started before biopsy. Colonoscopy was delayed for 14 days, and biopsy showed ischemic colitis without specific evidence of CMV.

Overall, the sensitivity and specificity of plasma CMV DNA qPCR for the diagnosis of CMV GI tract disease were 85%

Figure 2. Cytomegalovirus (CMV) plasma viral load (DNA) for each patient with suspected CMV gastrointestinal (GI) tract disease, measured within 15 days of GI tract biopsy. Solid circles represent patients with detectable CMV DNA; open circles, patients with undetectable CMV DNA, at the lower limit of detection of the assay in use at the time. Three quantitative polymerase chain reaction assays were used during the study period, with varying lower limits of detection (600, 300, and 50 copies/mL) represented by dotted lines.

(95% CI, 61%–96%) and 95% (95% CI, 85%–99%), respectively (Table 3). Sensitivity was 100% for liver (95% CI, 56%–99%), 100% for CMV D⁺/R[−] (95% CI, 56%–99%), and 100% for D[−]/R[−] (95% CI, 20%–95%) transplant recipients. The lowest sensitivity was observed for CMV D⁺/R⁺ recipients (72.7%; 95% CI, 39%–93%) and kidney transplant recipients, including 3 patients with kidney-heart kidney-pancreas transplants (75%, 95% CI, 43%–93%). Sensitivity and specificity for assays with different LODs were comparable but with large CIs because the assays with LODs of 300 and 50 copies/mL were used in very few patients (Table 3). Predictive values were modeled according to different disease prevalence values (Figure 3). Assuming a prevalence of 24.7% (20 of 81 patients) for CMV GI tract disease among SOT recipients who underwent biopsy in this study, the PPV and NPV of CMV DNA qPCR were 0.85 and 0.94, respectively.

Risk Factor Analysis

Univariable analyses to identify predictors for CMV GI tract disease were performed using the independent variables listed in Table 4. Detection of CMV DNA in plasma was identified as the most significant predictor of CMV GI tract disease in SOT recipients (odds ratio, 81.9; 95% CI, 8.8–765.1; $P < .001$ [Table 4]).

DISCUSSION

CMV disease remains the most common viral complication after SOT. Although CMV DNA qPCR is widely used, the data on utility in the diagnosis of GI tract disease are limited. Results of published studies with smaller cohorts and anecdotal clinical experience have suggested that the sensitivity of qPCR for detecting CMV GI tract disease is poor. To our knowledge, ours is the largest series of kidney and liver transplant recipients with suspected CMV GI tract disease who underwent both GI tract biopsy and plasma CMV DNA testing. We report a good overall sensitivity (85%) and excellent specificity (95%) of qPCR for plasma CMV DNA as an adjunct diagnostic method for CMV GI tract disease in abdominal transplant recipients. The sensitivity and specificity seemed to vary depending on the recipient CMV serostatus.

Several previous studies have demonstrated a poor sensitivity (range, 48%–73%) of qPCR for plasma CMV DNA in the detection of GI tract disease [9–11]. These studies were limited by several factors, including (1) a mixed cohort of transplant recipients and immunocompetent patients [10], (2) small numbers of patients with available qPCR data [9, 11], and/or (3) a mix of end-organ CMV diseases, not exclusively GI tract

Table 2. Presentation of Patients With Discordant Plasma CMV DNA qPCR and Biopsy Results

Presentation	CMV Biopsy Positive			CMV Biopsy Negative		
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age, y	55	33	52	77	36	53
Sex	Female	Female	Female	Female	Male	Male
Race	White	White	Black	Black	White	White
Transplant type	Kidney	Kidney	Kidney	Kidney	Kidney-heart	Kidney
Time after transplantation	5 mo	9 mo	12 mo	4 y	15 mo	9 mo
CMV serostatus	D ⁺ /R ⁺	D ⁺ /R ⁺	D ⁺ /R ⁺	D ⁺ /R ⁺	D ⁺ /R ⁻	D ⁻ /R ⁻
CMV DNA by qPCR, copies/mL	Undetectable ^a	Undetectable ^b	Undetectable ^b	307 (2.49 log ₁₀)	3660 (3.56 log ₁₀)	346 736 (5.54 log ₁₀)
Timing of qPCR relative to biopsy, d	-5	-13	10	-6	-19	-17
Location of GI tract disease	Upper GI tract	Lower GI tract	Lower GI tract	Upper and lower GI tract	Upper GI tract	Lower GI tract
Macroscopic findings	Acute or chronic gastritis	Normal	Erythematous, friable mucosa	Esophageal plaques; colon erythematous lesion	Chemical gastritis ^c	Ischemic changes ^d
Pathologic findings	Positive IC results	Positive IC results ^e	VCE	No VCE	No VCE	No VCE
Clinical diagnosis	CMV UGI tract disease	CMV LGI tract disease	CMV LGI tract disease	MMF toxicity	CMV UGI tract disease	CMV LGI tract disease; ischemic colitis
Treatment	Intravenous GCV	Intravenous GCV	Intravenous GCV	None; MMF dose reduced	Intravenous GCV	Intravenous GCV
Timing of antiviral treatment relative to biopsy	18 d after	1 d after	10 d after	No treatment	8 d before	14 d before

Abbreviations: CMV, cytomegalovirus; D⁺, donor CMV seropositive; D⁻, donor CMV seronegative; GCV, ganciclovir; GI, gastrointestinal tract; IC, immunohistochemistry; LGI, lower gastrointestinal; MMF, mycophenolate mofetil; qPCR, quantitative polymerase chain reaction; R⁺, recipient CMV seropositive; R⁻, recipient CMV seronegative; UGI, upper gastrointestinal; VCE, viral cytopathic effect.

^a Assay limit of detection, 50 copies/mL plasma.

^b Assay limit of detection, 600 copies/mL plasma.

^c Esophagogastroduodenoscopy was performed after 8 days of treatment with induction-dose intravenous GCV because of persistent nausea and vomiting.

^d Colonoscopy was attempted at 7 days, but bowel preparation was poor. Because of persistent diarrhea, another colonoscopy with biopsy was performed after 14 days of treatment with induction-dose intravenous GCV.

^e A few scattered cells were positive for CMV.

Table 3. Sensitivity and Specificity of Plasma CMV DNA for CMV GI Tract Disease

Patients	Sensitivity, % (95% CI) [No. of Patients]	Specificity, % (95% CI) [No. of Patients]
Overall	85.0 (61–96) [17/20]	95.1 (85–99) [58/61]
By CMV serostatus		
D ⁺ /R ⁺	72.7 (39–93) [8/11]	97.2 (84–100) [35/36]
D ⁺ /R [−]	100 (56–99) [7/7]	80 (30–99) [4/5]
D [−] /R [−]	100 (20–95) [2/2]	95 (73–100) [19/20]
By GI tract site involved		
Upper	87.5 (47–99) [7/8]	96.1 (85–99) [49/51]
Lower	86.7 (58–98) [13/15]	94.9 (81–99) [37/39]
By SOT type		
Kidney only ^a	81.8 (48–97) [9/11]	94.7 (81–99) [36/38]
Kidney, kidney-heart, and kidney-pancreas	75 (43–93) [9/12]	92.5 (79–98) [37/40]
Liver only ^b	100 (56–99) [7/7]	100 (78–99) [18/18]
Liver and liver-kidney ^c	100 (60–99) [8/8]	100 (81–99) [21/21]
By LOD of assay used		
600 copies/mL	81.8 (48–98) [9/11]	95.4 (85–99) [42/44]
300 copies/mL	100 (59–100) [7/7]	92.9 (66–100) [13/14]
50 copies/mL	50 (20–95) [1/2]	100 (29–100) [3/3]

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; D⁺, donor CMV seropositive; D[−], donor CMV seronegative; GI, gastrointestinal; LOD, limit of detection; R⁺, recipient CMV seropositive; R[−], recipient CMV seronegative; SOT, solid organ transplant.

^a Patients who received only a kidney transplant; patients who received kidney-heart (n = 1), kidney-pancreas (n = 2), or kidney-liver (n = 4) transplants were not included in these calculations.

^b Patients who received only a liver transplant; patients who received kidney-liver transplants (n = 4) were not included in these calculations.

^c Four patients who received kidney-liver transplants were included in these calculations.

disease [9]. To overcome these limitations, we focused exclusively on abdominal SOT recipients presenting with gastrointestinal symptoms, and we identified 81 patients with biopsy and qPCR data. In this more uniform patient population presenting with GI tract symptoms, qPCR for plasma CMV DNA had a higher sensitivity. This is consistent with an abstract report of 20 cases of CMV colitis in 18 kidney transplant recipients [13], which noted that qPCR for plasma CMV DNA had a sensitivity of 85%

CMV donor and recipient serostatus seemed to affect the performance of plasma CMV DNA qPCR, with a sensitivity of 100% in CMV R[−] SOT recipients compared with 72% in CMV D⁺/R⁺ recipients. With limited numbers in each subgroup, the 95% CIs overlap; however, a real difference in the test performance relative to serostatus could be explained by differences in disease pathogenesis and immune responses. In CMV R[−] cases, CMV GI tract disease often represents primary CMV infection, with active, high-grade CMV viremia and subsequent

tissue invasion. In contrast, in CMV R⁺ cases, CMV GI tract disease may occur because of reactivation of CMV in GI tract-associated lymphoid tissues. In addition, CMV-seropositive recipients are more likely to have a preexisting immune response and may be able to partially suppress CMV replication. In these settings, CMV DNA may be less likely to be found in plasma.

The specificity of qPCR for plasma CMV DNA was excellent. Of 61 transplant recipients, only 3 had GI tract symptoms significant enough to warrant a biopsy in which plasma CMV DNA was detected by qPCR and a biopsy was negative. In 2 of them, treatment for CMV GI tract disease with high-dose intravenous ganciclovir was initiated by the team empirically for presumptive disease, and diagnostic biopsies were delayed for >1 week. In these scenarios, it is possible that the negative biopsy results were due to eradication of the virus from GI tissue as a result of ganciclovir treatment or that prompt initiation of targeted treatment prevented CMV end-organ involvement.

When SOT recipients present with GI tract symptoms, CMV GI tract disease is always a consideration. The usefulness of positive or negative plasma CMV DNA qPCR results in the diagnosis of CMV GI tract disease depends on patient characteristics, because the PPV and NPV of any test are highly affected by disease prevalence. True CMV GI tract disease prevalence is difficult to determine, because patients are often treated for presumptive CMV GI tract disease without undergoing biopsy to prove the presence of tissue-invasive disease. During the study period, the overall incidence of biopsy-proven CMV GI tract disease in abdominal SOT recipients at our institution was <5%. However, considering patients presenting with GI tract symptoms significant enough to prompt endoscopy, the prevalence of biopsy-proven CMV GI tract disease was much higher, at 25%. In this context, the PPV and NPV of detectable plasma CMV DNA were quite good, 0.85 and 0.94, respectively. These results cannot be generalized to a screening setting, where disease prevalence would be much lower.

Having circulating detectable CMV DNA in plasma with a GI tract syndrome was found to be the major predictor of biopsy-proven CMV GI tract disease in this cohort. The effects of CMV D/R serostatus and administration of CMV prophylaxis on CMV GI tract disease were most likely diluted in the multivariable analyses because of the small number of patients included.

Our study had several limitations, including its relatively small number of patients. To capture the patient population in whom CMV GI tract disease was considered a possible diagnosis, we defined our study population as SOT recipients presenting with GI tract symptoms who underwent endoscopy with biopsy and CMV DNA qPCR testing. This may have excluded some patients with CMV GI tract disease. Our reference standard of biopsy may have missed cases of CMV GI tract disease because of sampling errors or delays in acquisition. Moreover,

Figure 3. Positive (A) and negative (B) predictive values calculated from the sensitivity and specificity for the detection of plasma cytomegalovirus (CMV) DNA by quantitative polymerase chain reaction over a range of hypothetical prevalence values. Dotted lines represent prevalence of CMV gastrointestinal tract disease among patients in this series in whom biopsy was performed (24.7%), in whom the positive and negative predictive values were 0.85 and 0.94, respectively.

Table 4. Predictors of CMV GI Tract Disease

Predictor	Univariable Analysis			Multivariable Analysis		
	OR	95% CI	PValue	OR	95% CI	PValue
Sex (male vs female)	0.91	.3–2.5	.85	...	...	...
Ethnicity (white vs other)	1.16	.4–3.7	.80	...	...	...
Age (<50 vs ≥50 y)	0.72	.2–2.1	.55	...	...	...
Prior transplant (yes vs no)	1.23	.4–4.0	.73	...	...	...
Type of SOT (kidney vs other)	1.15	.6–2.1	.65	...	...	...
Rejection (yes vs no) ^a	3.7	.9–14.6	.06	...	...	...
CMV serostatus (D ⁺ /R ⁺ vs other)	0.26	.1–.6	.004	0.34	.06–1.8	.20
CMV prophylaxis (yes vs no) ^a	0.10	.02–.5	.004	0.11	.01–1.9	.13
CMV DNA at qPCR (positive vs undetectable)	109.6	20.2–593.2	<.001	81.95	8.8–765.1	<.001
Creatinine (>2 vs ≤2 mg/dL) ^b	3.85	1.2–12.0	.02	5.3	.6–42.9	.12
WBC count (>3000 vs ≤3000 cells/μL) ^b	0.24	.07–.8	.02	5.0	.2–108.1	.30
Platelet count (>100 000 vs ≤100 000 cells/μL) ^b	0.27	.09–.8	.02	0.33	.04–2.8	.31

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; D⁺/R⁺, donor and recipient CMV seronegative; GI, gastrointestinal; OR, odds ratio; qPCR, quantitative polymerase chain reaction; SOT, solid organ transplant; WBC, white blood cell.

^a Within 30 days before biopsy.

^b Within 3 days before biopsy.

testing for CMV DNA was performed on plasma; some evidence suggests that testing whole blood may increase the sensitivity of qPCR [14], but it would reduce specificity because CMV DNA is found in peripheral blood mononuclear cells in asymptomatic individuals. We also used PCR assays with varying LODs (600, 300, and 50 copies/mL of plasma). These assays seemed similar in sensitivity and specificity, but relatively few patients were tested with the third assay (LOD, 50 copies/mL) and assays with lower LODs may be more sensitive. Finally, because sensitivity and specificity were not calculated within the entire population at risk for CMV GI tract disease, performance cannot be generalized to the asymptomatic, screening setting.

In conclusion, plasma CMV qPCR had good sensitivity and excellent specificity when used as an adjunct diagnostic method for CMV GI tract disease in kidney and liver transplant recipients. Its sensitivity was higher in CMV-seronegative and lower in CMV-seropositive recipients, which may reflect differences in disease pathogenesis between these groups.

Notes

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