

1 **Real-time quantitative PCR analysis of endoscopic biopsies for diagnosing CMV gastrointestinal disease**
2 **in non-HIV immunocompromised patients: a diagnostic accuracy study.**

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19 **Running title:** qPCR of endoscopic biopsies for CMV-GID

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Abstract

Purpose: Cytomegalovirus gastrointestinal diseases (CMV-GIDs) are end-organ diseases of the gastrointestinal (GI) tract caused by CMV in immunocompromised patients. We aimed to evaluate the performance of quantitative polymerase chain reaction (qPCR) on endoscopic biopsies.

Methods: We retrospectively reviewed the qPCR data on endoscopic biopsies in non-human immunodeficiency virus (HIV) immunocompromised patients between January 2009 and May 2015. The performance of the qPCR for CMV-GID was evaluated with the sensitivity, specificity, and area under the receiver-operating characteristic curve (AUROC).

Results: A total of 195 patients were included, and 28 patients with confirmed CMV-GID were identified. The AUROC of the qPCR was 0.935 (95% confidence interval [CI], 0.885 to 0.985), the sensitivity was 89.3% (95% CI, 71.8 to 97.7%), and the specificity was 85.6% (95% CI, 79.4 to 97.6%) with a cut-off value of 180 copies/ μ g DNA. The proportion of patients with inflammatory bowel disease in the histopathology-negative, PCR-positive group was smaller than that in the histopathology-positive group (10.7 vs 35.0%, $p=0.026$), but other characteristics were not significantly different.

Conclusions: The use of qPCR on endoscopic biopsies demonstrated good diagnostic performance for detecting CMV in non-HIV immunocompromised patients. It may increase the diagnostic yield when combined with a conventional histopathology.

Key words: “cytomegalovirus”, “gastrointestinal disease”, “real-time quantitative PCR”, “endoscopic biopsy”, “diagnostic accuracy”

Introduction

Cytomegalovirus gastrointestinal disease (CMV-GID) is a tissue-invasive, end-organ disease. It is a major cause of morbidity and mortality in immunocompromised patients, especially in transplant recipients and human immunodeficiency virus (HIV)-infected patients, as well as in those who receive immunosuppressive drugs [1-5]. A definitive diagnosis of CMV-GID requires a biopsy obtained by endoscopy with histologic or culture-based evidence of CMV [6]. However, a viral culture is not routinely performed, and the histopathology of biopsied tissue has limited sensitivity, as previously demonstrated in a study of CMV oesophagitis in HIV-infected patients, which showed 3 biopsy samples were diagnostic in 80% of patients, with as many as 10 biopsy samples needed for the diagnosis in the remaining patients [7].

Non-invasive molecular diagnostic tools, such as the CMV antigenaemia assay and polymerase chain reaction (PCR) on blood samples, are widely used, although the latter is not approved in Japan. Despite the high sensitivity of the CMV antigenaemia assay for detection of viral reactivation before the onset of CMV pneumonitis in haematopoietic stem cell transplant (HSCT) recipients, this assay does not necessarily provide positive results preceding the onset of other CMV diseases [8]. For example, Jang et al. [9] reported insufficient sensitivity of the CMV antigenaemia assay for the diagnosis of CMV-GID.

Real-time quantitative PCR (qPCR) on blood samples is considered to be more sensitive than the CMV antigenaemia assay [10]. However, Mori et al. [8] reported that the blood PCR assay yielded positive results for only 50% of HSCT recipients with CMV-GID. In solid organ transplant (SOT) recipients, the sensitivity of qPCR analysis of plasma samples has been reported to be 85% to 97% in CMV-positive donor (D+)/CMV-negative recipient (R-) patients, but it is decreased to 50% to 75% in R+ patients [11, 12].

Tissue diagnostics, such as viral culture and tissue PCR, are expected to increase the diagnostic yield for CMV-GID. Fisher et al. [12] reported that the addition of a viral culture to histopathology increases the diagnostic yield for CMV-GID in SOT recipients. PCR on tissue samples is also a promising tool due to its short turn-around-time (TAT) and presumptive high accuracy. Burston analysed the diagnostic accuracy of a real-time qualitative PCR assay on biopsies targeting DNA polymerase for the diagnosis of CMV disease in non-HIV patients [13]. In that study, the sensitivity was 100% (95% confidence interval [CI], 79.4 to 100%), and the specificity was 71.9% (95% CI, 65.1 to 78.1%); however, various organs other than the GI tract were included,

and the PCR was performed in a qualitative manner. In the latest consensus definition of CMV diseases in transplant recipients, “possible disease” was proposed that requires a positive result from qPCR performed on endoscopic biopsies but does not require evidence of CMV by histopathology [6]; the optimal cut-off value remains to be determined. The objective of our study was to evaluate the diagnostic accuracy and optimal cut-off value of qPCR performed on tissue samples for diagnosing CMV-GIDs in non-HIV immunocompromised patients.

Methods

Study Design

This single-centre, retrospective, cross-sectional study was approved by the Ethics Committee of Kyoto University Graduate School and the Faculty of Medicine (R0454). The need to obtain informed patient consent was waived.

Participants

The database was retrospectively reviewed to identify consecutive adult patients (aged >18 years) whose endoscopic biopsies of the GI tract (oesophagus, stomach, small bowel, or colon) were subjected to qPCR (limited to one episode per patient) between January 2009 and May 2015 at Kyoto University Hospital, which is a 1121-bed tertiary referral hospital in Kyoto, Japan. Non-HIV immunocompromised patients, including post-transplant patients, patients with haematological malignancy and patients receiving immunosuppressive therapy, were eligible. The index test was real-time qPCR performed on endoscopic biopsies, and the reference standard was CMV evidence in the tissue shown by histopathology or immunohistochemistry (IHC) using biopsies obtained at the same time but from a different site than that used for the PCR analysis. Patients who had a history of CMV-GID and those who did not have a concurrent histopathology examination were excluded from the study.

Definitions of CMV-GID

CMV-GID was defined as either proven, probable, or possible disease according to previously published consensus definitions of CMV disease in transplant recipients [6]. A definition of proven disease required upper

and/or lower GI symptoms, macroscopic mucosal lesions and evidence of CMV in the tissue shown by histopathology or IHC. Probable disease required upper and/or lower GI symptoms and CMV evidence in the tissue but did not require macroscopic mucosal lesions. Possible disease required upper and/or lower GI symptoms and CMV evidence based on qPCR performed on endoscopic biopsies. In this study, only patients with proven and probable disease were defined as having “CMV-GID” because in the possible disease, the index test result is included but the reference standard is not.

PCR Assay

The PCR assay was performed at the discretion of the endoscopist. DNA extraction from endoscopic biopsies was performed using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The typical tissue size for extraction is approximately 30 mm³. During the study period, 2 in-house real-time qPCR assays targeting the immediate-early (IE) genes were used at Kyoto University Hospital with the following primers and probe: forward (5'- GACTAGTGTGATGCTGGCCAAG -3') and reverse (5'- GCTACAATAGCCTCTTCCTCATCTG -3') primers and probe (5'-FAM- AGCCTGAGGTTATCAGTGTAATGAAGCGCC -TAMRA-3'). BLAST search was undertaken in the GenBank database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) in order to ensure that the primer and probe were not homologous with other viruses. During the first period, from January 2009 to June 2010, an assay using the ABI PRISM 7700 (Thermo Fischer Scientific, Waltham, MA, USA) was performed as previously described, with modifications [14]. In the second period, from July 2010 to May 2015, real-time qPCR was performed in a 25 µL reaction volume containing 0.2 µM of forward and reverse primers, 0.125 µM of probe, 2.5 µL of TaqMan Gold Buffer A (Thermo Fischer Scientific), and 125 ng of purified DNA. Thermal cycling was performed in a Step One Plus thermocycler (Thermo Fischer Scientific) as follows: 10 min at 95 °C followed by 50 cycles of 15 s at 95 °C and 1 min at 60 °C. The results are reported as copies/µg DNA. The limit of detection was 10 copies/µg DNA for both assays. The linear range of the both assays was 10 to 10⁶ copies/µg DNA.

Statistical Analysis

Descriptive statistics included counts and percentages for qualitative variables and quartiles (25th, 50th, and 75th percentile) for quantitative data. Categorical variables were compared using the Fisher exact test. Continuous variables were compared using the Mann-Whitney U-test. The diagnostic accuracy of qPCR was

evaluated by the area under the receiver operating characteristic curve (AUROC), and the optimal cut-off values were determined using the maximum Youden index. The sensitivity and specificity were evaluated at the cut-off value. Estimates of conditional probability were accompanied by 95% CIs. For patients with multiple specimens tested by qPCR, the maximum value reported was chosen for the analysis. A p value <0.05 was considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). The sample size was estimated as follows: using a pre-determined value of the AUROC of 0.90, to estimate the AUROC with 95% confidence and a degree of precision of approximately 0.10, the required sample size was calculated to be at least 27 patients in each disease and non-disease group, as previously described [15]. The results of this study are reported according to the Standards for Reporting Diagnostic Accuracy Studies (STARD) statement [16].

Results

Participants

Between January 2009 and May 2015, 516 patients from the qPCR database of the Department of Clinical Laboratory Medicine were assessed for initial eligibility and invited to participate. Figure 1 shows the flow of patients through the study. A total of 321 patients were excluded for the following reasons: 123 patients had duplicate data, 106 patients were not immunocompromised, 46 patients did not have concurrent histology, 41 patients were less than 18 years old, 3 patients had a past history of CMV-GID, 1 patient was HIV-positive and 1 patient had insufficient data (biopsy organ was unknown). Among the 195 eligible patients, 28 patients (14.3%) with proven CMV-GID (15 of the upper GI tract, and 13 of the lower GI tract), 40 patients (20.5%) with possible disease and 127 patients (65.1%) with non-CMV-GID were identified. There were no cases of probable disease. The baseline clinical characteristics of the patients are presented in Table 1. Thirty-six patients (18.5%) were given antiviral drugs within 30 days before the endoscopy. The number of patients with prior antiviral drug therapy markedly varied among underlying diseases as follows: 20 (42.6%) HSCT patients, 6 (28.6%) SOT patients, 7 (15.6%) connective tissue disease (CTD) patients, and 1 (1.3%) inflammatory bowel disease (IBD) patient. Among the 167 patients in the possible and non-CMV-GID groups, the alternate diagnoses were as follows: 67 (40.1%) with ulcerative colitis, 48 (28.7%) with non-specified GI diseases, 23 (15.6%) with graft-versus-host-disease (GVHD) and 7 (4.2%) with drug-induced ulcers.

Diagnostic Performance of qPCR on Biopsies

The qPCR results are presented in Figure 2. Among the 28 patients with proven disease, 27 patients were qPCR-positive. To discriminate the proven group from the possible and non-CMV-GID groups, the ROC analysis produced an AUROC of 0.935 (95% CI, 0.885 to 0.985) with a sensitivity of 89.3% (95% CI, 71.8 to 97.7%) and a specificity of 85.6% (95% CI, 79.4 to 97.6%) for a cut-off value of 180 copies/μg DNA. One patient in the proven disease group had a negative qPCR result. The patient was a 60-year-old man who received immunosuppressive therapy for granulomatosis with polyangiitis and was diagnosed with CMV gastritis by histopathology. He was not using antiviral drugs.

The subgroup analysis of patients without prior antiviral drug therapy resulted in an AUROC of 0.933 (95% CI, 0.864 to 1.000) with a sensitivity of 88.9% (95% CI, 65.3 to 98.6%) and a specificity of 87.2% (95% CI, 79.8 to 91.7%) for a cut-off value of 530 copies/μg DNA (Table 2). The subgroup analysis based on underlying disease category is also shown in Table 2. The cut-off value ranged from 180 to 600 copies/μg DNA, except for that of the IBD patient, which was 10,000 copies/μg DNA.

As shown in Figure 2, the PCR value of patients with proven disease was significantly higher than that of patients with possible disease. A significantly greater proportion of patients had IBD in the possible group (10.7 vs 35.0%, $p=0.026$), and significantly higher number of blood lymphocyte count were seen in the possible group (Table 3). A greater proportion of patients have received the antiviral treatment in the proven group (92.9 vs 57.5%, $p=0.002$), and a greater proportion of patients have demonstrated clinical response in the proven group (100.0 vs 73.9%, $p=0.007$). There were 2 patients who have not treated in the proven group. The one had been diagnosed as mucosa associated lymphoid tissue lymphoma (MALToma) later, and the other had been tapered the immunosuppressive drugs and improved clinically. In the possible group, 23 (57.5%) patients had treated and 16 (69.6%) demonstrated clinical response. Among 7 patient who did not have clinical response, 2 were diagnosed as GVHD, 2 as UC flare, 1 as amyloidosis, and 2 had no alternative diagnosis, but all of them clinically improved. Among 17 patients who did not receive antiviral treatment in the possible group, only 1 patient had exacerbation of the symptom 1 month later, and had improved after antiviral treatment.

Discussion

To the best of our knowledge, this study represents the largest cohort studied to date to evaluate the accuracy of qPCR performed on tissue samples to diagnose CMV-GIDs. The diagnostic performance of qPCR was excellent.

We showed that the sensitivity of qPCR was 89.3%, which was consistent with a previous study that assessed the performance of qPCR on formalin-fixed, paraffin-embedded (FFPE) tissue [17]. That study compared results from IHC and qPCR on FFPE tissue. Although the same tissue sample was used for IHC and qPCR, 3 cases (9.1%) were found to be PCR-negative among the 33 IHC-positive cases. The authors proposed sampling error as a cause of the false negatives. In our study, 1 case of CTD was found to be PCR-negative and histopathology-positive. Sampling error was likely the cause of this result because different single biopsy samples were obtained for histopathology and qPCR. The use of multiple biopsies might have lowered the incidence of sampling error.

Although the AUROC was comparable between patients with or without prior antiviral drug treatment, the cut-off value of the former group was greater than that of the latter group. It is expected that antiviral drugs may lower the sensitivity of diagnostic tests because these drugs affect the virus-infected GI tissue, which decreases the viral load within that tissue. To evaluate the true diagnostic accuracy of qPCR, only patients without exposure to antivirals before the biopsy should be included, although that introduces a selection bias. It is difficult to fully exclude the effect of prior exposure to antiviral drugs on the accuracy of qPCR in diagnosing CMV-GID because in the real world, it is necessary to begin treatment without waiting for endoscopic examination, especially for transplant patients.

The specificity of qPCR was 85.6%, which was also consistent with a study on FFPE tissue qPCR [17] and better than that demonstrated in a study with qualitative PCR of tissue [13]. In the guidelines for opportunistic infections in HIV-infected patients [18], tissue diagnostics, such as viral culture, are not recommended because asymptomatic viral shedding is common in HIV patients [19]. Data on asymptomatic viral shedding in the non-HIV patients are insufficient; however, CMV reactivation is frequently observed in IBD patients [20]. In this study, the cut-off value for IBD patients was as high as 10,000 copies/ μ g DNA (Table 2), which may reflect asymptomatic viral shedding, although the sample size is limited. Because IBD patients without immunosuppressive therapy were not included, further study is needed to evaluate the role of qPCR in discriminating true pathogenicity from non-specific reactivation in IBD patients.

The smaller qPCR value in the possible disease group may indicate that the viral burden differs between groups. Moreover, the difference of lymphocyte count between groups may also suggest that the risk for CMV disease differ between groups. Given a certain proportion of the patient who had treated in the possible group had demonstrated clinical response, it is worth evaluating the possible group as a distinct category. However, the greater proportion of IBD patients in the possible group and the existence of patient who have improved without treatment may indicate the possibility of non-pathogenic viral shedding in the possible group.

Although qPCR performed on endoscopic biopsies demonstrated good sensitivity and specificity, this procedure requires an endoscopic examination, which can be invasive for patients. In situations where patients can undergo an endoscopy, if qPCR is performed routinely it is useful for early therapy due to its shorter TAT. On the other hand, if qPCR is performed when histopathology is negative, it may increase the diagnostic yield for CMV-GID by defining patients who belong to the possible group. Therefore, it is expected that clinicians can make a clinical decision to start antiviral drugs based on the result of qPCR assays.

This study had other inherent limitations. First, it was a single-centre, retrospective analysis. Second, the sample size was not large enough to achieve an accurate estimation of diagnostic values. Third, not all patients who were suspected to have CMV-GID were included because the participants of this study were reviewed using the qPCR database. Finally, the methodology for qPCR performed on tissue samples is not standardized, unlike qPCR performed on plasma, which, according to a 2010 WHO International Standard, has a more reproducible methodology across laboratories [21]. Further study is warranted to determine standardized methods for qPCR performed on tissue samples.

In conclusion, qPCR performed on endoscopic biopsies of the GI tract achieved good results for diagnosing CMV-GID in non-HIV immunocompromised patients. It also may increase the diagnostic yield when performed in combination with conventional histopathological examinations, but we have to be aware of the possibility of non-pathogenic viral shedding.

Authors' Contributions

YT participated in the design of the study, reviewed the medical records, performed the statistical analysis, and drafted the manuscript. MN, SN, MY, YM, and SI participated in the design of the study, interpreted the data and revised the manuscript. MM and HS participated in the manuscript preparation.

Conflicts of interest

No conflicts of interest.

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Figure Legends

Figure 1. Flow of participants.

Abbreviations: CMV-GID, cytomegalovirus gastrointestinal disease. HIV, human immunodeficiency virus.
PCR, polymerase chain reaction.

Figure 2. Comparison of qPCR values between possible CMV-GID and proven CMV-GID.

CMV DNA qPCR copy numbers according to patient group. In the box and whisker plots, the boxes include 50% of the sample data, with the median value indicated by a horizontal bar. The whiskers include 1.5× the interquartile range. The copy numbers of proven CMV-GID were significantly higher than those of possible CMV-GID ($p < 0.001$).

Figure 3. ROC curve of CMV copy numbers for the diagnosis of CMV-GID.

The ROC analysis produced an AUROC of 0.935 (95% CI, 0.885 to 0.985), a sensitivity of 89.3% (95% CI, 71.8 to 97.7%) and a specificity of 85.6% (95% CI, 79.4 to 97.6%) using a cut-off value of 180 copies/μg DNA.

317 **Table 1. Clinical characteristics of all eligible participants.**

	All (n=195)	
Age, y	51	(34-64)
Male sex	113	(57.9)
Underlying condition or illness		
HSCT	47	(24.1)
Allo-BMT	21	(10.8)
Allo-PBSCT	6	(3.1)
Auto-PBSCT	2	(1.0)
CBT	18	(9.2)
SOT	21	(10.8)
Liver	13	(6.7)
Lung	5	(2.6)
Kidney	2	(1.0)
Small intestine	1	(0.5)
Connective tissue disease	45	(23.1)
Inflammatory bowel disease	75	(38.5)
Haematological malignancy	52	(26.7)
Others	22	(11.3)
Systemic steroid	98	(50.3)
Immunosuppressant	144	(73.8)
Anti-cancer chemotherapy	9	(4.6)
Endoscopically abnormal	184	(94.4)
Erythema	116	(59.5)
Erosion	80	(41.0)
Ulcer	104	(53.3)
Biopsied organ		
Oesophagus	11	(5.6)
Stomach	49	(25.1)
Duodenum	13	(6.7)
Jejunum/ileum	13	(6.7)
Caecum/colon/rectum	117	(60.0)
≥2 organs	10	(5.1)
Number of biopsied specimen		
1	165	(84.6)
2	28	(14.4)
3	2	(1.0)
Antigenaemia positive	45/131	(34.4)
CMV-IgG positive	96/109	(88.9)
Antiviral drug within 30 days	36	(18.5)
90-day mortality	3	(1.5)

318 Data represent No. (%) or median (IQR). Abbreviations: IQR, interquartile range. HSCT, haematopoietic stem
 319 cell transplant. BMT, bone marrow transplant. PBSCT, peripheral blood stem cell transplant. CBT, cord blood
 320 transplant. SOT, solid organ transplant. PCR, polymerase chain reaction.

Table 2. Subgroup analysis of the diagnostic accuracy of qPCR.

Patient group	n ^a /N ^b	Cut-off ^c	Sensitivity (95% CI)	Specificity (95% CI)	AUROC (95% CI)
All patients	28/195	180	0.893 (0.718-0.977)	0.856 (0.794-0.906)	0.935 (0.885-0.985)
Without prior antiviral drugs within 30 days	18/159	530	0.889 (0.653-0.986)	0.872 (0.798-0.917)	0.933 (0.864-1.000)
Transplant recipients	10/65	180	0.900 (0.555-0.997)	0.891 (0.778-0.959)	0.958 (0.906-1.000)
HSCT	7/47	10	1.000 (0.473-1.000)	0.800 (0.644-0.909)	0.954 (0.885-1.000)
HSCT with GI-GVHD	2/27	180	1.000 (0.094-1.000)	0.960 (0.781-0.999)	0.980 (0.925-1.000)
SOT	3/21	530	1.000 (0.194-1.000)	0.944 (0.653-0.986)	0.981 (0.930-1.000)
Patients other than transplant recipients	18/130	600	0.889 (0.653-0.986)	0.866 (0.789-0.923)	0.922 (0.850-0.995)
Inflammatory bowel disease	3/75	10000	1.000 (0.194-1.000)	0.986 (0.924-1.000)	0.991 (0.970-1.000)
Connective tissue disease	11/45	600	0.909 (0.587-0.998)	0.794 (0.621-0.913)	0.872 (0.744-0.999)

^a number of patients diagnosed with proven CMV-GID. ^b number of all patients among the groups. ^c copies/μg DNA.

Abbreviations: CMV-GID, cytomegalovirus gastrointestinal disease. qPCR, quantitative polymerase chain

reaction. CI, confidence interval. AUROC, area under the receiver-operating characteristic curve. CI, confidence

interval. HSCT, haematopoietic stem cell transplant. GI, gastrointestinal. GVHD, graft-versus-host disease. SOT,

solid organ transplant.

329 **Table 3. Comparison of patient characteristics between the proven and possible group.**

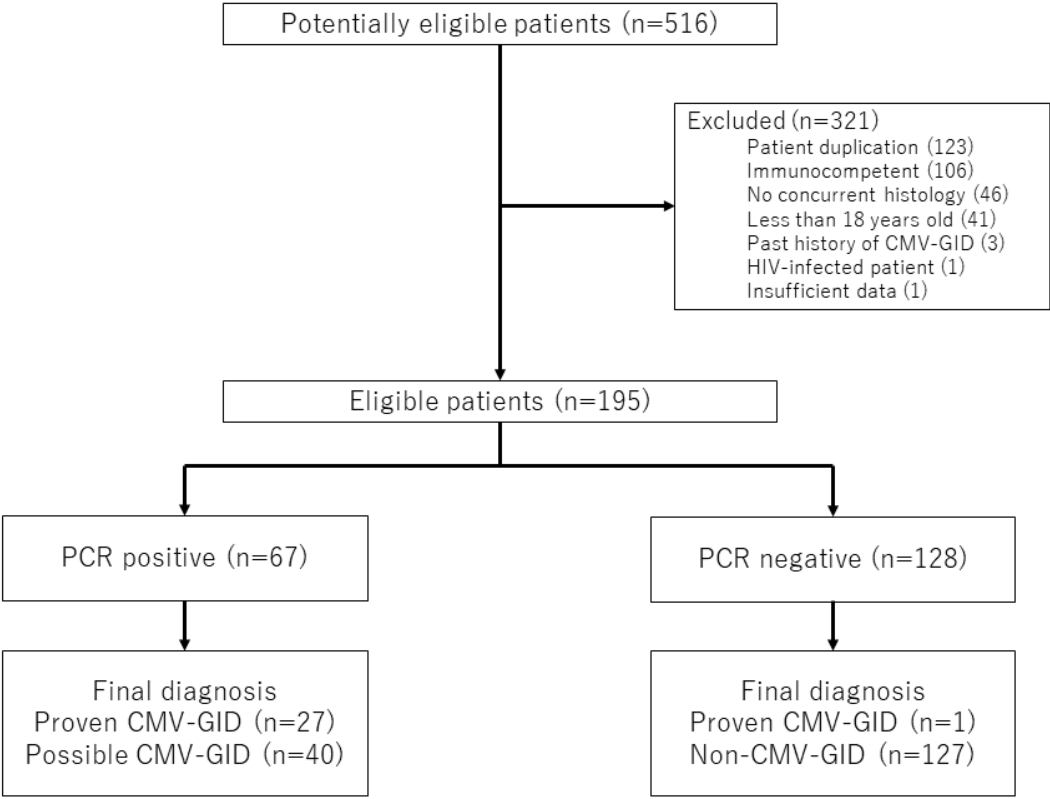
	Proven (n=28)		Possible (n=40)		p value
Age, y	59.5	(52-67.5)	58.5	(41-81)	0.497
Male sex	14	(50.0)	25	(62.5)	0.330
Underlying condition or illness					
HSCT	7	(25.0)	8	(20.0)	0.768
SOT	3	(10.7)	5	(12.5)	1.000
Connective tissue disease	11	(39.3)	10	(25.0)	0.287
Inflammatory bowel disease	3	(10.7)	14	(35.0)	0.026
Haematological malignancy	8	(28.6)	10	(25.0)	0.785
Others	4	(14.3)	3	(7.5)	0.435
Systemic steroid	20	(71.4)	23	(57.5)	0.310
Immunosuppressant	21	(75.0)	29	(72.5)	1.000
Anti-cancer chemotherapy	1	(3.6)	2	(5.0)	1.000
Endoscopically abnormal	28	(100.0)	39	(97.5)	1.000
Erythema	14	(50.0)	22	(55.0)	0.806
Erosion	10	(35.7)	16	(40.0)	0.803
Ulcer	19	(67.9)	27	(67.5)	1.000
Biopsied organ					
Oesophagus	2	(7.1)	1	(2.5)	0.564
Stomach	14	(50.0)	12	(30.0)	0.129
Duodenum	2	(7.1)	5	(12.5)	0.691
Jejunum/ileum	3	(10.7)	5	(12.5)	1.000
Caecum/colon/rectum	11	(39.3)	20	(50.0)	0.462
≥2 organs	4	(14.3)	4	(10.0)	0.708
Number of biopsied specimen					
1	20	(71.4)	30	(75.0)	0.644
2	7	(25.0)	10	(25.0)	
3	1	(3.6)	0	(0.0)	
WBC count, /μL	6550	(4245-8610)	6200	(4400-8850)	0.842
Lymphocyte count, /μL	542	(407.25-802.75)	856	(672.50-1586.00)	0.010
Antigenaemia positive	18/24	(75.0)	15/33	(45.5)	0.054
CMV-IgG positive	16/16	(100.0)	21/22	(95.5)	1.000
Antiviral drug within 30 days	10	(35.7)	10	(25.0)	0.421
Treated with antiviral drug	26	(92.9)	23	(57.5)	0.002
Clinical response to treatment	26/26	(100.0)	16/23	(69.6)	0.003
90-day mortality	2	(7.1)	0	(0.0)	0.166

330 Data represent No. (%) or median (IQR). Abbreviations: IQR, interquartile range. HSCT, haematopoietic stem

331 cell transplant. SOT, solid organ transplant. CMV, cytomegalovirus.

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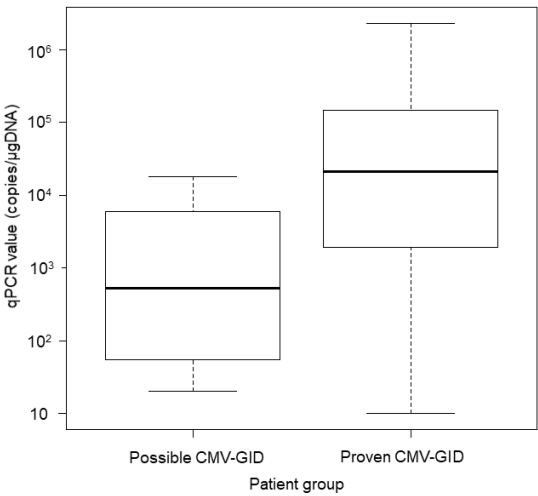
333 **Figure 1.**



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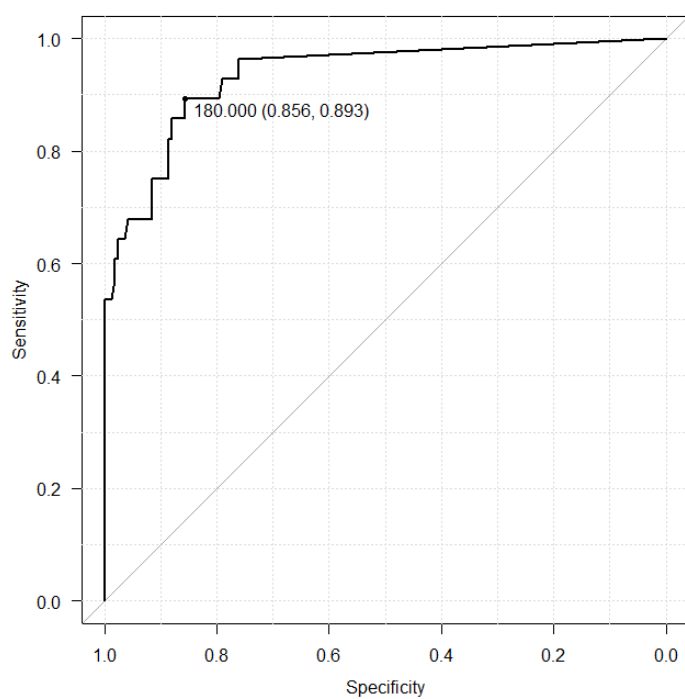
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336 **Figure 2.**



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338 **Figure 3.**



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