High prevalence of gastrointestinal manifestations among Cytomegalovirus end-organ disease in the combination antiretroviral era

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ARTICLE INFO

ABSTRACT

Background: Cytomegalovirus (CMV) end-organ disease (EOD) continues to pose a significant risk to patients with advanced HIV disease despite decreased incidence with combination anti-retroviral therapy (ART) and lower mortality with effective *anti*-CMV therapy. Subclinical CMV shedding may also contribute to ongoing inflammation and non-infectious comorbidities.
Methods: We examined the occurrence of CMV EOD and CMV shedding in a cohort of patients participating in a prospective observational study of severely immunosuppressed (CD4 ≤100 cells/μl), ART-naïve, HIV-1 infected adult participants.
Results: We studied 206 participants, of whom 193 (93.7%) were CMV IgG positive. Twenty-five participants (12.1%) developed confirmed CMV EOD. At baseline, 47 (22.8%) had CMV viremia detectable by PCR in the absence of clinical disease (CMV viremia). The remaining 134 (65%) had neither CMV EOD nor CMV viremia detected at baseline. Five participants with CMV EOD (2.4% of total cohort, 20% of CMV EOD) met AIDS Clinical Trials Group criteria for CMV immune reconstitution inflammatory syndrome (IRIS). Only one-third of CMV EOD patients had retinitis, while two-thirds presented with histologically confirmed gastrointestinal illness. CMV viremia was associated with higher percentages of activated CD8$^+$ T cells even after HIV suppression.
Conclusion: The manifestations of CMV EOD in advanced HIV disease before and after initiation of ART may be more diverse than previously described, with high incidence of gastrointestinal illness. Recognition and treatment of unusual clinical presentations of CMV infection remains important in reducing morbidity and mortality from HIV co-infections.

1. Introduction

In the first decade of the HIV epidemic, Cytomegalovirus (CMV)-associated end-organ disease (EOD) emerged as a leading cause of morbidity and mortality in patients with AIDS.[1,2] Although CMV-related gastroenteritis, colitis, esophagitis, pneumonitis and encephalitis were each described early on,[1] CMV retinitis was clearly the predominant manifestation of CMV reactivation in AIDS patients.[3] Fortunately, the availability of effective antiretroviral therapy (ART) led to a remarkable decrease in CMV retinitis[4] and CMV EOD overall.[5]

Even in the era of ART, however, severely immunosuppressed HIV-infected patients remain at risk for CMV EOD as well as CMV-related

Immune Reconstitution Inflammatory Syndrome (IRIS). IRIS is the paradoxical worsening of appropriately treated infection or the unmasking of a previously subclinical infection following the initiation of ART. In one U.S. cohort, IRIS affected approximately 10% of HIV-infected patients commencing ART.[6] Although the incidence of IRIS specifically attributable to CMV is not known, ophthalmologic disease (either retinitis or uveitis) has been the most commonly reported manifestation of CMV-IRIS.[7–10]

Despite considerable efforts, predicting which patients might develop CMV EOD or CMV-IRIS remains a challenge, especially because the vast majority of HIV-infected patients are latently infected with CMV.[11] Clinical predictors associated with progression to CMV EOD include CD4 <75 cells/μL and plasma HIV RNA level >10,000 copies/mL.[12] Although CMV DNA levels above the level of detection have also been recognized as a risk factor for CMV EOD,[5] molecular testing for CMV in the blood is not recommended as part of a clinical work-up of possible CMV EOD due to low specificity, low sensitivity, and low positive predictive values reported in this setting.[13]

The aim of this study was to examine the incidence and manifestations of CMV EOD and CMV-IRIS in a contemporary prospective cohort study of late presenters with HIV disease, naïve to ART, who were thoroughly evaluated and followed after ART initiation. We also sought to characterize CMV EOD in a recent cohort of patients with HIV and severe immunosuppression and re-evaluate whether CMV PCR results, potentially used in combination with other laboratory data, could be used to predict additional risk for CMV EOD.

2. Methods

2.1. Study design and population

This study was performed at the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) under an Institution Review Board approved protocol. All study participants signed informed consent and participated in a prospective observational study of severely immunosuppressed (CD4 ≤100 cells/μL), ART-naïve, HIV-1 infected adult participants (NCT #00286767). We included all patients who enrolled in the study at the NIH site.[14] All participants initiated ART according to Department of Health and Human Services guidelines and were thoroughly evaluated, including with an ophthalmologic examination, at the time of study entry. Demographic data were self-reported. The term "Latine" is used here to encompass persons of all genders with Latin American ethnicity, including Latino and Latina individuals.

2.2. Case definitions

The diagnosis of CMV retinitis was made clinically based on ophthalmologic examination of the retina. All other CMV EOD diagnoses required pathologic confirmation of clinical diagnosis. CMV-IRIS events were identified using the AIDS Clinical Trials Group IRIS criteria[15]: (1) symptoms consistent with an infectious or inflammatory condition that are temporally related to the initiation of ART; (2) associated with a decrease in HIV RNA level; and (3) not explained by a newly acquired infection or the expected clinical course of a previously diagnosed infection, or side effects of ART.

2.3. Laboratory methods

Plasma HIV viral load, CD4 counts, and routine safety laboratory evaluations (hemoglobin, white blood cell count [WBC], platelets, glucose) were performed in real time using FDA-approved assays. Batched cryopreserved plasma samples from all participants at the time of ART initiation were tested in the same laboratory by electrochemiluminescence for CRP (MesoScale Discovery, Rockville, MD) and by ELFA on a VIDAS instrument (bioMerieux, Marcy l'Etoile, France) for

D-dimer levels, according to the manufacturer's instructions. CMV was detected via quantitative real-time PCR amplification of whole blood using DNA hybridization probes specific for the CMV genome. Detectable CMV viremia was defined as CMV viral load >250 copies/mL.

For immunophenotyping, peripheral blood was drawn into ethylenediaminetetracetate [EDTA]-containing tubes according to the manufacturer's instructions, using a modification of the Centers for Disease Control and Prevention guidelines, in a Clinical Laboratory Improvement Act (CLIA)-certified laboratory. Cells were stained with monoclonal antibodies from BD Biosciences (San Jose, CA) then lysed after staining with Optilyse C (Beckman Coulter, Hialeah, FL), washed twice, and resuspended in 500 μl of phosphate-buffered saline (Cambrex, Walkersville, MD). Samples were analyzed immediately on a BD Facs-Canto flow cytometer (BD Biosciences, San Jose, CA). Flow cytometry data were analyzed with FACSDiva software version 6.1.3 (BD Biosciences, San Jose, CA).

2.4. Statistical analysis

We divided the cohort in three groups for analysis: participants with CMV EOD (CMV EOD), participants with CMV viremia in the absence of CMV EOD (CMV$^+$ PCR), and participants with neither CMV EOD nor viremia (CMV$^-$). Continuous variables were reported as median values and interquartile ranges (IQR) and compared using the Kruskal-Wallis test, with post hoc analyses using Dunn's multiple comparisons test. Categorical variables were reported as number and percentage and compared using Chi-square or Fisher's exact test, followed by pairwise comparisons if the initial test was significant. A *P*-value less than 0.05 was considered significant for all tests. All statistical comparisons were performed in GraphPad Prism version 8.4.3 for Macintosh (GraphPad Software, La Jolla, CA) or in R (R core team).

3. Results

3.1. The cohort

We studied a total of 206 participants. The median age of all study participants was 38 years (IQR 31-46), and the majority of patients (150/206, 72.8%) were assigned male at birth [Table 1]. Based on self-reported data, the most represented racial demographic groups were Black (120/206, 58.3%), White (68/206, 33%), and multiracial (11/206, 5.3%). Approximately one third of participants reported Hispanic or Latine ethnicity (71/206, 34.5%). All participants were late presenters with median absolute CD4 count of 19 cells/μL (IQR 8-46 cells/μL) and HIV viral load of 5.1 log copies/mL (IQR 4.7- 5.5 log copies/mL) at the time of enrollment.

Of the 206 participants, 25 (12.1%) had CMV EOD, 47 (22.8%) had detectable CMV viremia at baseline but no active end-organ disease (CMV$^+$ PCR), and 134 (65%) had neither CMV EOD nor detectable viremia at baseline (CMV$^-$) [Table 1]. CMV EOD participants did not significantly differ compared to CMV$^+$ PCR and CMV$^-$ participants in age, sex, race, ethnicity, CD4 count, or HIV viral load.

3.2. CMV end-organ disease and viremia

At baseline, 193 participants (93.7%) had positive CMV IgG, and 65 (31.6%) had detectable CMV viremia (viral load >250 copies/mL). Twenty-five participants (12.1%) were ultimately diagnosed with having active CMV EOD; 13 had disease at baseline while 12 developed disease after ART initiation, including 4 cases of unmasking IRIS. Seven (28% of CMV EOD, 3.4% of cohort,) had CMV retinitis, while 17 (68% of CMV EOD, 8.3% of cohort) had CMV involvement of the gastrointestinal tract [Fig. 1, Table 2]. Only two participants (8% of CMV EOD, 1% of cohort) had pulmonary CMV disease: one developed CMV pneumonitis alone, and the other had concurrent CMV gastritis and pneumonitis.

Three participants with CMV EOD were negative for CMV IgG at

Table 1
Baseline characteristics of all participants (N=206).

	All participants (N=206)[a]	Study group[a]			P-values[b]	Pairwise comparisons[b]		
		CMV EOD (N=25, 12.1%)	CMV+ PCR (N=47, 22.8%)	CMV- PCR (N=134, 65%)		EOD vs PCR+	EOD vs CMV-	PCR+ vs CMV-
Age, years	38 (31-46) [206]	41 (32-47) [25]	38 (30-45.5) [47]	38 (31-46) [134]	ns			
Male at birth	150 (72.8%)	15 (60%)	32 (68.1%)	103 (76.9%)	ns			
Race[c]								
White	68 (33%)	11 (44%)	22 (46.8%)	35 (26.1%)	ns			
Black	120 (58.3%)	12 (48%)	22 (46.8%)	86 (64.2%)				
Indigenous	1 (0.5%)	0 (0%)	0 (0%)	1 (0.7%)				
Multiracial	11 (5.3%)	2 (8%)	2 (4.3%)	7 (5.2%)				
Unknown	6 (2.9%)	0 (0%)	1 (2.1%)	5 (3.7%)				
Ethnicity[c]								
Hispanic or Latine	71 (34.5%)	9 (36%)	21 (44.7%)	41 (30.6%)	ns			
Non-Hispanic or Latine	133 (64.6%)	16 (64%)	25 (53.2%)	92 (68.7%)				
Unknown	2 (1%)	0 (0%)	1 (2.1%)	1 (0.7%)				
HIV Disease								
HIV viral load, $\log_{10}$ copies/mL	5.1 (4.7-5.5) [205]	5.1 (4.7-5.5) [25]	5.2 (4.8-5.7) [47]	5 (4.7-5.5) [133]	ns			
CD4 T cell count, cells/mm^3	19 (8-46) [205]	12 (5-42) [25]	14 (7.5-39) [47]	21 (11-51) [133]	ns			
CD4 T cell proportion, %	3 (1-7) [205]	2 (1-4) [25]	2 (1-4) [47]	3 (2-8) [133]	**0.0386**	ns	ns	ns
CD8 T cell count, cells/mm^3	412 (263-611) [205]	412 (263-540) [25]	428 (266-679) [47]	393 (248-614) [133]	ns			
CD8 T cell proportion, %	63 (53-72) [205]	64 (53-79) [25]	68 (56.5-74.5) [47]	61 (53-70) [133]	ns			
CMV								
CMV IgG positive	193 (93.7%)	22 (88%)	44 (93.6%)	127 (94%)	**0.0040**	ns	**0.0029**	ns
CMV viral load >250 copies/mL	65 (31.6%)	18[d] (72%)	47 (100%)	0 (0%)	**<0.0001**	**0.0003**	**<0.0001**	**<0.0001**
CMV viral load, copies/mL	0 (0-450) [205]	850 (250-7,750) [25]	1000 (450-3,125) [47]	0 (0-0) [133]	**<0.0001**	ns	**<0.0001**	**<0.0001**
CMV viral load, copies/mL untreated	0 (0-450) [196]	1,700 (625-7,263) [16]	1000 (450-3,125) [47]	0 (0-0) [133]	**<0.0001**	ns	**<0.0001**	**<0.0001**
IRIS								
All cases	49 (23.8%)	12[e] (48%)	13 (27.7%)	24 (17.9%)	**0.0040**	ns	**0.0029**	ns
Non-CMV IRIS cases	45 (21.8%)	8 (32%)	13 (27.7%)	24 (17.9%)	ns			

[a] Categorical data are presented as number (percentage). Continuous data are presented as median (interquartile range) [number of participants with available data].

[b] Chi-square or Fisher's exact test was used to calculate comparison between categorical groups followed by pairwise comparisons if significant. Continuous variables were compared using the Kruskal-Wallis test with post-hoc analyses using Dunn's multiple comparisons test.

[c] Participants with unknown data were excluded from analysis.

[d] There were 9 participants who were on treatment for CMV at time of baseline labs. Five of these 9 participants had CMV VL>250.

[e] One patient had IRIS due to CMV and Strongyloides and is counted as both CMV and non-CMV IRIS.

baseline [Table 1], but positive for CMV PCR. Eighteen of those with CMV EOD (72%) had detectable CMV viremia at baseline. Among the seven CMV EOD participants with negative CMV PCR at baseline, four were receiving CMV therapy at the time of the assessment.

3.3. IRIS

Forty-nine participants (23.8% of cohort) developed any form of IRIS, including five with CMV-IRIS (3.7% of IRIS cases, 20% of CMV EOD, 2.4% of cohort) [Tables 1 and 2]. The most common causes of IRIS were Mycobacterial species and human herpesviruses as previously reported.[10] Of the five participants who developed CMV-IRIS following initiating of ART, one had retinitis, one had sialoadenitis, one had ileitis, one had enteritis, and one had appendicitis.

3.4. Treatment

Of the 25 participants with CMV EOD, 23 (92%) received therapy directed against CMV [Table 2]. Nine of the treated participants were receiving anti-CMV therapy at the time of enrollment and baseline labs, and four of these had CMV viral loads below the limit of detection on this therapy at baseline measurement. Two participants, one with esophagitis and another with sialoadenitis, did not receive specific anti-CMV therapy and were monitored closely until symptoms improved with ART and supportive care.

Participants with retinitis typically received combination therapy with intravitreal foscarnet and ganciclovir and/or oral valganciclovir, while those with gastrointestinal disease received intravenous ganciclovir and/or oral valganciclovir [Table 2]. Of note, five of the 23 participants receiving treatment developed valganciclovir-related neutropenia requiring rescue therapy with filgrastim.

Other co-infections were also common among CMV EOD participants, particularly oral or esophageal Candida (18/25, 72%), genital or rectal Herpes Simplex Virus (7/25, 28%), and pulmonary or disseminated *Mycobacterium avium* complex (6/25, 24%) [Table 2]. In addition, approximately half of the CMV EOD group had received steroid treatment.

3.5. Immunophenotyping and immune activation markers

There was no difference in baseline CD4$^+$ T cell count, CD8$^+$ T cell count, or plasma HIV RNA between the groups at baseline [Table 1]. Compared to CMV$^-$, CMV$^+$ PCR participants had higher percentages of activated (CD38$^+$HLA-DR$^+$) CD8$^+$ T cells (67% vs 57%) and effector memory CD8$^+$ T cells (29% vs 25%), suggesting that even subclinical CMV shedding is associated with increased immune activation [Fig. 2, Supplemental Table 1]. Compared to CMV$^-$, both CMV EOD and CMV$^+$ PCR had lower percentages of plasmacytoid dendritic cells (0.9% in CMV EOD and 1.0% in CMV$^+$ PCR, compared to 1.4% in CMV$^-$). CMV$^+$ also had lower percentages of central memory CD4$^+$ T cells (41% vs 57.5% in CMV$^-$) and central memory CD8$^+$ T cells (30% vs 35% in CMV$^-$). No statistically significant differences in any of the immune
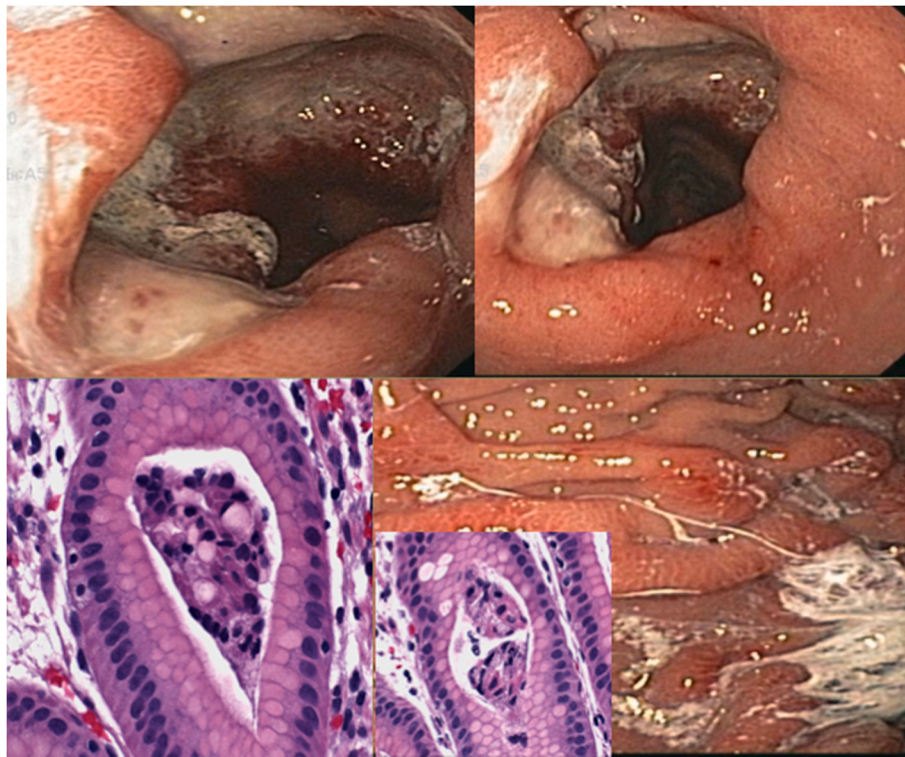
Fig. 1. Cytomegalovirus end-organ disease pathology.
Clockwise from top left: Gastroesophageal junction; pyloric channel; gastric body; and two hematoxylin and eosin stains in a patient with immune reconstitution inflammatory syndrome due to gastrointestinal Cytomegalovirus infection.

phenotypes studied were detected between CMV EOD and CMV$^+$ PCR participants.

After 48 weeks of ART, compared to CMV$^-$ participants, CMV$^+$ PCR participants had higher percentages of activated CD4$^+$ T cells (16.5% vs 12%), activated CD8$^+$ T cells (42% vs 29%), and effector memory CD8$^+$ T cells (36 vs 22.5%) [Supplemental Table 2], as well as lower levels of naïve CD8$^+$ T cells (14.5% vs 19.5%), indicating long-term immune activating effects of CMV replication. CMV EOD had higher median levels of activated CD4$^+$ and CD8$^+$ T cells compared to CMV$^-$ as well, but these results were not statistically significant in post hoc tests. No significant differences in immune phenotypes were detected between CMV EOD and CMV$^+$ PCR patients after 48 weeks of ART.

3.6. Biomarkers

We compared biomarkers of inflammation and coagulopathy between the three groups. CMV EOD participants had higher median levels of D-dimer (0.96 µg/ml vs 0.71 µg/ml in CMV$^+$ PCR and 0.76 µg/ml in CMV$^-$), CRP (5.4 mg/L vs 1.4 mg/L in CMV$^+$ PCR and 1.7 mg/L in CMV$^-$), and alkaline phosphatase (114 IU/L vs 88 IU/L in CMV$^+$ PCR and 84 IU/L in CMV$^-$), but these differences were not statistically significant. No statistically significant differences in levels of hemoglobin, platelets, D-dimer, CRP, or alkaline phosphatase were observed between CMV$^+$ PCR and CMV$^-$ participants, either, suggesting subclinical CMV shedding did not significantly increase biomarker levels. In addition, no differences in biomarkers were observed between any of the groups after 48 weeks of ART [Supplemental Fig. 4].

3.7. Outcomes

The majority of participants in all three groups had HIV viral loads below the limit of detection after 48 weeks of therapy, indicating viral suppression [Supplementary Table 5]. At 48 weeks post-ART, study participants had a median absolute CD4 count of 189 cells/µL, with a median increase of 152 cells/µL over baseline [Supplementary Table 5]. There were no significant differences between the groups in terms of viral suppression or CD4 reconstitution.

Of the 206 participants reported in this study, ten deaths occurred at any time during the study follow-up [Supplemental Table 6]. No deaths occurred among the CMV EOD participants. The participants were also affected by a high rate of adverse health outcomes, including malignancy, which occurred in 39 (18.9%) of all participants; deep venous thrombosis or pulmonary embolism, which occurred in 14 (6.8%) of all participants; and cardiac disease including myocardial infarction, cardiovascular disease, cardiomyopathy and congestive heart failure, which occurred in 13 (6.3%) of all participants [Supplemental Table 6]. Notably, four of the seven participants who developed CMV retinitis also continued to have significant visual impairment despite treatment.

4. Discussion

In this study we describe the occurrence of CMV EOD in a cohort of 206 adult participants with advanced HIV infection followed prospectively from the time of ART initiation. The prevalence of CMV EOD within this cohort was 12.1%, with 2.4% of participants overall developing CMV-IRIS. Our study demonstrates a surprisingly high proportion of gastrointestinal illness due to CMV (68% of all CMV EOD), in contrast to older literature emphasizing ocular disease as most common.[16,17] Furthermore, in our cohort, gastrointestinal disease accounted for the majority of CMV-IRIS cases as well (80%). Our results may in part be attributable to the close follow-up and low threshold for investigation into symptoms during both the pre- and post-ART period. In spite of the reported decline in the incidence of CMV EOD in the era of effective ART, these data support the need for continued vigilance in clinical monitoring and evaluation for CMV EOD in HIV-infected participants who are late presenters with low CD4 counts,[18] with particular attention to potential gastrointestinal manifestations. They also further highlight the important role of CMV coinfection in mucosal injury which is known

Table 2
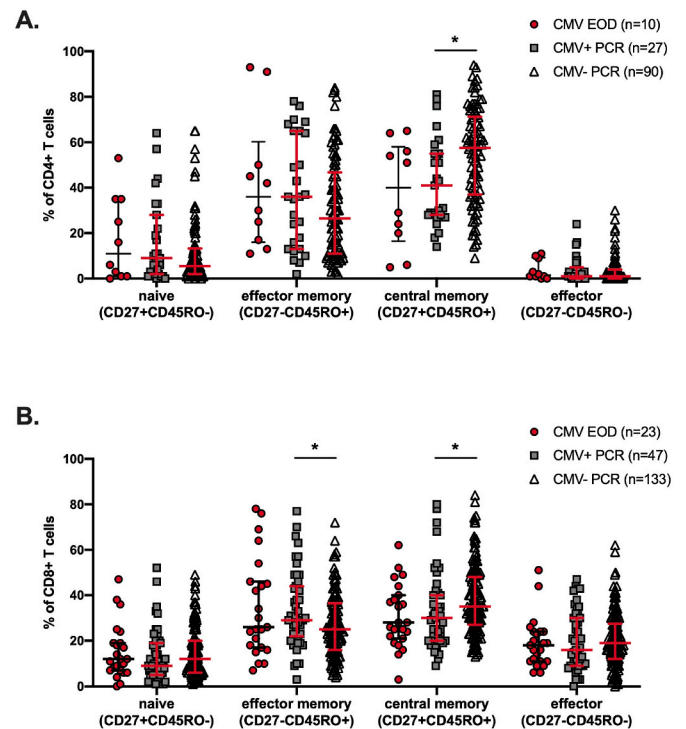Overview of participants with CMV EOD (*N*=25).

Organ system involved	Number (%)
Ophthalmologic	7 (28%)
Retinitis	7 [1 CMV-IRIS]
Gastrointestinal	17 (68%)
Sialoadenitis	1 (4%) [1 CMV-IRIS]
Esophagitis	3 (12%)
Gastritis	4[a] (16%)
Ileitis	2 (8%) [1 CMV-IRIS[b]]
Enteritis	1 (4%) [1 CMV-IRIS]
Enterocolitis	1 (4%)
Colitis	4 (16%)
Appendicitis	1 (4%) [1 CMV-IRIS]
Pulmonary	2 (8%)
Pneumonitis	2[a] (8%)
CMV therapy received	
Any	23 (92%)
Intravitreal foscarnet, intravitreal ganciclovir and oral valganciclovir	5 (20%)
Intravenous ganciclovir and oral valganciclovir	5 (20%)
Intravenous ganciclovir alone	2 (8%)
Oral valganciclovir alone	11 (44%)
None	2 (8%)
Opportunistic co-infections/conditions	
Oral or esophageal candidiasis	18 (72%)
Genital or rectal HSV	7 (28%)
Pulmonary or disseminated *Mycobacterium avium* complex	6 (24%) [4 MAC-IRIS]
CNS or disseminated toxoplasmosis	5 (20%)
Varicella Zoster	5 (20%) [1 VZV-IRIS]
Pneumocystis complex pneumonia	5 (20%)
HPV-related malignancies	3 (12%)
Diarrheal illness: Cryptosporidiosis, Microsporidiosis	2 (8%)
Lymphoma	2 (8%) [1 lymphoma-IRIS]
Histoplasmosis	2 (8%) [1 Histoplasmosis-IRIS]
Tuberculosis	2 (8%)
Hepatitis B or C Virus	2 (8%)
Cryptococcal meningitis	1 (4%)
Strongyloides	1 (4%) [1 Strongyloides-IRIS[b]]
History of systemic steroid use	
Any	
Oral prednisone for CMV disease	1 (4%)
Oral prednisone for emphysema	1 (4%)
Oral prednisone for *Pneumocystis* pneumonia	3 (12%, 3 at baseline)
Oral prednisone for chemotherapy	1 (4%)
Oral prednisone for IRIS	2 (8%)
Oral prednisone and intravenous hydrocortisone for adrenal insufficiency	1 (4%, 1 at baseline)
Oral dexamethasone for CNS toxoplasmosis	3 (12%, 2 at baseline)
None	13 (52%)

[a]One patient had CMV gastritis and CMV pneumonitis and is counted in both categories.

[a]One patient had IRIS due to CMV and Strongyloides and is counted as both CMV-IRIS and Strongyloides-IRIS.

Fig. 2. T cell phenotypes in patients with or without active Cytomegalovirus infection.

Participants with Cytomegalovirus end-organ disease (CMV EOD) or evidence of active replication (CMV+ PCR) had lower percentages of central memory CD4$^+$ T cells compared to those without detectable CMV replication (CMV$^-$ PCR). Compared to CMV$^-$ participants, CMV$^+$ PCR participants also had lower percentages of central memory CD8$^+$ T cells and higher percentages of effector memory CD8$^+$ T cells. *p<0.05 in post hoc analyses using Dunn's multiple comparison's test after significant Kruskal-Wallis test results.

to fuel immune activation in HIV.[19]

Serologic and virologic assays can reflect prior exposure to CMV and current reactivation, but they are poor predictors of the development of clinical disease. Although 93.2% of this cohort overall had positive CMV IgG, and 31.6% had detectable CMV viremia at baseline, only 12% had CMV EOD. Further, two of the fourteen participants with negative CMV IgG at baseline developed CMV EOD. We hypothesized that combining serologic and virologic assays with other common measurements may help improve their utility as predictors of individuals at risk of developing clinical disease. However, no statistically significant differences in immune phenotypes or biomarker levels were detected between CMV EOD and CMV$^+$ PCR participants, limiting the use of these metrics. The relatively small sample number of participants with CMV EOD compared to CMV$^+$ PCR and CMV$^-$ was a limitation for detecting these differences.

We noted an increase in activated (CD38$^+$HLADR$^+$) and effector memory (CD27$^-$CD45RO$^+$) CD8$^+$ T cells in the CMV$^+$ PCR group compared to CMV$^-$ group. The correlation between effective virus-specific cytotoxic response and recovery from CMV infection has long been known.[20] An increased proportion of CMV-specific effector CD8$^+$ T cells is necessary but not sufficient to protect against disease progression; multiple studies have shown that the role of IFN-γ production is critical.[21–24] In HIV-infected patients, CMV EOD has been associated with a preceding increase in CMV-specific CD8$^+$ T cell counts and a relative lack of CMV-specific IFN-γ-producing CD8 T cells.[22,24] After initiation of ART, CMV-specific IFN-γ production may in turn confer protection from CMV reactivation and unmasking CMV-IRIS.[25] Consistent with previous studies,[26,27] we found that CMV viremia was still associated with increased CD8$^+$ T cell activation even after ART. This chronic immune activation driven by subclinical CMV replication may contribute to the development of other adverse health effects and maintenance of the HIV reservoir.[28] CMV replication in the gut has been shown to disrupt epithelial integrity[19] and may have a synergistic effect on promoting bacterial translocation and persistent inflammation in people with HIV. Interestingly these observations were more evident in viremia than EOD, suggesting that *anti*-CMV treatment administered only in people with EOD, may have abrogated some of the T-cell activation.

Recent data showed that poor antiviral immune responses and a relative depletion of Th17 cells, rather than Treg cells, were characteristic of patients developing CMV immune recovery uveitis.[29] Treg cell compartments were intact in these patients, who developed no evidence of exaggerated systemic CMV-specific or polyclonal immune responses.

In our cohort, both CMV$^+$ PCR and CMV EOD participants had lower median proportions of Treg cells compared to CMV$^-$ participants; however, these differences were not statistically significant in post hoc analyses. In addition, Treg levels did not significantly differ between CMV$^+$ PCR and CMV EOD and thus did not help predict the development of disease in viremic persons. Involvement of other immune cell types, namely dendritic cells, in the control of CMV replication, disease progression, and immunosenescence is not well-defined. In our cohort, we observed a significantly lower proportion of plasmacytoid dendritic cells in CMV EOD and CMV$^+$ PCR participants, as compared to CMV$^-$, but the significance of this observation is unclear.

In our study, CMV$^+$ PCR participants had the highest incidence of cerebrovascular events. Our group has previously published findings supporting the association of CMV reactivation and elevated risk of thromboembolic in HIV co-infected patients.[30] The possible link between CMV reactivation and thromboembolic sequalae that warrants further investigation. Although not noted in this cohort, other non-infectious complications of HIV infection, such as atherosclerosis and cardiovascular disease, have been linked to persistent CMV activation and microvascular disease.[31,32] Treatment of subclinical CMV infection has been shown to reduce levels of CD8$^+$ T cell activation in treated people with HIV[33] and may reduce the prevalence of adverse health outcomes in this population.

In conclusion, manifestations of CMV EOD in advanced HIV disease may be more diverse than previously described. In our contemporary cohort of individuals with advanced HIV disease followed prospectively prior to and after ART initiation, we observed a predominance of CMV-associated gastrointestinal illness. As the use of anti-retroviral therapy expands, recognition and treatment of unusual clinical presentations of CMV infection, including CMV-IRIS, will become increasingly important in understanding, and ultimately in reducing, morbidity and mortality in HIV co-infection. Given the high prevalence of CMV infection among these patients, further investigation into factors predictive of increased risk of developing CMV EOD could help reduce the burden of this complication. Moreover our data provide a link between CD8 T cell activation and CMV viremia which may be relevant in chronic inflammation as well as persistence of reservoirs in treated HIV.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jve.2021.100052.

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