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ABSTRACT

Objective: Subclinical cytomegalovirus (CMV) viremia has been associated with other infections, prolonged hospitalization, and mortality in select immunosuppressed populations. We examined the incidence and outcomes of subclinical CMV viremia in hospitalized patients with systemic autoimmune diseases (AD) [systemic lupus erythematosus (SLE) or anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV)] using a highly sensitive CMV assay.

Methods: Prospectively collected samples were obtained from AD hospitalized patients at study entry with a second sample collected 1 week later or at hospital discharge. Controls included age- and gender- matched inpatients without AD and outpatients with AD. All samples were tested in batch using the Abbott RealTime CMV for investigational use assay (RT assay), with a LLOD (LLOQ) at 21 IU/mL (32 IU/mL).

Results: Twenty-three inpatients (10 SLE, 8 AAV, 5 controls), and 31 outpatient controls were recruited. Subclinical CMV viremia was found in 61% (11/18) of inpatient AD subjects, 3% (1/31) of outpatient AD subjects, and in none of the five inpatient controls ($p < 0.001$). CMV viremia was associated with increased median length of ICU stay (13 vs. 4 days, $p = 0.033$), hospital stay (17 vs. 9 days, $p = 0.014$) and increased nosocomial infections (7 vs. 1, $p = 0.007$). CMV viremia was not associated with overall severity of illness nor with disease-specific activity or damage.

Conclusion: Over one-half of hospitalized AD patients in our cohort had detectable CMV viremia, which was associated with increased length of hospital stay and nosocomial infections. These data suggest that further study of the immunomodulatory effects of subclinical CMV viremia in AD is warranted.

1. Introduction

Although the clinical manifestations and outcomes of CMV-associated disease in immunosuppressed populations are well-recognized, there is emerging evidence that subclinical reactivation of CMV, even in the absence of clinically manifest disease, may impart immunologic adverse effects. The negative clinical impact of subclinical CMV is established in the solid organ transplant population, where it is associated with infection risk, organ rejection, and mortality [1–3].

Although the majority of data regarding morbidity associated with CMV reactivation are from the transplant and HIV/AIDS literature, small studies suggest that patients with autoimmune diseases are vulnerable to CMV reactivation and CMV-associated adverse outcomes. We and other investigators have identified an increased prevalence of CMV IgM seropositivity in AAV and hospitalized SLE patients [4,5]. Furthermore, additional small studies found associations between prior CMV exposure with changes in T cell subsets, increased risk of other infections, and mortality in patients with autoimmune diseases [6–8]. We hypothesized

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that patients with AAV and SLE are also vulnerable to subclinical CMV reactivation and associated adverse outcomes, due to both disease-associated immune dysregulation and treatment with intensive immunosuppressive therapy. Our study examined the prevalence of subclinical CMV viremia in hospitalized patients with AAV and SLE using an investigational, highly sensitive CMV assay, and examined its association with subsequent clinical outcomes.

2. Materials and methods

Hospitalized patients meeting the American College of Rheumatology criteria for the diagnosis of SLE or patients fulfilling the reviewed Chapel Hill criteria for primary AAV were prospectively enrolled following informed consent. Disease activity and damage were assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and Systemic Lupus International Collaborating Clinics-ACR damage index (SLICC) for SLE patients, and the Birmingham Vasculitis Activity Score (BVAS) and Vasculitis Damage Index (VDI) for AAV patients. The Acute Physiology and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) scores were calculated for patients recruited from the intensive care units. Age- and gender-matched control patients were prospectively recruited. Samples were collected at study entry and one week later (if still hospitalized). Outpatient SLE and vasculitis patients were recruited at routine clinic visits, at which time a single blood sample was collected. Approval by the Henry Ford Hospital Institutional Review Board was obtained for this study prior to any study procedures.

2.1. Measurement of CMV viremia

Clinical whole blood samples were obtained and transported to the research laboratory for separation into aliquots of plasma and PBMCs within 2 hours. Plasma samples were stored at -80C until thawed for testing. Patient plasma samples were tested in duplicate with both the baseline and follow up sample tested in the same extraction and quantification procedures. CMV testing was performed using the Abbott RealTi*m*e CMV for investigational use assay (RT assay) on the Abbott *m*2000 instrument as per manufacturer recommendations. The Abbott RT assay has a LLOD & LLOQ of 21 and 32 IU/mL that corresponds adjusted to WHO standards to 13.5 and 20 copies/mL.

2.2. Statistical analysis

All data collected were analyzed descriptively, followed by univariate and multivariate analyses. Continuous variables with normal distributions were analyzed using means, standard deviations, Student's t-test, Pearson correlations and ANOVA. Non-parametric analyses were performed for all non-normally distributed continuous variables, ordinal and categorical variables. All p values presented were nominal and unadjusted for multiple comparisons. SPSS version 23 (IBM Corporation, Armonk, New York) was used for all the analyses.

3. Results

Eighteen patients with AD, 7 inpatient controls, and 31 AD outpatients were prospectively recruited (Table 1).

In both inpatient and outpatient AD groups, SLE was the most common diagnosis. There were 7 granulomatosis with polyangiitis (GPA) and 1 microscopic polyangiitis (MPA) patient in the inpatient AAV group, while the outpatient control group included patients with GPA, MPA, Behcet disease, and IgG4 disease. Notably, all hospitalized AAV patients enrolled were newly diagnosed and undergoing induction therapy for vasculitis with high dose glucocorticoid therapy (median dose > 60 mg/day), while all SLE patients had established disease, with 6/10 SLE patients with active disease (SLEDAI score > 0) and a median daily steroid dose of 50 mg/day at study entry. The control patient diagnoses included COPD exacerbation, worsening tracheal stenosis, lumbar radiculopathy, elective orthopedic surgical admission, and abdominal pain.

Table 1
Baseline patient characteristics.

	AD Inpatient N (%)	Inpatient Control N (%)	AD Outpatient N (%)
Participants	18	7	31
Age (mean)	47	47	49
Female gender	12 (67%)	5 (71%)	26 (84%)
Race			
Black	9 (50%)	2(29%)	12 (39%)
White	8 (44%)	5 (71%)	14 (45%)
Other	1 (6%)	0	5 (16%)
Disease			
AAV	8 (44%)	NA	7 (22.5%)
SLE	10 (56%)	NA	22 (71%)
Other	0	NA	2 (6.5%)

3.1. CMV viremia

Detectable CMV viremia by the RT assay was found significantly more often in inpatient AD patients (61%) than in inpatient controls (0%) or outpatient AD patients (3%) ($p < 0.001$) (Fig. 1).

63% of AAV patients had detectable viremia, all of whom had a BVAS score > 0, while 50% of SLE patients had detectable viremia, 60% of whom had a SLEDAI score > 0 at study entry.

The average level of CMV viremia for AD patients at entry was 51.8 IU/mL, and at follow up was 175.3 IU/mL. All 7 inpatients with detectable CMV viremia at baseline and a follow up sample had increased viral load (maximum level of 394 IU/mL). CMV IgG titers, as measured by standardized ELISA testing, were similar between controls, AD patients, and AD patients with CMV viremia (2.90 vs. 3.01 vs. 3.75, $p = 0.54$). The IgG titers for CMV tended to be higher in patients with detectable CMV viremia than those without viremia (median 3.8 vs. 3.0, $p = 0.02$) but IgG levels did not correlate with the level of CMV viremia ($p = 0.12$).

3.2. CMV viremia and disease scores

There were no statistically significant differences in the average APACHE II (18 vs 20, $p = 0.0.64$) and SOFA scores (5 vs 2, $p = 0.08$) between groups. CMV viremia was not significantly associated with APACHE II scores ($p = -0.327$), but there was a trend toward association with baseline SOFA scores ($p = 0.056$). Disease-specific activity measures (SLEDAI and BVAS scores) and damage indices (SLICC and VDI scores) were not significantly associated with CMV viremia.

3.3. Clinical impact

Both the median (mean) length of intensive care unit (ICU) stay and the total inpatient stay were significantly increased in patients with detectable CMV viremia: 13 (25) vs 4 (5) ICU days, $p = 0.033$; 17 (35) vs 9 (10) total days, $p = 0.014$); median comparisons shown in Fig. 2.

Patients with CMV viremia had more hospital-acquired infections than aviremic patients (7 vs 1, $p = 0.007$). Types of infections in the viremic patients included pneumonia (3 patients), urinary tract infection (3 patients), and one patient with meningitis. Although there was no difference in total white blood cell count between groups, absolute lymphocyte count in viremic patients was significantly lower than in aviremic patients ($p = 0.035$). Patients with viremia also had significantly lower hematocrit levels ($p = 0.024$).

Two AD inpatients, neither of whom were receiving immunosuppressive therapy prior to hospital admission, were treated for possible CMV disease, both of whom had subclinical viremia at study entry. The

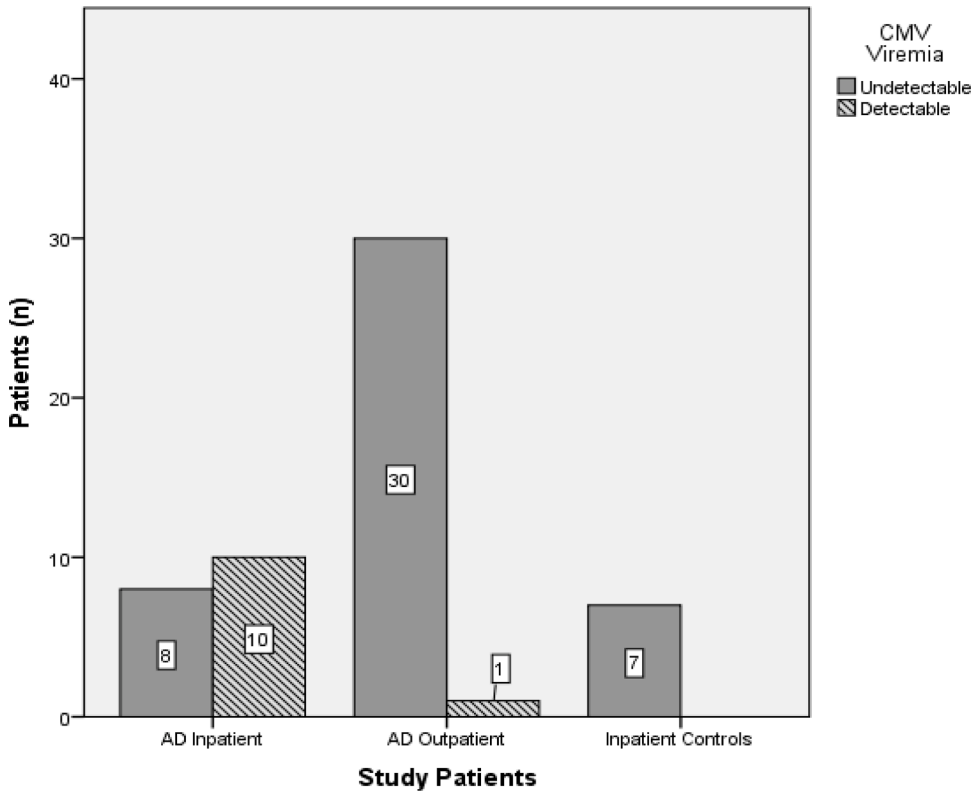

Fig. 1. The majority of AD inpatients had detectable CMV viremia.

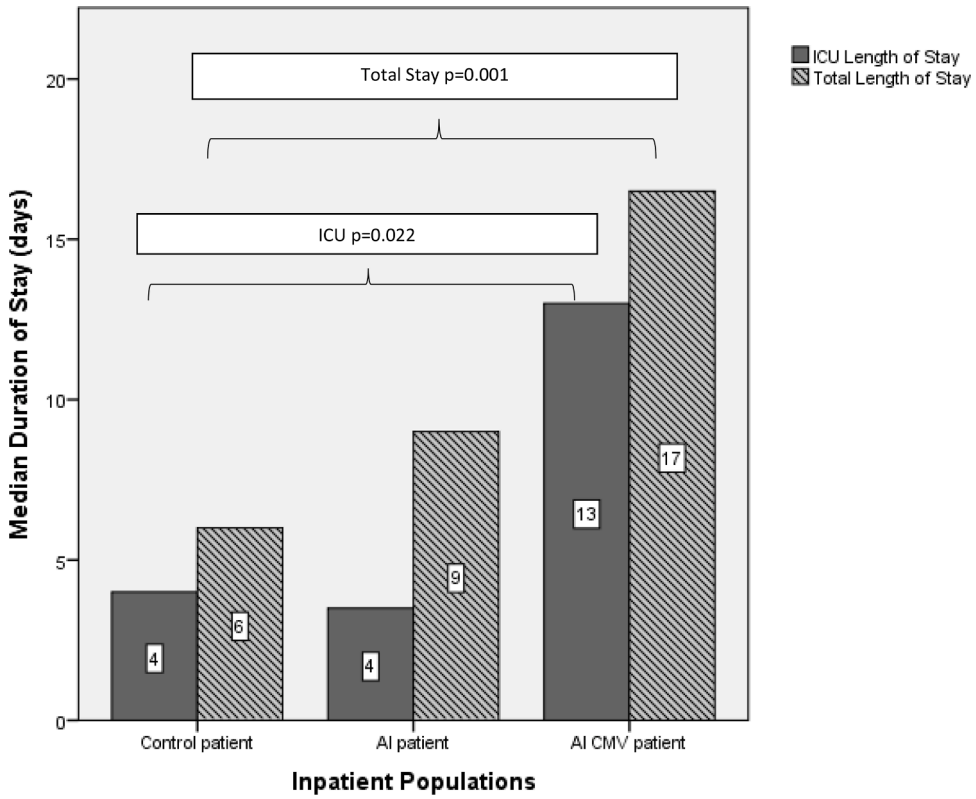

Fig. 2. CMV viremia is associated with increased ICU and total length of stay.

first patient was diagnosed with GPA during his hospitalization after presenting with pulmonary-renal syndrome, and developed CMV pneumonia, diagnosed by positive bronchioalveolar lavage viral culture, worsening pulmonary infiltrates, diffuse alveolar hemorrhage with hypoxia, and persistently detectable plasma CMV DNA by PCR. Despite systemic antiviral therapy, he had persistent viremia, subsequently

developed other systemic infections, and ultimately succumbed to multiorgan failure. The second patient had known SLE and had admitted to stopping her medication several months prior to presentation. She was admitted with active SLE (arthritis and nephritis) and developed meningitis, respiratory failure with pulmonary infiltrates, acute renal failure, and pancytopenia during her hospitalization. This patient also had CMV plasma DNA viremia detected at multiple time points by the hospital laboratory assay, and met CMV syndrome criteria (fever, fatigue/malaise, hepatic aminotransferase elevation, and CMV viremia). Ultimately, she received both systemic antiviral therapy and immunosuppressive therapy, and made a full recovery. However, her clinical picture was complicated by her active lupus in addition to concomitant herpes simplex and candida infections, and without tissue substantiation of end organ disease, could not be conclusively established to be CMV disease.

Three AD inpatients had disease flares during the 6 months following hospital discharge, all of whom had detectable CMV viremia during hospitalization. There was a trend toward new need for hemodialysis at hospital discharge in patients with CMV viremia, although it did not reach statistical significance (4 vs. 1, $p = .104$).

4. Discussion

In our study, 61% of AD inpatients developed low level CMV viremia during the first week of admission. Intriguingly, subclinical CMV viremia was associated with adverse clinical outcomes including increased length of ICU and hospital stay, rates of infections, and lower lymphocyte and hematocrit levels.

Recent studies in other AD patients support that the association between CMV viremia and adverse outcomes is more than just an epiphenomenon. In rheumatoid arthritis, psoriasis, and inflammatory bowel disease, CMV seropositivity or viremia has been associated with more aggressive and refractory disease [9–11]. Additionally, data examining reactivation rates of CMV in an AD cohort presenting with fever demonstrated that CMV antigenemia was detected in 50% of patients and was associated with increased risk of infection and death [12].

The presence of CMV viremia in the AAV group, all of whom received high dose induction glucocorticoid therapy for newly diagnosed vasculitis after admission to the hospital, is reminiscent of the solid organ transplant population, in which the risk for CMV reactivation is highest immediately post-transplant, when immunosuppressive therapy is at its peak intensity. Similarly, viremia occurred at almost an equal frequency in the SLE patients, all receiving 10mg of prednisone or less at home, but receiving higher doses of glucocorticoids (median dose of 50mg/day) after admission. These findings imply that treatment with glucocorticoids, in combination with other factors such as the immunologic aberrancies associated with AD, may lower the threshold for viral reactivation.

There is a clear lack of consensus regarding the need for and threshold for treating CMV viremia in AD. Even in the organ transplant realm, where the adverse effects of subclinical viremia have been conclusively established, the optimal threshold for and timing of interventions have not yet been unequivocally determined. Our pilot study demonstrates adverse clinical outcomes and potential long-term detrimental consequences of CMV viremia in AD inpatients; however, larger prospective studies are needed to understand the associated underlying immunologic perturbations and downstream effects, and to determine the need, timing, duration, and nature for any preventative or therapeutic interventions.

Lastly, the “chicken or egg” question-whether CMV viremia worsens AD and comorbid conditions, or disease activity/illness precipitates viral reactivation-remains unanswered. It appears that three proposed interrelationships for CMV with inflammation/inflammaging could readily be applied to CMV reactivation in AD patients. First, CMV has been proposed as the villain, where the immunologic response to viral reactivation propagates systemic inflammation. Secondly, immunosenescence (in the case of AD patients, their disease-associated and treatment-associated immunosuppressed state) has been proposed as the culprit, which allows CMV reactivation, promoting further immune impairment and a proinflammatory milieu. Finally, inflammation has been proposed as the inciting event, which favors CMV replication and activation, leading to adverse downstream immunologic consequences. All of these theories are supported by data from examining conditions that have been associated with CMV reactivation disease and/or viremia [13–15], but precisely how these hypothesized pathways impact outcomes in immunocompromised patients remains undefined. It is possible that any or all of these pathways may be important, and all certainly appear potentially relevant in the context of AD, where patients are frequently immunocompromised by medications, have underlying immune dysfunction related to their underlying disease, and have a disease state driven by ongoing systemic inflammation.

5. Conclusions

CMV viremia is frequently found in patients with systemic AD, and even at low levels is associated with adverse clinical outcomes. Data from other immunocompromised cohorts support the association between subclinical CMV reactivation with increased systemic inflammation and decreased immune function, suggesting that CMV viremia could potentially function to both intensify systemic inflammation (increasing disease activity) and further impair immunologic function (leading to higher risk of infection) in patients with AD. The precise timing, duration, and immunologic consequences of CMV viremia in patients with AD warrant further study, as CMV reactivation is a potentially preventable and treatable complication of impaired host immunity.

Author contributions: KM designed and oversaw study, obtained and recorded subject data, aided in study analysis design, reviewed study data, and was the primary contributor in writing the manuscript. JZ received, processed, and stored patient specimens, and performed the laboratory components of this study. JH recruited and consented subjects and recorded clinical data. SH recruited and consented subjects. JM was involved in study design, oversaw all laboratory procedures and proceedings, performed the data analysis for the study, and was a major contributor in writing the manuscript. All authors read, edited and approved the final manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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