**SUBCLINICAL CMV VIREMIA IS ASSOCIATED WITH INCREASED NOSOCOMIAL INFECTIONS AND PROLONGED HOSPITALIZATION IN PATIENTS WITH SYSTEMIC AUTOIMMUNE DISEASES**

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University of Pittsburgh, 2018

**Abstract**

**Background:** Subclinical cytomegalovirus (CMV) viremia is associated with adverse outcomes in select immunosuppressed populations, including other infections, prolonged hospitalization, and mortality. We examined the incidence and impact 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 the new Abbott RealTime CMV assay (RT assay).

**Methods:** Prospectively collected blood samples were obtained from AD hospitalized patients at study entry and 1 week later or at discharge from the hospital. All samples were tested in batch using the 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. Detectable CMV viremia was found in 61% (11/18) of inpatient AD subjects, 3% (1/31) of outpatient AD subjects, and no inpatient controls (p<0.001). Average CMV viremia for AD patients at entry was 51.8 IU/mL (33.1 copies/mL) and at 7 days was 175.3 IU/mL (112.4 copies/mL). CMV viremia was associated with increased ICU stay (25 vs. 5 days, p=0.033), hospital stay (35 vs. 10 days, p=0.014) and nosocomial infections (7 vs. 1, p=0.007). CMV viremia was not associated with overall severity of illness at entry nor disease activity or damage.

**Conclusions/Public Health Relevance:** Over 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 subclinical CMV viremia may wield significant adverse effects in hospitalized patients with SLE and AAV, and be a potentially modifiable risk factor to improve outcomes in this population.

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1. **INTRODUCTION**

**1.1 Epidemiology and Public Health Significance**

Cytomegalovirus (CMV) is a herpesvirus that occurs commonly worldwide, with infection rates impacted by gender, age, ethnicity, and socioeconomic status. Seroprevalence rates range are reported to be about 50% in the United States, but can be as high as 97% in developing countries[1](#_ENREF_1) [2](#_ENREF_2). Primary infection is typically acquired early in life, and may occur via various routes of transmission, including close contact, blood/tissue exposure, sexual exposure, and perinatal exposure. The clinical spectrum of CMV infection is broad, with disease manifestations directly impacted by the immune status of the individual. Healthy persons affected by CMV may have minimal or no symptoms at the time of infection, while in contrast, CMV infection in an organ transplant patient may lead to death from disseminated disease. Like other members of the herpesviridae family, CMV remains in human cells following primary infection in a latent state, with the potential to reactivate throughout the lifetime of an infected individual.

The public health impact of CMV was first appreciated in the study of children affected by congenital CMV infection. CMV remains a significant cause of sensorioneural hearing loss and neurologic impairment in affected children in the most vulnerable populations. Maternal seroprevalence is a primary risk factor for congenital CMV infection, with every 10% increase associated with a 0.26% increase in CMV birth prevalence [3](#_ENREF_3). Non-white race/ethnicity, socioeconomic status, high risk sexual behavior, and the care of young children are all associated with seropositivity in women, and thus may be contributory to risk of congenital CMV infection[4](#_ENREF_4). Although studies of pregnant women and children worldwide have advanced our understanding of risk factors for congenital CMV infection, it has been more challenging to identify effective means of decreasing its incidence. In the absence of any vaccine or antiviral therapy and lack of routine prenatal and newborn screening, the primary means of prevention is centered around changes in social behaviors, such as sexual contact, close personal nonsexual contact, close contact between mothers and their children, and breastfeeding, which require complex psychosocial alterations in behavior, with the potential to negatively impact other psychosocial interactions and relationships.

**1.2 IMMUNOSUPPRESSION: OPENING THE DOOR TO CMV REACTIVATION DISEASE**

The advent of solid organ transplantation with systemic immunosuppression created a new at-risk population for CMV, with CMV emerging as the most common post-transplant viral infection, occurring in up to 80% of recipients prior to the protocoled use of universal antiviral prophylaxis [5](#_ENREF_5). Although the routine use of antiviral prophylaxis has led to a significant decrease in post-transplant reactivation disease, with rates as low as 2-3% reported in the first year in CMV-negative patients receiving a kidney or liver from a CMV-negative donor, the one-year incidence of primary CMV infection in CMV-negative patients receiving organs from CMV-positive donors has been reported from 19-31% despite prophylactic therapy [6](#_ENREF_6). Further analysis of post-transplant CMV disease has identified a number of risk factors including recipient age, type and intensity of immunosuppression, type of organ transplanted, donor/recipient CMV serostatus, and the presence of allograft rejection[7](#_ENREF_7),[8](#_ENREF_8). Although antiviral prophylaxis is the standard of care following solid organ transplantation, there is a lack of consensus about risk stratification, best method of prevention, duration of therapy, and frequency and timing of sequential antiviral monitoring. Also relevant to this consideration is the risk of “late-onset” CMV disease, which occurs after discontinuation of universal prophylaxis, may be associated with gancyclovir-resistant CMV, and is associated with decreased patient and graft survival[9](#_ENREF_9),[10](#_ENREF_10).

**1.2.1 Prevention of CMV disease in transplant patients**

Universal prophylaxis refers to administration of antiviral therapy to individuals deemed to be at highest risk of CMV for a prescribed length of time, independent of any serologic measures of viremia, and is typically utilized in solid organ transplant recipients. Preemptive therapy, which involves routinely monitoring patients for the development of asymptomatic viremia and treating at the time viremia is identified, is an alternative means of preventing post-transplant CMV disease, most often utilized in recipients of hematopoietic stem cell transplantation. Both methods have clear benefits and limitations, but data from multiple cohort studies currently suggest better overall outcomes from universal prophylaxis. However, despite antiviral therapy, transplant patients remain at significant risk of both direct and indirect effects of CMV. Hence, preventative and therapeutic strategies against post-transplant CMV infection remain an area of active investigation.

**1.2.2 CMV in HIV patients**

Increased vulnerability to CMV disease was subsequently identified in other immunocompromised populations, including those with the human immunodeficiency virus (HIV). Prior to the advent of highly active antiretroviral therapy (ART), CMV end-organ disease occurred frequently in patients with advanced HIV disease, with CMV retinitis being the most common complication but also including gastrointestinal, neurologic, and pulmonary involvement[11](#_ENREF_11) . HIV patients are usually co-infected with CMV, which appears to exert additional adverse effects on T cell immunity and impairs response to HIV antiviral therapy[12](#_ENREF_12),[13](#_ENREF_13). Additionally, CMV was also identified as a risk factor for severe, non-AIDS related adverse clinical events in a recent longitudinal study, demonstrating its ability to remain immunomodulatory and relevant in contemporary times, even where ART is the standard of care [14](#_ENREF_14).

**1.2.3 CMV in chemotherapy patients**

Patients receiving cancer chemotherapy have also been found to be at risk for CMV disease. Rates of detectable CMV viremia following induction chemotherapy have been reported as high as 50-90% of patients[15](#_ENREF_15),[16](#_ENREF_16). Although viremia is symptomatic in many patients, it is more often self-limiting, with resolution of viremia seen following completion of therapy without requiring antiviral treatment. However, one recent study examining reactivation of CMV in children receiving chemotherapy demonstrated clinical CMV disease in 16% of patients, with the youngest patients being at highest risk [17](#_ENREF_17).

**1.2.4 The newest at-risk group for CMV**

The most recently identified at-risk group for CMV disease is in patients with systemic autoimmune diseases (AD). This is not surprising when considering that the immunosuppressive medications used to treat AD overlap significantly with those used post-transplantation and in those for cancer chemotherapy. Although the data in this area are based from small studies and are quite limited in scope, they imply that CMV infection in these patients is also associated with worse clinical outcomes. These data are reviewed and discussed in detail in a later section.

**1.3 CLINICAL MANIFESTATIONS OF CMV**

The close surveillance of solid organ transplant patients has been instrumental in the study of the natural history and effects of CMV infection longitudinally. In this cohort of patients, CMV disease most often is a result of reactivation or by transplantation of an organ from a CMV-positive donor to a CMV-negative recipient. Although the direct effects of acute infection were rapidly recognized and characterized, it has taken decades to identify other adverse effects that can be seen following CMV infection, characterized as “indirect effects”, as they are not a direct result of tissue invasion but appear to be a consequence of viral interference with immune function (Table 1)[4](#_ENREF_4),[18](#_ENREF_18).

**Table 1 Direct and indirect effects of CMV infection**

|  |  |
| --- | --- |
| **Direct Effects** | **Indirect Effects** |
| CMV disease | Acute and chronic rejection |
| Hepatitis | Infections |
| Pneumonitis | Opportunistic: fungal, other viral |
| Pancreatitis | Reactivation of chronic viral infections (hepatitis B, C) |
| Colitis | Bacterial superinfection |
| Meningioencephalitis. | Nocardiosis |
| Nephritis | Diabetes mellitus |
| Retinitis | Vascular thrombosis |
| Myocarditis | Atherosclerotic vascular disease  |
| Death (disseminated disease) | Post-transplant lymphoproliferative disease |

**1.3.1 Diagnosis of CMV infection**

The diagnosis of CMV disease is most often established currently by either nucleic acid testing from serum or plasma or by histopathology in the appropriate clinical scenario. However, the initial lack of assay standardization, variations in viral load depending on the sample type and by clinical laboratory at which the sample was tested, limited comparisons between studies and even in clinical management of patients until recently. The World Health Organization provided guidelines in 2010 that led to standardization of CMV viremia assays for commercial use [19](#_ENREF_19). Current limitations remain an undefined optimal timing of serial measurements and undefined viral load thresholds for treatment initiation and clinical response to therapy. CMV serology (IgG and IgM) is typically used for pre-transplant evaluation (donor/recipient +/-) and determination of prior exposure and risk stratification, but is not reliable for the diagnosis of CMV disease.

**1.3.2 Subclinical viremia-the tip of the iceberg?**

With recent laboratory technology allowing for detection of lower level viremia, it is now recognized that subclinical CMV viremia is frequent in immunosuppressed populations, which may not be clinically symptomatic and never ultimately progress to CMV disease. However, some studies suggest that subclinical viremia, even in the absence of CMV disease, may alter immunologic function, and may be associated with adverse clinical outcomes, such as increased risk of infection or mortality ([12](#_ENREF_12),[20-22](#_ENREF_20)). At present, there are no standardized recommendations for monitoring subclinical CMV viremia in at-risk cohorts, nor protocoled treatment for a specified level of asymptomatic subclinical viremia. However, this remains an area of interest and is under study in transplant and other immunosuppressed populations.

**1.4 CMV IS IMMUNOMODULATORY AND PROINFLAMMATORY**

CMV has the capacity to blunt or circumvent the normal immunologic responses to infection, promoting an immunosuppressed state that allows for viral latency. CMV is able to down regulate both cellular and humeral immunity by diverse and complementary mechanisms, which include modulation of HLA expression, MHC interference, antigen presentation, T cell proliferation and response, Fc receptor expression, cytokine and chemokine production and degradation, complement inhibition, and macrophage activity[23-25](#_ENREF_23). Furthermore, the effects of CMV infection and reactivation appear to be more complex and far-reaching than previously appreciated. In addition to the acute effects of infection or reactivation, there are data to suggest that CMV infection also may leave an immunologic “fingerprint”, with downstream changes in immune cell populations and activity that extend for years following acute viremia (Table 2)[26](#_ENREF_26).

**Table 2 Cellular effects of active and latent viremia**

|  |  |  |
| --- | --- | --- |
| **Host cell functions** | **Lytic infection** | **Latent infection** |
| Virus status | Active replication/gene expression | Limited to no gene expression, no replication |
| Cell cycle manipulation | Yes | No |
| Cell signaling modulation | Yes | Yes |
| Cell death inhibition | Yes | Yes |
| Immune surveillance inhibition | Yes | Yes |
| Cell stress inhibition | Yes | Yes |
| Modulation of cell protein translation | Yes | No |
| Modulation of cell transcription | Yes | Yes |
| Modulation of cell metabolism | Yes | No |

How these long-ranging changes may potentially impact immunologic function, vulnerability to reactivation disease, and other adverse effects has garnered much attention. There is circumstantial evidence to support CMV infection/exposure in the pathogenesis of chronic diseases, but causality and mechanisms for disease have not yet been conclusively established ([23](#_ENREF_23),[25](#_ENREF_25),[27-29](#_ENREF_27)). However, it is currently hypothesized that the immunomodulatory effects of infection in combination with chronic systemic inflammation are a driving force in disease pathogenesis.

**1.4.1 CMV: promoting a shift toward autoimmunity?**

CMV infection, similar to other viral infections, induces the production of multiple proinflammatory cytokines, including Il-1, Il-6, TGF-beta, MCP-1, MIP-1, and anti-TNF alpha[23](#_ENREF_23),[24](#_ENREF_24),[30](#_ENREF_30),[31](#_ENREF_31). Mechanisms promoting systemic inflammation include inducing translocation of NF-κβ, direct interaction with TLR-2 and CD14, and the production of chemokine receptor homologues which recruit neutrophils[24](#_ENREF_24),[30](#_ENREF_30). Following acute infection, there is a shift from the acute inflammatory response to an immunosuppressive and lower inflammatory state to promote sustained viral latency. These modulatory effects are purported to persist chronically and, in combination with other immunologic alterations induced by CMV, are hypothesized to play a role in the development of a number of diseases, including atherosclerotic vascular disease, acute myocardial infarction, neoplastic diseases, and AD (Figure 1)[24](#_ENREF_24), considered indirect manifestations of CMV infection.



**Figure 1: Mechanisms by which CMV can induce host immunopathology**

**1.4.2 Rationale for study in autoimmune diseases**

It has been recognized and reported for decades that immunosuppressed AD patients are at increased risk for infections. The advent of biologic therapies has brought closer scrutiny directed at short- and long-term adverse effects of treatment, with a greater focus on reactivation of chronic infections and risk of opportunistic infections. There have been numerous case reports documenting CMV reactivation disease in a number of AD. However, this closer scrutiny combined with the solid organ transplant data has highlighted the potential for chronic viral infections to not only cause morbidity in AD patients as a result of reactivation disease, but also the possibility that they may also influence immune activation and immunocompetence. Even though the studies examining CMV in AD are limited by small sample sizes, heterogeneous means of assessing CMV exposure/disease, and scarce immunologic data, they suggest that CMV may affect both disease activity and outcomes in a subset of these patients.

**1.4.2.1 CMV in patients with inflammatory bowel disease (IBD).** Evidence of CMV reaction in colonic tissue of IBD patients has been documented for decades. However, its relationship with disease and response to therapy has remained an area of controversy. Similar to other populations with autoimmune diseases, the immunosuppressive medications used to treat IBD increase the risk of both usual and opportunistic infections, including CMV. Furthermore, it has been proposed that malnutrition associated with active colitis may pose as yet another risk factor for CMV reactivation. Numerous studies have examined the relationship between CMV and IBD severity and treatment resistance, with varied results. One limitation in evaluating the literature is the heterogeneity of the studies; some looking at seropositivity only (exposure), while newer studies have included examining for reactivation disease specifically by identifying CMV DNA in colonic tissue or blood. More recently, several studies have identified CMV viremia (detectable in tissue or in blood) as a risk factor for more severe and steroid-resistant disease, with a few small studies documenting improvement in IBD following antiviral therapy, suggesting that CMV may have a role in disease activity in IBD[28](#_ENREF_28),[32-34](#_ENREF_32).

**1.4.2.2 CMV in psoriasis.** Infection has long been associated with flares of skin disease in patients with psoriasis, and several small studies have examined CMV as a potential culprit in patients given its prevalence, proinflammatory effects, and immunomodulatory potential. Although the prevalence of CMV in psoriasis patients is no greater than in the general population, one study found a significantly higher prevalence of CMV antigenemia in psoriasis patients (43%) when compared with controls (blood donors, 6%)[35](#_ENREF_35). In this cohort, antigenemia was associated with the presence of anti-CMV IgG, but only rarely with CMV DNA in tissue lesions and was asymptomatic, implying subclinical reactivation. Perhaps more interestingly, CMV antigenemia decreased with antipsoriatic therapy (which typically is immunosuppressive), which suggests that inflammation associated with autoimmune disease activity may be a trigger for subclinical viral reactivation. Similarly, another study examining the effects of CMV on peripheral cellular responses in patients with psoriasis demonstrated that more severe psoriatic skin disease was associated with the level of CMV antigenemia, and that the lower frequency of CMV-specific T cells seen in CMV seropositive patients increased with effective therapy for psoriasis[36](#_ENREF_36). More recently, Fornana *et al* found that both CMV-specific CD4+ and CD8+ cells increased following anti-tumor necrosis factor therapy (TNF) for psoriasis, which they postulated to represent a immunostimulatory effect on anti-CMV activity related to decreasing TNF-associated systemic inflammation[37](#_ENREF_37). However, they also remark that the immune suppression associated with anti-TNF therapy could also lead to subclinical viral reactivation, which could also lead to an increased cellular response against CMV. Although these data imply a possible relationship between CMV and psoriasis, the precise influence of either on the other remains unclear.

**1.4.2.3 CMV in rheumatoid arthritis.** The immunobiology of rheumatoid arthritis, which encompasses immunologic changes including accelerated immunosenescence of CD4+ and CD8+ cells, clonal expansions and increased frequencies of CD4+CD28null T cells with expression of killer cell immunoglobulin-like receptor, parallel changes that are seen following CMV infection, and have led to further study of the potential association between CMV infection and RA[29](#_ENREF_29). Although RA patients have a similar prevalence of anti-CMV seropositivity as healthy controls, seropositivity in RA patients is associated with higher frequencies of CD4+CD28null T cells and more severe joint disease (as measured by radiographic joint destruction and need for joint-related surgical procedures)[29](#_ENREF_29),[38](#_ENREF_38). Similarly, Davis *et al* found that a T-cell cytokine profile associated with CMV exposure was associated with lower improvement scores (less response to treatment) following disease-modifying anti-rheumatic therapy[39](#_ENREF_39).

**1.4.2.4 CMV in systemic lupus erythematosus**. CMV infection is prevalent in SLE, with several small studies demonstrating a significantly higher prevalence when compared with controls (97% vs. 67%; 100% vs. 77%)[40](#_ENREF_40),[41](#_ENREF_41). Furthermore, several small studies examining CMV reactivation in lupus found that increased antigenemia was associated with disease activity and mortality[22](#_ENREF_22),[42](#_ENREF_42). CMV infection has also been reported to trigger the onset of SLE or flare of disease [43](#_ENREF_43). To further study the potential role of CMV infection as a trigger for lupus-associated autoimmunity, Hsieh *et al* immunized mice with a fragment of the HCMVpp65 antigen and demonstrated the induction of multiple anti-nuclear antibodies, high levels of double stranded DNA antibodies, and immunoglobulin deposition on glomeruli, which suggests that CMV may induce cross-reactive antibodies that may play a role in the development of autoimmune disease[*44*](#_ENREF_44).

**1.4.2.5 CMV in systemic vasculitides**. Although the pathogenesis of the systemic vasculitides remains unknown, infectious pathogens remain under scrutiny as inciting factors and as potentially causative agents for disease flares. Case reports document associations between infection and reactivation of numerous viral pathogens, including CMV, with disease onset or flare.

It is known that patients with granulomatosis with polyangiitis (GPA) have an increased frequency of and higher levels of anti-CMV IgM [45](#_ENREF_45). However, whether this is a cause or effect of disease or treatment is not clear. The intensive immunosuppression used for induction therapy in the vasculitides places these patients at risk for CMV reactivation, similar to any other immunosuppressed populations. It has also been found that patients with GPA have altered T cell populations, including expansion of both CD4+CD28- and CD8+CD28- T cells, but only in patients who are CMV seropositive[46](#_ENREF_46). A study examining outcomes in GPA patients with expanded CMV-reactive CD4+CD28- T cell populations found associations with immunosuppression, decreased naïve T cells, impaired renal function, and risk of infection and mortality[47](#_ENREF_47). From this, the authors postulated that the presence and magnitude of CMV-specific immune response influences clinical outcomes in GPA, extrapolating this to suggest that antiviral therapy might serve as a disease-modifying therapy. The United Kingdom National Health Service is currently sponsoring a study of the use of antiviral therapy for the treatment of ANCA-associated vasculitis, perhaps heavily based on this presumption. However, this study was unable to address the question of whether these are downstream immunologic changes from prior viral activity, or a consequence of subclinical or clinically manifest viremia. In an aviremic vasculitis patient, there is a paucity of evidence to support that antiviral therapy would impart any beneficial effect on immune response or vasculitis disease activity.

**1.4.2.6 CMV in autoimmune diseases: unanswered questions and unmet needs.** In summary, there is evidence that CMV infection is prevalent in AD, that reactivation disease occurs in these patients and may be associated with worse outcomes, and that deleterious immunologic changes identified in these patients may be influenced by CMV infection or reactivation. That is, CMV infection may both increase the risk of infection in patients in AD by its immunosuppressive effects, and potentially may worsen disease activity by its proinflammatory effects. However, our overall understanding of the role of CMV in AD is limited, and it remains unclear what interventions, if any, should be undertaken at what time. To address these gaps in the field, larger longitudinal studies are needed to better identify what influence CMV infection wields on patients with AD, and whether these changes are a result of prior infection, subclinical viremia, or both. Next, it is necessary to identify and characterize associations between treatment, disease activity, and viral reactivation. Current questions include whether subclinical viremia leads to flares of disease, if disease-associated inflammation leads to viral reactivation, and whether some treatments impart higher risk for reactivation/viremia than others. Lastly, and perhaps most importantly will be to establish when an intervention (treatment) is beneficial. It seems unlikely that a one-size-fits all approach will work; in some patients, it may be that better disease control will aid in control of CMV, while in others, less immunosuppression may allow for decreased viremia by increasing host immune response.

Based on these limited data, data from the transplant literature, and our own clinical experiences with immunosuppressed patients, we hypothesized that AD patients are vulnerable to CMV reactivation, and that immunosuppressive and proinflammatory effects of viremia promote both infection and disease flare, further promoting viral persistence, potentially leading to an unremitting cycle of infection and inflammation. If subclinical CMV viremia is a risk factor for poor outcomes, then early identification and interventions to decrease subclinical CMV viremia may be a novel means to decrease infection- and disease-associated morbidity and mortality in AD patients. We chose to examine patients with ANCA-associated vasculitis and systemic lupus erythematosus for several reasons: first, these patients frequently receive combined immunosuppressive therapies, similar to solid organ transplant patients. Secondly, opportunistic infections, including CMV disease, have been widely reported in these disorders. In our clinical experience, we also had noted the vast heterogeneity in treatment response and disease control in patients with these diseases-that is, some patients tolerated highly intensive therapy without infectious complications or flares, while others with milder disease and/or less intensive therapy suffered infection after infection, often accompanied by treatment-refractory disease. We further hypothesized that this yet undetermined factor might be CMV reactivation, where viremia could simultaneously drive inflammation and risk of infection.

**2.0 OBJECTIVES**

**2.1. Research question**

Does subclinical CMV viremia in hospitalized patients with ANCA-associated vasculitis (AAV) or systemic lupus erythematosus (SLE) impact clinical outcomes?

**2.1.1 Hypotheses**

Hypothesis 1: Subclinical CMV viremia is associated with prolonged hospitalization in patients with AAV and SLE.

Hypothesis 2: Subclinical CMV viremia is associated with an increase in other infections in hospitalized patients with AAV and SLE.

Hypothesis 3: Subclinical CMV viremia is associated with increased disease activity in hospitalized patients with AAV and SLE.

**3.0 METHODS**

**3.1 Study population**

 Hospitalized patients meeting the American College of Rheumatology criteria for the diagnosis of SLE or patients fulfilling the 2012 revised Chapel Hill criteria for primary AAV were prospectively enrolled following informed consent. The patients were enrolled from 2013 to 2015. 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.

**3.2 Study design**

A prospective age, race, and gender-matched control study of patients with SLE and AAV were matched to patients without autoimmune disease being admitted to the hospital and intensive care unit was recruited. Samples were collected at study entry and one week later (if still hospitalized). The outpatient AD controls did not have an active infection or signs of CMV disease and were on stable therapy at enrollment.

**3.3 Measurements**

Demographic, laboratory, imaging, and clinical data obtained as part of routine clinical care were reviewed and collected. 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. 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 RealTime CMV assay (RT assay) on the Abbott *m*2000 instrument as per manufacturer recommendations (1 ml plasma/specimen).

**3.4 Statistical analysis**

All data collected were analyzed descriptively, followed by univariate and multivariate analyses. Continuous variables with normal distributions were analyzed using means, SD, 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, unadjusted for multiple comparisons. SPSS version 23 (IBM Corporation, Armonk, New York) was used for all analyses.

**4.0 RESULTS**

**4.1 Patient characteristics**

Eighteen patients with AD, 7 inpatient controls, and 31 AD outpatients were prospectively recruited. The groups were matched to and similar in mean age (47 years in AD inpatient and inpatient controls, 49 years in outpatient controls) and gender (inpatient AD 67% female, inpatient controls 71% female, and outpatient AD 84% female). There were no significant differences in mean age at study entry or gender between groups. The racial/ethnic distribution of patients in both AD groups was similar (44% vs. 45%), but there were a higher proportion of Caucasian patients in the inpatient control group (71%) in comparison.

In both inpatient and outpatient autoimmune disease (AD) groups, SLE was the most common diagnosis, with 10 inpatients and 22 outpatients recruited. There were 7 granulomatosis with polyangiitis (GPA) and 1 microscopic polyangiitis (MPA) patient in the inpatient AAV group, while the outpatient vasculitis control group (7 patients) 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) during their admission while in contrast, all SLE patients had established disease, but with 6/10 SLE patients with active disease as indicated by a SLEDAI score >0 and had a median steroid dose of 50 mg/day at study entry.

**4.2 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) (Figure 2).



**Figure 2: The majority of AD inpatients had detectable CMV viremia**

Sixty-three percent 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.

The average 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 and a follow up sample had increased viral load (max 394 IU/mL). CMV IgG titers 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 the IgG level did not correlate with the level of CMV viremia (p=0.12).

**4.3 CMV viremia, illness acuity, and disease-specific measures**

There were no significance difference in the average APACHE II (18 vs. 20, p=0.0.64) and SOFA scores (5 vs. 2, p=0.08) between inpatient 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 viremia.



**Figure 3: CMV viremia by patient group and testing timepoint.**

**4.4 Clinical impact**

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



**Figure 4: CMV viremia is associated with increased ICU and total length of stay**

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 each with colitis or 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 which was receiving immunosuppressive therapy prior to hospital admission, but required immunosuppressive therapy during admission, developed CMV disease following detectable viremia. The first 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 CMV meningitis during her hospitalization, both of which were successfully treated. The second patient was diagnosed with GPA during his hospitalization after presenting with pulmonary-renal syndrome, developed CMV pneumonia, and despite antiviral therapy, had persistent viremia, ultimately succumbing to multiorgan failure.

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

**5.0 DISCUSSION**

Given that patients with systemic AD have both immune dysregulation as part of their disease process and immunosuppression from agents used to treat these disorders, it is anticipated that CMV viremia frequently occurs in this population. In our study, 61% AD inpatients developed low level CMV viremia during the first week of admission. Intriguingly, in this population, low CMV levels were associated with adverse clinical outcomes including increased length of ICU and hospital stay, rates of infections, and lower lymphocyte and hematocrit levels.

As previously discussed, recent studies in other AD patients support that the association between CMV viremia and adverse outcomes may be more than just an epiphenomenon. 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, could be considered comparable to the risk identified in the solid organ transplant population (most notable in the era prior to antiviral prophylaxis), in which the rates of CMV reactivation are highest immediately post-transplant, when immunosuppressive therapy is at a maximal level. Similarly, viremia occurred at almost an equal frequency in the SLE patients, all whom had established disease with lower level disease activity (receiving 10 mg/day of prednisone or less at home) but received higher doses of glucocorticoids (median dose: 50mg/day) after admission. These findings suggest that treatment with glucocorticoids, likely in combination with other factors such as the immunologic perturbations associated with AD, may lower the threshold for CMV reactivation and viremia in AD patients.

The need for and threshold for treating CMV viremia in AD is undefined. Data from the cardiac transplantation literature demonstrate that patients with CMV viremia have a higher risk of transplant vasculopathy, and that the presence of CMV-specific CD4 T cells following transplant is associated with lower viral loads and with luminal preservation of the allograft coronary arteries, suggesting that the indirect effects of viremia, even in the absence of CMV disease, may pose significant adverse long-term vascular consequences[48](#_ENREF_48),[49](#_ENREF_49). Similarly, in the pediatric renal transplant population, subclinical CMV and Epstein-Barr viremia were associated with increased risk of acute rejection, hypertension, decreased 3-year graft function, and graft loss[21](#_ENREF_21). 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. This is highlighted by a recent randomized trial of empiric antiviral therapy in immunocompetent CMV seropositive ICU patients, which was prematurely halted because of higher mortality in the treated group. This suggests that more data are required to determine who might benefit from intervention and when it should be undertaken[50](#_ENREF_50).

Lastly, the “chicken or egg” question-whether viremia worsens AD and comorbid conditions, or disease activity/illness precipitates viral reactivation-remains unanswered. This question is particularly relevant when contemplating preventative interventions against CMV reactivation. It appears that three proposed interrelationships for CMV with inflammation/inflammaging could readily be applied to CMV reactivation in AD patients [4](#_ENREF_4). 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, this could be analogous to their immunosuppressed state) has been proposed as the culprit. This allows CMV reactivation, which promotes 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. 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 conditions driven by ongoing systemic inflammation.

The strengths of our study include the ability to measure lower level viremia using a noncommercial assay, the ability to look at the incidence of viremia in the context of clinical, disease specific, and other serologic data, and the inclusion of two distinct systemic autoimmune disorders for the basis of comparison. Our limitations include a small cohort size, measuring viremia at a single time point rather than longitudinal sequential assessment, and the inclusion of only two systemic autoimmune disorders.

**5.1 SUMMARY AND FUTURE DIRECTIONS**

In summary, CMV viremia is frequently found in patients with systemic AD, and even at low levels is associated with adverse 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 treatable, and perhaps preventable, complication of impaired host immunity. One future direction based on these findings could be to prospectively evaluate the effects of intensification vs. minimizing immunosuppression on subclinical viremia and outcomes in patients with systemic autoimmune diseases. It may also be of benefit to examine the role of newer commercial assays that measure CMV-specific immune responses in AD patients in order to aid in risk stratification.

Patients with AD suffer significant disease and treatment-associated morbidity, which frequently leads to chronic illness, loss of employment, high health care costs, and premature death. Subclinical viremia may be a modifiable risk factor for disease activity and infection, and intervention directed at this could impact public health by decreasing health care and societal costs by decreasing loss of employment, need for dialysis, need for hospitalizations, need for antimicrobial therapies, and lower mortality.

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