Title: Cytomegalovirus-associated Venous and Arterial Thrombotic Disease

Running Head: CMV infection associated with VTE & ACS

Amar H. Kelkar, M.D.[1]

Brian L. Loc, D.O.[2]

Michael D. Tarantino, M.D.[2,3,4]

Anita Rajasekhar, M.D., M.S.[1]

Huaping Wang, Ph.D.[2]

John J. Farrell, M.D.[2,5]

[1]University of Florida College of Medicine, Department of Medicine, Division of Hematology & Oncology,

Gainesville, FL, U.S.A.

[2]University of Illinois College of Medicine at Peoria, Department of Medicine, Peoria, IL, U.S.A.

[3]Bleeding & Clotting Disorders Institute, Peoria, IL, U.S.A.

[4]University of Illinois College of Medicine at Peoria, Department of Pediatrics, Peoria, IL, U.S.A.

[5]Microbiology & Immunology, OSF System Lab, Peoria, IL, U.S.A.

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Corresponding Author:

Amar H. Kelkar, M.D.

Department of Internal Medicine

Division of Hematology & Oncology

University of Florida College of Medicine

1600 SW Archer Rd, Box 100278, Gainesville, FL 32610

Phone: (352) 548-6000

Fax: (352) 273-7849

Email: Amar.Kelkar@medicine.ufl.edu

Alternate Corresponding Author:

Brian L. Loc, D.O.

Department of Internal Medicine

Division of Cardiology

University of Illinois College of Medicine at Peoria

1 Illini Dr, Peoria, IL 61605

Phone: (512) 497-6801

Fax: (309) 655-3297

Email: Brian.Loc1@gmail.com

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Key Points

- Cytomegalovirus (CMV) infection has been associated with venous thromboembolism (VTE).

- Incidence of acute VTE within one year of CMV testing was studied retrospectively in Peoria, IL.

- Control groups were less likely to develop VTE than the CMV-infected group within one year.

- Seronegative controls had lower incidence of acute coronary syndrome than the CMV-infected groups.

Abstract

Background: Cytomegalovirus (CMV) infection has been associated with venous thromboembolism (VTE) and acute coronary syndromes (ACS).

Methods: A retrospective study was conducted within the OSF HealthCare System in Peoria, Illinois. The objectives were to determine the incidence of acute VTE and ACS within one year of CMV testing. The "study group" included patients with positive CMV IgM or positive CMV Polymerase Chain Reaction (PCR). The "seropositive control" group included patients with positive CMV IgG and negative IgM. The "seronegative control" group included patients with negative CMV IgG and IgM, or negative PCR.

Results: Within one year of CMV infection, 38 of 379 patients (10.0%) developed VTE in the study group compared to 41 of 1334 patients (3.1%) in the seropositive control and 37 of 1249 (3.0%) in the seronegative control. Adjusting for age and gender, both control groups were less likely to have VTE than the study group within one year (Seropositive Control: OR = 0.3, 95% CI 0.2-0.5, p <0.0001; Seronegative Control: OR = 0.4, 95% CI 0.2-0.6, p <0.0001). ACS was more likely to occur in the study group, with incidence of 7.7% compared to 4.7% (p <0.0001) in the seropositive control and 1.9% (p <0.0001) in the seronegative control. Adjusting for age and gender, the seronegative control was less likely to develop ACS than the study group within one year (OR = 0.4, 95% CI 0.2-0.7, p = 0.003).

Conclusions: This retrospective study demonstrates that CMV infection may be a significant risk factor for VTE and ACS.

Introduction

Cytomegalovirus (CMV) is a heterogenous DNA virus in the herpesviridae family capable of infecting a broad range of tissue types within a host.[1] CMV is most often associated with heterophile-negative mononucleosis and invasive infections in primary immunocompromised hosts, patients on immunosuppressants, and infants.[1] CMV was first described in association with venous thromboembolism (VTE) in 1984.[2] Since that time, dozens of cases of CMV-associated VTE in both immunocompromised and immunocompetent hosts have been described.

Numerous risk factors have been linked to VTE. Irreversible risk factors include inherited thrombophilic disorders, advanced age, prior VTE, and underlying malignancy. Reversible risk factors include recent surgery, trauma, prolonged immobility, obesity, pregnancy, estrogen use, indwelling catheters, acute infection, and current or recent hospitalization.[3,4] However, few studies have explored CMV as a risk factor for VTE.

CMV has also been associated with acute coronary syndrome (ACS) and other arterial vascular diseases. CMV antigens were first identified in cultured human arterial smooth muscle cells from atherosclerotic carotid and aortic plaques in 1983.[5] This relationship has been further explored *in vitro*; however, results *in vivo* have been limited.[6,7] The relationship between CMV and thrombotic complications is particularly interesting given the unique biology of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and vascular disease being described during the COVID-19 pandemic.{Jean, 2020, COVID-19 and its implications for thrombosis and anticoagulation}

Herein, we present retrospective data collected from the OSF HealthCare System to determine the incidence of acute VTE and ACS within one year of CMV testing. The purpose was to determine whether biological models of CMV thrombogenicity translate to increased incidence of thrombotic events.

Methods

We conducted an institutional retrospective study of patients with prior CMV testing within the OSF HealthCare System, a network of 13 hospitals based in Peoria, IL, between January 2008 and September 2017. During this period of time, 2,144,725 patients were seen across these facilities. This study was performed by query of the Epic Systems™ (Verona, WI) patient electronic health record. Patients with CMV IgG, IgM, or Polymerase Chain

Reaction (PCR) testing, or an ICD-9 (078.5) or ICD-10 (B25.9) diagnosis code for CMV disease during the study period were included. CMV immunoglobulin testing was performed in the OSF System Laboratory on a BioPlex® 2200 automated analyzer (Bio-Rad Laboratories, Hercules, CA). CMV deoxyribonucleic acid (DNA) polymerase chain reaction (PCR) is a plasma-based assay performed in the OSF System Laboratory with the Cobas® AmpliPrep/Cobas® TaqMan® CMV quantitative PCR (Roche Diagnostics, Indianapolis, IN). A data collection form was developed for systematic retrieval of anonymized epidemiologic, historic, diagnostic, and treatment data. Patients of all ages including children and pregnant women were included, but incarcerated patients were excluded. The study was approved by the OSF HealthCare System Research Administration Department and the University of Illinois College of Medicine at Peoria Institutional Review Board.

The primary outcome was incidence of acute VTE within one year of CMV testing. These data were collected by database query of the sample population within one year of the CMV detection date, initially using ICD-9 (415.1, 452, 453.4, 453.8) and ICD-10 (I26.90, I26.99, I81, I82, I82.40, I82.90) codes for VTE and all subtypes to identify diagnosis and date. Manual review of the charts was performed to confirm diagnoses using available imaging or provider documentation. VTE was defined as deep vein thrombosis (DVT) of the upper or lower extremities, pulmonary embolism (PE), or splanchnic vein thromboses. Catheter-related DVTs were included. PE was confirmed by results of computed tomography with pulmonary angiography (CTPA). DVT or splanchnic vein thrombosis were confirmed by venous doppler ultrasound, abdominal ultrasound, or computed tomography with angiography. The majority events were confirmed by in-network studies; however, scanned reports in the electronic health record from outside facilities were also reviewed and incorporated. Demographic data were also collected.

Secondary outcome data included events of ACS within one year of CMV testing. These data were also collected by database query of the sample population within one year of the CMV detection date, initially using ICD-9 (410) and ICD-10 (I21) codes to identify diagnosis and date, followed by manual review of the charts to confirm these diagnoses using available imaging, provider documentation, or scanned reports from outside facilities. ACS was defined as ST-elevation myocardial infarction (STEMI) and non-ST elevation myocardial infarction (NSTEMI), confirmed by coronary angiography or a combination of elevated troponin levels with consistent electrocardiogram findings for NSTEMI.[8,9]

Data was de-identified after extraction and review. Univariate and multivariate analysis included logistic regression models, chi square test, Kruskal-Wallis test, and Wilcoxon two-sample test, followed by the post hoc test once statistical significance was determined, all performed using SAS® 9.4 software (Cary, NC). Age- and gender-adjusted analysis was also performed. Patient race was collected with initial demographic data but was not consistently recorded and was thus excluded from further analysis.

Results

The initial sample consisted of 3011 patients, and the medical records were manually reviewed for quality-control. Forty-nine patients were excluded due to lack of CMV IgG, IgM, or DNA PCR testing to confirm date of CMV diagnosis. The remaining population of 2962 patients was then divided into three groups. The "study group" included all patients with acute or recent CMV infection, defined as positive CMV IgM or detection of CMV DNA by PCR in plasma. The remaining patients were divided into two control groups. The "seropositive control" group was defined as patients with positive CMV IgG and negative IgM or PCR. The "seronegative control" group was defined as patients with negative CMV IgG, IgM, and/or PCR. Three patients included in the study group had positive CMV PCR, but negative IgM testing. The study design is depicted in *Figure 1*.

The study population of patients with acute CMV infection consisted of 379 patients. The seropositive control group included 1334 patients. The seronegative control group included 1249 patients.

Statistical differences between the groups were identified by the post hoc test. Patients in the study group (median 38.4 years, IQR 39.2) were older than both the seropositive control group (median 28.4 years, IQR 43.1, $p <0.0001$) and the seronegative control group (median 18.7 years, IQR 32.9, $p <0.0001$). The seropositive control group was older than the seronegative control group ($p < 0.0001$). The seropositive control group also had fewer male patients than the study group (41.8% versus 51.2%, $p = 0.001$) and the seronegative control group (41.8% versus 48.9%, $p = 0.0003$). Demographic data of age and gender can be seen in *Table 1*.

For primary outcome data, 38 of 379 patients (10.0%) in the study group were diagnosed with VTE within one year of acute CMV diagnosis. The vast majority of these events occurred within the first six months, as depicted in *Figure 2*. This is compared to 41 of 1334 patients (3.1%) in the seropositive control group and 37 of 1249 patients (3.0%) in the seronegative control group (*Table 2*). The seropositive control group had lower incidence of VTE than the study group (3.1% versus 10.0%, p <0.0001). The seronegative control group also had lower incidence of VTE than the study group (3.0% versus 10.0%, p <0.0001). There was no statistically significant difference between the incidence of VTE in the seropositive control group and seronegative control group (3.1% versus 3.0%, $p = 0.9$), suggesting that the effect of acute CMV infection on VTE incidence may be transient.

Multivariate analysis was performed after adjustment for age and gender. VTE in the study group was higher than either control group (*Table 2*). The seropositive control group was less likely to have VTE than the study group within one year (OR = 0.3, 95% CI 0.2-0.5, p <0.0001). The seronegative control group was also less likely to have VTE than the study group within one year (OR = 0.4, 95% CI 0.2-0.6, p <0.0001).

Secondary outcome data demonstrated that ACS was more likely to occur in the study group, with incidence at one year of 7.7%, compared to 4.7% (p <0.0001) in the seropositive control group and 1.9% (p <0.0001) in the seronegative control group (*Table 2*). Multivariate analysis of incidence adjusted for age and gender demonstrated that the seronegative control group was less likely to develop ACS than the study group within one year (OR = 0.4, 95% CI 0.2-0.7, $p = 0.003$). However, the seropositive control group failed to demonstrate significant differences compared the study group (OR = 0.7, 95% CI 0.4-1.2, $p = 0.2$). The difference between the two control groups in the secondary outcomes data was significant, with the seropositive control group more likely to develop ACS within one year than seronegative control group (OR = 1.8, 95% CI 1.1-2.9, $p = 0.03$).

Discussion

This study sought to retrospectively determine the incidence of acute VTE in the one-year period following CMV testing, dividing the results into groups of patients with active infection, seropositive IgG testing without active infection, and seronegative IgG and IgM testing. Differences in incidence of acute VTE between these groups

suggest that CMV may have contributed to a prothrombotic state in patients with active infection or seropositive state.

These data align with several studies in the past decade supporting the association between CMV and VTE. The MAISTHRO retrospective registry case-control study reported 4.3% with CMV IgM positivity amongst patients with acute VTE, compared to 0.6% with CMV IgM positivity in matched control patients without acute VTE (OR 7.3).[10] One large single-center prospective case-control study observed patients with acute CMV infection and matched CMV IgM negative controls for 6 months and found 3.06 cases of acute VTE per 1000 in those with positive IgM compared to 1.36 cases of acute VTE per 1000 in those with negative IgM (OR 2.25, 95% CI 1.38–3.66, $p = 0.003$).[11] A retrospective case-control study of 140 patients with acute CMV infection showed an incidence of concurrent VTE of 2.9%, which was significantly greater than 0% VTE in 140 matched controls without acute CMV infection.[12] A meta-analysis concluded that between 1.9% and 9.1% of patients hospitalized with acute venous thromboembolism had concurrent acute CMV infection.[13]

Our study is the second to look at the incidence of acute VTE following acute CMV infection over a prolonged period of time compared to incidence of acute VTE without acute CMV infection, following the work of Paran and colleagues.[11] We chose one-year follow-up, compared to six-month follow-up in prior studies, to increase the likelihood of capturing potential CMV-related VTE and ACS events based on existing data suggesting a durable effect of increased thrombosis, as well as the chronic nature of the viral infection. A unique feature was that our study demonstrated differences between acute CMV infection and seropositive status without active infection. Incidence of acute VTE in the seropositive control group was not significantly different than incidence in the seronegative control group. The data provide additional evidence for CMV being a reversible or transient risk factor of VTE. Current guidelines recommend transient or reversible causes of VTE be treated with anticoagulation for 3 months, whereas unprovoked VTE be treated with extended anticoagulation unless bleeding risk is high.[14] We suspect that CMV-associated VTE is likely underrecognized because CMV testing is not routinely performed outside of immunocompromised populations.[15] However, additional retrospective and prospective studies are needed to determine whether acute CMV infection is a reversible risk factor for acute VTE and can be used to change CMV screening, primary prophylaxis, and VTE treatment practices.

The secondary outcome data collected in this study similarly showed a measurable difference between the seronegative control group and the study group, with regard to incidence of ACS. However, incidence of ACS was not significantly different between the seropositive control group and the study group. While there are no prior studies of similar design to directly compare these data, the discrepancies might be explained by the different mechanisms involved in arterial thrombosis. Studies examining the role of CMV in arterial disease fall into two groups. CMV IgG seropositivity and chronic CMV endothelitis have been linked with the pathogenesis of atherosclerosis, while acute CMV infection has been associated with ACS.[6,7,16-18] Both primary ACS and restenosis were counted amongst the ACS incidence data in our study, possibly explaining the similar incidence rate of ACS in both the seropositive control group and the study group.

Our study data should be considered in the context of the following limitations to the study design. Data were collected retrospectively and without prejudice towards the immunocompromised status or reason for CMV testing. There was large variance in the justification for CMV testing across the spectrum of patients in the initial study population and clinical symptoms of infection were not consistently recorded. Both immunoglobulin and serum DNA testing were not always available, limiting differentiation between acute and recent CMV infections. When designing the study, we noted three patients in the study group that had positive CMV PCR, but negative IgM testing. These patients were included in the study group based on the study design and due to high serum PCR titers. The method of database query could not account for VTE or ACS events occurring outside of the OSF HealthCare System, conferring a form of attrition bias, though scanned records sent from out-of-system facilities were reviewed and included in the analysis. There was an unusually high rate of VTE incidence across all the groups within this study, likely due to other concurrent risk factors for VTE. However, other risk factors for VTE and CMV infection, including immunocompromised status, were not collected in the data set and thus could not be used for adjustment in a multivariate analysis. The data did not differentiate between primary ACS and restenosis, due to limitations in the database query. Furthermore, treatment with antiviral medications was not captured in the data and thus its impact could not be included in the analysis. These study limitations are also described in *Table 3*.

Despite these limitations, these data support the existence of CMV-associated VTE. There are also several known and proposed mechanisms for a causative relationship have been described *in vitro*. The majority of models are based on host monocyte and endothelial cell infection. Direct infection of vascular endothelial cells has been demonstrated to increase release of von Willebrand factor and increase cell-surface expression of tissue factor.[7,19] This has been shown to promote inhibition and depletion of natural anticoagulants as well as inhibition of antithrombin III and fibrinolysis.[7,20] CMV infection also increases leukocyte adhesion to CMV-infected endothelial cells, which has been shown to promote a procoagulant microenvironment through similar mechanisms, possibly acting as cofactors.[21,22] Direct infection of monocytes also resulted in increased secretion of tissue factor, thus promoting thrombosis.[7] Another model described an immunologic response to CMV envelope phospholipids, including phosphatidylserine, which has procoagulant prothrombinase properties, reducing the factor Xa clotting time by bypassing most of the coagulation cascade and directly activating thrombin.[20]

One proposed model describes transient elevations of antiphospholipid antibodies in the context of acute CMV and other viral infections including Epstein-Barr Virus, Hepatitis C Virus, Parvovirus, Varicella Zoster Virus, and Adenovirus.[23] A study in mice demonstrated that immunization with a CMV capsule peptide known as TIFI (an analog of human beta-2-glycoprotein I [β2GPI]), induced lupus anticoagulant activity and resulted in thrombotic complications.[24,25] Subsequent study demonstrated that co-injection of TIFI with exogenous antiphospholipid antibodies decreased incidence of vascular thrombosis in the setting of mechanical injury to the mice. These mice did not form endogenous antiphospholipid antibodies, suggesting a potentially protective effect of the exogenous antibodies.[26] Case reports in humans have described transient increases of serum antiphospholipid antibodies in acute CMV, specifically anticardiolipin IgM.[27,28] This would support the applicability of the findings in mice to humans. However, at this time there are no published studies connecting TIFI to formation of anti-β2GPI antibodies in human patients.[29] If this link can be established, the homology between CMV TIFI and the 5th domain of human β2GPI could explain why CMV has been more commonly associated with VTE than other viral infections.

Several prior studies have looked at associations between organ system-specific acute infections and thrombosis in the hospital and community settings. One study through the UK-based Health Improvement Network looked at a cohort of patients with first-time diagnoses of DVT (n = 7278) or PE (n = 3755) and looked at incidence of

preceding urinary tract and respiratory tract infections in the preceding six months. They found that the incidence

risk ratios of DVT (2.10, 95% CI 1.56–2.82) and PE (2.11, 95% CI 1.38–3.23) were significantly higher after

urinary tract infections, particularly in the first two weeks after infection. Respiratory tract infections also had

increased risk of VTE, but diagnostic data were more confounded.[30] One key difference in our study design was

following a single infection across a broad cohort of patients, rather than looking for incidence of preceding

infection in a cohort of patients with VTE. Additionally, past studies did not consider disease-specific causes of

VTE. They cite studies discussing transient alterations to endothelial function, increased inflammation measured by

surrogate markers such as C-reactive protein, the role of neutrophil activation and neutrophil extracellular traps,

tissue factor activation of thrombotic pathways, inhibition of endogenous anticoagulant pathways, and inhibition of

fibrinolysis.[4,30-32] While there are other known associations between acute infection and thrombosis, they neither

address nor negate the evidence presented for a potentially unique causal relationship between CMV and

thrombosis. This is further evidenced by the presence of increased odds of VTE in the CMV seropositive group of

patients suggesting a more prolonged prothrombotic state than has been presented in other infection models of

thrombosis.

Conclusions

Our findings support the hypothesis of a link between acute CMV infection and thrombotic events such as acute

VTE and ACS. In particular, the adjusted odds ratios for acute VTE were convincing of a difference between the

study and control populations related to CMV status. The secondary outcome data contribute to the limited existing

evidence for a role of acute CMV infection in ACS. Additional *in vivo* studies are needed to further clarify the

relationship between acute CMV infection and thrombosis and account for other known risk factors for VTE,

especially in high-risk populations. These studies should include data on incidence of anti-β2GPI antibody

formation based on *in vitro* findings.[28] No prior studies have evaluated VTE prophylaxis in patients with acute CMV

infection. These concepts should be considered in future clinical trials if an association between CMV infection and

VTE is confirmed.

Authorship Details

All authors had access to the data and had a role in writing the manuscript. Amar H. Kelkar M.D. and Brian L. Loc D.O. wrote the preliminary draft manuscript. The study was designed by Amar H. Kelkar M.D., Brian L. Loc D.O., and John J. Farrell M.D. Data collection and review were performed by Amar H. Kelkar M.D. and Brian L. Loc D.O. Data analysis was performed by Amar H. Kelkar M.D. and Huaping Wang, Ph.D. All authors made critical revisions related to the content of the manuscript and approved the final version of this article for submission.

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Conflict of Interest

None of the authors have any relevant conflicts of interest or disclosures. However, it should be noted that Anita Rajasekhar has served on an advisory board for Alexion, Baxter, Bayer, Kedrion Biopharma Octapharma Plasma, and her institution has received research support on her behalf from Alnylam (Sanofi Genzyme), Baxalta (Shire), Biomarin, Dimensions Therapeutics, Genetech, Janssen Pharmaceuticals, and Roche.

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Table 1. Demographics		Seropositive Control Group n = 1334	Seronegative Control Group n = 1249	Study Group n = 379
Age, years				
	Median (IQR)	28.4 (43.1)	18.7 (32.9)	38.4 (39.2)
Gender, n (%)				
	Female	776 (58.2)	638 (51.1)	185 (48.8)
	Male	558 (41.8)	611 (48.9)	194 (51.2)

Table 1. Demographics Description of age and gender demographics within the seropositive control group,

seronegative control group, and study group.

Abbreviations: IQR = Interquartile Range

Table 2. Incidence of VTE and ACS in Control Groups versus Study Group							
	Seropositive Control Group	Seronegative Control Group	Study Group		Unadjusted Results		*Age- and Gender-Adjusted* Results
	n = 1334	n = 1249	n = 379		*p* value	OR (95% CI)	*p* value	OR (95% CI)
VTE, n (%)	41 (3.1)	37 (3.0)	38 (10.0)	Seropositive Control Group vs. Study Group	<0.0001	0.3 (0.2-0.5)	<0.0001	0.3 (0.2-0.5)
				Seronegative Control Group vs. Study Group	<0.0001	0.3 (0.2-0.4)	<0.0001	0.4 (0.2-0.6)
				Seropositive Control Group vs. Seronegative Control Group	0.9	1.0 (0.7-1.6)	0.5	0.9 (0.5-1.4)
ACS, n (%)	62 (4.7)	24 (1.9)	29 (7.7)	Seropositive Control Group vs. Study Group	0.02	0.6 (0.4-0.9)	0.2	0.7 (0.4-1.2)
				Seronegative Control Group vs. Study Group	<0.0001	0.2 (0.1-0.4)	0.003	0.4 (0.2-0.7)
				Seropositive Control Group vs. Seronegative Control Group	0.0002	2.5 (1.5-4.0)	0.03	1.8 (1.1-2.9)

Table 2. Incidence of VTE and ACS in Control Groups versus Study Group Incidence of VTE and ACS in the seropositive control group, seronegative control group, and study group with calculated odds ratios (OR), 95% confidence intervals (CI), and *p* values between each of the control groups and the study group. Age- and gender-adjusted results were also calculated.

Abbreviations: VTE = Venous Thromboembolism; ACS = Acute Coronary Syndromes; OR = Odds Ratio; CI = Confidence Interval

Table 3. Study Limitations
Design Limitations
Retrospective study design   **Data were collected without prejudice towards the immunocompromised status or reason for CMV testing**   **Did not collect data on concurrent risk factors of VTE or immunocompromised status for multivariate analysis**
Database Limitations
Unusually high rate of VTE incidence across all the groups within this study   **Clinical symptoms of infection were not consistently recorded**   **Large variance in the justification for CMV testing**   **Both immunoglobulin and serum DNA testing were not always available, limiting differentiation between acute and recent CMV infections**   **Treatment with antiviral medications not consistently recorded**   **Could not account for VTE or ACS events occurring outside of the OSF HealthCare System**   **Attrition bias**   **Scanned records sent from out-of-system facilities were reviewed and included in the analysis**   **Could not differentiate between primary ACS and restenosis**

Table 3. Study Limitations The results of the study must be viewed within the context of its limitations, which

include its retrospective study design, large volume of patients with missing or untested data points, and database

search limitations.

Abbreviations: CMV = Cytomegalovirus; VTE = Venous Thromboembolism; DNA = Deoxyribonucleic Acid; ACS

= Acute Coronary Syndromes
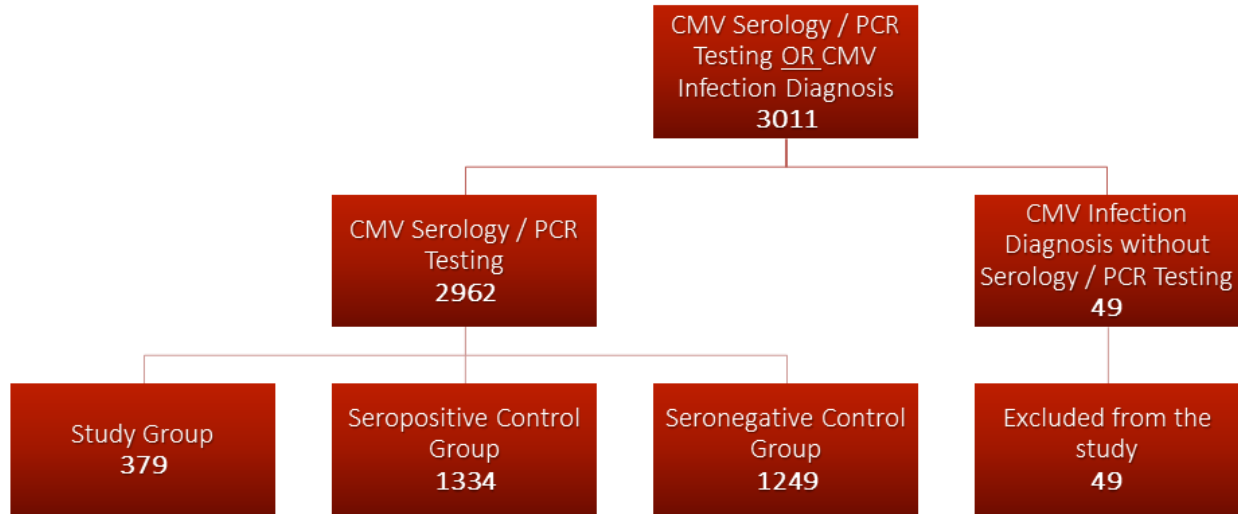
Figure 1. Study Design The patient population was divided into three groups after excluding patients with no

serology or PCR testing: "study group" (IgM+/PCR+), "seropositive control" group (IgG+/IgM-/PCR-),

"seronegative control" group (IgG-/IgM-/PCR-).

Abbreviations: PCR = Polymerase Chain Reaction
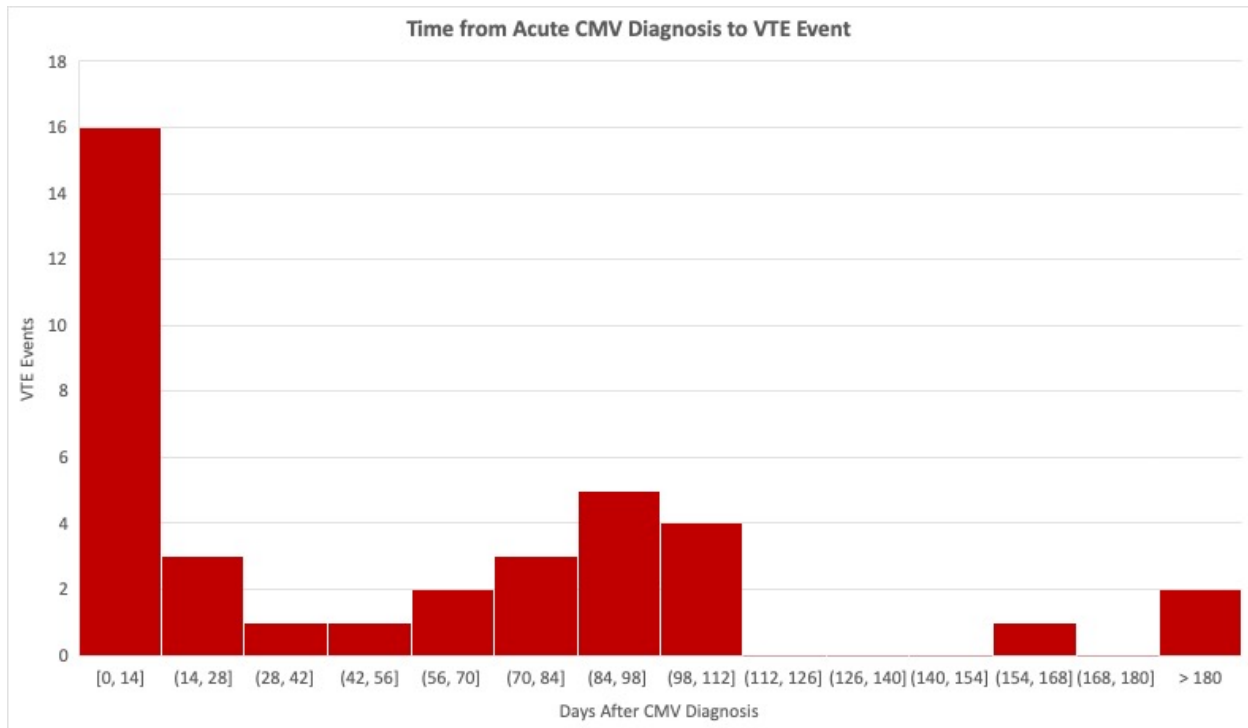
Figure 2. Time from Acute CMV Diagnosis to VTE Event Within the study group, the majority of VTE events

occurred within six months of the initial acute CMV diagnosis, supporting a temporal relationship.

Abbreviations: VTE = Venous Thromboembolism; CMV = Cytomegalovirus