



ISSN: 1813-1638

The Medical Journal of Tikrit University

Available online at: www.mjtu.tu.edu.iq

MJTU

The Medical Journal of
Tikrit University

Comparative Analysis of CRP, D-dimer and Platelet Count in CMV-Positive Versus CMV-Negative Patients

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Keywords: CMV, CRP, D. dimer, Platelets.

ARTICLE INFO

Article history:

Received	18/01/2026
Received revised	21/03/2026
Accepted	20/04/2026
Final Proofreading	17/05/2026
Available online	30/06/ 2026

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Citation

DOI: <http://dx.doi.org/10.25130/mjotu.32.1.10>

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ABSTRACT

Background: Cytomegalovirus (CMV) infection can induce inflammatory responses, but the relationship between specific inflammatory biomarkers and CMV infection is not fully understood. This study aims to evaluate the levels of C-reactive protein (CRP), D-dimer, and platelet counts in patients with CMV seropositivity.

Methodology: Fifty individuals were enrolled, divided equally between a CMV-infected group and a control group. The CMV diagnosis was based on serological tests. Exclusion criteria included co-infections, cardiovascular abnormalities, the use of anticoagulants, and hematological changes. The study measured CRP and D-dimer levels using the AFIAS-6 instrument and platelet counts via automated hematology analyzers. Data analysis included comparison of biomarker levels between groups and Receiver Operating Characteristic (ROC) analysis for diagnostic utility.

Results: CRP levels were significantly higher in the CMV group compared to the control group, with a mean of 31 mg/l versus 4 mg/l. D-dimer levels were also elevated in CMV patients (mean: 509 ng/ml) compared to controls (mean: 209 ng/ml). Platelet count differences were not significant. ROC analysis showed high specificity and moderate sensitivity for CRP and D-dimer in diagnosing CMV infection (AUC of 0.848 and 0.795 respectively), but platelet count was not a reliable indicator.

Conclusion: CRP and D-dimer are promising biomarkers for detecting CMV infection, but platelet counts do not offer significant diagnostic value. Further research is needed to explore the utility of these biomarkers in different clinical settings and populations.

INTRODUCTION

Cytomegalovirus (CMV) infections, initially documented in 1881, are prevalent in a range of 30% to 100% among individuals without any underlying health conditions [1]. The human cytomegalovirus (HCMV) is a highly prevalent pathogen that is accountable for predominantly asymptomatic and enduring infections in individuals with normal health conditions. In the absence of a robust immune response, such as in people with immature or weakened immune systems, the virus has the potential to induce serious illness. Moreover, it is the primary pathogenic microorganism responsible for congenital abnormalities [2]. Cytomegalovirus (CMV), is classified under the Herpesviridae family [3]. The process of diagnosing CMV infection necessitates the verification of the presence of the infection by the utilization of Serological testing for human cytomegalovirus (CMV) immunoglobulin G (IgG) [4].

Biomarkers, or biological markers, are objective measurements used to assess internal bodily processes, often aiding in medical diagnosis and treatment. They encompass a wide range, from blood pressure to imaging tests, such as X-rays. Frequently, when referencing biomarkers, people refer to blood tests which might assess components such as specific proteins or cell types [5]. Inflammatory biomarkers are a subset that provides information about inflammation and the immune system. Inflammation is the body's complex response to injury, infection, or toxins, and can be acute (short-lived) or chronic (long-term). Chronic inflammation is observed in conditions such as rheumatoid arthritis and lupus [6],[7].

Common inflammatory biomarkers include complete blood counts (CBC) or more specifically platelet count (PLT), C-

reactive protein (CRP), D-Dimer. The complete blood count (CBC) measures various cell types and components in the blood, while CRP gauge inflammation levels [8]–[10]. CRP, a calcium-dependent ligand-binding protein, indicates inflammation and is synthesized in the liver in response to IL-6 secretion due to tissue injury or pathogenic agents. Its levels rise quickly after IL-6 elevation and can be cost-effectively measured, although they lack specificity. Elevated CRP levels, found in 9% of individuals with CMV, correlate with increased mortality risk, especially from cardiovascular diseases. D-dimer, a fibrin-derived metabolite, signifies secondary fibrinolysis, aiding in diagnosing conditions like deep vein thrombosis and pulmonary embolism. Elevated D-dimer levels might also indicate inflammation and have been observed in CMV infections, suggesting a potential marker for CMV. Platelets, small cellular fragments crucial for blood clot formation and immune responses, undergo rapid changes in response to stimuli and release mediators affecting coagulation and inflammation. CMV infection might promote thrombosis by enhancing platelet and leukocyte adhesion to infected endothelial cells, increasing clot formation risk, which necessitates vigilance for thrombosis signs in acute CMV infections [11]–[14].

This study assesses the inflammatory biomarkers, namely CRP, D-Dimer and platelet count and their association with cytomegalovirus infection. By scrutinizing their roles, interactions, and significance, we aim to bridge the existing knowledge gaps and highlight the potential clinical implications of these biomarkers in managing CMV-related complications.

METHODOLOGY

Fifty (50) sample were included in this study , (25) Patients with a confirmed diagnosis of Cytomegalovirus and (25) control group. All patients with Cytomegalovirus were diagnosed based on characteristic serological findings as positive for Cassette method were evaluated to be included in the study. Data collection was from the 1st of November 2023 until the end of March 2024. Patient selection was performed based on :

Inclusion Criteria :

- Patients who have been tested positive for CMV infection .
- Patients who are pregnant or planning to become pregnant .

Exclusion Criteria :

- Records of patients with co-infections or positive history of viral infections
- Patients with myocardial infraction or any cardiovascular abnormalities .
- Patients who are on anti-coagulations .
- Patients with bone marrow or hematological changes .

Data Collection Tools and Procedure :

A standardized data collection sheet was designed, which captured the following :

- Patient's demographic details: age, gender, date of the test, etc .
- CMV infection status: positive or negative .
- Levels of inflammatory biomarkers .
- Any noted clinical manifestations or complications related to CMV.

RESULTS

The study included 50 participants, with 25 in the case group (infected with CMV) and 25 in the control group (not infected). The levels of C-reactive protein (CRP), D-

dimer, and platelets were compared between the two groups. As demonstrated in table 1, The mean CRP level in the case group was significantly higher (31 mg/l) compared to the control group (4 mg/l), with a p-value <0.01, indicating a strong association between CRP levels and CMV infection. Similarly, D-dimer levels were notably higher in the case group (mean: 509 ng/ml) compared to the control group (mean: 209 ng/ml), with the difference being statistically significant (p-value <0.01). The mean platelet count was slightly higher in the case group (328) compared to the control group (316), but this difference was not statistically significant (p-value >0.05). Figure 1 depicted a boxplot of CRP levels, illustrating a clear difference between the case and control groups, with the case group showing higher variability and outliers. Figure 2 showed the boxplot for D-dimer levels, again demonstrating higher levels and variability in the case group. Figure 3 presented the boxplot for platelet counts, where the distributions were more similar between the two groups.

The Receiver Operating Characteristic (ROC) analysis provided interesting insights as demonstrated in table 2, for CRP, the area under the curve (AUC) was 0.848, indicating good diagnostic ability. At the cutoff level of 11.50 mg/l, CRP had a high specificity of 100% and a sensitivity of 72%. The AUC for D-dimer levels was 0.795, with a cutoff level of 244.00 ng/ml yielding a sensitivity of 72% and specificity of 80%. In contrast, The AUC for platelet count was 0.551, indicating poor discriminative power, with a cutoff of 326.00 and sensitivity of 60% and specificity of 64%. Figure 4 presented the ROC curve for CRP, showing excellent specificity (100%) but moderate sensitivity (72%) for detecting CMV infection. Figure

5 illustrated the ROC curve for D-dimer, which also had good specificity (80%) and sensitivity (72%). Figure 6 displayed the ROC curve for platelet count, which had lower sensitivity (60%) and specificity (64%), suggesting it is not a good discriminator for CMV infection.

DISCUSSION

In the current study, CRP levels were significantly higher in the CMV cases group compared to the control group, this is consistent with the findings of Barlik et al (35), who found that elevated CRP levels were positively correlated with seropositivity for CMV infection indicating that a marked elevation in CRP in case suspected of CMV exposure should raise the index of suspicion. This is further backed by older observations by Zhu et al (36) who identified an association between elevated CRP and CMV seropositivity. Furthermore, elevated CRP is also associated with Positive CMV DNA in serum as demonstrated by the findings of Rollag et al (37). For diagnosing CMV infection, CRP showed an area under the curve (AUC) of 0.848 with a cutoff level of 11.50 mg/l, demonstrating 72% sensitivity and 100% specificity, which adds up to the conclusions of Costalonga et al who demonstrated 100% sensitivity and 90.63% specificity to detect CMV disease and distinguish it from tuberculosis and bacterial infections when $CRP < 44.5$ mg/L (38). Which suggests that CRP could be utilized as a diagnostic measurement for detecting CMV infection.

In the current study its levels were also significantly higher in the case group. This is consistent with the results of the study conducted by Shi et al, that found that plasma D-dimer levels in the CMV hepatitis group were markedly higher than those in control group [39]. This was also

evident in a study by Pérez-Granda et al, which observed CMV reactivation in severe COVID-19 infected cases was also associated with elevated D-dimer levels compared to case where no reactivation of CMV infection occurred [40]. These findings warrant the consideration of CMV infection in patients who present with elevated d-dimer as summarized by the review conducted by Burkey et al who emphasized that physicians must consider cytomegalovirus-associated portal vein thrombosis as a potential diagnosis when patients present with abdominal pain and viral symptoms especially with elevated D-dimer [41]. The current findings state that D-Dimer had an AUC of 0.795 with a cutoff level of 244.00 ng/ml, showing 72% sensitivity and 80% specificity. Suggesting it could be utilized as a diagnostic tool for detecting CMV infection.

Platelet count differences were not significant between the groups and had an AUC of 0.551 with a cutoff level of 326.00, indicating 60% sensitivity and 64% specificity, but this was not statistically significant either. The insignificance of platelet count was also noted in a study by Phasuk et al (42). This finding indicates that platelet count is not a reliable measure to detect CMV infection. However, observations done by Zhan et al who demonstrated that CMV infection cases had significantly lower platelet count in their study. In addition, their investigation demonstrated that the best measure to utilize platelet count in the context of detecting CMV infection wasn't platelet count directly, but instead they measured platelet to lymphocyte (PLR) ratio which tends to be lower in CMV infected cases, with an AUC for of 0.69 which is significantly more reliable than direct measurement of platelet count [43]. Which raises the question of whether our findings

would've been significant if we did utilize the same measure.

CONCLUSION

1. C-Reactive Protein (CRP) Levels: There was a significant elevation in CRP levels in the CMV-infected group compared to the control group. This finding aligns with previous research indicating a strong correlation between elevated CRP levels and CMV infection. CRP's diagnostic ability, as indicated by the area under the curve (AUC) of 0.848, was notable, suggesting its potential utility as a diagnostic marker for CMV infection.
2. D-Dimer Levels: Similar to CRP, D-dimer levels were significantly higher in the CMV-infected group. This observation is consistent with other studies, suggesting a potential role of D-dimer as a diagnostic tool in CMV infection, especially in cases with elevated levels.
3. Platelet Count: The study found no significant difference in platelet count between the CMV-infected group and the control group. This finding contrasts with some studies that have found an association between CMV infection and altered platelet count. However, the platelet to lymphocyte ratio (PLR) may be a more reliable indicator, as suggested by other research.

CONFLICT OF INTEREST

- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Authors sign on ethical consideration's approval

- Ethical Clearance: The project was approved by the local ethical committee in Al-mustansiriyah university.

ACKNOWLEDGEMENTS

Dr.Zainab Fadhel Ashour /Head of Microbiology dep. college of medicine /Mustansiriyah university.

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TABLES

Table 1. CRP, D-Dimer and platelet levels between case and control groups (N=50)

Variable	Sample				P-value
	Case (N=25)		Control (N=25)		
	Mean	Standard Deviation	Mean	Standard Deviation	
CRP mg/l	31	39	4	3	<0.01**
D-dimer ng/ml	509	281	209	46	<0.01**
Platelet count	328	117	316	81	>0.05 N.S.

FIGURE

Table 2. CRP, D-Dimer and platelet levels AUC, sensitivity, and specificity for CMV infection (N=50)

Parameters	CRP mg/l	D-Dimer ng/ml	Platelet count
AUC (95% CI)	0.848 (0.732,0.964)	0.795 (0.660,0.931)	0.551 (0.387,0.715)
Cutoff level	11.50	244.00	326.00
Sensitivity	72%	72%	60%
Specificity	100%	80%	64%
P-value	<0.01**	<0.01**	>0.05 N.S.

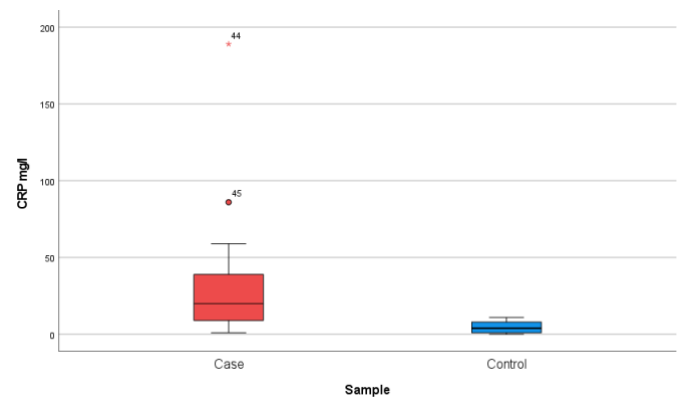


Figure 1. CRP levels between case and control groups

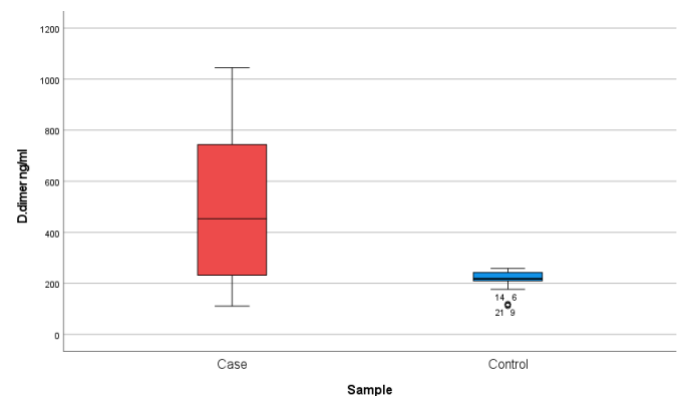


Figure 2. D-Dimer levels between case and control groups

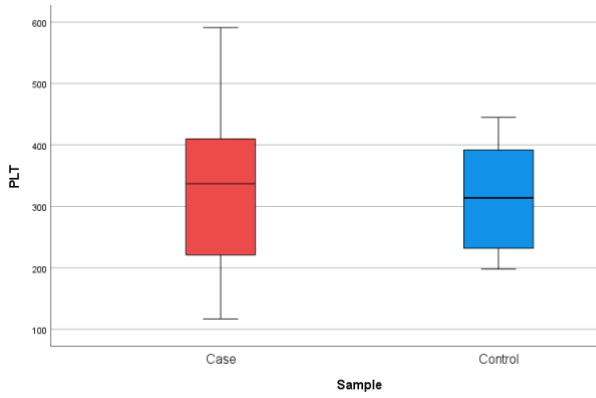


Figure 3. Platelet levels between case and control groups

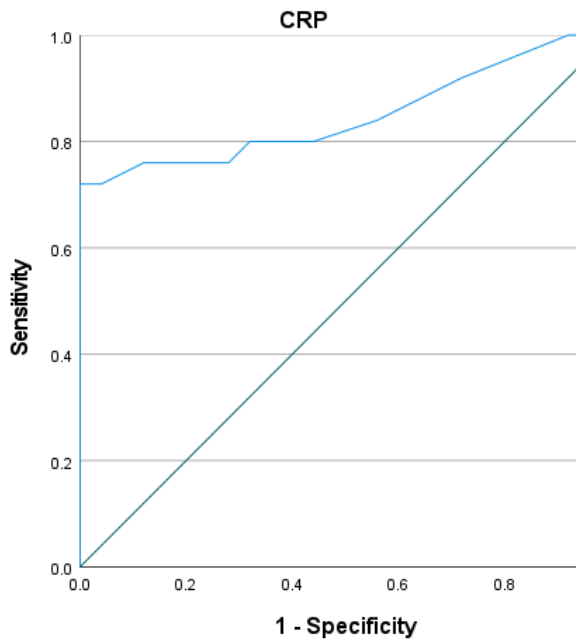


Figure 4. ROC curve of CRP levels sensitivity and specificity to CMV infection

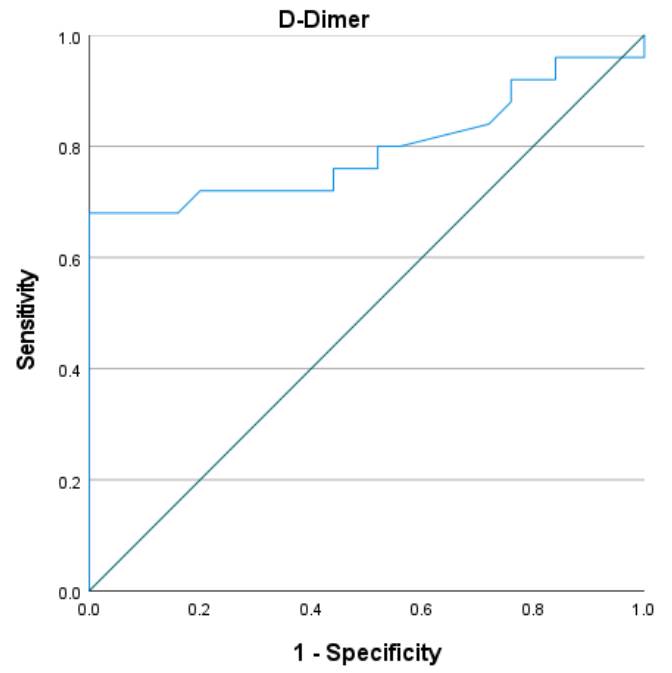


Figure 5. ROC curve of D-Dimer levels sensitivity and specificity to CMV infection

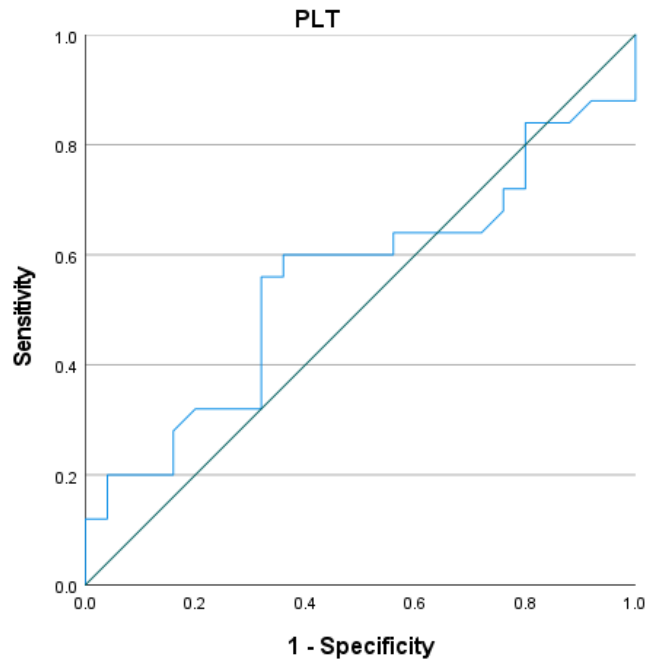


Figure 6. ROC curve of platelet count sensitivity and specificity to CMV infection