Hepatogastroentrology

Concordance between Anti-CMV IgM ELISA and IgG Avidity Testing of Serum Immunoglobulin in FUO Patients

Abdelrahman Mohamed Nagy[1,*] M.B.B.CH., El Sayed Mohamed Mohy El deen[1] PhD., Mostafa Mahmoud El nakib[2] PhD., and Mohamed Gharib Mohamed[1] MD.

** Corresponding Author:*
Abdelrahman Mohamed Nagy
abdalrahmanalnagy@gmail.com

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[1]*Department of Hepatogastroentrology and infectious diseases Medicine, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.*

[2]*Department of Microbiology and Immunology, Military Medical Academy, Cairo, Egypt.*

ABSTRACT

Background: Primary cytomegalovirus (CMV) illness is underdiagnosed in clinical practice, particularly in the setting of fever of unexplained origin (FUO). CMV IgM can last for months after original infection, and can be positive in reactivated CMV infections.The IgG avidity of (CMV) is a useful tool for identifying people who have recently been infected. IgG avidity matures over six months after primary infection .Low CMV IgG avidity predicts recent infection within the previous 3 to 4 months, but high avidity rules out the main infection within the last 3 months.

Aim of the work: To determine concordance between Anti-CMV IgM ELISA and IgG Avidity Testing of Serum Immunoglobulin in FUO Patients.

Patients and Methods: This was a retrospective cross-sectional study of 170 patients who presented with FUO and were admitted to the inpatient section of a military fever hospital; all included patients underwent routine laboratory and imaging examinations.56 patients were tested to evaluate the CMV IgM and IgG avidity tests.

Result: Out of 56 patients tested, 10 patients (17.9%) were CMV-IgM positive, 6 patients (60%) of them were CMV low avidity, and 4 patients (40%) were CMV high avidity, which indicates that most patients with CMV IgM positive tests had significant low avidity tests. 21.4% of patients were positive as regards the CMV avidity test. The degree of concordance between CMV IgM positive patients and CMV avidity according to kappa agreement was 44%.

Conclusion: Our findings show the critical relevance of incorporating CMV-IgG avidity into the CMV primary infection screening criterion.

Keywords: *CMV (cytomegalovirus); FUO (fever of unknown origin); IgG-avidity.*

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INTRODUCTION

Fever is a main ailment for which people need medical attention. Fortunately, fever is usually self-limiting, or the cause of fever can be determined. Fever of unknown origin (FUO) is described as a chronic, unexplained fever that persists despite a thorough examination. It is a common clinical problem. [1]

Fever of unknown origin (FUO) is considered as a fever of 101°F (38.3°C) or higher that remains unidentified after 3 days and for at least 2 days after microbiological culture incubation [2].

Infectious, malignant/neoplastic,autioimmune and miscellaneous other illnesses are the four etiologic categories of FUOs. About a third of FUO cases are caused by infection. Infection with the human herpesvirus cytomegalovirus (CMV) is a frequent in healthy adults and children. Seroprevalence studies have found that in healthy people, the prevalence ranges from 50% to 90%, with the prevalence increasing with age.[3]

CMV infection is frequently asymptomatic or causes mild flu-like symptoms. A primary CMV infection could be the source of an unexplained fever.[4]

CMV is a common causative pathogen that causes serious morbidity and mortality in immunosuppressed individuals. Infection can cause a feotus to develop a symptomatic congenital illness if it occurs during pregnancy.

In immunocompetent people, however, initial CMV infection is generally asymptomatic or causes just a moderate illness. Recent illnesses in adults are frequently reported as mononucleosis.[5]

Anti-CMV IgM antibodies can last for months or even years following the original illness. In addition, they can also be detected after reactivation or reinfection with a new strain of CMV, making anti-CMV IgM's accuracy in predicting primary infection more difficult. [6]

Furthermore, false-positive CMV IgM findings can arise during non-CMV diseases like primary Epstein-

Barr virus infection. Furthermore, investigations have revealed that CMV IgM tests have a wide range of specificity and sensitivity, as well as a significant percentage of discordance.[7]

Antibody avidity, an effective indicator of how strongly an antibody attaches to its antigen of interest, has recently been discovered to rise in the weeks following initial infection. Low-avidity IgG antibodies to CMV last for up to 20 weeks following a primary CMV infection. After that, high-avidity antibodies replace the low-avidity ones. [8 - 9]

The anti-CMV IgG avidity test is currently an important and robust test to determine primary CMV illness. During an acute or recent primary CMV infection, a low avidity index demonstrates the existence of low-avidity IgG antibodies in the serum. A high avidity index could be regarded as a reliable sign of previous infection. [2]

In this study, we sought to evaluate the compatibility between the detection of serum anti-CMV IgM antibodies by ELISA and the results of the IgG avidity testing in patients with FUO, as well as to identify the possible frequency of CMV illness in FUO patients.

PATIENTS AND METHODS

This is a retrospective cross-sectional study that was performed on 170 cases that were presented with FUO and admitted to the inpatient section of a military fever hospital from May to November 2018. A detailed history has been taken, including personal data, the presenting complaint, and general and neurological symptoms. A thorough clinical examination, including general and neurological examination, was performed. Routine laboratory investigations include complete blood counts, liver function tests, renal function tests, and urine analysis. Routine imaging includes chest X-rays and abdominal ultrasonography. 56 patients were subjected to CMV IgG, IgM, and IgG avidity, and their age ranged between 16 and 46 years, with a mean age of 29.21 ± 7.55 years. 52 cases were males (92.9%) and 4 were females (7.1%)

The patients were enrolled according to the following criteria:

Inclusion criteria: Immune-competent Patients who have been hospitalised because they have fever > 38.3 for more than 7 days of unknown origin after performing routine investigations after hospitalization

Exclusion criteria: Patients aged ≤ 18 and ≥60, Patients with end-organ failure, Patients with absolute neutropenia and/or absolute leucopenia or any evidence of immune suppression, Patients with a history of immunosuppressive drugs, Patients with autoimmune disorders, Patients with malignancy, even during remission

Study design: Immune-competent Patients who were hospitalized with a fever greater than 38.3 for more than 7 days of unknown origin were identified after routine investigations.

Patients who were to be accepted in this study were selected after obtaining the ethical approval of the military fever hospital and El-Hussein University hospital. The effectiveness of the CMV avidity test using the fully automated Vidas analyzer (bioMérieux) as a function of the concentration of CMV-specific IgG in the patient's blood was investigated. [10] 56 patients were studied employing a modified ELISA test using an ELFA (enzyme-linked fluorescent assay) phase and immune concentration technology for the detection of CMV antibodies IgG, IgM, and IgG avidity.

The VIDAS is an automated instrument that performs an enzyme-linked fluorescent immunoassay (ELFA). The instrument controls all assay steps and assay temperature. Each CMV assay kit contains controls, standards, reagent strips, and SPR (Solid Phase Receptacle). After a sample dilution step, the sample is cycled in and out of the SPR for a specified length of time. Anti-CMV antibodies present in the specimen will bind to the purified CMV antigen coating the interior of the SPR. Unbound sample components are washed away. A monoclonal anti-human IgG conjugated with alkaline phosphatase is cycled in and out of the SPR and will attach to any human IgG bound to the SPR wall. A final wash step removes unbound conjugate. A fluorescent substrate, 4-methylumbelliferyl phosphate, is introduced into the SPR. Enzyme remaining on the wall of the SPR will catalyze the conversion of the substrate to the fluorescent product, 4-methylumbelliferone (450 nm). The intensity of the fluorescence is measured by the optical scanner in the instrument; it is proportional to the quantity of CMV IgG found in the sample. When the VIDAS CMV Assay is completed, the results are analyzed automatically by the computer. The quantity of anti-CMV IgG present in the sample is calculated in reference to a calibration curve stored in the instrument. A report is printed for each sample.

IgG antibodies with avidities of 50% were termed low avidity antibodies, those with avidities of 50.0 and 59.9% were regarded grey as zone antibodies, and those with avidities of 60% or more were termed high avidity antibodies [(2)].

Statistical analysis: The data were revised and analyzed using version 24 of the SPSS software package. The mean and standard deviation (SD) were used to express quantitative data. The frequency and proportion of qualitative data were calculated. Statistical analysis was done using the Mann–Whitney U test. The Fisher's extract was used to compare qualitative data and the Chi-square test to compare quantitative data.

A P-value of less than 0.05 was considered statistically significant.

Cohen's kappa is a measure of the agreement between two ratters who have recorded a categorical outcome for a number of individuals. Cohen's kappa factors out agreement due to chance and the two ratters either agree or disagree on the category that each subject is assigned to (the level of agreement is not weighted). Cohen's kappa is a metric often used to assess the agreement between two raters. It can also be used to assess the performance of a classification model .The Kappa statistic is calculated using the following formula:

$$\frac{\text{Observed agreement - chance agreement}}{}$$

1-chance agreement

A kappa value of 0.57 indicates moderate to good agreement between observers

RESULTS

This is a retrospective cross-sectional study that was performed on 170 cases that were presented with FUO and admitted to the inpatient section of a military fever hospital. 56 patients were tested to evaluate the IgG, IgM, and IgG avidity tests, and their age ranged between 16 and 46 years, with a mean age of 29.21 ± 7.55 years. 52 cases were males (92.9%) and 4 were females (7.1%). There is no important data in this table in terms of demographic outcomes (Table 1).

		Total no. = 56
Age (years)	Mean ± SD	29.21 ± 7.55
	Range	19 – 46
Gender	Females	4 (7.1%)
	Males	52 (92.9%)
Duration of admission (days)	Mean ± SD	7.29 ± 1.57
	Range	5 – 12
Residence	Rural	28 (50.0%)
	Urban	28 (50.0%)

Table 1: Demographic analysis of results.

The results of complete blood counts, liver function tests, renal function tests, and urine analysis in relation to the CMV IgM positive and negative patients are presented in (Table 2). The blood transaminases and bilirubin levels of CMV IgM-positive patients are significantly elevated (Table 2).

		CMV-IgM		Test value	P-value	Sig.
		Negative	Positive			
		No. = 46	No. = 10			
HB	Mean ± SD	13.00 ± 2.98	11.66 ± 1.21	1.388•	0.171	NS
	Range	4.3 – 15.7	10.5 – 13.6			
RBCs	Mean ± SD	5.68 ± 2.32	4.42 ± 0.44	1.698•	0.095	NS
	Range	4.3 – 14.9	3.7 – 4.8			
WBCs	Mean ± SD	6.51 ± 1.87	6.18 ± 2.58	0.470•	0.640	NS
	Range	2.6 – 9.8	2.6 – 9.8			
Neutrophils	Mean ± SD	60.55 ± 11.64	53.02 ± 15.61	1.742•	0.087	NS
	Range	38.7 – 88.5	26.5 – 67.8			
Lymphocytes	Mean ± SD	32.96 ± 10.72	36.92 ± 16.23	-0.962•	0.341	NS
	Range	10.1 – 57.4	24.2 – 65.8			
Platlet count	Mean ± SD	199.04 ± 58.93	204.20 ± 48.36	-0.258•	0.797	NS
	Range	72 – 314	154 – 291			
Erethrocyte sedimentation rate (ESR)	Median (IQR)	15 (11 – 21)	20 (11 – 22)	-0.736≠	0.462	NS
	Range	8 – 22	5 – 48			
Alanine Aminotransferase (AST)	Median (IQR)	20 (12 – 28)	68 (11 – 97)	-1.416≠	0.157	NS
	Range	8 – 172	11 – 155			
Aspartate Aminotransferase (AST)	Median (IQR)	20 (18 – 32)	52 (26 – 67)	-2.275≠	0.023	S
	Range	11 – 78	17 – 92			
Total Bilirubin	Mean ± SD	0.65 ± 0.29	0.94 ± 0.46	-2.589•	0.012	S
	Range	0.25 – 1.5	0.5 – 1.7			
Direct Bilirubin	Mean ± SD	0.25 ± 0.14	0.47 ± 0.34	-3.274•	0.002	HS
	Range	0.08 – 0.7	0.1 – 0.9			
Urea	Median (IQR)	28 (24 – 33)	28 (23 – 30)	-0.086≠	0.932	NS
	Range	13 – 44	22 – 122			
Creatinine	Median (IQR)	1 (0.7 – 1.1)	1 (1 – 1.1)	-1.137≠	0.255	NS
	Range	0.5 – 1.3	0.8 – 5			

Table 2: Comparison between CMV IgM positive and negative patients regarding laboratory investigations.

Out of 56 patients tested to evaluate the CMV IgM and IgG avidity tests, 10 patients (17.9%) were CMV-IgM positive, 6 patients (60%) were CMV low avidity, and the remaining 4 patients (40%) were CMV high avidity, which indicates that most patients with CMV IgM positive tests were significant low avidity tests (Table 3**)**. Hepatomegaly (20%) and hepatospienomegaly (20%) were highly significant imaging findings in patients with CMV low avidity (Table 3).

		CMV-IgM		Test value	P-value	Sig.
		Negative	Positive			
		No. = 46	No. = 10			
CMV-IgG	Negative	2 (4.3%)	0 (0.0%)	0.451*	0.502	NS
	Positive	44 (95.7%)	10 (100.0%)			
CMV-IgG Av	High	40 (87.0%)	4 (40.0%)	10.757 *	0.001	HS
	Low	6 (13.0%)	6 (60.0%)			
Imaging (U/S)	Normal	44 (95.7%)	6 (60.0%)	10.914 *	0.001	HS
	Hepatosplenomegaly	0 (0.0%)	2 (20.0%)	9.541*	0.002	HS
	Hepatomegaly	0 (0.0%)	2 (20.0%)	9.541*	0.002	HS
	Cervical Lymph Node enlargement	2 (4.3%)	0 (0.0%)	0.451*	0.502	NS

Table 3: Evaluation of CMV IgM positive patient according to specific laboratory investigation and imaging.

17.9% of patients were positive as regards the CMV IgM test, and 21.4% of patients were positive as regards the CMV avidity test. The degree of concordance between CMV IgM positive patients and CMV avidity according to kappa agreement was 44% (Table 4).

	CMV-IgM No. (%)	CMV-Av No. (%)	Test value	P-value	Sig.	Kappa agreement (95% CI)
Negative	46 (82.1%)	44 (78.6%)	0.226	0.635	NS	0.435
Positive	10 (17.9%)	12 (21.4%)				(0.143 to 0.728)

Table 4: Concordance of CMV IgM positive patient and CMV avidity according to kappa agreement.

DISCUSSION

Out of 56 patients, 10 patients (17.9%) were CMV-IgM positive, while 6 patients (60%) of them were CMV low avidity, and the remaining 4 patients (40%) were CMV high avidity (P-value = 0.001), which indicates that most patients with CMV IgM positive test had significant low avidity test.

Our findings matched those of Dollard, who were the first to determine the prevalence of CMV IgM and low IgG avidity in a random sample of the U.S. population. High IgM antibody levels were closely related to low avidity and likely early CMV infection, according to IgG avidity testing of 170 IgM-positive sera .[3]

Our results were also compatible with the work of Prince and Leber who evaluated IgG avidity in 64 CMV IgM-positive sera, most of which were low or high titer, and found that the high IgM-titer samples had a 93 percent lower IgG avidity.[11]

Our findings matched those of Prince *et al.*, who tested CMV IgG avidity in 369 CMV IgG-positive serum samples sent to the Focus Diagnostics Reference Laboratory. All of the CMV IgM testing methods studied accurately determined >80% of low-avidity serum samples as IgM positive, indicating the significant relationship between low IgG avidity and IgM reactivity. [2]

The findings of Chakravarti *et al.*, were consistent with our findings, as they studied sera from 50 women, 26 of whom had Ig M antibodies, out of which 15 had low avidity antibodies, implying that a 40 percent avidity index as well as the existence of IgM antibodies strongly indicate an initial primary infection. [12]

Our results differ from the findings of Seo *et al.*, in which 744 pregnant women were consecutively enrolled over 2 months and serum samples from the women who were positive for CMV IgG and IgM were assigned for CMV IgG avidity testing to distinguish primary from non-primary CMV infection. CMV IgG and IgM seroprevalence were calculated to be 98.1 percent and 1.7 percent, respectively. All of the women who tested positive for CMV IgM or had results in the grey zone exhibited high avidity for CMV IgG in their samples. This discrepancy may be because Seo's study involved a large number of pregnant women, whereas our study involved a diverse sample of mostly males. [13]

In our study, 10 patients (17.9%) were CMV-IgM positive, with 6 patients (60%) having CMV IgG low avidity and the remaining 4 patients (40%) having CMV high avidity (P-value = 0.001). Thus, the degree of concordance between CMV IgM positive patients and CMV avidity according to kappa agreement was 44 percent.

Revello *et al.*, investigated diagnostic findings, alternatives, and pregnancy outcomes in 735 women with primary CMV infection over a 20-year period (1990–2009). It was discovered that seroconversion was used to diagnose primary CMV infection in 44.4 percent of cases, as well as various combinations of virus-specific IgM and low IgG avidity, which validates our findings.[14]

Our findings are also consistent with those of Prince and Leber who examined a group of 64 CMV IgG- and IgM-positive samples with no indication on the time since seroconversion. All values were highly distributed; 41 of 64 sera (64 percent) showed low avidity, 8 of 64 sera (13 percent) showed intermediate avidity, and 15 of 64 sera (23 percent) showed high avidity. This serum panel was then utilized to study the association between CMV IgG avidity and CMV IgM levels. Nearly all sera with IgM index values of 3.0 had low IgG avidity. [11]

Our findings matched those of Guisasola *et al.,* who evaluated a panel of 85 blood samples from 85 patients submitted for CMV infection diagnosis. All 85 samples were analyzed to determine CMV IgG avidity. Following a specified consensus criterion, samples were categorized as having high, low, or indeterminate avidity. According to this criterion, primary infections were defined as those with high IgM avidity but low IgG avidity. Non-primary infections were classified as those with positive IgM samples but low IgG avidity, whereas secondary infections were characterised as those with positive IgM but uncertain avidity. [15]

In our study, patients with CMV low IgG avidity were admitted for fewer days (5-7 days) than patients with CMV high IgG avidity (5-12 days) because they were treated earlier and avoided unnecessary medications and labs. This finding corroborated that of Persson *et al.,* who found that rapid recognition of a primary CMV infection helps exclude alternative diagnoses, avoid unnecessary antibiotic exposure, and assure patients that their disease is self-limiting and benign. [16]

In terms of demographic data, our results identified no differences among CMV low avidity patients, which are corroborated by Dollard, who found that CMV recent infection among patients varied from 2.3 to 4.5 percent across age groups and did not demonstrate obvious age-related results. There was no link found between CMV primary infection and the study participants' age, race, or household income. [2]

These findings support the notion that CMV IgM can be generated throughout life after primary CMV infection or as a consequence of a reactivation or reinfection, and they show that elderly people are just as likely as younger people to experience a recurring infection.

Our findings contradict the findings of Schimanski *et al.,* who found that the rate of seropositive people rises with age, where adult prevalence varies from 30% to more than 90%, relying upon a variety of characteristics, such as socioeconomic level. This could be because our study only included individuals in their adult years and did not include a wide range of ages or socioeconomic status. [17]

In our results, two patients (20%) presented with hepatosplenomegaly and two patients (20%) presented with hepatomegaly, indicating that hepatosplenomegaly and hepatomegaly are related to CMV infection. The main symptom of primary CMV infection is "CMV syndrome," which includes fever, leucopenia, atypical lymphocytes, hepatomegaly, myalgia, and arthralgia according to Dolin *et al.,* which was consistent with our findings. [18]

LIMITATION

Our study depend on diagnosis of CMV only by detection of CMV antibodies IgG, IgM, and IgG avidity by ELISA test using an ELFA (enzyme-linked fluorescent assay) in spite of the gold standard of diagnosis is the quantitative CMV-PCR assay.

CONCLUSION

Our findings show the critical relevance of incorporating CMV-IgG avidity into the CMV primary infection screening criterion.

Conflict of interest : none

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