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ORIGINAL ARTICLE

Seromolecular Detection of Cytomegalovirus Infection in Immunocompromised Versus Immunocompetent Pediatric Patients

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ABSTRACT

Objective: Our study aimed to assess the effectiveness of combining serological markers and PCR assays for diagnosing CMV in children, particularly in immunocompromised patients.

Study Design: Observational cross-sectional study.

Place and Duration of Study: Tertiary University Hospital from 2019-2023.

Material and Methods: 203 pediatric patients' records were collected and divided into Group 1, Immunocompromised, and Group 2, Immunocompetent, who underwent PCR and Electrochemiluminescence Immunoassay simultaneously.

Results: Positive CMV IgG patients were 82.3% and 81.3% in Group 1 and Group 2, respectively. Patients with positive CMV IgM and negative PCR were recorded in only (6.3%) of children from Group 1 and (3.7%) of Group 2. Patients with positive CMV PCR were divided into patients with positive CMV IgM representing 4/96 (4.2%) in Group 1, 4/107 (3.7%) in Group 2 and others with negative CMV IgM 6/96 (6.3%) in Group 1, 5/107 (4.7%) in Group 2 respectively. Regarding median viral load it was greater in Group 1 (1189.00 copies/ml) than in Group 2 (492 copies/ml). Total positive CMV by both PCR and IgM (ECLIA) were 11/96 (11.4%) patients in Group 1 giving a sensitivity of 27.2% and a specificity of 90.59% for (ECLIA) versus PCR in this group. Total positive CMV by both PCR and IgM was 9.3% in Group 2 giving a higher sensitivity of 40% and a specificity of 96.91% for (ECLIA) versus PCR in this group.

Conclusion: Our study points out the limitations of relying solely on serology without PCR or vice versa, especially in immunocompromised cases, to avoid missing positive cases.

Key Words: *CMV, immunocompetent, immunocompromised, PCR, Serology*

Abbreviations: AU/ml: arbitrary units/mL CMV: Cytomegalovirus DNA: Deoxyribonucleic acid ECLIA: Electrochemiluminescence Immunoassay EDTA: Ethylenediaminetetraacetic acid FE: Fisher Exact HCMV: Human cytomegalovirus IQR: Interquartile range NPV: negative predictive value PCR: polymerase chain reaction PPV: positive predictive value SPSS: Statistical Package for the Social Sciences

INTRODUCTION

Cytomegalovirus (CMV) is a double-stranded DNA virus belonging to the Herpesviridae family.[1] About 59% of individuals >6 years old have encountered

CMV.[2] In immunocompetent children, CMV can lead to asymptomatic or mild disease, while immunocompromised ones may face severe, potentially life-threatening issues.[2] CMV spreads through blood products during transfusions, organ transplants, and body fluids like urine or breast milk during breastfeeding.[3]

CMV complications usually occur in immunocompromised patients, leading to CMV retinitis, hepatitis, esophagitis, colitis and fulminant liver failure or subacute encephalitis. Detecting CMV early through laboratory diagnosis is crucial for prompt and accurate treatment, especially in immunocompromised individuals, as it significantly reduces morbidity and mortality rates.[4]

Diagnosing CMV infection involves direct methods using quantitative PCR or indirect methods using serological assays. A positive IgM suggests a recent or acute infection, while a positive IgG indicates a past infection. Serological testing may not provide an accurate picture in at-risk patients due to immunodeficiency. Furthermore, IgM antibody tests might not reliably indicate a primary infection, as IgM can remain detectable for months after the initial infection, potentially causing false-positive results.[5]

PCR is widely used to detect CMV DNA in different samples due to their high sensitivity in identifying even small amounts of nucleic acid. Quantitative PCR enables continuous monitoring in immunocompromised individuals, helping identify those at risk for CMV disease and assess treatment response.[6] It also distinguishes between low and high viral loads, supporting in predicting disease severity and observing treatment efficacy. Despite its effectiveness, PCR can be expensive and may not be readily accessible in all healthcare units.[7]

In immunocompromised patients, the antibody response, particularly IgM production, may be reduced or delayed, making it challenging to detect an active infection through IgM tests alone. Accordingly, combining serological and molecular assays can enhance accurate diagnosis.[8] This reduced or delayed antibody production often renders serological testing unreliable. PCR addresses this limitation by directly detecting viral DNA, ensuring no infections are overlooked. Meanwhile, serology complements PCR by providing critical insights into the timing and type of infection—information that PCR alone cannot offer. Despite its high sensitivity, qualitative PCR detects CMV DNA in both active infections and latent viral states, making it less useful as a standalone diagnostic tool. By integrating both methods, we hypothesize that this combined approach could significantly improve diagnostic accuracy, particularly in cases where serological responses are weakened or when PCR results lack sufficient clinical context. By applying both methods simultaneously to the same patient, our approach seeks to bridge these gaps, offering a more comprehensive and accurate diagnostic framework.

Our study aimed to assess the effectiveness of combining serological markers and PCR assays for diagnosing CMV in children, particularly in immunocompromised patients.

MATERIALS AND METHODS

This study was conducted at the Molecular and Serology labs at our Tertiary University Hospital. Ethical Committee Approval (with Federal Wide Assurance number: 000017585, R15/2024) was obtained from the Ethics Committee Center of the University. Measures were taken to ensure data confidentiality and privacy. In the four-year cross-sectional observational study from December 2019 to December 2023, electro-chemiluminescence immunoassay (ECLIA) performance was evaluated in conjunction with PCR for CMV detection in both immunocompromised and immunocompetent pediatric populations. The study included records of 203 patients across various age groups. Only those patients with serological and PCR testing were conducted simultaneously, as determined by the laboratory record, were included in the study.

Patients were divided into immunocompromised: Group 1 and immunocompetent: Group 2. Patients with organ transplantation and/or receiving chemotherapy, systemic corticosteroids, or immunosuppressive agents were defined as immunocompromised (according to the WHO ICD 10 classification) coupled with the patient's lab having lymphopenia. All the other patients were grouped as immunocompetent. The study excluded adults aged ≥18 years, duplicate

samples, and patients lacking either PCR or ECLIA results.

CMV Detection Assays: The Roche Elecsys CMV IgG and IgM assays (Roche Diagnostics AG, Rotkreuz, Switzerland) were assessed in comparison with QIAGEN Artus CMV RG PCR kit (QIAGEN GmbH, GERMANY).

A) Elecsys CMV IgG and IgM ECLIA using Cobas e411 autoanalyser: ECLIA used for in-vitro qualitative determination of IgM and IgG CMV antibodies in human serum and plasma. Results obtained assist in the diagnosis of recent/active or past CMV infections.

A total of 203 serum samples were collected and processed immediately or stored at 2-8°C until next day. Samples were centrifuged then processed at 20-25°C using the Cobas e411 autoanalyzer following the manufacturer's protocol. Elecsys CMV IgM results were determined using instrument-specific cut-off values from positive and negative calibrators, with a cut-off index (COI) <0.7 considered negative, >1.0 positive, and 0.7 to 1.0 indeterminate. For Elecsys CMV IgG, results were normalized against an internal Roche standard, with <0.5 AU/mL considered negative, >1.0 AU/mL positive, and 0.5 to 1.0 AU/mL indeterminate.

B) Qiagen Artus CMV RG PCR: The Artus CMV RG PCR Kit is designed for detecting CMV DNA through polymerase chain reaction (PCR) on Rotor-Gene Q Instruments from Corbett Research Pty Limited, Sydney, Australia. Results from this assay aid in diagnosing recent or active CMV infections.

A total of 203 EDTA plasma samples were collected simultaneously with serological samples. Within 6 hours of collection, whole blood underwent centrifugation for 20 minutes at 800–1600 x g to separate plasma that was processed immediately or stored at 2-8°C until the next day. DNA extraction utilized the QIAamp DSP Virus Spin Kit from Qiagen, Hilden, Germany, following the manufacturer's instructions. Subsequent DNA amplification and human cytomegalovirus detection were performed using real-time PCR with the Artus CMV RG PCR kit, adhering to the manufacturer's protocol. An internal control was included in all samples. The analytical detection limit, considering the purification of the Kit with the Rotor-Gene 3000, is 57.1 copies/ml (p = 0.05). This indicates a 95% probability of detecting 57.1 copies/ml.

A high viral load was determined to be more than or equal to 500 copies as this is viral threshold for starting treatment in CMV infection in our tertiary university hospital.

There were no specific funding sources for the simultaneous serological and PCR testing; the testing was performed solely based on the ordering clinician's discretion.

Statistical Analysis: Data analysis was done using the SPSS program version 24. Quantitative data were presented using the mean and standard deviation or median and interquartile range for non-parametric data. Qualitative data were presented using count and percentage. Student t-test and Mann-Whitney U tests were used to compare quantitative data between two independent groups. Chi-square and Fisher's exact tests were used to compare the qualitative data. Sensitivity, specificity, positive and negative predictive values were used to measure the validity of different qualitative data. These values were calculated for IgM detection using ELISA, with PCR as the gold standard for CMV diagnosis. A p-value less than or equal to 0.05 was considered statistically significant

RESULTS

This study was conducted on 203 pediatric patients. Divided into two groups; Group 1: Immunocompromised patients and Group 2: Immunocompetent patients. Group 1 represented 96/203 (47.3%) and 107/203 (52.7%) were from Group 2. Males were a total of 54 patients (56.3%) and there were 42 (43.8%) females in Group 1, whereas in Group 2, males were 66 (61.7%) and females were (38.3%). Being categorized into 6 age groups, the lowest percentage was detected in neonates, where only one was immunocompromised (neonatal leukemic patient), representing (1%) in Group 1, and 6 immunocompetent, representing (5.6%) in Group 2. The most prevalent of all patients were children 6-11 years old, representing 31/96 (32.3%) from Group 1 and 33 (30.8%) in Group 2, as shown in table 1.

TABLE 1: Demographic data of immunocompromised compared to immunocompetent pediatrics

		Group1		Group 2		p value
		N=96	Percentage	N=107	Percentage	
Age	<28 days	01	1.0	6	5.6	0.44
	28 days-12 months	15	15.6	16	15.0	
	13 months-2 years	06	6.3	8	7.5	
	2-5 years	24	25.0	30	28.0	
	6-11 years	31	32.3	33	30.8	
	12-18 years	19	19.8	14	13.1	
Sex	Male	54	56.3	66	61.7	0.46
	Female	42	43.8	41	38.3	

*Chi-square test

Patients with only positive CMV IgG represented 79/96 (82.3%) in Group 1 and 87/107 (81.3%) in Group 2. Patients with positive CMV IgM and negative PCR were recorded in only six immunocompromised children (6.3%) and 4 (3.7%) immunocompetent ones. On the other hand, patients who had positive PCR coupled with negative CMV IgM 6/96 (6.3%) in Group 1 were higher than in Group 2, 5/107 (4.7%), with a p-value of 0.42. Indeterminate IgM was detected only in Group 2 (1.9%) but was undetected in Group 1. Moreover, patients with negative CMV PCR and serology results for IgG and IgM were 5/107 (4.7%) in Group 2 and only one (1%) in Group 1. The sero-molecular assay was statistically non-significant between the two groups, as shown in table 2.

TABLE 2: Sero-molecular assay of Group 1 compared to Group 2

		Group 1		**Group 2**		**p value**
		N=96	**Percen-tage**	**N=107**	**Percen-tage**	
Sero-molecular assay	Positive CMV IgG	79	82.3	87	81.3	0.42
	Positive CMV IgM / Negative CMV PCR	6	6.3	4	3.7	
	Positive CMV IgM / Positive CMV PCR	4	4.2	4	3.7	
	Negative CMV IgM /Positive CMV PCR	6	6.3	5	4.7	
	Positive CMV IgG /Indeterminate CMV IgM	0	0.0	2	1.9	
	Negative CMV IgG/IgM/PCR	1	1.0	5	4.7	

*Chi-square test (FE: Fisher Exact)

Patients with high viral load and negative CMV IgM included 5 out of 96 (5.2%) in Group 1 and 1 out of 107 (0.9%) in Group 2. Although of non-statistical significance, it's important to note that the number of immunocompromised patients having high viral load with negative IgM was much higher than in the immunocompetent group, as shown in table 3.

TABLE 3: Frequency of patients with high viral load and negative IgM in both groups

		Group 1		**Group 2**		**p value**
		N	**Percentage**	**N**	**Percentage**	
High viral load and negative CMV IgM	Yes	05	5.2	1	0.9	0.10
	No	91	94.8	106	99.1	

Table 4 shows that regarding PCR and (ECLIA) results, non-statistically significant difference was found between the two groups regarding both CMV IgG, IgM and CMV PCR viral loads, although notably median high viral load was greater in Group 1 (1189.00 copies/ml) which is considered higher viral load than Group 2 (492 copies/ml) which is considered low viral load.

TABLE 4: Comparing PCR and (ECLIA) results in both groups

	Group 1		Group 2		p value
	Median	IQR	Median	IQR	
CMV PCR Viral Load (copies/ml)	1189.00	135-6108	492.00	168-2003	0.73
CMV IgG Value (AU/ml)	109.20	31.60-258.95	147.60	35-324	0.48
CMV IgM Value (COI)	0.19	0.16-0.28	0.19	0.16-0.25	0.97

*Mann Whitney U test **Chi-square test (FE: Fisher Exact)

Regarding evaluation of ECLIA performance versus PCR in group 1, total positive CMV by both PCR and IgM (ECLIA) were 11/96 (11.4%) patients. Showing sensitivity 27.2%, specificity 90.59%, positive predictive value (PPV): 27.27% and negative predictive value (NPV): 90.59% as shown in table 5.

TABLE 5: Evaluation of (ECLIA) compared to PCR in Group 1

		CMV PCR Result		
		Positive	Negative	Total
CMV IgM	Positive	3	8	11
	Negative	8	77	85
	Total	11	85	96

Sensitivity: 27.27%; Specificity: 90.59%; PPV: 27.27%; NPV:90.59%

Regarding the evaluation of ECLIA performance versus PCR in Group 2, total positive CMV by both PCR and IgM (ECLIA) were 10/107 (9.3%) in Group 2, giving a higher sensitivity (40%) than in Group 1, specificity of (96.91%), PPV: (57.14%) and NPV (94%) as shown in table 6.

TABLE 6: Evaluation of (ECLIA) compared to PCR in Group 2

		CMV PCR Result		
		Positive	Negative	Total
CMV IgM	Positive	4	3	7
	Negative	6	94	100
	Total	10	97	107

Sensitivity: 40%; Specificity: 96.91%;PPV: 57.14%; NPV: 94%

As shown in table 6: The performance of ECLIA was notably different between immunocompromised and immunocompetent groups. The lower sensitivity and PPV observed in immunocompromised patients highlight the limitations of relying solely on serological methods in this population. These findings underscore the necessity of incorporating PCR as a diagnostic tool for accurately detecting CMV in immunocompromised individuals. However, the higher sensitivity and PPV in immunocompetent patients suggest that ECLIA may be a reasonable alternative in settings where PCR is unavailable or resource-limited.

DISCUSSION

In children, the disease caused by CMV infection ranges from asymptomatic or mild disease in immunocompetent children to severe and potentially life-threatening disease in newborns and immunocompromised ones with significant elevation in morbidity and mortality rates. Therefore, reliable seromolecular CMV assays is essential for early and accurate detection of infection and treatment initiation. Thus, this four-year retrospective study was conducted with the primary objective of a comprehensive assessment of CMV detection methodologies across varying immunological states aiming to provide a clearer understanding of CMV infection dynamics.

Our study was conducted on 203 pediatric patients of various pediatric age groups. The most prevalent age group was children 6-11 years old, representing 31/96 (32.3%) from the immunocompromised group and 33 (30.8%) in the immunocompetent ones. This aligns with a study done by the National Center for Health Statistics of the CDC where CMV infection was 58.9% in individuals ⩾6 years old and 36.3% in 6–11 years old.[9] This may be due to high day care and school attendance which is a risk for CMV infection. In our study the least percentage of CMV patients was detected in neonates where only one was immunocompromised representing (1%) in Group 1 and 6 immunocompetent representing (5.6%) in Group 2. This aligns with a study done in Brazil where 87 /8047 live born infants had a confirmed diagnosis of congenital CMV infection, giving an overall prevalence of 1.08%.[10]

In our research, a substantial 82.3% of immunocompromised patients and 81.3% immunocompetent patients, demonstrated evidence of past CMV having positive CMV IgG. This indicates that there is a high prevalence of CMV infection. In African countries the prevalence of CMV infection in general population as well as in transplant recipients is notably high. Showing 90% prevalence in Eritrea, 77.6% in Ghana and 36.2% in Egypt. This may be attributed to the low socioeconomic status with poor living conditions and bad hygienic practices.[11] This high percentage highlights the vulnerability of these immunocompromised individuals to CMV reactivation or reinfection which can be life threatening. This is mainly due to the fact CMV is not cleared from the host after the initial infection establishing lifelong latent infection by evading the host innate immune response.[12] According to the CDC's guidelines, a positive CMV IgG test signifies a historical CMV infection, without specifying the time of initial infection, particularly applicable to individuals aged 12 months or older, where maternal antibodies are no longer present[13] It is important to note that immunocompromised patients besides having reactivation of latent CMV infection can also have either primary infection or re infection with a different CMV strain.[14]

Furthermore, our results revealed that among immunocompromised children, 6.3% showed positive IgM paired with negative PCR, while in the immunocompetent group, 3.7% displayed a similar pattern and they showed no statistically significant difference. IgM antibodies indicate the presence of acute or recent infection. Serological IgM assays a various with different principles.[15] However, studies have shown poor correlation of results obtained with different commercial kits for IgM testing.[16] In addition, assays for IgM antibody lack specificity for primary infection because of false-positive results, because IgM can persist for months after primary infection, and because IgM can be positive in reactivated CMV infections.[17] Therefore, even in the case of positive IgM this patient may not have active infection needing treatment.

On the other hand, we found patients having both positive PCR and IgM were only 4.2% in immunocompromised and 3.7% in immunocompetent patients whereas 6.3% of the studied immunocompromised patients were PCR positive & IgM negative in comparison to 4.7 % in immunocompetent patients. This aligns with a study done on 226 patients whom were kidney transplant patients where, IgM-anti-CMV was detected positive in only four out of sixteen (25%) having invasive symptomatic CMV infection, while the remaining 75% were found negative.[18] This might be explained in patients having serology testing done before the first 2 weeks of infection where IgM antibody synthesis takes place after this period.

It's important to note that immunocompromised patients were grouped depending on history of taking chemotherapy and/or immunosuppressive drugs and lymphopenia which might explain the weak immune response and inability to produce IgM anti CMV due to decrease in both T and B cell. Therefore, an antiviral prophylaxis is recommended for these patients to avoid increased mortality and morbidity of having high viral load while serology showing negative results. This aligns with a retrospective study done in France where they recommended that lymphocyte count less than < 1,000/µL for kidney transplant patient to take antiviral prophylaxis.[19]

Our findings revealed that 6.3% of immunocompromised and 3.7% of immunocompetent patients had positive CMV IgM results but negative PCR. This may reflect the persistence of IgM antibodies for weeks to months following an acute CMV infection. Additionally, low-level viral replication below the PCR detection threshold could explain negative PCR results, even in cases of recent or resolving infections. Similar findings are documented in the literature, where IgM antibodies are observed to persist during the convalescent phase or during reactivations without significant viremia.[20] The variability in IgM assay performance is also influenced by the testing principles employed by different commercial kits. Studies have shown discrepancies between IgM results obtained with different kits, attributed to differences in assay sensitivity and specificity.[16] These factors highlight the need to interpret IgM results in conjunction with PCR and clinical findings, particularly in immunocompromised patients where serological responses may be unreliable. Thus, integrating molecular and serological approaches remains

critical for accurate CMV diagnosis. Accurate diagnosis of CMV infection in immunocompromised patients is crucial for early treatment of those patients thus supporting sustainable developmental goal “3” by ensuring health and wellbeing for all.

In our current study we have 5.2% immunocompromised patients whom had positive PCR with high viral load and negative IgM whereas in immunocompetent patients it was only 0.9%. Although our results showed non statistical significance this may be due to our small sample size of positive CMV PCR thus, false negative IgM results is of a high clinical significance. A study done in Sudan showed that two out of the six patients displaying a high viral load (mean value 17532.83 copies/ml) having signs and symptoms of active infection have died.[11] Therefore, missing high viral load in immunocompromised patients by depending only on the false negative serology result may lead to serious morbidities even mortality with wrong clinical decisions. According to systematic review[21] antiviral pre-emptive therapy started at cytomegalovirus viral load thresholds between 2 and 3 log10 IU/ml and according to our tertiary university hospital policy it was 500Iu/units which is what we considered an appropriate cut off value for starting treatment.

Although there was no statistically significant difference, the median viral load in immunocompromised group was much higher (1189 viral copies) and is considered in the high viral load. Whereas, the median range in immunocompetent group was 492 viral copies which is considered low viral load. This is aligning with a retrospective study where the median viral load in immunocompetent patients and immunocompromised patients was 370 and 2736 copies/mL, respectively ($p = 0.01$).[22]

Our study assessed the validity of ECLIA compared to PCR in immunocompromised patients, it revealed a sensitivity of 27.2% and a specificity of 90.59%. The positive predictive value (PPV) and negative predictive value (NPV) were 27.27% and 90.59%, respectively. In contrast a previous cross-sectional study, reported a higher sensitivity of 84.4% and specificity of 99.3% for the detection of CMV-IgM in neonatal serum, in comparison to urine PCR particularly for the diagnosis of congenital CMV infection. The PPV in their study was notably high at 96.4%.[23] The discrepancy between our findings and previous results can be attributed to several factors. First, they focused exclusively on congenital CMV infection, whereas our study encompassed a diverse range of immunocompromised patients with varying ages and immunological statuses. Additionally, the difference in sample types may contribute to the variation in results; as they utilized urine samples, while our study used plasma samples for PCR.

Previous case study described symptomatic CMV disease in immunocompetent adults and reported that symptoms generally resolve with few sequelae. However, it can cause severe and relapsing symptoms that can last for several weeks.[24] This aligns with a systematic review and meta-analysis who highlighted that critically ill, non-immunosuppressed patients admitted to ICUs often exhibit a high incidence of CMV infection.[25] In this retrospective analysis, we incorporated an immunocompetent group to evaluate and validate (ECLIA) against PCR which showed 40% sensitivity, 96.91% specificity, a positive predictive value of 57.14%, and a negative predictive value of 94%. There was a noticeable enhancement in both sensitivity and specificity compared to the immunocompromised group. These results underscore the potential utility of IgM serological test as a diagnostic method in immunocompetent cases, especially when PCR is challenging to perform.

Our study's retrospective design limited us to analyzing available diagnostic data without access to comprehensive clinical information, such as disease severity or treatment outcomes. This lack of clinical data restricts the ability to correlate diagnostic findings with the clinical presentation of CMV in these patients. Future studies should include prospective data collection that integrates laboratory diagnostics with detailed clinical profiles to better understand the implications of diagnostic performance in different patient groups.

CONCLUSION

Our research emphasizes the value of a dual diagnostic strategy that combines PCR and IgM ECLIA, especially for patients with impaired immune systems. IgM testing can offer more information about the patient's immunological

history and assist in identifying recent or resolving infections, even if PCR is still the gold standard for detecting current viral replication and measuring viral load. This supplementary approach is essential to reduce missed cases and provide a thorough evaluation of CMV status, particularly in vulnerable populations. Future studies should use a prospective approach that combines laboratory and clinical data to build on these findings. This will help us better understand the clinical consequences of CMV diagnosis and guide the development of more efficient management and methods for diagnosis.

Our study's strengths include its large dataset and focus on comparing serological and molecular diagnostic methods for CMV in immunocompromised and immunocompetent pediatric patients, providing practical guidance for clinicians managing CMV in diverse patient populations. However, the retrospective design limited access to detailed clinical data and introduced variability due to clinician-driven testing requests. These limitations highlight the need for prospective studies to correlate diagnostic findings with clinical outcomes better. This limits our capacity to relate clinical results to test results. Finally, although the study concentrated on diagnosis accuracy, it neglected to assess these methods' accessibility or cost-effectiveness, two crucial factors for clinical practice.

Conflict of Interests: All authors declare that they have no conflict of interest

Ethical Approval: This is a retrospective study conducted at Microbiology Laboratory, Ain Shams University, Cairo, Egypt. Ethical committee Approval (with Federal Wide Assurance number: 000017585, R15/2024) were taken from Ethics Committee Center at Ain Shams University. Measures were taken to ensure confidentiality and privacy of data.

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