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Cytomegalovirus reactivation in seropositive critically ill patients with liver cirrhosis: A hospital-based longitudinal study

Dhara Shah[a], Ekta Gupta[a,*], Sukriti Baweja[b], Samba Siva Rao Pasupuleti[c,d], Rakhi Maiwall[e], Archana Ramalingam[f], Lalita Gouri Mitra[g], Shiv Kumar Sarin[e]

[a] Department of Virology, Institute of Liver and Biliary Sciences, New Delhi, India
[b] Department of Molecular and Cellular Medicine, Institute of Liver and Biliary Sciences, New Delhi, India
[c] Department of Bio-Statistics, Institute of Liver and Biliary Sciences, New Delhi, India
[d] Department of Statistics, Mizoram University, Pachhunga University College Campus, Aizawl, India.
[e] Department of Hepatology, Institute of Liver and Biliary Sciences, New Delhi, India
[f] Department of Epidemiology and Clinical Research, Institute of Liver and Biliary Sciences, New Delhi, India
[g] Department of Critical Care Medicine, Institute of Liver and Biliary Sciences, New Delhi, India

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ABSTRACT

Background: Cytomegalovirus (CMV) reactivation is known to occur among intensive care unit (ICU) patients. CMV-reactivation is not well-evaluated among critically ill cirrhotic adults who are not overtly immunocompromised.

Objectives: Primary objective was to estimate the CMV-reactivation incidence rate among seropositive/latently infected critically ill cirrhotic adults. The secondary objective was to study the risk factors, host-related cytokine responses, and ICU outcomes associated with CMV-reactivation.

Methods: In this longitudinal study conducted between November 2018 and June 2019, all consecutive anti-CMV-IgG-positive cirrhotic Liver-ICU patients were assessed at day 0/ICU-admission, day 7, 14, and 21 for CMV-reactivation/plasma-DNAemia (≥500 IU/ml), cytokines, clinical, laboratory and outcome parameters.

Results: Fifty-five (48 male) cirrhosis patients consecutively admitted to liver-ICU were prospectively studied. Twenty (36%) adults developed CMV-reactivation. Majority (n=17/55, 30.9%; 95% CI: 19.1 - 44.8) showed CMV-reactivation on ICU-day 7. CMV-reactivation incidence rate during 21-day follow-up was 2.75% per person-day (95% CI: 1.68-4.26% per person-day). None of the risk factors studied was independently associated with CMV-reactivation. Acute respiratory distress syndrome (p=0.04), systemic inflammatory response syndrome (p=0.01), secondary (bacterial and/or fungal) infections (p=0.009), and raised pro-inflammatory cytokines (IFN γ, p=0.012; TNFα, p=0.052) were observed concomitantly to CMV-reactivation on ICU-day 7. ICU-Mortality (n=34/55, 61.8%) did not vary with a presence or absence of CMV-reactivation (55% versus 65.7%; p=0.43). Length of stay (LOS) in liver-ICU did not differ concerning CMV-reactivation (5 days versus 4.5 days; p=0.17)

Conclusions: CMV-reactivation incidence rate was considerable among seropositive non-immunosuppressed critically ill cirrhotic adults. Mortality and LOS in Liver-ICU were not significantly influenced by CMV-reactivation.

1. Background

Human Cytomegalovirus (CMV), tends to maintain latency within monocytes/macrophages and dendritic cells following primary infection [1,2]. CMV-reactivation in immunosuppressed hosts could lead to CMV disease [3]. CMV-reactivation is demonstrated in non-immunosuppressed hosts under stressful conditions like acute lung injury, sepsis, major trauma, burns, and intensive care unit (ICU) stay/hospitalization [2,4,5]. A high CMV seroprevalence (>95%) [6] is observed in the Indian population. ICU-patients with chronic systemic diseases may transiently have immunocompromised states [7,8]. Patients with cirrhosis are often associated with immune dysfunction prior to immunosuppressive therapy [9].

A few authors [2,4,10] have evaluated CMV-reactivation and associated clinical outcomes among non-immunosuppressed ICU-patients. Although CMV-reactivation has been evaluated in cirrhosis patients by some researchers [11,12], it has not been entirely studied in latently infected/seropositive critically ill cirrhotic adults.

* Corresponding author at: Ekta Gupta, Department of Virology, Institute of Liver and Biliary Sciences, New Delhi, India.
E-mail address: ektagaurisha@gmail.com (E. Gupta).

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2. Objectives

Primary objective was to investigate the CMV-reactivation incidence rate by quantitative real-time polymerase chain reaction (qPCR) based CMV-plasma-DNAemia monitoring among critically ill seropositive cirrhotic adults. Secondary objectives were to study the risk factors, host-related cytokine responses, and ICU-outcomes associated with CMV-reactivation.

3. Study design

3.1. Patient selection

All consecutive cirrhotic adults were screened upon liver-ICU admission for enrolment in a longitudinal observational study conducted at the Institution of Liver and Biliary Sciences (ILBS), New Delhi between November 2018 and June 2019. Inclusion criteria were: (a) Adult (>18 years) with cirrhosis requiring critical care; (b) CMV-seropositivity/anti-CMV-IgG detection at baseline/ICU-admission. Exclusion criteria were: (a) Less than 72 hours of liver-ICU stay; (b) pregnant and/or breast-feeding woman; (c) Baseline/day 0 CMV-plasma-DNAemia/viral load of 500 (2.69 $\log_{10}$) International Units (IU) per millilitre (ml) or higher (d) Death or ICU-discharge/transfer before follow-up CMV-plasma-DNAemia by qPCR on ICU-day 7; (e) patients with ICU-readmissions; (f) Neutropenia (neutrophils<500/μl), immunodeficiency (congenital, primary or acquired), solid organ/bone marrow transplantation, immunosuppressive therapy/long-term corticosteroids (prednisolone or equivalent more than 0.5 mg/kg/day for > six weeks prior to ICU-admission), solid/hematologic malignancy, anticancer radiotherapy/chemotherapy and antiviral therapy within 10 days prior to ICU-admission.

3.2. Definitions

CMV-reactivation: For this study, patients with CMV latency (a positive anti-CMV IgG serology at ICU-admission) and an active CMV-replication on ICU-Day 7, 14, and 21 indicated by CMV viremia/plasma-DNAemia i.e. CMV viral load in plasma estimated to be at least 500 (2.69 $\log_{10}$) IU/ml or higher [2,3,13-15]. **Cirrhosis:** Based on the clinical, radiological, endoscopic, and/or histological criteria [16,17]. **Critically ill cirrhotic:** Any patient with cirrhosis requiring intensive care with at least two organ-dysfunction/failure and necessitating mechanical ventilation and/or inotropic support/vassopressors [17]. **Acute-on-chronic liver failure (ACLF):** Any patient with an acute hepatic insult manifesting as jaundice (serum bilirubin $\geq$ 5 mg/dL (85 micromol/L) and coagulopathy (INR $\geq$ 1.5 or prothrombin activity < 40%) complicated by clinical ascites and/or encephalopathy within 4 weeks in a patient with previously diagnosed or undiagnosed chronic liver disease/cirrhosis [18]. **Secondary infection (bacterial/fungal):** A positive laboratory-confirmed bacterial and/or fungal culture (after 72 hours of ICU-admission) co-relating with the relevant clinical manifestations and in accordance with the well-established criteria [21] for specific types of health care-associated infections.

Severity scores [Model for End-Stage Liver Disease (MELD) and Sequential Organ Failure Assessment (SOFA)], Systematic Inflammatory Response Syndrome (SIRS), septic shock, and Acute Respiratory Distress Syndrome (ARDS) were defined according to well-established criteria [14,19-21].

3.3. Study protocol

Ethical clearance and approval were obtained from the ILBS Institutional Ethics Committee (IEC)/Institutional Review Board (IRB) (Ethical clearance number: IEC/2018/64/MA07). Written informed consent was obtained for blood specimen collection and recording of clinical and laboratory parameters from Liver-ICU patients meeting the eligibility criteria. The first set of virology testing included anti-CMV-IgG and CMV-DNA-qPCR. Consecutive cirrhotic with positive anti-CMV-IgG and viral load non-quantifiable (i.e. loads less than the lower detection limit of assay) or CMV-plasma-DNAemia (viral load) less than 500 (2.69 $\log_{10}$) IU/ml at ICU-admission (day 0) were included for further follow-up with clinical and laboratory data obtained from Hospital Information System (HIS) and CMV-plasma-DNAemia monitoring on ICU-day 7, 14, and 21 or until death in ICU or discharge/transfer from ICU whichever was earlier. Broncho-alveolar lavage or any site-specific biopsy/tissue samples were not included in this study. Details of interventions were not analysed in the current study.

3.4. CMV-plasma-DNAemia detection

CMV-DNA was measured by qPCR targeting a CMV major immediate-early (MIE) gene (CMV UL123MIE; 105 base-pair region), using artus CMV QS- RGQ kit (Qiagen, Germany) performed and interpreted as per the manufacturer's instructions/recommendations (analytical sensitivity of 69.7 IU/ml and linear range of detection; 1.30×10^2 -1.64×10^8 IU/ml). A water sample was used as a negative control during sample extraction.

3.5. Cytokines detection

Based on the pro-inflammatory and anti-inflammatory cytokine profiles studied by several authors [2,9,13,22] and considering the access/availability of relevant laboratory test material, mainly interferon γ (IFN-γ), Tumour Necrosis Factor α (TNF-α), interleukin (IL) - 4 and, IL-10 were evaluated in this study. Plasma cytokine concentrations of IFN-γ, TNF-α, IL - 4 and, IL-10 were determined on ICU-day 0 and 7, by ELISA (ELabsciences, Texas, USA) according to the manufacturer's protocol.

3.6. Sample size

CMV-reactivation was seen in approximately one-third of non-immunosuppressed ICU patients in previous studies [2,22,23] targeting various ICU-population. A minimum sample size of 54 patients was estimated, allowing 0.125 (12.5%) error in estimating the unknown incidence probability, taking α as 0.05 (5%); equivalent to a 95 % confidence level.

3.7. Statistical analysis

Categorical and continuous variables were analysed by the Chi-square test (or Fisher's exact test, if applicable) and the Student's t-test/Mann-Whitney test, respectively. CMV incidence rates for ICU day 7, 14, and 21 were provided. The risk factors and parameters related to severity of illness/organ- dysfunction were assessed using binary logistic regression analysis. Kaplan-Meier (K-M) survival estimation (Log-rank test) and Cox-regression analysis with Hazard Ratio (HR) were used to assess the length of stay (LOS) and mortality in ICU relating to CMV-reactivation. All reported P values are 2-sided and $P < 0.05$ was considered significant. SPSS 22.0 (SPSS Inc. Chicago, Illinois 60606, USA) and SAS® On Demand for Academics (SAS Institute, NC, USA) were used.

4. Results

A total of 55 patients out of 94 consecutive cirrhotic ICU-patients screened for CMV (IgG and DNA) were enrolled and 137 blood samples were collected at specific time points determined as per study protocol (Fig. 1). Chronic Liver Disease (CLD) patients with cirrhosis and its complications requiring intensive care regardless of extra-hepatic organ failure were primarily admitted to Liver-ICU. The Baseline status/characteristics of the study population are depicted in Table 1.

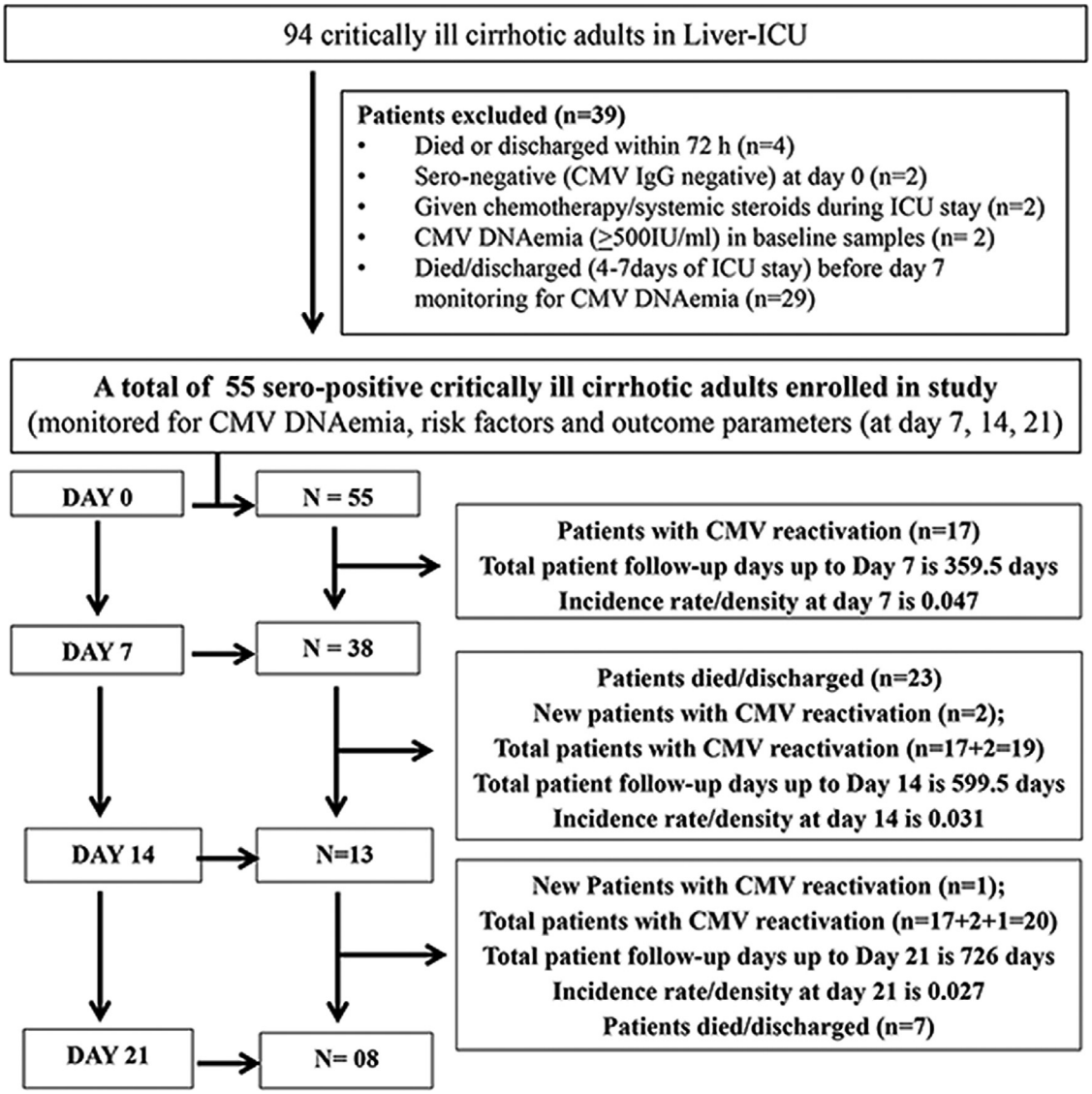

Fig. 1. Patient population (at risk) for monitoring of Cytomegalovirus reactivation in Liver-Intensive Care Unit

4.1. Incidence of CMV-reactivation

CMV-reactivation occurred in 36.4% (n=20/55) of seropositive cirrhotic adults with median time for follow up of 11 ICU-days (IQR: 8-18 days). At 7-day follow-up, cumulative incidence was 30.9% (95% CI: 19.1 - 44.80) for CMV-reactivation. Of the patients with CMV-reactivations, maximum (n=17/55, 30.9%; CI: 19.1- 44.8) developed CMV reactivation on ICU-day 7; two patients (n=2/18, 11.1%; CI= 1.4 - 3.5) on ICU-day 14 and only one (n=1/9, 11.1%; CI= 0.3 - 48.2) on ICU-day 21 (Fig. 1). High CMV-plasma-DNAemia (≥ 4 $\log_{10}$ IU/ml) was observed in 11.1% (n=2/18) on ICU- day 14. Median CMV load (range) in $\log_{10}$ IU/ml were 2.71 (1.42 – 3.06), 2.62(1.73 – 3.13) and 2.72 (2.63 – 3.27) for liver-ICU-day 7, 14 and 21, respectively (Supplementary Table S1, S2). Incidence rate of CMV-reactivation during 21-day follow-up was 2.75% per person-day (95% CI: 1.68 to 4. 26% per person-day). (Supplementary Table S3).

4.2. Risk factors

Risk factor analysis of CMV-reactivation is shown in Table 2. Based on the univariate analysis, patients with ACLF (p=0.004), reduced albumin levels (p=0.04), higher serum total bilirubin level (p=0.04), and raised Total Leukocyte Count (TLC) at ICU-admission/enrolment (p=0.01) were associated with CMV-reactivation (Table 2).

4.3. Host inflammatory status

Cytokine levels upon ICU-admission/day-0 and ICU-day 7 were measured among patients with CMV-reactivation (n=17) and those without CMV-reactivation (n=22). Among patients with CMV- reactivation, a rising trend was observed in the pro-inflammatory cytokine levels, on day 0 vs. day 7, for IFN-γ and TNF-α, (4.14±1.22 vs. 11.8±4.12 pg/mL; p=0.012 and 9.4±2.12 vs. 14.78±5.22 pg/mL; p=0.052, respectively) (Supplementary figure S1). ICU-day 7 levels of TNF-α were significantly elevated in patients with CMV-reactivation (p=0.023) (Supplementary figure S2).

4.4. Association between CMV reactivation and ICU outcomes

Univariate analysis showed increased ARDS (p=0.04), secondary bacterial/fungal infections (p=0.009) and SIRS (p=0.01) concurring with the CMV-reactivation at ICU-day 7 (Table 3). In ICU-mortality (n=34/55, 61.8%) was not influenced with regard to the presence or absence of CMV-reactivation (n=11/20, 55% versus n=23/35,

Table 1
Baseline characteristics of study population on ICU-admission (day 0) (N = 55).

Characteristic	All patients (N=55) (%)	With CMV- Reactivation (N=20) (%)	Without CMV-Reactivation (N=35) (%)	p-value
Age, median (IQR)	49 (40-57)	47(40-52.50)	49(39-58)	0.81
Male sex, No. (%)	48 (87.30)	17(35.42)	31(64.58)	0.69
Aetiology of Chronic Liver Disease				0.14
Alcoholic Liver Disease	28 (50.9%)	11 (55.0%)	17 (48.6%)	
Hepatitis B(HBV)	8 (14.5%)	4 (20.0%)	4 (11.4%)	
Hepatitis C(HCV)	3 (5.5%)	0 (0.0%)	3 (8.6%)	
NAFLD	12 (21.8%)	2 (10.0%)	10 (28.6%)	
AIH	4 (7.3%)	3 (15.0%)	1 (2.9%)	
Type of Clinical Presentation (at ICU-admission)				0.01
Decompensated CLD	37 (67.3%)	9 (45.0%)	28 (80.0%)	
ACLF	18 (32.7%)	11 (55.0%)	7 (20.0%)	
Clinical Variables				
Ascites	45 (81.8)	16(35.56)	29(64.4)	1.00
SBP	4 (7.3)	1(25)	3(75)	1.00
Variceal bleed	14 (25.5)	7(50)	7(50)	0.33
AKI	34 (61.8)	14(41.2)	20(58.82)	0.39
SIRS	31 (56.4)	9(29.0)	22(71.0)	0.26
HE	45 (81.8)	18(90.0)	27(77.1)	0.29
Septic Shock	17 (30.9)	6(35.29)	11(64.71)	1.00
ARDS	30 (54.5)	14(46.67)	16(53.33)	0.09
Mechanical Ventilation	43 (78.2)	14(70)	29(82.9)	0.31
Severity Predictor Scores[a]				
MELD-Na	29.7±6.9	30.2±5.9	29.5±7.5	0.72
SOFA	9.3±3.0	9.3±2.6	9.4±3.2	0.88
Laboratory Parameters				
Total Bilirubin (mg/dl)[b]	7.9(3.3–17.3)	14.2(4.7–20.6)	6.0(2.9–15.3)	0.04
INR[a]	2.4±0.8	2.4±0.8	2.4±0.8	0.80
AST (IU/L)[b]	80.0 (55–124)	91.5 (75.5–181.5)	69.0 (54 - 103)	0.01
ALT(IU/L)[b]	35.0(24–54)	35.5(23.5–68.0)	32.0(24 - 47)	0.43
Serum albumin (gm/dl)[a]	2.5±0.6	2.3±0.5	2.6±0.6	0.04
Haemoglobin (gm/dl)[a]	9.1±2.4	9.0±2.2	9.1±2.5	0.79
TLC (10^9/L)[a]	14.4±9.2	19.0±12.0	11.7±5.9	0.003
Platelet count (10^9/L)[b]	83.0 (57–111)	94.5(71–143)	72.0(45– 96)	0.03
Serum Creatinine (mg/dl)[a]	1.9±1.2	2.0±1.4	1.8±1.0	0.42
Sodium levels (Na) (mmol/L)[a]	133.2±8.7	133.2±10.0	133.3±8.0	0.97
Potassium levels (K) (mmol/L)[a]	4.0±0.8	3.7±0.6	4.1±0.8	0.03

Abbreviations: CMV, Cytomegalovirus; NAFLD, Non-Alcoholic Fatty Liver Disease; AIH, Autoimmune Hepatitis; CLD, Chronic Liver Disease; ACLF, Acute-on-Chronic Liver Failure; SBP, Spontaneous Bacterial Peritonitis; AKI, Acute Kidney Injury; SIRS, Systemic Inflammatory Response Syndrome; HE, Hepatic Encephalopathy; ARDS, Acute Respiratory Distress Syndrome; MELD Score, Model for End-stage Liver Disease Score; SOFA Score, Sequential Organ Failure Assessment Score; INR, International Normalized Ratio; AST, Aspartate transaminase; ALT, Alanine transaminase; TLC, Total Leukocyte count.

[a] (Mean ± SD)

[b] Median (interquartile range).

65.7%; p=0.43) among Liver-ICU-patients enrolled. Patients with CMV-reactivation in liver-ICU were observed to have an early death (3 days versus 7 days; Log-rank test p=0.067) (Fig. 2A). Among 21 patients transferred/discharged from Liver-ICU, there was no difference in LOS with reference to CMV-reactivation i.e. 5 days versus 4.5 days (p=0.17) (Fig. 2B). CMV reactivation was not independently associated with time to death (HR =0.70, 95% CI: 0. 31 to 1.61, p=0.40) [Supplementary Tables-S4, S5a and S5b].

5. Discussion

Our study found that CMV-reactivation occurs frequently in seropositive, non-immunosuppressed, critically ill, cirrhotic adults, with a peak number of CMV reactivations by ICU-day 7. Liver-ICU mortality and ICU stay were not significantly affected by CMV- reactivation.

CLD patients with cirrhosis often show immune dysfunctions [9,24]. A higher sero-prevalence of CMV in our country (> 95%) [6] may put this distinct ICU-population with cirrhosis at risk for developing CMV-reactivation. In agreement with previous studies [2,4,25,26] assessing CMV reactivation during the first 12 ICU days, we also observed CMV-plasma-DNAemia occurring with a median time of 11 days post-ICU-admission. Timing of monitoring also influences CMV-plasma-DNAemia detection because CMV-reactivation tends to occur between 1 and 3 weeks post ICU-admission and is not observed in the initial three days of ICU-stay [2,4,25,26,27]. CMV-reactivation occurred in 36.4% of our study population similar to other studies [2,27] which demonstrated a CMV-reactivation rate of 22–42%. No cases of primary CMV infection and/or CMV end-organ disease were identified in this study, as one of the inclusion criteria was CMV seropositivity.

No significant association was found between CMV- reactivation and severity scores (MELD and SOFA) at day 0/liver-ICU-admission, agreeing with review literature [2]. Factors other than disease severity/underlying organ dysfunction may be the main risk factors in this Liver-ICU population, requiring further investigation of immunological predisposition and predictive markers/factors for CMV-reactivation. Previous study [28] has revealed the role of SIRS during ACLF in the causation of organ dysfunction among these patients. Our results showed that patients with ACLF were 4.89-fold more likely to have CMV reactivation at ICU-admission/Day 0 than patients with decompensated CLD, requiring further understanding to identify CMV reactivation as a possible trigger for ACLF. Our study presents some risk factors, such as higher total bilirubin levels, lower serum albumin levels, and higher TLC representing increased liver injury and/or underlying host inflammation, making it clearer that greater inflammation at ICU-admission

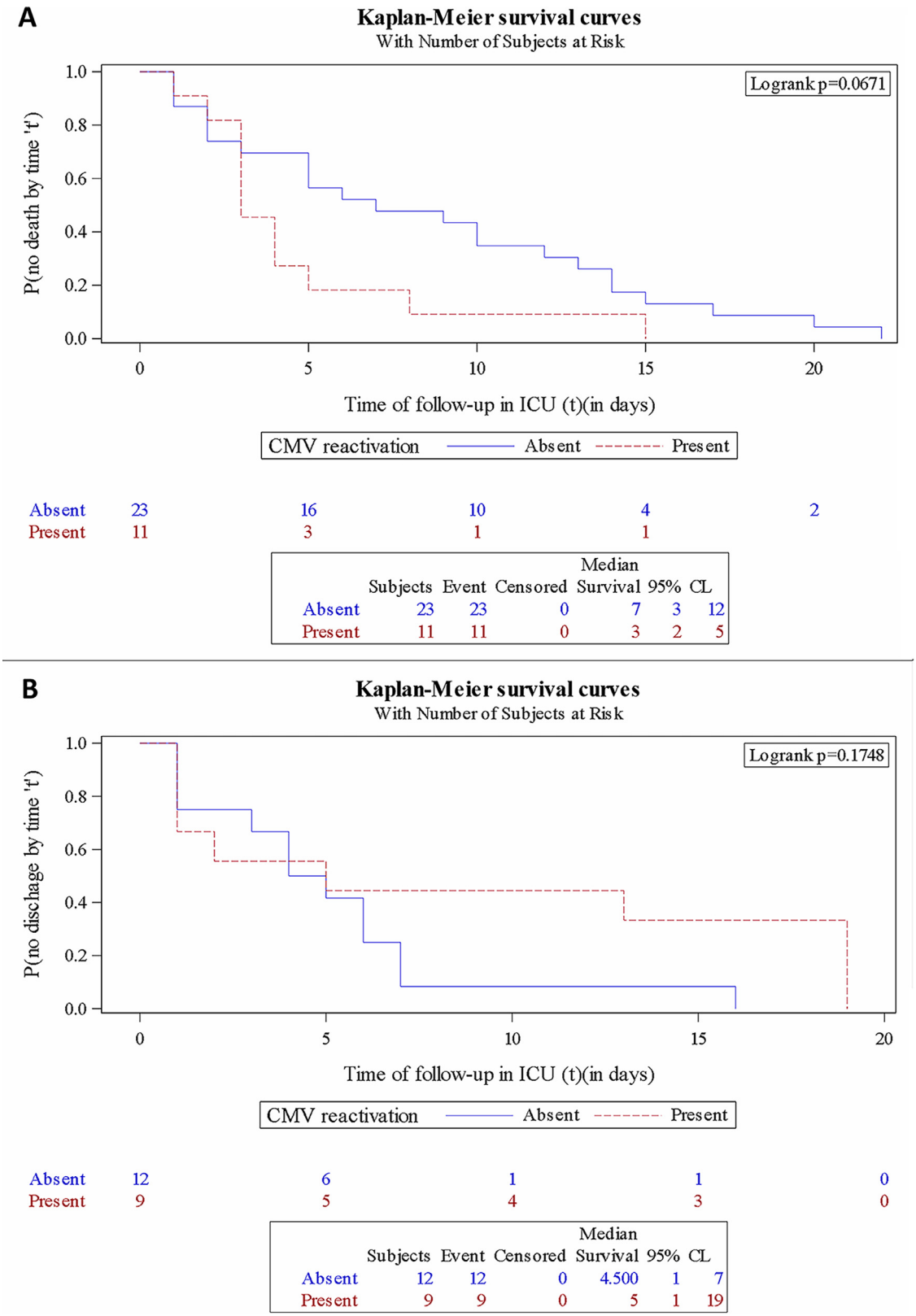

Fig. 2. (A) K-M survival curve showing no significant difference in ICU mortality (n=34) among patients with CMV reactivation Vs patients without CMV reactivation. (B): Liver-ICU stay (in days) for CMV-reactivation among surviving patients discharged/transferred from ICU (n=21), after study enrolment.

Table 2
Risk Factors (day 0/baseline status) associated for CMV-reactivation (N = 55).

Risk factors (Day 0/Baseline status on ICU-admission)	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Age,	0.99 (0.95-1.04)	0.81		
Male sex	0.73 (0.15-3.66)	0.70		
Aetiology of Chronic Liver Disease				
Alcoholic Liver Disease	3.23 (0.59-17.65)	0.175		
Hepatitis B (HBV)	5.00(0.64-39.05)	0.125		
Hepatitis C (HCV)	1(1.00-1.00)	*a		
NAFLD	1(Reference)			
AIH	15.00 (0.98-228.89)	0.051		
Type of Clinical Presentation (at ICU-admission)				
Decompensated CLD	1(Reference)			
ACLF	4.89*(1.46-16.38)	**0.01***	1.65 (0.33-8.40)	0.54
Clinical Variables				
Ascites	0.83(0.20-3.37)	0.792		
SBP	0.56(0.05-5.79)	0.628		
Variceal bleed	2.15(0.63-7.42)	0.224		
AKI	0.48(0.16-1.48)	0.20		
SIRS	1.75(0.54-5.62)	0.34		
HE	0.94(0.28-3.08)	0.91		
Septic Shock	2.67(0.51-14.03)	0.24		
ARDS	2.77(0.86-8.88)	0.08		
Mechanical Ventilation	0.48(0.13-1.77)	0.27		
Severity Predictor Scores				
MELD-Na	1.01(0.94-1.10)	0.71		
SOFA	0.99(0.82-1.19)	0.88		
Laboratory Parameter				
Total Bilirubin (mg/dl)	1.06(1.00-1.13)	**0.04***	1.01(0.93-1.09)	0.76
INR	1.09(0.56-2.14)	0.80		
AST (IU/L)	1.00(1.00-1.01)	0.41		
ALT(IU/L)	1.01(0.99-1.02)	0.50		
Serum albumin (gm/dl)	0.35(0.13-0.99)	**0.04***	0.40(0.09-1.62)	0.20
Haemoglobin (gm/dl)	0.97(0.76-1.23)	0.78		
TLC (10^9/L)	1.11(1.02-1.20)	**0.01***	1.09(0.98-1.22)	0.09
Platelet count (10^9/L)	1.01(1.00-1.02)	0.11		
Serum creatinine (mg/dl)	1.22(0.76-1.95)	0.41		
Sodium levels (Na) (mmol/L)	1.00(0.94-1.06)	0.97		
Potassium levels (K) (mmol/L)	0.39(0.16-0.96)	**0.04***	0.34(0.11-1.01)	0.05

* denotes statistical significance at the 5% level. *a denote corresponding p-value cannot be determined, due to no events in this group, and the corresponding odds ratio estimate and confidence interval may be incorrect and they cannot be determined exactly. Abbreviations: CI, Confidence interval; CMV, Cytomegalovirus; OR, Odds Ratio; NAFLD, Non-Alcoholic Fatty Liver Disease; AIH, Autoimmune Hepatitis; CLD, Chronic Liver Disease; ACLF, Acute-on-Chronic Liver Failure; SBP, Spontaneous Bacterial Peritonitis; AKI, Acute Kidney Injury; SIRS, Systemic Inflammatory Response Syndrome; HE, Hepatic Encephalopathy; ARDS, Acute Respiratory Distress Syndrome; MELD Score, Model for End-stage Liver Disease Score; SOFA Score, Sequential Organ Failure Assessment Score; INR, International Normalized Ratio; AST, Aspartate transaminase; ALT, Alanine transaminase; TLC, Total Leukocyte count.

has a greater likelihood of CMV reactivation later. Although the magnitude of the association between CMV-reactivation and specific risk factors was in the expected direction in the univariate analysis, no independent risk factor/trigger was identified in the multivariate analysis, possibly due to the relatively small sample size.

CMV reactivation could be triggered during ICU-acquired immunosuppression as a result of the compensatory anti-inflammatory response syndrome along with the production of anti-inflammatory cytokines (IL-4 and 10) [2,22,29]. Although higher levels of anti-inflammatory cytokines (IL-10 and 4) were observed at ICU admission/Day 0 in patients with CMV reactivation than in patients without CMV reactivation, the differences failed to reach statistical significance, contrary to what was reported in a previous study[13]. Alternatively, cytokine release could be due to inflammation-mediated by CMV-reactivation [2,13,27,30]. Simultaneously with CMV-reactivation on ICU-day 7, an enhanced inflammatory response with significantly elevated levels of pro-inflammatory cytokines (IFN-γ and TNF-α) was observed in patients with CMV reactivation in our study. TNF-α might be playing a role in CMV-reactivation as a result of stimulation of the immediate-early enhancer/promoter region of CMV [30]. Therefore, our results may have compelling implications and warrant further investigations.

Survival was not significantly impaired in our study population of Liver-ICU, in contrast to the results of previous studies [4,10] targeting different ICU populations. Our results, being consistent with some investigators [13,25], also showed that CMV reactivation was not significantly associated with LOS in the Liver-ICU; however, these results did not agree with other researchers [8,23,26]. As described in a systematic review [22], we also found that CMV- reactivations were associated with the severity of organ dysfunction/critical illness in the Liver-ICU-day 7 mainly in relation to SIRS (OR:12.67, 95% CI:1.52-105.69; p=0.01), increased secondary bacterial/fungal infections (OR: 5.08; 95% CI: 1.50-17.24; p=0.009), ARDS (OR:4.25; 95% CI:1.05-17.20; p=0.04), and total bilirubin levels (OR:1.06, 95% CI:1.01-1.12; p=0.02). The immunomodulatory effect of CMV may be responsible for the increased risk of secondary infections in critically ill patients [1,2,7,27]. Our results suggest that CMV-reactivation could be a possible consequence of immune modulation induced by a severe liver disease during critical illness, leading to increased disease severity/organ dysfunction but not directly affecting survival. This observational study was unable to distinguish whether CMV-reactivation is responsible for increased disease severity or exaggerated disease severity leading to CMV-reactivation.

The main strengths of our study are the qPCR-based monitoring of CMV-plasma-DNAemia, the prospective/longitudinal observational study design that determines the incidence rate of CMV reactivation, and potential associations with host cytokine responses. There is currently no prospective study in the context of critically ill cirrhosis patients. Re-

Table 3
Association between parameters for severity of Critical illness/organ-dysfunction and CMV-reactivation (Day 7) (N = 55).

Parameter	At Day 7 of Liver-ICU stay			
	Unadjusted OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Ascites	0.53 (0.10-2.92)	0.46		
SBP	0.86 (0.14-5.18)	0.87		
Variceal Bleed	1.12 (0.31-4.06)	0.85		
Secondary (bacterial/fungal) Infection	5.08(1.50-17.24)	**0.009***	2.85 (0.74, 11.03)	0.129
SIRS	12.67(1.52-105.69)	**0.01***	7.67 (0.84, 70.02)	0.071
AKI	3.12(0.60-16.15)	0.17		
Septic Shock	0.55(0.15-2.02)	0.36		
HE	3.17(0.34-29.23)	0.30		
ARDS	4.25(1.05-17.20)	**0.04***	2.13(0.42, 10.92)	0.363
Mechanical Ventilation	1.20(0.34-4.19)	0.77		
MELD-Na score	0.99(0.91-1.08)	0.85		
SOFA score	1.01(0.84-1.21)	0.92		
Total Bilirubin(mg/dl)	1.06(1.01-1.12)	**0.02***	1.04(0.98, 1.10)	0.216
INR	0.77(0.47-1.24)	0.27		
AST(IU/L)	1.00(1.00-1.01)	0.18		
ALT(IU/L)	1.00(0.99-1.01)	0.57		
Serum albumin(gm/dl)	0.61(0.27-1.37)	0.23		
Haemoglobin(gm/dl)	1.26(0.77-2.09)	0.35		
TLC(10^9/L)	1.06(1.00-1.12)	0.05		
Platelet count(10^9/L)	1.02(1.00-1.03)	0.08		
Serum creatinine(mg/dl)	0.98(0.67-1.43)	0.90		
Sodium levels(mmol/L)	1.04(0.96-1.13)	0.36		
Potassium levels(mmol/L)	1.40(0.48-4.11)	0.53		

Abbreviations: CI, confidence interval; CMV, Cytomegalovirus; OR, Odds Ratio; Spontaneous Bacterial Peritonitis; AKI, Acute Kidney Injury; SIRS, Systemic Inflammatory Response Syndrome; HE, Hepatic Encephalopathy; ARDS, Acute Respiratory Distress Syndrome; MELD Score, Model for End-stage Liver Disease Score; SOFA Score, Sequential Organ Failure Assessment Score; INR, International Normalized Ratio; AST, Aspartate transaminase; ALT, Alanine transaminase; TLC, Total Leukocyte count.
*Denotes statistical significance at the 5% level.

markably, therefore, the prospectively analysed data in our study recognize seropositive critically ill cirrhotic adults as a distinct subset among diverse ICU patients who are at high risk of CMV- reactivation.

This study had a few limitations. This was a single-centre study with a relatively small sample size to address the secondary objective of confirming the impact of CMV-reactivation on the causality of adverse clinical outcomes. Therefore, our results cannot be generalized to different ICU centres. There was also a limited frequency of once-a-week CMV-DNAemia estimation until ICU day 21. Because most of the enrolled patients died or were discharged by ICU-day 14 and 21, the entire study population could not undergo weekly CMV-DNAemia testing at specific time-points (ICU- day 7, 14, and 21). Consequently, the transient occurrence of CMV-plasma-DNAemia may have been overlooked. An optimal frequency should have been at least twice a week throughout ICU-stay to detect subsequent CMV-reactivations according to previous results [5,10]. Also, a parallel study of host-related cell-mediated immunity may reveal the effects of CMV reactivation on the stress response and/or host-immune function and vice versa.

To conclude, the incidence rate of CMV-reactivation was substantial among seropositive critically ill cirrhotic adults. CMV-reactivation occurred more frequently on ICU day-7. Elevated pro-inflammatory cytokines and more severe organ dysfunction were observed concomitantly with CMV reactivation. CMV-reactivation among cirrhotic adults did not influence mortality and LOS in liver-ICU.

Furthermore, the pathogen versus bystander question for CMV warrants an adequately powered prospective/longitudinal cohort studies and/or larger clinical trials in the critically ill cirrhotic adult populations with frequent assessments of CMV reactivations for optimal duration along with clinical outcomes evaluated even after discharge from ICU. Therefore, the potential harm and benefit of antiviral therapy/prophylaxis in critically ill non-immunosuppressed cirrhosis patients should be carefully considered.

Institution/place of work

Institute of Liver and Biliary Sciences, New Delhi-110070, India

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Author contribution statement

Dhara Shah: Writing – original Draft, conceptualization, study design, data acquisition, data analysis, methodology, interpretation, and manuscript drafting. **Ekta Gupta:** Project administration, conceptualization, study design and resources, and reviews. **Rakhi Maiwall, Samba Shiva Rao Pashupuleti and Archana Ramalingam:** Study design, formal analysis, interpretation, reviewing, and/or editing. **Sukriti Baveja and Lalita Gauri Mitra:** collaboration and review. **Shiv Kumar Sarin:** Supervision/project administration and resources.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcvp.2022.100087.

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