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Active *Cytomegalovirus* Infection In Critically Ill Immunocompetent Patients Admitted To ICU Of Dr. Kariadi Hospital Semarang-Indonesia: A Molecular Diagnostic Approach

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Abstract

Background : Active *Cytomegalovirus* (CMV) infection has long been related to immunocompromised conditions such as malignancy, HIV-AIDS, longterm use of corticosteroids and organ transplantation. Nowadays, several studies showed that active CMV infection also frequently found in formerly immunocompetent patients during critically ill condition. Alteration of immune system in critically ill condition might become the most possible reason underlying this adverse event.

Aim : To document the prevalence of active CMV infection in critically ill immunocompetent patient admitted to ICU and to find out the difference of the disease severity between group of patients with and without active CMV infection.

Method : This was a cross sectional study. Study conducted from April 1st - June 30th 2013. Subjects were patient aged ≥14 years, hospitalized in the ICU of Dr. Kariadi Hospital, Semarang, Indonesia. Patients who had history of malignancy, HIV-AIDS, use of corticosteroids and organ transplatation were excluded from the study. Disease severity was calculated using APACHE II score in the first 24 hours of ICU admission. EDTA sample for qualitative PCR examination (procedure as described elsewhere) collected after 4 days of ICU admission. Primer for CMV were as follow CMV-F: CATGAAGGTCTTTGCCCAGTAC, CMV-R: GGCCAAAGTGTAGGCTACAATAG. Datas were analyzed using bivariate analysis.

Result : Active CMV infection was detected in 16 out of 50 subjects. Mean score of disease severity in all subjects (based on APACHE II scoring system) was 11.8±6.43. Mean of APACHE score was higher in infected group than non-infected group, but the difference was not significant (12.75 vs. 11.47; *p*=0,510).

Conclusion : The prevalence of active CMV infection in critically ill immunocompetent patient is relatively high (16/50; 32%) in the ICU of Dr. Kariadi Hospital, Semarang, Indonesia. Degree of disease severity might influence the occurance of CMV infection. Qualitative PCR testing was an aqurate tool for diagnosing active CMV infection.

Key words: immunocompetent, critically ill, active CMV infection, PCR

INTRODUCTION

It is surprising to know that alteration in immune system both innate and adaptive take place in critically ill immunocompetent patient.[1] Definition of immunocompetent patient is patient that do not possess clear evidence of immunocompromise condition.[2]

Active CMV infection, particularly reactivation from latency, was reported prevalent among critically ill immunocompetent patients such as patient with severe trauma, sepsis, shock, burns, chirrosis, myocardial infarction and other critical conditions that made a patient treated in Intensive Care Unit (ICU). The highest incidence of active CMV infection found in patient with septic shock.[3]
According to previous research, active CMV infection was mostly detected in day 4 until day 12 of hospitalization in ICU. Risk factors for active CMV infection include sepsis, use of mechanical ventilation and history of blood transfusion.[4]

Active CMV infection define as detection of CMV either through culture, detection of pp65 antigen or detection of CMV DNA by PCR technique from either blood, urine or Bronkho-Alveolar Lavage (BAL) specimen. Published data showed that the rate of active CMV infection in ICU was between 0-36%.[5] PCR technique considered as a gold standard in diagnosing active CMV infection since it possessed high sensitivity to detect DNA virus in a very early state of the infection. Thus, PCR technique is very suitable as a tool for early detection of active CMV infection.[4,6]

Disease severity in every patient hospitalized in ICU quantified using a systematic scoring system. Based on many literatures, scoring systems that most frequently applied in the ICU were Acute Physiology and Chronic Health Evaluation (APACHE) II, Simplified Acute Physiology Score (SAPS), Mortality Probability Model (MPM), Multiple Organ Dysfunction Score (MODS) dan Therapeutic Intervention Scoring System (TISS).[7] Among those, APACHE II was the most common used due to its reliability and simplicity.[8,9]

Disease severity in critically ill immunocompetent patient would influence the prevalence of active CMV infection either new or reactivation from latency. Data of CMV infection in critically ill immunocompetent patient in Indonesian population had never been found before. Researcher thought that it would be very important to find out the prevalence of active CMV infection among critically ill immunocompetent patients treated in ICU of Dr. Kariadi Hospital. We also found out whether the disease severity differ significantly between groups of patient with and without active CMV infection.
Immunocompromised condition could be caused by congenital or acquired aspect. Critical illness was the example of acquired immunocompromise condition. Critical illness define as every disease process that cause physiologic instability or death within minutes or hours. Neurologic and cardiorespiratoric disorder had the most threadfull effect to the patient's life.[10]
Cytomegalovirus is a member of Herpesviridae family that include Ebstein-Barr virus (EBV), Herpes simplex virus, Varicella-zoster virus dan herpesvirus 6, 7, and 8.[11,12] Primary CMV infection usualy invisible or unknown. Like other herpes virus, CMV remain latent and would re-activate once host's immune system supressed.[11]

There were three kinds of active CMV infection: (1) primary infection, occur when the virus infect CMV-naive host, (2) endogenous infection, reactivation of latency from CMV-seropositive host and (3) exogenous reinfection, reinfection by a new strain of CMV.[13]

Reactivation of CMV occured through many processes and not all of the processes known clearly. Eventhough, it was believed that the activation of IE region of CMV was the beginning of the reactivation. IE region was a region consists of NF κB which in normal condition should be in a non active state. IE region could be activated by proinflammatory cytokines, chemokines, adhesion molecules, inflammatory enzimes and many receptors emerged during sepsis, burns, operation, trauma and multi organ failure.[14]

Active CMV infection both primary or re-activation from latency might cause tissue injury through 2 mechanisms: (1) cytopathology and (2) immunopathology. Cytopathology caused by direct effect of virus that re-activate in the organs, whereas immunopathology was a tissue disarrangement caused by sequential immunological response againts the viral, particularly in the form of proinflammatory cytokine production.[14,15]

Critically ill condition would activate inflammatory cytokines that further would activate NF κB in IE region of CMV. These event caused activation of prior viral infection (re-activation). Prolonged critically ill condition may also caused polarity shifting from inflammatory response to anti-inflammatory response dominance.

METHOD

This was a cross sectional study, performed from April 1st until June 30th 2013 in ICU of Dr. Kariadi Hospital Semarang, Indonesia. This study was approved by The Ethical Committee of Faculty of Medicine-Diponegoro University.
Subjects were surgical and non-surgical patients admitted to ICU. The inclusion criteria were: (1) age more than 14 years, (2) fulfilled the criteria of critical illness, (3) APACHE II score could be assessed within the first 24 hours of admission and (4) patient and/or family agreed to take a part in the study (informed consent).

Exclusion criteria were: (1) longterm use of corticosteroids or immunosupressive drugs, (2) HIV-AIDS or clinically suspected HIV-AIDS, (3) malignancy, (4) organ transplantation and (5) died or allowed to exit from ICU before 4 days of treatment. Minimum sample size was 50 patients. Disease severity calculated using APACHE II score in the first 24 hours admission in ICU.

PCR technique was used to diagnose active CMV infection. Sera for qualitative PCR examination (procedure as described elsewhere) collected after 4 days admission in ICU. Primer for CMV were as follow CMV-F: CATGAAGGTCTTTGCCCAGTAC, CMV-R: GGCCAAAGTGTAGGCTACAATAG. Finally, datas were analyzed using bivariate analysis.

RESULTS

Sixty subjects were included in this study but 10 of them must be excluded due to malignancy and mortality before 4 days of treatment. Subjects were predominantly males (60%). Age were not normally distributed; median of age was 55 year (range 17-81 year old).

Subjects consist of 16 surgical cases and 34 non-surgical cases. From the surgical group there were 14 patients undergoing operation procedures. Subjects were classified into 11 diagnosis (See **Table 1**). Number of subject receiving blood transfusion was 19 (38%) and subject using mechanical ventilator was also 19 (38%).

Tabel 1. Clinical diagnosis underlying patients to enter the Intensive Care Unit (ICU)

Surgical	n (%)
Gastrointestinal operation	2 (4)
Thorax-cardiovascular operation	8 (16)
Trauma (traffic accident)	1 (2)
Burns	1 (2)
Other*	4 (8)
Total	**16 (32)**
Non Surgical	
CVA/Cardiovascular accident	16 (32)
Cardiogenic shock	4 (8)
Sepsis	7 (14)
Resp. Failure**	4 (8)
Hemorrhagic stroke	1 (2)
Eclampsia	2 (4)
Total	**34 (68)**

*1 case of craniotomy, 1 case of amputation, 1 case of ORIF/*open reduction internal fixation*, 1 case of pyelolithotomy, **3 cases of overhidration due to ESRD, 1 case of heart failure due to thyroid disease.

Qualitative PCR examination showed 16 out of 50 (32%) patients possitive for active CMV infection. APACHE II score among subjects were normally distributed. Mean of APACHE II score was 11,88±6,439. Mean of APACHE II score in infected group was higher than non-infected group, but the differences was not statistically significant [12,75 vs.11,47 (95%CI; *p*=0,510)].

Length of hospitalization in ICU was not normally distributed with median of 14 days (range 5-69 days). Infected and non-infected group spent relatively same length of stay [14 days (5-58) vs.13,5 days (5-69); *p*=0,535]. Median age of infected group was younger than uninfected group, but the differences was not statistically significant [53 (18-81) vs.56 (17-76); *p*=0,693]. Eventhough the prevalence of active CMV infection much higher in female group, but the difference was not statistically significant [11/20 (55%) vs.5/30 (16,7%); 95%CI, *p*=0,055].

In this research, operation procedure, use of mechanical ventilator and administration of blood transfusion did not influence the occurance of active CMV infection significantly. The prevalence of active CMV infection in surgical group vs. non-surgical group was 5/14 (35,7%) vs.11/36 (30,5%); 95%CI, *p*=0,989]. The prevalence of active CMV infection in subject using mechanical ventilator vs. not using mechanical ventilator was 7/19 (36,8%) vs. 9/31 (29%); *p*=0,793. The prevalence of active CMV infection in subject receiving transfusion vs. not receiving transfusion was 6/19 (31,6%) vs. 6/31 (19,3%); *p*=1,000 (see **Table 2**).

	Possitive PCR CMV	Negative PCR CMV	*Significance*
Number of patients	16	34	
Sex			
Male (n)	5	25	*p* 0,055*
Female (n)	11	9	
Age (year)	53	56	*p* 0,693**
Surgical (n)	8	8	
Non surgical (n)	8	26	
Surgical			
Gastrointestinal operation	2	0	
Thorax-CV operation	1	7	
Trauma (traffic accident)	1	0	
Burns	1	0	
Others	3	1	
Non surgical			
CVA	4	12	
Cardiogenic shock	1	3	
Septic condition	0	7	
Respiratory failure	2	2	
Hemorrhagic stroke	1	0	
Eclampsia	0	2	
Surgery			
Yes	5	9	*p* 0,989*
No	1	25	
Ventilator			
Yes	7	12	*p* 0,793*
No	9	22	
History of transfusion			
Yes/No	6	13	*p* 1,000*
No	6	25	
Score of APACHE II	12,75±1,804(3-30)	11,47±1,048(2-23)	*p* 0,510***
Length of stay in ICU	14(5-58)	13,5(5-69)	*p* 0,535**

*Chi Square test, **Mann Whitney test, *** T test

DISCUSSION

In this study, the point of prevalence for active CMV infection in critically-ill immunocompetent patient hospitalized in ICU was relatively high (32%). Point of prevalence means that the datas taken in a specified short time period (3 months). However, this result similar to previously published datas from other researchs [32% (Muller, 2006), 33% (Limaye, 2008) and 40,69% (Heininger, 2011)].[2,9,16]

The variability of datas might be caused by: (1). variability of method used in detecting CMV infection (PCR, antigenemia and serology). Meta-analysis done by Ryosuke Osawa et al stated that PCR examination detect CMV infection earlier than other methods[3]; (2). variation of disease onset. Most re-activation took place between day 4 until day 12 of hospitalization in the ICU. Thus, serial PCR examination might give more precise data regarding the prevalence of CMV infection but this serial examination of course would be very expensive.

Disease severity between the two groups (quantified by APACHE II scoring system) was not differ significantly [12,75 vs. 11,47 (95%CI; *p*=0,510)]. This result also similar to previous study showed that disease severity and the mortality between the two groups not differ significantly [SAP II score was 43 (33-47) vs. 44 (33-37); *p*=0,15 in infected vs. non infected group].[16] In this research, it seems that the disease severity had a weak correlation with the occurance of active CMV infection. It could be caused by time gap in examining those two variables. Disease severity quantified in the first 24 hour, whereas re-activation of CMV infection occur in day 4-12 of ICU admission.[3] Eventhough, it was remain too early to make this conclusion.

We gained datas from both surgical and non surgical patients because this was an early study so we wanted to collect data of active CMV infection

among all group of patients. On the other hand, due to short period of study, it was impossible for us to gain sufficient datas from homogenous subject.

Blood transfusion and usage of mechanical ventilator did not give significant difference in the occurance of active CMV infection. This result differ from previous study showed that history of blood transfusion [OR 6,7 (1,1-42,7)] and use of mechanical ventilator [OR 8,5 (1,1-66,5)] considered as risk factor for CMV re-activation.[2]

Length of stay between infected and non-infected group did not differ significantly [14 (5-58) vs. 13,5 (5-69); *p*=0,535]. This result differ from previous study stated that CMV re-activation had a possitive corelation with the length of stay in ICU [30,0 (14-48) vs.12,0 (7-19) HR 3,365; 95%CI 1,233-9,183, *p*=0,018].[16]

REFERENCES

1. Morris AC, Kefala K, Wilkinson TS, Dhaliwal K, Farrel L, *et al*. C5a mediates peripheral blood neutrophil dysfunction in critically ill patient. *Am J Respir Crit Care Med* 2009;180:19-28.

2. Limaye AP, Kirby KA, Rubenfeld GD, Leisenring WM, Bulger EM, *et al*. Cytomegalovirus reactivation in critically ill immunocompetent patient. *JAMA* 2008;300(4):413–22.

3. Osawa R, Singh N. Cytomegalovirus infection in critically ill patient: a systematic review. *Critical Care* 2009;13(3):1-10.

4. Bhatia J, Shah BV, Mehta AP, Deshmukh M, Sirsat RA, *et al*. Comparing serology, antigenemia assay and polymerase chain reaction for the diagnosis of Cytomegalovirus infection in renal transplant patients. *JAPI* 2004;52:297-300.

5. Cohen JI. CMV in ICU: pathogen or passenger? *Crit Care Med* 2009;37(6):2095–6.

6. Tang CH, Yang CM, Chuang CY, Chang ML, Huang YC, *et al*. Comparative study of clinical severity scoring systems in ICU in Taiwan. *Tzu Chi Med J* 2005;17(4):239-45.

7. Safavi M, Honarmand A. Comparison of infection probability score, APACHE II, and APACHE III scoring systems in predicting need for ventilator and ventilation duration in critically ill patients. *Arch Iranian Med* 2007;10(3):354-60.

8. Le Gall JR. The use of severity scores in the intensive care unit. *Intensive Care Med* 2005;31:1618-23.

9. Muller, Klemm A, Weiss M, Schneider M, Wiedeck HS, *et al*. Active Cytomegalovirus infection in patients with septic shock. *Emerging Infectious Diseases* 2006;12(10):1517-22.

10. Frost P, Wise MP. Recognition and early management of the critically ill ward patient. *British Journal of Hospital Medicine* 2007;68(10):M180-3.

11. Taylor GH. Cytomegalovirus. *Am Fam Physician* 2003;67(3):519-24.

12. Rafailidis PI, Mourtzoukou EG, Varbobitis IC, Falagas ME. Severe Cytomegalovirus infection in apparently immunocompetent patients: a systematic review. *Virology Journal* 2008;5(47):1-7.

13. Varani S, Landini MP. Cytomegalovirus-induced immunopathology and its clinical consequences. *Herpesviridae* 2011;2(6):1-14

14. Jain M, Duggal S, Chugh TD. Cytomegalovirus infection in non-immunosupressed critically ill patient. *J Infect Dev Ctries* 2011;5(8):571-9.

15. Cook CH, Trgovcich J. Cytomegalovirus reactivation in critically ill immunocompetent hosts: a decade of progress and remaining challenges. *Antiviral Res* 2011;90(3)151-9.

16. Heininger A, Haeberle H, Fischer I, Beck R, Riessen R, *et al*. Cytomegalovirus reactivation and associated outcome of critically ill patients with severe sepsis. *Critical Care* 2011;15:1-10.