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Original Article

Invasive pulmonary aspergillosis is associated with cytomegalovirus viremia in critically ill patients - A retrospective cohort study

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Abstract *Background/Purpose:* Cytomegalovirus (CMV) viremia is associated with a higher mortality rate and prolonged intensive care unit (ICU) stay for critically ill patients. CMV infection causes transient but substantial immunosuppression for transplant recipients, increasing risk of fungal infection. The association between CMV viremia and invasive pulmonary aspergillosis (IPA) for critically ill patients is still unknown.
Methods: We retrospectively analyzed patients received bronchoalveolar lavage (BAL), galactomannan test, influenza survey and blood CMV viral load test in ICUs of a university hospital

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between April 2017 and May 2020. Independent risks for IPA were analyzed by multivariable logistic regression.
Results: A total of 136 patients were included. Twenty-one patients had IPA, 48 patients had CMV viremia and 22 patients had influenza. In a multivariable logistic regression model, patients with CMV viremia or influenza had higher IPA risk (adjusted odds ratio, 3.98 and 8.72; 95% CI, 1.26—12.60 and 2.64—28.82; p value = 0.019 and <0.001, respectively.). Patients with detectable CMV in BAL fluid did not have higher IPA risk (crude odds ratio, 0.95; 95% CI, 0.33—2.79; p value = 0.933). After stratifying patients by CMV viral load, the IPA risk is higher for patients with higher viral loads. There is an additive synergistic effect on IPA risk between CMV viremia and influenza infection.
Conclusion: For critically ill patients, CMV viremia is an independent risk factor of IPA. Patients with higher blood CMV viral loads have a higher risk of IPA. CMV viremia and influenza have an additive synergistic effect for IPA risk in critically ill patients.

Introduction

Cytomegalovirus (CMV) is an omnipresent virus existing in healthy people. In the general population, CMV serology tests suggest that about two-thirds of people have a positive test result.[1] There are increasing evidence shown that critically-ill patients are at risk of CMV viremia or infection, the CMV infection rate ranged from 0 to 36% with the median rate of 25% among these patients by previous studies.[2,3] CMV viremia is associated with a higher mortality rate, prolonged intensive care unit (ICU) and hospital stay, prolonged mechanical ventilation use and a higher rate of nosocomial infection for critically ill patients and a lower ventilator weaning rate for acute respiratory distress syndrome patients.[2—6] In addition, CMV infection causes transient but substantial immunosuppression.[7] Clinical evidence has shown that CMV infection induces significant immunosuppression in transplant recipients, increasing the risk for opportunistic fungal infection.[8,9]

Invasive pulmonary aspergillosis (IPA) is more prevalent in critically ill patients than the general population, with the IPA incidence among ICU patients ranging from 0.33% to 19%.[10] In the past, IPA was mainly reported in immunocompromised patients or patients with structural lung disease.[10] The established risk factors for IPA include prolonged neutropenia, solid organ transplantation, allogeneic stem cell transplantation, systemic corticosteroids administration, severe inherited immunodeficiency or immune modulator agent use.[11,12] Recently, retrospective studies have found influenza is an independent risk factor for IPA.[13—15] Nevertheless, IPA is sometimes diagnosed in critically ill patients without established risk factors,[16] and it's possible that there are undiscovered risk factors for IPA.

Previous studies have shown that CMV infection is associated with IPA in immunocompromised patients.[17,18] However, to the best of our knowledge, there is no published study concerning the association between CMV viremia and IPA in critically ill patients. To explore the association, we conducted a retrospective cohort study in our ICUs to analyze the relationship between IPA, CMV viremia and other IPA risk factors, such like influenza infection, organ transplantation and corticosteroid use.

Methods

Study design and patient recruitment

We conducted a retrospective cohort study in the ICUs of National Cheng Kung University Hospital (NCKUH) from April 1, 2017 to May 31, 2020. The inclusion criteria stipulated patients 20 years and older and having received bronchoscopy-guided bronchoalveolar lavage (BAL) in the ICU. Exclusion criteria included patients had no pulmonary infiltration in chest radiologic examinations; had previous diagnosed IPA; did not receive an influenza test (including influenza polymerase chain reaction or enzyme-linked based rapid antigen test on nasopharynx swab, oropharynx swab, sputum or BAL) or BAL galactomannan (GM) test; and, had no blood CMV viral load data within 7 days of the BAL test day. In addition, we chose one BAL data if the patient received several BAL in one ICU course. The Institutional Review Board of NCKUH approved this retrospective cohort study (A-ER-108-231) before commencement.

Data collection

Data were collected from electronic medical records in the NCKUH database. Collected data included age, sex, BMI, acute physiology and chronic evaluation (APACHE) II score on the ICU admission day, proven IPA risk factor based on European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group definition,[12] the bronchoscopy procedure, and the results of the virologic and mycologic surveys. The tests for the

pathogen survey included the values of the serum and BAL GM test, the PCR (polymerase chain reaction)-based CMV nucleic acid test for BAL, quantitative PCR for the blood CMV viral load, the influenza nucleic acid amplified test (NAAT) and the rapid antigen test on nasopharynx swab, oropharynx swab, tracheal aspirated sputum and BAL.

Definition of invasive pulmonary aspergillosis

We used the modified *AspICU* algorithm to define patients with IPA in our cohort (Supplementary Table 1).[19] The modified *AspICU* algorithm is revised from *AspICU* algorithm,[20] aiming to increase test sensitivity for critically ill patients by adding galactomannan value in mycological criteria.[19] Based on the modified *AspICU* algorithm, IPA is diagnosed according to the presence of clinical, radiological, and microbiologic criteria. We used Platelia Aspergillus test (Bio-Rad Laboratories, Marnes-la-Coquette, France) to detect galactomannan optic density index (ODI). We reviewed medical charts, chest radiologic examinations and laboratory results to make an IPA diagnosis for every patient in our cohort.

Definition of CMV viremia and other IPA risk factors

Patients who have detectable blood CMV virus within 7 days of the BAL day were defined as having CMV viremia in our cohort. We performed a Real-Time PCR assay using the artus CMV RG PCR kit (QAIGEN GmbH, Germany) on the BD MAX™ System (BD Diagnostics, Sparks, MD, USA) for CMV blood and BAL viral load measurement.[21] Influenza was diagnosed based on positive result real-time PCR or commercial rapid test assay (BD Veritor™ System, BD Diagnostics, Sparks, MD, USA) on nasopharynx swab, oropharynx swab, sputum or BAL specimens. Patients who received more than 10 mg prednisolone equivalent dose of corticosteroid per day for 14 days or longer were defined as having prolonged systemic corticosteroid use. Patients with an absolute neutrophil count less than 500 per microliter for 10 days or longer were defined as having prolonged neutropenia. Lastly, patients with a body mass index of less than 18.5 were defined as having malnutrition.

Statistical analysis

We set models of univariate and multivariable analyses to predict IPA risk factors. In the univariate analysis, continuous variables were compared by Student *t* test or Mann–Whitney U test, and the categorical variables were compared by Fisher's exact test or the $\chi 2$ test. For established independent risk of IPA, a univariate analysis was constructed with binary logistic regression. The dependent variable was the modified *AspICU* algorithm-diagnosed IPA, and the independent variables included influenza, CMV viremia, detectable CMV in BAL fluid, and other known risk factors for IPA. Firth logistic regression was performed for independent risk factors with rare events. Previously identified high risk factors of IPA for critically-ill patients[10,15] and variables with a $p < 0.05$ in univariable analysis were entered into multivariable logistic regression model. The estimated association was presented by crude and adjusted odds ratios with confidence intervals of 95%. We used the G*power 3.1.9.7 software to estimate sample size and the achieved power of multivariable logistic regression model.[22] When setting the model is two-tailed tested with a significance level α of 0.05, incidence of IPA to 0.15, prevalence of CMV viremia to 0.35 and the sample size to 136, the calculated power is 84% if we assume odds ratio is 3.5. To test the robustness of association between CMV viremia and IPA, we performed sensitivity analyses by including patients who were not tested for influenza, and by stratifying blood CMV viral load. Synergy index was calculated to check the synergistic effect between CMV viremia and other variables with statistical significance in the multivariable analysis.[23,24] In addition, we grouped patients by age for subgroup analysis by logistic regression. *P* value less than 0.05 was considered statistically significant. All data were analyzed by SAS software (version 9.4, Cary, NC: SAS Institute Inc; 2014).

Results

A total of 792 patients who received BAL in the ICUs of NCKUH were recruited (Fig. 1). Of these, 356 patients were excluded due to the following reasons: no BAL GM test (n = 135), no influenza test (n = 108), repeated BALs in one ICU course (n = 107), and previous pulmonary aspergillosis (n = 6). Of the remaining 436 patients, 300 more were excluded because of the lack of blood CMV viral load data within 7 days of the BAL day. Finally, data from a total of 136 patients with BAL GM test, blood CMV viral load and influenza test results were included for statistical analyses. There were 36 (26.5%) of 136 patients received one type of test for influenza survey (NAAT: 27 patients, included 22 BAL samples and 5 oropharyngeal or nasopharyngeal swab sample; rapid antigen test: 9 patients, included 2 BAL samples and 7 oropharyngeal or nasopharyngeal swab samples), 57 (41.9%) of 136 patients received two different types of tests (all of them received at least one NAAT) and 43 (31.6%) of 136 patients received more than three different types of tests for influenza survey.

Characteristics of included patients

Characteristics of the included patients are listed in Table 1. The median age was 65 (IQR, 54.5–74.5), 91 (66.81%) patients were male and 68 (50.00%) patients were older than 65 years old. The median APACHE II score was 23 (IQR 18.0–30.0). Twenty-two (16.18%) patients had influenza, 48 patients (35.29%) had CMV viremia and 72 (62.61%) of 115 patients had detectable CMV nucleic acid in the BAL fluid. Furthermore, 21 (15.44%) patients were diagnosed with IPA by the modified *AspICU* algorithm. Compared to patients without IPA, patients with IPA have a higher risk of experiencing CMV viremia (14 of 21, 66.67% vs 34 of 115, 29.57%, $p = 0.003$) and influenza infection (10 of 21, 47.62% vs 12 of 115, 10.43%, $p < 0.001$), but are not prone to having detectable CMV in the BAL fluid (13 of 21, 68.42% vs 78 of 115, 67.83%, $p = 0.962$). Regarding other known independent risk factors for IPA, there were no statistical differences between those with and without IPA.

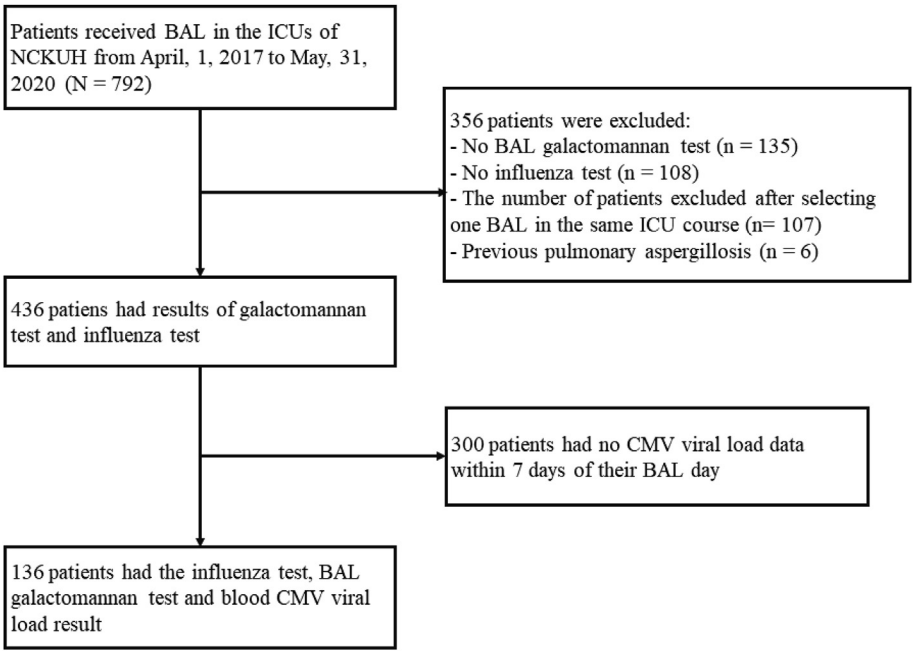

Figure 1. Overview of case inclusion process and the corresponding number of excluded and included cases. BAL = bronchoalveolar lavage, CMV = cytomegalovirus, ICU = intensive care unit, NCKUH=National Cheng Kung University Hospital.

Independent risk factors of IPA

Table 2 shows the results of the univariate logistic regression. CMV viremia (OR, 4.76; 95% CI, 1.77–12.84, *p*-value = 0.002) and influenza (OR, 7.80; 95% CI 2.75–22.18; *p*-value < 0.001) were the independent risk factors of IPA. Patients who received bone marrow transplantation had a trend of increased IPA risk, but it was not statistically significant (OR, 4.63; 95% CI, 0.95–22.40, *p*-value = 0.057). Detectable CMV in BAL, age older than 65 years and prolonged systemic corticosteroid use are not independent risk factors for IPA. After analysis of the multivariable logistic regression model (Table 2), CMV viremia and influenza remained as statistically significant increased risk factors of IPA (for CMV viremia: adjusted OR 3.98; 95% CI 1.26–12.60; *p*-value = 0.019; for influenza: adjusted OR, 8.72, 95% CI, 2.64–28.82, *p*-value < 0.001).

Sensitivity analyses

After including patients who were not tested for influenza for multivariable analysis, CMV viremia is still associated with IPA (adjusted OR, 3.39; 95% CI, 1.39–8.26; *p*-value = 0.007) (Supplementary Table 2 and Supplementary Table 3). After stratifying patients by CMV viral load, the multivariable regression model showed that patients with higher blood CMV viral loads had a higher IPA risk (blood CMV viral load >10,000 IU/mL, adjusted OR 9.18; 95% CI 1.62–52.11; *p* value = 0.012, reference: blood CMV viral load ≤1000 IU/mL) (Table 3). There is an additive synergistic effect on IPA risk between CMV viremia and influenza (Synergy index, 2.54) (Table 4).

Subgroup analysis for old-age, critically ill patients

After stratifying patients by age, patients younger than 65 years with CMV viremia did not have an increased IPA risk (adjusted OR, 1.68; 95% CI 0.32–8.86; *p*-value = 0.542). In univariable analysis, patients aged 65 years or older had higher risk of IPA, and the effect was not statistically significant in multivariable analysis (crude OR, 12.60; 95% CI, 2.44–65.06; *p*-value = 0.003; adjusted OR, 4.82; 95% CI, 0.86–27.06; *p*-value = 0.074) (Supplementary Table 4).

Discussion

To the best of our knowledge, this is the first study to focus on the association between CMV viremia and IPA in critically ill patients. In this retrospective cohort study, we included 136 critically ill patients who received the BAL fluid GM test, blood CMV viral load test, and influenza test. According to the logistic regression model, our data demonstrated that CMV viremia and influenza infection are independent risk factors of IPA for critically ill patients. Furthermore, the risk of IPA rises with increased blood CMV viral loads. In contrast, detectable CMV in BAL fluid was found not to be an independent risk factor of IPA for critically ill patients. Previous studies targeting CMV and invasive fungal infection have been performed in patients with bone marrow transplantation, solid organ transplantation and hematologic malignancy.[17] In our cohort, only a few patients received either bone marrow (n = 7) or solid organ transplantation (n = 9). Additionally, there were 32 (23.53%) patients with hematologic malignancy in

Table 1 Characteristics of study population.

Characteristic	Invasive pulmonary aspergillosis			*p*-value[b]
	All (N = 136)	Negative (N = 115)	Positive (N = 21)	
	Number (%)	Number (%)	Number (%)	
CMV viremia	48 (35.29)	34 (29.57)	14 (66.67)	0.003
Influenza	22 (16.18)	12 (10.43)	10 (47.62)	<0.001
Detectable CMV in BAL[a] (N = 115)	72 (62.21)	59 (61.46)	13 (68.42)	1.000
Age (years), median (IQR)	65 (54.5, 74.5)	64.0 (55.0, 74.0)	66.0 (53.0, 72.0)	0.962
Age ≥65 years	68 (50.00)	57 (49.57)	11 (52.38)	1.000
Male sex	91 (66.91)	78 (67.83)	13 (61.90)	0.781
APATCHE II score, median (IQR)	23 (18.0, 30.0)	23.0 (18.0, 29.0)	27.0 (20.0, 32.0)	0.141
Comorbidity				
Hematologic malignancy	32 (23.53)	25 (21.74)	7 (33.33)	0.269
Received bone marrow transplantation	7 (5.15)	4 (3.48)	3 (14.29)	0.074
Solid-organ malignancy	35 (25.74)	31 (26.96)	4 (19.05)	0.624
Chemotherapy in recent one month	24 (17.65)	18 (15.65)	6 (28.57)	0.209
Prolonged neutropenia[c]	11 (8.09)	8 (6.96)	3 (14.29)	0.375
AIDS with CD4 <200	4 (2.94)	3 (2.61)	1 (4.76)	0.493
Connective tissue disease	13 (9.56)	10 (8.70)	3 (14.29)	0.424
Solid-organ transplantation	9 (6.62)	9 (7.83)	0 (0.00)	0.354
Prolonged systemic corticosteroid use[d]	46 (33.82)	37 (32.17)	9 (42.86)	0.484
Other immunosuppressant agent use	19 (13.97)	17 (14.78)	2 (9.52)	0.737
Inherited severe immunodeficiency	0 (0)	0 (0.00)	0 (0.00)	–
Malnutrition[e]	22 (16.18)	21 (18.26)	1 (4.76)	0.196
Diabetes mellitus	35 (25.74)	31 (26.96)	4 (19.05)	0.624
Chronic kidney disease[f]	29 (21.32)	26 (22.61)	3 (14.29)	0.565
Liver cirrhosis	8 (5.88)	6 (5.22)	2 (9.52)	0.358
Pre-existing structural lung disease	29 (21.32)	22 (19.13)	7 (33.33)	0.155

[a] There are 19 and 2 missing data in patients with and without invasive pulmonary aspergillosis, respectively.
[b] Chi-square test or Fisher's exact test for categorical variables/T test or Mann-whitney U test for continuous variables.
[c] Prolonged neutropenia is defined as an absolute neutrophil count less than 500 for 10 days or longer.
[d] Prolonged systemic corticosteroid use is defined as patients having received more than 10 mg prednisolone equivalent dose of a corticosteroid per day for 14 days or longer.
[e] Malnutrition is defined as a body mass index less than 18.5 kg/m^2.
[f] Chronic kidney disease is defined as an estimated Glomerular filtration rate of less than 60 mL/min/1.73^2.
AIDS = acquired immunodeficiency syndrome, BAL = bronchoalveolar lavage, CMV = cytomegalovirus.

our cohort; however, hematologic malignancy was not an independent risk factor for IPA in our statistical model.

In this study, we used modified *AspICU* algorithm to define patients with IPA. As aforementioned, the modified *AspICU* algorithm has been used in previously published studies with critically ill patients.[15,20] In our cohort, the mean optical density index (ODI) of the GM index was 3.83 (SD, 2.30). D'Haese J et al.[25] showed that if the BAL fluid GM ODI is greater than 0.8, the sensitivity, specificity, positive predictive value and negative predictive value for IPA diagnosis are 86.4%, 90.7%, 81% and 93.6%, respectively. An ODI value higher than 3.0 corresponds to a 100% specificity, irrespective of the pretest probability. Although the revised EORTC/MSG criteria require a microbiologic and/or histopathologic finding of fungal invasion in lung tissue to define proven IPA,[12] surgical lung biopsy carries a relatively high complication rate and morbidity in critical-ill patients.[26,27] Besides, the accuracy of histopathologic and cytopathologic examination for Aspergillus species is low.[28]

Whether CMV is the culprit or simply a bystander in critically ill patients remains controversial.[29] Previous observational studies have revealed that critically ill patients with CMV viremia have a higher mortality rate, prolonged ICU and hospital stay, prolonged mechanical ventilation use and more frequent infections.[1,6,30,31] However, the levels of several inflammatory cytokines tend not to be significantly different between patients with CMV viremia and those without.[32,33] A randomized control trial conducted by Limaye et al. found that ganciclovir or valganciclovir did not reduce the IL-6 level of CMV-seropositive critically ill patients, and there was no improvement for clinical outcomes in patients who received anti-viral treatment.[34] A prospective study about prophylaxis of CMV viremia in critically ill patients by using valganciclovir or valacyclovir was halted prematurely because of higher mortality in the valganciclovir group.[35] Nevertheless, CMV viremia increases the IPA risk in immunocompromised patients.[18] Evidence from *in vitro* study showed that CMV impaired the function of antigens presenting cell, proliferation of lymphocytes and activity of NK cells.[36]

In our cohort, 127 (93.4%) of 136 patients received at least one NAAT for diagnosis of influenza. Meta-analysis

Table 2 Univariate and multivariate logistic regression for invasive pulmonary aspergillosis (N = 136).

	Crude OR (95% CI)	*p*-value	Adjusted OR[a] (95% CI)	*p*-value
CMV viremia	4.76 (1.77–12.84)	0.002	3.98 (1.26–12.60)	0.019
Influenza	7.80 (2.75–22.18)	<0.001	8.72 (2.64–28.82)	<0.001
Detectable CMV in BAL	0.95 (0.33–2.79)	0.933		
Age ≥65 years	1.12 (0.44–2.84)	0.812		
Male sex	0.77 (0.29–2.02)	0.597	1.00 (0.32–3.14)	0.997
APATCHE II score	1.04 (0.98–1.10)	0.216	1.03 (0.96–1.11)	0.363
Comorbidity				
Hematologic malignancy	1.80 (0.66–4.94)	0.254	0.49 (0.10–2.47)	0.385
Received bone marrow transplantation	4.63 (0.95–22.40)	0.057	5.54 (0.48–63.85)	0.170
Solid-organ malignancy	0.64 (0.20–2.04)	0.449		
Chemotherapy in recent one month	2.16 (0.74–6.30)	0.160	1.64 (0.32–8.44)	0.557
Prolonged neutropenia[b]	2.23 (0.54–9.20)	0.268	4.20 (0.42–41.98)	0.222
AIDS with CD4 <200	1.87 (0.18–18.86)	0.597		
Connective tissue disease	1.75 (0.44–6.98)	0.428		
Solid-organ transplantation	0.26 (0.01–5.41)	0.385[f]		
Prolonged systemic corticosteroid use[c]	1.58 (0.61–4.08)	0.344	1.27 (0.39–4.14)	0.698
Other immunosuppressant agent use	0.61 (0.13–2.85)	0.526		
Malnutrition[d]	0.22 (0.03–1.76)	0.155		
Diabetes mellitus	0.64 (0.20–2.04)	0.449		
Chronic kidney disease[e]	0.57 (0.16–2.09)	0.397		
Liver cirrhosis	1.91 (0.36–10.19)	0.448		
Pre-existing structural lung disease	2.11 (0.76–5.86)	0.150		

[a] Multivariable logistic regression analysis of variables, variables included CMV viremia, influenza, male sex, APATCHE II score, hematologic malignancy, received bone marrow transplantation, chemotherapy in recent one month, prolonged neutropenia and prolonged systemic corticosteroid use.
[b] Prolonged neutropenia is defined as absolute neutrophil count less than 500 for 10 days or longer.
[c] Prolonged systemic corticosteroid use is defined as patients having received more than 10 mg prednisolone equivalent dose of a corticosteroid per day for 14 days or longer.
[d] Malnutrition is defined as body mass index less than 18.5 kg/m^2.
[e] Chronic kidney disease is defined as an estimated Glomerular filtration rate of less than 60 $mL/min/1.73^2$.
[f] Firth logistic regression analysis of variables.
AIDS = acquired immunodeficiency syndrome, BAL = bronchoalveolar lavage, CMV = cytomegalovirus.

conducted by Merckx et al. showed the sensitivities of nucleic acid amplified were 91.6% for influenza A and 95.4% for influenza B.[37] The specificity of NAAT was more than 98%. For critically-ill patients, real-time PCR testing of lower respiratory tract specimens yield higher detecting rate than upper respiratory tract specimens.[38] One-hundred and twenty-two (89.7%) of 136 patients in our cohort had at least one test performed on lower respiratory tract specimen. The inconsistency of diagnosis between different influenza test was low, only 3 (2.2%) of 136 patients had inconsistent results among the influenza tests.

The prevalence of detectable CMV in BAL in our study population and initially included population were 62.21% (72 of 115) and 45.85% (94 of 205), respectively. In a prospective longitudinal double-blinded observational study for non-immunosuppressed CMV-seropositive ICU patients with severe sepsis, 25 (32.5%) of 77 patient had positive CMV PCR in tracheal secretion.[39] In another prospective

Table 3 Logistic regression analysis for invasive pulmonary aspergillosis after stratifying by blood CMV viral load.

	Crude OR (95% CI)	*p*-value	Adjusted OR[a] (95% CI)	*p*-value
bCMV viral load (IU/mL)				
≤1000	Ref.		Ref.	
>1000～≤10,000	1.77 (0.51–6.11)	0.367	1.29 (0.29–5.65)	0.739
>10,000	4.04 (1.04–15.74)	0.044	9.18 (1.62–52.11)	0.012

[a] Multivariable logistic regression analysis of variables, variables included CMV viremia, influenza, male sex, APATCHE II score, hematologic malignancy, received bone marrow transplantation, chemotherapy in recent one month, prolonged neutropenia and prolonged systemic corticosteroid use.
bCMV: blood cytomegalovirus.

Table 4 Logistic regression analysis for invasive pulmonary aspergillosis after grouping by CMV viremia and influenza.

Characteristic	Invasive pulmonary aspergillosis						
	Negative (N = 115)	Positive (N = 21)	*p*-value[b]	Crude OR (95% CI)	*p*-value[c]	Adjusted OR (95% CI)	*p*-value[d]	Synergy Index
	number (%)	number (%)						
Group [a]								2.54
CMV (−) and Influenza (−)	74 (64.35)	4 (19.05)	<0.001	Ref.		Ref.		
CMV (+) and Influenza (−)	29 (25.22)	7 (33.33)		4.47 (1.22—16.41)	0.024	4.40 (1.09—17.81)	0.038	
CMV (−) and Influenza (+)	7 (6.09)	3 (14.29)		7.93 (1.47—42.78)	0.016	10.39 (1.74—62.18)	0.010	
CMV (+) and Influenza (+)	5 (4.35)	7 (33.33)		25.90 (5.63—119.16)	<0.001	33.49 (5.98—187.47)	<0.001	

[a] CMV: CMV viremia; (−): no; (+): yes.
[b] Fisher's exact test for categorical variables.
[c] Logistic regression analysis of variables.
[d] Multivariable logistic regression analysis of variables, variables included CMV viremia, influenza, male sex, APATCHE II score, hematologic malignancy, received bone marrow transplantation, chemotherapy in recent one month, prolonged neutropenia and prolonged systemic corticosteroid use.

observational study for CMV-seropositive patients in surgical and trauma ICUs, 42 (42%) of 99 patients had positive CMV PCR by tracheal aspiration.[40] Because the retrospective designed of the study, the high prevalence of detectable CMV in BAL in our cohort might not represent the true prevalence of CMV in BAL sample in the ICUs.

This study has several limitations. First, this study was conducted retrospectively and the BALs were arranged based on clinical judgement. The study population have higher prevalence of hematologic malignancy, organ transplantation, immunocompromised status and CMV viremia than the excluded patients. The study population may not be representative of the entire ICU population. However, we have adjusted these variables in the multivariable analysis, and the result was still robust. With respect to the prevalence of CMV viremia and IPA, the APACHE II scores of the patients in our cohort are similar to other studies,[10,31] the selection bias in our study might be minor. Second, the diagnosis of IPA was made by the modified *AspICU* algorithm rather than histopathology. Nevertheless, the diagnostic value of galactomannan test for IPA had been validated,[25] and modified *AspICU* algorithm has been used in several published studies for critically ill patients.[15,20] Besides, the GM ODI values of IPA patients is significantly higher than standard value, and so the sensitivity and specificity of IPA diagnoses in our cohort are reliable. Third, not every patient in our cohort received an examination for all pathogenic viruses, and we did not analyze the association between IPA and other virus. Some investigators have proposed an association between IPA and respiratory syncytial virus (RSV) in patients with hematologic malignancy.[41,42] Nevertheless, thirty-two (23.53%) of 136 patients had hematologic malignancy, and 25 (78.13%) of those 32 patients had the PCR-based RSV test in our study. The effect of RSV infection was small because there were only 2 (8.0%) of 25 patients that tested positive for RSV PCR. Forth, we only included patients had BAL-GM test in the study population. Patients had serum GM test but had no BAL-GM were not included for analysis. However, only 18 ICU patients were under this situation during the study period. After added these patients into analysis, the association between CMV viremia and IPA were still robust (Supplementary Table 5).

Conclusion

This study demonstrated that CMV viremia is associated with IPA for critically ill patients. For patients with CMV viremia, the risk of IPA rises with increases in the blood CMV viral load. CMV viremia and influenza have an additive synergistic effect on IPA risk. Nevertheless, further prospectively designed studies are warranted to confirm whether CMV viremia is an independent risk factor of IPA for critically ill patients.

Ethical approval

The Institutional Review Board of NCKUH approved this retrospective cohort study (A-ER-108-231) before commencement.

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Declaration of competing interest

All authors declare no conflicts of interest.

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Appendix A. Supplementary data

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