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Detection of Human Cytomegalovirus in Bronchoalveolar Lavage of Intensive Care Unit Patients

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To the Editor:

The seroprevalence of human cytomegalovirus (CMV) is very high worldwide (1,2). Spectrum of disease by CMV ranges from asymptomatic state or a mononucleosis-like syndrome to severe disease including pneumonia, retinitis or gastrointestinal infection. The most severe disease occurs in congenital infection and in immunosuppressed patients, in whom the virus act as an opportunistic pathogen.

Its role in other populations is less clear and controversial (3). Some studies in critical patients describe a relation between CMV and increase mortality rates, longer length of stay and prolonged needed of mechanical ventilation (3, 4, 5). The incidence of active CMV infection depends on the diagnostic method used. Using the most sophisticated available biological tools, the incidence can reach 15–20 % of (intensive care unit) ICU patients (6). We aimed to assess the incidence, clinical characteristics, risk factors, and outcomes of ICU patients with CMV detection in BAL.

We performed a prospective observational cohort study of consecutive adult patients admitted to two ICUs within 24 hours of admission to the Emergency Department. This study has been conducted at Hospital Clínic of Barcelona, Barcelona, Spain, between January 2013 and November 2015. Inclusion criteria were: 1) ICU admitted patients, 2) Practice of BAL. The decision of performing bronchoscopy was made by the attending physicians. Due to the nature of the study, the researchers had no impact in this decision.

The following parameters were recorded at admission: demographic, co-morbidities, immunodepressed status, antibiotic treatment in the previous 30 days before hospital admission, treatment with oral and inhaled corticosteroids, clinical symptoms,

laboratory parameters, diagnostic procedures, ventilatory support, length of hospital stay, length of ICU stay, and 30-day mortality. Immunosuppression was defined as presence of solid organ or bone marrow transplantation, human immunodeficiency virus (HIV) infection, cancer under chemotherapy, and/or treatment with corticosteroids (daily doses >20 mg prednisolone-equivalent for more than two weeks). Sepsis-related organ failure assessment (SOFA) score was calculated at ICU admission (7). BAL were cultured for bacteria, fungi and mycobacteria. One hundred microliters of BAL were inoculated onto sheep blood, chocolate, blood charcoal yeast extract (BCYE) and Sabouraud agar. All cultures were incubated at 37°C under aerobic conditions and in CO_2-enriched atmosphere, except for Sabouraud agar that was incubated at 25ºC. Cultures were evaluated for growth 24h and 48h later and discarded if negative. Bacterial identification and antibiotic susceptibility tests were performed according EUCAST guidelines and breakpoints (version 5.0, 2015; http://www.eucast.org). *Pneumocystis jirovecii* was detected by methenamine silver stain. For the detection of herpes simplex virus 1 (HSV-1) and 2 (HSV-2) on BAL, we used human fibroblast cells monitored for up to 1 week for sign of infection. A BAL was considered positive when a cytopathic effect was observed on conventional cell cultures and then confirmed by inmunofluoresce detection of the antigen. Molecular detection of CMV in BAL and blood was performed by real-time quantitative PCR (ELITechGroup, Italy), after extraction of DNA with DSP Virus/Pathogen Midi kit (Hilden, Germany) on a QIAsymphony automated platform (Qiagen, Germany). This technique has a detection limit of 20 copies/mL and a quantitative limit of 282 copies/mL. Other respiratory virus were detected by two multiplex reverse transcription nested-PCR assays as previously described (8).

We report the number and percentage of patients for categorical variables and the median (interquartile range [IQR]) for continuous. Categorical variables were compared using the χ^2 or the Fisher exact test. Continuous variables were compared using the Mann-Whitney test. Logistic regression analyses were performed to identify variables associated with positive detection of CMV; variables that showed a $p<0.20$ in the univariate analyses were included in the multivariate model (backward stepwise procedure). The Hosmer-Lemeshow goodness-of-fit test was performed to assess the overall fit of the multivariate model (9). The area under the receiver operating characteristic (ROC) curve of the multivariate model to predict positive detection of CMV was calculated. Internal validation was conducted using ordinary nonparametric bootstrapping with 1,000 bootstrap samples and bias-corrected, accelerated 95% confidence intervals (CIs) (10). The level of significance was set at 0.05 (2-tailed). All analyses were performed using IBM SPSS Statistics 22.0 (Armonk, New York, USA).

During the study period 880 patients were admitted to the two ICUs. BAL was practice in 133 (15%) patients. The three main causes of ICU admission in these 133 patients were: respiratory failure (n=83, 62%), septic shock (n=23, 17%) and cardiac failure (n=11, 8%). The main cause for BAL practice was suspected respiratory infection. Detection of CMV in BAL sample was positive in 26% (35/133) of the samples, corresponding to a 4% (35/880) of the ICU patients, with a median (IQR) of 7,637 (2604-47249) copies/mL. The detection of CMV was performed in 19 samples of blood from patients with CMV in BAL, being positive in 13/19 (68%) cases with a median of 4,323 (433-2272) copies/mL.

In 18/133 (14%) BAL CMV was the only microorganism detected, in 17/133 (13%) CMV and other microorganisms, in 49/133 (37%) only other microorganisms different from CMV and in 49/133 (37%) no microorganism was detected. *Pseudomonas aeruginosa* (16/133, 12%) was the most frequent microorganism isolated, followed by *Stenotrophomona maltophilia* (8/133, 6%), rhinovirus (7/133, 5.3%) and influenza virus A (7/133, 5.3%). Demographics and clinical characteristics are presented in Table 1. Patients with CMV had received more previous systemic corticosteroids (49% vs. 21%, p=0.002), were more frequently immunosuppressed (71% vs. 48%, p=0.017), had longer hospital stay (35 vs. 46 days, p=0.017), and had higher 30-day mortality (64% vs. 41%, p=0.024). Multivariate logistic regression analysis revealed that previous use of corticosteroids (OR 3.46, 95% CI 1.53 to 7.86) was the only risk factor for positive detection of CMV. The area under the ROC curve was 0.64 (95% CI 0.52 to 0.75) for the predictive model of positive detection of CMV. The only variable included in the model demonstrated robust results, with a small 95% CI around the original coefficient.

What we learned from our study is that the detection of CMV in BAL was positive in 4% (35/880) of the ICU patients, which is a lower incidence than previously published (6), but it is remarkable that 29% of the patients with CMV detection in BAL were immunocompetent.

Immunosuppression was associated with CMV detection in BAL, at the expense of systemic corticosteroid.

The detection of CMV in BAL was associated to a longer hospital stay and higher mortality, as describe previously (3, 11). The mechanism that could explain it is complex: direct CMV pathogenicity (12) or indirect CMV effects (13), such as CMV

mediated inmunosuppression (14) and CMV-mediated lung injury. ICU stay length was longer in the group of patients with CMV but not statistically significant, probably because of the sample number.

All of these studies are observational, which leads us to the question of whether there is a causal relationship between CMV infection and unfavourable outcomes. However, some studies show that treatment with ganciclovir or foscarnet have improved the outcome in UCI patients with CMV pneumonia (15).

Negative associated outcomes suggest that detection screening for CMV would be necessary in all patients with suspicion of respiratory infection in ICU. Additional prospective trials are necessary to confirm this hypothesis.

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16. Table 1. Baseline characteristics and clinical outcomes depending on the CMV PCR result.

Variable	Negative CMV (n=98)	Positive CMV (n=35)	p-Value
Demographic			
Age, median (IQR), years	62.0 (23.0)	61.0 (24.0)	0.37
Men, n (%)	63 (64)	23 (66)	0.88
Current smoker, n (%)	31 (36)	7 (25)	0.28
Current alcohol consumer, n (%)	8 (10)	2 (7)	>0.99
Immunocompromised, n (%)[a]	47 (48)	25 (71)	**0.017**
HIV	8 (8)	3 (9)	>0.99
Transplant	19 (19)	9 (26)	0.43
Cancer	16 (16)	7 (20)	0.62
Systemic corticosteroid[b]	21 (21)	17 (49)	**0.002**
Diagnosis at ICU admission			0.73
Respiratory failure	59 (60)	24 (69)	
Septic shock	18 (18)	5 (14)	
Cardiac failure	9 (9)	2 (6)	
Sepsis	5 (5)	1 (3)	
Other	7 (7)	3 (9)	
Previous antibiotic, n (%)	7 (7)	3 (9)	0.72
Comorbidities, n (%)[c]	76 (78)	26 (74)	0.70
Chronic respiratory disease	27 (28)	8 (23)	0.55
Chronic cardiovascular disease	23 (24)	6 (17)	0.44
Diabetes mellitus	12 (12)	6 (17)	0.57

Variable	Negative CMV (n=98)	Positive CMV (n=35)	p-Value
Neurological disease	11 (11)	7 (20)	0.25
Chronic renal disease	12 (12)	7 (20)	0.27
Chronic liver disease	4 (4)	2 (6)	0.65
Arterial hypertension	38 (39)	18 (51)	0.19
Laboratory tests, median (IQR)			
Creatinine, mg/mL	1.0 (1.0)	1.2 (1.1)	0.98
C-reactive protein, mg/dL	12.5 (15.9)	11.9 (20.0)	0.87
White blood cell count, 10^9/L	10.0 (9.0)	9.0 (12.0)	0.41
SOFA score (ICU admission), median (IQR)	5 (6)	5 (5)	0.91
Outcomes			
Pulmonary complications, n (%)	16 (39)	5 (33)	0.70
NIMV, n (%)	59 (94)	17 (94)	>0.99
IMV, n (%)	72 (92)	26 (93)	>0.99
Hospital stay days, median (IQR)	35.0 (33.0)	46.0 (62.0)	**0.017**
ICU stay days, median (IQR)	19.5 (21.5)	31.5 (69.0)	0.070
30-day mortality, n (%)	38 (41)	21 (64)	**0.024**

17. BAL: bronchoalveolar lavage; HIV: human immunodeficiency virus;ICU: intensive unit care; IMV: invasive mechanical ventilation; IQR: interquartile range; NIMV: non-invasive mechanical ventilation. [a] Could have more than 1 immunodeficiency condition. [b] Daily doses >20 mg prednisolone-equivalent for more than two weeks. [c]Could have more than 1 comorbid condition.

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