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Prevalence of Pandemic Influenza a (H1N1) Virus and Cytomegalovirus Co-Existence at an Early Stage in Evangelismos Hospital-ICU Patients

Magira EE[1*], Asimakos A[1], Grispou E[2] and Zakynthinos S[1]

[1]*First Department of Critical Care, Evangelismos Hospital, School of Medicine, University of Athens, Athens, Greece*

[2]*Department of Molecular Biology, Evangelismos Hospital, School of Medicine, University of Athens, Athens, Greece*

[*]**Corresponding author:** Eleni E Magira, First Department of Critical Care, National and Kapodistrian University of Athens Medical School, 50 Marathonos St, Vrilisia Athens 15235, Greece, Tel: +302107488164; Fax: 011302107488164; E-mail: elmagira@yahoo.com

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Abstract

Objective: Cytomegalovirus (CMV) infections in immunocompetent ICU patients have been associated with poor outcomes. We determined the distribution of cytomegalovirus coinfection among patients admitted in the ICU1 of Evangelismos Hospital of Athens with or diagnosed with pandemic influenza A (H1N1) respiratory infection.

Patients: We examined retrospectively 33 ICU consequently patients with community or hospital-acquired pulmonary infection. Investigation of viral-viral coinfection rate was determined at the first 48 h after ICU admission.

Results: A total of 13 patients that received a diagnosis of influenza A (H1N1) had their diagnosis confirmed by influenza A rapid antigen testing and/or by influenza A (H1N1) polymerase chain reaction (PCR). Only three out of the 13 H1N1 positive patients were cytomegalovirus bronchoalveolar lavage (BAL)-PCR positive and 10 were negative. Additionally 2 out of the 20 H1N1 negative patients were CMV-BAL-PCR positive.

Conclusion: We did not find significant CMV distribution in our H1N1 positive patients at least at the early 4-5 days period after H1N1 infection. However, the study is interesting because patients with a combination of H1N1 and CMV infections have been described only rarely. The scarce presence of CMV in H1N1 patients may suggest that endogenous reactivation of CMV infection within the lungs does occur and that these patients may profit from antiviral therapy. The interplay between host and viruses is very complex and therefore the triggering for the CMV disease activation following H1N1 pneumonia is possible to require more than the absolute presents of the H1N1 virus especially in the ICU patients.

Keywords: Coinfection; Influenza; Cytomegalovirus; Early infection

Introduction

Bacterial co-infection with the pandemic influenza A (H1N1) is an important contributor to morbidity and mortality during influenza pandemics [1]. Viral co-infections, such as cytomegalovirus (CMV) the most prevalent viral disease globally, may also play a very important role in the overall outcome of critically ill patients who were admitted because of H1N1 pulmonary infection. However CMV co-infections with H1N1 pulmonary infection are poorly investigated and warrant more studies. The immunopathogenesis of CMV infection is highly complex as it engages both the innate and adaptive immunity, in addition to humoral immunity from B cells and plasma cells [2]. Cytomegalovirus can be pathogenic by a direct organ insult (such as for the lung), by decreasing host defenses against other microorganisms and/or by enhancing the body's inflammatory response (as in acute respiratory distress syndrome). Therefore CMV infections needs to be diagnosed early and placed on appropriate treatment since they associated with poor outcomes.

On the other hand influenza A viruses of the H1N1 subtype have an important epidemiologic impact in humans by causing seasonal epidemics of various degrees of severity. Upon each infection subjects develop innate and adaptive immune responses. Innate immunity is the first crucial step for the limitation of the initial viral replication and antigen load and also assists through activation of co-stimulatory molecules the production of antigen-specific lymphocytes of the adaptive immune response [3]. The cytokine-chemokine storm resulting by the severe dysregulation of the host innate immune response leads to `collateral' damage to the lung tissue [4].

We were looking at patients who were admitted in the ICU with a community or hospital-acquired respiratory infection. We determined the frequency of the H1N1 pulmonary infection and the distribution of CMV infection among the H1N1 positive patients. Cytomegalovirus becomes latent after primary infection, being dormant until some unrelated stimulus precipitates reactivation. The hypothesis is that influenza infection could predispose patients to CMV susceptibility infection. Moreover investigational studies of CMV and other upper respiratory virus's co-infection are rare and extensive further work needs to be done. The prevalence and the associated findings of the CMV co-infection with H1N1 virus are discussed in these preliminary results.

Definition

CMV pulmonary infection is defined as the detection of CMV in the lungs by culture or polymerase chain reaction (PCR), irrespective of symptoms or signs of disease [5]. CMV end organ disease like CMV

pneumonia is defined as the occurrence of clinical and radiographic evidence of pneumonia, in association with the isolation of CMV in BAL, or lung-tissue specimens or with the identification of CMV in lung tissue by histopathology, immunohistochemistry or PCR [6].

Statistical Analysis

Two-tailed Fisher's exact test (contingency table) was used to determine whether there was a statistical difference between the CMV frequencies of patients with H1N1 positive and negative pulmonary infection using the Instat version 3.05 (GraphPad, San Diego, CA) statistical analysis program for the Windows 97. Results were considered statistically significant when $p<0.05$.

Methods and Results

A total of 33 consecutive patients with respiratory community or hospital -acquired infection disease between November 2013 and April 2014 were retrospectively enrolled in the study that was conducted at the ICU1 of Evangelismos Hospital of Athens. All of the admitted adult patients with mechanical ventilation were screened for eligibility. Patients were included if ICU stay was longer than 3 days, lack of use of antiviral agents against CMV within the last 60 days and no previous treatment with immunosuppressive drugs. Patients were excluded for any of the following reasons: younger than 18 years old, history of immunodeficiency (including HIV infection), pulmonary malignancy, bronchiectasis or known or suspected pulmonary infection caused by Pneumocystis jirovecii. Patients with ventilator-associated pneumonia were also excluded. PCR-BAL for CMV was evaluated, in all patients who proved H1N1 positive, within the first 48 h after ICU admission. CMV PCR is more reliable than the CMV antibodies at the onset of CMV infection.

Among the 33 patients a total of 13 patients received a diagnosis of influenza A (H1N1) (Table 1) and had their diagnosis confirmed in the laboratory positive test result by influenza A rapid antigen testing and or by influenza A (H1N1) PCR. All patients were suffering respiratory symptoms (fever, dyspnea, and cough) for at least 48 h before ICU admission. All 13 patients were mechanically ventilated and they presented with a paO_2/FiO_2 ratio <150. BAL fluids after bronchoscopy were analyzed by PCR for CMV DNA in H1N1 positive patients. We do not tested CMV antibodies in serum.

CAP/HAIs (N=33)			
	H1N1 patients (+) N=13	**H1N1 patients (-) N=20**	**p-value**
Age (mean)	57	62	
Gender (F *vs.* M)	5	11	
Underlying diseases			
Transplantation	-	2(10%)	0.5
Severe obesity	1 (7.6%)	2 (10%)	1.0
Diabetes mellitus	3 (15.3%)	4 (20%)	1.0
Cancer	4 (30%)	4 (20%)	0.68
COPD	2 (15,4)	7 (35%)	0.2
Autoimmune disease	2 (15.4%)	2 (10%)	1.0
Outcome (died)	8 (61%)	10 (50%)	0.7
CMV BAL positive	3 (15.3%)	2 (10%)	0.36
CMV BAL negative	10 (77%)	18 (90%)	0.7

Table 1: Viral co-infection with H1N1 infection in patients admitted with community or hospital - acquired pneumonia. Cytomegalovirus (CMV), H1N1 (Influenza type A) BAL (Bronchoalveolar lavage), CAP (Community-acquired Infection), HAIs (Hospital- acquired Infections).

In patients with positive CMV DNA in lavage fluid, we further analyzed peripheral blood for the presence of CMV DNA and they were proved negative. The interpretation of CMV DNA viral load values can be complex since CMV viral load tests are considered laboratory-developed tests that are developed and validated by an individual laboratory to the standard of the laboratory-inspecting agencies [7].

Quantitative PCR that provides an estimate of the number of CMV among the 13 H1N1 patients were detected in only 3 patients (Table 2). These patients (2 males and 1 female) all had an increased risk of CMV disease (1 with rheumatoid arthritis, 2 with cancer). The other 10 H1N1 (+) patients were all PCR-BAL-CMV negative although they had identifiable concurrent illnesses that might have been associated with compromised immunity (Table 1). Among the 20 H1N1 (-) patients similarly 2 patients (1 renal transplant and 1 with cancer) were PCR-BAL-CMV positive. In most of the patients who were H1N1 negative PCR CMV was searched in bronchial secretions and it proved negative. The H1N1 patients had a mean age of 58 years *vs.* 62 years in controls. There were no statistically significant differences between the two groups with respect to gender and the comorbidities. No statistically significant difference in mortality was observed between

H1N1 positive and H1N1 negative patients (61% *vs.* 50%, p=0.7) (Table 1).

	H1N1 (+)			H1N1 (-)	
	Pt#1	**Pt#2**	**Pt#3**	**Pt#1**	**Pt#2**
BAL CMV (copies/ml)	1 x 10^4	1 x 10^3	1 x 10^3	8 x 10^3	1 x 10^3
Blood CMV (copies/ml)	undetected	undetected	undetected	undetected	undetected
Comorbidities	Rheumatoid Arthritis	Cancer	Cancer	Renal Transplant	Cancer

Table 2: The PCR-CMV results obtained from BAL samples. Among the 13 H1N1(+) patients CMV copies were detected in only 3 patients (Pt#1, Pt#2 and Pt#3) and among the H1N1 (-) patients CMV copies were detected in only 2 patients (Pt#1 and Pt#2). Cytomegalovirus (CMV), H1N1 (Influenza type A), BAL (Bronchoalveolar lavage).

The in-house quantitative CMV-DNA-real time PCR assays were performed as previously described [8]. Identification of the H1N1 virus was done by the RespiFast RG Panel kit (PathoFinder B.V., The Nethelands, distributed by Qiagen GmbH, Hilden, Germany), which is a qualitative multiplex PCR test appropriate for the detection and differentiation of 22 pathogens that cause respiratory tract infections in humans. It provides highly sensitive, accurate results for a broad range of pathogens – 18 viruses and four bacteria. No other respiratory viruses or bacteria in the study were detected.

Patients were followed-up from admission until their discharge from the ICU or their death. We compared cases with and without CMV co-infection.

Discussion

This retrospective study was looking at the impact of H1N1 virus on CMV activation in the period of the first 48 h after ICU admission and the first 4-5 days after influenza infection. In a small number of cases object of this study no differences emerged concerning the frequency of CMV pulmonary infections between patients with H1N1 infection hospitalized in ICU from about 48 h and patients hospitalized in ICU without H1N1 pulmonary infection.

Co-infection with CMV may prolong the status of persistent immunosuppression during severe H1N1 infection, which subsequently could associated with a high risk of death. This may be true since the 3 PCR-BAL-CMV positive cases identified among the H1N1 positive patients finally succumbed. However the death was due to septic shock and occurred after almost twenty days of ICU stay. Although the number of cases in this study is small, the presence of more than one virus at an early ICU stage did not seem to have a major clinical impact in terms of severity of illness in these patients. However, intensivists managing patients with pandemic H1N1 influenza virus infection should be alert to the possibility of co-infection or sequential infection with CMV. The pathophysiological and immunopathological effects of CMV co-infections are still to large extent clinical speculations.

Cytomegalovirus like H1N1 virus can infect a variety of cell types in the lungs. Immunosenescence, dysfunction of the immune system associated with aging, has been implicated in the CMV infection although there are many inconsistent findings [9]. In our total population the five CMV BAL (+) patients was 70, 72, 68, 70 and 59 years-old (H1N1 patients had a mean age of 58 years *vs.* 62 years in controls) and therefore age-related changes in the immune system could also attributed in the reactivation of the CMV. This is in line with the study of Chiche et al., reported that age was a risk factor for CMV infection [10].

Moreover the three H1N1 patients in this study that were detected with BAL positive load for CMV had an underline pathophysiology that influenced the immunological status of the patients. Among the H1N1 positive patients even CMV colonization which is usually transient may increases the risk of invasive CMV lung disease. The three H1N1 (+) CMV BAL (+) patients were finally succumb (100%) instead of the 5 out of the 10 H1N1 (+) CMV BAL (-) patients (50%). As it was mentioned co-infection had little impact on severity of illness or outcome in those admitted to intensive care. This is consistent with the finding that viral coinfection had little impact on morbidity and mortality in a sample consisting mostly of adult patients (79.3%) admitted to an intensive care unit (ICU) in Australia [11]. In a retrospective observational study with 142 critically ill children patients, showed that the co-infection of swine origin influenza A (H1N1) with respiratory syncytial virus (RSV) is a risk factor for respiratory failure and increased mortality [12]. None of these studies were mentioning the time frame of acquiring the second viral infection except, a recently published retrospective cohort study [13] reported that co-infection with H1N1 and another respiratory virus within 72 h of admission was not associated with worse clinical outcomes. Unfortunately occurrence of CMV-H1N1 coinfection was not reported [13]. The impact of early identification of viral coinfection will be particularly interesting in the course of critically ill H1N1 positive patients and may influence the outcome.

It should also be emphasized that the search of co-existence CMV and H1N1 infection in our study, was related to the first 48 h after ICU admission and the first 4-5 days after initiation of influenza symptoms. The negative association at an early stage may imply that other factors like length of ICU stay, ventilator days, and organ system dysfunction are necessary to influence the CMV activation. Moreover cytomegalovirus can cause pneumonia in patients with suspected VAP but this appears to occur after a median ICU stay of 18 days [14] and therefore the presence of CMV in our patients could not be attributed to VAP since CMV was searched soon after admission.

Although the 13 H1N1 (+) patients were all lymphopenic and could facilitate CMV infection predisposition some authors claims that the temporary presence of relative lymphopenia that usually the virus H1N1 causes does not significantly alter the quality of the immune status of the patients with H1N1 acute respiratory distress syndrome [15] On the other hand Corona-Nakamura Al et al., reported that

lymphopenia showed relative risk of 14.75 times for the appearance of CMV infection [16].

Several limitations of this study merit discussion including the fact that it is a retrospective study. This is a very small sample of patients who are H1N1 positive and CMV-BAL negative that we cannot extract conclusions. CMV pneumonitis infection is followed after a bacterial respiratory infection rather than another virus respiratory infection. A definite diagnosis of CMV pneumonitis requires a biopsy for the histological confirmation this is rarely performed in our ICU patients. One other limitation is that, our sample size lacked the power to show a difference in mortality, so the observed lack of difference may not be present in a larger study. Finally this study is limited by its retrospective design and it was conducted at a single center in a single ICU over a few months and thus may not be fully representative of other hospitals. However a key advantage of this study was the investigation of a possible correlation between the two viruses (H1N1 and CMV) at a very early ICU stage when the patient is not burdened by the length of hospitalization time, by other hospital-acquired infections and by extended ventilator use, factors that substantially affect the prevalence of CMV infection.

Conclusion

Based on this report the association between the pandemic influenza A (H1N1) and the cytomegalovirus is scarce. Viruses possibly differ in their ability to facilitate other viral confection. Pandemic influenza A (H1N1) causes direct disruption in the lower respiratory tract whereas CMV lies latent in cells of the myeloid. Initiation of lung CMV disease at least at the first 4-5 days of the initiation of the H1N1 lung infection symptoms has limited representation. Thus, it seems that infection by H1N1 virus does not cause early pulmonary CMV reactivation. This could indicate that the research of CMV in the first days after hospitalization may not be useful. However larger and deeper studies are required to draw reliable conclusions. It would have been important to perform molecular assays to control the length of H1N1 and CMV infections.

References

1. Cillóniz C, Ewig S, Menéndez R, Ferrer M, Polverino E, et al. (2012) Bacterial co-infection with H1N1 infection in patients admitted with community acquired pneumonia. J Infect 65: 223-230.
2. Taylor DO, Edwards LB, Boucek MM, Trulock EP, Aurora P, et al. (2007) Registry of the International Society for Heart and Lung Transplantation: twenty-fourth official adult heart transplant report-2007. J Heart LungTransplant 26: 769-781.
3. Mogensen TH (2009) Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev 22: 240-273.
4. Oslund KL, Baumgarth N (2011) Influenza-induced innate immunity: regulators of viral replication, respiratory tract pathology & adaptive immunity. Future Virol 6: 951-962.
5. Osawa R, Singh N (2009) Cytomegalovirus infection in critically ill patients: a systematic review. Crit Care 13: R68.
6. Ramanan P, Raymund R (2013) Cytomegalovirus Infections in Solid Organ Transplantation: A Review. Infect Chemother 45: 260-271.
7. Burd EM (2010) Validation of laboratory-developed molecular assays for infectious diseases. Clin Microbiol Rev 23: 550-576.
8. Wolf DG, Spector SA (1993) Early diagnosis of human cytomegalovirus disease in transplant recipients by DNA amplification in plasma. Transplantation 56: 330-334.
9. Brunner S, Herndler-Brandstetter D, Weinberger B, Grubeck-Loebenstein B (2010) Persistent viral infections and immune aging. Ageing Res Rev 10: 362-369.
10. Chiche L, Forel JM, Papazian LL (2011) The role of viruses in nosocomial pneumonia. Curr Opin Infect Dis 24: 152-156.
11. Blyth CC, Webb SA, Kok J, Dwyer DE, van Hal SJ, et al. (2013) The impact of bacterial and viral coinfection in severe influenza. Influenza Other Respi Viruses 7: 168-176.
12. Torres SF, Iolster T, Schnitzler EJ, Farias JA, Bordogna AC, et al. (2012) High mortality in patients with influenza A pH1N1 2009 admitted to a pediatric intensive care unit: a predictive model of mortality. Pediatr Crit Care Med 13: e78-83.
13. Echenique IA, Chan PA, Chapin KC, Andrea SB, Fava JL, et al. (2013) Clinical Characteristics and Outcomes in Hospitalized Patients with Respiratory Viral Co-Infection during the 2009 H1N1 Influenza Pandemic. PLoS One 8: e60845.
14. Papazian L, Fraisse A, Garbe L, Zandotti C, Thomas P, et al. (1996) Cytomegalovirus: An unexpected cause of ventilator-associated pneumonia. Anesthesiology 84: 280-287.
15. Cunha BA (2010) Cytomegalovirus pneumonia: community-acquired pneumonia in immunocompetent hosts. Infect Dis Clin North Am 24: 147-158.
16. Corona-Nakamura AL, Monteón-Ramos FJ, Troyo-Sanromán R, Arias-Merino MJ, Anaya-Prado R (2009) Incidence and predictive factors for cytomegalovirus infection in renal transplant recipients. Transplant Proc 41: 2412-2415.