Received: 23 July 2017 | Revised: 30 July 2018 | Accepted: 15 August 2018

DOI: 10.1111/crj.12961

ORIGINAL ARTICLE

WILEY

The association between *Cytomegalovirus* co-infection with *Pneumocystis* pneumonia and mortality in immunocompromised non-HIV patients

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Abstract

Introduction: Impact of Cytomegalovirus (CMV) co-infection pneumonia in non-HIV patients with *Pneumocystis jirovecii* pneumonia (PCP) is unclear.

Objectives: The aim of our study was to determine whether CMV co-infection is associated with an increased risk of mortality.

Methods: Our study was conducted at Ege University Hospital, Turkey. We used molecular assays to diagnose *Pneumocystis jirovecii* in respiratory samples, and CMV in both respiratory and blood samples. We compared morbidity and mortality stratified by CMV co-infection status.

Results: Between 2009 and 2015, 43 patients (mean age: 56.7 ± 15.3 years) were diagnosed with PCP. Only 3 of 43 patients had received PCP prophylaxis. We microbiologically confirmed CMV co-infection in 28 of 43 (65.1%) patients. Acute respiratory distress syndrome (ARDS) and requirement of mechanical ventilation were more common in the CMV co-infection group ($P = .019$ and $P = .031$ respectively), and duration of intensive care unit was also longer ($P = .006$). In univariate analyses, mortality at 30 days was higher in the CMV co-infection group as compared to the group with PCP alone (78.6% and 46.7% respectively; $P = .046$). In multivariate analyses, mortality was independently associated only with the presence of ARDS [OR: 6.22 95% CI 1.3-29.32] and the association with CMV co-infection was no longer significant [OR: 2.6 95% CI 0.49-13.72, $P = .257$].

Conclusion: The risk of mortality appears to be increased in the setting of CMV and PCP co-infection in HIV-uninfected immunocompromised patients. PCP prophylaxis use was lower than expected, suggesting low physician awareness of the risks of PCP in this population.

KEYWORDS

cytomegalovirus (CMV), HIV-negative (non-HIV), mortality, *Pneumocystis jirovecii*, pneumonia

1 | INTRODUCTION

Pneumocystis jirovecii is an opportunistic organism responsible for *Pneumocystis jirovecii* pneumonia (PCP). Patients with immunodeficiencies (such as HIV, hematological malignancies, solid tumors, organ transplants or connective tissue diseases) and those taking immunosuppressive treatment (such as corticosteroids or chemotherapeutic agents) are at greatest risk.[1-7] PCP carries a high mortality rate that is paradoxically higher in HIV-uninfected patients, for whom it ranges from 30% to 60%,[8,9] as compared to HIV-infected patients, for whom mortality ranges from 9.7% to 16.9%.[10]

The impact of *Cytomegalovirus* (CMV) co-infection pneumonia in HIV-uninfected patients with PCP is not well understood and it remains unclear whether antiviral therapy to treat CMV is indicated when PCP is already being adequately treated. A small retrospective study addressing this issue found high mortality rates regardless of the presence of CMV co-infection.[11] Another study found that pulmonary CMV co-infection does not increase risk of death.[12] These studies used less sensitive diagnostic techniques for detecting CMV, including a shell vial culture from bronchoalveolar lavage (BAL), which may have resulted in misclassification of patients. More recently, real-time polymerase chain reaction (PCR) has emerged as the preferred diagnostic technique for detecting both *P. jirovecii* and CMV in respiratory specimens.[13,14]

In this study, we aimed to determine whether there is an association between CMV co-infection and risk of 30-day mortality in immunocompromised, non-HIV patients with PCP. We used real-time PCR methods for detection of CMV and *P. jirovecii* to diagnose the respective pathogens with high sensitivity.[13,14]

2 | MATERIALS AND METHODS

2.1 | Study population

We conducted a retrospective cohort study with hospitalized patients in the Chest Disease Department of Ege University Hospital, Izmir, Turkey. We included all immunocompromised adult (18 years or older) patients without HIV infection diagnosed with PCP between January 2009 and December 2015.

2.2 | Sampling methods

We performed microbiological examinations to detect *P. jirovecii* on a variety of respiratory sample types, with the vast majority of cases being diagnosed using BAL or mini-BAL. We investigated sputum and endotracheal aspiration material in only 2 patients. In these cases, 1 patient was not intubated, and provided sputum samples, while the other patient provided sufficient respiratory material via endotracheal aspiration for examination.

2.3 | Microbiologic assessments

All respiratory samples were tested for bacteria, mycobacteria, fungi, viruses and for parasites. Blood was assayed for CMV DNA and *Aspergillus* galactomannan antigen levels.

Microscopic examination with Giemsa, Gram Weigert staining methods[15-17] and real-time PCR[18,19] were applied to all respiratory samples. DNA was extracted from raw respiratory fluid (BAL, mini-BAL, tracheal aspiration material and sputum). The QIAamp DNA mini kit was used for DNA isolation in accordance with the manufacturer's protocol (Qiagen). The definition of PCP was based on detecting *P. jirovecii* in respiratory samples using microscopy and molecular assays, in the setting of consistent clinical and radiological findings.

CMV detection was based on a commercial real-time PCR assay (CMV DNA kit, Abbott Diagnostics, USA and CMV QNP 2.0, Fluorion, Iontek, Turkey). Nucleic acid isolation from BAL was performed using the Roche High Pure Viral nucleic acid isolation kit (Boehringer Mannheim, Switzerland). The definition of CMV infection was based on detecting CMV DNA in respiratory samples, using molecular tools, in the setting of consistent clinical and radiological findings. If a plasma CMV viral load of higher than 2000 IU/ml was detected or a rapidly increasing CMV-load in the plasma, we also considered these findings as diagnostic for CMV infection.

The threshold values for bacterial quantitative evaluation were 10^5 cfu/mL for endotracheal aspirate material and 10^4 cfu/mL for mini-BAL and BAL. Mycological investigation employed direct microscopy with Sabouraud dextrose agar used as culture.

2.4 | Demographic, clinical and radiographic data

Two investigators (PKE and ZNT) reviewed medical records of the patients and abstracted data using a standardized case report form. At admission, data on age, gender, comorbid diseases, underlying immunosuppressive diseases, immunosuppressive treatment agent, presence of prophylaxis against *P. jirovecii*, clinical and radiological characteristics were collected. We classified disease severity of PCP as mild ($PaO_2 > 70$ mm Hg or $AaDO_2 < 35$), moderate ($PaO_2 \leq 70$ mm Hg or $AaDO_2 \geq 35$) or severe ($PaO_2 < 60$ mm Hg or $AaDO_2 \geq 45$).[20] We abstracted data on the PCP treatment regimen, antibiotics at admission, development of hospital-acquired infection, length of hospital stay and 30-day overall mortality.

2.5 | Statistical methods

We performed descriptive analysis for the demographic characteristics of the patients. The Mann-Whitney U test was performed to compare continuous variables with abnormal distribution and given the small number patients in subgroups. Categorical variables were compared by chi-square test. We performed univariate analysis for mortality. Covariates with a P value of <.05 and clinically important variables were used for multivariate logistic regression analyses. Models were constructed using the forward stepwise method. The Hosmer and Lemeshow test was used to assess the goodness of fit for logistic regression models. We considered $P < .05$ as statistically significant. SPSS version18 (SPSS, Chicago, IL, USA) was used for data recording and analysis.

3 | RESULTS

A total of 45 patients with a diagnosis of PCP were hospitalized at the chest department during the study period. We excluded 2 patients from the study as they were HIV-infected, leaving 43 patients with non-HIV-related PCP for analyses. Mean age was 56.7 ± 15.3 years and 69.8% were male. Identification of *P. jirovecii* was confirmed via microscopy (n = 2), real-time PCR (n = 30) or both methods (n = 11). CMV co-infection was confirmed in 28 patients, and of these, 18 were positive in both blood and respiratory samples, 5 only had a CMV DNA-positive result in their respiratory specimens and another 5 were diagnosed only from their blood examination.

Table 1 presents demographic characteristics and CMV co-infection status for all groups and subgroups. The most common etiology for immunosuppression was related to the use of immunomodulating drugs targeting connective tissue diseases. There was no significant difference in the rate of patients showing CMV co-infection between the various types of immunosuppressive drugs prescribed.

Table 2 shows clinical characteristics, results of blood analyses, microbiological examination results, disease severity, treatment of PCP, ratio of using methylprednisolone, stratified by CMV infection status. There were no significant differences between the two groups in terms of radiological findings or symptoms at admission. The primary treatment regimen for PCP consisted of trimethoprim/sulfamethoxazole and methylprednisolone. Seven patients did not receive treatment during their hospitalization, because they died or were discharged prior to diagnostic results being available. Three patients reported allergies to trimethoprim/sulfamethoxazole and alternative regimens were used. Triple therapy (trimethoprim/sulfamethoxazole, Clindamycin and Primaquine) was administered to 1 patient in the setting of resistance to trimethoprim/sulfamethoxazole. Outcomes of follow up complications and morbidity in the patients based on CMV co-infection status are shown in Table 3.

Table 4 shows the patient's microbiological and follow-up characteristics base on their 30-day mortality outcome. Among the group who died, there were 28 patients with severe disease versus nine in the surviving group ($P = .074$). There was no significant difference between two groups in terms of both the number of CMV-coinfected patients not treated for CMV (2 patients vs 2 patients, $P = .191$) and the number of PCP-infected patients not accordingly treated (5 patients vs 2 patients, $P = 1.0$).

Multivariate logistic regression analysis showed that mortality was associated with the presence of ARDS at hospital admission [OR: 6.22 (95% CI 1.32-29.32), $P = .021$]. Development of hospital-acquired infection during follow-up was another predictor of mortality, but its P value was found on limit and as unremarkably at the end of the mortality analysis [OR: 5.83 (95% CI 0.98-34.44), $P = .051$]. After adjustment, age and CMV co-infection were not found to be significantly associated with mortality; CMV co-infection's odds ratio was 2.6 [(95% CI 0.49-13.72), $P = .257$].

4 | DISCUSSION

In this study, we have shown that mortality was higher in non-HIV immunocompromised patients with PCP pneumonia when CMV co-infection was present, but that, after adjustment for other clinical characteristics, CMV co-infection was no longer an independent risk factor. We also found that development of ARDS and need for mechanical ventilation was more common in the CMV co-infection group, requiring a significantly longer duration of intensive care. Although complications occurred more frequently in the patients with CMV co-infection, presence of ARDS at admission was the only important independent predictor of mortality after adjustment for all pertinent clinical variables.

PCP carries significant risks for morbidity and mortality, especially in solid organ or hematological stem cell-transplanted patients and others receiving immunosuppressive therapy for connective tissue diseases or interstitial lung diseases. Despite the seriousness of this opportunistic infection, we were surprised to find that only three out of the 43 patients included in our study were prescribed appropriate prophylaxis against *P. jirovecii*. This suggests that additional training is needed in our hospital to raise physician awareness of the risks of PCP in HIV-uninfected, immunocompromised patient populations.

Due to severe respiratory failure, another challenge in HIV-uninfected populations is to apply invasive diagnostic approach for attaining an accurate diagnosis and thereby

TABLE 1 Demographic and clinical characteristics of patients with PCP

Parameters	Total (n = 43)	CMV-Positive (n = 28)	CMV-Negative (n = 15)	*P*-Value
Gender (male), n (%)	30 (69.8)	18 (64.3)	12 (80)	.49
Age, *years (mean ± SD)*	56.7 ± 15.3	57.5 ± 15.4	55.2 ± 15.4	
Underlying immunosuppressive disease, n (%)				
Connective tissue disease	14 (32.6)	10 (35.7)	4 (26.7)	.74
Hematological malignancy	9 (20.9)	4 (14.3)	5 (33.3)	.24
İnterstitial lung disease	6 (14.0)	4 (14.3)	2 (13.3)	1.0
Solid organ tumor	6 (14.0)	4 (14.3)	2 (13.3)	1.0
Stem cell transplantation	4 (9.3)	1 (3.6)	3 (20)	.11
Solid organ transplantation	4 (9.3)	1 (3.6)	3 (20)	.11
Vasculitis	4 (9.3)	4 (14.3)	0	.28
Other disease	5 (11.6)	5 (17.9)	0	.15
Comorbid disease, n (%)	15 (34.8)			
Cardiovascular disease	11 (25.6)	4 (14.3)	5 (33.3)	.24
Diabetes mellitus	4 (9.3)	2 (7.1)	2 (13.3)	.60
Chronic renal failure	3 (7)	3 (10.7)	0	.54
HBV	1 (2.3)	0	1 (6.7)	.35
Immunosuppressive therapy, n (%)[a]	40 (93)			
Steroid	28 (65.1)	19 (67.9)	9 (60)	.74
Anti-proliferative agent	18 (41.9)	11 (39.3)	7 (46.7)	.75
Anticancer agent	12 (27.9)	6 (21.4)	6 (40)	.28
T-cell immunosuppressant	5 (11.6)	3 (10.7)	2 (13.3)	1.0
Prophylaxis for PCP, n (%)	3 (7.0)	0	3 (20)	**.037**

Abbreviations: HBV, hepatitis B infection; PCP, *Pneumocystis jirovecii* pneumonia.
Other diseases: Nephritis (3), Inflammatory bowel disease (1), Common variable immunodeficiency (1).
[a]In total, 40 patients were receiving immunosuppressive therapy.

ensuring the correct treatment. In our study, for instance, if we had not performed BAL or mini-BAL, 5 patients who had pneumonia with four different microbial agents might have been given inappropriate or insufficient infection therapy. Some experts report that viral loads in blood samples should be assessed for CMV infection in immunocompromised patients.[21,22] According to our findings, 5 patients with CMV co-infection were correctly identified by their only blood CMV load.

Deciding if and when to initiate treatment for CMV has been another clinical challenge. Kim et al.[12] have suggested that anti-CMV treatment is not essential when CMV is co-isolated from the BAL in non-HIV immunocompromised patients. But in our study population, severe respiratory failure

TABLE 2 Clinical characteristics, microbiological examination and follow-up of patients based on CMV co-infection status

Parameters	CMV-Positive (n = 28)	CMV-Negative (n = 15)	*P*-Value
Admission at the hospital			
APACHE II score (n = 34)[a]	20 [16-25]	19 [15-23]	.74
PaO_2/FiO_2(n = 34)	151 [119-186]	172 [117-221]	.47
Presence of ARDS (n = 42), n (%)	20 (74.1)	8 (53.3)	.19
Presence of septic shock, n (%)	6 (21.4)	3 (20)	1.0
Results of blood analysis			
Leukocyte count (10^3/μL)[a]	11.2 [6.7-15.2]	5.8 [3.1-9.8]	**.017**
Neutrophil count (103/μL)[a]	10.0 [5.8-13.1]	4.3 [2.0-7.5]	**.001**
Presence of neutropenia, n (%)[a]	1 (3.6)	3 (20)	.11
CRP (mg/dL)[a]	16.1 [7.2-31.3]	11.1 [7.0-29.6]	.84
Albumin (g/dL)[a]	2.7 [2.6-3.0]	2.9 [2.6-3.6]	.26
Lactate dehydrogenase (U/L)[a]	869 [509-1327]	966 [411-1190]	.80
Pulmonary co-infection, n (%)[b]			
Bacteria	6 (21.4)	4 (26.7)	.71
Bacteria and fungus	4 (14.3)	0	.28
Fungus	6 (21.4)	1 (6.7)	.39
Fungus and virus other than CMV	1 (3.6)	0	1.0
Presence of severe disease in terms of PCP, n (%)	25 (89.2)	12 (0.80)	.12
PCP Treatment, n (%)			
Non-treated	4 (14.3)	3 (20)	.68
TMP-SMX	20 (71.4)	12 (80)	.71
TMP-SMX + Clindamycin + Primaquine	1 (3.6)	0	1.0
Clindamycin + Primaquine	1 (3.6)	0	1.0
Caspofungin + Clindamycin	1 (3.6)	0	1.0
Caspofungin	1 (3.6)	0	1.0
Using methylprednisolone, n (%)	23 (82.1)	13 (86.7)	1.0

Abbreviations: APACHE II score, acute physiology and chronic health evaluation II score; ARDS, acute respiratory distress syndrome; ICU, intensive care unit.
[a]Median [IQR].
[b]Pulmonary co-infection was detected in 17 (60.7%) patients with CMV co-infection and in 5 (33.3%) patients without CMV co-infection.

was common with 18 patients having PCR results positive for CMV in both their blood and respiratory samples. We chose not to ignore these positive bronchoalveolar results, and all patients with CMV infection were treated with intravenous ganciclovir administered as 5 mg/kg every 12 hours.

In non-HIV immunocompromised patients with PCP infection, there is a significant risk for morbidity and mortality, and as such we advocate that a rigorous diagnostic approach with comprehensive microbiologic assessments are critically important to help guide treatment decisions. In our study, 86% of patients required admission to the intensive care unit, and 74% required mechanical ventilation and a high proportion were treated for both CMV and PCP infections. These values are higher than reported in prior studies of non-HIV patients with PCP.[12,23] This difference may be explained by our aggressive diagnostic approach in patients with severe respiratory failure at our hospital, and because we used more rapid and sensitive real-time PCR assays to identify PCP and CMV. Prior studies relied on direct immune fluorescent test,[12,23,24] modified May-Giemsa stain[25] for PCP diagnosis and CMV culture for CMV infection,[12,24,25] which may have delayed diagnoses as well as the initiation of treatment. These more traditional techniques have been shown to have low sensitivity, potentially leading to under-diagnosis of PCP

TABLE 3 Outcomes of patients based on CMV co-infection status

Parameters	CMV-Positive (n = 28)	CMV-Negative (n = 15)	*P*-Value
Follow-up complication, n (%)			
ARDS	25 (89.3)	8 (53.3)	**.019**
Requirement of mechanical ventilation	24 (85.7)	8 (53.3)	**.031**
Hospital-acquired infection	14 (50)	4 (26.7)	.19
Morbidity, *day*[a]			
Treatment duration	15 [7-20]	14 [5-21]	.84
ICU duration	15 [9-19]	5 [1-11]	**.006**
Length of hospital stay	17 [10-24]	12 [10-25]	.49
Mortality, n (%)	22 (78.6)	7 (46.7)	**.046**

[a]Median [IQR].

and misclassification of patients as CMV-uninfected.[13,14] We believe the CMV co-infection rates, we found (65%), were higher than in the literature (17%-29%) because we used more sensitive diagnostic techniques.[12,24,25]

The overall mortality rate in our patients with PCP was 67.4%. Follow-up complications, such as development of ARDS and requirement of mechanical ventilation, as well as the mortality ratio, were significantly higher in the CMV co-infection group. In contrast to prior studies, mortality rate in the CMV co-infection group was higher than for those without CMV co-infection. This difference may be explained by CMV misclassification in the other studies.[11,12] As noted, CMV infection appeared to impact the prognosis of patients with PCP in our study,however, after adjustment only ARDS at hospital admission remained as an independent risk factor for 30-day mortality.

Our study has limitations. First, this was a single center study with a small sample size. Despite this limitation, our study adds to the literature, which has historically had small-to-modest population sizes and less severe disease as compared to our study. Secondly, the etiologies for immunosuppression in our patient population were predominantly due to medications that target connective tissue disorders. We caution generalization of our findings to other more diverse causes of immunosuppression. Third, we did not monitor clearance of *P. jirovecii* and CMV after treatment. Future studies should include assays to evaluate for pathogen clearance as a risk factor for outcomes.

TABLE 4 Univariate analysis of 30-day overall mortality

Parameters, *n (%)*	Dead (n = 29)	Alive (n = 14)	*P*-value
CMV co-infection	22 (75.9)	6 (42.9)	**.046**
Other pulmonary co-infection[a]			
Bacteria	8 (27.6)	2 (14.3)	.45
Bacteria and fungus	3 (10.3)	1 (7.1)	1.0
Fungus	6 (20.7)	1 (7.1)	0.39
Fungus and virus other than CMV	1 (7.1)	0	1.0
Duration of PCP treatment, *day* [b]	9 [4-16]	21 [0]	**<.001**
Follow-up complications			
ARDS	28 (96.6)	5 (35.7)	**<.001**
Requirement of mechanical ventilation	28 (96.6)	4 (28.6)	**<.001**
Hospital-acquired infection	16 (55.2)	2 (14.3)	**.019**

Abbreviations: ARDS, acute respiratory distress syndrome; PCP, *Pneumocystis jirovecii* pneumonia.
[a]Rate of other pulmonary co-infections was 62.1% in the dead group and 28.6% in the alive group.
[b]Median [IQR].

In summary, we show that the presence of CMV co-infection is associated with increased mortality among immunocompromised non-HIV patients with PCP. However, after adjustment for other co-variables, presence of ARDS at time of admission was the only independent predictor for mortality at 30 days. We also found that the use of prophylaxis against PCP was low in our setting, suggesting the need to increase physician awareness of the risks of PCP in immunocompromised HIV-uninfected populations.

ACKNOWLEDGMENT

The authors acknowledge and thank the American Thoracic Society (ATS); the ATS Methods in Epidemiologic, Clinical, and Operations Research (MECOR) Program; and especially David H. Au. We are also grateful to Mark Woodhead for his support.

CONFLICTS OF INTEREST

The authors declare no conflict of interest to declare relevant to the content of this manuscript.

AUTHOR CONTRIBUTIONS

Study concept and design: Bacakoglu, Korkmaz Ekren
Acquisition, analysis or interpretation of data: Korkmaz Ekren, Töreyin, Nahid, Doskaya, Caner, Turgay, Zeytinoglu, Toz, Bacakoglu, Guruz and Erensoy
Drafting of the manuscript: Korkmaz Ekren, Nahid
Critical revision of the manuscript for important intellectual content: Doskaya, Turgay, Toz, Caner, Bacakoglu
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Study supervision: Bacakoglu

ETHICS

The study was approved by the Ege University Ethics Committee (Approval number: B.30.2.EGE.0.20.05.00/OY/591/300).

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How to cite this article: Korkmaz Ekren P, Töreyin ZN, Nahid P, et al. The association between *Cytomegalovirus* co-infection with *Pneumocystis* pneumonia and mortality in immunocompromised non-HIV patients. *Clin Respir J*. 2018;12:2590–2597. https://doi.org/10.1111/crj.12961