Pneumocystis jirovecii pneumonia infections in HIV-uninfected patients hospitalized for COVID-19

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Introduction: Coronavirus Infectious Disease 19 (COVID-19) represents the infection caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Today is know that the invasive fungal infections (IFIs) are a non-negligible complication of COVID-19, especially among critically ill patients, and are also associated with a significantly higher mortality.
The most common IFIs that cause severe complication during COVID-19 are invasive candidiasis, pulmonary aspergillosis and mucormycosis. Literature data highlight that other IFIs, such as *Pneumocystis jirovecii* pneumonia (PJP), are only sporadically reported, and mostly in patients with concomitant HIV infection or in solid-organ transplant recipients. In the present work we conducted a case-control study to identify the risk factors for PJP in HIV-negative patients hospitalized for COVID-19 at Federico II University Hospital in Naples, Italy.

Materials and Methods: Retrospectively have been evaluated the clinical data of all the patients admitted for COVID-19 at the Infectious Disease Operative Complex Unit and Intensive Care Unit of the Federico II University Hospital from 1 November 2021 to 30 September 2022 and who were then diagnosed with PJP during the hospital stay.
The cases of PJP were matched with controls, selected among patients admitted for COVID-19 in the same period who did not receive a diagnosis of PJP, with a 1:2 ratio, based on age ± 10 years, solid-organ transplantation (SOT), hematological malignancies, and in the setting of PJP development (ICU vs. non-ICU).
The diagnosis of PJP was considered "proven" if *P. jirovecii* was detected with a direct immunofluorescence assay (DFA) on respiratory samples (Figure 1).

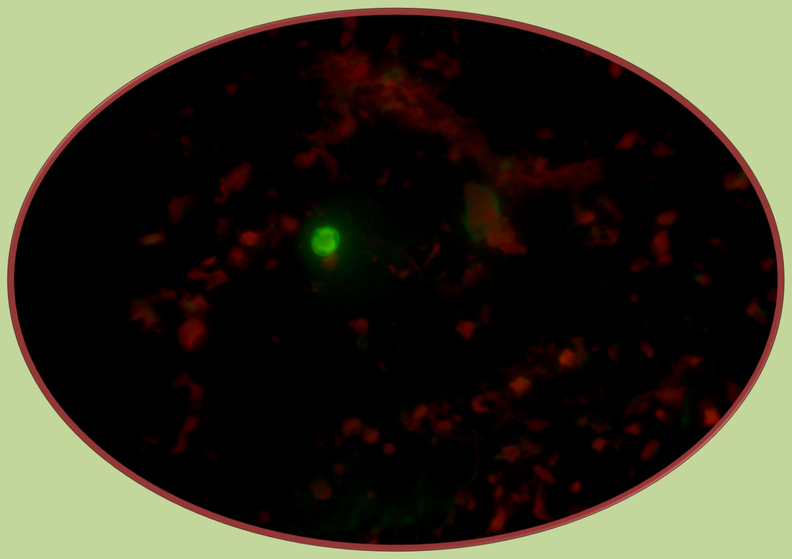

Figure 1: DFA from bronchoalveolar lavage fluid, showing a typical PJP cyst exhibiting apple-green fluorescence

	Overall N = 54	Cases N = 18	Controls N = 36	*p*-Value	OR (95%CI)	*p*-Value
Age, years, median (IQR)	60 (51–68)	60 (49.75–70)	60 (49–78)	0.98	0.994 (0.995–1.034)	0.754
Females, *n* (%)	19 (34.5)	7 (39)	12 (32)	0.764	0.754 (0.234–2.433)	0.637
Pregnant, *n* (%)	7 (12.7)	4 (22)	3 (8)	0.2	3.2 (064–16.32)	0.155
Charlson Comorbidity Index, median (IQR)	3 (1–5)	3 (0.75–5)	3 (1–5)	0.389	0.876 (0.685–1.12)	0.291
Deaths, *n* (%)	14 (25.5)	6 (33)	8 (21.6)	0.51	-	-
ICU admission, *n* (%)	20 (37)	9 (50)	11(30)	0.232	-	-
SARS-CoV-2 vaccination, *n* (%)						
1 dose	33 (60)	7 (39)	26 (70)	0.027	0.269 (0.083–0.877)	0.029
2 doses	29 (52)	6 (33)	23 (62)	0.042	0.304 (0.093–0.994)	0.049
3 doses	16 (29)	3 (16.7)	13 (35)	0.135	0.369 (0.9–1.5)	0.166
4 doses	2 (3.6)	0 (0)	2 (5.4)	1	0 (0–0)	-
Pneumocystis jirovecii prophylaxis recipients, *n* (%)	8 (14.5)	1 (5.6)	7 (19)	0.25	0.252 (0.029–2.226)	0.215
Length of stay, days, median (IQR)	16 (12–34)	20.5 (14.5–50.25)	15 (10.5–27)	0.058	1.033 (0.998–1.068)	0.062
Days of SARS-CoV-2 positivity, median (IQR)	20 (14–26)	25 (20–37)	16 (13–23)	0.014	1.141 (0.993–1.322)	0.062
Use of immunomodulatory drug for COVID-19, *n* (%)	6 (11)	3 (16.7)	3 (8)	0.381	2.267 (0.0409–12.5)	0.349
Hematologic malignancy, *n* (%)	29 (53)	5 (27.8)	10 (27)	0.39	0.564 (0.166–1.915)	0.359
Use of anti-CD20, *n* (%)	6 (11)	3 (16.7)	3 (8)	0.381	2.267 (0.409–12.5)	0.349
Chronic steroidal treatment, *n* (%)	8 (14.5)	3 (16.7)	5 (13.5)	1	1.28 (0.270–6)	0.756
Solid organ transplant recipients, *n* (%)	6 (11)	2 (11)	4 (10.8)	1	1.031 (0.171–6.23)	0.973
Cumulative steroid dose during hospital stay, milligrams, median (IQR)	84 (55–190)	178.5 (68–513)	78 (46–158)	0.026	1.004 (1–1.008)	0.042
Number of days of steroid, median (IQR)	15 (12–27)	15 (12–20)	16.5 (12–29.25)	0.718	0.984 (0.929–1.043)	0.596
Worst WHO grade, median (IQR)	4 (4–6)	5 (4–6)	4 (4–6.5)	0.434	1.287 (0.7–2.378)	0.421
Lowest PaO2/FiO2 ratio, median (IQR)	135 (89–235)	100 (65–191)	150 (100–269)	0.081	0.995 (0.988–1.002)	0.13
Days of highest O2, *n* (%)	7 (5–10)	7 (5–10)	6 (4.75–10)	0.849	0.965 (0.873–1.068)	0.5
Lowest lymphocyte value, cells/mm^3, median (IQR)	620 (320–1480)	540 (217–772)	780 (415–2313)	0.033	1 (0.999–1)	0.732
Highest CRP value, mg/dL, median (IQR)	10 (2.9–20)	14.4 (10–28.6)	6.3 (2.3–15)	0.005	1.076 (1.016–1.140)	0.012
Highest LDH value, IU/mL, median (IQR)	384 (289–524)	368 (289–452)	384 (258–533)	0.788	0.999 (0.996–1.002)	0.4
Ferritin on admission, median (IQR)	595 (246–1335)	740 (279–1342)	520 (205–1073)	0.332	1 (0.999–1.001)	0.588
Highest D-dimer value, median (IQR)	765 (533–1524)	761 (497–1392)	1051 (570–1526)	0.554	1 (1)	0.641

Table 1: Overall demographic and clinical characteristics of the population, group comparison, and univariate and multivariate analysis.

Results: 54 patients (18 cases and 36 matched controls) have been enrolled from a total of 380 patients admitted during the study period (14%). Among 18 cases of PJP, 16 were diagnosed as "proven", of which 10 were diagnosed with DFA on BAL, while 6 were diagnosed with DFA from nonbronchoscopic-obtained lower respiratory tract samples (bronchial aspiration or mini-BAL).
Seven of the eighteen cases were immunocompromised, of which five (27.8%) suffered from hematologic conditions and two (11%) were SOT recipients, while the other patients had no previous immunological impairment. Nine out of eighteen cases of PJP required ICU admission; among them, four were diagnosed before ICU admission and five during the ICU stay.
Patients with PJP had significantly lower median lymphocyte values (540 vs. 780 cells/mm3, p = 0.033), longer COVID-19 disease duration (25 vs.16 days, p = 0.014), a higher cumulative dose of steroid received (178.5 vs.78 mg, p = 0.026), higher CRP values (14.4 vs. 6.3 mg/dL, p = 0.005), and a lower SARS-CoV-2 vaccination rather than the controls (7 patients with at least one dose vs. 26 patients with no history of vaccination, p = 0.029) (Table 1).

Conclusions: According to our results, despite that we could not find any particular subgroup of COVID-19 patients at high risk of PJP development, we can say that PJP can occur in any COVID-19 patient who received corticosteroids for at least 2 weeks, regardless of previous immunological status (immunosoppressive conditions).
Nevertheless, the present study has some limitations: the retrospective design; the small number of subjects involved given the rarity of the disease; data collection from a single center in a limited period of time; and maybe potential selection bias.
All together these findings however strongly suggest a need for greater attention to potential risk factors for *P. jirovecii* development in COVID-19 patients.

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