Brief Report

Prevalence of Pneumocystis jirovecii Colonization in Non-critical Immunocompetent COVID-19 Patients: A Single Center Prospective Study

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Brief Report

Prevalence of *Pneumocystis jirovecii* Colonization in Non-Critical Immunocompetent COVID-19 Patients: A Single Center Prospective Study

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Abstract: BACKGROUND: *Pneumocystis jirovecii* pneumonia (PJP) is an invasive fungal infection (IFI) that occurs mainly in immunocompromised hosts. As we observed a high prevalence of PCP as a complication of COVID-19 in immunocompetent patients, we conducted a study to evaluate the prevalence of *P. jirovecii* colonization with PCR on oral washing samples (OWS) among non-immunocompromised and non-critical patients admitted for COVID-19 pneumonia at our University Hospital. METHODS: All patients over 18 years of age admitted to Infectious Diseases Unit for SARS-CoV-2 pneumonia between July 2021 and December 2022 were included. Patients undergoing invasive mechanical ventilation or ECMO, those with risk factors for developing PCP, and those receiving prophylaxis for *P. jirovecii* were excluded. Samples were collected by gargling with 10mL of 0.9% NaCl on day 14 of hospital stay or at discharge. RESULTS: Of 290 screened patients, 59 (20%) met the inclusion criteria and were enrolled. Only one of 59 patients (1.7%) resulted positive for *P. jirovecii* detection with PCR and the same patient was the only one to develop PCP in the follow up period. CONCLUSION: Our results are in line with the previous findings of other studies that confirmed a very low prevalence of *P. jirovecii* colonization on OWS in the immunocompetent population. Despite the limitation of the study, the fact that the only patient who tested positive *P. jirovecii* was the only one in our cohort to develop PCP leads us to reflect on the role of this non-invasive sample in predicting the risk of PCP in patients with COVID-19.

Keywords: COVID-19; Pneumocystis jirovecii; SARS-CoV-2; immunocompromised; pneumonia

1. Background

Pneumocystis jirovecii pneumonia (PCP) is an invasive fungal infection (IFI) that occurs mostly in immunocompromised patients, especially in HIV positive patients with a CD4+ lymphocyte count lower than 200 cells/mm3, solid organ transplant recipients and patients with hematologic malignancies or rheumatic conditions receiving prolonged doses of steroids or lymphocyte depleting agents.[1–3]

Despite less frequent than invasive aspergillosis, PCP can complicate the course of COVID-19 also in immunocompetent individuals, even though the exact prevalence of such IFI is not well established due to lack of standardized criteria among published studies.[4,5]

Pneumocystis jirovecii can colonize the respiratory tract of asymptomatic individuals, especially when affected by chronic respiratory diseases, and can spread to non-colonized individuals with an

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airborne route- in clinically evident disease in case of impairment of the immune system of the host.[6]

The definitive diagnosis of PCP can be made by detecting *P. jirovecii* on respiratory tract samples with direct immunofluorescence or traditional staining or by histopathological evidence, according to the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC).[7]

Polymerase chain reaction (PCR) for *P. jirovecii* on respiratory sample is a fast and very sensitive alternative to direct microbiology, although cannot discriminate between infection and colonization, therefore its positivity is considered as a minor microbiological criterion, together with beta-D-glucan (BDG) serum detection, to diagnose a probable PJP in patient with risk factors and clinical-radiologic signs of PCP.[7]

Despite the preferable sample to diagnose PCP is the bronchoalveolar lavage (BAL), non-invasive sampling of respiratory tract with induced sputum, nasopharyngeal aspirate and oral washing samples (OWS) have shown high sensitivity, especially with the aim of detecting colonization with molecular analysis such as PCR. [8,9] In fact, several studies aimed to describe the prevalence of *P. jirovecii* colonization have been carried out with the use of PCR on oral wash specimens also in healthy immunocompetent individuals, but never in COVID-19 patients.[8,10]

In our University Hospital we observed and reported an unexplainable high prevalence of PCP as complication of COVID-19 even in immunocompetent patients, while other published studies reported PCP mostly in HIV or transplanted individuals with COVID-19.[11–13] Therefore, we conducted a study to evaluate the prevalence and the features of *P. jirovecii* colonization with PCR on oral wash samples among non-immunocompromised and non-critical patients admitted for COVID-19 pneumonia at our institution.

2. Methods

2.1. Aim of the study

To establish the prevalence of *P. jirovecii* colonization in non-critical, immunocompetent patients admitted to medical wards for COVID-19 pneumonia and its relation to clinical, virological and individual variables.

2.2. Population

All the patients over 18 years of age with SARS-CoV-2 pneumonia admitted to medical units were screened for enrollment.

2.2.1. Inclusion criteria

- Patients over 18 years of age.
- Hospitalized in internal medicine and infectious diseases wards for SARS CoV-2 pneumonia.

2.2.1. Exclusion criteria

- Invasive mechanical ventilation or ECMO at enrollment.
- Patients on *P. jirovecii* prohylaxis or who are chronically taking or who have taken active drugs against *P. jirovecii* (trimethoprim-sulfamethoxazole, pentamidine, atovaquone, dapsone) within the last month.
- HIV infection.
- Solid organ or hematogenous stem cell transplant recipients.
- Active hematologic malignancies.

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- Connective tissue diseases with history of prolonged steroid therapy (>20 mg of prednisone or equivalents for >3 weeks) and/or lymphocyte depleting agents.
- Previous diagnose of PCP during their lifetime.
- Patients unable to express consent to participate.
- Patients unable to produce OWS.
- Patient not fulfilling criteria for PCP according to EORTC/MSGERC.

2.3. Definitions

- PCP was defined as "proven" if *P. jirovecii* was detected with direct immunofluorescence assay (DFA) on respiratory samples, "probable" in the presence of host factors, clinical features and microbiological evidence (PCR for *P. jirovecii* on respiratory samples or detectable serum BDG in two consecutive blood samples), and "possible" in the presence of host and clinical factors with the absence of microbiological evidence (EORTC/MSGERC criteria).[7]
- Colonization from *P. jirovecii* was defined as: i) absence of signs and symptoms of PCP; ii) respiratory specimen with detectable *P. jirovecii* DNA by nested PCR; iii) no criteria fulfilled for definitive or probable PJP according to EORTC/MSGERC.
- Charlson Comorbidity Index was calculated to evaluate patient's comorbidity.[14] Steroid dose was calculated as equivalent to dexamethasone, since dexamethasone was the most frequently used steroid drug in the studied population.
- COVID-19 severity was assessed with the World Health Organization 9-point severity scale as follows: 0: no clinical or virological evidence of infection; 1: ambulatory, no activity limitation; 2: ambulatory, activity limitation; 3: hospitalized, no oxygen therapy; 4: hospitalized, oxygen mask or nasal prongs; 5: hospitalized, noninvasive mechanical ventilation (NIMV) or high-flow nasal cannula (HFNC); 6: hospitalized, intubation and invasive mechanical ventilation (IMV); 7: hospitalized, IMV + additional support such as pressors or extracardiac membranous oxygenation (ECMO); 8: death.[15]

2.4. Sampling and data collection

As per routine clinical protocol at out Institution, all the patients admitted for SARS-CoV-2 pneumonia undergo a full blood picture and complete biochemical blood and urine analysis, HIV antibody test with 5th generation ELISA assay, chest X-ray and/or chest CT scan and arterial blood gas analysis.

Patients meeting inclusion and exclusion criteria were asked to sign and informed consent and to produce an OWS by gargling with 10 mL of sterile physiologic serum (0.9% NaCl) for a period of 2 min on the 14th day of hospital stay or at discharge, whichever came first. A serum sample for BDG detection was collected on the same day of oral wash sample.

Included patients were followed up for a period of 3 months with monthly scheduled visits at the post-COVID outpatient clinic of our institution and they were provided with a 12/24h telephone contact with the study center to report any symptoms or worsening of their status. Patients requiring medical attention were visited at the outpatient clinic within 48 hours from the call.

OWS were stored at -80°C after collection and only analyzed after the end of the follow-up study.

2.5. Laboratory analysis

The DNA of *Pneumocystis jirovecii* was searched out in oral wash samples by real-time PCR. The specimens were equilibrated at room temperature (RT) and 2ml of samples were centrifuged for 10 min at 10000 rpm at RT and suspended in 190 µL of Buffer G2 (EZ1 DNA Tissue Kit, QIAGEN GmbH,

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Hilden Germany) and in 10 µL of Proteinase K (EZ1 DNA Tissue Kit, QIAGEN GmbH, Hilden Germany). The samples were incubated at 56°C in a thermostatic bath (HAAKE Shaking Water Bath (SWB25), Germany) for 30 min and then the DNA was extracted from the specimens using the instrument EZ1 Advanced XL (QIAGEN GmbH), according to the manufacturer's instructions. The detection and quantification of DNA of *P. jirovecii* was performed with the RealStar® *Pneumocystis jirovecii* PCR Kit 1.0 (Altona Diagnostics GmbH, Hamburg, Germany), an *in vitro* diagnostic test, based on real-time PCR technology. The whole process was monitored adding to each sample 5 µL of Internal Control (IC) (Altona Diagnostics GmbH, Hamburg, Germany), before the DNA extraction to confirm the nucleic acid extraction and to exclude PCR inhibition. The Real-time PCR tests were performed according to the manufacturer's protocol. Briefly, the amplification was carried out in a CFX96 Real-Time thermocycler (Bio-Rad, Hercules, CA, USA). Each PCR was performed with 10 µL of extracted DNA in a in a final reaction volume of 30 µL. The thermal cycling conditions consisted of a denaturation at 95 °C for 2 min, followed by 45 cycles of alternating incubations: denaturation at 95 °C for 15 s, annealing at 58 °C for 45 s and extension at 72 °C for 15 s. Negative and positive controls, provided in the kit, were included in each assay. The final results were analyzed using the CFX96 Real-Time fluorescence quantitative PCR software (Bio-Rad, Hercules, CA, USA). The samples were positive if there were the detection of the IC in the JOE™ detection channel and of *P. jirovecii* DNA in the FAM™ detection channel. For positive samples, a quantification standards curve contain standardized concentrations of *P. jirovecii* specific DNA (Altona Diagnostics GmbH, Hamburg, Germany), was used to determine the concentration of *P. jirovecii* specific DNA in the sample.

2.6. Statistical analysis

The statistical analysis was performed using SPSS version 27 (SPSS Inc. Chicago, IL). Continuous variables were reported as median and interquartile range and categorical variables as frequency and percentages. Categorial variables were confronted with Chi-squared test and Fisher's exact test when appropriate. Continuous variables were confronted with Mann-Whitney U test. A significance level of 0.05 was set for the interpretation of the results.

3. Results

We screened for inclusion criteria all the patients hospitalized for COVID-19 between July 2021 and December 2022. Of 290 patients screened, 59 (20%) met the inclusion and exclusion criteria and were enrolled. Median age of the study population was 62 years (IQR 35-68), with 61% of females and 11 on 59 (18.6%) pregnant women. Only 4 (6.8%) patients have had an intensive care unit admission in the 20 days before the enrolment. The most reported comorbidity was chronic lung disease (17%), followed by chronic kidney disease (13.6%). Most of the patients required low flow oxygen support with Venturi mask (37.3%) or nasal cannula (25.4%) and therefore the median of the highest WHO grading reached was 4 (IQR 4.5). Detailed demographic and clinical characteristics of the patients are displayed in **Table 1**.

Table 1. Demographic and clinical characteristics of the population. .

	N=59
Age, years, median (IQR)	62 (35-68)
Females, n (%)	36 (61)
Pregnancy, n (%)	11 (18.6)
Lenght of stay, days, median (IQR)	16 (11-22)
Days from admission to sampling, median (IQR)	13 (9-13)
Admitted to ICU in the last 20 days, n (%)	4 (6.8)
Myocardial infarction, n (%)	6 (10.2)
Congestive heart failure, n (%)	4 (6.8)
Peripheral vascular disease, n (%)	2 (3.4)
Cerebrovascular disease, n (%)	5 (8.5)

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Dementia, n (%)	0 (0)
Chronic lung disease, n (%)	10 (17)
Connective tissue disease, n (%)	1 (1.7)
Peptic ulcer, n (%)	1 (1.7)
Diabetes with organ damage, n (%)	1 (1.7)
Moderate/severe kidney disease, n (%)	8 (13.6)
Hemiplegia, n (%)	0 (0)
Moderate/severe liver disease, n (%)	2 (3.4)
Solid tumor in the last 5 years, n (%)	7 (12)
Metastatic tumor, n (%)	2 (3.4)
Charlson Comorbidity Index, median (IQR)	2 (0-6)
Days on steroid therapy, median (IQR)	13.5 (10-16.25)
Cumulative dose of steroid, mg, median (IQR)	70 (50-86)
Worst WHO grade, median (IQR)	4 (4-5)
Lowest PaO2/FiO2 ratio, median (IQR)	180.5 (130.25-269.75)
Days on highest oxygen support, median (IQR)	5 (5-8.5)
Lowest lymphocyte count cells/mm3, median (IQR)	655 (432-950)
Ferritin on admission, ng/mL, median (IQR)	204 (114-583)
CRP on admission, mg/dL, median (IQR)	5.5 (2.1-12.8)
Highest oxygen support required	
Nasal cannula, n (%)	15 (25.4)
Venturi mask, n (%)	22 (37.3)
CPAP, n (%)	3 (5)
HFNC, n (%)	9 (15.3)
NIV, n (%)	4 (6.8)

ICU: intensive care unit; WHO: World Health Organization; CRP: C-reactive protein; CPAP: continuous positive airway pressure; HFNC: high flow nasal cannula; NIV: non-invasive ventilation.

Out of 59 oral washing samples, collected on a median of 13 (IQR 9-13) days from admission, only one (1.7%) resulted positive for *Pneumocystis jirovecii* genome detection with PCR and the same patient was the only one to develop clinically evident *Pneumocystis jirovecii* pneumonia 10 days after hospital discharge. The patient, a 77 years-old man, vaccinated for SARS-CoV-2 with two doses, obese and affected by hypertension, was admitted at our institution for COVID-19 pneumonia 22 days before PCP diagnosis. He was treated with intravenous remdesivir and dexamethasone 6 mg daily for 5 days and he received oxygen with Venturi mask with the highest need for FiO2 of 60%. He was discharged 12 days later on room air and in good condition with negative nasopharyngeal swab for SARS-Cov-2 detection. Then, 10 days after hospital admission, he presented to the outpatient clinic with fever, exertional dyspnea, cough and a peripheral oxygen saturation of 89 on room air. A bronchoalveolar lavage fluid (BALF) was collected that resulted positive for *P. jirovecii* detection with direct immunofluorescence, while other tests on BALF and serum/urine for respiratory viruses, bacteria and fungi, including mycobacteria, resulted negative. He was treated with intravenous trimethoprim-sulfamethoxazole (TMP-SMX) at the dose of 15 mg/kg divided in 4 daily doses for 3 days (with switch to oral therapy after reaching clinical improvement) plus prednisone 40 mg twice daily for the first 5 days, followed by 40 mg daily for 5 days and 20 mg daily for the remaining 11 days. He was discharged at home 5 days after starting therapy for PJP in good clinical conditions with no oxygen requirement.

No other patients developed any signs or symptoms of PCP in the follow-up period. No patients, including the one who developed PCP, had detectable beta-D-glucan in serum on the day of the collection of the oral washing, as well as at the time of PCP diagnosis.

4. Discussion

The prevalence of *P. jirovecii* colonization detected with OWS in an immunompetent cohort of COVID-19 patients is very low (1.7%). According to the largest review available on the topic, published in 2021 by Vera C. and Rueda Z.V., the prevalence of *P. jirovecii* colonization detected with PCR on various respiratory sample (including OWS), is extremely variable across the included studies, ranging from 0% in healthy non-pregnant women to 50% in immunocompetent pregnant women and 70% in newborns and in patient with chronic obstructive pulmonary disease (COPD) or HIV infection.[6]

On the other hand, according to a 2008 review by Morris A. and colleagues, among 7 published studies specifically aimed to search for *P. jirovecii* colonization in healthy immunocompetent hosts, 5 out of 7 studies found a prevalence of 0% with PCR on several respiratory specimens, including BAL fluid and lung specimen from autopsies, on a number of subjects ranging from 10 to 30.[16] Conversely, one study published in 1997 by Nevez and colleagues found a prevalence of positive PCR of 20% among 169 patients that underwent a BAL for any reason (the largest cohort among the 7 studies), while another paper of 2005 by Medrano and colleagues found the same prevalence with PCR on OWS on 50 healthy workers of a Spanish hospital with no underlying lung conditions.[10,17]

Such differences in colonization prevalence in immunocompetent hosts seem related to different risk factors in the included populations, hospitalized patients, in the case of Nevez et al., and healthcare workers in the case of Medrano et al.[10,17] Transmission of *P. jirovecii* from the hospital environment or from other colonized or infected patients is in fact a known route of colonization also of immunocompetent hosts.[6]

For this exact reason we decided not to test our study population at hospital admission, but after 14 days of hospital stay or at hospital discharge, whichever occurred first, since we expected a very low prevalence of *P. jirovecii* colonization in immunocompetent patients coming from the community. Nonetheless, we still found that the prevalence of colonization from *P. jirovecii* on OWS in non-critical immunocompetent patients with COVID-19 is very low.

On the other hand, despite the small number of our population, the fact that the only patient that tested positive for *P. jirovecii* on OWS was the only one in our cohort that developed clinically significant PCP, leads us to reflect about the role of this non-invasive sample in predicting the risk of PCP in COVID-19 patients.

According to EORTC/MSGERC criteria, a positive PCR for *P. jirovecii* on a respiratory sample, together with the presence of a host factor and a typical clinical-radiologic picture, is sufficient for a "probable" diagnosis of PCP.[7] Less is known about the role of PCR in the preclinical context to stratify at risk patients that may benefit of a close follow-up or prophylaxis, especially in atypical populations, such as COVID-19, where classic risk factors for PCP can be absent and the clinical-radiologic picture is very similar to SARS-CoV-2 pneumonia. Moreover, it is still debated whether the *P. jirovecii* PCR load can discriminate between colonization and infection, using a cut-off of 1000 copies/mL, according to several published studies. [8,18]

5. Conclusions

According to the result of our work, despite its limitations, we can speculate that PCR on oral washing samples, other than the well-established diagnostic role in defining probable PCP, can have a role in the early identification of patients at risk of developing clinically significant PCP during the course of COVID-19, also contributing to selecting those patients that might benefit from PCP chemoprophylaxis. Nonetheless, further studies on larger populations are required to evaluate the predictive value of this test on COVID-19 population.

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethical Committee of the University of Naples Federico II (protocol n. 180/21).

Consent for publication: patients signed informed consent for participation to the study and for publication of the results.

Availability of data and materials: The data that support the findings of this study are available Federico II University Hospital, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Federico II University Hospital.

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References

1. de Boer MGJ, Kroon FP, le Cessie S, de Fijter JW, van Dissel JT. Risk factors for Pneumocystis jirovecii pneumonia in kidney transplant recipients and appraisal of strategies for selective use of chemoprophylaxis. Transplant Infectious Disease. 2011 Dec;13(6):559–69.
2. Maertens J, Cesaro S, Maschmeyer G, Einsele H, Donnelly JP, Alanio A, et al. ECIL guidelines for preventing Pneumocystis jirovecii pneumonia in patients with haematological malignancies and stem cell transplant recipients. Journal of Antimicrobial Chemotherapy. 2016 Sep;71(9):2397–404.
3. Wolfe RM, Peacock JE. Pneumocystis Pneumonia and the Rheumatologist: Which Patients Are At Risk and How Can PCP Be Prevented? Curr Rheumatol Rep. 2017;19(6):35.
4. Sasani E, Bahrami F, Salehi M, Aala F, Bakhtiari R, Abdollahi A, et al. Pneumocystis pneumonia in COVID-19 patients: A comprehensive review. Heliyon [Internet]. 2023 Feb 1;9(2). Available from: https://doi.org/10.1016/j.heliyon.2023.e13618
5. Amstutz P, Bahr NC, Snyder K, Shoemaker DM. Pneumocystis jirovecii Infections Among COVID-19 Patients: A Case Series and Literature Review. Open Forum Infect Dis [Internet]. 2023 Feb 1;10(2):ofad043. Available from: https://doi.org/10.1093/ofid/ofad043
6. Vera C, Rueda ZV. Transmission and Colonization of Pneumocystis jirovecii. Journal of Fungi 2021, Vol 7, Page 979 [Internet]. 2021 Nov 18 [cited 2023 Mar 5];7(11):979. Available from: https://www.mdpi.com/2309-608X/7/11/979/htm
7. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, et al. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clinical Infectious Diseases [Internet]. 2020 Sep 12 [cited 2020 Dec 29];71(6):1367–76. Available from: https://academic.oup.com/cid/article/71/6/1367/5645434
8. Hviid CJ, Lund M, Sørensen A, Eriksen SE, Jespersen B, Dam MY, et al. Detection of Pneumocystis jirovecii in oral wash from immunosuppressed patients as a diagnostic tool. PLoS One [Internet]. 2017 Mar 1 [cited 2023 Mar 5];12(3):e0174012. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0174012

9. Senécal J, Smyth E, Del Corpo O, Hsu JM, Amar-Zifkin A, Bergeron A, et al. Non-invasive diagnosis of Pneumocystis jirovecii pneumonia: a systematic review and meta-analysis. Clinical Microbiology and Infection. 2022 Jan 1;28(1):23–30.
10. Medrano FJ, Montes-Cano M, Conde M, De La Horra C, Respaldiza N, Gasch A, et al. Pneumocystis jirovecii in General Population - Volume 11, Number 2—February 2005 - Emerging Infectious Diseases journal - CDC. Emerg Infect Dis [Internet]. 2005 [cited 2023 Jan 24];11(2):245–50. Available from: https://wwwnc.cdc.gov/eid/article/11/2/04-0487_article
11. Viceconte G, Buonomo AR, Lanzardo A, Pinchera B, Zappulo E, Scotto R, et al. Pneumocystis jirovecii pneumonia in an immunocompetent patient recovered from COVID-19. Infect Dis. 2021;
12. Gentile I, Viceconte G, Lanzardo A, Zotta I, Zappulo E, Pinchera B, et al. Pneumocystis jirovecii Pneumonia in Non-HIV Patients Recovering from COVID-19: A Single-Center Experience. International Journal of Environmental Research and Public Health 2021, Vol 18, Page 11399. 2021 Oct 29;18(21):11399.
13. Viceconte G, Buonomo AR, D'Agostino A, Foggia M, Di Fusco A, Pinchera B, et al. Risk Factors for Pneumocystis jirovecii Pneumonia in Non-HIV Patients Hospitalized for COVID-19: A Case-Control Study. Journal of Fungi. 2023 Aug 11;9(8):838.
14. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40(5):373–83.
15. WHO R&D Blueprint novel Coronavirus COVID-19 Therapeutic Trial Synopsis [Internet]. 2020 [cited 2022 Feb 18]. Available from: https://www.who.int/blueprint/priority-diseases/key-action/COVID-19_Treatment_Trial_Design_Master_Protocol_synopsis_Final_18022020.
16. Morris A, Norris KA. Colonization by pneumocystis jirovecii and its role in disease [Internet]. Vol. 25, Clinical Microbiology Reviews. American Society for Microbiology Journals; 2012 [cited 2021 Feb 9]. p. 297–317. Available from: http://cmr.asm.org/
17. Nevez G, Jounieaux V, Linas MD, Guyot K, Leophonte P, Massip P, et al. High Frequency of Pneumocystis carinii sp.f. hominis Colonization in HIV-Negative Patients. Journal of Eukaryotic Microbiology [Internet]. 1997 [cited 2023 Apr 24];44(6):36s–36s. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1550-7408.1997.tb05760.x
18. Gioia F, Albasata H, Hosseini-Moghaddam SM. Concurrent Infection with SARS-CoV-2 and Pneumocystis jirovecii in Immunocompromised and Immunocompetent Individuals. Vol. 8, Journal of Fungi. 2022.