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COVID-19–related Respiratory Failure and Lymphopenia Do Not Seem Associated with Pneumocystosis

To the Editor:

We read with great interest the letter "A Case of COVID-19 and *Pneumocystis jirovecii* Coinfection" by Menon and colleagues (1) that reports a cooccurrence of coronavirus disease (COVID-19) and pneumocystosis in an 83-year-old non–HIV-infected female. The authors hypothesize that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection led to a state of functional immune suppression related to lymphocytopenia (absolute lymphocyte count 1,090 cells/μl), predisposing the patient to *Pneumocystis jirovecii* infection. In this case, mycological arguments for pneumocystosis were a positive qualitative real-time PCR assay on a tracheal aspirate and a serum (1,3)-β-D-glucan at 305 pg/ml. Also, subtle cystic images were observed on her computed tomographic scan and the patient was receiving inhaled and low-dose oral corticosteroid therapy for a history of asthma and ulcerative colitis.

A follow-up serum obtained 1 week after initiating treatment showed an important decrease in the amount of β-glucan. This is surprising, as it is usually known to diminish very slowly or even increase (median decline of 17 pg/ml; range, −343 to 205) (2, 3). The patient was treated and promptly extubated (on Day 7 of hospitalization); it would therefore be interesting to know on which day the anti-*Pneumocystis* treatment was initiated because clinical improvement is usually expected after 4–8 days (4).

PCR is of great interest for the diagnosis of pneumocystosis in non–HIV-infected patients. However, as stated by the authors, its great sensitivity can lead to the detection of low fungal loads and has made the distinction between colonization and infection a regular problem.

We have recently seen hundreds of patients with COVID-19 in our institution (La Pitié-Salpêtrière hospital, a 1,850-bed tertiary care center in Paris, France), many of whom were managed in ICUs. In line with previous data indicating that severe forms of COVID-19 are associated with lymphopenia (5), many of our patients had an absolute lymphocyte count below 1,000/μl. Taking into account that this condition represents a documented risk factor for pneumocystosis (6) and the lack of knowledge concerning the susceptibility of these patients to fungal complications, we performed *P. jirovecii* PCR assays (targeting the mitochondrial large subunit ribosomal RNA) on all respiratory samples obtained from patients under mechanical ventilation or venovenous extracorporeal membrane oxygenation (ECMO) support.

A total of 423 PCR assays were performed on respiratory samples obtained from 145 patients with severe, proven SARS-CoV-2 infections (mean, 2.9 samples per patient; range, 1–11) between March 12 and April 27 (Table 1). Among them, 22 patients had preexisting recognized risk factors for pneumocystosis, 6 other patients were HIV infected but with relatively abundant $CD4^+$ cells, and 22 other patients received corticosteroids as treatment for their COVID-19. Most of them (79%; 113/143) had lymphocytopenia (<1,000 cells/μl). Almost all *P. jirovecii* PCR assay results were strictly negative (99.3%; 420/423).

We found three positive results in 2 among the 145 patients (1.4%). The first patient was a 78-year-old woman with diabetes and hypertension admitted to the ICU (March 12, Day 1) for COVID-19–related respiratory failure. She had lymphocytopenia (nadir: 410/μl), was not tested for β-D-glucan, and had a low fungal load in BAL sampled at Day 3 (740 copies/ml; 2.9 log). Her respiratory state improved. She later developed bacterial and thrombotic complications that lead to her death on Day 43 from hemorrhagic shock with no evidence of respiratory failure.

The second patient was a pregnant woman with obesity (body mass index, 40.4 kg/m^2), type 2 diabetes, and chronic hypertension. She was admitted to the ICU (March 20, Day 1) in a severe respiratory state ($Pa_{O_2}/F_{I_{O_2}} < 100$ mm Hg; SAPS II score = 65) that required venovenous ECMO support. She presented concomitant transient lymphocytopenia (770–1,420/μl). A low *P. jirovecii* load was detected in two BAL samples

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Table 1. Characteristics of ICU Patients with Severe COVID-19 for Whom a Specific Research for *P. jirovecii* Pneumonia Has Been Conducted

Demographic characteristics and underlying conditions	
Number of patients	145
Age, mean (±SD), yr	54 (±12)
Sex, M/F	104/41
Hypertension, *n* (%)	83/143 (58)
Diabetes, *n* (%)	46/143 (32.2)
Overweight (BMI >25 kg/m^2), *n* (%)	99/140 (70.7)
Preexisting risk factors for *P. jirovecii* pneumonia	
Solid organ transplant, *n* (%)	14/143 (9.8)
HIV infection, *n* (%)*	6/142 (4.2)
Corticosteroid therapy (>0.3 mg/kg/d), *n* (%)	4/143 (2.8)
Hematological malignancies, *n* (%)	4/143 (2.8)
ICU management and clinical characteristics	
Corticosteroid therapy (>20 mg/d), *n* (%)†	22/132 (16.7)
Nadir absolute lymphocytes count/μl, median (IQR) (number of patients with available data)	690 (435–940) (*n* = 143)
SAPS II score, median (IQR) (number of patients with available data)	47 (32–63) (*n* = 108)
Venovenous ECMO, *n* (%)	73/135 (54%)
Worst $Pa_{O_2}/F_{I_{O_2}}$, median (IQR) (number of patients with available data)	60 (51–73) (*n* = 135)
ICU stay, d, median (IQR) (number of patients with available data)	28 (15–47) (*n* = 129)
Intubation period, d, median (IQR) (number of patients with available data)	27 (14–45) (*n* = 129)
P. jirovecii PCR, % of positive samples (*n*)	
BAL	1% (3/312)
Tracheal aspiration	0% (0/110)
Pleural liquid	0% (0/1)
Patients with positive PCR	1.4% (2/145)

Definition of abbreviations: BMI = body mass index; COVID-19 = coronavirus disease; ECMO = extracorporeal membrane oxygenation; IQR = interquartile range; *P. jirovecii* = *Pneumocystis jirovecii*.
*All HIV-infected patients received antiretroviral therapy at the time COVID-19 was diagnosed. Five patients had absolute CD4$^+$ lymphocytes cells >200/μl; one patient had 184 CD4$^+$ lymphocytes cells/μl.
†Dexamethasone 20 mg/d or high-dose prednisone (3–5 mg/kg/d).

performed on Day 2 and Day 6 (753 copies/ml, 2.9 log, and 162 copies, 2.2 log, respectively). Serum β-D-glucan was negative (18 pg/ml; Fungitell assay; Associates of Cape Cod). After a slow improvement and the explantation of ECMO on Day 4, other respiratory samples (Day 23, Day 32, and Day 35) came back with negative *Pneumocystis* PCR results. Finally, the patient later presented multiple bacterial superinfections and mechanical ventilation–acquired pneumonia and died on Day 61.

As their respiratory state improved despite any anti–*Pneumocystis*-specific treatment, and because of the absence of other relevant immunosuppression factors and a low fungal burden (<3 log), both patients were considered to have colonization.

Consistent with the fact that only chronic and deep prolonged lymphocytopenia constitutes a risk factor for pneumocystosis, our results indicate a very low risk for patients with severe COVID-19 to develop *Pneumocystosis jirovecii* pneumonia. Of note, none of our immunocompromised patients developed pneumocystosis either.

It is expected that most or all patients with severe COVID-19 will have computed tomographic scan abnormalities featuring ground-glass opacities with or without consolidations (7). Because COVID-19 and pneumocystosis share certain radiographic anomalies, pneumocystosis should therefore be kept in mind in the initial diagnostic workup of all those patients. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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Reply to Blaize *et al.*

From the Authors:

We thank Dr. Blaize and colleagues for their interest and letter in response to our recent publication detailing a case of suspected coronavirus disease (COVID-19) and *Pneumocystis jirovecii* coinfection (1). The authors correctly note that the decline in serum (1,3)-β-D-glucan level observed in our patient was more rapid than is typically expected following initiation of appropriate therapy (2). Several studies have demonstrated that (1,3)-β-D-glucan is not a reliable biomarker of response to *P. jirovecii* treatment (3); however, in some patients, such as ours, decreasing (1,3)-β-D-glucan levels have been shown to correlate with improved clinical outcomes in *Pneumocystis* pneumonia (4). In our patient, the serum (1,3)-β-D-glucan level was elevated at 305 pg/ml on admission, remained persistently elevated at 268 pg/ml on hospital Day 3, at which point trimethoprim-sulfamethoxazole treatment was initiated, and then declined to 90 pg/ml 1 week after initiating treatment.

The diagnosis of *P. jirovecii* infection can be challenging, particularly in patients without HIV, in whom the fungal burden is generally lower (5). Furthermore, owing to the high sensitivity of *P. jirovecii* PCR (6), it may be difficult to differentiate between *Pneumocystis* colonization versus infection, particularly in the setting of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which may have similar clinical manifestations as *Pneumocystis* pneumonia. Nevertheless, the constellation of an elevated (1,3)-β-D-glucan level on two occasions, positive tracheal aspirate qualitative PCR assay, presence of typical cystic changes of *P. jirovecii* on chest computed tomography, and rapid clinical improvement following initiation of trimethoprim-sulfamethoxazole therapy support our conclusion that our patient indeed had a true *P. jirovecii* infection. It is worth noting that several studies have suggested that quantitative *P. jirovecii* PCR and (1,3)-β-D-glucan may be used to distinguish between *Pneumocystis* pneumonia and colonization (7, 8). Specifically, a positive quantitative PCR from a BAL fluid sample ($>1.6 \times 10^3$ DNA copies/μl) and a (1,3)-β-D-glucan level cutoff of 100 pg/ml has been suggested to discriminate between *P. jirovecii* colonization versus infection (8). Gold-standard staining methods to detect *P. jirovecii* in respiratory specimens are also useful to confirm *P. jirovecii* infection, although the sensitivity is low compared with PCR, particularly in HIV-negative immunocompromised patients (9–11). Unfortunately, we do not have quantitative PCR or cytologic/immunofluorescent staining data from our patient, which would have further strengthened our argument for true *P. jirovecii* infection.

Dr. Blaize and colleagues performed quantitative *P. jirovecii* PCR testing on 423 respiratory samples obtained from 145 patients at their center with confirmed SARS-CoV-2 infection who required either mechanical ventilation or venovenous extracorporeal membrane oxygenation support. Despite the high prevalence of lymphocytopenia in this population of critically ill patients with COVID-19, there were no true cases of *P. jirovecii* coinfection. In our patient with SARS-CoV-2 and *P. jirovecii* coinfection, other than COVID-19–associated $CD4^+$ lymphocytopenia, she did not have a known underlying immunodeficiency. She was on oral budesonide for treatment of her ulcerative colitis, which was well controlled, and an albuterol inhaler as needed for mild intermittent asthma (1), but not inhaled corticosteroids as suggested by the authors. Oral budesonide formulations provide topical antiinflammatory activity in the colon but have very little systemic bioavailability owing to their high first-pass hepatic metabolism. As such, oral budesonide formulations have not been associated with increased risk of *P. jirovecii* infection in patients with ulcerative colitis (12). Although our patient did not have any other classical risk factors for *P. jirovecii* infection, it is certainly possible that her coinfection was a coincidence (a proof of principle of "Hickam's dictum" [13]) or that an unbeknownst underlying immune defect predisposed the patient independently to SARS-CoV-2 and *P. jirovecii* infection.

Given the inherent risk of overextending conclusions from a single case, we were conservative in the interpretation of our case and suggested that it may be reasonable to consider additional diagnostic testing for *P. jirovecii* by assaying serum (1,3)-β-D-glucan in patients with COVID-19 if there are additional clinical findings, such as cystic findings on chest computed tomography or elevated lactate dehydrogenase that may support coinfection (1). This approach should be considered particularly in patients with classical *P. jirovecii* risk factors such as HIV, as coinfection with SARS-CoV-2 and *P. jirovecii* has been reported in both well-controlled (14) and severely immunocompromised patients with HIV (15, 16). We commend the authors for their work and for expanding the COVID-19 evidence

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