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

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Author's contributions: MB, AL and AF collected microbiological data, drafted the manuscript and revised the final version. JM and CEL participated in patients' care and clinical data collection. All authors revised and contributed to the final version.

AJRCCM Articles in Press. Published September 17, 2020 as 10.1164/rccm.202007-2938LE

Descriptive number: 10.13 Pneumonia: Fungal Infections

Short running head: Absence of Pneumocystosis during COVID-19

Source of support: none

Word count: 800

Keywords: SARS-CoV-2, COVID-19, Pneumocystosis, *Pneumocystis jiroveccii*, broncho-alveolar lavage, intensive care unit

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To the Editor,

We read with great interest the Letter "A Case of COVID-19 and *Pneumocystis jirovecii* Coinfection" by Menon *et al* (1) that reports a co-occurrence of COVID-19 and pneumocystosis in a 83-year old non-HIV infected female. The authors hypothesize that 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 *P. 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)-b-D-glucan at 305 pg/mL. Also, subtle cystic images were observed on her CT-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 beta-glucan. This is surprising, as it is usually known to diminish very slowly or even increase (median decline of 17 pg/mL; range: −343, 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 as 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 centre in Paris, France), many of whom

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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 *Pneumocystis jirovecii* PCR assay (targeting the mitochondrial large subunit ribosomal RNA) on all respiratory samples obtained from patients under mechanical ventilation or veino-venous extra-corporeal membrane oxygenation (vvECMO) support.

A total of 423 PCR 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, 12th and April 27th (Table 1). Among them, 22 patients had pre-existing recognized risk factors for pneumocystosis, 6 other patients were HIV-infected but with relatively abundant CD4+ cells, and 22 other patients received corticosteroid as treatment for their COVID-19. Most of them (79%; 113/143) had lymphocytopenia (<1000 cells/µL). Almost all *Pneumocystis jirovecii* PCR were strictly negative (99.3%; 420/423).

We found 3 positive results in 2 among the 145 patients (1.4%). The first patient was a 78 year-old woman with diabete and hypertension admitted to the ICU (March 12th, Day 1) for COVID-19 related respiratory failure. She had lymphocytopenia (nadir: 410/µL), was not tested for beta-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 choc with no evidence of respiratory failure.

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The second patient was a pregnant woman, obese (BMI: 40.4 kg/m^2), with type 2 diabetes and chronic hypertension. She was admitted to the ICU (March 20th, day 1) in a severe respiratory state (PaO_2/FiO_2 < 100 mm Hg; SAPS II score = 65) that required vvECMO support. She presented concomitant transient lymphocytopenia (770 to 1,420/µL). A low *P. jirovecci* load was detected in two BAL performed on day 2 and day 6 (753 copies/mL; 2.9 log and 162 copies; 2.2 log respectively). Serum beta-D-glucan was negative (18 pg/mL; Fungitell® assay; Associates of Cape Cod). After a slow improvement and the explantation of the ECMO on day 4, other respiratory samples (day 23, day 32 and day 35) came back with negative *Pneumocystis* PCR. Finally, the patient later presented multiple bacterial superinfections, mechanical ventilation acquired pneumonia and died on Day 61.

As their respiratory state improved despite any anti-*Pneumocystis* specific treatment, adding the absence of other relevant immunosuppression factors and a low fungal burden (<3 log), both were considered as 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 severe COVID-19 patients to develop *Pneumocystosis jirovecci* 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 CT scan abnormalities featuring ground glass opacities with or without consolidations (7). As COVID-19 and pneumocystosis share certain radiographic anomalies, pneumocystosis should therefore be kept in mind in the initial diagnostic work up of all those patients.

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Demographic characteristics and underlying conditons	Number of patients	145
	Age (years) mean (standard deviation)	54 (+/-12)
	Sex (male/female)	104/41
	Hypertension	83/143 (58%)
	Diabetes	46/143 (32.2%)
	Overweight (BodyMass Index > 25 kg/m²)	99/140 (70.7%)
Preexisting Risk factors for *Pneumocystis jirovecii* pneumonia	Solid Organ Transplant	14/143 (9.8%)
	HIV infection[1]	6/142 (4.2%)
	Corticosteroid Therapy (>0.3 mg/kg/day)	4/143 (2.8%)
	Hematological malignancies	4/143 (2.8%)
ICU management and clinical characteristics	Corticosteroid Therapy[2] (>20 mg/day)	22/132 (16.7%)
	Nadir absolute lymphocytes count/µL; median [interquartile] (number of patients with available data)	690 [435 - 940] (n=143)
	SAPS II score; median [interquartile] (number of patients with available data)	47 [32 - 63] (n=108)
	veinovenous Extra Coroporeal Membrane Oxygenation	73/135 (54%)
	Worst PaO2/FiO2; median [interquartile] (number of patients with available data)	60 [51 - 73] (n=135)
	ICU Stay (days); median [interquartile] (number of patients with available data)	28 [15 - 47] (n=129)
	Intubation period (days); median [interquartile] (number of patients with available data)	27 [14 - 45] (n=129)
***Pneumocystis jirovecii* PCR; % of positive samples**	Broncho-alveolar lavage	1% (3/312)
	Tracheal aspiration	0% (0/110)
	Pleural liquid	0% (0/1)
	Patients with positive PCR	1.4% (2/145)

Table 1: characteristic of ICU patients for severe COVID-19 for whom a specific research for *Pneumocystis jirovecii* pneumonia has been conducted

[1] 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

[2] Dexamethasone 20 mg/day or high dose prednisone (3-5 mg/kg/day)

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