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The presence of *Pneumocystis jirovecii* in critically ill patients with COVID-19

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Letters to the Editor

Dear Editor,

We read with interest the recent review on co-infections in coronavirus disease-2019 (COVID-19) patients[1] and believe that fungal co-infections as evaluated from selected studies are underestimated. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV2) is still spreading pandemically. Approximately 5-10% of COVID-19 patients may require intensive care unit (ICU) management and 30% may develop secondary pneumonia without identified etiology.[2] Hospital-acquired bacterial or fungal superinfections, as described in critically ill patients with *Influenza* virus, can be suspected.[3] Since pneumocystosis is usually reported in patients with T-cell immunodepression,[4] less attention has been paid to *Pneumocystis jirovecii* in non-immunocompromised ICU patients although it accounts for 7% of the co-infections reported in those admitted with Influenza.[5] Interestingly, COVID-19 patients may develop lymphocytopenia and acute respiratory distress syndrome (ARDS) requiring adjunctive steroids and/or immunomodulatory therapies, well-known susceptibility factors for developing pneumocystosis.[5] We designed this observational cohort study to investigate the prevalence of *P. jirovecii* acid nucleic detection in respiratory specimens sampled to identify co-infections in COVID-19 patients in the ICU.

All consecutive patients admitted to the ICU between 2020/03/15 and 2020/05/01 with a positive SARS-CoV-2 PCR (Cobas® SARS-CoV-2 Test, Roche, France) and ≥1 respiratory sample (bronchoalveolar lavage (BAL), tracheal aspirate, sputum) sent to the mycology department. This study was part of the COVID-ICU and French COVID-19 cohort registries. Our institutional ethics committee approved the study (IDRCB, 2020-A00256-33; CPP, 11-20-20.02.04.68737). When possible, signed informed consent was obtained from the patients or the next of kin.

Whenever possible, bronchoalveolar lavage (BAL) was performed in the middle lobe by a trained pneumologist in the ICU using at least 120 ml saline (yield, ~50%). Upon reception, specimens including BAL fluids and dTT-treated aspirations (dTT 1X at 37°C for 15min) were centrifuged, suspended in 200µL of water and submitted to extraction (whole nucleic acids extraction) using the GeneLead-VIII extractor-thermocycler™ (Precision System Science, Japan). *P. jirovecii* reverse transcriptase quantitative PCR (RTqPCR) was performed to amplify mtSSU and mtLSU RNA and DNA of *P. jirovecii* using the new R-DiaPnJ kit™ (Diagenode, Belgium). Serum β-D-glucan was tested using the Fungitell kit™ (Cape

Cod Inc, US) as recommended by the manufacturer. Data are presented as median [25th-75th percentiles] or percentages as appropriate. Comparisons were performed using Mann-Whitney or exact Fisher tests as required. *P*-values ≤0.05 were considered as significant.

One hundred-and-eight successive HIV-negative COVID-19 patients (Male/Female sex ratio, 4.4; age, 62 years [56-68]) with the usual risk factors for severe COVID-19 presentation were included (Table1). All except three patients were intubated on admission. Thirty-four patients (31.4%) who developed ARDS received at least one day of corticosteroids before BAL sampling. Respiratory samples included 80 BALs (74.1%), 22 tracheal aspirates (20.4%), 4 sputa (3.7%) and two bronchial aspiration fluids (1.9%). In 10/108 patients (9.3%), *P. jirovecii* RTqPCR was positive. Median delay between sampling and ICU admission was 2days [1-2]. The median quantitative cycle value was 32.6 [30.8-34.7]. Serum β-D-glucan were measured in nine patients and was negative (>80pg/mL) in seven patients.

Clinical characteristics of the patients carrying *P. jirovecii* did not significantly differ from the other patients except for lower plasma D-dimer (1,270ng/mL [750-2,390] *vs* 2,610ng/mL [1,405-4,700], *P*=0.03) and more frequent lopinavir/ritonavir administration (40.0% vs 12.2%, *P*=0.04), while long-term corticosteroid prescription tended to be more frequent (30.0% vs 8.2%, *P*=0.06). Of note, among our 10 *P. jirovecii* carriers, five concomitantly met the criteria for COVID-19-associated pulmonary aspergillosis.[6] Out of these patients, four (40%) received co-trimoxazole as prophylaxis (80/400mg once daily) whereas six including four who rapidly improved did not. One co-trimoxazole-treated and two non-treated patients died while the seven remaining patients were discharged. Mortality was similar in both groups.

We found an unexpectedly high proportion of critically ill COVID-19 patients detected with *P. jirovecii* (10/108 patients; 9.3%), similarly to previous findings in influenza patients (3/45; ~7%).[5]

The presence of *P. jirovecii* in the healthy adult population has been measured using oropharyngeal wash samples obtained by gargling and examined by conventional or nested PCR methods.[7] However, experts agree that the reported prevalence (~20%) has been overestimated due to technical issues such as contamination with amplicons responsible for false positives.[4] In our center managing almost exclusively immunocompromised patients, prevalence of qPCR-positive respiratory specimens with fungal load as low as in our COVID-19 patients, is ~13% (unpublished data), as reported elsewhere.[3] COVID-19

patients mostly exhibited marked lymphopenia and alterations in lymphocyte functions,[8] likely explaining the high-rate of *P. jirovecii* detection.

Since serum β-D-glucan is advocated in pneumocystosis diagnosis,[4] we measured its concentrations in four of our five *P. jirovecii* RTqPCR-positive patients and obtained low values (<120pg/mL) in accordance with the low nucleic acids fungal loads in the lung alveoli.[9] Of note, in two out of our nine tested *P. jirovecii* RTqPCR-negative patients, higher B-D-glucan concentrations (450 and 500pg/ml) lead to the diagnosis of pulmonary aspergillosis, another fungal infection of risk in COVID-19 patients.[6] Although a recent meta-analysis questioned its sensitivity in non-HIV patients,[10] β-D-glucan has been widely used to rule out pneumocystosis because of its high negative predictive value. This finding may support the hypothesis that our patients were carrying *P. jirovecii*, yet not being infected *per se*. Thus, although interesting in the context of invasive fungal infections diagnosis, serum β-D-glucan should be interpreted with caution when excluding the diagnosis of pneumocystosis.

Here, four out of ten *P. jirovecii* RTqPCR-positive patients received co-trimoxazole as prophylactic regimen, based on the treating physician's decision. Whether a positive result should be an indication to consider administering co-trimoxazole, at least at prophylactic dosage in COVID-19 patients remains questionable.

Our study limitations include the relatively small number of patients, the bi-center setting, and the short study period. However, to the best of our knowledge, this is the first study evaluating the prevalence of *P. jirovecii* in COVID-19 patients. Because we focused on critically ill COVID-19 patients, *P. jirovecii* prevalence in less severe patients remains to be determined.

In conclusion, an unexpectedly high proportion of *P. jirovecii*-positive pulmonary samples is observed in critically ill COVID-19 patients. Based on our findings, we advocate systematically searching for *P. jirovecii* in deep respiratory specimens in these patients. We believe that this strategy may be useful in limiting enhanced inflammation due to the presence of *P. jirovecii* in the lung and avoiding inter-patient *P. jirovecii* transmission.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

This study was part of the French COVID-19 cohort registry conducted by the REACTing consortium (REsearch and ACTion targeting emerging infectious diseases) and directed by INSERM (Institut national de la santé et de la recherche médicale) and ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium). Our institutional ethics committee approved the study (N°, IDRCB, 2020-A00256-33; CPP, 11-20-20.02.04.68737).

Availability of data and materials

Drs. Alanio, Mégarbane and Bretagne conceived of and designed the study. Drs. Voicu, Azoulay and Mégarbane managed the patients. Drs. Alanio, Dellière and Bretagne performed the microbiological analysis. All authors acquired, analyzed the data and interpreted the results. Drs. Alanio, Mégarbane and Bretagne drafted the manuscript. All authors participated to the critical revision of the manuscript for important intellectual content. Dr. Alanio has full access to all data and takes responsibility for the data integrity and its analysis accuracy.

Consent for publication

All the authors agree to publish.

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None. The case investigations, analysis, and manuscript preparation were completed as part of official duties at the university hospital.

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138 **Table 1**

139 Characteristics of hundred-and-eight critically ill COVID-19 patients according to *Pneumocystis jirovecii*
140 detection in the respiratory samples. Data are presented as percentages or medians [25th-75th percentiles].
141 Comparisons were performed using Mann-Whitney (∘) or Fisher exact tests (*), as appropriate.

142

	Total (N=108)	**No detection of *P. jirovecii* (N=98)**	**Detection of *P. jirovecii* (N=10)**	***P***
Male gender, N (%)	88 (81.5%)	80 (81.6%)	8 (80.0%)	1*
Age (years)	62 [56-68]	62 [56-68]	59 [46-68]	0.40∘
COVID-19 risk factors				
Past hypertension, N (%)	64 (59.3%)	58 (59.2%)	6 (60.0%)	1*
Diabetes, N (%)	40 (37.0%)	37 (37.8%)	3 (30.0%)	0.74*
Obesity, N (%)	35 (32.4%)	32 (32.7%)	3 (30.0%)	1*
Coronary disease, N (%)	15 (13.9%)	14 (14.3%)	1 (10.0%)	1*
Body-mass index (kg/m^2)	28 [25-31]	28 [25-31]	28 [27-32]	0.61∘
Other remarkable comorbidities				
Asthma, N (%)	5 (4.6%)	4 (4.1%)	1 (10.0%)	0.39*
Chronic obstructive pulmonary disease, N (%)	2 (1.9%)	2 (2.0%)	0 (0.0%)	1*
Immunocompromised patient, N (%)	10 (9.3%)	10 (10.2%)	0 (0.0%)	0.59*
Long-term corticosteroids, N (%)	11 (10.2%)	8 (8.2%)	3 (30.0%)	0.06*
Biological data of interest on admission				
PaO_2/FiO_2 (mmHg)	137 [83-247]	134 [83-239]	177 [108-253]	0.60∘

Serum creatinine (µmol/L)	80 [64-111]	80 [63-111]	80 [67-104]	0.99◦
Plasma D-dimer (ng/mL)	2,2395 [1,193-4,635]	2,610 [1,405-4,700]	1,270 [750-2,390]	0.03◦
Serum lactate dehydrogenase (IU/L)	687 [540-901]	687 [568-903]	708 [436-893]	0.65◦
Bronchoalveolar lavage characteristics				
% BAL macrophages	28 [15-46]	27 [14-42]	51 [49-55]	0.13◦
% BAL polymorphonuclear cells	37 [26-81]	46 [26-81]	29 [18-32]	0.54◦
% BAL Lymphocytes	13 [6-32]	14 [5-34]	13 [10-23]	0.81◦
Specific anti-COVID-19 therapy, N (%)				
Azithromycin, N (%)	34 (31.5%)	30 (30.6%)	4 (40.0%)	0.72*
Hydroxychloroquine, N (%)	34 (31.5%)	30 (30.6%)	4 (40.0%)	0.72*
Hydroxychloroquine + Azithromycin, N (%)	29 (26.9%)	26 (26.5%)	3 (30.0%)	1*
Lopinavir-ritonavir, N (%)	16 (14.8%)	12 (12.2%)	4 (40.0%)	0.04*
Polyvalent immunoglobulins, N (%)	3 (2.8%)	3 (3.1%)	0 (0.0%)	1*
Sarilumab, N (%)	1 (0.9%)	1 (1.0%)	0 (0.0%)	1*
Eculizumab, N (%)	6 (5.6%)	4 (4.1%)	2 (20.0%)	0.10*
Tocilizumab, N (%)	4 (3.7%)	4 (4.1%)	0 (0.0%)	1*
Dexamethasone, N (%)	53 (49.1%)	46 (46.9%)	7 (70.0%)	0.19*
Dexamethasone cumulative dose >100mg, N (%)	16 (14.8%)	15 (15.3%)	1 (10.0%)	1*
Severity during hospitalization and outcome				
SAPS II on admission	37 [31-49]	38 [31-51]	34 [28-37]	0.16◦
SOFA on admission	6 [3-8]	6 [3-8]	5 [2-7]	0.43◦

Lowest PaO_2/FiO_2 (mmHg)	71 [58-89]	71 [59-89]	65 [53-102]	0.79◦
Vasopressors, N (%)	89 (82.4%)	81 (82.7%)	8 (80.0%)	1*
Renal replacement therapy, N (%)	38 (35.2%)	35 (35.7%)	3 (30.0%)	1*
ECMO, N (%)	10 (9.3%)	9 (9.2%)	1 (10.0%)	1*
SAPS II on admission	37 [31-49]	38 [31-51]	34 [28-37]	0.16◦
ICU length of stay (days)	20 [12-32]	20 [12-33]	10 [6-19]	0.03◦
Mortality, N (%)	47 (43.5%)	44 (44.9%)	3 (30.0%)	0.51 *

143 BAL, bronchoalveolar lavage; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; SOFA, Sepsis-
144 related Organ Failure Assessment; SAPS II, Simplified Acute Physiology Score II.

Table 1

Characteristics of hundred-and-eight critically ill COVID-19 patients according to *Pneumocystis jirovecii* detection in the respiratory samples. Data are presented as percentages or medians [25th-75th percentiles]. Comparisons were performed using Mann-Whitney (∘) or Fisher exact tests (*), as appropriate.

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