**RESEARCH LETTER**

**Pneumocystis pneumonia risk among viral acute respiratory distress syndrome related or not to COVID 19**

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Lymphopenia, corticosteroids and immunomodulatory therapeutics frequently used in COVID-19 patients with acute respiratory distress syndrome (C-ARDS) may be contributing factors to opportunistic infection such as *Pneumocystis jirovecii* pneumonia (PCP).

We conducted a retrospective study to compare the prevalence of PCP between patients with C-ARDS and those with non-SARS-CoV-2 viral ARDS (NC-ARDS).

Methods and some data from this cohort have been previously published [1]. There was no systematic protocol to search for PCP but in case of suspicion of PCP (respiratory symptoms with any consistent radiographic features), several analyses were performed on respiratory samples, such as broncho-alveolar lavage (BAL), blind protected sample, or sputum. It included direct examination (using May-Grünwald Giemsa (MGG), or immunofluorescence staining), detection of *Pneumocystis jirovecii* DNA by real-time polymerase chain reaction (qPCR) [2], and serum (1–3)-β-D-glucan. During the COVID-19 outbreak, immunofluorescence staining was not performed. PCP was defined as per the revised EORTC/MSGERC definition [3] as follows: proven in case of suspicion with positive direct examination; possible in case of suspicion with positive qPCR and positive BDG in ≥2 consecutive serum samples provided other etiologies have been excluded. SARS-CoV-2 and other viruses were not considered a priori as host factors. Patients with positive qPCR but lacking the other criteria for possible PCP were classified as colonized.

The primary endpoint was the difference in prevalence of PCP between C-ARDS and NC-ARDS patients.

No statistical sample size calculation was performed a priori, and sample size was equal to the number of patients treated during the study period. All patients were included only once.

Between October 1, 2009, and April 29, 2020, ninety patients had C-ARDS (positive RT PCR test for SARS-CoV-2), while 82 patients had viral NC-ARDS. Our study comprises 120 patients with fungal analyses on respiratory samples obtained from 81 C-ARDS and 39 NC-ARDS patients. NC-ARDS patients had more comorbidities were more often immunocompromised, and had lower lymphocyte counts than C-ARDS patients (Table 1). C-ARDS patient received less steroid than NC-ARDS patients because they were included before randomized trials demonstrating decreased mortality with dexamethasone.

**Pneumocystis** analyses were performed on a mean of 3.1 respiratory sample per patient (range 1–15). Direct examination was performed in a total of 72 samples, with two positive cases. qPCR was performed in a total of 368 samples (294 blind protected samples, 72 BAL, and three sputum). All qPCR were negative in C-ARDS patients, while five (13%) NC-ARDS patients had at least...
Table 1  Characteristics of patients with *Pneumocystis jirovecii* research according to C-ARDS and NC-ARDS patients

|                          | NC-ARDS (n = 39) | C-ARDS (n = 81) | p-value  |
|--------------------------|------------------|-----------------|----------|
| Age, median [IQR]        | 61.8 [56.1–69.3] | 58 [52–69.5]    | 0.32     |
| Male gender              | 28 (72%)         | 65 (80%)        | 0.30     |
| Medical history          |                  |                 |          |
| McCabe                   |                  |                 | <0.001   |
| No underlying condition  | 13 (33%)         | 70 (86%)        |          |
| Ultimately fatal         | 16 (41%)         | 10 (12%)        |          |
| Rapidly fatal disease    | 10 (26%)         | 1 (1%)          |          |
| Charlson comorbidity index | 3 [2–4]       | 1 [0–2]         | <0.0001  |
| Diabetes mellitus        | 11 (28%)         | 38 (47%)        | 0.051    |
| Congestive heart failure (NYHA 3–4) | 3 (8%) | 6 (7%) | >0.99 |
| Supraventricular arrhythmia | 5 (13%)     | 8 (10%)         | 0.76     |
| Hypertension             | 16 (41%)         | 52 (64%)        | 0.016    |
| COPD                     | 2 (5%)           | 8 (10%)         | 0.50     |
| Chronic renal failure    | 8 (21%)          | 13 (16%)        | 0.55     |
| Dialysis                 | 3 (8%)           | 2 (3%)          | 0.33     |
| Stroke                   | 1 (3%)           | 3 (4%)          | >0.99    |
| Liver cirrhosis (Child C)| 1 (3%)           | 0 (0%)          | 0.33     |
| Current smoking          | 8 (21%)          | 21 (26%)        | 0.52     |
| Immunosuppression conditions |              |                 |          |
| Overall                  | 32 (82%)         | 13 (16%)        | <0.0001  |
| Solid cancer             | 2 (5%)           | 4 (5%)          | >0.99    |
| Blood cancer             | 15 (38%)         | 0 (0%)          | <0.0001  |
| Organ transplant         | 9 (23%)          | 5 (6%)          | 0.013    |
| HIV infection            | 3 (8%)           | 3 (4%)          | 0.39     |
| Sickle cell disease      | 1 (3%)           | 3 (4%)          | >0.99    |
| Others                   | 4 (10%)          | 1 (1%)          | 0.038    |
| Clinical characteristics upon ICU admission | | | |
| IGS2                     | 51 [37–68]       | 35 [27–43]      | <0.0001  |
| Baseline SOFA, median [IQR] | 9 [6–12]     | 7 [4–8]         | <0.0001  |
| ARDS classification (Berlin definition) | | | 0.046 |
| Mild                     | 12 (31%)         | 10 (12%)        |          |
| Moderate                 | 18 (46%)         | 44 (54%)        |          |
| Severe                   | 9 (23%)          | 27 (33%)        |          |
| Norepinephrine, n (%)    | 20 (51.3%)       | 35 (43.2%)      | 0.41     |
| Serum creatinine (µmol/L) | 147 [83–226]   | 82 [66–124]     | 0.001    |
| White blood cell count (x 10⁹/L) | 5.4 [3–14.8] | 8.1 [5.5–11.9] | 0.44     |
| Lymphocyte count (x 10⁹/L) | 0.4 [0.2–0.9] | 0.8 [0.5–1.2]  | 0.01     |
| Documented bacterial co-infections | 18 (46%)      | 13 (16%)        | <0.0001  |
| Treatments during the first 24 h | | | |
| Antibiotics              | 39 (100%)        | 81 (100%)       | >0.99    |
| Antiviral treatment      | 26 (67%)         | 65 (80%)        | 0.10     |
| Corticosteroids (any dose) | 21/38 (55%)   | 10/79 (13%)*   | <0.0001  |
| Corticosteroids (low dose) | 20/38 (53%) | 8/79 (10%)*   | <0.0001  |
| Corticosteroids (high dose) # | 1/38 (3%)    | 2/79 (3%)*      | >0.99    |
| ARDS treatment during ICU stay | | | |
| Corticosteroids (any dose) | 24 (63%)        | 32 (41%)*       | 0.02     |
| Corticosteroids (low dose) | 22 (58%)         | 22 (28%)*       | 0.002    |
| Corticosteroids (high dose) # | 2 (5%)          | 10 (13%)*       | 0.22     |
| Prone position           | 20 (51%)         | 71 (88%)        | <0.0001  |
Table 1 (continued)

|                                | NC-ARDS (n = 39) | C-ARDS (n = 81) | p-value |
|--------------------------------|------------------|-----------------|---------|
| Neuroumuscular blockade        | 25 (64%)         | 74 (91%)        | <0.0001 |
| Inhaled nitric oxide           | 6 (15%)          | 28 (35%)        | 0.03    |
| Extra-corpooreal membrane oxygenation | 5 (13%)      | 20 (25%)        | 0.13    |
| Organ support and outcome during ICU stay |                  |                 |         |
| Renal replacement therapy during ICU stay | 19 (49%)        | 29 (36%)        | 0.18    |
| Norepinephrine, n (%)          | 32 (82%)         | 61 (75%)        | 0.41    |
| ICU length of stay among survivors, days | 17 [10–28]      | 30 [22–46]      | 0.09    |
| Death at day 28                | 15 (30%)         | 30 (37%)        | 0.88    |
| Death in the ICU               | 17 (44%)         | 32 (40%)        | 0.67    |

COPD = chronic obstructive pulmonary disease, HIV = human immunodeficiency virus, SAPS II = Simplified Acute Physiology Score II, SOFA = sequential organ failure assessment, ICU = intensive care unit; *two missing values because two patients received dexamethasone or placebo in a randomized controlled trial; #denotes more than 1 mg/kg of prednisone or equivalent

Table 2

| Patient, age, sex | Underlying disease | Date of PCP diagnosis | Viral association | Respiratory sample | Direct examination (IFI or MGG) | Pneumocystis qPCR | BDG (pg/ml)* | Time between ICU admission and positive sample (day) | Treatment |
|------------------|--------------------|-----------------------|-------------------|--------------------|-------------------------------|-------------------|-------------|------------------------------------------------|-----------|
| P1, 58y, M       | Diabetes mellitus  | 14/01/2014            | Coronavirus, Rhinovirus | BAL                | Negative                      | 36.7              | NA          | 1                                              | No        |
| P2, 73y, M       | Renal transplantation | Renal transplantion diabetes mellitus | 29/08/2015 | Coronavirus | BAL | Positive | 32 | 106 | 0 | Yes (sulfamethoxazole) |
| P3, 52y, M       | Myasthenia (steroid, azathioprine) | 04/07/2012 | Respiratory syncytial virus | BAL | Negative | 39.8 | NA | 1 | No |
| P4, 32y, F       | Acute lymphoblastic leukemia (methotrexate and ara-cytosine) | 08/01/2019 | Metapneumovirus | BAL | Positive | 27.9 | 188 | 0 | Yes (sulfamethoxazole) |
| P5, 67y, M       | Cirrhosis, rheumatoid polyarthritis (steroid) | 22/04/2019 | Coronavirus NL63 | BAL | Negative | 36.6 | NA | 1 | No |

M = male, F = female, P = patient; BDG = (1–3)-β-D-glucan,*BDglucan not performed in the lab before 2013. BDglucan was performed using the Fungitell kit™ (Cape Cod Inc, USA) with a positivity threshold of 80 pg/mL; qPCR of P. jirovecii was performed using a region of the mitochondrial large subunit rRNA gene (LSU) after DNA extraction with a Qiasympohy kit (Qiagen, Courtaboeuf, France)
one positive PCR, with a median cycle threshold of 36.6 [30–38.3].

Two NC-ARDS patients fulfilled proven PCP diagnostic criteria, with a positive direct examination, a single β-D-glucan > 80 pg/mL (Table 2), and received treatment for PCP.

Three other NC-ARDS patients were classified as colonized, but no patient fulfilled possible PCP diagnostic criteria. Time between ICU admission and positive sample for PCR (Table 2) was short (<2 days) like in other invasive fungal infections (i.e. invasive pulmonary aspergillosis) in severe influenza infection or ARDS.

In this study of patients with viral ARDS, we found a low risk for possible or proven PCP. Our findings are in accordance with two smaller studies in France [4, 5] retrieving a low risk of Pneumocystis colonisation in COVID-19 patients. In our cohort, qPCR was positive in 13% of NC-ARDS. This result is in accordance with a previous study showing 7% of positive qPCR in ICU-admitted influenza patients [6]. The strengths of our study are the analysis of a large ARDS cohort with fungal analyses. Our study also has limitations: monocentric design, NC-ARDS patients more frequently immunocompromised, and a long cohort period.

Abbreviations
C-ARDS: Coronavirus disease 19 related acute respiratory distress syndrome; NC-ARDS: Non-coronavirus disease 19 viral ARDS; PCP: Pneumocystis jirovecii Pneumonia.

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Authors’ contributions
KR, RA and FB contributed to the study design, analysis and interpretation of data. KR, RA and AMD drafted the initial manuscript and approved the article final version. AFH, SF, and the COVID-PCP group contributed to the interpretation of data, critical revision of intellectual content and approval of the submitted version of the article. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets supporting the conclusions are included within the article.

Declarations

Ethics approval and consent to participate
This observational study was approved by the Ethical Review Board of the French Society for Intensive Care Medicine (Société de Réanimation de Langue Française). As per the French law, no informed consent was required for this type of studies.

Consent for publication
Not applicable.

Competing interests
All authors report no conflict of interest relevant to this study.

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