Acronym[1]	**MY-CO-VID**
Title of the proposal	Characterization of fungal infections in COVID-19 infected and mechanically ventilated patients in ICU
Name and firstname of the coordinator	Pr GANGNEUX Jean-Pierre
Coordinator institution	Identification de l'établissement : CHU de Rennes Adresse : 2 rue Henri le Guilloux, 35033 Rennes Cedex 09
Requested funding	196 994.16 euros
Total cost :	196 994.16 euros
Duration	12 months
Key words of the proposal	Invasives mycoses, aspergillosis, pneumocystosis, mucormycosis.

[1] Il est ici rappelé que le choix des noms et acronymes des projets (notamment de l'absence de violation de droits des tiers) relève de la seule responsabilité des porteurs de projets.

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Proposal summary

Among various causes of morbidity and mortality in COVID-19 patients, the impact of fungal co-infections has still been poorly studied and understood, particularly in patients with an acute respiratory distress syndrome (ARDS). However, we must consider two important facts:
(i) few Chinese publications that described the COVID-19 patients characteristics reported up to 10% of co-infections in patients hospitalized in intensive care units (ICU) for ARDS (Yang et al, Lancet Respir Med 2020),
(ii) our recent knowledge on fungal co-infections during influenza infection underlined that mortality can reach between 16 and 23% between centers (Verweij P et al., CID 2020).

In this work, we propose two main objectives:
1. to set up a unique and simple protocol to prospectively follow COVID-19 patients with ARDS in ICU. The aim is to early diagnose invasive fungal infections (IFI) with a wide syndromic fungal molecular panel that will detect *Aspergillus*, *Pneumocystis jirovecii* and mucormycetes and to estimate the incidence of opportunistic fungal co-infections.
2. to decipher the dynamic of colonization and of fungal infection in these patients from admittance to discharge from ICU, with the aim to optimize the management and reduce the mortality. The systematic follow up will indeed allow to adapt the management with a rapid and targeted curative treatment. Depending on the data obtained, preventive strategies such as antifungal chemoprophylaxis and environmental measures could be envisaged with the aim to decrease morbidity and mortality.

Expected results are to provide original and still unknown epidemiological data on IFI during COVID-19, to improve the diagnosis with an efficient syndromic molecular approach for fungal respiratory infection during ARDS and to optimize immediate COVID-19 patients management with the introduction of a targeted treatment and discussion on preventive strategies with the aim to decrease morbidity and mortality.

Investigators from 13 participating centers are internationally recognized for their contribution to the diagnosis and management of fungal infections in ICUs that are in front line of the COVID-19 pandemic and constitute an unprecedented consortium.

1. Proposal's context, positioning and objective(s)

General context of the proposal

The epidemic of respiratory infection due to the new coronavirus SARS-CoV-2 that emerged in December 2019 in China is now pandemic all over the world with a huge mortality. The mortality rate greatly varies between countries, with an unexplained high rate in Italy.

Among various causes of morbidity and mortality in COVID-19 patients, the impact of bacterial and fungal co-infections has still been poorly studied and understood, particularly in patients with an acute respiratory distress syndrome (ARDS). However, we must consider two important facts:
(i) Few Chinese publications that described the COVID-19 patients characteristics reported up to 10% of co-infections in patients hospitalized in intensive care units (ICU) for ARDS (Yang et al, Lancet Respir Med 2020),
(ii) Our recent knowledge on fungal co-infections during influenza infection underlined that mortality can reach between 16 and 23% between centers (Verweij P et al., CID 2020).

In this context, we propose a **national multicentric study that aims to explore the risk of fungal co-infection during COVID-19 in patients with ARDS**, and to optimize the early diagnosis in order to allow a prompt specific antifungal treatment and the management of the patients. A consortium of ICU and Mycology specialists has been constituted to elaborate a simple and unique protocol that will allow to improve the management in real time. **This study fits perfectly with the ANR - AAP Flash COVID-19 within the topic "Etudes épidémiologiques et translationnnelles, caractéristique clinique et prise en charge".**

State of the art

Invasive fungal infections (IFI) during COVID-19 are still rarely reported and are probably underdiagnosed. To date, in France, one invasive aspergillosis (IA) case in the intensive care unit (ICU) has been diagnosed in a Parisian hospital. However, the high aggressive feature of the SARS-CoV-2 virus to the lung tissue and the large bilateral alveolo-interstitial lesions make the occurrence of IFI very likely, specifically those with a primary pulmonary entry and an airborne route of infection such as IA, Pneumocystosis (PCP) and mucormycosis (He F, J Med Virol. 2020).

The incidence of IA in ICU patients admitted for severe influenza A and B is high, reaching 19% versus 5% in patients with severe pneumonia other than flu. The 3-month mortality rate of influenza is 51 % when associated with IA and 28% without IA (Schauwvlieghe A, Lancet Respir Med 2018). Besides, recent Chinese publications reported at least 10% of co-infection during COVID-19, among them *Aspergillus* infections (Yang Lancet Respir Med 2020).

In France, IFI account for a higher risk of mortality in weak patients from 9 to 40% (Bitar C, Emerg Infect Dis 2014). IA is notably diagnosed in neutropenic patients, patients under chemotherapy, particularly for hematological malignancies, prolonged corticosteroid therapy or biotherapy, HSCT allografted or solid organ transplantation, or chronic respiratory diseases. PCP is an opportunistic infection diagnosed in lymphopenic patients, patients co-infected with HIV, and patients suffering from hematological malignancies, solid organ transplantation or chronic respiratory diseases. Invasive mucormycosis is increasingly reported (thanks to the improvement of diagnostic tools) in susceptible patients such as those suffering from diabetes, hematological malignancies, solid organ transplantation or chronic respiratory diseases and superficial injuries in burned patients or after local traumatism.

Using local and literature data, the global burden of severe fungal infection is estimated approximately 1,000,000 (1.47%) cases in France each year (Gangneux et al., J Mycol Med 2016).

Two gripping points must be underlined:

- Patients hospitalized in ICU for COVID-19 share all the risk factors described for IFI,
- We still don't know exactly how fungal co-infection impacts on morbidity and mortality but are aware of the dramatic impact of influenza/IA co-infection with a mortality reaching 23% in some European centers (Verweij et al, CID 2020, Schauwvlieghe Lancet Respir Med 2018, van de Veerdonk Am J Respir Crit Care Med 2017). In all cases, fungal co-infection was responsible for obvious attributable mortality. Besides, recent Chinese publications reported until 10% of co-infection during COVID-19, among them *Aspergillus* infections (Yang Lancet Respir Med 2020).
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Required measures to limit the risk of professional contamination during lab process of respiratory samples induce a gradation of our diagnostic tests with sometimes the exclusion of some classical tools for the diagnosis of fungal infections such as direct examination, stained smears or even the way to culture. That's why, in order to give all chances for an efficient diagnosis as part of a personalized patient management, new molecular tools as well as blood biomarkers are of prime interest.

We propose two main objectives to this work:

1. Set up a unique and simple protocol to prospectively follow COVID-19 patients with ARDS in ICU. The aim is to early diagnose IFI with a wide syndromic fungal molecular panel that will detect *Aspergillus*, *Pneumocystis jirovecii* and mucormycetes and to estimate the incidence of opportunistic fungal co-infections.
2. Decipher the dynamic of colonization and of fungal infection in these patients from the entry to the exit of ICU, with the aim to optimize the management and reduce the mortality. The systematic follow up will indeed allow to adapt the management with a rapid and targeted curative treatment. Depending on the data obtained, preventive strategies such as antifungal chemoprophylaxis and environmental measures could be envisaged with the aim to decrease morbidity and mortality.

A considerable scientific dynamic within a few days on this topic allowed to constitute an unprecedented consortium of specialists from all France to design a multicenter study

- ICU specialists involved in this study are skilled in the routine management of ARDS and some of them has already start to manage many COVID-19 patients
- Mycology specialists are skilled in the diagnostic tools. All of them routinely realize *Aspergillus* and *Pneumocystis* PCR in respiratory samples. This study is the opportunity to standardized and share similar protocol for PCR Mucorales and specific diagnostic fungal markers.

Study design, tasks, and methodology

National prospective multicentric study with consecutive inclusion of patients

Primary objective	Prospective respiratory screening of IFI in COVID-19 patients with ARDS in ICU using a wide syndromic fungal molecular panel that will detect *Aspergillus*, *Pneumocystis jirovecii* and mucormycetes.
Primary evaluation criterion	Incidence of opportunistic fungal co-infections.
Secondary objectives	- Evaluation of the dynamic of respiratory colonization and fungal infection in COVID-19 patients with ARDS in ICU, - Optimization of the management through a targeted antifungal therapy and proposition for prevention
Secondary evaluation criteria	- Determination of the median time between entry in ICU and beginning of ARDS and (i) colonization and (ii) probable/proven infection with *Aspergillus*, *Pneumocystis jirovecii* and mucormycetes - Evaluation of the time between diagnosis and targeted treatment - Proposal for evaluation of preventive strategies if necessary, because of high incidence, in terms of chemoprophylaxis and/or environmental measures
Criteria for inclusion of subjects	- ICU patients with COVID-19 infection - Intubated and mechanically ventilated patients - Adult patients - Patients (or family) informed on the research without opposition
Non-inclusion criteria of subject	- Patients <18 years old - Unability to follow or to understand the study procedures

Methodology – description of the protocol

Currently, ICU patients with ARDS, whatever the etiology, are not systematically screened for the detection of respiratory fungal infections.

Here, the protocol will be in two steps:

First step
COVID-19 patients hospitalized in ICU for ARDS will benefit for a systematic screening with a fungal respiratory syndromic panel twice a week from the entry to the exit of ICU:

- Sample: tracheal aspiration, bronchial aspiration, BAL
- Fungal respiratory panel: samples will be processed in each lab for culture without direct examination nor stained smears, and real-time PCR will be performed for *Aspergillus*, *Pneumocystis jirovecii* and mucormycetes
- Results will be given to ICU in order to optimize the management of the patient

Second step
Complementary analysis will be performed in order to finalize the diagnostic and to differentiate between colonization and infection, with:

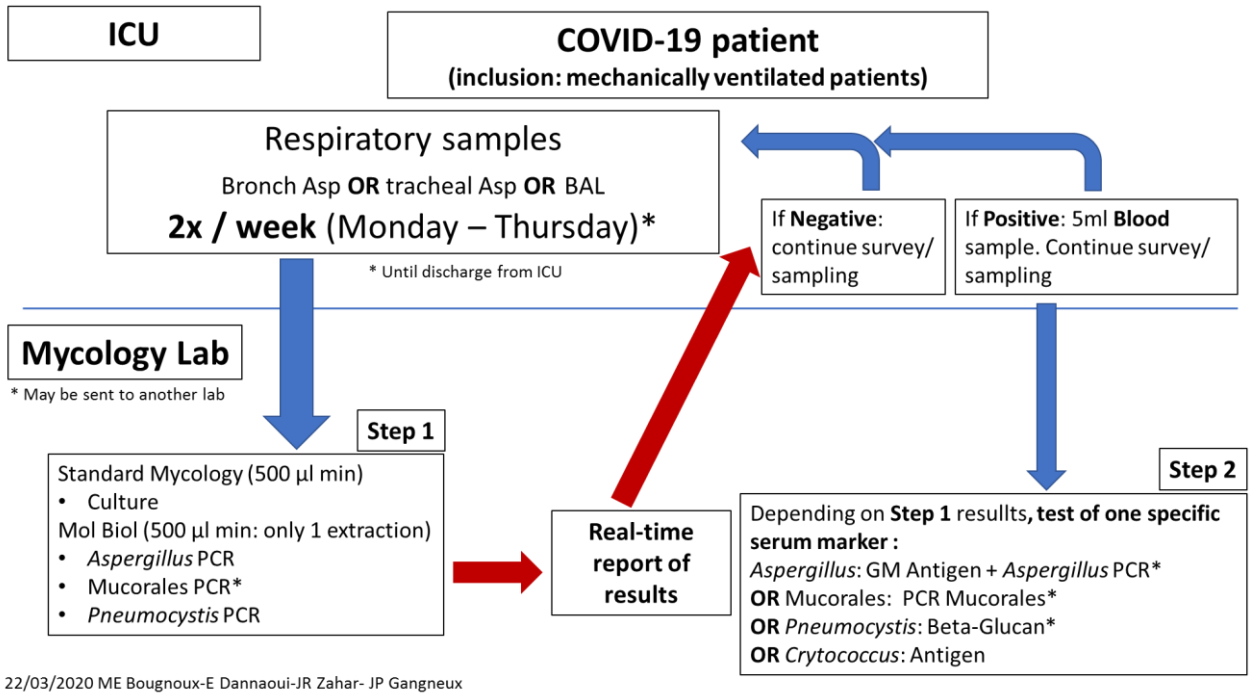
- Serum detection of galactomannan and serum *Aspergillus* PCR in case of positive respiratory sample for *Aspergillus*

- Serum/plasma beta-D-glucan detection in case of positive respiratory sample for *Pneumocystis jirovecii*
- Serum mucorales PCR in case of positive respiratory sample for mucorales

This second step is possible in most of the labs, but when necessary a confirmation test can be externalized. A process of DNA transmission to a reference lab within each region will be implemented.
This second step will allow to classify infections as probable or proven according to international recommendations (Donnelly et al., CID 2019).

Case report form (CRF)
A short but standardized CRF will be proposed to all centers in order to collect demographic data and the essential clinical and laboratory data during the survey.

Flow-chart depicting the study design



Justification of the sample size

- Estimated incidence of IFI in high-risk populations or during influenza was estimated between 5 and 23%.
- Our hypothesis is that the incidence of colonization and/or fungal infection during COVID-19 infection is at least 10%.
- In this context, our objective for this exploratory study is to enroll 250 patients (25 to 30 consecutive patients by ICU), thus allowing if the percentage of positive patients is 10% to obtain a precision of the estimation (95% confidence interval) between 6.3 and 13.7%.

Statistical analysis

- Descriptive statistics will consist of percentage (number of patients) with the corresponding 95% confidence interval for categorical variables and mean, median, standard deviation, and quartiles for continuous variables.
- Comparisons between sugroups (for ex postive and negative patients) will use standards statistical approaches: chi² for categorical variables and student t test or wilcoxon rank sum test for continuous variables.

Gantt diagram showing milestones of the proposal

Months					
	M1	M 3	M6	M9	M12
Patient inclusion					
Mycological work-up of samples					
Specific blood markers					
Collection of clinical data					
Result analysis					
Final report					

Previously obtained resources
There is currently no financial resources for this proposal.

2. Consortium

A consortium has been constituted with the aim to reach the objectives of the project.

- Principal investigators: Jean-Pierre Gangneux (CHU de Rennes), Marie-Elisabeth Bougnoux (CHU de Necker & Institut Pasteur, Paris), Eric Dannaoui (CHU HEGP, Paris), Jean-Ralph Zahar (CHU Avicennes, Bobigny).
- ICU partners: All participating ICU physicians have already experience in management of COVID-19 infected patients. All participating centers are located in areas with high incidence of COVID-19 infections.
- Mycology partners: All participating mycologists are routinely involved in diagnosis of fungal infections in University Hospitals and are skilled in the management of research protocols for IFI.

Principal investigators are internationally recognized for their contribution to the diagnosis and management of fungal infections.

Scientific coordinators

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Participating centers :

Center	ICU specialists	Mycologists
CHU Rennes	Jean-Marc Tadié / Yves Le Tulzo	Jean-Pierre Gangneux / Florence Robert-Gangneux
CHU Paris - HEGP	Anna Novara	Eric Dannaoui
CHU Paris - Tenon	Muriel Sarrah Fartoukh	Christophe Hennequin
CHU Paris - Mondor	Nicolas de Prost / Keyvan Razazi	Francoise Botterel
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CHU Strasbourg	Feraht Meziani	Valérie Letcher-Bru
CHU Angers	Achille Kouatchet / Alain Mercat	Jean-Philippe Bouchara
CHU Lyon	Jean Christophe Richard/ Laurent Argaud	Martine Wallon / Florence Persat
CHU Toulouse	Beatrice Riu-Poulenc	Antoine Berry / Xavier Iriart
CHU Tours	Pierre-François Dequin	Guillaume Desoubeaux
CHU Nantes	Emmanuel Canet	Florent Morio / Patrice Le Pape

This complementary partnership will benefit from the synergic expertise of the ICU physicians with the mycologists:

- Tasks for ICU physicians: inclusion of patients; perform bi-weekly systematic respiratory samples in patients, and provide clinical data.
- Tasks for mycologists: perform mycological work-up on the respiratory samples; perform a real-time reporting of culture and qPCR results for optimal management of fungal infections diagnosed in these patients.
- Tasks for the methodologist and coordinator: data collection, record and monitor CRFs, and analyze data.

Feasibility of the project
- This protocol is feasible in the participating centers because ICU and mycology physicians are skilled in the monitoring of fungal infections in patients with ARDS. Most of them are/have been principal or associated investigators for national or international research protocol.
- Participating centers are all university hospitals and among the most concerned by the COVID-19 epidemic in France.
- All laboratories involved are from University hospitals and specialized in medical mycology. Laboratory processes will be shared in order to upgrade techniques if necessary, in some centers.

3. Impact of the proposal

Expected results are:
- To provide original and still unknown epidemiological data during COVID-19. This study is not a simple register but a national prospective study with a proactive bi-weekly screening. It will be a unique opportunity to evaluate the incidence and the dynamic of fungal infection in the course of the stay in ICU.
- To improve the diagnosis. This protocol will allow the validation of an efficient syndromic molecular approach for fungal respiratory infection during ARDS that can be shared with all hospitals receiving COVID-19 patients
- To optimize immediate COVID-19 patients management. Patients included will benefit from a particular large and real-time screening in order to introduce as early as possible a targeted treatment. First-line treatment for aspergillosis, pneumocystosis and mucormycosis are far different and empirical treatments will be avoided as much as possible. Depending on the data obtained, preventive strategies such as antifungal chemoprophylaxis and environmental measures could be envisaged with the aim to decrease morbidity and mortality.

4. Requested resources to reach the objectives

- Requested funding: 196 994.16 euros as detailed in the budget form

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