Appel à projets SARS-Cov2 – Sorbonne Université - Faculté de médecine

COVID-METAFLAM.SU

A TARGETED **META**BOMOLIC APPROACH TO DESIGN A PREDICTIVE MODEL FROM THE MONITORING OF THE IN**FLAM**MATORY MEDIATORS KINETICS IN PATIENTS ADMITTED TO INTENSIVE CARE UNIT WITH COVID-19 PNEUMONIA.

UNE APPROCHE MÉTABOMOLIQUE CIBLÉE À RÉPONSE RAPIDE POUR CONCEVOIR UN MODÈLE PRÉDICTIF DE LA CINÉTIQUE DES MÉDIATEURS INFLAMMATOIRES CHEZ LES PATIENTS COVID-19 ADMIS EN SERVICE DE RÉANIMATION.

L'objectif de ce projet consiste à développer, à partir des signatures métabolomiques et cytokiniques, un modèle prédictif du statut inflammatoire au cours de la prise en charge de patients COVID-19 dans les services de réanimation d'AP-HP.SU. Cette stratégie pourra être aussi bien utilisée dans le cadre diagnostic que dans celui de biomarqueurs compagnons pour l'évaluation des essais cliniques en cours. D'un point de vue méthodologique, le projet est dimensionné à l'échelle des DMU d'AP-HP.SU en particulier (APPROCHES-Pr. Fartoukh et BioGeM-Pr. Levy) pour maximiser la synergie entre les équipes tout en évitant les projets « doublons ». En outre, notre projet transversal et multidisciplinaire s'appuiera sur les expertises d'équipes de réanimateurs, pneumologues, virologues, biologistes médicaux et biostatisticiens ayant déjà travaillées ensemble sur des problématiques combinant soins intensifs, métabolomique et inflammation (Centre de recherche Saint Antoine / Département Métabolisme – Inflammation). Enfin, les aspects éthiques et réglementaires seront particulièrement renseignés, en collaboration avec les structures AP-HP d'appui à la recherche (URC-Est, CRB).

A noter que ce projet a été déposé auprès de l'ANR Flash COVID et vient d'être retenu par le comité d'évaluation et de pilotage pour recevoir un financement initial de démarrage. La présente demande, centrée sur le périmètre de la Faculté de Médecine SU, permettra de compléter ce financement pour accélérer sa réalisation et augmenter son dimensionnement.

1. RÉSUMÉ DU PROJET

Beginning in December 2019, a novel coronavirus, designated SARS-CoV-2, has caused an international outbreak of respiratory illness termed Covid-19, requiring for a small proportion of patients, admission to Intensive Care Unit (ICU). However, despite the urgent needs of therapeutic guidelines, little is known about the exact immune and inflammatory response of patients with COVID-19 and no robust, sensitive and accurate biomarkers are available. The main objective of the present COVID-METAFLAM.SU project is to characterize the kinetic of metabolomic inflammatory profiles associated with the inflammatory-immune response during COVID-19 pneumonia in ICU patients. We also plan to help for the prediction of the successive phases (acute phase, resolution phase) of the inflammatory-immune response; to search for an association between metabolomic profiles and clinical outcome (morbidity, mortality); to focus on metabolomic profiles in patients receiving immunomodulatory, chloroquine and/or anti-inflammatory treatments. We hypothesize that the integration and machine learning modeling of inflammatory targeted metabolomics in addition to cytokine profiles and bio-clinical data should demonstrated that some metabolites highly correlated with the pathogenic phase of the infection, whereas other are associated with the resolution phase. Our project is thus aimed at stratifying COVID-19 patients according to their inflammatory and metabolic status to help the management of this patients and to provide guidelines for patient treatment. This project will benefit from combined expertise of teams of physicians, virologists, medical biologists and biostatistician working on intensive care and metabolomics/lipidomics. We will also pay a particular attention to the ongoing therapeutic assays using anti-viral, steroids, immunomodulators and other drugs such as hydroxychloroquine. The project will last 12 months and will be organized into four tasks: (i) Recruitment of patients and collection usual demographic, anamnestic, clinical, radiological, biological and microbiological data; (ii) Collection and transport of biological samples; (iii) Biological explorations; (iv) Analysis of results. COVID-METAFLAM is ancillary to the COVID-ICU

Study (REVA Network), a French prospective multicenter study (>70 ICUs), which gathers more than 10 ICUs in the Paris area. COVID-METAFLAM is a candidate study to use biological samples collected for the Biobank COVID AP-HP.

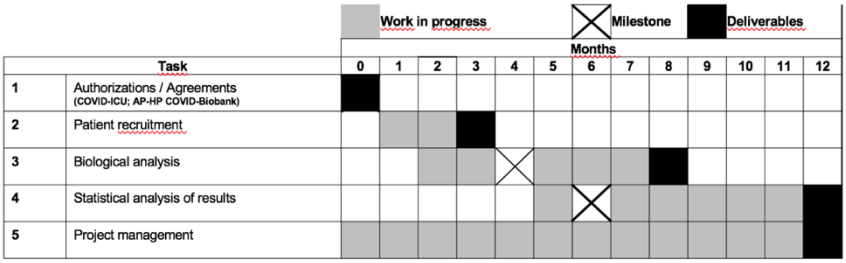
DURÉE DU PROJET

To explore our hypothesis, we propose the COVID-METAFLAM project, a prospective non-interventional multicenter study of translational research. We will study patients with COVID-19 pneumonia admitted to ICUs in the Paris area. Biological samples will include plasma and serum collected at D0, D3 and D7 of the ICU stay.

The project will last 12 months and will be organized into four tasks :

- Task 1: Recruitment of patients and collection usual demographic, anamnestic, clinical, radiological, biological and microbiological data;
- Task 2: Collection and transport of biological samples;
- Task 3: Biological explorations;
- Task 4: Analysis of results;

The coordination of tasks 1 and 2 and the coordination of task 3 will be assumed respectively by Dr Guillaume VOIRIOT (ICU, Tenon Hospital, DMU APPROCHES) and Dr Antonin LAMAZIERE (department of clinical metabolomics, DMU BioGeM). Task 4 will be shared.



2. CONTEXTE & JUSTIFICATIF DE LA RECHERCHE

Covid-19 physiopathology: from immune disorder to over inflammation

As a new type of highly contagious disease in human, the pathophysiology of unusually high pathogenicity for COVID-19 has not been completely understood yet. Several studies have shown that increased amounts of proinflammatory cytokines in serum were associated with pulmonary inflammation and extensive lung damage in SARS [3], MERS-CoV infection [4], and recently in COVID- 19 [1, 2]. Given that higher expression of proinflammatory cytokines and chemokines in COVID-19 patients, especially in the severe cases, the consumption of CD4+ and CD8+ T cells, and the decrease of regulatory T cells, presented in some studies, might result in aggravated inflammatory responses, the production of cytokine storm and make damaged tissue worse [5]. Correlative evidence from those severe patients with lower number of lymphocytes might suggest a role for dysregulated immune responses in COVID-19 pathogenesis [5]. Therefore, dysregulated immune response in COVID-19 patients suggests that SARS-CoV-2 might mainly act on lymphocytes, especially T lymphocytes, induce a cytokine storm in the body, and generate a series of inflammatory and immune responses that damage the corresponding organs; thus, surveillance of neutrophil-lymphocyte-ratio (NLR) and lymphocyte subsets is helpful in the early screening of critical illness, diagnosis and treatment of COVID-19.

Moreover, in a recent feedback from China, it was reported that patients infected with SARS-CoV-2 also had high amounts of IL1b, IFNγ, IL10, and MCP1, probably leading to activated T-helper-1 (Th1) cell responses [6]. Moreover, patients

requiring intensive care unit (ICU) admission had higher concentrations of GCSF, IP10, MCP1, MIP1A, and TNFα than did those not requiring ICU admission, suggesting that the cytokine storm was associated with disease severity [6]. However, SARS-CoV-2 infection also initiated increased secretion of T-helper-2 (Th2) cytokines (eg, IL4 and IL10) that suppress inflammation, which differs from SARS-CoV infection[3].

Controlled immune responses to infection and injury involve complex molecular signaling networks with coordinated and often opposing actions. Eicosanoids and related bioactive lipid mediators derived from polyunsaturated fatty acids constitute a major bioactive lipid network that is among the most complex and challenging pathways to map in a physiological context. Eicosanoid signaling, similar to cytokine signaling and inflammasome formation, has primarily been viewed as a pro-inflammatory component of the innate immune response; however, recent advances in lipidomics have helped to elucidate unique eicosanoids and related docosanoids with anti-inflammatory and pro-resolution functions. This has advanced our overall understanding of the inflammatory response and its therapeutic implications [7].

There is, therefore, in that particular situation, an urgent need to elucidate more into details the kinetic of a broad inflammatory mediator profile including cytokines, eicosanoids and other associated lipid mediators, in COVID-19 patients in order to give more accurate biological views of what is going on at the circulating level, in order to guide ICU physicians on both patient follow-up and treatment guidelines.

Potential lipid biomarkers

When SARS-CoV-2 infects the upper and lower respiratory tract it can cause mild or highly acute respiratory syndrome with a consequent release of pro-inflammatory cytokines (including interleukin (IL)-1b and IL-6) [8] as well as other lipidic inflammatory related species. Bioactive lipid mediators are also known to play a crucial role in the induction and resolution of inflammation [9]. They include eicosanoids, sphingolipids and steroids and they can be now very rapidly and accurately quantified in bio-clinical departments such as ours at Saint Antoine fully equipped with new generation mass spectrometry. Eicosanoids refer to a family of bioactive lipid mediators that regulate a wide variety of physiological as well as pathophysiological responses and often exhibit potent inflammatory properties [10]. Their profiles vary over time with an initial proinflammatory response followed by a distinct anti-inflammatory response [11]. These mediators are generated from arachidonic acid and related polyunsaturated fatty acids after their enzymatic release from membrane phospholipids via complex metabolic mechanisms involving over 50 unique enzymes [12]. Several bioactive lipid mediators have also been appreciated for their anti-inflammatory activity and their participation in the resolution phase of inflammation [11]. Arachidonic acid-derived lipoxins via the lipoxygenase pathway and docosahexaenoic acid (DHA)- and eicosapentaenoic acid (EPA)-derived resolvins, protectins, and maresins have anti-inflammatory and proresolution activities. These lipid mediators can prevent further infiltration of immune cells to the site of infection as well as signaling the nonphlogistic phagocytosis of apoptotic immune and epithelial cells, therefore allowing the system to return to homeostasis after microbial infection.

Moreover, in the situation, such as Covid-19, where the inflammation period tends to last, apoptotic processes appear. In a recent lipidomic study, it was shown that ceramide can play important functions in the initiation or maintenance of pathogen-induced immune responses. For instance, an increase in the production of 24-carbon ceramide has been demonstrated to occur in vitro during LPS-induced dendritic cell maturation which suggests roles for sphingolipids, ceramide or ceramide metabolizing enzymes during immune responses [13, 14].

Repeated profiles allow direct monitoring of the action of pharmacological agents (immunoregulators and possibly anti-inflammatories according to the updated indications) at the level of their targets. The fatty acid profiles circulating during septic shock will also allow the quantification of insulin resistance (acceleration of hepatic lipogenesis), a reaction correlated with the increase in the HOMA index. For this work combining the simultaneous measurement in patients infected with SARS-CoV2 of insulin resistance (index of lipogenesis in the profile of circulating fatty acids and HOMA) and the steroid profile (corticosterone and cortisol) the correlation with the severity of metabolic syndrome can be tested.

COVID-19 treatments

To date, no anti-viral agent has been approved in COVID-19 pneumonia. Therefore, apart from therapeutic trials, management of ICU patients with COVID-19 pneumonia is symptomatic, based on organ failure support therapies [15].

However, considering the huge cytokine storm, described in the more severe patients, immunomodulatory therapies are of growing interest. Corticosteroids, widely used during the outbreaks of SARS-CoV and MERS-CoV [16], have been administered in COVID-19 patients in China [17] and are investigated in ongoing trials (NCT04273321). Other therapies are under investigation, *e.g.* anti-IL6 antibody (Tocilizumab, NCT0431709, NCT04315480; Sarilumab, NCT04315298), thalidomide (NCT04273581) and IFNα2β (NCT04293887) and Hydrochloroquine (NCT04303299). For this later drug, the Discovery assay has been launched on March 21. We hypothesized that this drug may act on the intracellular assembly of the virus, as previously shown by our team. [18, 19, 20, 21, 22, 23]. From the data available, treatment of COVID-19 likely requires a combination of both antivirals and non-antivirals-based approaches such as corticosteroids or immunomodulator therapies. Corticosteroids were widely used during the outbreaks of severe acute respiratory syndrome SARS-CoV and Middle East respiratory syndrome (MERS)-CoV [16] and are being used in patients with SARS-CoV2 in addition to other therapeutics [24]. However, current interim guidance from WHO on clinical management of severe acute respiratory infection when novel coronavirus (SARS-CoV2) infection is suspected (released Jan 28, 2020) advises against the use of corticosteroids unless indicated for another reason [25]. Understanding the evidence for harm or benefit from corticosteroids in 2019-nCoV is of immediate clinical importance. Corticosteroids suppress lung inflammation but also inhibit immune responses and pathogen clearance. Eventually, the role of the immune defense in COVID-19 is a complex, ever-changing matter of balance. Immunosuppressed individuals are at elevated risk of infection, yet people who die from it often undergo cytokine storms, succumbing to an overzealous immune response that can rapidly lead to sepsis. Existing drugs that alter immune function in clinical trials include the multiple sclerosis drug, Fingolimod to tackle COVID-19 pneumonia and PD-1 blockers used to treat cancer to treat cytokine storms.
To conclude, there is an urgent need to describe profiles and kinetics of the inflammatory-immune response during COVID-19 pneumonia. Such data may identify therapeutic targets, give scientific rational for immunomodulatory agents, and guide their use in terms of indication and timing of administration.

3. OBJECTIFS DU PROJET

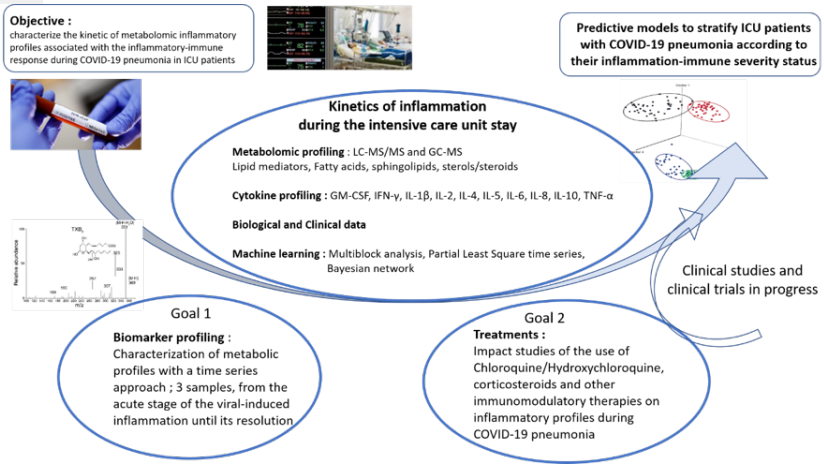
Beginning in December 2019, a novel coronavirus, designated SARS-CoV-2, has caused an international outbreak of respiratory illness termed Covid-19. The full spectrum of COVID-19 ranges from mild, self-limiting respiratory tract illness to severe pneumonia (COVID-19 pneumonia), requiring admission to Intensive Care Unit (ICU), and ultimately leading to multiorgan failure and death [1, 2]. It has been reported that COVID-19 pneumonia was more likely to occur in older men with comorbidities, especially those with altered immune functions. Several studies have shown that increased amounts of proinflammatory cytokines in serum were associated with massive pulmonary inflammation and extensive lung damage in COVID-19 patients. However, although they are absolutely required to ensure a precise follow-up of ICU patients, little is known about the exact immune and inflammatory response of patients with COVID-19 and no robust, sensitive and accurate biomarkers are available.

Objectives

The main objective is to characterize the kinetic of metabolomic inflammatory profiles associated with the inflammatory-immune response during COVID-19 pneumonia in ICU patients.

Second objectives are :

- to describe kinetics of the metabolic and inflammatory processes for the prediction of the successive phases (acute phase, resolution phase) of the inflammatory-immune response;
- to search for an association between metabolomic profiles and clinical outcome (morbidity, mortality);
- to focus on metabolomic profiles in patients receiving immunomodulatory, chloroquine and/or anti-inflammatory treatments.



We hypothesize that the integration and machine learning modeling of inflammatory targeted metabolomics (including eicosanoids and lipid mediators, sphingolipids and steroid species) in addition to cytokine profiles and bio-clinical data should demonstrated that some metabolites highly correlated with the pathogenic phase of the infection, whereas other are associated with the resolution phase.

To explore our hypothesis, we propose the Covid-METAFLAM.SU project, a prospective non-interventional multicenter study of translational research. We will study patients with COVID-19 pneumonia admitted to ICUs in the Paris area. **The goal of our project** is to identify potential biomarker profiles paralleling the kinetic of metabolic processes for the prediction of the immune and inflammatory status during an active infection. To elucidate their involvement during COVID-19 infection, liquid chromatography/mass spectrometry lipidomic profiling of circulating lipid species combined with machine learning modelling approach will be performed on ICU patients.

In conclusion**, our project is aimed at stratifying COVID-19 patients according to their inflammatory and metabolic status in order to help the management of this patients from pulmonary to systemic phase.** This project will benefit from combined expertise of teams of physicians, virologists and medical biologists working on intensive care, metabolomics/lipidomics and inflammation issues.

4. MÉTHODOLOGIE & MISE EN ŒUVRE

TASK 1

Recruitment of patients and collection of usual demographic, anamnestic, clinical, radiological, biological and microbiological data (based on the COVID-ICU Study – REVA)

COVID-ICU Study is a French prospective multicenter (>70 ICUs, >10 ICUs in the Paris area) non-interventional study performed by the REVA network (*Réseau Européen de Recherche en Ventilation Artificielle)*. All the regulatory and ethical preliminary procedures have already been performed. This ongoing study includes all the patients admitted to ICU for COVID-19 pneumonia (inclusion criteria: ICU adult patients with PCR-confirmed COVID-19 infection, with or without mechanical ventilation; no exclusion criteria).

Data, collected on the Cleanweb plateform by the REVA investigators in each center, include:

- Anamnestic and demographic data;
- Clinical data on admission;
- Radiological data;
- Biological data;
- Microbiological data: COVID-19 PCR, bacterial blood cultures, respiratory tract specimens for usual bacterial analysis;
- Antibiotics and antiviral therapies;
- Corticosteroids, immunomodulatory drugs: timing and dose regimen;

- Complications and organ failures during the ICU stay;
- Outcomes: dates of ICU and hospital discharge, vital status at 28 days.

All the 10 ICUs of the Paris area participating in COVID-ICU Study (REVA) will be asked to participate to the COVID-METAFLAM Study.

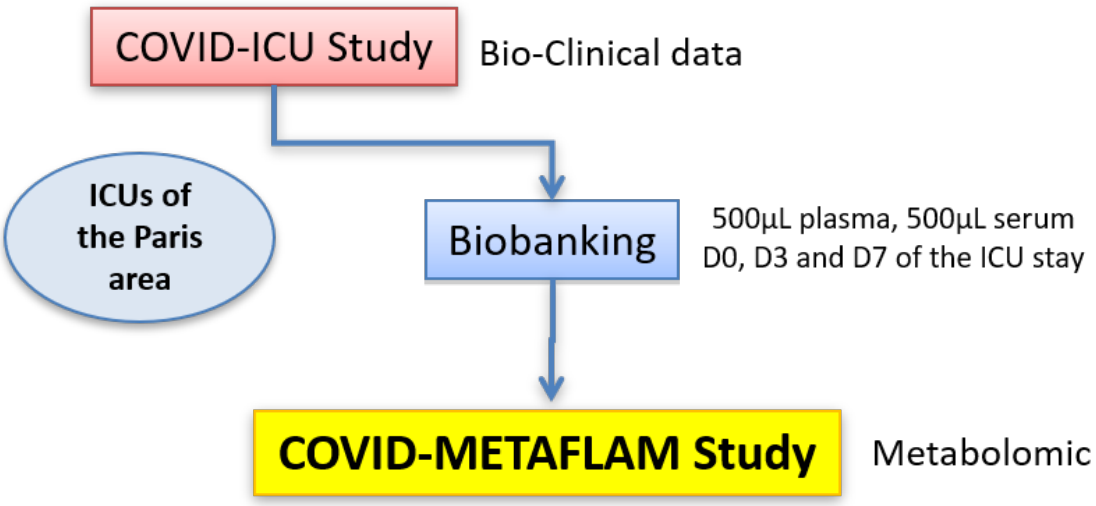
TASK 2

Collection and transport of biological samples (based on the AP-HP COVID-Biobank)

The AP-HP Biobank is an active Biobank that collect and conserve plasma/serum from COVID-19 pneumonia patients hospitalized in AP-HP Hospitals (Paris area). All the regulatory and ethical preliminary procedures have already been performed. All the procedures of sampling, transport and conservation of the samples are already been defined, organized and financed. Blood tubes are collected at D0, D3 and D7, transported at ambient temperature, centrifuged, and the supernatant (plasma and serum) aliquoted and stored at -80°C in each center, then centralized to a Certified BioBank Research Center. All the 10 ICUs of the Paris area participating in COVID-ICU Study (REVA) might be involved in the Biobank COVID AP-HP.

Therefore, **the COVID-METAFLAM Study propose to use samples (one aliquot of plasma, 500µL; one aliquot of serum, 500mL; at D0=ICU admission, D3 and D7)** from patients included in both the COVID-ICU study (Clinical data) and the Biobank COVID AP-HP (blood samples). Aliquots would be transported each month from the Centre of Biological Resources to the Laboratory of Dr Antonin LAMAZIERE.

The use of samples from the Biobank is subject to the agreement of the Biobank's Scientific Committee, which will be requested in the next future.



Number of centers, number of subjects/samples

The duration of inclusion in COVID-METAFLAM Study is 3 months.

The estimated number of participating ICUs is 10 (Paris aera).

Over the 3-month period of inclusion, we estimate that 900 COVID-19 pneumonia patients will be admitted to the 10 participating ICUs (30 patients/month/center).

We estimated that 80% of these 900 patients will be included in the COVID-ICU Study. Among them, we estimate that 50% will be included in the Biobank COVID AP-HP. Altogether, we estimate that 360 patients will be included in the COVID-METAFLAM Study.

Among these 360 patients, we estimate that 15% will be dead before D3, and 30% before D7. We do not anticipate any hospital discharge before D7. Altogether, with anticipating a 10% unavailability of samples for technical reasons, we estimate a number of analyzed samples of 826 (306 D0, 275 D3 and 227 D7)

Regulatory and Ethical Framework

All patients participating to the COVID-METAFLAM Study will have previously gave their consent (or next of kin) for inclusion in both the COVID-ICU Study (REVA) and the Biobank COVID AP-HP. Thus, there is no need for additional consent for the COVID-METAFLAM Study.

The COVID-METAFLAM Study is endorsed by the COPIL COVID AP-HP, a special Research Committee of the AP-HP, which centralized all the ethical and regulatory procedures, aiming to accelerate clinical and translational researches on COVID-19 conducted in the institution.

TASK 3

From a methodological point of view, all these molecular species are routinely quantified by Liquid Chromatography-Mass Spectrometry (LC-MS/MS) and/or Gas Chromatography-Mass Spectrometry (GC-MS) in our certified department of clinical metabolomic at Saint Antoine hospital (Paris). In our department 4 LC-MS/MS (ABSciex QTrap 6500, QTrap 5500, TQ5500 and Shimadzu8060) and 3 GC-MS (Agilent 5973 and 5975) are available for the analysis.

In details 4 blocks of classes will be considered:

1- Eicosanoids and related inflammation species
2- Fatty acids
3- Sphingolipids
4- Steroids

In total, it will comprise approximately 200 molecular species. In terms of feasibility, we will be able to achieve the analysis with the programmed sampling (500µL plasma, 500µL de serum for each patient/time point) Our platform is equipped with robot and preanalytical steps are fully automatized and are suitable for the considered analysis. The process is now designed for high throughput metabolomic analysis and thus adapted to intensive unit timing. It should be mentioned that all these studies are achieved within a very short time (10 minutes for each block of classes) as we previously reported. [26, 27, 28]
For cytokine measurements GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNF-α will be analysed : this kit is comprised of components for the measurement of human cytokines in serum, plasma, or tissue culture supernatant.

TASK 4

Modelling the time-related behaviour of biological situation, such as inflammation in COVID-19 patients, is essential for understanding their dynamic responses to perturbations. Thanks to our expertise, several approaches could be achieved, from simple to more complex statistical methods, but also advanced machine learning algorithms [29, 30].

We experienced for a long time supervised multivariate modelling approach with the objective to model the time-related variation in the data for short and sparsely sampled time-series. A set of piecewise Orthogonal Projections to Latent Structures (OPLS) models could be indeed a powerful tool, describing changes between successive time points. The idea is to combine data a wide variety of origins, clinical and imagery data (qualitative or quantitative data), biological results

and metabolomic to provide a statistical model with prognostic value. Moreover, Joint and Unique Multiblock Analysis (JUMBA) will also be achieved. In a viral infection which severity is extremely variable like COVID-19 pneumonia, we want to obtain a modeling with a predictive quality in terms of individual kinetic. Currently the JUMBA method would also be used to integrate all the protein profiles of serum, the profiles of eicosanoids and the clinical data of patients. The method can be generalized to n blocks (OnPLS) as it has been discussed recently to integrate the applications of various platforms in clinical biochemistry [31]. Thanks to our experience in the field of anti-doping research, we propose to transpose the concept of the athlete biological passport (ABP) follow-up on the microbiota exploration and its impact on metabolism over time. The ABP is an individual electronic record for professional athletes, in which profiles of biological markers of doping and results of doping tests are collated over a period of time. Bayesian models (Anti-Doping Administration & Management System = ADAMS) are to monitor longitudinal variations of different biomarkers, which are targeted to define the profile of an individual. The deviation from normal range of a profile may indicate either doping practices or medical/pathological conditions of the athlete that requires closer examination. When studying the inflammation processes, the notion of time is a critical issue as we are facing individual evolutive processes and variations (age, treatment, immune status…). If we now agree to consider the time scale as a parameter, the statistical power of our models will be significantly improved as demonstrated in many examples such as anti-doping strategies or pharmacokinetic studies. Finally, machine learning approaches, and more recently advanced deep learning (such as LSTM, "Long Short-Term Memory") have been used in time series problems in a medical context. Several studies have shown the ability of these different techniques to accurately predict or identify the patient deterioration (even mortality) in ICU, as a rapid or real-time response system. Secondly, we plan to retrospectively model our data with these new approaches [32, 33, 34]

5. RÉSULTATS ATTENDUS

The main expected results of the present COVID-METAFLAM.SU project is to obtain the kinetic of metabolomic inflammatory profiles associated with the inflammatory-immune response during COVID-19 pneumonia in ICU patients. We also plan to help for the prediction of the successive phases (acute phase, resolution phase) of the inflammatory-immune response; to search for an association between metabolomic profiles and clinical outcome (morbidity, mortality); to focus on metabolomic profiles in patients receiving immunomodulatory, chloroquine and/or anti-inflammatory treatments. We hypothesize that the integration and machine learning modeling of inflammatory targeted metabolomics in addition to cytokine profiles and bio-clinical data should demonstrated that some metabolites highly correlated with the pathogenic phase of the infection, whereas other are associated with the resolution phase. Our project is thus aimed at stratifying COVID-19 patients according to their inflammatory and metabolic status to help the management of this patients and to provide guidelines for patient treatment

6. ÉQUIPES IMPLIQUÉES

- **Équipes pilotes :**

Dr Guillaume Voiriot	**Dr Antonin Lamaziere**
Service de Réanimation et USC Médico-Chirurgicale	Département de Métabolomique Clinique
Hôpital Tenon	Hôpital Saint Antoine
DMU APPROCHES	DMU BioGeM

- **Équipe(s) partenaire(s) :**

1) Centre de recherche Saint Antoine / Département Métabolisme – Inflammation : **Équipe Pr. Seksik-Pr. Sokol Microbiote, intestin et Inflammation**
2) GRC RESPIRE RE animation et Soins intensifs du Patient en Insuffisance Respiratoire aigüE (site Trousseau-Saint Antoine-Tenon-Pitié)
3) DMU BioGeM -Pr. Rachel Levy
4) DMU APPROCHES- Pr. Muriel Fartoukh

7. BUDGET

- **Consumables :** ***Subtotal: 1.000€***
  - Small equipment (syringes, tubes ...)
- **Biological measures :**
  - LC-MS/MS and GC/MS quantitative circulating profiles : **subtotal 20 000€**
    Lipid mediators, Fatty acids, sphingolipids, sterols/steroids = 50euros/sample *400 samples
  - Cost of the Cytokins dosages : GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNF-α 10 cytokins to be tested on 400 serums : **subtotal 7 500€**

- **Other expenses:** Administrative fees/Publication / translation costs **Subtotal: 4.000 €**

Budget calculated 32 500 €

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