Acronyme[1]	**COVID-METAFLAM**
Titre de la proposition	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.
Nom et prénom des responsables (coordinateurs)	Guillaume VOIRIOT Antonin LAMAZIERE
Organisme du coordinateur	Identification de l'établissement : Sorbonne Université Adresse :
Aide demandée	60.000 euros
Coût complet :	60.000 euros
Durée	12 mois
Mots clés de la proposition	COVID-19, intensive care, inflammatory mediators, metabolomic, machine learning

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Summary

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 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 five tasks: (i) Recruitment of patients and collection usual demographic, anamnestic, clinical, radiological, biological and microbiological data (based on the COVID-ICU Study, REVA); (ii) Collection and transport of biological samples (based on the AP-HP COVID-Biobank); (iii) Biological explorations; (iv) Analysis of results; (v) Project management. It will be supported by existing networks: COVID-ICU Study, a French prospective multicenter (>70 ICUs, 10 ICUs in the Paris area) performed by the REVA network and the AP-HP COVID-Biobank. 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 AP-HP COVID-Biobank. Altogether, we estimate that 360 patients will be included in the COVID-METAFLAM Study. A total of more than 200 molecular species will be quantified using 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). We will 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 and predictive values, as previously made in our laboratory for specific pathologies and anti-

doping research. The project coordination will be ensured by URC –EST, the Clinical Research Center (CRC-EST), the Certified BioBank Research Center (CRB) of Sorbonne University, and the Clinical Research Unit. Our project is ambitious and innovative: (i) this is the first European project which propose a metabolomic approach to monitor kinetics of inflammatory mediators in patients with COVID-19 pneumonia; (ii) the short delay to get results will guaranty a short-term impact, with a quick translation into clinical practice and therapeutics.

1. PROJECT DESCRIPTION

Thematic (regarding of the ANR Call for Proposal)
Pathophysiology of the disease (immune-inflammatory response)
➔ Identification of therapeutics targets

## 2. CONTEXT, POSITION AND OBJECTIVES OF THE PROPOSAL

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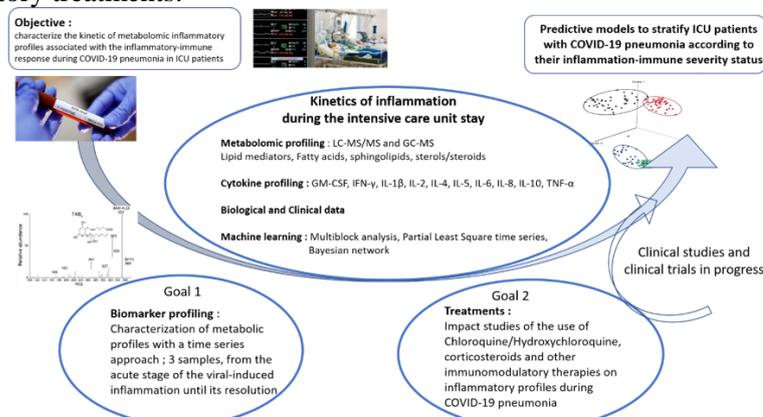
We hypothesize that the integration and machine learning modeling of inflammatory targeted metabolomics (including eicosanoids and lipid mediators, sphingolipids and steroid species) in addition to cytokine profiles and bio-clinical data should demonstrated that some metabolites highly correlated with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

To explore our hypothesis, we propose the Covid-METAFLAM 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.

3. STATE OF THE ART

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, IP10, 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 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]. The three major metabolic pathways for enzymatic eicosanoid biogenesis are the cyclooxygenase pathway (COX-1 and COX-2), producing the prostaglandins and thromboxanes; the lipoxygenase pathway (5-LOX, 12-LOX, and 15-LOX), producing the leukotrienes as well as numerous hydroperoxy and hydroxy fatty acids; and the cytochrome P450 pathway, producing epoxide and corresponding dihydroxy metabolites of arachidonic acids. Polyunsaturated fatty acids are susceptible to lipid peroxidation, especially under condition of oxidative stress during inflammation producing isoprostanes as well as other nonenzymatic oxidation products, including hydroxy fatty acids. In addition to arachidonic acids, the same enzymes can effectively metabolize other polyunsaturated fatty acids, such as linoleic and linolenic acids. 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].

Eventually, the profile of circulating total fatty acids also tracks the amount of arachidonic acid consumed by inflammation. 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]. As the newest global medical emergency, SARS-CoV2 infection exhibits features that are unlikely ameliorated by antivirals-based approaches alone. First, although the new coronavirus (SARS-CoV-2) infect lung and intestine, many patients suddenly take a turn for the worse even when the viral replication appears to be under control [6]. Second, as previously described, patients with serious or critical clinical symptoms show remarked T cell lymphopenia. Functional exhaustion of T cells is suggested by high expression of T-cell exhaustion markers. Third, multiple cytokines are elevated among patients with severe clinical symptoms, which potentially explains the multiple organ failure associated with COVID-19. For these reasons, 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. In SARS-CoV infection, as with influenza, systemic inflammation is associated with adverse outcomes. 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

4. DESCRIPTION OF THE PROJECT

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 five tasks :
- Task 1: Recruitment of patients and collection usual demographic, anamnestic, clinical, radiological, biological and microbiological data (based on the COVID-ICU Study, REVA);
- Task 2: Collection and transport of biological samples (based on the AP-HP COVID-Biobank);
- Task 3: Biological explorations;
- Task 4: Analysis of results;
- Task 5: Project management.

The coordination of tasks 1 and 2 and the coordination of task 3 will be assumed respectively by Dr Guillaume VOIRIOT and Dr Antonin LAMAZIERE. Task 4 will be shared. Tasks 5 will be performed by the URC-EST, under supervision of Prof. Tabassome SIMON.

		Work in progress					Milestone		Deliverables			
							Months					
	Task	0	1	2	3	4	5	6	7	8	9	10	11	12
1	Authorizations / Agreements (COVID-ICU; AP-HP COVID-Biobank)	■												
2	Patient recruitment		▓	▓	■									
3	Biological analysis			▓	▓	✕	▓	▓	▓	■				
4	Statistical analysis of results						✕	▓	▓	▓	▓	▓	▓	■
5	Project management		▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	■

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 and radiologically-confirmed pneumonia, 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: age, gender, comorbidities, date of hospital admission / date of ICU admission;
- Clinical data on admission: heart rate, blood pressure, temperature, respiratory rate, pulse oxymetry, PaO2/FiO2 ratio, state of consciousness, urine output;
- Radiological data: Radiological infiltrate, alveolar or interstitial, uni or bilateral, uni or multifocal;
- Biological data: arterial blood gas analysis, blood cell count, CRP, procalcitonin, D-dimères, LDH, ions, urea and creatinine;
- Microbiological data: COVID-19 PCR, bacterial blood cultures, respiratory tract specimens for usual bacterial analysis, *Mycoplasma/Chlamydia* serologies, *Legionella pneumophila* and *Streptococcus pneumoniae* urine antigen assays;
- 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 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.

For the AP-HP COVID-Biobank, EDTA and dry 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 the Certified BioBank Research Center (Hôpital de la Pitié-Salpétrière, AP-HP). All the 10 ICUs of the Paris area participating in COVID-ICU Study (REVA) are also involved

in the AP-HP COVID-Biobank. Therefore, **the COVID-METAFLAM Study will 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 AP-HP COVID-Biobank (blood samples). Aliquots will be transported each month from the Centre of Biological Resources to the Laboratory of Dr Antonin LAMAZIERE.
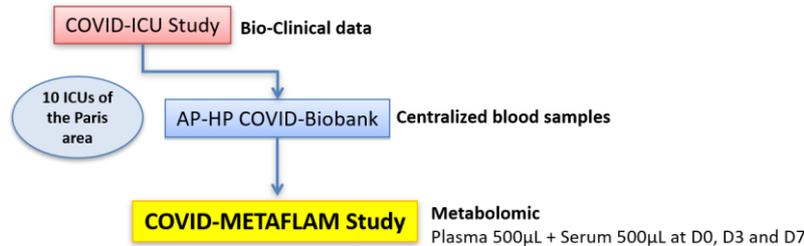
Number of centers, number of subjects/samples

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

The number of participating ICUs is 10 (Paris aera): all these ICUs participate to both the COVID-ICU study and the AP-HP COVID-Biobank.

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 AP-HP COVID-Biobank. 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 (REVA) Study and the AP-HP COVID-Biobank. Thus, there is no need for additional consent for the COVID-METAFLAM Study.

The COVID-METAFLAM Study is endorsed by the COVID-COPIL 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 (approxymatly 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 citokines 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 individual OPLS models are linear, but the piecewise combination of several models accommodates modelling and prediction of changes which are non-linear with respect to the time course. 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. This "multiblock" modeling has been used recently in the laboratory for metabolomic (lipidomic) and hormonal profiles (circulating steroids) studies with a O2PLS statistical regression method for 2 data blocks. 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]. Eventually, highlight the importance of monitoring patients over time. 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. Obviously, a transposition of these longitudinal approaches needs to be considered to model efficiently the individual variations of the inflammatory kinetic of COVID-19 patients. 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]

TASK 5
Project management

The project coordination will be ensured by URC –EST in order to ensure the coherence of actions in the research actions. A report will be drawn up every month to assess the project progress, potential difficulties encountered and corrective actions implemented. Recruitment reporting will be planned at the end of the 3-month inclusion period. Three meetings with all partners is planned (at M3, M6 and M12), but can be more frequent when appropriate.

PREVIOUS GRANT(S) FOR THIS PROPOSAL
The COVID-META-FLAM Study has not been funded so far. Nevertheless, the supports ongoing studies COVID-ICU and AP-HP COVID-Biobank have already been funded. Thus, the grant that we request here for the COVID-META-FLAM Study will be entirely dedicated to the expenditures of research laboratory.

5. **TEAM**

   - Dr Guillaume Voiriot is Associate Professor, vice-head of the Intensive Care Unit of Tenon Hospital (Hospital Group AP-HP.Sorbonne Université), and member of the REVA Network (*Réseau Européen de Recherche en Ventilation Artificielle)* for 7 years. GV has already developed, coordinated or participated in more than 20 multicenter prospective clinical and/or translational studies.
   - Dr Antonin Lamazière is Associate Professor, head of the department of clinical metabolomic at Saint-Antoine Hospital (Hospital Group AP-HP.Sorbonne Université) has a high expertise in the field of lipid metabolism, mass spectrometry and machine learning. All the biological studies planned in the COVID-METAFLAM study are routinely performed in his clinical Lab at Saint Antoine Hospital.
   - Prof Tabassome SIMON is Professor of Clinical Pharmacology in the Department of pharmacology, Saint-Antoine Hospital (Hospital Group AP-HP.Sorbonne Université). TS is the Director of the Clinical Research platform of the East of Paris, including the Clinical Research Center (CRC-EST), the Certified BioBank Research Center (CRB) of Sorbonne University, and the Clinical Research Unit.

6. **IMPACTS AND FEASIBILITY OF THE PROJECT**

Several factors supports the feasibility of our project:
   - The COVID-METAFLAM study is ancillary to the ongoing COVID-ICU Study. This French prospective multicenter non-interventional study is performed by the REVA network (*Réseau Européen de Recherche en Ventilation Artificielle),* which gathers more than 70 ICUs, including 10 ICUs in the Paris area. Patient screening and inclusion, and collection of bio-clinical data will be entirely performed by the REVA investigators.
   - The COVID-METAFLAM study will use biological samples collected for and conserved in the AP-HP (*Assistance Publique-Hôpitaux de Paris)* COVID-Biobank. This biobank is under supervision of Pr Tubach and Pr Hausfater (Hôpital Pitié-Salpétrière, Paris). All participating centers in the Paris area collect and address serum and plasma (D0, D3 and D7) from all their patients with COVID-19 pneumonia. This Biobank is designed to serve a large panel of translational researches, including the COVID-METAFLAM Study.

Our project is ambitious and innovative:
   - This is the first European project which propose a metabolomic approach to monitore kinetics of inflammatory mediators in patients with COVID-19 pneumonia.
   - The short delay to get results will guaranty a short term impact, with a quick translation into clinical practice and therapeutics.

7. BUDGET

- **Consumables :** *Subtotal: 1.000€*

- Small equipment (syringes, tubes ...)

- **Biological measures :**
  - LC-MS/MS and GC/MS quantitative circulating profiles : **subtotal 40 000€**
    Lipid mediators, Fatty acids, sphingolipids, sterols/steroids = 50euros/sample *800 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 800 serums   : **subtotal 15 000€**

- **Other expenses: Subtotal: 5.000 €**

- Frozen shipment for blood samples /paper, reprography/Administrative fees/Publication / translation costs

Budget calculated	**60.100 €**

8. REFERENCES

1. Huang, C. *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**, 497-506 (2020).
2. Wang, D. *et al.* Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *Jama* (2020).
3. Wong, C.K. *et al.* Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. *Clin Exp Immunol* **136**, 95-103 (2004).
4. Mahallawi, W.H., Khabour, O.F., Zhang, Q., Makhdoum, H.M. & Suliman, B.A. MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile. *Cytokine* **104**, 8-13 (2018).
5. Qin, C. *et al.* Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clinical Infectious Diseases* (2020).
6. Guan, W.J. *et al.* Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* (2020).
7. Dennis, E.A. & Norris, P.C. Eicosanoid storm in infection and inflammation. *Nat Rev Immunol* **15**, 511-523 (2015).
8. Conti, P. *et al.* Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. *J Biol Regul Homeost Agents* **34** (2020).
9. Tam, V.C. *et al.* Lipidomic profiling of influenza infection identifies mediators that induce and resolve inflammation. *Cell* **154**, 213-227 (2013).
10. Quehenberger, O. & Dennis, E.A. The human plasma lipidome. *N Engl J Med* **365**, 1812-1823 (2011).
11. Serhan, C.N., Chiang, N. & Van Dyke, T.E. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol* **8**, 349-361 (2008).
12. Buczynski, M.W., Dumlao, D.S. & Dennis, E.A. Thematic Review Series: Proteomics. An integrated omics analysis of eicosanoid biology. *J Lipid Res* **50**, 1015-1038 (2009).
13. Pritzl, C.J. *et al.* A ceramide analogue stimulates dendritic cells to promote T cell responses upon virus infections. *J Immunol* **194**, 4339-4349 (2015).
14. Santinha, D.R. *et al.* Profiling changes triggered during maturation of dendritic cells: a lipidomic approach. *Anal Bioanal Chem* **403**, 457-471 (2012).
15. Bouadma, L., Lescure, F.X., Lucet, J.C., Yazdanpanah, Y. & Timsit, J.F. Severe SARS-CoV-2 infections: practical considerations and management strategy for intensivists. *Intensive Care Med* (2020).
16. Stockman, L.J., Bellamy, R. & Garner, P. SARS: systematic review of treatment effects. *PLoS Med* **3**, e343 (2006).
17. Zhou, F. *et al.* Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* (2020).
18. Chwetzoff, S. & Trugnan, G. Rotavirus assembly: an alternative model that utilizes an atypical trafficking pathway. *Curr Top Microbiol Immunol* **309**, 245-261 (2006).
19. Delmas, O. *et al.* Different ways to reach the top of a cell. Analysis of rotavirus assembly and targeting in human intestinal cells reveals an original raft-dependent, Golgi-independent apical targeting pathway. *Virology* **327**, 157-161 (2004).
20. Enouf, V., Chwetzoff, S., Trugnan, G. & Cohen, J. Interactions of rotavirus VP4 spike protein with the endosomal protein Rab5 and the prenylated Rab acceptor PRA1. *J Virol* **77**, 7041-7047 (2003).
21. Sapin, C. *et al.* Rafts promote assembly and atypical targeting of a nonenveloped virus, rotavirus, in Caco-2 cells. *J Virol* **76**, 4591-4602 (2002).
22. Jourdan, N. *et al.* Rotavirus is released from the apical surface of cultured human intestinal cells through nonconventional vesicular transport that bypasses the Golgi apparatus. *J Virol* **71**, 8268-8278 (1997).
23. Condemine, W. *et al.* The C Terminus of Rotavirus VP4 Protein Contains an Actin Binding Domain Which Requires Cooperation with the Coiled-Coil Domain for Actin Remodeling. *J Virol* **93** (2019).
24. Russell, C.D., Millar, J.E. & Baillie, J.K. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. *Lancet* **395**, 473-475 (2020).
25. Organization, W.H. Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected: interim guidance, 28 January 2020: World Health Organization; 2020.
26. Fiet, J. *et al.* A Liquid Chromatography/Tandem Mass Spectrometry Profile of 16 Serum Steroids, Including 21-Deoxycortisol and 21-Deoxycorticosterone, for Management of Congenital Adrenal Hyperplasia. *J Endocr Soc* **1**, 186-201 (2017).
27. Lamaziere, A., Wolf, C. & Quinn, P.J. Perturbations of lipid metabolism indexed by lipidomic biomarkers. *Metabolites* **2**, 1-18 (2012).
28. Humbert, L. *et al.* Bile acid profiling in human biological samples: comparison of extraction procedures and application to normal and cholestatic patients. *J Chromatogr B Analyt Technol Biomed Life Sci* **899**, 135-145 (2012).

29. Lamaziere, A., Wolf, C. & Quinn, P.J. How lipidomics provides new insight into drug discovery. *Expert Opin Drug Discov* **9**, 819-836 (2014).
30. Burban, A. *et al.* Predictive Value of Cellular Accumulation of Hydrophobic Bile Acids As a Marker of Cholestatic Drug Potential. *Toxicol Sci* **168**, 474-485 (2019).
31. Surowiec, I. *et al.* Joint and unique multiblock analysis of biological data - multiomics malaria study. *Faraday Discuss* **218**, 268-283 (2019).
32. Yu, K., Zhang, M., Cui, T. & Hauskrecht, M. Monitoring ICU Mortality Risk with A Long Short-Term Memory Recurrent Neural Network. *Pac Symp Biocomput* **25**, 103-114 (2020).
33. Cho, K.-J. *et al.* Detecting Patient Deterioration Using Artificial Intelligence in a Rapid Response System. *Read Online: Critical Care Medicine | Society of Critical Care Medicine* **48**, e285-e289 (2020).
34. Kim, J. *et al.* Development of a Real-Time Risk Prediction Model for In-Hospital Cardiac Arrest in Critically Ill Patients Using Deep Learning: Retrospective Study. *JMIR Med Inform* **8**, e16349 (2020).