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Angélique Blangy-Letheule, Amandine Vergnaud, Thomas Dupas, Damien Habert, Walid Oulehri, et al.. Value of a secretomic approach for distinguishing patients with COVID-19 viral pneumonia among patients with respiratory distress admitted to intensive care unit. *Journal of Medical Virology*, 2024, 96 (6), pp.e29756. 10.1002/jmv.29756 . hal-04834745

HAL Id: hal-04834745

<https://hal.science/hal-04834745v1>

Submitted on 23 Jan 2025

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Value of a secretomic approach for distinguishing patients with COVID-19 viral pneumonia among patients with respiratory distress admitted to intensive care unit

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Funding information

Agence Nationale de la Recherche; Association sauve ton Cœur; Fondation d'entreprises Genavie; Société Française d'Anesthésie et de Réanimation; Région Pays de la Loire; European Union's Horizon 2020 research and innovation program

Abstract

In intensive care units, COVID-19 viral pneumonia patients (VPP) present symptoms similar to those of other patients with Nonviral infection (NV-ICU). To better manage VPP, it is therefore interesting to better understand the molecular pathophysiology of viral pneumonia and to search for biomarkers that may clarify the diagnosis. The secretome being a set of proteins secreted by cells in response to stimuli represents an opportunity to discover new biomarkers. The objective of this study is to identify the secretomic signatures of VPP with those of NV-ICU. Plasma samples and clinical data from NV-ICU ($n = 104$), VPP ($n = 30$) or healthy donors (HD, $n = 20$) were collected at Nantes Hospital (France) upon admission. Samples were enriched for the low-abundant proteins and analyzed using nontarget mass spectrometry. Specifically deregulated proteins (DEP) in VPP versus NV-ICU were selected. Combinations of 2 to 4 DEPs were established. The differences in secretome profiles of the VPP and NV-ICU groups were highlighted. Forty-one DEPs

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were specifically identified in VPP compared to NV-ICU. We describe five of the best combinations of 3 proteins (complement component C9, Ficolin-3, Galectin-3-binding protein, Fibrinogen alpha, gamma and beta chain, Proteoglycan 4, Coagulation factor IX and Cdc42 effector protein 4) that show a characteristic receptor function curve with an area under the curve of 95.0%. This study identifies five combinations of candidate biomarkers in VPP compared to NV-ICU that may help distinguish the underlying causal molecular alterations.

KEYWORDS

biomarkers, intensive care unit, secretome, viral pneumonia

1 | INTRODUCTION

Pneumonia, along with other lower respiratory tract infections, is the fourth leading cause of mortality worldwide with approximately 2.38 million deaths in 2016.¹ In the European Union, 131.450 people died of pneumonia which represents nearly 3% of all deaths.² These infections, which can be acquired outside or within the hospital environment, can be caused by a wide variety of microorganisms, including viruses.^{3,4} One of the great issues at stake is to rapidly identify the causes of respiratory pneumonia among intensive care unit (ICU) patients to more appropriately treat the patients. In fact, patients in ICU, may often present symptoms similar to viral pneumonia, such as pulmonary failure, that are not related to viral infection, but that occur in response to various stimuli, such as bacterial infection, trauma or burns, making management challenging. Direct isolation of the possible causal agent from the lower respiratory tract is invasive and may be limited by the patient's clinical condition, thus increasing the diagnosis difficulty. The incidence of viral pneumonia is likely to increase dramatically with the onset of viral epidemics, as in the recent years for COVID-19. Improvements in the sensitivity of detection techniques such as PCR have improved the ability to identify viral pneumonia.⁵ However, it can take several hours to obtain these results, a timeframe that delays appropriate patient care. It remains essential to distinguish viral pneumonia patients from other Nonviral ICU patients (NV-ICU) for several reasons: (1) the obligation to control the spread of the virus in a hospital environment and (2) the necessity to adapt the management of the disease as a function of its causes. In parallel, the similarities in clinical symptoms observed between VPP and NV-ICU patients argue for common pathogenic mechanisms that require a better fundamental analysis. In this context, the study of the secretome, defined as the set of proteins secreted by a cell, a tissue or an organism under physiological and pathological conditions, has been proposed as a relevant strategy to identify the deregulated proteins (DEPs) during COVID-19 viral pneumonia (VPP) or NV-ICU itself.⁶ This protocol enables noninvasive collection of biological samples, facilitating patient monitoring. In addition, biomarkers identified in the secretome may help to distinguish the different cause of pneumonia. The study of the secretome was favored over

the use of stem cell derivatives because it is less time consuming, more cost-effective and should provide more relevant information regarding the pathological conditions of the patients (direct context and not indirect upon cell culturing). Investigating the secretome has already proved successful for the identification of DEPs in rat models of endotoxemic shock.⁷ It is expected that viral infection, that leads to pneumonia, does not modify in a similar manner the signaling pathways of lung cells undergoing nonviral infection. As a consequence, it is expected that the DEPs of the secretome of VPP should be different in some extent to the DEPs of the secretome of NV-ICU patients. A comparison of both secretomes should allow an early and accurate distinction between these two conditions, as well as informing on the possible existence of common determinants. The objective of this study was to identify a combination of biomarkers to distinguish VPP from NV-ICU patients. For this purpose, the plasma secretome of VPP, NV-ICU patients and healthy donors (HD) was studied using a large-scale, nontargeted, mass spectrometry (MS) approach.

2 | METHODS

2.1 | Patients and samples

Plasma samples and clinical data of patients NV-ICU ($n = 104$) and VPP patients ($n = 30$) were collected at the Nantes University Hospital upon patients' admission (Figure 1). Plasma samples and clinical data of healthy donors (HD, $n = 20$) were collected at the Établissement Français du Sang (EFS). All donors had given written informed consent and the study was approved by the regional ethical review board of Nantes. Inclusion criteria were established as follows for each group.

2.1.1 | Healthy donors

Sample and clinical data were collected from healthy blood donors, recruited at the Blood Transfusion Center (Établissement Français du Sang, Nantes, France).

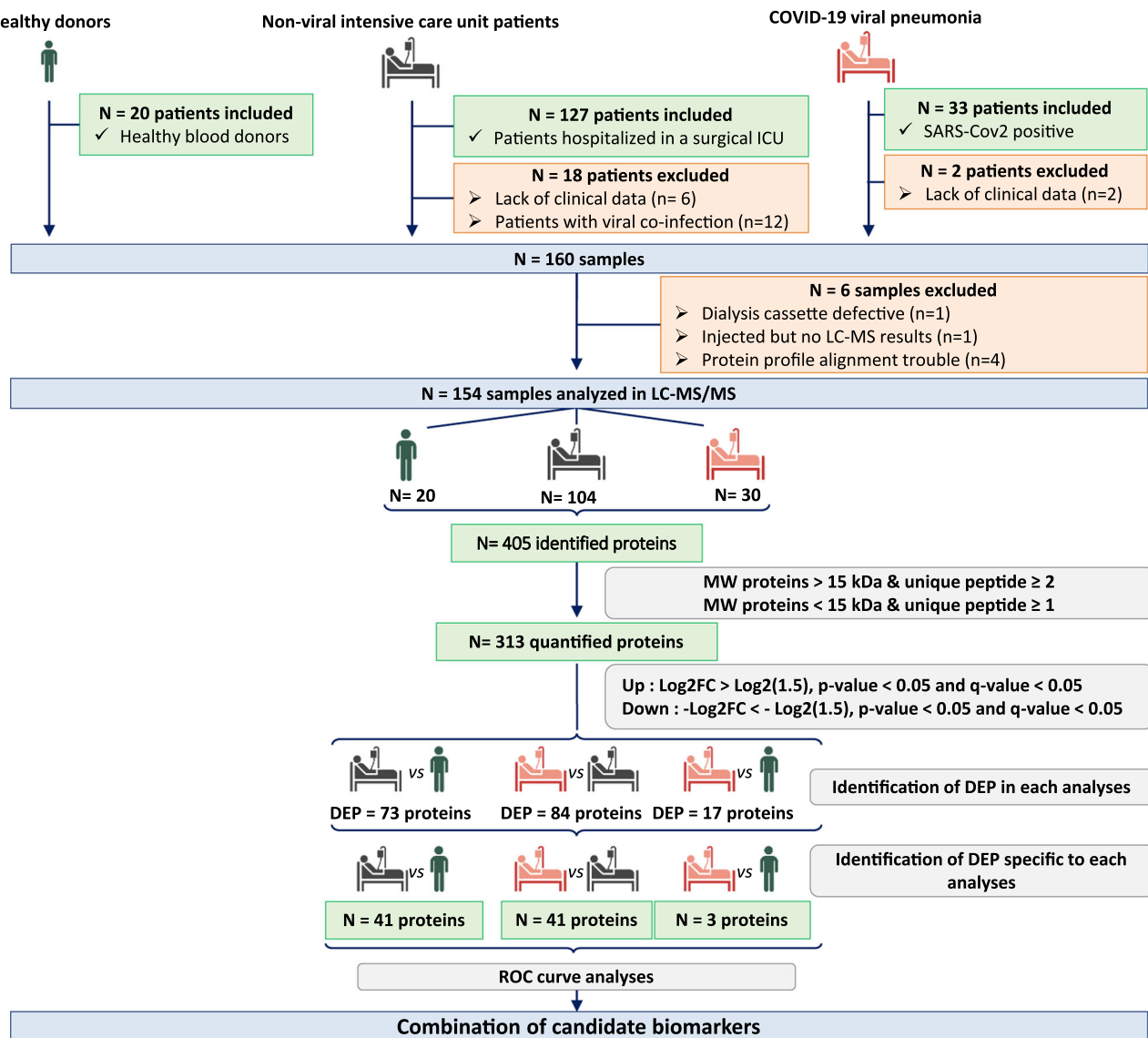


FIGURE 1 Flow chart of the study. Patients of the three following groups were used for this study: Healthy donors (HD), nonviral intensive care unit patients (NV-ICU) and COVID-19 viral pneumonia (VPP). All NV-ICU patients and VPP were hospitalized at the Nantes University Hospital. Each plasma sample was analyzed by mass spectrometry, and proteomic results were analyzed by Rstudio software. A total of 405 proteins were identified. Proteins were considered identified if: two single peptides were identified for proteins with a molecular weight (MW) higher than 15 kDa and with only a single peptide if the molecular weight was lower than 15 kDa. Using these parameters, 313 proteins were included. The quantified proteins with a p-value and q-value lower than 0.05 and a fold change lower than -1.5 ($\text{Log}_2\text{FC} \leq -\text{Log}_2\text{FC}(1.5)$) or greater than 1.5 ($\text{Log}_2\text{FC} \geq \text{Log}_2\text{FC}(1.5)$) were considered deregulated. For each analysis (NV-ICU vs. HD; VPP vs. HD and VPP vs. NV-ICU), all combinations of 2, 3 or 4 DEP were established. To identify the most discriminating combination accuracy, confidence interval, specificity and sensitivity values were calculated.

2.1.2 | NV-ICU patients

Bioresources - IBIS-sepsis (severe septic patients) and IBIS (brain-injured patients), Nantes, France. Patients were enrolled from September 2014 to November 2020 in a surgical intensive care unit of University Hospital. Twelve patients with viral co-infection were excluded from the study. Samples were collected in accordance with the guidelines of standardization (CoBRA).⁸ Nonviral infection was established on (i) clinical grounds such as isolated or non-isolated respiratory involvement, fever, (ii)

biological grounds such as hyperleukocytosis, and inflammatory syndrome, (iii) radiological grounds such as radio-thoracic, acute lobar frank lung disease and (iv) microbiological grounds.

The study by Ferreira and collaborators found that a SOFA score of 9 or above is a good predictor of mortality risk.⁹⁻¹¹ Therefore, a SOFA score 9 was selected as the cut off for triaging of patients increased risk of death.

The collection of human samples has been declared to the French Ministry of Health (DC-2011-1399), and it has been approved

by an institutional review board. Written informed consent from a next-of-kin was required for enrollment. Retrospective consent was obtained from patients.

2.1.3 | VPP patients

Patients hospitalized with diagnosis of COVID-19 ($n = 30$) confirmed with detection of SARS-CoV-2 by reverse transcriptase-polymerase chain reaction (RT-PCR) assays on nasopharyngeal smear or bronchoalveolar lavage were enrolled from March 2020 to October 2020. All plasma samples were stored at -80°C .

2.2 | Label-free quantitative mass spectrometry analyses

Samples were prepared for nanoLC-MS analyses (nano-liquid chromatography - mass spectrometry) by using Proteominer[®] low-abundance proteins enrichment kits as described previously.⁷ Twenty-two μg of enriched low-abundance proteins were submitted to trypsin/lys-C digestion and desalting as described previously by adding 700 fmol of internal standard (*Enolase S. Ceravisae*, P00924).⁷ Two microliters of each desalting digest were pooled to prepare a quality control (QC) sample. Samples and QC were analyzed by nano-LC/HDMS^E using the NanoAcquity-ESI-SynaptG2-Si (Waters, Milford, United Kingdom) system, operating in nanoESI positive mode. Following loading on the ACQUITY UPLC peptide BEH130 C18 nanoACQUITYTM column, $100\ \mu\text{m} \times 100\ \text{mm}$ with $1.7\ \mu\text{m}$ diameter (Waters) at a flow rate of $0.45\ \mu\text{L}/\text{min}$, peptides were separated with a gradient increasing from 1% Buffer B (acetonitrile with 0.1% formic acid)/99% Buffer A (H_2O with 0.1% formic acid) to 40% Buffer B/60% Buffer A, and then to 85% Buffer B/15% Buffer A, over 120 min. MS spectra were recorded in a 50–2000 m/z mass range at 10,000–20,000 resolution. The nano LC/HDMS^E method included 120 min of acquisition time, positive polarity, HDMS^E acquisition range of 50–4000 Da, collision energy range of 20–55 V.

Identification and quantification of proteins were performed by using the software Progenesis for proteomics QI (Waters) with the ion accounting algorithm and the following parameters: trypsin digestion, a maximum of 2 missed cleavages, a maximum of protein mass of 250 kDa, fixed modification (carbamodimethyl (C)), variable modification (oxidation (M)), false discovery rate (FDR) of 1%, automatic mass tolerance, Human-Swissprot-UniprotKB (21649 main entries + 9923 isoforms) concatenated with yeast enolase (*Enolase S. Ceravisae*, P00924) and SARS-CoV-2 protein (Release 2021_02; <https://www.uniprot.org/>); label-free relative quantitation with a normalization on the internal standard (*Enolase S. Ceravisae*, P00924). The list of quantified proteins was refined based on the number of unique peptides and their molecular weight. Proteins with a molecular weight (MW) greater than 15 kDa had to present at least 2 unique peptides, while proteins with a MW less than 15 kDa had to present at least 1 unique peptide (Figure 1).

2.2.1 | Differential expressed proteins analyses

The quantified proteins with a p-value, a q-value lower than 0.05 and a Fold-change lower than -1.5 ($\text{Log}_2\text{FC} \leq -\text{Log}_2\text{FC}$ (1.5)) or greater than 1.5 ($\text{Log}_2\text{FC} \geq \text{Log}_2\text{FC}$ (1.5)) were considered deregulated (Figure 1). These thresholds were defined according to volcano plot profiles. The gene ontology (GO) of the biological processes (BP) and cellular components (CC) of the deregulated proteins were then analyzed with the R software “ClusterProfiler” package (4.2.2 version). A Venn diagram was created from the DEPs identified in the analyses (i) VPP versus HD, (ii) VPP versus NV-ICU and (iii) NV-ICU vs HD to identify the common and distinct DEPs for each analysis.

2.2.2 | Selection of combination of candidate biomarkers

Using the differentially expressed proteins exclusively in the VPP group, all combinations of 2, 3 or 4 selected DEP were evaluated through the combinations function from the R package “gtools” (version 4.2.2). To predict the diagnostic relevance of each combination, a Generalized Linear Model (GLM) was constructed using the ‘caret’ R packages to train the model on 30% of the original data set. Subsequently, the model was validated on the remaining 70% of the data set and specificity, sensitivity, and accuracy were extracted for evaluation. The 5 combinations with the best parameters (sensitivity, specificity and accuracy) were selected.

2.3 | Statistical analyses

The numerical and categorical variables of the clinical data of patients included in the study were respectively analyzed with a Student's t test and a Chi² test using the “tableby” function of the R package “arsenal” (R software version 4.2.0). A $p < 0.05$ was considered as significant. Bioinformatics and statistical proteomic data processing were performed using the Progenesis QI for proteomics software (Waters). LC-MS analyses were performed for each sample in 3 technical replicates. All graphs were created using R software (version 4.2.0).

3 | RESULTS

3.1 | Clinical data

Comprehensive demographic data were collected and are provided in Table 1 and Supporting Information S1: Table S1. Mean age of healthy donors was 39.5 ± 14.14 years, the percentage of males was 40%. There was no difference in sex ratio between NV-ICU and VPP patients (NV-ICU: 74% of male vs VPP: 70% of male, $p < 0.83$). Patients with VPP were significantly older than patients in NV-ICU (NV-ICU: 41.96 ± 17.78 years vs. VPP: 58.55 ± 15.10 years, $p < 0.01$).

TABLE 1 Baseline characteristics and clinical data of patients hospitalized in ICU without sign of viral infection and those with viral pneumonia.

	NV-ICU (N = 104)	VPP (N = 30)	p value
Baseline characteristics			
Sex (male)	74.0%	70.0%	0.83
Age			<0.01
- Mean (SD)	41.96 (17.78)	58.55 (15.10)	
- Range (min-max)	18.10-77.30	25.00-85.00	
Presence of comorbidities			
Neurological history	15.4%	6.7%	0.26
Chronic kidney failure	1.9%	0.0%	1.00
Pulmonary disease	1.0%	6.7%	0.16
Cardiac insufficiency	1.0%	10.0%	0.03
Diabetes	6.7%	26.7%	<0.01
Clinical data			
Respiratory failure: PaO ₂ /FiO ₂ < 200 and need of invasive mechanical ventilation)	25.7%	16.7%	0.37
Bacterial hospital-acquired pneumonia (yes)	42.3%	0.0%	<0.01
ICU length of stay (days)			<0.01
- Mean (SD)	21.67 (16.02)	3.53 (7.61)	
- Range (min-max)	4.00-70.00	0.00-30.00	
ICU duration of mechanical ventilation (days)			<0.01
- Mean (SD)	15.69 (13.62)	3.12 (7.38)	
- Range	1.00-70.00	0.00-30.00	
ICU noninvasive ventilation (Yes)	10.0%	20.0%	0.18
SOFA (≤9)	59.6%	100.0%	<0.01
Antibiotics	90.4%	76.7%	0.07
Corticosteroid	0.0%	46.7%	<0.01

Note: N represent the number of patients in each cohort. SD, standard deviation. The numerical and categorical variables of the clinical data of patients included in the study were respectively analyzed with a Student's t test and a Chi² test using the "tableby" function of the R package "arsenal" (R software version 4.2.0). The critical point of SOFA at 9 was used as an indicator for triaging of patients increased risk of death.

Neurological history, chronic kidney failure, or pulmonary disease showed no disparity between NVICU and VPP patients. Patients in the VPP group exhibited a higher prevalence of heart failure and diabetes when compared to those in the NV-ICU group (Table 1).

The duration of hospitalization in ICU and the duration of mechanical ventilation were significantly higher in NV-ICU patients than in VPP (duration of stay in ICU: NV-ICU: 21.67 ± 16.02 days vs. VPP: 3.53 ± 7.61 days, $p < 0.01$; duration of mechanical ventilation: NV-ICU: 15.69 ± 13.62 days vs. VPP: 3.12 ± 7.38 days, $p < 0.01$) (Table 1).

In terms of treatment, the proportion of patients receiving antimicrobial therapy in ICU was not statistically different between NV-ICU and VPP (NV-ICU: 90.4% vs VPP: 76.7%, $p = 0.07$). However, VPP received corticosteroids far more frequently (46.7%, Table 1).

3.2 | Study of secretomic profiles of NV-ICU patients, VPP and healthy donors

3.2.1 | Analysis of global secretome profile

Secretome analysis of the 154 samples included in the study allowed the identification of 405 proteins. Based on our quality criteria described in material and method section, 313 proteins were retained for quantification analyses (Figure 1). Heatmap and principal component analyses (PCA) indicated that the global protein expression patterns were not different between groups (Figure 2A and Figure 2B).

3.2.2 | Identification of deregulated proteins

To determine whether the patterns of protein expression is different in VPP compared to other groups, differential expression analyses of secretome profiles were performed between (i) VPP versus HD group, (ii) VPP versus NV-ICU, and (iii) NV-ICU versus HD (Figure 1). The volcano plot shown in Figure 2C revealed 84 proteins significantly deregulated in NV-ICU compared to VPP patients. Among these proteins, 35 (41.6%) were significantly upregulated and 49 (58.4%) were significantly downregulated in VPP patients (Figure 2C and Supporting Information S1: Table SIIA). With the same approach, 17 DEPs were identified in VPP compared to HD. Of the DEPs identified in the VPP compared to HD analysis, 2 (11.8%) were significantly upregulated and 35 (88.2%) were significantly downregulated (Figure 2D and Supporting Information S1: Table SIIB). Finally, 73 DEPs were identified in NV-ICU compared to HD. Of the DEPs, 5 (6.8%) were significantly upregulated and 68 (93.1%) were significantly downregulated (Figure 2E and Supporting Information S1: Table SIIC).

To establish a protein signature for the comparison between VPP and NV-ICU, the DEP common to these two groups, as opposed to the HD group, were investigated. The Venn diagram illustrate that C-reactive protein (CRP) is the only DEP in either (i) HD versus NV-ICU, (ii) HD versus VPP or (iii) NV-ICU versus VPP. There are more proteins specifically deregulated specifically in NV-ICU vs HD patients (41 DEP) compared to proteins deregulated specifically in VPP versus ICU (3 DEP). Focusing on the DEP found only in VPP versus NV-ICU, 41 DEPs were identified (Figure 2F).

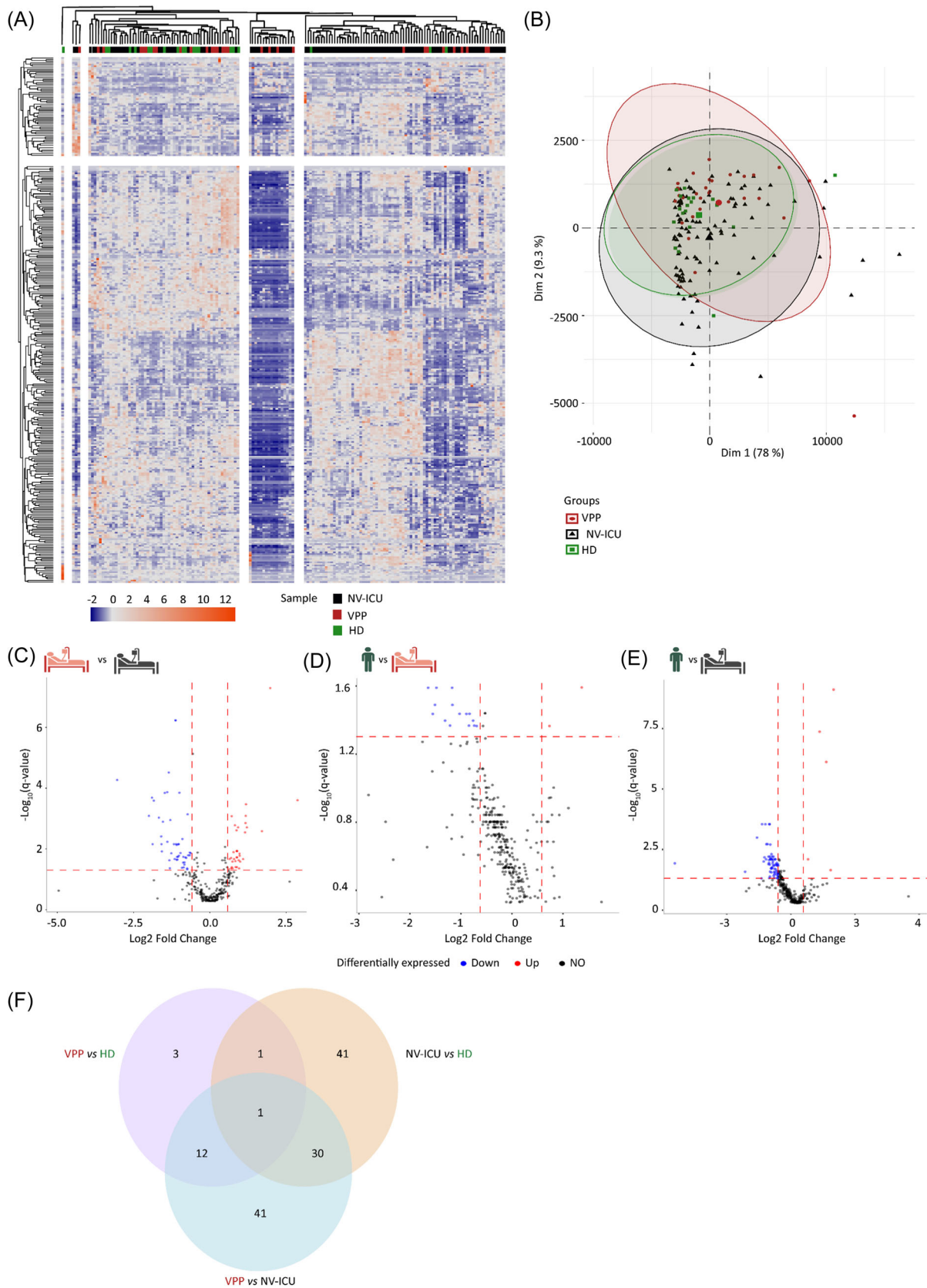


FIGURE 2 (See caption on next page).

3.2.3 | DEPs study specifically deregulated in VPP compared to HD

Visualization of the 3 DEPs specifically identified in VPP compared with HD, using a heatmap, highlighted a group of 14 VPP over the 30 studied (Figure 3A). A Gene Ontology of biological process (GO-BP) analysis was performed to decipher the impact of the changes induced by COVID-19 viral pneumonia. This gene ontology study

highlights that proteins specifically deregulated in VPP group were associated with the acute phase response (UNIPROT code: **P02765** - Alpha-2-HS-glycoprotein), the innate immune response (UNIPROT code: **A0A0A0MS15** - Immunoglobulin heavy variable 3-49) and muscle contraction (UNIPROT code: **P14649** - Myosin light chain 6B) (Figure 3B). While these proteins are significantly deregulated, the normalization of their level obtained with MS highlights a high heterogeneity between VPP patients (Figure 3C).

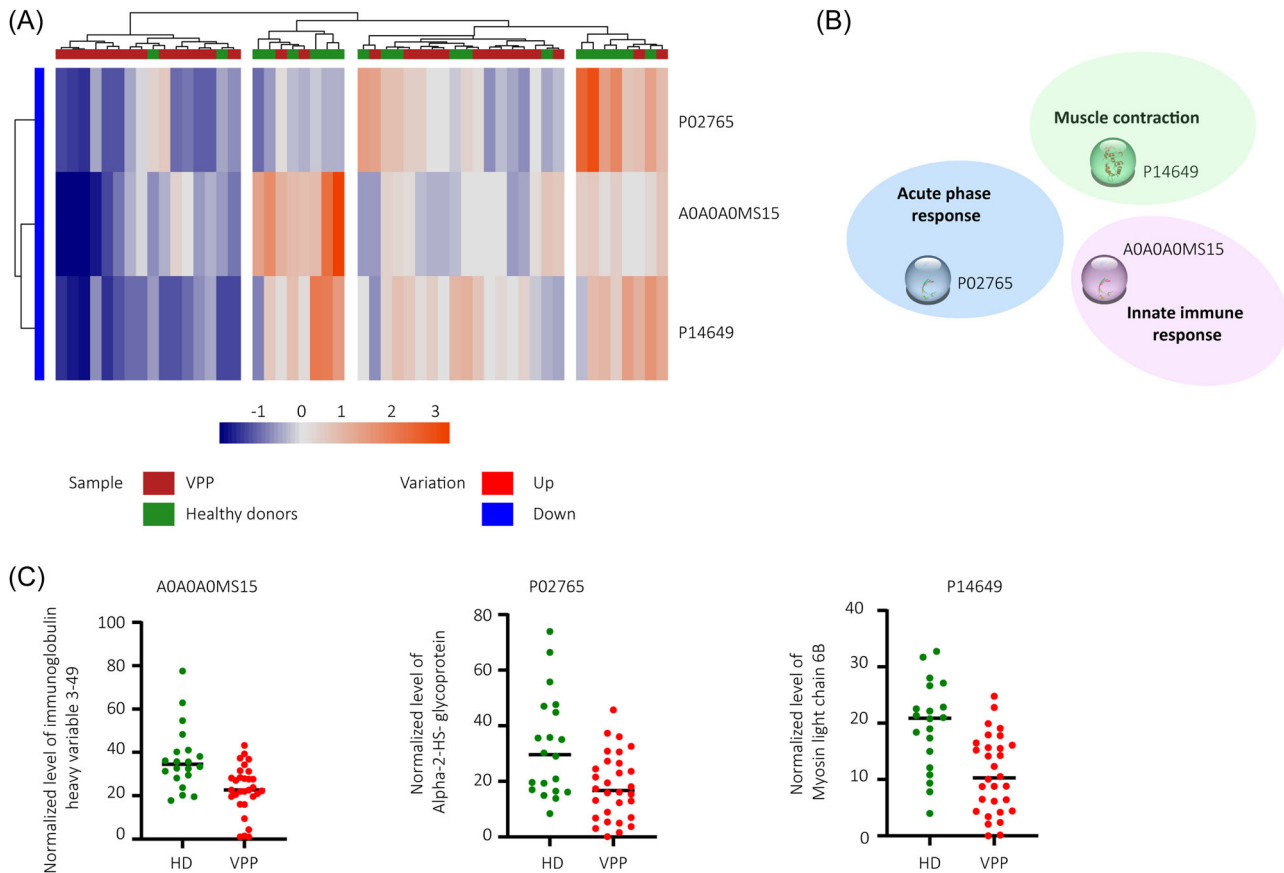


FIGURE 3 Study of the secretomic profiles of the three proteins specifically deregulated in viral pneumonia patients compared to healthy. (A) Heatmap visualization of the three proteins specifically dysregulated in viral pneumonia patients (VPP) and healthy donors (HD) showed clustering between these different groups. Upregulated and downregulated proteins were represented in red and blue, respectively. (B) The network of dysregulated proteins in VPP compared to HD. The analysis using Gene Ontology (GO) databases revealed the most modified biological processes (BP) following viral infection. String protein was used to clustered dysregulated proteins. (C) Boxplot representing the normalized levels obtained in mass spectrometry (MS) of the three specifically DEPs in VPP patients compared to HD.

FIGURE 2 Secretomic profiles analyses from samples of ICU patients without viral infection, COVID-19 viral pneumonia patients and healthy donors. Secretome analysis was performed on plasma from nonviral infection intensive care unit patients (NV-ICU) or hospitalized with viral pneumonia patients (VPP) and healthy donors (HD) and identified 313 proteins. (A) Heatmap visualization of the 313 proteins quantified by mass spectrometry in the groups of HD, NV-ICU and VPP patients showed no clustering to distinguish these different groups. (B) Patient plot from principal component analysis (PCA) of secretomic data shows that VPP, NV-ICU and HD are not separated. The graph was obtained with the function "fviz_pca_ind" Package R "factoextra". Volcano plot representing results obtained from differential expression analysis between (C) VPP patients and NV-ICU patients, (D) VPP and HD, (E) NV-ICU and HD. Proteins significantly decreased according to the p -value have been represented in blue and proteins significantly increased according to the p -value have been represented in red. The function "ggplot" has been used to make this figure (R version 4.2.0). (F) Venn diagram representing the proteins deregulated (DEPs) common or specific to VPP, NV-ICU and HD groups.

3.2.4 | DEP study specifically deregulated in VPP compared to NV-ICU

Visualization of 41 DEPs identified in NV-ICU compared to VPP with a heatmap highlight a cluster of NV-ICU patients. The heatmap did not distinguish NV-ICU from VPP, neither did it distinguish the severity of the pathology based on their secretomic profiles (Figure 4A). A GO-BP analysis was performed to decipher the impact of the SARS-CoV-2 infection-induced modification compared to the NV-ICU. Gene ontology study highlighted that the upregulated proteins were associated with immune response process while the downregulated proteins in VPP were involved in with coagulation regulation process (Figure 4B–D). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichments for *homo sapiens* ("hsa") species were conducted to decipher most impacted pathways. This enrichment of DEPs showed that coagulation and complement pathway (hsa04610) was altered in the secretome between NV-ICU and VPP (Figure 4D). Concerning the complement cascade, complement A4, ficolin-3, MASP1 protein and complement factor B were identified as downregulated in VPP compared to NV-ICU. In the intrinsic and extrinsic pathway of coagulation, factor 9, 7 and serpin F2 were observed to be downregulated in VPP compared to NV-ICU patients (Figure 4D).

3.3 | Identification of a protein combination distinguishing patients with viral pneumonia from those hospitalized in intensive care unit without viral infection

The 41 DEPs specifically identified in NV-ICU compared to VPP were grouped by combination of 2, 3 or 4 proteins resulting in 280, 10,660 and 101,270 possible DEPs combinations respectively. For each of these combinations, specificity and sensitivity values were calculated, which allowed us to identify the top 5 promising combinations of three deregulated proteins in VPP versus NV-ICU (Table 2). Interestingly, Ficolin-3 (FCN-3) and proteoglycan 4 (PRG4) are present among the 3 most promising combinations. These combinations present a high Receiver Operator Characteristic value equal to 95% which shows the robustness of this biomarker combination.

4 | DISCUSSION

Although a major proportion of community-acquired pneumonia is of bacterial origin, about a quarter is considered to be of viral origin.¹² There is evidence that viral respiratory infections play an increasing role in respiratory disease, particularly because of the COVID-19 pandemic. Diagnosis of viral pneumonia is a global challenge for all countries in adult populations who need better diagnostics to improve antibiotic management and mortality outcomes. Large studies have been performed in pediatric and adult populations to distinguish between viral and bacterial pneumonias, but, to our knowledge, studies devoted to identify biomarkers to distinguish

carriers of viral pneumonias among patients hospitalized in ICU have never been carried out. For the first time, using a secretomic approach, we have demonstrated that the secretome profile of patients with COVID-19 viral pneumonia can be distinguished from ICU patients without viral infection.

4.1 | Basic biomarkers to distinguish VPP and NV-ICU patients

In ICU, biomarkers, as CRP and procalcitonin (PCT), are commonly used to assess the clinical status of critically-ill patients. In our study, CRP is the only protein differentially expressed in the three comparisons but downregulated specifically in VPP compared with NV-ICU patients. It may indeed seem troubling to find that proteins involved in the pro-inflammatory response evolve differently between the two groups. However, this result is not so contradictory given that the inflammatory response and associated signaling pathways can vary depending on the source of infection. Indeed, around half the patients in the NV-ICU group had a bacterial infection, whereas all patients in the VPP group had a viral infection. Articles in the literature have already demonstrated the role of CRP in distinguishing between viral and bacterial. CRP is a nonspecific inflammatory marker commonly used in ICU to assess the presence and monitoring of inflammation in critically-ill patients. These arguments agree with our results. In contrast to CRP, PCT was not identified by mass spectrometry and is most probably not deregulated in our study. PCT is described as a nonspecific biomarker of inflammation but PCT levels are not elevated during viral infections. In contrast, PCT levels increase during bacterial infections and, for this reason, it has been proposed as a biomarker to differentiate viral from bacterial etiology.¹³ Indeed, an American study has shown that PCT values in patients with a typical bacterial etiology are significantly higher than those in patients with a viral etiology.¹⁴ However, in our study, 60% of patients in the NV-ICU cohort lacked bacterial infection, which may explain the absence of PCT deregulation between the NV-ICU and VPP groups.

4.2 | Length of ventilation support in VPP compared to NV-ICU

In this study, the length of ventilation support was shorter in VPP than in NV-ICU. This difference can be explained by the intrinsic difference in the natural history of the disease. The variation in ventilation duration can be attributed to several factors among VPP patients. Firstly, they mainly presented isolated respiratory dysfunction requiring intensive pulmonary care, potentially leading to protocol adaptations. Moreover, the context of a pandemic contributed to a need of rapid turnover of patients in hospital wards.^{15,16} In contrast, NV-ICU patients experienced respiratory issues intertwined with other organ dysfunctions, likely prolonging their need for respiratory support.

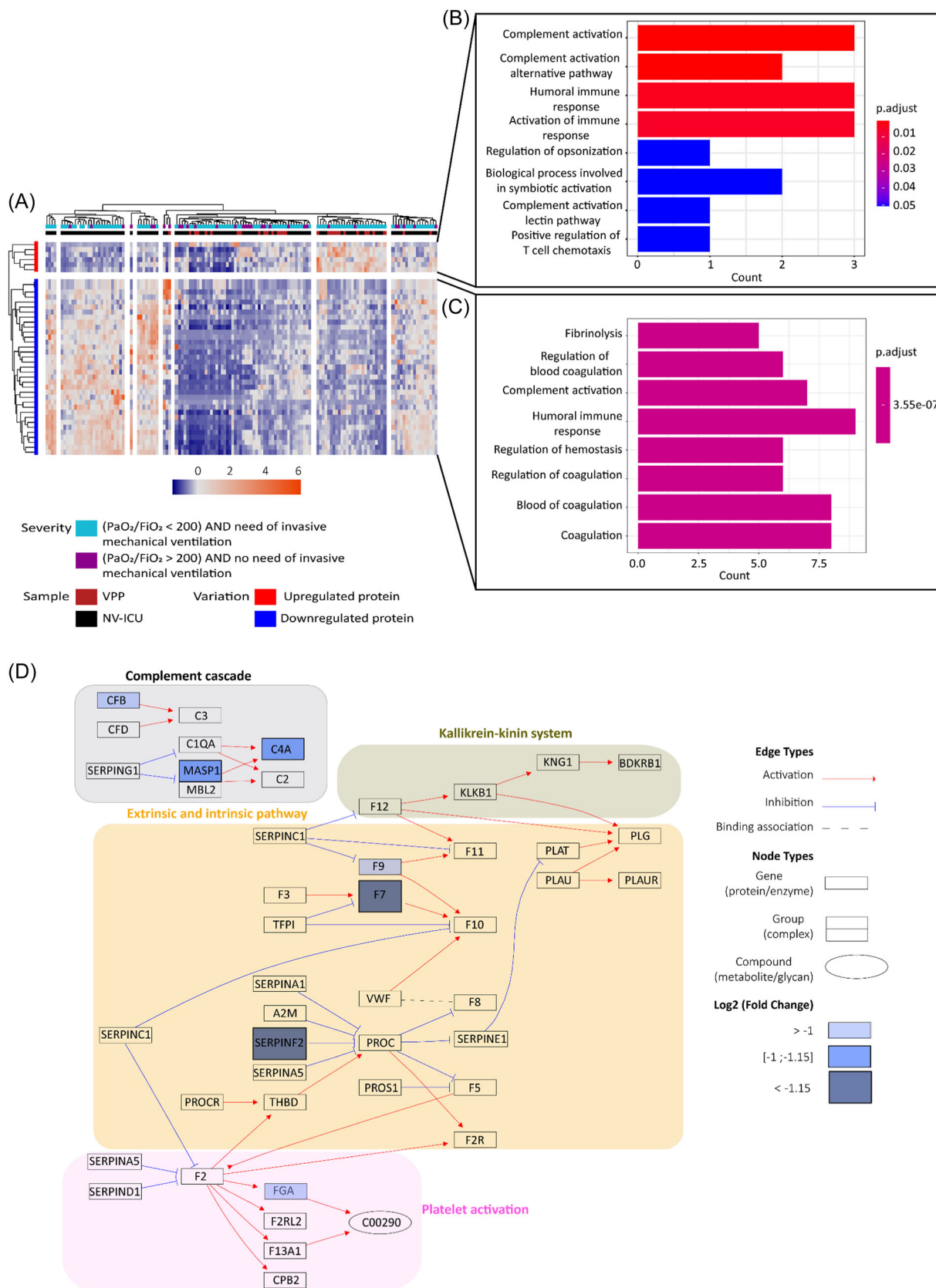


FIGURE 4 Study of the secretomic profiles of the 41 proteins specifically deregulated in viral pneumonia patients compared to those hospitalized in ICU without sign of viral infection respectively. (A) Heatmap visualization of the 41 proteins specifically dysregulated in VPP and patients hospitalized in intensive care unit without sign of viral infection (NV-ICU). The graph was obtained with the R package “pheatmap”. (B) Gene Ontology (GO) enrichment were conduct to decipher biological process (BP) in which upregulated proteins were mostly involved. The graph was obtained with the R package “ggplot”. (C) GO enrichment were conduct to decipher BP in which downregulated proteins were mostly involved. The graph was obtained with the R package “ggplot”. (D) Viral pneumonia promotes coagulation and complement pathways. Identified DEPs are shown in blue.

TABLE 2 Summary of five most promising protein combinations of biomarkers to distinguish patient hospitalized in intensive care unit without sign of viral infection from viral pneumonia patients.

Protein 1	Protein 2	Protein 3	Accuracy	CI ₉₅ [min-max]	Sensitivity	Specificity
Complement component C9 P02748	Galectin-3-binding protein Q08380	Cdc42 effector protein 4 Q9H3Q1	0.950	[0.831-0.994]	0.935	1.000
Ficolin-3 O75636	Fibrinogen gamma chain P02679	Proteoglycan 4 Q92954	0.950	[0.831-0.994]	1.000	0.778
Ficolin-3 O75636	Proteoglycan 4 Q92954	Fibrinogen alpha chain P02671	0.950	[0.834-0.994]	1.000	0.778
Complement component C9 P02748	Fibrinogen beta chain P02675	Cdc42 effector protein 4 Q9H3Q1	0.950	[0.831-0.994]	1.000	0.778
Complement component C9 P02748	Coagulation factor IX P00740	Apolipoprotein D P05090	0.925	[0.7961-0.984]	0.935	0.889

4.3 | FCN-3 and PRG4 as interesting candidate biomarkers to distinguish viral pneumonia patients

This study allows us to propose combinations of three proteins specifically deregulated in VPP adult patients to distinguish VPP from NV-ICU patients. FCN-3, also known as H-Ficoline,¹⁷ is upregulated in VPP patients compared to NV-ICU patients. FCN-3 has previously been shown to be highly expressed in human lung tissue and to have a higher complement-activating capacity than other lectin complement pathway initiators.¹⁸ Plovsing and colleagues have shown that acute lung inflammation is accompanied by a progressive increase in alveolar FCN-3 levels.¹⁹ The combined results from Plovsing and colleagues and our present data indicate that the elevated FCN-3 levels in the plasma may arise from a leakage from the lungs into the circulation. Nevertheless, to assure that this may be the case, a continuous and parallel monitoring of alveolar and plasma levels of FCN-3 during pulmonary inflammation should be performed. It cannot be ruled out however that the elevated plasma FCN-3 levels have a different origin. This protein can activate complement via the lectin pathway after forming complexes with MASP family proteins.²⁰ In addition, FCN-3 has been identified in patients with viral pneumonia, particularly in the context of influenza A virus (IAV) infection. Several studies demonstrated that FCN-3 possess antiviral activity against IAV.^{21,22} It would be interesting to test its antiviral activity in other viral infections. FCN-3 has been proposed as a diagnostic biomarker of lung infection.²³

Our study shows that PRG4, also known as lubricin, is decreased in VPP patients compared to NV-ICU patients. This protein has been studied primarily as a lubricating molecule essential to articular cartilage homeostasis. PRG4 has been studied mainly in joints, but this protein is expressed in the liver, heart, lungs or kidneys. It is becoming clear that PRG4 is involved in a variety of biological processes in parallel with lubrication, and that the loss/absence of this protein can lead to a loss of normal

homeostasis in many tissues/organ systems. Indeed, studies have shown that PRG4 is excreted by immune cells during infection to enable propagation of the inflammatory response and its systemic and cellular regulation.^{24,25} Thus, it has been shown that the absence of PRG4 switches the macrophage population to a pro-inflammatory phenotype, while the addition of exogenous PRG4 switches macrophages to an anti-inflammatory phenotype.²⁶ To our knowledge, the present study is the first identifying PRG4 as a candidate biomarker to distinguish between VPP and NV-ICU patients. On its own, PRG4 may not be able to distinguish between VPP and NV-ICU patients, but when coupled with other proteins, it may become more sensitive.

Interestingly, in our study, when comparing VPP vs NV-ICU, FCN-3 is upregulated while PRG4 is downregulated. The different directions of variation of these two pro-inflammatory proteins could be explained by the development of a compensatory inflammatory response. The increase in FCN-3 may reflect a pro-inflammatory response, while the decrease in PRG4 expression indicates a form of inflammation regulation by negative control mechanisms to limit tissue damage.

Furthermore, stressful stimuli, such as SARS-CoV-2 infection, may affect cellular functions in several tissues and organs by inducing inflammation, cell death and/or senescence. In this context, the senescence-associated secretory phenotype (SASP), which is a subpart of the secretome, can significantly modify the latter and thus have important consequences on the progression of pathology. For example, SAPS can contain proteins linked to inflammation, notably via interleukins such as IL-1, IL-4, IL-12 or NF- κ B.²⁷ However, these proteins were not identified as deregulated in our proteomic study, which is in agreement with a previous study.⁷ This absence of interleukin deregulation in the proteomic data in a context of inflammatory pathology is potentially explained by the use of ProteoMiner[®] technology that tends to eliminate low molecular weight proteins.

4.4 | Requirement to use protein combinations

Studies frequently focus on the identification of biomarkers to discriminate VPP from other conditions. However, most studies attempt to use a single biomarker as the reference. This approach, while seductive, does not seem realistic, as no single biomarker appears to be sensitive and specific enough. Such an approach has never been used to diagnose patients with COVID-19-induced viral pneumonia among NV-ICU patients, although in the context of other pathologies, such as sepsis, an increase in predictive efficiency was shown to occur with the use of protein combinations.²⁸ Therefore, we decided to identify combinations of proteins to increase COVID-19 viral pneumonia diagnosis and prognosis accuracy. Our study identifies 5 promising combinations of 3 biomarkers. Once refined and validated by a biochemical approach, these combinations could be transposed into routine clinical use.

4.5 | Limitations of the study

In our study viral pneumonia were all induced by SARS-CoV-2. However, it should be noticed that, in clinical practice, several viruses are susceptible to cause viral pneumonia, particularly influenza. It would therefore be interesting to use other viral pathogens to validate or invalidate that the protein combination identified herein is specific or not to infection by SARS-CoV-2. This study is also single-center and performed on a limited cohort. It would be interesting to confirm the results obtained herein on a larger multicenter cohort. Thirdly, in our study, VPP patients have a lower SOFA severity score than patients in the NV-ICU group. This score is a universal clinical score that addresses a large number of patients and may therefore lack sensitivity. It is logically expected that different SOFA scores should be accompanied with differences in the DEPs of the secretomes. However, at this stage, given the heterogeneity of the patients' condition and the still limited size of the cohort, this study was unable to establish a satisfactory correlation between SOFA scores and DEPs. Cohort size increase and better classification of patients during cohort constitution will solve this issue at a later stage. Finally, the proposed combinations have not been validated by a biochemical approach in an external cohort, due to the lack of reliable biochemical tools for these proteins. The aim is to develop such tools for test and potential routine clinical use.

5 | CONCLUSION

In conclusion, this article presents an innovative and promising secretomic analysis to distinguish VPP patients from NV-ICU patients using combinations of protein markers. Among the identified proteins, ficolin-3 and proteoglycan-4 appear to be deregulated specifically in VPP compared to NV-ICU patients. Additional studies are needed to confirm the results of this research and to evaluate the effectiveness of this method in real-world clinical situations.

AUTHOR CONTRIBUTIONS

Conceptualization: Angélique Blangy-Letheule, Antoine Roquilly, Michel De Waard, Bertrand Rozec and Benjamin Lauzier. *Investigation:* Angélique Blangy-Letheule, Jérôme Montnach, Sandrine Bourgoïn-Voillard, Damien Habert, Thomas Dupas, Amandine Vergnaud, Walid Oulehri, Dorian Hassoun, Manon Denis, Jules Lecomte, Antoine Persello, José Courty, Michel Seve, Antoine Roquilly, Aurélie A. Leroux, Bertrand Rozec, Michel De Waard and Benjamin Lauzier. *Writing—original draft preparation:* Angélique Blangy-Letheule, Thomas Dupas and Amandine Vergnaud. *Writing—review and editing:* Angélique Blangy-Letheule, Jérôme Montnach, Sandrine Bourgoïn-Voillard, Damien Habert, Amandine Vergnaud, Thomas Dupas, Walid Oulehri, Dorian Hassoun, Manon Denis, Jules Lecomte, Antoine Persello, José Courty, Michel Seve, Antoine Roquilly, Aurélie A. Leroux, Bertrand Rozec, Michel De Waard and Benjamin Lauzier. *Funding acquisition:* Bertrand Rozec, Michel De Waard and Benjamin Lauzier. All authors have read and approved the final manuscript.

ACKNOWLEDGMENTS

We thank the patients and blood donors who contributed to this study and the hospitals at Nantes for sample and data collection. We thank the biological resource center for biobanking (CHU Nantes. Nantes Université, Centre de ressources biologiques (BB-0033-00040), F-44000 Nantes, France). This work was supported by "Société Française d'Anesthésie et de Réanimation" (Paris, France), "Fondation d'entreprises Genavie" (Nantes, France), "Association sauve ton Cœur", and "Agence nationale de la recherche" (20-ASTC-0032-01-hErOiSmE) (Paris, France). A.R. received a grant (N°847782) from the European Union's Horizon 2020 research and innovation program and two other ones from Agence National de la Recherche (ANR) and Région Pays de la Loire – Flash COVID-19: COVARDS project.

CONFLICT OF INTEREST STATEMENT

No conflict of interest declared. The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

DATA AVAILABILITY STATEMENT

The proteomic data were deposited to the ProteomeXchange Consortium with the MassIVE identifier MSV000092749 (<https://massive.ucsd.edu/> accessed on 25 August 2023) and ProteomeXchange PXD044859 (<https://proteomecentral.proteomexchange.org/> accessed on 25 August 2023).

ETHICS STATEMENT

The work described in this manuscript was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The manuscript complies with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aims to include representative human populations (sex, age and ethnic origin) in accordance with these recommendations. Samples were

collected with the informed consent of patients and their right to privacy was respected.

REFERENCES

- Naghavi M, Abajobir AA, Abbafati C, et al. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390:1151–1210. doi:10.1016/S0140-6736(17)32152-9
2022. Deaths from pneumonia in EU regions n.d. <https://ec.europa.eu/eurostat/web/products-eurostat-news/-/edn-20191112-1>
- Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases society of America/American Thoracic Society Consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis*. 2007;44:S27–S72. doi:10.1086/511159
- Metlay JP, Waterer GW, Long AC, et al. Diagnosis and treatment of adults with community-acquired pneumonia. an official clinical practice guideline of the American Thoracic Society and infectious diseases society of America. *Am J Respir Crit Care Med*. 2019;200:e45–e67. doi:10.1164/rccm.201908-1581ST
- Ruuskanen O, Lahti E, Jennings LC, Murdoch DR. Viral pneumonia. *Lancet*. 2011;377:1264–1275. doi:10.1016/S0140-6736(10)61459-6
- Chenau J, Michelland S, Seve M. Le sécrétome: définitions et intérêt biomédical. *La Revue de Médecine Interne*. 2008;29:606–608. doi:10.1016/j.revmed.2007.12.011
- Blangy-Letheule A, Persello A, Michelland S, et al. Study of secretome deregulation of a rat model of endotoxemic shock using a non-targeted mass spectrometry approach. *Arch Cardiovasc Dis Suppl*. 2021;13:213. doi:10.1016/j.acvdsp.2021.04.158
- Bravo E, Calzolari A, De Castro P, et al. Developing a guideline to standardize the citation of bioresources in journal articles (CoBRA). *BMJ Med*. 2015;13:33. doi:10.1186/s12916-015-0266-y
- Ferreira FL. Serial evaluation of the SOFA score to predict outcome in critically ill patients. *JAMA*. 2001;286:1754–1758. doi:10.1001/jama.286.14.1754
- Fayed M, Patel N, Angappan S, et al. Sequential organ failure assessment (SOFA) score and mortality prediction in patients with severe respiratory distress secondary to COVID-19. *Cureus* 14:e26911. doi:10.7759/cureus.26911
- Esmaili Tarki F, Afaghi S, Rahimi FS, Kiani A, Varahram M, Abedini A. Serial SOFA-score trends in ICU-admitted COVID-19 patients as predictor of 28-day mortality: A prospective cohort study. *Health Sci Reports*. 2023;6:e1116. doi:10.1002/hsr2.1116
- Burk M, El-Kersh K, Saad M, Wiemken T, Ramirez J, Cavallazzi R. Viral infection in community-acquired pneumonia: a systematic review and meta-analysis. *Eur Respir Rev*. 2016;25:178–188. doi:10.1183/16000617.0076-2015
- Covington EW, Roberts MZ, Dong J. Procalcitonin monitoring as a guide for antimicrobial therapy: a review of current literature. *Pharmacother J Human Pharmacol Drug Therapy*. 2018;38:569–581. doi:10.1002/phar.2112
- Self WH, Balk RA, Grijalva CG, et al. Procalcitonin as a marker of etiology in adults hospitalized with community-acquired pneumonia. *Clin Infect Dis*. 2017;65:183–190. doi:10.1093/cid/cix317
- Collange O, Sammour Y, Soulié R, Castelain V, Mertes PM. ICU re-organisation to face the first COVID-19 epidemic wave in a tertiary hospital. *Anaesth Crit Care Pain Med*. 2020;39:731–732. doi:10.1016/j.accpm.2020.09.005
- Castelnuovo F, Marchese V, Cristini G, et al. Discharge ward during the SARS-CoV-2 pandemic: an effective way to increase patient turnover when human resources are scarce. *Infez Med*. 2020;28:539–544.
- Trolborg A, Thiel S, Mistegaard CE, et al. Plasma levels of H- and I-ficolin are increased in axial spondyloarthritis: improvement of disease identification. *Clin Exp Immunol*. 2020;199:79–87. doi:10.1111/cei.13374
- Hummelshoj T, Fog LM, Madsen HO, Sim RB, Garred P. Comparative study of the human ficolins reveals unique features of Ficolin-3 (Hakata antigen). *Mol Immunol*. 2008;45:1623–1632. doi:10.1016/j.molimm.2007.10.006
- Plovsing RR, Berg RMG, Munthe-Fog L, et al. Alveolar recruitment of ficolin-3 in response to acute pulmonary inflammation in humans. *Immunobiology*. 2016;221:690–697. doi:10.1016/j.imbio.2015.11.015
- Świerzko AS, Cedzyński M. The influence of the lectin pathway of complement activation on infections of the respiratory system. *Front Immunol*. 2020;11:585243. doi:10.3389/fimmu.2020.585243
- Verma A, White M, Vathipadikal V, et al. Human H-Ficolin inhibits replication of seasonal and pandemic influenza A viruses. *J Immunol*. 2012;189:2478–2487. doi:10.4049/jimmunol.1103786
- White MR, Tripathi S, Verma A, et al. Collectins, H-ficolin and LL-37 reduce influenza viral replication in human monocytes and modulate virus-induced cytokine production. *Innate Immun*. 2017;23:77–88. doi:10.1177/1753425916678470
- Bidula S, Sexton DW, Yates M, et al. H-ficolin binds *Aspergillus fumigatus* leading to activation of the lectin complement pathway and modulation of lung epithelial immune responses. *Immunology*. 2015;146:281–291. doi:10.1111/imm.12501
- Qadri M, Jay GD, Zhang LX, et al. Recombinant human proteoglycan-4 reduces phagocytosis of urate crystals and downstream nuclear factor kappa B and inflammasome activation and production of cytokines and chemokines in human and murine macrophages. *Arthritis Res Ther*. 2018;20:192. doi:10.1186/s13075-018-1693-x
- Das N, Schmidt TA, Krawetz RJ, Dufour A. Proteoglycan 4: from mere lubricant to regulator of tissue homeostasis and inflammation. *BioEssays*. 2019;41:1800166. doi:10.1002/bies.201800166
- Krawetz RJ, Abubacker S, Leonard C, et al. Proteoglycan 4 (PRG4) treatment enhances wound closure and tissue regeneration. *NPJ Regen Med*. 2022;7:32. doi:10.1038/s41536-022-00228-5
- Oguma Y, Alessio N, Aprile D, et al. Meta-analysis of senescent cell secretomes to identify common and specific features of the different senescent phenotypes: a tool for developing new senotherapeutics. *Cell Commun Signaling*. 2023;21:262. doi:10.1186/s12964-023-01280-4
- Huang N, Chen J, Wei Y, et al. Multi-marker approach using C-reactive protein, procalcitonin, neutrophil CD64 index for the prognosis of sepsis in intensive care unit: a retrospective cohort study. *BMC Infect Dis*. 2022;22:662. doi:10.1186/s12879-022-07650-6

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Blangy-Letheule A, Vergnaud A, Dupas T, et al. Value of a secretomic approach for distinguishing patients with COVID-19 viral pneumonia among patients with respiratory distress admitted to intensive care unit. *J Med Virol*. 2024;96:e29756. doi:10.1002/jmv.29756