



Key factors of the deranged antiviral response in elderly patients with COVID-19: a machine-learning analysis

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Abstract Age is a well-known risk factor to develop severe viral respiratory infections, including severe COVID-19. This study aimed to identify the biological alterations linked to severe disease in

elderly patients with COVID-19. For this purpose, we employed a derivation cohort with 450 SARS-CoV-2 infected and unvaccinated patients admitted to hospital wards and a validation cohort with 244 SARS-CoV-2 infected and unvaccinated patients admitted to hospital Intensive Care Unit (ICU). Twenty-one biomarkers were measured in plasma samples from patients upon admission, including SARS-CoV-2 RNA, IgG antibodies, and protein biomarkers. Patient cohorts were divided into two groups based on age:

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adult (≤ 70 years old) and elderly (> 70 years old) patients. In the derivation cohort, 90-day mortality rate observed in the adult group was 6.0% whereas in the elderly group it rises to 31.6%, same trend was noticed regarding the validation cohort, with 11.2% versus 40.3% 90-day mortality rates for adult and elderly groups, respectively. The machine-learning framework XGBoost-SHAP, fed with the plasma biomarkers information, was used to profile an age-related host response to SARS-CoV-2 infection. Based on SHAP plot, elderly patients had a strong

thrombo-inflammatory response profile (significantly elevated plasma levels of: lipocalin-2, endothelin-1, D-dimer) combined with deficient adaptive and cytotoxic antiviral responses. Model performance evaluated with the validation cohort confirmed the robustness and generalizability of the model developed (AUC=0.710). In conclusion, the machine learning approach we built allowed us to identify the presence of a deranged host response in elderly patients with COVID-19 linked to poor viral control and increased mortality.

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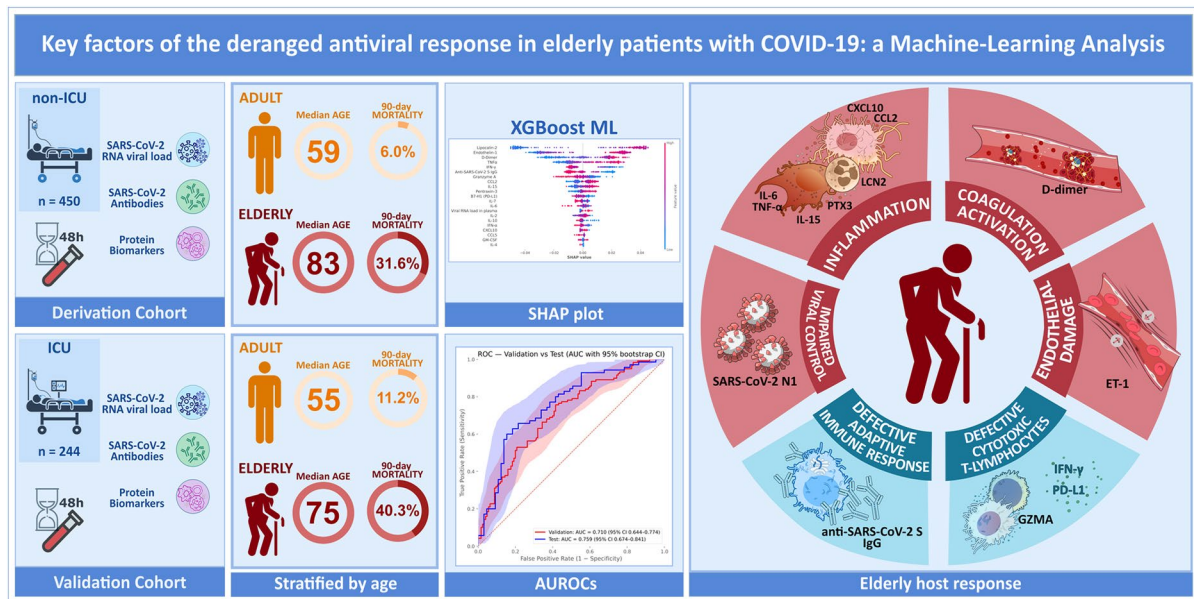
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Graphical Abstract



Keywords SARS-CoV-2 · Elderly · Machine Learning · Deranged response · Viral load

Introduction

In 2016, 3 years before the onset of the COVID-19 pandemic, a study conducted on the global burden of disease (Global Burden of Disease Study 2016) [1] estimated that lower respiratory tract infections were a leading cause of mortality in children under 5 years of age and in elderly adults, with a particularly high mortality burden in adults aged over 70 years. In that year, more than 1 million deaths were reported in people over 70 years of age due to lower respiratory tract infections, of which 13 to 31% were caused by viruses [2].

Since the aforementioned study was conducted in 2016, the world has faced a global pandemic caused by SARS-CoV-2 infection, resulting in more than 7.0 million confirmed COVID-19 deaths to date [3]. This fact has highlighted the burden and impact that viral respiratory infections have on the population, especially the elderly population.

Age has been found to be the one of the most important risk factors for the development of severe

COVID-19. The higher prevalence of chronic diseases in the elderly population plays an important role in this increased SARS-CoV-2 infection susceptibility [2].

Many studies have shown a strong correlation between age and COVID-19 severity and related mortality [4–6], and this association becomes stronger in those aged 70 years and older [6–9].

Much of the global initiative to reduce the burden of lower respiratory tract infections has been focused primarily on children under 5 years of age [1]. The United Nations (UN) predicts that the number of people over 60 years of age will double worldwide from 901 million in 2015 to 2.1 billion in 2050 [10], reinforcing the need to foster research and care of this population group.

During aging, immunosenescence and low-grade chronic inflammation (inflammaging) lead to an imbalance of the immune system. Immunosenescence, the age-related gradual deterioration of the immune system, along with inflammaging may indirectly increase the predisposition and vulnerability of the elderly to respiratory viral infections [11–14].

The presence of immunosenescence and chronic diseases predisposes this population to develop more severe diseases and to suffer poorer outcomes when they get infected by a respiratory virus [14–16].

The aim of this study was to identify the specific alterations that the elderly have in their biological response to SARS-CoV-2, by using machine learning (ML) approaches. For this purpose, we developed a multicentric study recruiting hospitalized patients who lacked prior immunity and profiled a wide range of biomarkers involved in the innate and adaptive immunity along with viral load in plasma. Results of this study could contribute to understanding the impact of age on host responses to SARS-CoV-2 and other epidemic and emerging respiratory viral infections.

Methods

Study design

This study included a cohort of 450 patients admitted to three hospitals located in Spain with SARS-CoV-2 infection confirmed by a positive nasopharyngeal swab reverse-transcription polymerase chain reaction (RT-PCR) test.

The eligibility criteria were as follows: adult patients (aged 18 and above), with no concomitant infection and without SARS-CoV-2 vaccination that were admitted to hospital wards within the period ranged from July 2nd, 2020 to March 10th, 2021, corresponding to the 2nd and 3rd waves of COVID-19 when most prevalent variants of concern (VOCs) were B.1.1.7 and BA.5.

A different cohort with 244 SARS-CoV-2 infected patients hospitalized in the Intensive Care Unit (ICU) were recruited, with the same eligibility criteria, across 23 Spanish hospitals between March 16th, 2020, and February 27th, 2021. The non-ICU cohort of patients was used as the derivation cohort for the machine learning analysis pipeline while the ICU cohort was used for the external validation of the final machine learning model.

The study protocol was approved by the ethics committees of the participating hospitals, adhering to the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans and in adherence to Good Clinical Practice. Samples

were processed at the BioSepsis laboratory and at the IRB-Lleida Biobank (B.0000682)/"Biomodels Platform ISCHII PT23/00032". All participants or their legal representatives signed study consent forms for the use of data and stored samples in the research.

Blood samples

Patients were sampled within the first 48 h after hospital admission. Blood samples were collected in EDTA tubes, plasma was separated by centrifugation and stored at -80°C until analysis.

Data collection

Demographics, medical history and clinical course, laboratory values, and outcomes of all patients were collected retrospectively from electronic medical records by the investigators.

Laboratory essays

Blood panel

Routine clinical biomarkers, including biochemistry, and hemogram were promptly analyzed at the central laboratory of each hospital.

Blood SARS-CoV-2 viral load and antibodies titers

Viral RNA load in plasma was evaluated using droplet digital PCR as previously described [17]. In the derivation (non-ICU) cohort, IgG antibodies against SARS-CoV-2 S proteins were assessed by using the SARS-CoV-2 IgG II Quant assay on an Alinity platform (Abbott Laboratories Inc.). In the validation (ICU) cohort, anti-SARS-CoV-2 S IgG antibodies in plasma were measured as previously described [18].

Biomarker's profile

A panel of 19 protein biomarkers was profiled using the Ella-SimplePlex™ immunoassay (San Jose, California, USA). Those biomarkers included are: TNF- α (Tumor Necrosis Factor α ; inflammation), Interleukin (IL)–2 (T helper 1 response), IL-4 (T helper 2 response), IL-6 (inflammation), IL-15, IL-7 (T and B cell activation and proliferation), C–C motif chemokine ligand 2/Monocyte Chemoattractant Protein-1 (CCL2/MCP-1), C–X–C motif

chemokine ligand 10/Interferon gamma-induced protein 10 (CXCL10/IP-10), Granulocyte–Macrophage Colony-Stimulating Factor (GM-CSF), C–C motif chemokine ligand 5/Regulated on Activation, normal T cell expressed and secreted (CCL5/RANTES; chemotaxis), Endothelin-1 (ET-1; vasoconstriction), Programmed Death-Ligand 1 (PD-L1/B7-H1; immunosuppression), IL-10 (immunosuppression), Lipocalin-2 (LCN2/NGAL; Neutrophil Gelatinase-Associated Lipocalin), D-dimer (coagulation), Pentraxin-3/TSG14, Granzyme A (cytotoxicity), Interferon (IFN)- α , and IFN- γ (interferon response).

Statistical analysis

Data were analyzed by using R software (version 4.0.5; R Foundation for Statistical Computing, Vienna, Austria), Python Programming Language (version 3.7.9; Python Software Foundation, Wilmington, DE, USA), and IBM SPSS® version 20 (IBM, Armonk, NY, USA).

Categorical variables were reported as total counts and proportions (n (%)) and χ^2 or Fisher's exact test were used for comparisons. Continuous variables were presented as medians and interquartile range (IQR) and, assuming nonparametric data distribution, Mann–Whitney U test was used to compare between patient groups. All statistical tests were conducted as two-sided at the 0.05 significance level.

In the derivation cohort, we performed a receiver operating characteristic (ROC) curve analysis to evaluate the impact of age on the combined outcome: ICU admission and/or 90-day mortality. The optimal operating point (OOP) in the ROC curve was calculated as the point on the curve with the minimum distance to the upper left corner by Pythagoras' theorem [19, 20]. According to OOP for age, patients were distributed into two groups, creating a categorical variable.

Kaplan Meier survival analysis and log-rank test were used to illustrate differences in 90-day mortality rates among these age groups in both cohorts.

Development of the predictive model using a XGBoost-SHAP framework to compare the response to SARS-CoV-2 across age groups

Model training

Ln-transformed biomarker concentrations at baseline were used to develop a machine learning approach using the Extreme Gradient Boosting (XGBoost)

algorithm model. XGBoost was used to associate the levels of the 21 biomarkers analyzed with an elderly or adult-specific host response to SARS-CoV-2 infection. XGBoost is a machine learning algorithm proposed by Chen and Guestrin [21]. Boosting algorithms are based on the idea of building a strong and reliable classifier by sequentially combining multiple weak classifiers, usually using shallow decision trees.

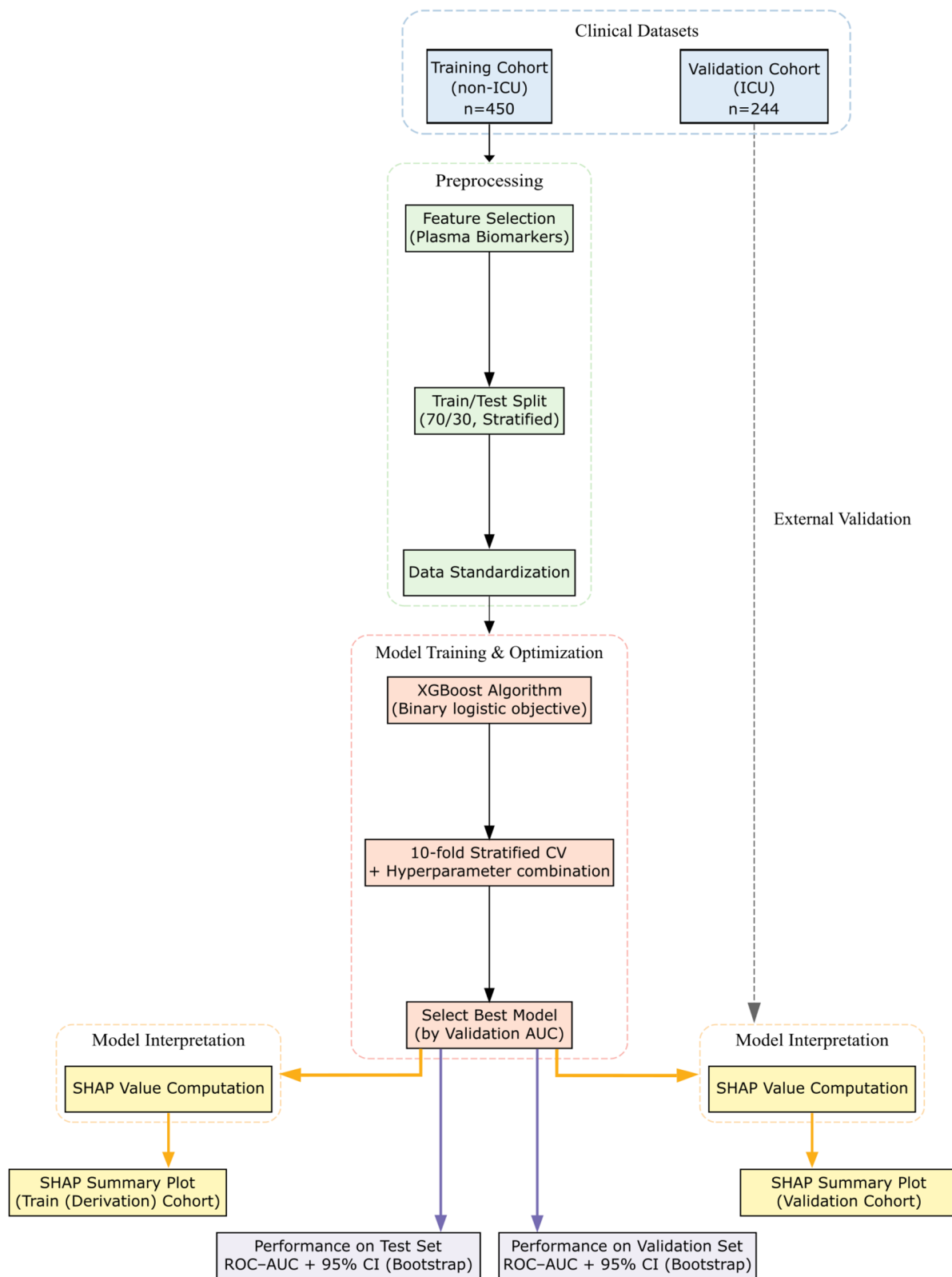
The derivation (non-ICU) cohort was randomly split into training (70%) and test (30%) datasets. A 10-fold cross-validation (CV) was applied to the training dataset using the area under the receiver operating characteristic curve (AUROC) as the metric to be optimized by selecting the best values of different hyperparameters that, when combined, yielded the highest AUC. Those best hyperparameters were used to train the final model.

Model performance

The AUROC was used to evaluate discrimination performance of the XGBoost final model selected for both the test subset and the external validation cohort. Also, a bootstrap resampling with replacement (5000 iterations) procedure was computed to estimate the 95% confidence intervals for the AUC values reported. The AUROCs and 95% bootstrap confidence interval for the internal and external validation datasets were plot for visual analysis.

Model explainability

The SHAP (SHapley Additive exPlanations) method was initially developed by Shapley in 1953 [22] and then proposed by Lundberg and Lee [23] to overcome the black-box problem of diverse machine learning methods as XGBoost. SHAP assigns each feature an importance value for a particular prediction (elderly-specific host response, in this study). The SHAP values are calculated by carefully perturbing the input features and observing how these changes correspond to the final model prediction. The final values are then obtained by summing the marginal contributions of all possible feature value combinations. The SHAP summary plot gives an overview of the magnitudes and directions of predictions, with the size of each SHAP value indicating the contribution of a specific feature to the model's performance, where larger absolute SHAP values correspond to higher contributions [24–26].



◀Fig. 1 Workflow followed to build and develop the machine learning model

Influence of confounding factors on the ML model: multivariable analysis to address the impact of sex on the most important biomarkers resulting from the ML analysis

A binomial logistic regression analysis was performed to identify plasma biomarkers associated with the elderly host response to SARS-CoV-2 infection in both derivation (non-ICU) and validation (ICU) cohorts. Those variables tested in the machine learning analysis yielding p values < 0.050 in univariable analyses were tested for multivariable analyses where sex was used as adjusting variable. All statistical tests were conducted as two-sided at the 0.05 significance level.

Protein–protein interaction network and functional pathway enrichment analysis

Protein–protein interactions and functional enrichment analysis were performed using STRING [27] (version 12.0; <https://string-db.org/>), Cytoscape (version 3.10.4), and Enrichr (<https://maayanlab.cloud/Enrichr/>). Methodology used explained in further detail in Supplementary Text S1, Online Resource 1.

Multivariable analysis to predict severe outcomes in each age group

We performed a binomial logistic regression analysis to identify the factors associated with the combined outcome ICU admission and/or 90-day mortality in the derivation (non-ICU) cohort and with 90-day mortality in the validation (ICU) cohort for each group of patients. Variables used in the machine learning analysis yielding p values < 0.050 in the univariable analyses were tested for the final multivariable analyses and age, sex and SOFA score were used as adjusting variables. All statistical tests were conducted as two-sided at the 0.05 significance level.

Results

Clinical characteristics of the patients

This study recruited 450 SARS-CoV-2 infected patients admitted to three different hospitals located in Spain. We used an independent dataset from a different patient population as a validation cohort with a total of 244 patients with the same infection hospitalized in the ICU (Fig. 1).

Patients were grouped as described in the Methods section to identify the age of those patients at higher risk of developing adverse outcomes. The Optimal Operating Point (OOP) obtained from the ROC analysis resulted in a cutoff age value of 71 years old (Supplementary Fig. S1, Online Resource 2).

According to OOP, patient cohorts were divided into two distinct groups: adult patients (≤ 70 years old) and elderly patients (> 70 years old) (Supplementary Fig. S1, Online Resource 2), representing 48.0% and 52.0% of the total derivation cohort, respectively, and 51.2% and 48.8% in the validation cohort.

Baseline characteristics of the included patients in both derivation and validation cohorts, and comparisons between age-groups are shown in Table 1 and Table 2.

In both cohorts, derivation (non-ICU) and validation (ICU), elderly patients (> 70 years old) displayed a higher prevalence of most comorbidities at admission. In the derivation cohort, hypertension (72.6% (170/234) vs. 36.6% (79/216); $p < 0.001$), diabetes (28.2% (66/234) vs. 12.9% (28/216); $p < 0.001$), and chronic cardiovascular diseases (25.6% (60/234) vs. 5.56% (12/216); $p < 0.001$) were the most frequent conditions in the elderly group. Similar trends were observed in the validation cohort where hypertension (62.2% (74/119) vs. 35.2% (44/125); $p < 0.001$), diabetes (37.0% (44/119) vs. 14.4 (18/125); $p < 0.001$) and chronic cardiovascular diseases (19.3% (23/119) vs. 10.4% (13/125); $p = 0.008$) were also the most prevalent conditions in this group of patients. In addition, older patients presented with higher median Charlson index in the derivation (5.00 [2.00] vs. 2.00 [3.00]; $p < 0.001$) and validation (4.00 [2.00] vs. 1.00 [2.00]; $p < 0.001$) cohorts as well as higher SOFA score (2.00 [1.00] vs. 1.00 [2.00]; $p < 0.001$) and (4.00 [1.00] vs. 3.00 [0.00]; $p < 0.001$), respectively (values provided as median [IQR]) (Table 1).

Table 1 Baseline characteristics of non-ICU hospitalized patients. Statistics: Continuous variables are represented as median (interquartile range) and categorical variables as absolute count (n (%)). *P*-values were assessed by using the Mann–

Whitney test for continuous variables, and Chi-squared test for categorical variables. Statistically significant differences are shown in bold

Baseline characteristics (Derivation Cohort: non-ICU patients)

Clinical characteristics and outcomes	All cohort	Adult patients (≤ 70 yrs)	Elderly patients (> 70 yrs)	<i>P</i> value
Demographics and underlying conditions				
No. [n (%)]	450	216 (48.0)	234 (52.0)	-
Age [median years (IQR)]	72.0 (25.0)	59.0 (23.0)	83.0 (10.0)	0.003
Male sex [n (%)]	244 (54.2)	133 (61.6)	111 (47.4)	0.003
Smoking [n (%)]	16 (3.60)	10 (4.60)	6 (2.60)	0.237
Comorbidities				
Hypertension [n (%)]	249 (55.3)	79 (36.6)	170 (72.6)	<0.001
Dyslipidemia [n (%)]	164 (36.4)	61 (28.2)	103 (44.0)	0.001
Diabetes [n (%)]	94 (20.9)	28 (13.0)	66 (28.2)	<0.001
Obesity [n (%)]	93 (20.7)	47 (21.8)	46 (19.7)	0.582
Chronic cardiovascular disease [n (%)]	72 (16.0)	12 (5.56)	60 (25.6)	<0.001
Chronic cerebrovascular disease [n (%)]	25 (5.56)	3 (1.39)	22 (9.40)	<0.001
Chronic atrial fibrillation [n (%)]	54 (12.0)	8 (3.70)	46 (19.7)	<0.001
Chronic renal disease [n (%)]	53 (11.8)	11 (5.09)	42 (18.0)	<0.001
Chronic Respiratory disease [n (%)]	63 (14.0)	23 (10.7)	40 (17.1)	0.049
Cancer [n (%)]	50 (11.1)	12 (5.56)	38 (16.2)	<0.001
Admission status and scores				
Day since symptoms onset to hospital admission [median years (IQR)] *447	6.00 (6.00)	7.00 (5.00)	4.50 (5.00)	<0.001
SOFA score [median (IQR)]	2.00 (2.00)	1.00 (1.00)	2.00 (2.00)	<0.001
Sepsis [n (%)]	262 (58.2)	106 (49.1)	156 (66.7)	<0.001
Bilateral pneumonia in the Chest X-ray [n (%)]	284 (63.1)	146 (67.6)	138 (59.0)	0.058
PaO ₂ /FIO ₂ (< 400) [n (%)]	325 (72.2)	151 (69.9)	174 (74.7)	0.292
MAP (< 70 mmHg) [n (%)]	24 (5.30)	4 (1.90)	20 (8.50)	0.002
Charlson score *447	4 (3.00)	2.00 (2.00)	5.00 (3.00)	<0.001
Laboratory parameters at hospital admission				
Hyperglycemia (Glucose > 126 mg/dl) [n (%)]	199 (44.2)	91 (42.1)	108 (46.2)	0.390
Creatinine > 1.2 mg/dL [n (%)]	78 (17.3)	16 (7.40)	62 (26.5)	<0.001
Bilirubin > 1 mg/dL [n (%)] *449	29 (6.5)	12 (5.56)	17 (7.30)	0.453
Hypertransaminasemia (ALT > 40 UI/L) [n (%)] *445	129 (29.0)	94 (43.9)	35 (15.2)	<0.001
Hyperuremia (Urea > 50 mg/dL) [n (%)] *422	177 (41.9)	40 (20.1)	137 (61.4)	<0.001
Hypernatremia (Na > 145 mmol/L) [n (%)]	26 (5.8)	5 (2.31)	21 (8.97)	0.002
LDH > 280 UI/L [n (%)] *445	278 (62.5)	134 (62.0)	144 (62.9)	0.854
Lactate > 2 mmol/L [n (%)] *436	89 (20.4)	30 (14.4)	59 (26.0)	0.003
Hemoglobin < 12 g/dL [n (%)]	107 (23.8)	33 (15.3)	74 (31.6)	<0.001
Thrombocytopenia (Platelets < 150 cell × 10 ³ /μl) [n (%)]	143 (31.8)	62 (28.7)	81 (34.6)	0.178
C-reactive protein > 100 mg/L [n (%)]	173 (38.4)	74 (34.3)	99 (42.3)	0.080
Lymphopenia (< 1000 cells/mm ³) [n (%)]	281 (62.4)	115 (53.2)	166 (70.9)	<0.001
Neutrophilia (> 7500 cells/mm ³) [n (%)] *449	79 (17.6)	35 (16.2)	44 (18.9)	0.456
Monocytopenia (< 200 cells/mm ³) [n (%)] *449	95 (21.2)	45 (20.8)	50 (21.5)	0.871
Treatments				
Remdesivir [n (%)]	47 (10.4)	37 (17.1)	10 (4.27)	<0.001

Table 1 (continued)*Baseline characteristics (Derivation Cohort: non-ICU patients)*

Clinical characteristics and outcomes	All cohort	Adult patients (≤ 70 yrs)	Elderly patients (> 70 yrs)	P value
Heparin [n (%)]	354 (78.7)	180 (83.3)	174 (74.4)	0.020
Corticoids [n (%)]	361 (80.2)	169 (78.2)	192 (82.1)	0.311
Tocilizumab [n (%)]	83 (18.4)	49 (22.7)	34 (14.5)	0.026
Azithromycin [n (%)] *296	95 (32.1)	47 (35.3)	48 (29.5)	0.28
Oxygenotherapy [n (%)]	360 (80.0)	160 (74.1)	200 (85.5)	< 0.001
Complications				
ARDS [n (%)]	125 (27.8)	68 (31.5)	57 (24.4)	0.092
Acute cardiac failure [n (%)] *448	30 (6.70)	3 (1.39)	27 (11.6)	< 0.001
Acute renal failure [n (%)] *448	27 (6.02)	8 (3.70)	19 (8.20)	0.046
Acute arrhythmia [n (%)]	37 (8.22)	12 (5.56)	25 (10.7)	0.048
Nosocomial infection [n (%)] *448	58 (12.9)	23 (10.7)	35 (15.1)	0.162
Length of hospital stay [median days (IQR)]	8.00 (10.0)	7.00 (7.00)	9.00 (9.25)	0.015
Outcomes				
ICU admission [n (%)]	52 (11.6)	36 (16.7)	16 (6.84)	0.001
Mortality [n (%)]	87 (19.3)	13 (6.00)	74 (31.6)	< 0.001

Data are presented as No. (%) or median [IQR]. *P* values were assessed by Mann-Whitney, χ^2 or Fisher's test when appropriate

SOFA Sequential Organ Failure Assessment, *MAP* Mean Arterial Pressure, *ARDS* Acute Respiratory Distress Syndrome

Asterisk ("*") indicates the actual number of samples available (n) for that specific parameter, which may be less than the total sample size of the study

Among laboratory parameters measured in blood, elderly patients exhibited more frequently lymphopenia at admission in the derivation (70.9% (166/234) vs. 53.2% (115/216); $p < 0.001$) and validation (87.4% (97/119) vs. 68.5% (85/125); $p = 0.001$) cohorts (Table 1 and Table 2). In the derivation cohort, presence of SARS-CoV-2 RNAemia was reported in 42.1% (98/234) and 30.1% (65/216) of elderly and adult patients, respectively ($p = 0.008$) (Table 3), while in the validation cohort, although it was higher in both groups compared with patients who were not admitted to ICU, elderly group still showed a significantly higher SARS-CoV-2 RNAemia prevalence (77.3% (92/119) vs. 62.4% (78/125); $p = 0.011$) (Table 4).

Kaplan–Meier curves in Fig. 2 and log-rank test evidenced a significantly higher 90-day mortality rate in the elderly group compared to the adult one in both derivation (31.6% (74/234) vs. 6.00% (13/216); $p < 0.001$) and validation (40.3% (48/119) vs. 11.2% (14/125); $p < 0.001$) cohorts.

Machine learning-based identification of biomarker patterns in adult vs. elderly patients

To assess the different patterns of host response to SARS-CoV-2 infection in adult and elderly patients, we profiled 19 protein biomarkers, viral RNA load and anti-SARS-CoV-2 S antibodies in plasma and applied a Machine Learning approach in the derivation cohort as described in the Methods section.

The SHAP summary plot for the derivation cohort is shown in Fig. 3, which ranked features according to their importance in profiling the different host response to SARS-CoV-2 infection between the groups of adult and elderly patients.

Detailed explanations of the meaning of SHAP values, their obtention, and a guide for SHAP plot interpretation are provided in Supplementary Text S2, Online Resource 3.

In our model, plasma levels of lipocalin-2 ranked first, since the mean of its absolute SHAP values is

Table 2 Clinical characteristics of Intensive Care Unit (ICU) hospitalized patients. Statistics: Continuous variables are represented as median (interquartile range) and categorical vari-ables as absolute count (n, (%)). *P*-values were assessed by using the Mann-Whitney test for continuous variables, and Chi-squared test for categorical variables*Baseline characteristics (Validation Cohort: ICU patients)*

Clinical characteristics and outcomes	All cohort	Adult patients (≤ 70 yrs)	Elderly patients (> 70 yrs)	<i>P</i> value
Demographics and underlying conditions				
No. [n (%)]	244	125 (51.2)	119 (48.8)	-
Age [median years (IQR)]	70.0 (21.0)	55.0 (18.0)	75.0 (5.00)	< 0.001
Male sex [n (%)]	159 (65.2)	80 (64.0)	79 (66.4)	0.696
Smoking [n (%)]	14 (5.70)	11 (8.80)	3 (2.50)	0.035
Comorbidities				
Hypertension [n (%)]	118 (48.4)	44 (35.2)	74 (62.2)	< 0.001
Diabetes [n (%)]	62 (25.4)	18 (14.4)	44 (37.0)	< 0.001
Obesity [n (%)]	86 (35.2)	54 (43.2)	32 (26.9)	0.008
Chronic cardiovascular disease [n (%)]	36 (14.8)	13 (10.4)	23 (19.3)	0.049
Chronic cerebrovascular disease [n (%)]	15 (6.15)	3 (2.40)	12 (10.1)	0.012
Chronic renal disease [n (%)]	15 (6.15)	2 (1.60)	13 (10.9)	0.002
Chronic Respiratory disease [n (%)]	20 (8.20)	11 (8.80)	9 (7.60)	0.725
Cancer [n (%)]	11 (4.50)	5 (4.00)	6 (5.00)	0.695
Admission status and scores				
Day since symptoms onset to hospital admission [median years (IQR)]	6.00 (5.00)	7.00 (4.00)	6.00 (4.00)	0.337
SOFA score [median (IQR)]	3.00 (1.00)	3.00 (0.00)	4.00 (1.00)	< 0.001
PaO ₂ /FIO ₂ (< 400) [n (%)] *209	208 (99.5)	106 (99.1)	102 (100)	0.328
MAP (< 70 mmHg) [n (%)] *209	10 (4.10)	4 (3.20)	6 (5.00)	0.468
Charlson score	3.00 (3.00)	1.00 (2.00)	4.00 (2.00)	< 0.001
Laboratory parameters at hospital admission				
Hyperglycemia (Glucose > 126 mg/dl) [n (%)] *233	173 (74.2)	83.3 (68.6)	90 (80.4)	0.040
Creatinine > 1.2 mg/dL [n (%)] *240	14 (5.8)	4 (3.20)	10 (8.70)	0.070
Bilirubin > 1 mg/dL [n (%)] *199	8 (3.30)	2 (1.90)	6 (6.30)	0.115
Hypertransaminasemia (ALT > 40 UI/L) [n (%)] *219	103 (47.0)	63 (56.3)	40 (37.4)	0.005
Hyperuremia (Urea > 50 mg/dL) [n (%)] *226	107 (47.3)	46 (38.3)	61 (57.5)	0.004
Hypernatremia (Na > 145 mmol/L) [n (%)] *241	7 (2.90)	2 (1.60)	5 (4.31)	0.211
LDH > 280 UI/L [n (%)] *212	181 (85.2)	87 (79.1)	94 (92.2)	0.007
Lactate > 2 mmol/L [n (%)] *156	38 (24.4)	16 (18.4)	22 (31.9)	0.051
Hemoglobin < 12 g/dL [n (%)] *237	56 (23.6)	21 (16.8)	35 (31.3)	0.009
Thrombocytopenia (Platelets < 150 cell × 103/μl) [n (%)] *240	13 (5.40)	4 (3.20)	9 (7.80)	0.114
C-reactive protein > 100 mg/L [n (%)] *231	112 (48.5)	50 (41.7)	62 (55.9)	0.031
Lymphopenia (< 1000 cells/mm ³) [n (%)] *235	182 (77.4)	85 (68.5)	97 (87.4)	0.001
Neutrophilia (> 7500 cells/mm ³) [n (%)] *231	136 (58.9)	66 (52.8)	70 (63.6)	0.161
Monocytopenia (< 200 cells/mm ³) [n (%)] *230	54 (23.5)	23 (19.0)	31 (28.4)	0.092
Treatments				
Remdesivir [n (%)]	55 (22.5)	30 (24.0)	25 (31.0)	0.576
Heparin [n (%)] *243	237 (97.5)	123 (98.4)	114 (96.6)	0.369
Corticoids [n (%)] *243	237 (97.5)	121 (96.8)	116 (98.3)	0.450
Tocilizumab [n (%)]	57 (23.4)	35 (28.0)	22 (18.5)	0.079
Antibiotics [n (%)]	225 (92.2)	111 (88.8)	114 (95.8)	0.041
Non invasive mechanical ventilation [n (%)] *243	70 (28.8)	29 (23.2)	41 (34.7)	0.047

Table 2 (continued)*Baseline characteristics (Validation Cohort: ICU patients)*

Clinical characteristics and outcomes	All cohort	Adult patients (≤ 70 yrs)	Elderly patients (> 70 yrs)	P value
Invasive mechanical ventilation [n (%)]	157 (64.3)	64 (51.2)	93 (78.2)	< 0.001
Complications				
ARDS [n (%)]	181 (74.2)	86 (68.8)	95 (79.8)	0.049
Acute cardiac failure [n (%)]	5 (2.00)	3 (2.40)	2 (1.70)	0.692
Acute renal failure [n (%)]	62 (25.4)	19 (15.2)	43 (36.1)	< 0.001
Acute arrhythmia [n (%)]				
Nosocomial infection [n (%)] *242	110 (45.5)	46 (37.1)	64 (54.2)	0.007
Length of hospital stay [median days (IQR)]	21.0 (19.0)	18.0 (15.0)	26.0 (24.0)	< 0.001
Outcomes				
Mortality [n (%)]	62 (24.4)	14 (11.20)	48 (40.3)	< 0.001

Data are presented as No. (%) or median [IQR]. *P* values were assessed by Mann-Whitney, χ^2 or Fisher's test when appropriate

SOFA Sequential Organ Failure Assessment, *MAP* Mean Arterial Pressure, *ARDS* Acute Respiratory Distress Syndrome

Asterisk ("*") indicates the actual number of samples available (n) for that specific parameter, which may be less than the total sample size of the study

the highest in the plot (Fig. 3). Here, the blue points in the lipocalin-2 row correspond to negative SHAP values, while the red points correspond to positive SHAP values, meaning that high values of the feature (elevated lipocalin-2 plasma levels) were associated with the main output of the model, the elderly host response to SARS-CoV-2 infection. Conversely, the blue points and negative SHAP values indicate that lower lipocalin-2 levels were not related to the model's main output, being then much more representative of an adult host response to SARS-CoV-2 infection (Fig. 3).

Levels of lipocalin-2, endothelin-1, D-dimer, TFN α , IFN- γ , anti-SARS-CoV-2 S IgG antibodies, and granzyme A had the greatest effects on the model. Among them, lipocalin-2, endothelin-1, D-dimer, and TFN α showed the same pattern in which elevated levels of these biomarkers were associated with an elderly host response (red points and positive SHAP values) (Fig. 3). In contrast, IFN- γ , anti-SARS-CoV-2 S IgG antibodies, and granzyme A showed a different pattern in which low levels of these biomarkers were related to the elderly host response in the model (blue points and positive SHAP values) (Fig. 3).

In addition, to a lesser extent but still with a highly positive association with the model's main output

(elderly host response), there were inflammation-related signatures represented by elevated plasma levels of biomarkers such as CCL2, IL-15, and pentanxin-3 (Fig. 3). Elevated plasma viral RNA loads were also positively related to the elderly host response (Fig. 3).

Among biomarkers associated to an elderly host response when decreased, besides IFN- γ , anti-SARS-CoV-2 S IgG antibodies, and granzyme A, were immunomodulators such as PD-L1 and IL-10, and type I interferon, IFN- α (Fig. 3).

To assess the validity of the model obtained, we applied the final trained model and its adjusted hyperparameters to the validation dataset and visualize how the model interprets it. As seen, the SHAP plot for the validation cohort exhibited a similar pattern of features' importance and contribution to that observed in the validation cohort (Fig. 4).

In addition, Area Under the Receiver Operating Characteristic (AUROC) curves used to evaluate model's performance were good and comparable: AUC for the subset test=0.759 (95% CI: 0.674–0.841) and AUC for validation cohort=0.710 (95% CI: 0.644–0.774) (Fig. 5).

These results support the generalizability and biological relevance of the model's learned relationships.

Table 3 Biomarkers' profile of non-ICU hospitalized patients. Statistics: Continuous variables are represented as median (interquartile range) and categorical variables as absolute count (n (%)). *P*-values were assessed by using the Mann–Whitney test for continuous variables, and Chi-squared test for

categorical variables. Statistically significant differences are shown in bold. Asterisk ("*") indicates the actual number of samples available (n) for that specific parameter, which may be less than the total sample size of the study

Biomarkers' profile (Derivation Cohort: non-ICU patients)

Clinical characteristics and outcomes	All cohort	Adult patients (≤ 70 yrs)	Elderly patients (> 70 yrs)	<i>P</i> value
Demographics and underlying conditions				
No. [n (%)]	450	216 (48.0)	234 (52.0)	-
Age [median years (IQR)]	72.0 (25.0)	59.00 (23.0)	83.00 (10.0)	0.003
Male sex [n (%)]	244 (54.2)	133 (61.6)	111 (47.4)	0.003
Smoking [n (%)]	16 (3.60)	10 (4.60)	6 (2.60)	0.237
Virological and serological status				
RNAemia (YES), [n (%)]	163 (36.3)	65 (30.1)	98 (42.1)	0.008
Viral RNA load in plasma (copies N1/mL) [median (IQR)]	0.00 (280)	0.00 (146)	0.00 (514)	0.001
anti-SARS-CoV-2 S1 antibodies (AU/mL) [median (IQR)]	33.8 (313)	98.9 (467)	14.7 (207)	<0.001
Protein biomarkers' profile				
IL-2 (pg/mL) [median (IQR)]	0.260 (0.328)	0.209 (0.298)	0.299 (0.375)	0.003
GMCSF (pg/mL) [median (IQR)]	0.00 (0.209)	0.00 (0.213)	0.00 (0.208)	0.736
CCL2 (pg/mL) [median (IQR)]	281 (277)	248 (217)	324 (297)	<0.001
Granzyme A (pg/mL) [median (IQR)]	122 (77.9)	132 (90.3)	112 (63.9)	0.001
IFN-γ (pg/mL) [median (IQR)]	3.40 (10.9)	3.99 (11.8)	2.89 (10.2)	0.027
IL-4 (pg/mL) [median (IQR)]	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.269
TNFα (pg/mL) [median (IQR)]	13.4 (7.13)	12.7 (5.08)	14.8 (8.50)	<0.001
B7H1 (PD-L1) (pg/mL) [median (IQR)]	213 (134)	200 (122)	224 (141)	0.032
Endothelin-1 (pg/mL) [median (IQR)]	2.86 (2.04)	2.44 (1.98)	3.27 (2.13)	<0.001
IFN-α (pg/mL) [median (IQR)]	6.20 (25.2)	6.26 (25.9)	6.20 (23.8)	0.917
IL-10 (pg/mL) [median (IQR)]	8.84 (10.5)	8.61 (9.91)	9.73 (11.4)	0.225
IL-7 (pg/mL) [median (IQR)]	3.38 (4.84)	4.06 (5.59)	2.93 (4.45)	0.056
IL-15 (pg/mL) [median (IQR)]	4.04 (3.15)	3.60 (2.99)	4.44 (3.00)	<0.001
CXCL10 (pg/mL) [median (IQR)]	1237 (1083)	1140 (1030)	1408 (1266)	0.016
IL-6 (pg/mL) [median (IQR)]	17.3 (39.2)	14.3 (33.1)	22.0 (46.1)	0.007
D-dimer (pg/mL) [median (IQR)] *448	1,099,467 (1,256,139)	863,817 (820,639)	1,427,894 (1,858,032)	<0.001
RANTES/CCL5 (pg/mL) [median (IQR)] *448	14,471 (18,404)	16,094 (19,562)	12,964 (17,666)	0.026
Pentraxin-3 (pg/mL) [median (IQR)] *448	10,541 (19,526)	8443 (16,653)	11,692 (25,980)	0.001
Lipocalin-2 (pg/mL) [median (IQR)]	94,505 (58,198)	79,740 (38,037)	113,130 (79,532)	<0.001

Data are presented as No. (%) or median [IQR]. *P* values were assessed by Mann-Whitney, χ^2 or Fisher's test when appropriate

Influence of confounding factors on the ML model: multivariable analysis to address the impact of sex on the most important biomarkers resulting from the ML analysis

To acknowledge the fact that, not only age, but also sex influences severe outcomes in respiratory

infection diseases such as SARS-CoV-2, additional logistic regression analyses were performed. Age distribution was uniform across sex groups in both cohorts (Supplementary Figure S2, Table S1 and Table S2, Online Resource 4).

Multivariable analyses using sex as an adjusting variable also identified lipocalin-2, endothelin-1,

Table 4 Biomarkers' profile of Intensive Care Unit (ICU) hospitalized patients. Statistics: Continuous variables are represented as median (interquartile range) and categorical variables as absolute count (n, (%)). *P*-values were assessed by using the

Mann-Whitney test for continuous variables, and Chi-squared test for categorical variables. Asterisk ("*") indicates the actual number of samples available (n) for that specific parameter, which may be less than the total sample size of the study

Biomarkers' profile (Validation Cohort: ICU patients)

Clinical characteristics and outcomes	All cohort	Adult patients (≤ 70 yrs)	Elderly patients (> 70 yrs)	<i>P</i> value
Demographics and underlying conditions				
No. [n (%)]	244	125 (51.2)	119 (48.8)	-
Age [median years (IQR)]	70.0 (21.0)	55.0 (18.0)	75.0 (5.00)	< 0.001
Male sex [n (%)]	159 (65.2)	80 (64.0)	79 (66.4)	0.696
Smoking [n (%)]	14 (5.70)	11 (8.80)	3 (2.50)	0.035
Virological and serological status				
RNAemia (YES), [n (%)]	170 (69.7)	78 (62.4)	92 (77.3)	0.011
Viral RNA load in plasma (copies N1/mL) [median (IQR)]	628 (2910)	314 (1230)	1360 (6848)	< 0.001
Anti-SARS-CoV-2 S1 antibodies (AU/mL) [median (IQR)]	198 (829)	183 (779)	212 (902)	0.892
Protein biomarkers' profile				
IL-2 (pg/mL) [median (IQR)] *239	0.201 (0.497)	0.123 (0.339)	0.294 (0.589)	0.002
GMCSF (pg/mL) [median (IQR)] *240	1.07 (1.01)	1.02 (0.859)	1.085 (1.09)	0.386
CCL2 (pg/mL) [median (IQR)] *239	361 (412)	307 (378)	426 (457)	0.001
Granzyme A (pg/mL) [median (IQR)] *236	71.9 (40.4)	75.3 (39.3)	66.5 (44.9)	0.017
IFN-γ (pg/mL) [median (IQR)] *239	1.77 (4.04)	1.66 (4.48)	1.87 (3.58)	0.696
IL-4 (pg/mL) [median (IQR)] *239	0.141 (0.237)	0.141 (0.235)	0.140 (0.238)	0.825
TNFα (pg/mL) [median (IQR)] *239	10.9 (5.86)	10.2 (5.93)	12.0 (6.17)	0.002
B7-H1 (PD-L1) (pg/mL) [median (IQR)] *240	199 (101)	196 (113)	206 (88.3)	0.197
Endothelin-1 (pg/mL) [median (IQR)] *236	2.19 (1.76)	2.01 (1.39)	2.51 (2.11)	0.001
IFN-α (pg/mL) [median (IQR)] *236	1.17 (3.41)	1.39 (5.20)	0.971 (2.16)	0.061
IL-10 (pg/mL) [median (IQR)] *240	11.3 (14.3)	9.78 (13.9)	13.1 (13.9)	0.030
IL-7 (pg/mL) [median (IQR)] *240	6.70 (6.57)	6.65 (6.48)	6.71 (6.31)	0.797
IL-15 (pg/mL) [median (IQR)] *239	5.83 (3.09)	5.54 (3.17)	6.38 (3.73)	0.001
CXCL10 (pg/mL) [median (IQR)] *239	1473 (1362)	1347 (1439)	1498 (1334)	0.081
IL-6 (pg/mL) [median (IQR)] *240	39.0 (139)	27.0 (119)	53.4 (160)	0.006
D-Dimer (pg/mL) [median (IQR)] *240	1,809,251 (3,025,492)	1,343,230 (2,211,967)	2,282,017 (3,232,829)	< 0.001
RANTES/CCL5 (pg/mL) [median (IQR)] *237	23,068 (29,280)	24,020 (30,843)	21,844 (27,285)	0.035
Pentraxin-3 (pg/mL) [median (IQR)] *238	22,612 (35,434)	19,085 (27,441)	31,436 (49,723)	0.029
Lipocalin-2 (pg/mL) [median (IQR)] *240	94,604 (53,830)	86,030.5 (47,543.5)	101,401 (52,840)	< 0.001

Data are presented as No. (%) or median [IQR]. *P* values were assessed by Mann-Whitney, χ^2 or Fisher's test when appropriate

D-dimer, TFNα, anti-SARS-CoV-2 S IgG antibodies, and granzyme A, among others, as the biomarkers with the greatest impact on the elderly host response, in agreement with the profile made by the ML model (Supplementary Table S3 and Table S4, Online Resource 4).

Protein–protein interaction-guided functional enrichment analysis

Network plot representing protein–protein interactions and colored according to fold-change by age-group showed a similar weight-pattern as provided

Fig. 2 Kaplan–Meier survival curves with 95% CI region for each group of patients according to age: adult group (orange) and elderly group (red). **a)** Derivation (non-ICU) cohort and **b)** validation (ICU) cohort

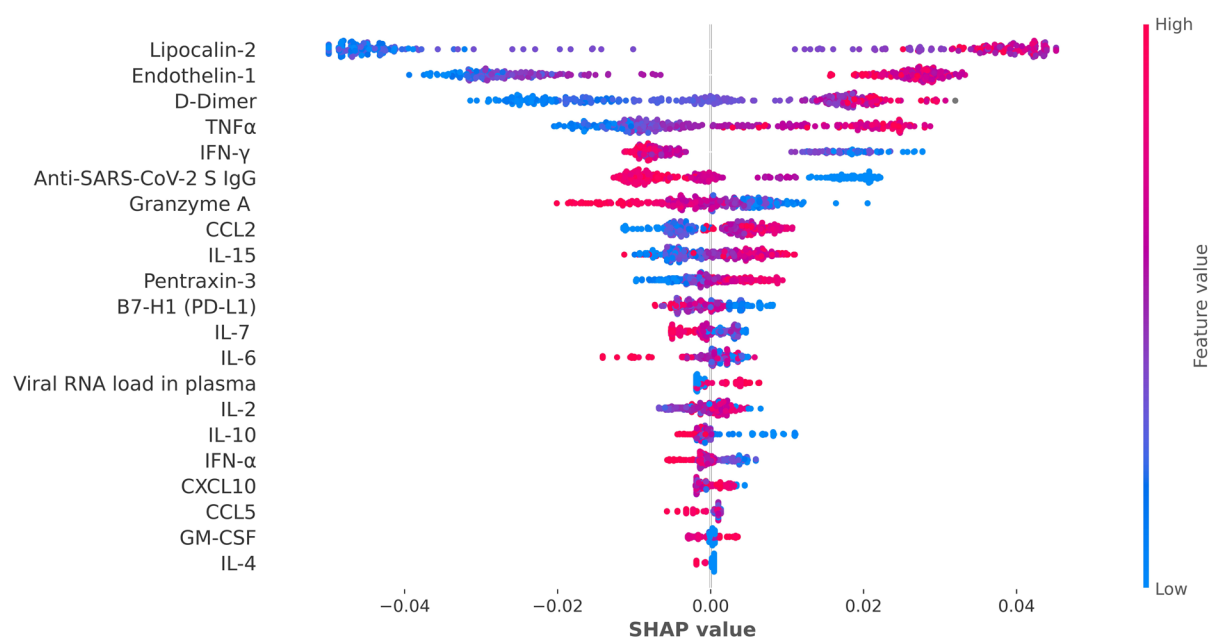
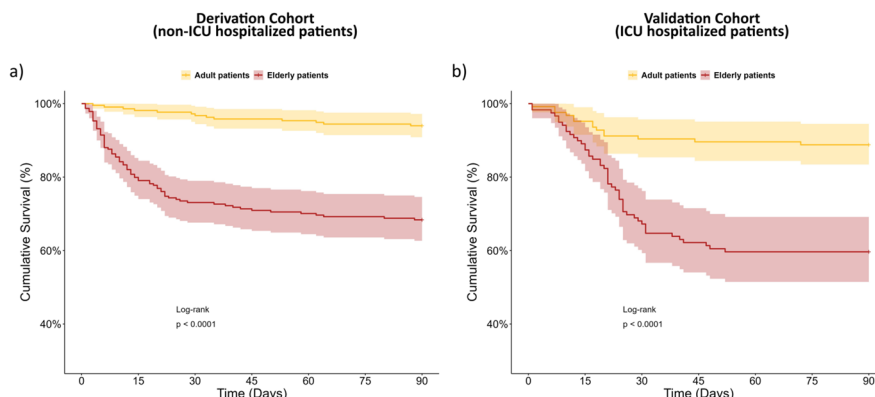


Fig. 3 SHAP plot obtained from the machine model trained with the XGBoost algorithm in the derivation cohort, illustrating the age-dependent features of host response to SARS-CoV-2 infection in non-ICU hospitalized patients. [Abbreviations: SHAP, SHapley Additive exPlanations; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IgG,

Immunoglobulin G; IL, interleukin; IFN, interferon; TNF- α , Tumor Necrosis Factor α ; PD-L1, Programmed Death-Ligand 1; CCL5, chemokine (C–C motif) ligand 5; CCL2, chemokine (C–C motif) ligand 2; CXCL10, C–X–C motif chemokine ligand 10; GM-CSF, Granulocyte–Macrophage Colony-Stimulating Factor]

by the SHAP plot (Supplementary Figure S3, Online Resource 5).

Functional enrichment analyses were performed on those biomarkers with the greatest importance in the SHAP plot. Proteins were grouped according to the sign of their SHAP values (positive or negative) associated with the elderly host response (Supplementary Figures S4 and S5, Online Resource 5).

For those proteins with high importance and positive SHAP values (CCL2, EDN1, TNF, IL15, D-dimer, PTX3, LCN2), the most significantly enriched pathways obtained from “Gene Ontology (GO) Biological process” database included defense response (FDR=0.00013), positive regulation of phagocytosis (FDR=0.00013), and inflammatory response (FDR=0.0012) (Supplementary Figure S6 and Table S5, Online Resource 5).

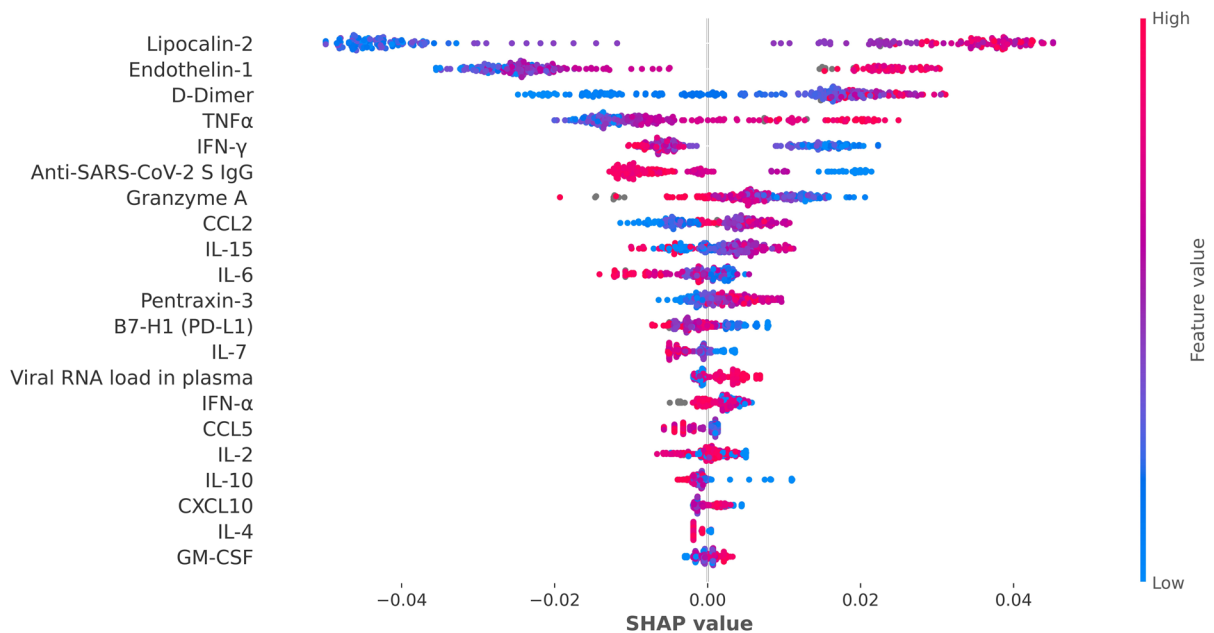


Fig. 4 SHAP plot obtained from the machine learning model developed and applied to the validation cohort, illustrating the age-dependent features of host response to SARS-CoV-2 infection in ICU hospitalized patients. [Abbreviations: SHAP, SHapley Additive exPlanations; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IgG, Immunoglobulin G;

IL, interleukin; IFN, interferon; TNF- α , Tumor Necrosis Factor α ; PD-L1, Programmed Death-Ligand 1; CCL5, chemokine (C-C motif) ligand 5; CCL2, chemokine (C-C motif) ligand 2; CXCL10, C-X-C motif chemokine ligand 10; GM-CSF, Granulocyte-Macrophage Colony-Stimulating Factor]

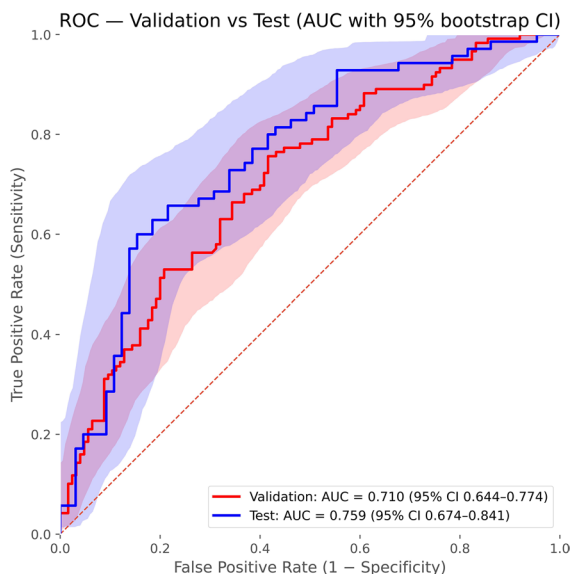


Fig. 5 Area Under the Receiver Operating Characteristic (AUROC) curves obtained to evaluate the final machine learning model performance on each testing set. Internal validation was done with the test subset (in blue) from the derivation (non-ICU) cohort, and the external validation was carried out with the validation (ICU) cohort (in red)

Functional enrichment analysis for this group of proteins by using “DISEASES” database indicated an overrepresentation of these proteins in conditions such as respiratory failure (FDR=0.00014), artery disease (FDR=0.00016), and ischemia (FDR=0.00023) (Supplementary Figure S7 and Table S6, Online Resource 5).

For those proteins with high importance and negative SHAP values (Granzyme A, IFN- γ , IL-7, and PD-L1), the terms most enriched by these proteins were found in the “TISSUES” database, which reports on the expression of these proteins in cytotoxic T-lymphocytes (FDR=0.0000249) (Supplementary Table S9, Online Resource 5).

Impact of host and viral factors on the combined severe outcome in each age group

The two groups of patients obtained by age stratification in the derivation cohort were again divided into two different groups according to their status in the combined outcome: ICU admission and/or 90-day mortality.

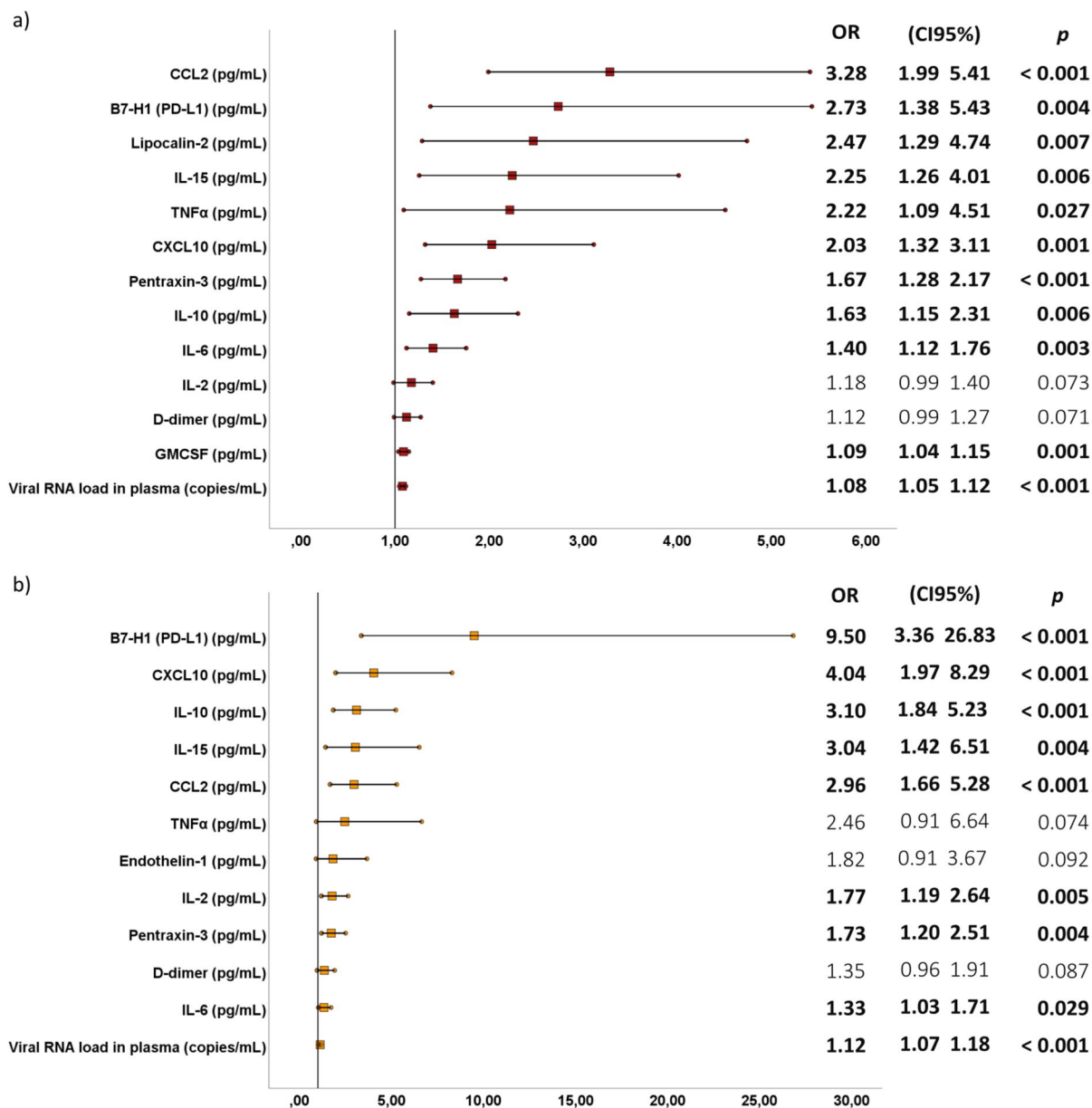


Fig. 6 Forest plot of multivariable logistic regression analysis carried out in the derivation cohort for factors associated with the combined outcome ‘ICU admission and/or 90-day mortality’ in each group of patients: **a)** elderly group and **b)** adult group. [Abbreviations: IL, interleukin; TNF- α , Tumor Necro-

sis Factor α ; PD-L1, Programmed Death-Ligand 1; CCL2, chemokine (C-C motif) ligand 2; CXCL10, C-X-C motif chemokine ligand 10; GM-CSF, Granulocyte-Macrophage Colony-Stimulating Factor]

In the elderly group, patients who were admitted to the ICU and/or died within 90 days after admission were admitted in wards earlier after symptoms onset (median [IQR]: 4.00 [5.75] vs. 5.00 [7.00] days; $p=0.048$). Elderly patients

with this poorer outcome also had higher SARS-CoV-2 plasma viral loads (median [IQR]: 394 [1945] vs. 0.00 [144] copies N1/mL plasma; $p<0.001$) (Supplementary Tables S10 and S11, Online Resource 6).

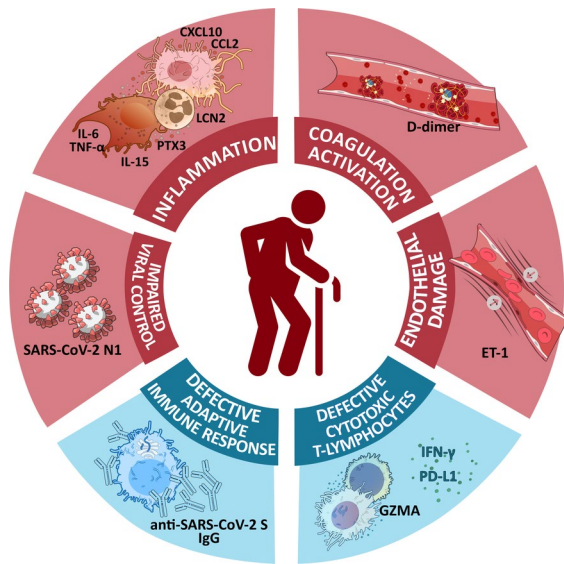


Fig. 7 Summary of key features representing elderly host response to SARS-CoV-2 infection

Patients in the adult group that were admitted to the ICU and/or died within 90 days following hospital admission had higher prevalence of hypertension (53.7% (22/41) vs. 32.6% (57/175); $p=0.012$) and obesity (43.9% (18/41) vs. 16.6% (29/175); $p<0.001$), and were also admitted earlier after symptoms onset (median [IQR]: 5.00 [3.00] vs. 7.00 [5.00] days; $p=0.039$). Besides, those patients had higher blood SARS-CoV-2 viral loads (median [IQR]: 421 [2485] vs. 0.00 [0.00] copies N1/mL plasma; $p<0.001$) (Supplementary Tables S12 and S13, Online Resource 6).

Multivariable analysis in the elderly group showed that higher levels of CCL2, PD-L1, lipocalin-2, IL-15, TNF- α , CXCL10, pentraxin-3, IL-10, IL-6, GM-CSF and SARS-CoV-2 viral load in plasma at hospitalization were associated with higher odds of UCI admission and/or 90-day mortality (Fig. 6 and Supplementary Table S14, Online Resource 6), whereas in the adult group the multivariable analysis showed that higher levels of PD-L1, CXCL10, IL-10, IL-15, CCL2, IL-2, pentraxin-3, IL-6 and SARS-CoV-2 viral load in plasma were associated with higher odds of UCI admission and/or 90-day mortality (Fig. 6 and Supplementary Table S15, Online Resource 6).

Results of the multivariable analysis performed in the validation (ICU) cohort for 90-day mortality are shown in Supplementary Figure S10 and Supp. Table S20 and S21, Online Resource 7.

Discussion

While SARS-CoV-2 poses a serious risk at any age, it is especially life-threatening for elderly individuals, defined in this study as older than 70 years old. In the Kaplan Meier analysis, we indeed observed a dramatic difference between the 90-day mortality rates in the elderly and adult groups. Our machine learning-based analysis suggests that this difference can be mainly explained by the presence of imbalanced antiviral responses accompanied by a predominant thrombo-inflammatory profile along with a significant endothelial dysfunction in the elderly with COVID-19, which translates into a poorer control of the virus, more severe disease and worse prognosis (Fig. 7).

The results obtained from the evaluation of the model's performance supported its biological relevance, generalizability and robustness.

In our model, the predominant thrombo-inflammatory response profiled in older individuals was fundamentally characterized by higher lipocalin-2 (acute-phase protein present in neutrophil extracellular traps (NETs) [28]) and D-dimer plasma levels. Several studies have shown that plasma levels of both biomarkers correlate positively with age [28–32], and COVID-19 severity [33–38]. These procoagulant and proinflammatory states observed in advanced age, are intimately linked to endothelial injury and dysfunction [39–41]. The SHAP values of endothelin-1 (vasoconstrictor) reported in our model are in line with the results observed in many studies where endothelin-1 plasma levels were also associated with advanced age [42–44] and worse outcomes in SARS-CoV-2 infected patients [40, 45, 46].

Chronic procoagulant and proinflammatory states observed in older individuals are closely related to persistent and dysregulated activation of nuclear factor kappa B (NF- κ B), a transcription factor that plays an important role in the induction of genes involved in immune and inflammatory responses [47] (Supplementary Fig. S11, Online Resource 8). Furthermore, NF- κ B is known as a central player in the pathogenesis of COVID-19, since SARS-CoV-2 bonds to Angiotensin II (Ang II)-converting enzyme 2 receptor (ACE2) to infect cells, free Ang II plasma levels increase, and, via Ang II type 1 receptor (AT1R), boosts the activation of the NF- κ B pathway [48, 49] (Supplementary Fig. S12, Online Resource 8).

TNF- α , a proinflammatory cytokine, is primarily produced by immune cells like macrophages and monocytes and its transcription is triggered mainly by microbial components and other cytokines. TNF- α is both an activator and a transcriptional target of NF- κ B, fueling the inflammatory response [50–52] (Supplementary Fig. S13 and Fig. S14, Online Resource 8).

The transcription of other proinflammatory proteins related to the elderly host response to SARS-CoV-2 in this study, such as lipocalin-2, endothelin-1, CCL2, IL15, and pentraxin-3, are also regulated via NF- κ B [50, 53, 54] (Supplementary Fig. S12 and Fig. S13, Online Resource 8). Ultimately, these signaling pathways drive to an amplified inflammation (hypercytokinemia), tissue damage, and coagulopathy, shaping a distinctive profile of the most severe cases of COVID-19, which are more common in elderly subjects.

The inadequate antiviral response we observed in older patients in our model was mainly composed of a weak humoral adaptive immune response and a defective induction of cytotoxic/Th1 immunity. Older individuals struggled to mount an efficient adaptive immune response against SARS-CoV-2, resulting in lower plasma antibody levels in our model. Also, the decrease of IL-7 plasma levels because of age-related thymic involution [55–57] could have affected T-cell development in these patients [58] and, thus, blunted the coordination of new immune responses necessary to control viruses such as SARS-CoV-2.

Antiviral cytotoxic responses, mediated principally by key effector molecules such as granzyme A produced by NK cells and cytotoxic T lymphocytes (CTL), are usually altered in elderly individuals by the ongoing immunosenescence [57, 59, 60]. Aging-associated cytotoxic lymphocyte depletion may contribute to the higher rates of lymphopenia found in the elderly group, favoring the lower plasma levels of granzyme A we observed in these patients and the development of more severe forms of COVID-19, as reported in other studies [61–63]. CTL exhaustion eventually could also lead to a situation where INF- γ production is compromised, as we saw for elderly in the SHAP plot [64, 65]. Alternatively, lymphopenia could be explained by migration of the lymphocytes to a more inflamed lung in the elderly.

In the multivariable logistic regression analysis performed on the derivation cohort, in both groups, the common biomarkers with the highest impact on

the combined outcome included the immunomodulatory proteins PD-L1 and IL-10, chemoattractant proteins such as CCL2 and CXCL10, the pro-inflammatory cytokines IL-15 and IL-6 and, also, SARS-CoV-2 RNA viral loads.

While SHAP plot showed that immunosuppressive mediators like PD-L1 and, to a less extent, IL-10, appeared more prominently in the adult group host response (red points and negative SHAP values), these proteins remain key drivers of mortality and ICU admission in elderly patients in both derivation and validation cohorts. Both proteins promote and maintain T cell exhaustion and modulate inflammation [66, 67]. Interleukin-10 is a pleiotropic cytokine produced by diverse immune cells during viral infection, its secretion generally occurs after the initial proinflammatory host response, and its dysregulation (excess or early secretion) promotes an early resolution of the inflammatory phase favoring viral persistence [66, 68–70]. In COVID-19, increased IL-10 and PD-L1 plasma levels correlate with worse outcomes [71, 72]. PD-L1 blockade could restore T cell exhaustion and is a promising therapy for treating COVID-19 severe cases, but its effectiveness depends on the same critical parameters observed for IL-10 secretion, such as timing or dosage [73].

We have several limitations in our study, such as sample size and the lack of a quantification of different immune cell populations and T cell subsets. Furthermore, because of the sample size and, since age distribution was uniform across sex groups in both cohorts, data from men and women were pooled together and we did not profile each host response based on sex using the machine learning approach. Still, sex influence was considered in the regression model, supporting the results provided by the ML model. The potential role of sex must be addressed in future studies.

Nonetheless, to our current knowledge, we present the first XGBoost-SHAP model developed involving virological, serological, and protein biomarkers data to define an age-dependent host response profile to SARS-CoV-2 infection in naïve hospitalized patients. The model ranked lipocalin-2, endothelin-1, D-dimer, TNF α , IFN- γ , IgG antibodies against SARS-CoV-2 S proteins, and granzyme A as the top factors deviated in the elderly host response, which support that these components should be playing central roles in the pathogenesis of COVID-19 in this population.

In conclusion, our study identifies the most relevant host and viral factors altered in the elderly patients with COVID-19 who need hospitalization. Elderly individuals, one of the most vulnerable populations to respiratory viral infections, show deranged responses to SARS-CoV-2, characterized by a defective antiviral immunity along with an exacerbated thrombo-inflammatory profile and endothelial dysfunction. This deranged response may contribute to explaining the poorer outcomes observed in these patients. Since we have verified the robustness, generalizability and biological relevance of our model, it is worthwhile developing further research to elucidate whether this host-response profile is also observed in elderly individuals suffering infections by other respiratory viruses causing yearly epidemics or with pandemic potential.

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Data availability The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declarations

Competing interests The authors declare no competing interests.

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