Heliyon 10 (2024) e37115

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

# Differential analysis of lipoprotein and glycoprotein profiles in bacterial infections and COVID-19 using proton nuclear magnetic resonance and machine learning

Simona Iftimie [a,b], Núria Amigó [b,c,d], Neus Martínez-Micaelo [b,c,d], Ana F. López-Azcona [a,b], Cristian Martínez-Navidad [e], Helena Castañé [e], Andrea Jiménez-Franco [e], Josep Ribalta [b,c,f], Sandra Parra [a,b], Antoni Castro [a,b,**], Jordi Camps [b,e,*], Jorge Joven [b,e]

[a] Department of Internal Medicine, Hospital Universitari de Sant Joan, Institut d'Investigació Sanitària Pere Virgili, Universitat Rovira i Virgili, Reus, Spain
[b] Department of Medicine and Surgery, Universitat Rovira i Virgili, Reus, Spain
[c] CIBER of Diabetes and Associated Metabolic Disease (CIBERDEM), Instituto de Salud Carlos III, Madrid, Spain
[d] Biosfer Teslab, Reus, Spain
[e] Unitat de Recerca Biomèdica, Hospital Universitari de Sant Joan, Institut d'Investigació Sanitària Pere Virgili, Universitat Rovira i Virgili, Reus, Spain
[f] Unitat de Recerca de Lípids i Arteriosclerosi, Hospital Universitari de Sant Joan, Institut d'Investigació Sanitària Pere Virgili, Universitat Rovira i Virgili, Reus, Spain

ARTICLE INFO

*Keywords:*
Bacterial infections
COVID-19
Infectious diseases
Lipoproteins
Metabolism
Proton nuclear magnetic resonance

ABSTRACT

*Background:* We scrutinized variations in the proton nuclear magnetic resonance ($^1$H NMR) lipoprotein and glycoprotein profiles among hospitalized individuals with infectious diseases.
*Methods:* We obtained sera from 124 patients with COVID-19, 50 patients with catheter-related bacterial infections, and 50 healthy volunteers. Results were interpreted using machine learning.
*Results:* COVID-19 patients had bigger and more abundant VLDL particles than the control group and higher VLDL-cholesterol and VLDL-triglyceride concentrations. Patients with bacterial infections showed similar trends, but differences often did not reach statistical significance. Both types of patients showed lower LDL-cholesterol concentrations than the controls. LDL were larger, and the number of particles was lower than that of the healthy individuals. HDL particles had decreased cholesterol and increased triglycerides. Small particles were reduced. Glycoproteins were increased in both groups of patients. All these alterations were more pronounced in COVID-19 patients than those with bacterial infections. The diagnostic accuracy of these profiles exceeded 90 % when distinguishing between healthy individuals and patients, and 85 % when differentiating between the two patient groups.
*Conclusion:* Our findings highlight the potential of $^1$H NMR analysis for lipoproteins and glycoproteins as infection biomarkers. Additionally, they reveal differences between viral and bacterial infections, shedding light on an area with promising clinical significance.

* Corresponding author. Hospital Universitari de Sant Joan, C. Sant Joan s/n, 43201 Reus, Spain.
** Corresponding author. Hospital Universitari de Sant Joan, C. Sant Joan s/n, 43201 Reus, Spain.
*E-mail addresses:* antoni.castro@urv.cat (A. Castro), jorge.camps@salutsantjoan.cat (J. Camps).

https://doi.org/10.1016/j.heliyon.2024.e37115
Received 7 February 2024; Received in revised form 27 August 2024; Accepted 27 August 2024
Available online 28 August 2024

## 1. Introduction

Infectious diseases are commonly related to increased oxidative stress and an inflammatory reaction [1]. Infection and inflammation set off a sequence of responses within the host, recognized as the acute-phase response. This reaction is linked to notable adjustments in the distribution of circulating lipoproteins [2,3]. Infectious diseases are associated with reduced levels of high-density lipoprotein (HDL) and elevated levels of very low-density lipoprotein (VLDL), accompanied by increased concentrations of plasma triglycerides. Moreover, the constitution of lipoproteins also undergoes modifications. For instance, HDL particles experience a reduction in the levels of proteins engaged in reverse cholesterol transport, as well as those that hinder the oxidation of plasma lipids. Additionally, there is a decline in cholesterol esters and a rise in free cholesterol, triglycerides, and free fatty acids [4]. These changes cause HDL particles to lose their anti-atherogenic and anti-inflammatory attributes, potentially adopting a pro-atherogenic and pro-inflammatory nature [5].

The advent of the COVID-19 pandemic has brought about a global tragedy but, at the same time, provided an opportunity to explore a novel disease utilizing advanced technological tools. COVID-19, for instance, serves as an exemplary model for unraveling the intricate connections between infectious mechanisms, metabolism, and inflammation. This disease is characterized by a strong inflammatory reaction to infection, often called the "cytokine storm," which is the primary driver of morbidity and mortality [6]. COVID-19 patients burdened with pre-existing cardiovascular conditions are more susceptible to developing severe illness and facing heightened mortality risks [7,8]. Conversely, COVID-19 can also trigger myocardial injury in patients, and the cardiovascular alterations found in individuals grappling with the persistent effects of COVID-19, termed "long-COVID," might endure for prolonged periods in survivors of the acute viral infection [9].

Recent technological advancements have facilitated swift, dependable, and detailed assessments of circulating lipoproteins. Using proton nuclear magnetic resonance ($^1$H NMR) offers the unique benefit of concurrently assessing lipoprotein particles' quantity, dimensions, and constitution, thereby affording a more comprehensive insight into alterations linked to metabolic dysregulations [10]. Furthermore, $^1$H NMR spectroscopy enables the quantification of distinct categories of glycoproteins, a clinically pertinent aspect in patient evaluations, as heightened protein glycation indicates inflammatory processes [11,12].

This study aimed to investigate alterations in the profiles of lipoproteins and glycoproteins using $^1$H NMR among hospitalized patients with infectious diseases. Specifically, we selected a cohort of patients with COVID-19 to represent viral infections with pronounced metabolic effects, along with patients with bacterial infections as a result of being urinary catheter carriers. Subsequently, these profiles were compared between the two groups and against a control group composed of healthy volunteers. This data could offer valuable insights into the protracted complications of infectious diseases and potentially aid in evaluating patients during future outbreaks or novel pandemics.

## 2. Materials and methods

### 2.1. Study design and participants

We conducted a post hoc retrospective cohort study in 124 patients admitted for COVID-19 during the period spanning from March to October 2020 at the Hospital Universitari Sant Joan de Reus. The inclusion criteria encompassed individuals aged $\geq$18 years and with a confirmed positive result for COVID-19 via PCR testing obtained within 24 h before collecting specimens for the study. Those with a life expectancy of less than or equal to 24 h, compromised liver function, or in a state of pregnancy were excluded from the study. Additionally, we analyzed specimens from 50 patients with bacterial infections (BI) but negative for COVID-19, who had been hospitalized earlier than in the pandemic era, and that were initially gathered for a prospective investigation focused on patients with bacterial infections linked to urinary catheters [13]. For control purposes, we assessed samples from 50 healthy volunteers without clinical or biochemical indications of conditions such as diabetes, cancer, kidney dysfunction, liver disorders, infectious diseases, or neurological impairment [14]. Serum samples were procured from all participants and preserved in our Biobank at −80 °C until the analysis. Alongside this, we documented clinical and demographic details. We computed the McCabe score, indicating disease severity [15], and the Charlson index to stratify their comorbidities [16].

To investigate whether any of the analyzed parameters could serve as a prognostic marker for the severity, we divided the patients into two groups, moderate and severe. We considered as "severe" those patients who met at least one of the following criteria: to have received invasive mechanical ventilation, to be admitted to the Intensive Care Unit, or to die up to 30 days after admission. According to these criteria, 3 of 50 (6 %) BI and 55 of 124 (44.4 %) COVID-19 patients were classified as "severe" Thirty-one COVID-19 patients died (Supplementary Table 1). No deaths were recorded in BI patients.

This study was approved by the *Comitè d'Etica i Investigació en Medicaments* (Institutional Review Committee) of the *Institut d'Investigació Sanitària Pere Virgili*, with the resolution number CEIM 040/2018, which was subsequently modified on April 16, 2020. All participants were informed verbally and in writing and provided written informed consent. The authors confirm that all methods were performed in accordance with the relevant guidelines and regulations and were performed in line with the Declaration of Helsinki.

### 2.2. Lipoprotein and glycoprotein analyses by $^1$H NMR spectroscopy

We employed serum samples from hospitalized patients within a standardized clinical practice framework. Electronic requests

facilitated analytical data acquisition, with each sample allocated a unique code for our study. Concurrently, upon drawing blood on admission, the nursing team executed extraction procedures, ensuring that the barcode-equipped tubes designated for our research were utilized. Blood extraction followed standardized protocols, utilizing gel-treated tubes to facilitate clot separation. After immediate homogenization, serum collection ensued. The serum acquisition protocol conforms to established norms employed by clinical biochemistry laboratories. Precisely, blood tubes were positioned upright at room temperature (18–22 °C) for a minimum of 30 min to induce clot formation. Subsequent centrifugation was conducted at 1600×*g* for 15 min at room temperature. Post-centrifugation, the supernatant serum was meticulously transferred into 0.5 mL Eppendorf tubes (Hamburg, Germany) and preserved at −80 °C. Frozen serum samples were shipped on dry ice to Biosfer Teslab (Reus, Spain) for the $^{1}$H NMR analysis. Before the analysis, 200 μL of serum was mixed with 50 μL deuterated water (D2O) and 300 μL of 50 mM phosphate buffer solution at pH 7.4 into 5-mm NMR glass tubes. The $^{1}$H NMR spectra were recorded at 310 K on an Avance III™-600 Bruker spectrometer (Bruker BioSciences Española S.A., Madrid, Spain) operating at a proton frequency of 600.20 MHz (14.1 T). We used the double stimulated echo pulse program with bipolar gradient pulses and a longitudinal eddy current delay (LED: ledbpgp2s1d: LED-bipolar gradients pulse sequence), and 1D Nuclear Overhauser Effect SpectroscopY (NOESY). The sequences run at 310 K in quantitative conditions. Refer to Supplementary Fig. 1 for the $^{1}$H NMR spectra of five different aliquots of a control serum sample, analyzed over five weeks using the LED pulse program. The relaxation delay was 2 s, and the finite impulse decays were collected into 64K complex data points per scan on each sample.

The advanced lipoprotein profile was analyzed using the NMR-based Liposcale® test. This analysis included measuring the lipid concentrations (triglycerides and cholesterol), size, and particle number of the four main classes of lipoproteins (VLDL, intermediate-density lipoprotein or IDL, low-density lipoprotein or LDL, and HDL), as well as the particle number of nine subclasses (large, medium, and small VLDL, LDL, and HDL) [10]. Each subclass particle concentration was calculated by dividing the lipid volume by the particle volume of a given class. Lipid volumes were determined using common conversion factors to convert concentration units into volume units [17]. Finally, weighted average VLDL, LDL, and HDL particle sizes were calculated from various subclass concentrations by summing the known diameter of each subclass multiplied by its relative percentage of subclass particle number.

The glycoprotein profile was determined by analyzing the region of the $^{1}$H NMR spectrum where the glycoproteins resonate (2.15–1.90 ppm) using several analytical functions according to a previously published procedure [11,18]. The GlycA and Glyc B areas signals originate from N-Acetylneuraminic acid and N-Acetylglucosamine moieties from N-glycans, respectively. The GlycF area measured the concentrations of the acetyl groups of N-acetylglucosamine, N-acetylgalactosamine and N-acetylneuraminic acid not bound to proteins (unbound, free fraction). Height-to-width (H/W) ratios of GlycA and GlycB (a parameter associated with the aggregation state of the sugar–protein bonds) were also reported. Height was measured as the difference from the baseline to the maximum of the corresponding $^{1}$H NMR peaks. Width values correspond to the peak width at half height [12]. The Supplementary Analytical Methods 1 provide a detailed description of quality assurance.

### 2.3. Statistical analysis

Quantitative results were shown as medians and 25–75 interquartile ranges, and qualitative or dichotomous variables were presented as percentages. To compare proportions and study relationships between qualitative variables, we used the $\chi$2-square test and Fisher's exact test, depending on the size and characteristics of the variables. We employed the Mann-Whitney *U* test or the Kruskal-Wallis test to estimate differences between two or more groups, and Spearman's $\rho$ correlation coefficients were calculated to estimate correlations between parameters.

The cutoff for determining the most significant variables was based on the variable importance scores from the Random Forest analysis. We selected the top 5 variables with the highest importance scores to classify patients between the indicated groups, as shown in the corresponding figures. Separation between groups was studied using Principal Component Analysis (PCA). To ensure model generalizability and prevent overfitting, we performed 10-fold cross-validation. The data set was randomly divided into training (70 %) and test (30 %) sets. During the model construction, we conducted 10-fold cross-validation with 100 replicates on the training data, and we assessed the model's performance on the hold-out test data. We began with a total of 32 variables related to complete lipoprotein and glycoprotein profiling. The data were normalized for the Random Forest analysis, and centered and scaled for PCA. Additionally, we constructed a linear fitting model. We evaluated its discriminatory ability between groups using the area under the curve (AUC) of the receiver operating characteristics (ROC) curves, along with a 95 % confidence Interval. Cross-validation predictions of the selected variables were plotted. The statistical analysis was conducted using R statistical software version 4.1.1. The main R packages used were caret 6.0–94 for machine learning, pROC 1.18.5 for ROC curves and calculating the AUC, mixOmics 6.25.0 for multivariate analysis, and ggforestplot 0.1.0 for generating and visualizing forest plots.

## 3. Results

### 3.1. Patient characteristics

There were no significant differences in the sex distribution of the different groups of participants, but the BI patients were older than the COVID-19 ones and the control group. Regarding associated comorbidities, COVID-19 patients had a higher incidence of chronic lung disease than negative patients and a lower incidence of type 2 diabetes mellitus, chronic kidney disease, and cancer. The Charlson and McCabe indices indicated that patients with bacterial infections had more comorbidities and a worse prognosis than patients with COVID-19 (Table 1).

### 3.2. Lipoprotein and glycoprotein changes are distinct and more profound in COVID-19 compared to bacterial infections

The analytical results are indicated numerically in Supplementary Table 2 and are graphically represented in Figs. 1–4. COVID-19 patients showed a significantly higher concentration of VLDL-cholesterol and VLDL-triglycerides than the control group. The size of these lipoproteins and the number of large, medium, and small particles were also higher. BI patients showed a similar trend, but differences were minor and often did not reach statistical significance (Fig. 1). COVID-19 and BI patients showed both a lower concentration of LDL-cholesterol than the control group, and that of triglycerides was higher only in the BI. LDL particle size was larger, and the number of particles was lower than that of the healthy individuals (Fig. 2). The HDL particles presented marked alterations in the two groups of infected patients, with a decrease in cholesterol concentration, an increase in triglycerides, and an increase in size, with a marked reduction in the concentration of small HDL particles (Fig. 3). Changes in IDL were minor and are not graphically represented although can be seen in Supplementary Table 2. On the other hand, all the parameters of the glycoprotein profile were increased in the two groups of patients (Fig. 4). In general terms, lipid and glycoprotein alterations were more marked in COVID-19 patients than in BI ones. These changes were maintained when adjusted for age, sex, and comorbidities (Supplementary Figs. 1–3).

Random forest analysis identified H/W Glyc-B, Glyc-B concentration, HDL diameter, small HDL particle concentration, and the ratio between the concentrations of triglycerides and cholesterol in LDL, as the five parameters with the highest capacity to discriminate BI patients from the control group (Fig. 5A). Conversely, small HDL concentration, HDL diameter, total HDL particle concentration, the ratio between the concentrations of triglycerides and cholesterol in HDL, and small LDL particle concentration, were the parameters with the highest capacity to discriminate COVID-19 patients from the control group (Fig. 5B). Finally, the parameters that best discriminated between the two groups of patients were VLDL diameter, medium LDL particle concentration, large HDL particle concentration, the ratio between the concentrations of triglycerides and cholesterol in VLDL, and LDL triglycerides concentration (Fig. 5C). The diagnostic accuracy of the combinations of these parameters measured by the ROC curves was higher than 90 % when the two groups of patients were compared with the control group and exceeded 80 % when comparing the patient groups with each other.

### 3.3. The alterations in lipoproteins and glycoproteins did not efficiently discriminate between severe and moderate COVID-19 patients

Severe COVID-19 patients showed a non-significant trend of having fewer HDL particles than moderates and significant increases in the ratios between the total number of particles and HDL and between the number of LDL particles and HDL particles (Supplementary Table 3). No significant differences were found in any other parameter. However, these differences were minor, and the PCA showed a high overlap between both groups. ROC curves did not demonstrate sufficient diagnostic accuracy to propose any prognostic marker of severity (Supplementary Fig. 4).

## 4. Discussion

The existence of considerable alterations in the characteristics of lipoproteins in infectious diseases is well known. Infection and inflammation trigger the acute phase response, eliciting pro-atherogenic modifications in lipid and lipoprotein metabolism [19–22]. Increased VLDL secretion may be attributable to adipose tissue lipolysis, heightened hepatic *de novo* fatty acid synthesis, and

**Table 1**
Demographic and clinical characteristics of the patients and the control group.

| | Control Group (n = 50) | COVID-19-negative (n = 50) | COVID-19-positive (n = 124) | *p*-value |
|---|---|---|---|---|
| Male sex, n (%) | 24 (48) | 20 (40) | 57 (46) | 0.693 |
| Age, years | 72.5 (69.0–79.0) | 83.5 (75.2–89.0) | 71.0 (58.8–83.0) | <0.001 |
| Type 2 diabetes mellitus, n (%) | NA | 26 (52.0) | 30 (24.2) | <0.001 |
| Cardiovascular disease, n (%)[a] | NA | 25 (50.0) | 68 (54.8) | 0.340 |
| Chronic liver disease, n (%) | NA | 0 (0.0) | 1 (0.8) | 0.713 |
| Chronic lung disease, n (%) | NA | 1 (2.0) | 17 (13.7) | 0.014 |
| Chronic kidney disease, n (%) | NA | 20 (40) | 22 (17.7) | 0.002 |
| Chronic neurological disease, n (%) | NA | 7 (14.0) | 29 (23.4) | 0.118 |
| Cancer, n (%) | NA | 15 (30) | 16 (12.9) | 0.009 |
| Charlson index | | | | |
| No comorbidity, n (%) | NA | 9 (18.0) | 81 (65.3) | <0.001 |
| Low comorbidity, n (%) | | 18 (36.0) | 29 (23.4) | |
| High comorbidity, n (%) | | 23 (46.0) | 14 (11.3) | |
| McCabe index | | | | |
| RFD, n (%) | NA | 24 (48.0) | 7 (5.6) | <0.001 |
| UFD, n/%) | | 25 (50.0) | 31 (25.0) | |
| NFD, n (%) | | 1 (2.0) | 86 (69.4) | |

Statistical analyses were performed by the Kruskal-Wallis test (quantitative) or the $\chi^2$ test (qualitative). Results are given as median and interquartile range or as numbers and percentages. NA: Not applicable; NFD: Non-fatal disease; RFD: Rapidly fatal disease; UFD: Ultimately fatal disease.
[a] Including hypertension.

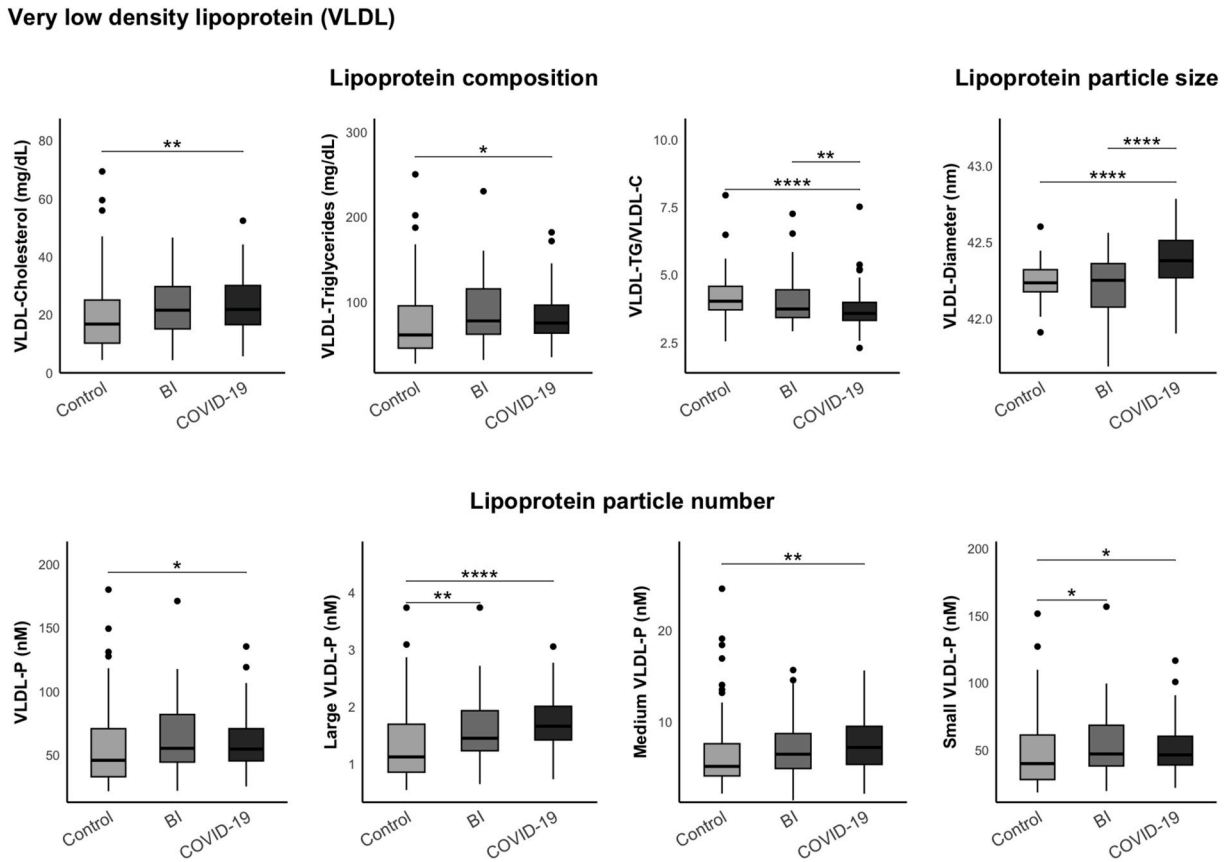


**Fig. 1.** Box plots representing the very low-density lipoprotein (VLDL) profile in the control group, the patients with bacterial infections (BI), and the COVID-19 patients. Boxes represent medians and interquartile ranges (IQR). Solid points are individual values outside the IQR.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

suppression of fatty acid oxidation. Furthermore, reduced lipoprotein lipase and apolipoprotein E activities result in diminished VLDL clearance. Concomitantly, HDL particles undergo substantial alterations, manifesting decreased cholesterol, apolipoprotein A1, and antioxidant enzyme levels, and enhanced concentrations of serum amyloid A. Notably, HDL assumes a pro-inflammatory character during this process [19,21]. Recent investigations suggest that COVID-19 follows a pattern consistent with these shared features, albeit with a likelihood of more pronounced manifestations than other infectious diseases. Several studies using routine biochemical methods have established that a low serum HDL-cholesterol concentration is a common feature of COVID-19 and have discussed the implications this may have on the effectiveness of the innate immune response of these patients [22–29]. Viral infections, including COVID-19, exhibit a reduction in lipid and apolipoprotein A1 synthesis and a potential increase in cholesterol absorption by cells, including immune cells, contributing to the observed decline in serum cholesterol levels [30].

Recently, some studies have performed a comprehensive metabolic analysis of lipoprotein alterations in COVID-19 using $^{1}$H NMR. A study using one-dimensional NMR (LipoProfile®) in patients with severe COVID-19 admitted to the Intensive Care Unit in two hospitals in Baltimore (USA) reported markedly reduced HDL particle numbers, meager numbers of the small HDL particles, and high levels of triglyceride-rich lipoprotein particles, together with high lipoprotein X and Z concentrations [31]. Another study using the same technology found similar alterations in children with severe acute symptoms of COVID-19 but not in asymptomatic children [32]. One study employing the AVANCE™ IVD method (Bruker, Billerica, MA, USA) found higher cholesterol and triglycerides in VLDL particles and lower cholesterol concentrations in LDL and HDL in hospitalized patients with COVID-19 [33]. Two more studies reported that patients with severe COVID-19 presented an increase in VLDL, IDL, and LDL particles and a decrease in HDL. Most lipoprotein subpopulations were generally enriched in triglycerides and depleted in cholesterol [34,35]. Most $^{1}$H NMR studies agree that COVID-19 patients show a pro-atherogenic lipoprotein profile characterized by elevated triglycerides in all lipoprotein fractions, with those in LDL showing the largest increase. Conversely, total cholesterol, LDL-cholesterol, and HDL-cholesterol are reduced, particularly in HDL subfractions 4 and 3 [36–39]. Furthermore, the analysis of COVID-19-positive subjects during acute infection versus recovery showed that lipoprotein alterations persist in many post-acute patients, indicating a delayed return to normality [40,41].

In the present study, we chose to use the Liposcale® test to analyze lipoprotein alterations in COVID-19-positive patients and COVID-19-negative patients suffering with bacterial infections. The Liposcale® method is based on 2D diffusion-ordered $^{1}$H NMR that, in addition to measuring the size and lipid composition of the lipoprotein subfractions, allows characterization of their size through the

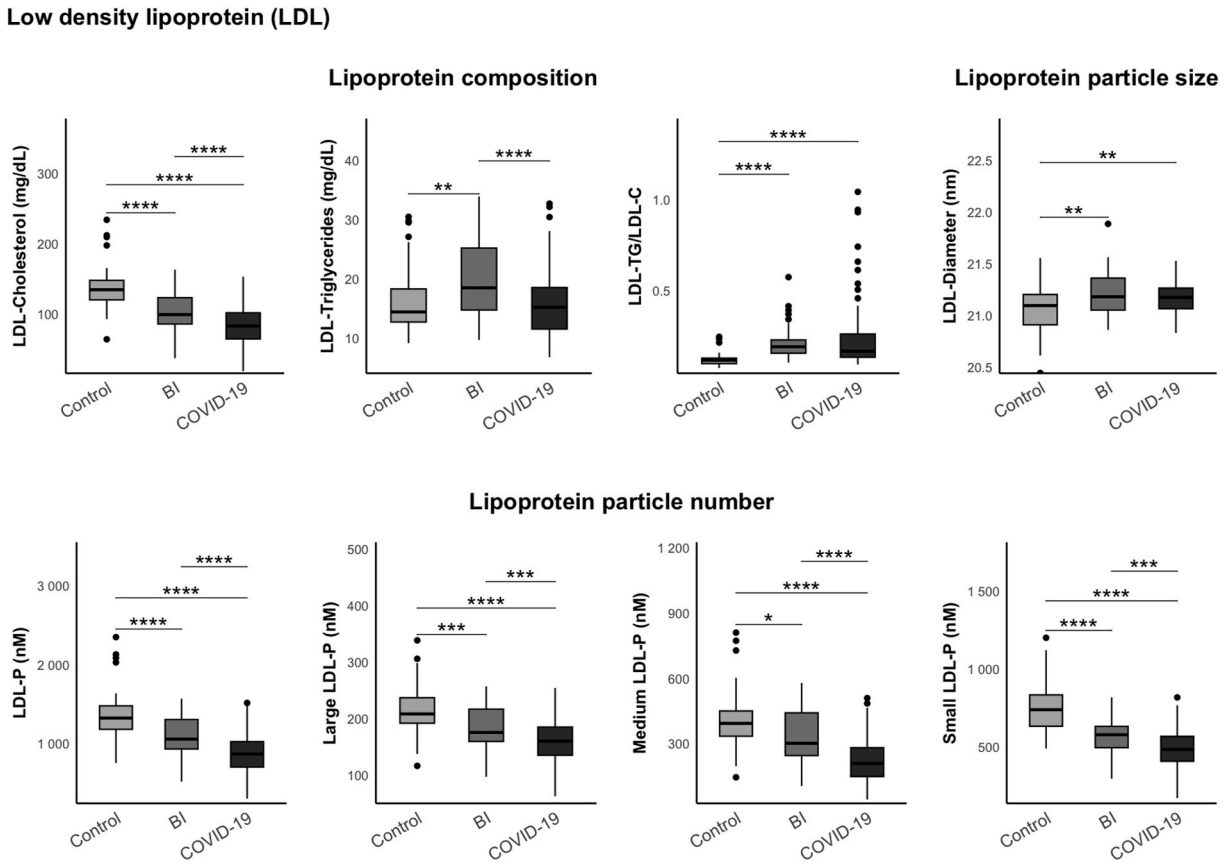


**Fig. 2.** Box plots representing the low-density lipoprotein (LDL) profile in the control group, the patients with bacterial infections (BI), and the COVID-19 patients. Boxes represent medians and interquartile ranges (IQR). Solid points are individual values outside the IQR.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

measurement of the diffusion coefficients from the Stokes-Einstein equation [10]. We found that specific parameters, namely small HDL particle concentration, HDL diameter, total HDL particle concentration, the ratio of triglycerides to cholesterol in HDL, and small LDL particle concentration, proved high efficacy in discerning COVID-19 patients from the control group. Conversely, parameters encompassing H/W Glyc-B, Glyc-B concentration, HDL diameter, small HDL particle concentration, and the ratio of triglycerides to cholesterol in LDL exhibited effectiveness in discriminating BI patients from the control group. The alterations in the lipoprotein signature of both groups of patients were unambiguous when compared to healthy subjects. The PCA showed almost complete discrimination between groups, and the AUCs of the ROC plots demonstrated a high diagnostic accuracy. These results are consistent with previous studies that have found an increase in triglyceride-rich lipoproteins and a marked decrease in HDL in patients with infectious diseases [22–35]. Lipoprotein levels are dependent on age and sex [42] and therefore our results have been statistically adjusted for these differences, as well as for comorbidities (See Supplementary Materials).

Glycoprotein results agree with the pro-inflammatory nature of the lipoprotein profile. Previous research has demonstrated the strength of the determination of glycoproteins by $^1$H NMR as biomarkers of systemic inflammation, and significant correlations have been observed between these parameters and several acute-phase response proteins [43]. Our study found higher GlycA, GlyB, and GlycF concentrations in patients with bacterial infections or COVID-19 than in healthy subjects. Similar results on GlycA and GlycB have recently been reported in COVID-19 [31–33,35]. Furthermore, we have also found higher H/W GlycA and H/W GlycB ratios, where H depends on the concentration, and W depends on the aggregation of the molecules that emit the signal. Elevated ratios suggest that links are in a state of looser aggregation, resulting from the presence of more easily accessible glycosylation zones in the proteins. This phenomenon is linked to increased inflammation, as inflammation triggers the formation of additional glycosylation branches. Consequently, the H/W ratio has been suggested as a potential marker for inflammation [11,44].

Compared to previous studies, our research is novel for simultaneously investigating two distinct infection categories: bacterial and viral. This dual focus allows a comparative assessment of outcomes within the two patient cohorts in addition to the classical comparison with healthy individuals. Including COVID-19 as the viral infection of interest adds considerable value, considering its profound metabolic impact and the persisting scientific interest in unraveling various aspects of this disease. Shared characteristics between both groups of patients encompass an increase in glycoprotein concentrations, a decrease in the number of LDL particles of all sizes and small HDL, an enrichment in triglycerides, and a depletion of HDL cholesterol. Notably, these modifications assume a

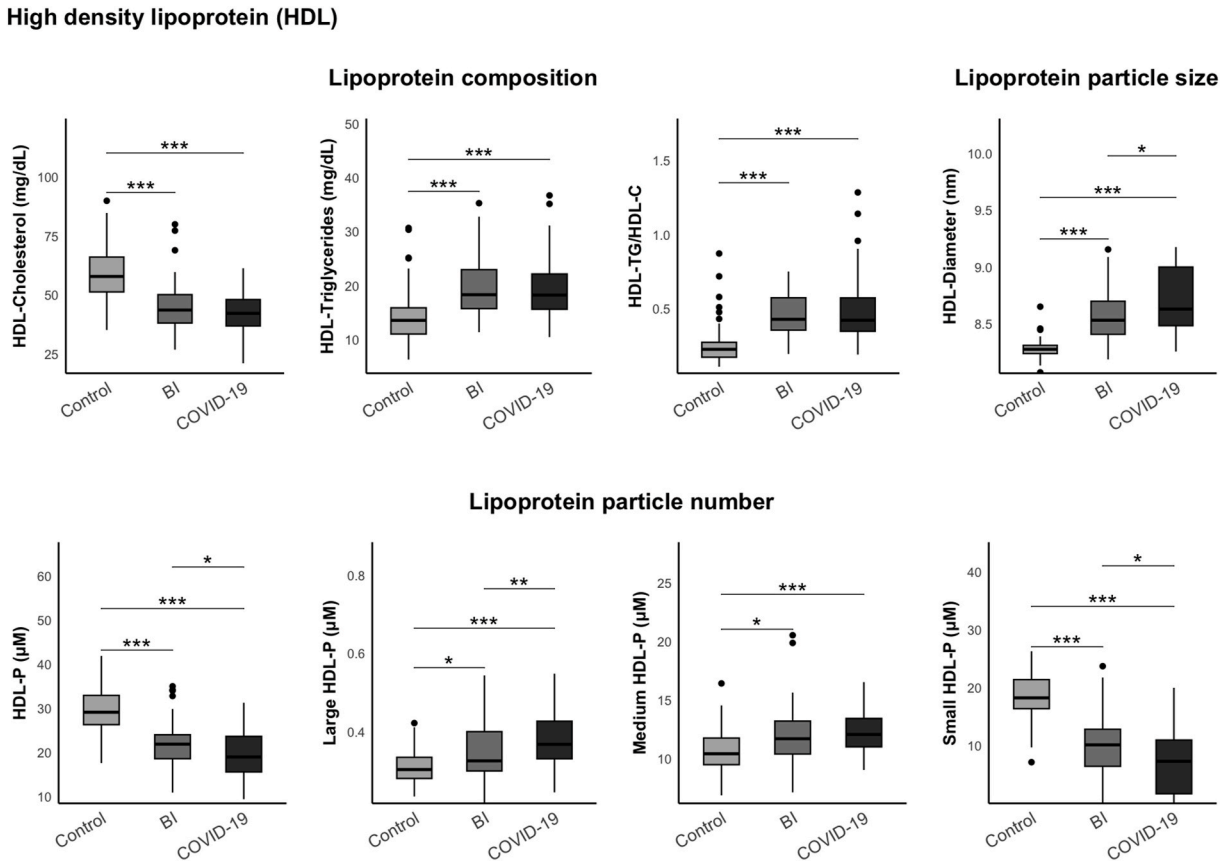


**Fig. 3.** Box plots representing the high-density lipoprotein (HDL) profile in the control group, the patients with bacterial infections (BI), and the COVID-19 patients. Boxes represent medians and interquartile ranges (IQR). Solid points are individual values outside the IQR.
*$p < 0.01$; **$p < 0.001$; ***$p < 0.0001$.

heightened significance in COVID-19 patients despite indications from the Charlson and McCabe indices showing fewer comorbidities and a less severe clinical condition. Conversely, COVID-19 diverges from bacterial infections primarily due to the larger diameter of VLDL particles, a reduced count of medium-sized LDL particles, and an increased number of large HDL particles. Whether these distinctive features are exclusive to COVID-19 or are found in other viral infections like influenza or respiratory syncytial virus warrants further investigation. Indeed, prior research utilizing traditional clinical laboratory techniques has revealed the existence of similar alterations in individuals infected with the human immunodeficiency virus [45] or hepatitis C [46]. Our findings align with those previously reported [34], indicating that a rise in the number of VLDL particles constitutes a fundamental characteristic distinguishing individuals with COVID-19 from counterparts with cardiogenic shock admitted to the Intensive Care Unit. Together, both the prior studies and our results suggest that modifications in VLDL metabolism may be specific to COVID-19 and serve as valuable indicators for discriminating this disease from others.

Although the metabolic profiles analyzed can be proposed as markers for the diagnosis of COVID-19 and bacterial infections, we have not identified any prognostic marker indicating a patient's need to receive invasive respiratory assistance or admission to the Intensive Care Unit. We found tendencies to have a low number of HDL particles in relation to other particles, which agrees with previous reports [22–24,26–28,31–33]. However, the differences we found were smaller than those reported by most authors [26,27,31]. These differences can probably be explained by our definition of "severe" and "moderate." We have not compared admitted patients with outpatients or asymptomatic patients. All our patients were collected during the first wave of COVID-19 when the aggressive alpha variant was predominant, and all required hospital admission. The clinical and demographic characteristics of these patients, and those of the dominant strain of the virus at the time of study may explain this discrepancy [28].

Several limitations warrant consideration in interpreting the findings of our study. Differences in ethnicity, age, and comorbidity prevalence among patients may introduce confounding factors when comparing our data with other studies. Variability in sampling time and follow-up durations could impact the comparability of prognostic information based on lipid and lipoprotein data across studies. It is essential to acknowledge the potential influence of corticosteroid therapy on lipoprotein levels, particularly in the context of severe COVID-19 cases. The retrospective nature of the study introduces inherent biases and limitations associated with data collection and analysis, potentially influencing the generalizability of our findings. The availability of limited outcome data further restricts the depth of our analysis and the comprehensiveness of the insights derived from our study. Moreover, the temporal aspect of

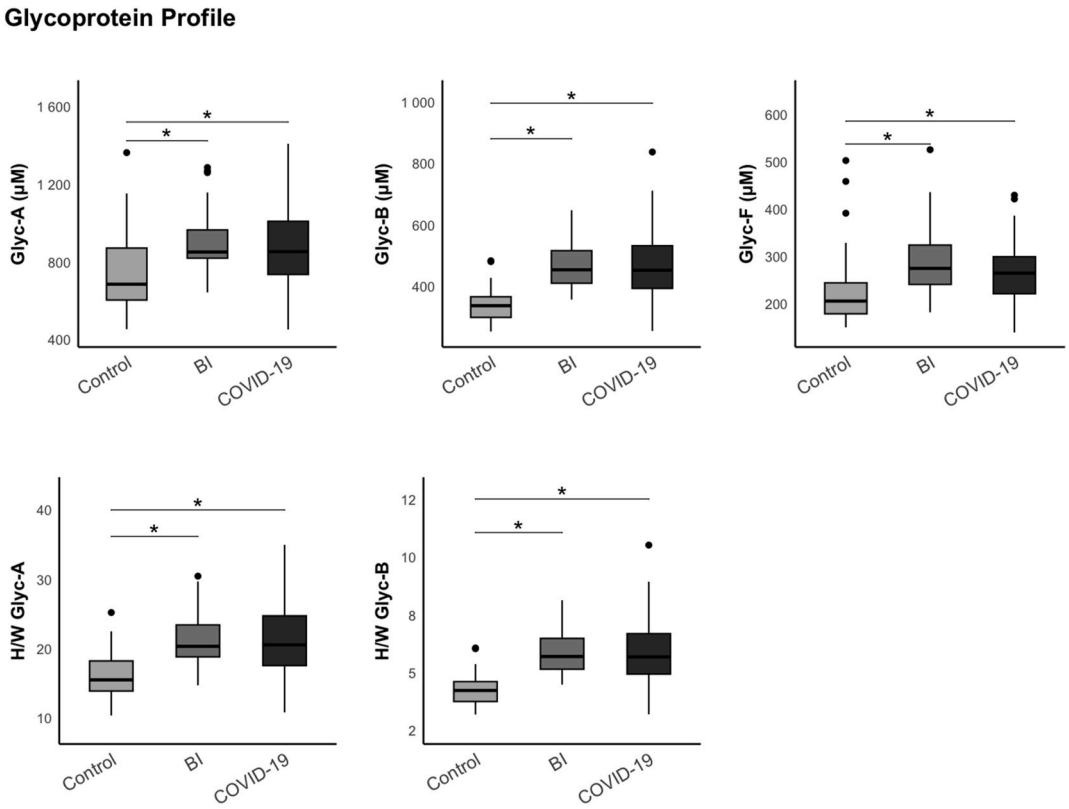


**Fig. 4.** Box plots representing the glycoprotein profile in the control group, the patients with bacterial infections (BI), and the COVID-19 patients. Boxes represent medians and interquartile ranges (IQR). Solid points are individual values outside the IQR. $^{*}p < 0.0001$.

our data collection, initiated at the onset of the pandemic, introduces a potential temporal bias. Some studies have highlighted the evolving nature of SARS-CoV-2 infection, with altered symptom expression and decreased respiratory severity observed over successive waves and corresponding to the emergence of variants [47,48]. Consequently, the dynamic nature of the infection and its evolving risks may impact the generalizability of our findings and introduce potential variations in metabolic sequelae over time. Finally, while the distinctions observed in the signatures of both groups of patients may be attributed to variations between viral and bacterial infections, it is equally plausible that they reflect the severity of the patients' conditions or the degree of inflammatory response.

## 5. Conclusion

Our findings underscore the potential of $^{1}$H NMR analysis for lipoproteins and glycoproteins as invaluable infection biomarkers. Additionally, they reveal intriguing differences between COVID-19 and bacterial infections, shedding light on a previously unexplored area with promising clinical significance. Further investigations are imperative to elucidate the clinical relevance of these findings and their potential implications for patient care and management.

## Funding

This study was funded by a grant from the Fundació La Marató de TV3 (201807-10), Barcelona, Spain.

## Data availability

Data included in article/supp. material/referenced in article.

## CRediT authorship contribution statement

**Simona Iftimie:** Writing – review & editing, Visualization, Validation, Resources, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Núria Amigó:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Neus Martínez-Micaelo:** Writing – review & editing, Software, Methodology, Investigation. **Ana F. López-Azcona:**

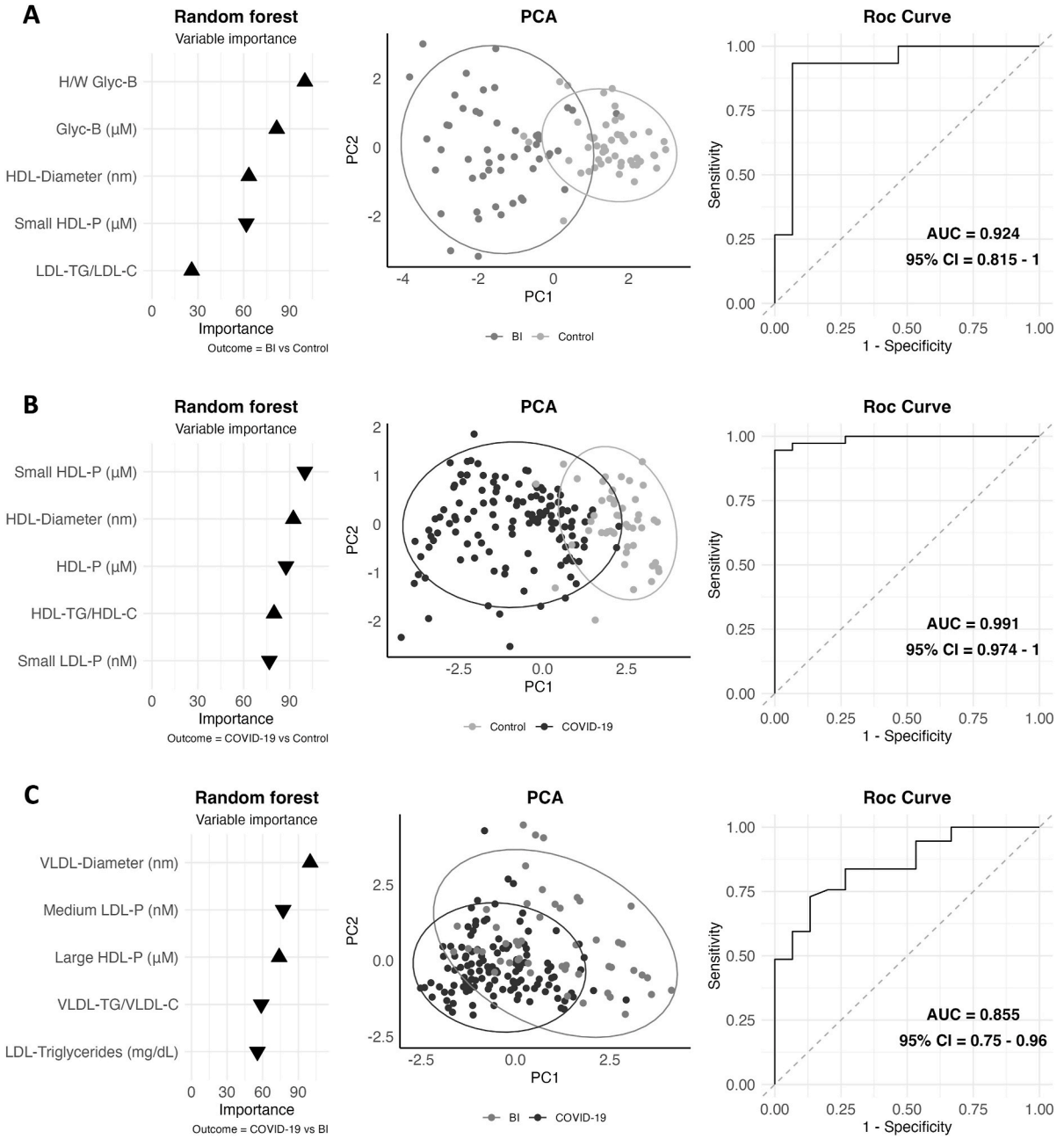


**Fig. 5.** From left to right, random forest plots, principal component analysis (PCA) and receiver component characteristics (ROC) curves when comparing patients with bacterial infections (BI) and the control group (A), COVID-19 patients with the control group (B) and COVID-19 with BI patients (C). In the random forest plots, unturned triangles indicate a higher value and inverted triangles indicate a lower value in the comparison group. AUC: Area under the curve; C: Cholesterol; CI: Confidence interval; HDL: High-density lipoprotein; H/W: Height to width ratio; LDL: Low-density lipoprotein; P: Particles; TG: Triglycerides; VLDL: Very low-density lipoprotein.

Investigation. **Cristian Martínez-Navidad:** Investigation. **Helena Castañé:** Investigation. **Andrea Jiménez-Franco:** Investigation. **Josep Ribalta:** Methodology, Investigation. **Sandra Parra:** Investigation. **Antoni Castro:** Supervision, Resources, Funding acquisition. **Jordi Camps:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Jorge Joven:** Supervision, Resources, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37115.

## References


[1] B.F. Bale, A.L. Doneen, P.P. Leimgruber, D.J. Vigerust, The critical issue linking lipids and inflammation: clinical utility of stopping oxidative stress, Front. Cardiovasc. Med. 9 (2022) 1042729. https://doi:10.3389/fcvm.2022.1042729.

[2] N.R. Webb, High-density lipoproteins and serum amyloid A (SAA), Curr. Atherosclerosis Rep. 23 (2021) 7. https://doi:10.1007/s11883-020-00901-4.

[3] K.E. Herbert, C. Erridge, Regulation of low-density lipoprotein cholesterol by intestinal inflammation and the acute phase response, Cardiovasc. Res. 114 (2018) 226–232. https://doi:10.1093/cvr/cvx237.

[4] V.G. Cabana, J. R Lukens, K.S. Rice, T.J. Hawkins, G.S. Getz, HDL content and composition in acute phase response in three species: triglyceride enrichment of HDL a factor in its decrease, J. Lipid Res. 37 (1996) 2662–2674.

[5] S.T. Chiesa, M. Charakida, High-density lipoprotein function and dysfunction in health and disease, Cardiovasc. Drugs Ther. 33 (2019) 207–219. https://doi:10.1007/s10557-018-06846-w.

[6] M.A. Fernández-Rojas, G. Ávila, M. Romero-Valdovinos, T. Plett-Torres, A.M. Salazar, M. Sordo, M. Chávez-Vargas, C.J. Coeto Ángeles, M. Cruz-Rivera, C. Santiago-Olivares, J.P. Ramírez Hinojosa, P. Maravilla, A. Flisser, P. Ostrosky-Wegman, F. Mendlovic, Elevated levels of cytotoxicity, cytokines, and anti-SARS-CoV-2 antibodies in mild cases of COVID-19, Viral Immunol. 36 (2023) 550–561. https://doi:10.1089/vim.2023.0012.

[7] S. Iftimie, A.F. López-Azcona, M. Vicente-Miralles, R. Descarrega-Reina, A. Hernández-Aguilera, F. Riu, J.M. Simó, P. Garrido, J. Joven, J. Camps, A. Castro, Risk factors associated with mortality in hospitalized patients with SARS-CoV-2 infection. A prospective, longitudinal, unicenter study in Reus, Spain, PLoS One 15 (2020) e0234452. https://doi:10.1371/journal.pone.0234452.

[8] M.V. Brizneda, A. Bansal, V. Jain, S. Kapadia, L.G. Svensson, V. Menon, P. Cremer, G. Reed, P. Rampersad, R. Grimm, B.P. Griffin, B. Xu, Coronavirus disease and the cardiovascular system: a narrative review of the mechanisms of injury and management implications, Cardiovasc. Diagn. Ther. 11 (2021) 939–953. https://doi:10.21037/cdt-20-779.

[9] S. Zhou, A. Zhang, H. Liao, Z. Liu, F. Yang F, Pathological Interplay and Clinical Complications between COVID-19 and Cardiovascular Diseases: an Overview in 2023, Cardiology, 2023. Aug 16, https://doi:10.1159/000533359. Online ahead of print.

[10] R. Mallol, N. Amigó, M.A. Rodríguez, M. Heras, M. Vinaixa N. Plana, E. Rock, J. Ribalta, O. Yanes, L. Masana, X. Correig, Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered $^{1}$H NMR spectroscopy, J. Lipid Res. 56 (2015) 737–746. https://doi:10.1194/jlr.D050120.

[11] R. Fuertes-Martín, D. Taverner, J. C Vallvé, S. Paredes, L. Masana, X. Correig Blanchar, Characterization of $^{1}$H NMR plasma glycoproteins as a new strategy to identify inflammatory patterns in rheumatoid arthritis, J. Proteome Res. 17 (2018) 3730–3739. https://doi:10.1021/acs.jproteome.8b00411.

[12] R. Fuertes-Martin, S. Moncayo, M. Insenser, M.A. Martínez-García, M. Luque-Ramírez, N.A. Grau, X.C. Blanchar, H.F. Escobar-Morreale, Glycoprotein A and B height-to-width ratios as obesity-independent novel biomarkers of low-grade chronic inflammation in women with polycystic ovary syndrome (PCOS), J. Proteome Res. 18 (2019) 4038–4045. https://doi:10.1021/acs.jproteome.9b00528.

[13] S. Iftimie, A. García-Heredia, I. Pujol, F. Ballester, I. Fort-Gallifa, J.M. Simó, J. Joven, J. Camps, A. Castro, Preliminary study on serum paraoxonase-1 status and chemokine (C-C motif) ligand 2 in hospitalized elderly patients with catheter-associated asymptomatic bacteriuria, Eur. J. Clin. Microbiol. Infect. Dis. 35 (2016) 1417–1424. https://doi:10.1007/s10096-016-2679-8.

[14] I. Fort-Gallifa, A. García-Heredia, A. Hernández-Aguilera, J.M. Simó, J. Sepúlveda, V. Martín-Paredero, J. Camps, J. Joven, Biochemical indices of oxidative stress and inflammation in the evaluation of peripheral artery disease, Free Radic. Biol. Med. 97 (2016) 568–576. https://doi:10.1016/j.freeradbiomed.2016.07.011.

[15] B.E. Kreger, D.E. Craven, P.C. Carling, W.R. McCabe, Gram-negative bacteremia. III. Reassessment of etiology, epidemiology and ecology in 612 patients, Am. J. Med. 68 (1980) 332–343. https://doi:10.1016/0002-9343(80)90101-1.

[16] L.F. Berkman, L. Leo-Summers, R.I. Horwitz, Emotional support and survival after myocardial infarction. A prospective, population-based study of the elderly, Ann. Intern. Med. 117 (1992) 1003–1009. https://doi:10.7326/0003-4819-117-12-1003.

[17] E.J. Jeyarajah, W.C. Cromwell, J.D. Otvos, Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy, Clin. Lab. Med. 26 (2006) 847–870. https://doi:10.1016/j.cll.2006.07.006.

[18] I. Amich, E. Anguita, S. Escribano-Serrat, C. Alvarez, D. Rodríguez-Muñoz, V. García, R. Bello, J.A. Peña-Pedrosa, N. Martínez-Micaelo, N. Amigó, P. Ortiz, M. J. Torrejón, L. Boscá, J. Martín-Sánchez, A. Aranda, S. Alemany, Free triiodothyronine levels and age influences the metabolic profile and COVID-19 severity parameters in euthyroid and levothyroxine-treated patients, Front. Endocrinol. 13 (2022) 1025032. https://doi:10.3389/fendo.2022.1025032.

[19] W. Khovidhunkit, M.S. Kim, R.A. Memon, J.K. Shigenaga, A.H. Moser, K.R. Feingold, C. Grunfeld, Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host, J. Lipid Res. 45 (2004) 1169–1196. https://doi:10.1194/jlr.R300019-JLR200.

[20] B. De Geest, M. Mishra, Impact of high-density lipoproteins on sepsis, Int. J. Mol. Sci. 23 (2022) 12965. https://doi:10.3390/ijms232112965.

[21] K.R. Feingold, The bidirectional interaction of COVID-19 infections and lipoproteins, Best Pract. Res. Clin. Endocrinol. Metabol. 37 (2023) 101751. https://doi:10.1016/j.beem.2023.101751.

[22] H.M. Al-Kuraishy, N.R. Hussien, M.S. Al-Niemi, E.H. Fahad, A.K. Al-Buhadily, A.I. Al-Gareeb, S.M. Al-Hamash, C. Tsagkaris, M. Papadakis, A. Alexiou, G. E. Batiha, SARS-CoV-2 induced HDL dysfunction may affect the host's response to and recovery from COVID-19, Immun, Inflamm. Dis. 11 (2023) e861. https://doi:10.1002/iid3.861.

[23] J. Mohammadshahi, H. Ghobadi, G. Matinfar, M.H. Boskabady, M.R. Aslani, Role of lipid profile and its relative ratios (cholesterol/HDL-C, triglyceride/HDL-C, LDL-C/HDL-C, WBC/HDL-C, and FBG/HDL-C) on admission predicts in-hospital mortality COVID-19, J, Lipids 2023 (2023) 6329873. https://doi:10.1155/2023/6329873.

[24] M. Lalosevic, J. Kotur-Stevuljevic, J. Vekic, M. Rizzo, T. Kosanovic, I.P. Blagojevic, A. Zeljkovic, D. Jeremic, M. Mihajlovic, A. Petkovic, L. Hajdarpasic, M. Djordjevic, V. Dobrilovic, S. Erceg, S. Vujcic, J. Marjanovic, J.M. Jovanovic, J. Saponjski, N. Bogavac-Stanojevic, Alteration in redox status and lipoprotein profile in COVID-19 patients with mild, moderate, and severe pneumonia, Oxid. Med. Cell. Longev. 2022 (2022) 8067857. https://doi:10.1155/2022/8067857.

[25] A. Aparisi, M. Martín-Fernández, C. Ybarra-Falcón, J.F. Gil, M. Carrasco-Moraleja, P. Martínez-Paz, I. Cusácovich, H. Gonzalo-Benito, R. Fuertes, M. Marcos-Mangas, C. Iglesias-Echeverría, J.A. San Román, E. Tamayo, D. Andaluz-Ojeda, A. Tamayo-Velasco, Dyslipidemia and inflammation as hallmarks of oxidative stress in COVID-19: a follow-up study, Int. J. Mol. Sci. 23 (2022) 15350. https://doi:10.3390/ijms232315350.

[26] I.D. Capraru, D.D. Vulcanescu, I.C. Bagiu, F.G. Horhat, I.M. Popescu, L.M. Baditoiu, D. Muntean, M. Licker, M. Radulescu, I.C. Mot, M.M. Diaconu, C. Marian C, COVID-19 Biomarkers comparison: children, adults and elders, Medicina (Kaunas) 59 (2023) 877. https://doi:10.3390/medicina59050877.

[27] Y. Chang, J. Jeon, T.J. Song, J. Kim, Association of triglyceride/high-density lipoprotein cholesterol ratio with severe complications of COVID-19, Heliyon 9 (2023) e17428. https://doi:10.1016/j.heliyon.2023.e17428.

[28] M.H. Li, R. Kulkarni, N. Koizumi, A. Andalibi, On behalf of the N3C Consortium, the association of the levels of high-density lipoprotein and apolipoprotein A1 with SARS-CoV-2 infection and COVID-19 severity: an analysis of the N3C database, Biology 12 (2023) 852. https://doi:10.3390/biology12060852.

[29] H. Sharma, K. Mossman, R.C. Austin, Fatal attractions that trigger inflammation and drive atherosclerotic disease, Eur. J. Clin. Invest. (2024) e14169. https://doi:10.1111/eci.14169.
[30] Y. Huang, S. Wang, Y. Liu, Y. Zhang, C. Zheng, Y. Zheng, C. Zhang, W. Min, H. Zhou, M. Yu, M. Hu, A preliminary study on the ultrasonic manifestations of peripulmonary lesions of non-critical novel coronavirus pneumonia (COVID-19), Available at: SSRN: https://ssrn.com/abstract=3544750, https://doi.org/10.2139/ssrn.3544750, February 26, 2020. (Accessed 18 January 2024).
[31] R.A. Ballout, H. Kong, M. Sampson, J.D. Otvos, A.L. Cox, S. Agbor-Enoh, A.T. Remaley, The NIH Lipo-COVID Study: a Pilot NMR investigation of lipoprotein subfractions and other metabolites in patients with severe COVID-19, Biomedicines 9 (2021) 1090. https://doi:10.3390/biomedicines9091090.
[32] M. Mietus-Snyder, W. Suslovic, M. Delaney, M.P. Playford, R.A. Ballout, J.R. Barber, J.D. Otvos, R.L. DeBiasi, N.N. Mehta, A.T. Remaley, Changes in HDL cholesterol, particles, and function associate with pediatric COVID-19 severity, Front. Cardiovasc. Med. 9 (2022) 1033660. https://doi:10.3389/fcvm.2022.1033660.
[33] S. Lodge, P. Nitschke, T. Kimhofer, J.D. Coudert, S. Begum, S.H. Bong, T. Richards, D. Edgar, E. Raby, M. Spraul, H. Schaefer, J.C. Lindon, R.L. Loo, E. Holmes, J. K. Nicholson, NMR spectroscopic windows on the systemic effects of SARS-CoV-2 infection on plasma lipoproteins and metabolites in relation to circulating cytokines, J. Proteome Res. 20 (2021) 1382–1386. https://doi:10.1021/acs.jproteome.0c00876.
[34] F. Schmelter, B. Föh, A. Mallagaray, J. Rahmöller, M. Ehlers, S. Lehrian, V. von Kopylow, I. Künsting, A.S. Lixenfeld, E. Martin, M. Ragab, R. Meyer-Saraei, F. Kreutzmann, I. Eitel, S. Taube, N. Käding, E. Jantzen, T. Graf, C. Sina, U.L. Günther, Metabolic and lipidomic markers differentiate COVID-19 from non-hospitalized and other intensive care patients, Front. Mol. Biosci. 8 (2021) 737039. https://doi:10.3389/fmolb.2021.737039.
[35] T. Rössler, G. Berezhnoy, Y. Singh, C. Cannet, T. Reinsperger, H. Schäfer, M. Spraul, M. Kneilling, U. Merle, C. Trautwein, Quantitative serum NMR spectroscopy stratifies COVID-19 patients and sheds light on interfaces of host metabolism and the immune response with cytokines and clinical parameters, Metabolites 12 (2022) 1277. https://doi:10.3390/metabo12121277.
[36] C. Bruzzone, M. Bizkarguenaga, R. Gil-Redondo, T. Diercks, E. Arana, A. García de Vicuña, M. Seco, A. Bosch, A. Palazón, I. San Juan, A. Laín, J. Gil-Martínez, G. Bernardo-Seisdedos, D. Fernández-Ramos, F. Lopitz-Otsoa, N. Embade, S. Lu, J.M. Mato, O. Millet, SARS-CoV-2 infection dysregulates the metabolomic and lipidomic profiles of serum, iScience 23 (2020) 101645. https://doi:10.1016/j.isci.2020.101645.
[37] R. Masuda, S. Lodge, P. Nitschke, M. Spraul, H. Schaefer, Bong S.H, T. Kimhofer, D. Hall, R.L. Loo, M. Bizkarguenaga, C. Bruzzone, R. Gil-Redondo, N. Embade, J.M. Mato, E. Holmes, J. Wist, O. Millet, J.K. Nicholson, Integrative modeling of plasma metabolic and lipoprotein biomarkers of SARS-CoV-2 infection in Spanish and Australian COVID-19 patient cohorts, J. Proteome Res. 20 (2021) 4139–4152. https://doi:10.1021/acs.jproteome.1c00458.
[38] G. Meoni, V. Ghini, L. Maggi, A. Vignoli, A. Mazzoni, L. Salvati, M. Capone, A. Vanni, L. Tenori, P. Fontanari, F. Lavorini, A. Peris, A. Bartoloni, F. Liotta, L. Cosmi, C. Luchinat, F. Annunziato, P. Turano, Metabolomic/lipidomic profiling of COVID-19 and individual response to tocilizumab, PLoS Pathog. 17 (2021) e1009243. https://doi:10.1371/journal.ppat.1009243.
[39] V. Ghini, W. Vieri, T. Celli, V. Pecchioli, N. Boccia, T. Alonso-Vásquez, L. Pelagatti, M. Fondi, C. Luchinat, L. Bertini, V. Vannucchi, G. Landini, P. Turano, COVID-19: a complex disease with a unique metabolic signature, PLoS Pathog. 19 (2023) e1011787. https://doi:10.1371/journal.ppat.1011787.
[40] M. Bizkarguenaga, C. Bruzzone, R. Gil-Redondo, I. SanJuan, I. Martin-Ruiz, D. Barriales, A. Palacios, S.T. Pasco, B. González-Valle, A. Laín, L. Herrera, A. Azkarate, M.A. Vesga, C. Eguizabal, J. Anguita, N. Embade, J.M. Mato, O. Millet, Uneven metabolic and lipidomic profiles in recovered COVID-19 patients as investigated by plasma NMR metabolomics, NMR Biomed. 35 (2022) e4637. https://doi:10.1002/nbm.4637.
[41] V. Ghini, G. Meoni, L. Pelagatti, T. Celli, F. Veneziani, F. Petrucci, V. Vannucchi, L. Bertini, C. Luchinat, G. Landini, P. Turano, Profiling metabolites and lipoproteins in COMETA, an Italian cohort of COVID-19 patients, PLoS Pathog. 18 (2022) e1010443. https://doi:10.1371/journal.ppat.1010443.
[42] E. Slade, M.R. Irvin, K. Xie, D.K. Arnett, S.A. Claas, T. Kind, D.W. Fardo, G.A. Graf, Age and sex are associated with the plasma lipidome: findings from the GOLDN study, Lipids Health Dis. 20 (2021) 30. https://doi:10.1186/s12944-021-01456-2.
[43] A. Mallagaray, L. Rudolph, M. Lindloge, J. Mölbitz, H. Thomsen, F. Schmelter, M.W. Alhabash, M.R. Abdullah, R. Saraei, M. Ehlers, T. Graf, C. Sina, A. Petersmann, M. Nauck, U.L. Günther, Towards a precise NMR quantification of acute phase inflammation proteins from human serum, Angew. Chem. Int. Ed. Engl. 62 (2023) e202306154. https://doi:10.1002/anie.202306154.
[44] N. Amigó, R. Fuertes-Martín, A.I. Malo, N. Plana, D. Ibarretxe, J. Girona, X. Correig, L. Masana L, Glycoprotein profile measured by a $^{1}$H-nuclear magnetic resonance based on approach in patients with diabetes: a new robust method to assess inflammation, Life 11 (2021) 1407. https://doi:10.3390/life11121407.
[45] S. Sarkar, T.T. Brown, Lipid disorders in people with HIV, in: K.R. Feingold (Ed.), Endotext [Internet], MDText.com, Inc., 2023 Jan 21. South Dartmouth, 2000. PMID: 33528966.
[46] W. Elgretli, T. Chen, N. Kronfli, G. Sebastiani, Hepatitis C virus-lipid interplay: pathogenesis and clinical impact, Biomedicines 11 (2023) 271. https://doi:10.3390/biomedicines11020271.
[47] W.T. Harvey, A.M. Carabelli, B. Jackson, R.K. Gupta, E.C. Thomson, E.M. Harrison, C. Ludden, R. Reeve, A. Rambaut, COVID-19 Genomics UK (COG-UK) Consortium; S.J. Peacock, D.L., SARS-CoV-2 variants, spike mutations and immune escape, Nat. Rev. Microbiol. 19 (2021) 409–424. https://doi:10.1038/s41579-021-00573-0.
[48] A.M. Carabelli, T.P. Peacock, L.G. Thorne, W.T. Harvey, J. Hughes, COVID-19 Genomics UK Consortium; S.J. Peacock, W.S. Barclay, T.I. de Silva, G.J. Towers, D.L. Robertson, SARS-CoV-2 variant biology: immune escape, transmission and fitness, Nat. Rev. Microbiol. 21 (2023) 162–177. https://doi:10.1038/s41579-022-00841-7.