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(54) Title: METHOD OF IDENTIFYING AND TREATING LONG COVID IN SUBJECTS

(57) Abstract: Provided herein is a method for diagnosing and treating Long COVID, comprising: (a) providing a biological sample obtained from the subject; (b) measuring concentration levels from the obtained sample of one or more COVID-related metabolites described herein and/or one or more Long COVID-related proteins described herein; (c) comparing the concentration levels of the Long COVID-related metabolites and/or proteins from the obtained sample to the concentration levels or corresponding reference Long COVID-related metabolites and/or proteins from an Long COVID- negative sample; (d) identifying the subject as having Long COVID if the concentration levels of the Long COVID-related metabolites and/or proteins from the obtained sample are different relative to the concentration levels of the reference Long COVID-related metabolites and/or proteins from the Long COVID-negative sample; and (e) causing treatment of the subject.



METHOD OF IDENTIFYING AND TREATING LONG COVID IN SUBJECTS

TECHNICAL FIELD

[0001] The present disclosure relates generally to the field of identifying whether a subject has Long COVID and treatment thereof.

BACKGROUND

[0002] COVID-19, caused by the SARS-CoV-2 virus, is a highly contagious respiratory illness that has become a global pandemic. The virus primarily spreads through respiratory droplets when an infected individual talks, coughs, or sneezes. Once a person is infected, the virus attaches to cells in the respiratory tract and uses a specific protein called the spike protein to enter and infect these cells. The virus then replicates in the cells and causes them to produce cytokines, which trigger an immune response that can lead to inflammation and damage to lung tissue. This immune response can also result in a cytokine storm, an overactive immune response that can cause severe inflammation and organ damage in severe cases of COVID-19. The exact mechanisms behind COVID-19 are still being studied, but understanding the disease mechanisms can help not only uncover clues as to the disease process but also future consequences resulting from infection and re-infection.

[0003] Post-COVID-19 condition, often referred to as Long COVID can be described as the persistence of symptoms for weeks or months following acute COVID-19 infection. These symptoms can range from fatigue, shortness of breath, and joint pain to neurological problems such as headaches, cognitive impairment, mood disorders, among other symptoms. The exact mechanisms behind Long COVID are still not fully understood, but it is believed to be related to ongoing inflammation and immune response following the initial infection. Some people may also have an ongoing viral infection or other underlying medical conditions that contribute to their symptoms.

[0004] Several studies have suggested that individuals with certain underlying medical conditions, such as obesity, diabetes, and cardiovascular disease, may be more susceptible to Long COVID. Additionally, individuals with a severe initial infection or a prolonged duration of symptoms are also at a higher risk of developing Long COVID. Furthermore, early studies suggest that

individuals with Long COVID may also be at higher risk of developing chronic diseases, including diabetes, cardiovascular disease, and neurodegenerative disorders. Additional studies have shown that individuals with Long COVID may have ongoing symptoms that affect their quality of life, including fatigue, difficulty concentrating, and persistent body aches. While there is currently no specific treatment for Long COVID, managing symptoms through lifestyle modifications, physical therapy, and medication may help improve quality of life for affected individuals.

[0005] Presently, there is no reliable test to determine whether an individual suffers from Long COVID. The World Health Organization has proposed a case definition for long COVID in which a patient is identified as having Long COVID if symptoms linger beyond 3 months of a probable or confirmed SARS-CoV-2 infection, which last at least 2 months and cannot be explained by an alternative diagnosis. However, the sensitivity, specificity and positive and negative predictive value of definitive diagnostic criteria have yet to be determined. (See Quinn et al., 2023, Canadian Medical Association Journal, 195(2) E78-E79).

[0006] Accordingly, an improved method is needed that can offer a more reliable identification of Long COVID and treatment thereof.

SUMMARY

[0007] The present disclosure provides a method for identifying and treating long COVID.

[0008] According to the present disclosure, a new metabolomic profile is used to identify a subject as having long COVID and to treat and/or ameliorate the disease.

[0009] According to the present disclosure, a unique combination of metabolites and/or proteins, peptides or fragments thereof has been identified with high predictive value for identifying long COVID. The disclosed methods thereby significantly improve the treatment of the disease relative to previous methods. In some embodiments, and without being limiting, the combination of metabolites and proteins, peptides or fragments thereof described herein are a subset of COVID markers that have a predictive value of at least 80%, 85%, 90% or more, as determined by a VIP plot. In one non-limiting example, at least one, two, three, four, five, six, seven or all metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin

(SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, two, three or all proteins, peptides or fragments thereof are selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5 are used to identify a subject having long COVID via a metabolomic and/or proteomic analysis carried out on a biological sample obtained from a subject. After identifying the subject as having long COVID, appropriate regimes to treat, prevent and/or ameliorate the disease may be carried out.

[0010] In one non-limiting example, at least one, two, three, four or five metabolites are selected from 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); Hydroxysphingomyelin (SM (OH)) C22:2; Dodecanedioylcarnitine (C12-DC); Succinic acid; and Propionic acid. In another example, at least one, two, three, four or five metabolites are selected from 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); and Lysophosphatidylcholine C16:1 (lysoPC C16:1).

[0011] In one non-limiting example, at least one, two, three, four or five proteins (including peptides and protein fragments thereof) are selected from Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; Extracellular matrix protein 1; Complement Component C7; Plasminogen; Attractin; Vitamin K-dependent protein S; and Complement factor H. In another example, at least one, two, three, four or five proteins (including peptides and protein fragments thereof) are selected from Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; and Extracellular matrix protein 1.

[0012] In additional or alternative embodiments, the new metabolomic and/or proteomic profile is used to monitor disease progression or provide improvements in the disease condition.

[0013] According to one aspect, there is provided a method for diagnosing and treating Long COVID in a subject, the method comprising: (a) providing a biological sample obtained from the subject; (b) measuring concentration levels from the obtained sample, at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin C22:2 (SM (OH) C22:2); Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, at least two, at least three, at least four or at least five Long COVID-related proteins and/or peptides selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5; (c) comparing the concentration levels of the Long COVID-related metabolites and/or proteins from the obtained sample to the concentration levels of corresponding reference Long COVID-related metabolites and/or proteins from a Long COVID- negative sample; (d) identifying the subject as having Long COVID if the concentration levels of the Long COVID-related metabolites and/or proteins from the obtained sample are different relative to the concentration levels of the reference Long COVID-related metabolites and/or proteins from the Long COVID-negative sample; and (e) treating or causing treatment of the subject so identified with a Long COVID treatment regime.

[0014] According to one embodiment, the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); Hydroxysphingomyelin (SM (OH)) C22:2; Dodecanedioylcarnitine (C12-DC); Succinic acid; and Propionic acid.

[0015] According to one embodiment, the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine

(C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); and Lysophosphatidylcholine C16:1 (lysoPC C16:1).

[0016] According to one embodiment, the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; Extracellular matrix protein 1; Complement Component C7; Plasminogen; Attractin; Vitamin K-dependent protein S; and Complement factor H.

[0017] According to one embodiment, the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; and Extracellular matrix protein 1.

[0018] According to another aspect, there is provided a method for diagnosing and treating Long COVID in a subject, the method comprising: (a) providing a biological sample obtained from the subject; (b) measuring from the obtain sample or having measured in a spectroscopy unit the concentration levels of at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5; (c) comparing or having compared concentration levels of the Long COVID-related metabolites and/or proteins as determined in the spectroscopy unit to the concentration levels of reference Long COVID- related metabolites and/or proteins from an Long COVID-negative sample; (d) identifying the subject as having Long COVID if the concentration levels of the Long COVID-related metabolites and/or proteins from the obtained sample are different relative to the concentration levels of the reference Long COVID-

related metabolites and/or proteins from the Long COVID-negative sample; and (e) treating or causing treatment of the subject so identified with a Long COVID treatment regime.

[0019] According to one embodiment of the above aspect, the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); Hydroxysphingomyelin (SM (OH)) C22:2; Dodecanedioylcarnitine (C12-DC); Succinic acid; and Propionic acid.

[0020] According to one embodiment of the above aspect, the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); and Lysophosphatidylcholine C16:1 (lysoPC C16:1).

[0021] According to one embodiment of the above aspect, the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; Extracellular matrix protein 1; Complement Component C7; Plasminogen; Attractin; Vitamin K-dependent protein S; and Complement factor H.

[0022] According to one embodiment of the above aspect, the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; and Extracellular matrix protein 1.

[0023] According to one embodiment of the above aspects, wherein the Long COVID treatment regime comprises adjusting the blood levels of one or more of the Long COVID-related metabolites and/or proteins in the subject diagnosed as having the Long COVID or predisposed of developing the Long COVID or a combination thereof.

[0024] According to one embodiment of the above aspect, the adjustment of the blood levels of one or more of the Long COVID-related metabolites and/or proteins in the subject occurs until an improvement in the Long COVID symptoms in the subject is observed.

[0025] According to one embodiment of the above aspect, the adjustment of the blood levels of one or more of the Long COVID-related metabolites comprises adjusting the composition of gut microbiota in the subject.

[0026] According to one embodiment of the above aspect, the identifying step occurs upon determination that the concentration levels of at least one, at least two, at least three, at least four or at least five of the Long COVID-related metabolites and/or proteins from the obtained sample differ by about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, or about 70% or more relative to the concentration levels of the reference Long COVID-related metabolites and/or proteins from the Long COVID-negative sample.

[0027] According to one embodiment of the above aspect, the obtained sample is blood or urine, preferably serum, plasma or urine.

[0028] According to one embodiment of the above aspect, the Long COVID-related metabolites and/or proteins are measured by a spectroscopic technique, wherein the spectroscopic technique is selected from the group consisting of liquid chromatography, gas chromatography, liquid chromatography mass spectrometry, gas chromatography mass spectrometry, high performance liquid chromatography mass spectrometry, capillary electrophoresis mass spectrometry, nuclear magnetic resonance spectrometry (NMR), raman spectroscopy, and infrared spectroscopy.

[0029] According to one embodiment of the above aspect, the comparison of the concentration levels of the Long COVID-related metabolites and/or proteins from the obtained sample to the concentration levels of the reference Long COVID-related metabolites and/or proteins from the Long COVID-negative sample comprises using multivariate statistical analysis.

[0030] According to one embodiment of the above aspect, the multivariate statistical analysis is selected from principal component analysis (PCA), or partial least squares projects to latent structures discriminant analysis (PLS-DA).

[0031] According to another aspect, there is provided a method of monitoring Long COVID progression and treating the Long COVID in a subject, the method comprising: (a) providing a first biological sample obtained from the subject at a first time; (b) assessing a first Long COVID-related metabolite and/or proteomic profile by measuring concentration levels of at least

one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5 from the first obtained sample; (c) comparing the first Long COVID-related metabolite and/or proteomic profile with a reference Long COVID-related metabolite profile and/or proteomic profile from an Long COVID-negative sample; (d) determining that there is a first difference between the first Long COVID-related metabolite and/or proteomic profile and the reference Long COVID-related metabolite and/or proteomic profile from the Long COVID-negative sample, the first difference being indicative of Long COVID; (e) providing a second biological sample obtained from the subject at a second time that is after the first time; (f) assessing a second Long COVID-related metabolite and/or proteomic profile by measuring concentration levels of the Long COVID-related metabolites and/or proteins from the second obtained sample; (g) comparing the second Long COVID-related metabolite and/or proteomic profile with the reference Long COVID-related metabolite and/or proteomic profile from the Long COVID-negative sample; (h) determining that there is a second difference between the first Long COVID-related metabolite and/or proteomic profile and the reference Long COVID-related metabolite and/or proteomic profile from the Long COVID-negative sample, the second difference being indicative of Long COVID; (i) determining Long COVID progression based on at least in part on the first and second differences; and (j) treating the subject as identified with an Long COVID treatment regime.

[0032] According to another aspect, there is provided a kit for use in the method of any one of the foregoing aspects or embodiments thereof comprising reagents for measuring concentration levels

of the Long COVID-related metabolites and/or the Long COVID-related proteins, optionally together with instructions for use.

[0033] According to another aspect, there is provided a kit for diagnosis Long COVID comprising: (a) a detector configured to detect concentration levels of at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5 in an obtained biological sample; (b) a composition comprising the corresponding Long COVID-related metabolites and/or Long COVID-related proteins in control levels corresponding to a control group of Long COVID-negative subjects; (c) a multivariate analysis system configured to analyze a difference in the concentration levels of the Long COVID-related metabolites and/or proteins and the control levels, and (d) optionally, instruction for an Long COVID diagnosis method; wherein the method comprises measuring, using the detector, the levels of the Long COVID-related metabolites and/or proteins from the obtained biological sample, and comparing the levels of the obtained Long COVID-related metabolites and/or proteins to the control levels of the Long COVID-related metabolites and/or proteins obtained from Long COVID-negative subjects.

[0034] According to an embodiment of the above aspect, the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine C16:1

(lysoPC C16:1); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); Hydroxysphingomyelin (SM (OH)) C22:2; Dodecanedioylcarnitine (C12-DC); Succinic acid; and Propionic acid.

[0035] According to an embodiment of the above aspect, the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); and Lysophosphatidylcholine C16:1 (lysoPC C16:1).

[0036] According to an embodiment of the above aspect, the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; Extracellular matrix protein 1; Complement Component C7; Plasminogen; Attractin; Vitamin K-dependent protein S; and Complement factor H.

[0037] According to an embodiment of the above aspect, the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; and Extracellular matrix protein 1.

[0038] According to an embodiment of the above aspect, the detector comprises a multi-metabolite detector and/or multi-proteomic detector configured to measure the levels of the Long COVID-related metabolites and/or the proteins.

[0039] According to another aspect, there is provided a computer-implemented method for processing a biological sample of a subject, diagnosing Long COVID and causing the treating the Long COVID, the computer-implemented method comprising: (a) receiving a biological sample obtained from the subject; (b) processing the sample in a spectroscopy unit directly or wirelessly linked to a processing device, the processing device having memory for storing measurement data from the spectroscopy unit; (c) in the spectroscopy unit, measuring levels of at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC

aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5 and storing the measurement data in the processor; (d) comparing the stored measurement data to a value in the memory representing an Long COVID- negative sample using multivariate statistical analysis; and (e) storing on the processing device a result corresponding to at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites and/or proteins from the obtained sample, wherein the result identifies the subject as having Long COVID if the measurement data representing the levels of the Long COVID-related metabolites and/or proteins are different relative to a concentration levels of reference Long COVID-related metabolites and/or proteins from an Long COVID-negative sample; and (f) displaying a Long COVID treatment regime on an electronic display connected directly or wirelessly to the processor for the subject identified as having Long COVID or as having predisposition of developing Long COVID, the displayed treatment regime comprising electronic text on a graphical user interface; and (g) causing the adjusting of blood and/or microbiome levels of one or more of the Long COVID-related metabolites and/or proteins in the subject diagnosed as having or predisposed of developing the Long COVID until an improvement in the cognitive performance in the subject is observed.

[0040] According to one embodiment of the above aspect, the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); Hydroxysphingomyelin (SM (OH)) C22:2; Dodecanedioylcarnitine (C12-DC); Succinic acid; and Propionic acid.

[0041] According to one embodiment of the above aspect, the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-

Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); and Lysophosphatidylcholine C16:1 (lysoPC C16:1).

[0042] According to one embodiment of the above aspect, the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; Extracellular matrix protein 1; Complement Component C7; Plasminogen; Attractin; Vitamin K-dependent protein S; and Complement factor H.

[0043] According to one embodiment of the above aspect, the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; and Extracellular matrix protein 1.

[0044] According to another aspect, there is provided a method for diagnosing and treating Long COVID comprising: (a) obtaining a signature of metabolites and proteins from a biological sample of a patient, the signature obtained by measuring Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC) and/or at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5; (b) classifying the patient into a Long COVID risk class or healthy class using a statistical analysis that has at least 85% predictive value comprising comparing the signature so obtained with a control signature, the classifying comprising a computer-implemented statistical analysis; and (c) treating or causing the treatment of the patient with a drug effective to treat, ameliorate or reduce the symptoms Long COVID, wherein the treating optionally comprises

adjusting the levels of one or more of the metabolites and/or proteins identified in the signature as being present at levels different than the control.

[0045] According to one embodiment of the foregoing aspect, the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); Hydroxysphingomyelin (SM (OH)) C22:2; Dodecanedioylcarnitine (C12-DC); Succinic acid; and Propionic acid.

[0046] According to one embodiment of the foregoing aspect, the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); and Lysophosphatidylcholine C16:1 (lysoPC C16:1).

[0047] According to one embodiment of the foregoing aspect, the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; Extracellular matrix protein 1; Complement Component C7; Plasminogen; Attractin; Vitamin K-dependent protein S; and Complement factor H.

[0048] According to one embodiment of the foregoing aspect, the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; and Extracellular matrix protein 1.

[0049] According to one embodiment of the foregoing aspect, the method further comprises adjusting or causing the adjustment of the levels of the metabolites and/or proteins comprises nucleic acid therapy.

[0050] According to one embodiment of the foregoing aspect, the nucleic acid therapy comprises reducing the level of a protein expressed or metabolite produced by siRNA or antisense therapy.

[0051] According to one embodiment of the foregoing aspect, the nucleic acid therapy comprises increasing the level of a protein expressed or metabolite produced by mRNA therapy.

[0052] According to one embodiment of the foregoing aspect, the metabolite and/or proteome profile measured is based on a profile identified in a previous computer-implemented statistical analysis model that has a predictive value of at least 90% and comprises at least one of means comparison, PCA, PLS-DA or recursive SVM data analyses.

[0053] According to one embodiment of the foregoing aspect, the metabolite and/or proteome profile has been previously identified as having the predictive value by classifying disease and non-disease samples into the two groups by a computer-implemented method based on the predicted metabolites and/or proteins used in the computer model and assessing whether the groups are separated.

[0054] According to another aspect of the disclosure, there is provided a method of identifying a subject's risk of developing Long COVID or identifying the subject as having Long COVID comprising: (a) obtaining one or more biological samples from the subject; (b) conducting a metabolomic and proteomic analysis of the one or more biological samples; (c) measuring at least at least one, two or all metabolites selected Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC) and at least one, two, three or all proteins, peptides or fragments thereof selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5, optionally wherein the metabolite(s) and/or protein(s) measured have a predictive score of at least 0.8 (80% or more) as determined on a VIP plot; and (d) identifying the subject as having Long COVID or a risk of development thereof if the levels of the metabolite(s) and/or protein(s) differ from a control reference; and (e) optionally providing results of the

metabolomic and/or proteomic analysis or a treatment regime based on said analysis via an on-line platform.

[0055] According to another aspect of the disclosure, there is provided a computer system comprising: (a) a high throughput spectroscopy unit for receiving one or more biological samples from a subject, and conducting a metabolomic and proteomic analysis of a panel of at least 25 metabolic and proteomic markers in the one or more biological samples; (b) optionally a computer processor for outputting a digital metabolomic and proteomic signature of the subject; (c) a computer processor for determining whether at least three Long COVID-related predictive metabolites are present at levels that differ from a control reference, thereby indicative of Long Covid, the predictive metabolites selected from 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); and Lysophosphatidylcholine C16:1 (lysoPC C16:1); (d) a computer processor for determining whether at least three Long COVID-related predictive proteins are present at levels that differ from a control reference, thereby indicative of Long Covid, the predictive proteins selected from Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; Extracellular matrix protein 1; Complement Component C7; Plasminogen; Attractin; Vitamin K-dependent protein S; and Complement factor H; wherein the metabolites and proteins measured in steps (c) and (d) have a predictive score of at least 0.8 (80% or more) as determined on a VIP plot; optionally, the computer processor compiling results of the step (c) and (d) in a database; (e) a wireless smart device comprising an application for receiving results from steps (c) and (d), the wireless smart device displaying results of the metabolomic and proteomic analysis via the application, the application having a dashboard displaying an assigned score for the Long COVID-related metabolites and proteins, and optionally displaying a treatment regime based on the assigned scores of the predictive metabolites and proteins, optionally the smart device receiving data from the database; and (f) optionally a computer processor for displaying a recommended treatment regime for the subject for treating symptoms of the Long Covid.

BRIEF DESCRIPTION OF THE DRAWINGS

[0056] While the specification concludes with claims particularly pointing out and distinctly claiming the disclosure, it is believed that the disclosure will be better understood from the following description of the accompanying figures wherein:

[0057] **Fig. 1A and 1B** show box plots for the short COVID vs. health control metabolites tested in Example 1.

[0058] **Fig. 2A and 2B** show box plots for the short COVID vs. health control proteins tested in Example 1.

[0059] **Fig. 3A and Fig. B** show box plots for the short COVID vs. long COVID control metabolites tested in Example 2.

[0060] **Fig. 4** shows box plots for the short COVID vs. long COVID control proteins tested in Example 2.

[0061] **Fig. 5** shows a PCA scatter plot for short COVID vs. healthy controls metabolomics data of Example 3.

[0062] **Fig. 6** shows a PCA scatter plot for short COVID vs. healthy controls proteomics data of Example 3.

[0063] **Fig. 7** shows a PCA scatter plot for short COVID vs. long COVID controls metabolomics data of Example 3.

[0064] **Fig. 8** shows a PCA scatter plot for short COVID vs. long COVID controls proteomics data of Example 3.

[0065] **Fig. 9** shows a Partial Least Squares Discriminate Analysis (PLS-DA) scatter plot for short COVID vs. healthy controls metabolomics data of Example 4.

[0066] **Fig. 10** shows a ROC curve for short COVID vs. healthy controls metabolomics data of Example 4.

[0067] **Fig. 11** shows VIP scores for the metabolites identified by the short COVID vs. healthy controls metabolomics data of Example 4.

[0068] **Fig. 12** shows a Partial Least Squares Discriminate Analysis (PLS-DA) scatter plot for short COVID vs. healthy controls proteomics data of Example 4.

[0069] **Fig. 13** shows a ROC curve for short COVID vs. healthy controls proteomics data of Example 4.

[0070] **Fig. 14** shows VIP scores for the proteins identified by the short COVID vs. healthy controls proteomics data of Example 4.

[0071] **Fig. 15** shows a Partial Least Squares Discriminate Analysis (PLS-DA) scatter plot for long COVID vs. short COVID metabolomics data of Example 5.

[0072] **Fig. 16** shows a ROC curve for long COVID vs. short COVID controls metabolomics data of Example 5.

[0073] **Fig. 17** shows VIP scores for the proteins identified by long COVID vs. short COVID metabolomics data of Example 5.

[0074] **Fig. 18** shows a Partial Least Squares Discriminate Analysis (PLS-DA) scatter plot for long COVID vs. short COVID proteomics data of Example 5.

[0075] **Fig. 19** shows a ROC curve for long COVID vs. short COVID controls proteomics data of Example 5.

[0076] **Fig. 20** shows VIP scores for the proteins identified by long COVID vs. short COVID proteomics data of Example 5.

[0077] In the drawings, exemplary embodiments are illustrated by way of example. It is to be expressly understood that the description and drawings are only for the purpose of illustrating certain embodiments and are an aid for understanding. They are not intended to be construed as limiting to the invention in any manner.

DETAILED DESCRIPTION

[0078] A detailed description of one or more embodiments of the invention is provided below along with accompanying figures that illustrate the principles of the invention. The invention is described in connection with such embodiments, but the invention is not limited to any particular embodiment described herein. The scope of the invention is limited only by the claims and equivalents thereof. Numerous specific details are set forth in the following description in order to

provide a thorough understanding of the invention. These details are provided for the purpose of providing non-limiting examples and the invention may be practiced according to the claims without some or all of these specific details. For the purpose of clarity, certain technical material that is known in the technical fields related to the invention has not been described in detail so that the invention is not unnecessarily obscured by such descriptions.

Definitions

[0079] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art to which the present invention pertains. As used herein, and unless stated otherwise or required otherwise by context, each of the following terms shall have the definition set forth below.

[0080] Articles such as “a” and “an” when used in a claim, are understood to mean one or more of what is claimed or described.

[0081] The terms “Long COVID” and “Post-COVID Condition” are used interchangeably to generally describe a condition caused or suspected of being caused by an earlier infection with the Sars-Cov-2 virus.

[0082] The term “Long COVID-negative” generally refers to a biological sample from an individual that does not suffer from Long COVID.

[0083] The term “Long COVID treatment regime” generally refers to an intervention made in response to a subject suffering from Long COVID. The aim of the regime may include, but is not limited to, one or more of the alleviation or prevention of symptoms, slowing or stopping the progression or worsening of Long COVID and the remission of Long COVID. In some embodiments, “Long COVID treatment regime” refers to therapeutic treatment (e.g., changing Long COVID-related metabolite levels and/or protein levels), dietary adjustments, and/or nutritional supplements.

[0084] The terms “comprises”, “comprising”, “include”, “includes”, “including”, “contain”, “contains” and “containing” are meant to be non-limiting, i.e., other steps and other sections which

do not affect the end of result can be added. The above terms encompass the terms “consisting of” and “consisting essentially of”.

[0085] The term “metabolite” generally refers to any molecule involved in metabolism. Metabolites can be products, substrates or intermediates in metabolic processes. Metabolites may include, without limitation, amino acids, peptides, acylcarnitines, monosaccharides, lipids and phospholipids, lysophospholipid, sphingolipids, glycerophospholipids, glucose, prostaglandins, hydroxy eicosatetraenoic acids, hydroxyoctadecadienoic acids, steroids, bile acids, glycolipids and phospholipids, including but not limited to lysophospholipids.

[0086] The term “protein” generally refers to a protein, fragment or peptide thereof unless otherwise specified.

[0087] The term “Long COVID-related metabolite” or “metabolomic profile” generally refers to metabolites associated with Long COVID comprising one, two or more metabolites described herein or a combination thereof.

[0088] The term “Long COVID-related protein” or “proteomic profile” generally refers to a profile of proteins, protein fragments and/or peptides associated with Long COVID comprising two or more, three or more, four or more, or five or more proteins described herein or a combination thereof. As would be appreciated by those of skill in the art, quantification of a protein can comprise quantifying a fragment or peptide thereof.

[0089] The terms “preferred”, “preferably” and variants generally refer to embodiments of the disclosure that afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful, and is not intended to exclude other embodiments from the scope of the disclosure.

[0090] The term “preventing”, and “prevention” are used interchangeably and generally refer to any activity that leads to a reduction in risk of developing Long COVID in the subject.

[0091] The term “subject” or “patient” is used without limitation and generally refers to a vertebrate, such as a mammal. The term “mammal” is defined as individual belonging to the class

Mammalia and includes, without limitation, humans, domestic and farm animals, and zoo, sports or pet animals, such as sheep, dogs, horses, cats or cattle. In some embodiments, the subject is human.

[0092] The term “treating”, or “treatment” generally refers to an intervention made in response to Long COVID or associated symptoms manifested by a subject. The aim of treatment may include, but is not limited to, one or more of the alleviation or prevention of Long COVID, slowing or stopping the progression or worsening of Long COVID and the remission of Long COVID. In certain embodiments, “treatment” refers to therapeutic, dietary and/or supplemental therapy.

[0093] In all embodiments of the present disclosure, all percentages, concentrations, parts and ratios are based upon the total weight of the compositions of the present disclosure, unless otherwise specified. All such weights as they pertain to listed ingredients are based on the active level and, therefore do not include solvents or by-products that may be included in commercially available materials, unless otherwise specified.

[0094] All ratios are weight ratios unless specifically stated otherwise. All temperatures are in Celsius degrees (°C), unless specifically stated otherwise. All dimensions and values disclosed herein (e.g., quantities, percentages, portions, and proportions) are not to be understood as being strictly limited to the exact numerical values recited. Instead, unless otherwise specified, each such dimension or value is intended to mean both the recited value and a functionally equivalent range surrounding that value. For example, a dimension disclosed as “40 mm” is intended to mean “about 40 mm.”

Metabolite and Protein Combinations with High Predictive Value

[0095] In one embodiment, the present disclosure relates to methods for diagnosis and treatment of Long COVID, and any associated symptoms, in a subject. The disclosure is based, at least in part, on the identification of new metabolites and/or new proteins that provide for metabolite-based and/or protein/peptide-based identification of Long COVID in a subject that can lead to more effective therapy.

[0096] Given the complexities of the interactions between genetics and the environment, metabolic and/or proteomic profiling as described herein can provide molecular-based tests that

aid in individualized treatment regimes. In one embodiment, metabolomic and/or proteomic based analyses as disclosed herein have the advantage of identifying marker profiles derived from an individual's inherited genes and/or the interactions of the individual's current lifestyle behaviors (e.g., smoking, alcohol consumption, sleep behaviours, physical activity and the like), gut microbiome, dietary, and environmental factors that contribute to the unique metabolic profile and/or proteomic profile of a subject with Long COVID. Therefore, the present disclosure provides an advancement in the art.

[0097] The metabolomic profile for Long COVID comprises at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC).

[0098] According to one embodiment, the metabolomic profile for Long COVID comprises at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from spermine, pyruvic acid; 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPPA); 3-Hydroxyoleoylcarnitine (C18:1-OH); phosphatidylcholine diacyl C32:2 (PC aa C32:2); hydroxysphingomyelin (SM (OH)) C22:2; lysophosphatidylcholine, C18:0 (lysoPC C18:0); and succinic acid.

[0099] According to another embodiment, the metabolomic profile for Long COVID comprises at least one, at least two or all of the Long COVID-related metabolites selected from spermine, pyruvic acid; 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPPA) and 3-Hydroxyoleoylcarnitine (C18:1-OH).

[00100] According to a further aspect of the present disclosure, a new proteomic profile for Long COVID is identified in a subject having Long COVID. The proteomic profile for Long COVID comprises at least one, at least two, at least three, at least four or at least five Long COVID-related proteins, peptides or fragments thereof selected from Fibronectin; Ficolin-2; Hemoglobin subunit

alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5.

[00101] According to one embodiment, the proteomic profile for Long COVID comprises at least one, at least two, at least three, at least four or at least five Long COVID-related proteins, peptides or fragments thereof selected from carboxypeptidase N subunit 2; inter-alpha-trypsin; extracellular matrix 1; zinc-alpha-2-glycoprotein; coagulation factor X; complement C2; lumican; and complement factor H.

[00102] According to one embodiment, the proteomic profile for Long COVID comprises at least one, at least two or all of the Long COVID-related proteins selected from carboxypeptidase N subunit 2; inter-alpha-trypsin; and extracellular matrix 1.

[00103] It has been surprisingly discovered that the metabolomic profile and/or proteomic profile are altered in a subject suffering from COVID as compared to non-COVID individual. In particular, the levels of the Long COVID-related metabolites and/or Long COVID-related proteins may be altered in circulation of the subject having Long COVID as compared to a non-Long COVID individual. In certain embodiments, the levels of the Long COVID-related metabolites and/or Long COVID-related proteins are altered in the blood (e.g., serum, plasma), body fluids (e.g., cerebrospinal fluid, pleural fluid, amniotic fluid, semen, or saliva), urine, and/or feces of the subject having Long COVID. Without wishing to be bound by theory, it is believed that the Long COVID-related metabolites and/or Long COVID-related proteins play a causative role in the development of Long COVID-related symptoms in the subject having Long COVID.

[00104] In one example, the present disclosure provides for a method for diagnosing and treating Long COVID in a subject. The method comprises step (a) providing a biological sample obtained from the subject, preferably a human. In accordance with the methods disclosed herein, any type of biological sample that originates anywhere from the body of a subject may be tested, including but not limited to, blood (including, but not limited to serum or plasma), cerebrospinal fluid ("CSF"), pleural fluid, urine, stool, sweat, tears, breath condensate, saliva vitreous humour, a tissue sample, amniotic fluid, a chorionic villus sampling, brain tissue, a biopsy of any solid tissue

including tumor, adjacent normal, smooth and skeletal muscle, adipose tissue, liver, skin, hair, brain, kidney, pancreas, lung or the like may be used. Preferably, the biological sample obtained from a live subject is urine. The Long COVID-related metabolites and/or proteins may be extracted from their biological source using any number of extraction/clean-up procedures that are typically used in quantitative analytical chemistry.

[00105] The method further comprises step (b) measuring from the obtained sample, concentration levels of at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC).; and/or Long COVID-related proteins, peptides or fragments thereof selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5.

[00106] In certain embodiments, the method comprises measuring at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 Long COVID-related metabolites and/or proteins from the obtained sample.

[00107] In certain embodiments, the measurement of the concentration levels of the Long COVID-related metabolites and/or proteins may be through mass spectrometry, including but not limited to gas chromatography mass spectrometry (GC-MS) GC and liquid chromatography mass spectrometry (e.g., LC-MS, LC-MS-MS, LC-MRM, LC-SIM, and LC-SRM). Preferably, the Long COVID-related metabolites are measured by a spectroscopic technique, wherein the spectroscopic technique is selected from the group consisting of liquid chromatography, gas chromatography, liquid chromatography mass spectrometry, gas chromatography mass spectrometry, high performance liquid chromatography mass spectrometry, capillary electrophoresis mass

spectrometry, nuclear magnetic resonance spectrometry (NMR), raman spectroscopy, and infrared spectroscopy. The measurement may also be performed under other methodology, such as for example, a colorimetric, enzymatic, immunological methodology, and gene expression analysis including, for example, real-time PCR, RT-PCT, northern analysis, and in situ hybridization.

[00108] In some embodiments, the mass spectrometry process for determining whether the Long COVID-related proteins are elevated comprises enzymatic or chemical digestion of the proteins or peptide fragments thereof of a sample obtained from a subject into peptide fragments. The peptide fragments are optionally separated and/or ionized and captured by mass spectrometry. The digestion may comprise a proteolytic digestion involving treating a preparation comprising the Long COVID-related proteins with an acid, base, or an enzyme such as trypsin or other proteolytic enzyme. One embodiment comprises a shotgun proteomics quantification in which the whole proteins in a complex mixture, such as serum, urine, and cell lysates, are hydrolyzed or otherwise cut into peptides and followed by multidimensional HPLC-MS, which aims to generate a global profile of protein mixtures as genome “shotgun” sequencing.

[00109] Thus, according to one aspect of the disclosure, there is provided a method for determining whether Long COVID-related proteins, or peptide fragments thereof, are elevated in a sample obtained from a subject, the Long COVID-related proteins, peptides or fragments thereof being selected from at least one of Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5, the method comprising enzymatic or chemical digestion of the proteins or peptide fragments thereof of the sample obtained from a subject into peptide fragments to produce peptide fragments thereof; introducing a solution comprising the peptide fragments to a mass spectrometer, optionally after one or more treatments comprising liquid chromatography or other treatments, to quantify the peptide fragments; determining the concentration of peptide fragment(s) relative to a baseline, such as a standard(s) (e.g., peptide standards); and assessing whether the fragment(s) are elevated relative to the baseline or standard(s); identifying the subject as having Long COVID or a being predisposed to developing same if one or more of the peptides are elevated relative to the baseline or standard; and treating or causing the treating of the subject with an Long COVID treatment,

optionally comprising an agent that is approved for use to treat Long COVID in a relevant jurisdiction.

[00110] According to one example, the peptide fragments include at least one, at least two, at least three, at least four or at least five Long COVID-related protein fragments selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5.

[00111] According to a further example, the peptide fragments include at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5.

[00112] According to a further example, the peptide fragments for Long COVID comprises at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5.

[00113] In certain embodiments, with any of the methods described herein, the methods may further include measuring the concentration levels of one or more additional Long COVID-related metabolites and/or proteins, including, but not limited to, any of those described herein and/or additional markers known in the art. The novel approach of the present disclosure identifies biomarkers that have high predictive value for a subset of the diagnostic class (i.e., Long COVID in this case). In one embodiment, the advantage of including additional Long COVID-related metabolites and/or proteins is to increase the overall sensitivity of the diagnostic method.

[00114] The method described herein further comprises step (c) comparing the concentration levels of the Long COVID-related metabolites and/or proteins from the obtained sample to the concentration levels of reference Long COVID-related metabolites and/or proteins from an Long COVID-negative sample. One skilled in the art will appreciate that references can be established as a value representative of the level of Long COVID-related metabolites and/or proteins in a non-Long COVID population that do not suffer from Long COVID for the comparison. Various criteria may be used to determine the inclusion and/or exclusion of a particular subject in the reference population, including age of the subject (e.g., the reference subject can be within the same age group as the subject in need of treatment) and gender of the subject (e.g., the reference subject can be the same gender as the subject in need of treatment).

[00115] The method described herein further comprises step (d) identifying the subject as having Long COVID if the concentration levels of the Long COVID-related metabolites and/or proteins, peptides or fragments thereof from the obtained sample are different relative to the concentration levels of the reference.

[00116] In certain embodiments, the identifying step (d) occurs upon determination that the concentration level of the at least one Long COVID-related metabolite and/or protein, peptide or fragments thereof from the obtained sample differs by about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, or about 70% or more relative to the concentration level of the at least one reference Long COVID-related metabolites and/or proteins from the Long COVID-negative sample. In certain embodiments, the identifying step (d) occurs upon determination that the concentration levels of at least two, at least three, at least four or at least five Long COVID-related metabolites and/or proteins from the obtained sample differ by about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, or about 70% or more relative to the concentration levels of the reference Long COVID-related metabolites and/or proteins from the Long COVID-negative sample.

[00117] The method described herein further comprises step (e) treating the subject so identified as having Long COVID with a Long COVID treatment regime.

[00118] In certain embodiments, with any of the methods described herein, the comparison of the concentration level of the at least one Long COVID-related metabolite and/or protein from the

obtained sample to the concentration level of the reference Long COVID-related metabolite and/or protein from the Long COVID-negative sample comprises using multivariate statistical analysis. Preferably, the multivariate statistical analysis is selected from principal component analysis (“PCA”), or partial least squares projects to latent structures discriminant analysis (“PLS-DA”). In certain embodiments, a computer is used for statistical analysis. Data for statistical analysis can be extracted from chromatograms (i.e., spectra of mass signals) using software for statistical methods known in the art.

[00119] In some aspects, the present disclosure relates to a method of monitoring Long COVID progression and treating Long COVID in a subject. In some embodiments, the method includes quantifying the Long COVID-related metabolites and/or proteins at one or more time points after the initiation of treatment to monitor Long COVID progression (e.g., rate of decline or rate of improvement of Long COVID progression) in a subject.

[00120] Accordingly, the method comprises: (a) providing a first biological sample obtained from the subject at a first time; (b) assessing a first Long COVID-related metabolite profile by measuring concentration levels of at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, two, three or all proteins, peptides or fragments thereof are selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5 ; from the first obtained sample; (c) comparing the first Long COVID- related metabolite and/or proteomic profile with a reference Long COVID-related metabolite profile from an Long COVID-negative sample; (d) determining that there is a first difference between the first Long COVID-related metabolite and/or proteomic profile and the reference Long COVID-related metabolite and/or proteomic profile from the Long COVID-

negative sample, the first difference being indicative of Long COVID; (e) providing a second biological sample obtained from the subject at a second time that is after the first time; (f) assessing a second Long COVID-related metabolite profile and/or proteomic profile by measuring concentration levels of the Long COVID-related metabolites and/or proteins from the second obtained sample; (g) comparing the second Long COVID-related metabolite and/or proteomic profile with the reference Long COVID-related metabolite and/or proteomic profile from the Long COVID-negative sample; (h) determining that there is a second difference between the first Long COVID-related metabolite profile and/or proteomic profile and the reference Long COVID-related metabolite and/or proteomic profile from the Long COVID-negative sample, the second difference being indicative of Long COVID; (i) determining Long COVID progression based on at least in part on the first and second difference; and (j) treating the subject as identified with an Long COVID treatment regime.

[00121] In certain embodiments of the above method, the period between the first time and the second time is at least 1 month, at least 2 months, at least 3 months, at least 6 months, at least 9 months, or at least 12 months, preferably at least 3 months. In some embodiments, the treatment has been administered to the subject before the first two biological samples have been obtained. In other embodiments, the treatment has been administered to the subject in the interval(s) between the taking of the biological samples. In certain embodiments, the first biological sample, the second biological sample, or both are blood or urine, preferably serum, plasma or urine.

[00122] According to one embodiment, the metabolomic profile for Long COVID comprises at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from spermine, pyruvic acid; 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPPHA); 3-Hydroxyoleoylcarnitine (C18:1-OH); phosphatidylcholine diacyl C32:2 (PC aa C32:2); hydroxysphingomyelin (SM (OH)) C22:2; lysophosphatidylcholine, C18:0 (lysoPC C18:0); and succinic acid.

[00123] According to one embodiment, the metabolomic profile for Long COVID comprises at least one, at least two or all of the Long COVID-related metabolites selected from spermine, pyruvic acid; 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPPHA) and 3-Hydroxyoleoylcarnitine (C18:1-OH).

[00124] According to embodiments of the above method, the proteomic profile for Long COVID comprises at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from carboxypeptidase N subunit 2; inter-alpha-trypsin; extracellular matrix 1; zinc-alpha-2-glycoprotein; coagulation factor X; complement C2; lumican; and complement factor H.

[00125] According to embodiments of the above method, the proteomic profile for Long COVID comprises at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from carboxypeptidase N subunit 2; inter-alpha-trypsin; and extracellular matrix 1.

Long COVID treatment regime

[00126] In one embodiment, the Long COVID treatment regime has the effect of adjusting the concentration levels of one or more of the Long COVID-related metabolites and/or proteins in the subject identified as having Long COVID towards the corresponding levels of the reference Long COVID-related metabolites and/or proteins from the Long COVID-negative sample.

[00127] Various methods can be used to adjust the concentration level, for example blood level (e.g., serum level), of the Long COVID-related metabolite and/or protein in the subject. Preferably, the adjustment of the concentration level of the one or more Long COVID-related metabolites in the subject occurs until an improvement in symptoms in the subject is observed.

[00128] In certain embodiments, an antibody that specifically binds the Long COVID-related metabolite, an intermediate for the in vivo synthesis of the Long COVID-related metabolite, or a substrate for the in vivo synthesis of the Long COVID-related metabolite can be administered to the subject. For example, an antibody that specifically binds one or more of metabolites and/or proteins on the metabolomic and/or proteomic profile can be used to reduce the levels thereof in the subject.

[00129] In certain embodiments, the concentration level, for example blood level (e.g., serum level), of the one or more Long COVID-related metabolites is adjusted by adjusting the composition of gut microbiota in the subject.

[00130] In further embodiments, nucleic acid therapy can be used to reduce the concentration of a protein (which includes a peptide) by using, for example, siRNA or antisense oligonucleotides to reduce expression of the protein. Likewise, nucleic acid therapy can be used to express a protein that is present at lower levels in the Long COVID-free reference using mRNA therapy. The therapeutic nucleic acid can be encapsulated in a suitable delivery vehicle. Nucleic acid therapy can also be used to increase the level of a metabolite described herein by using, for example, mRNA, antisense or siRNA therapy by, for example, modulating the activity of a protein that is involved in metabolism.

[00131] In another embodiment, the subject diagnosed or identified as being predisposed to developing Long COVID is treated or caused to be treated with an approved Long COVID therapeutic, such as a drug. The drug may be approved by any applicable regulator. An example of a Long COVID drug is nirmatrelvir/ritonavir (Paxlovid). Other examples include beta blockers or metformin. Thus, according to one embodiment, the Long COVID drug is selected from Paxlovid or metformin.

Kits

[00132] The metabolomic and/or proteomic profile described herein may be utilized in tests, assays, methods, kits for diagnosing, predicting, modulating or monitoring Long COVID, including ongoing assessment, monitoring and/or susceptibility assessment. The present disclosure includes a kit for diagnosis of Long COVID by measuring and identifying at least one or more Long COVID-related metabolites and/or protein associated with Long COVID. Preferably, the kit may comprise appropriate Long COVID treatment regime to be initiated upon the determination of Long COVID. Accordingly, the kit comprises (a) a detector configured to detect concentration levels of at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC).; and/or at least one, at least

two, at least three, at least four or at least five Long COVID-related proteins (e.g., peptides) selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5 control levels corresponding to control group of Long COVID-negative subjects, (c) a multivariate analysis system configured to analyze a difference in the concentration levels of the Long COVID-related metabolites and/or proteins and the control levels, and (d) optionally, instruction for an Long COVID diagnosis method, wherein the method comprises measuring, using the detector, the levels of the Long COVID-related metabolites and/or proteins from the obtained biological sample, and comparing the levels of the obtained Long COVID-related metabolites and/or proteins to the control levels of the Long COVID-related metabolites and/or proteins obtained from Long COVID-negative subjects. In one embodiment, the Long COVID diagnosis method comprises a multi-metabolite or protein (e.g., peptide) detector configured to measure the levels of Long COVID-related metabolites and/or proteins.

[00133] In some aspects, the kit may be for the measurement of the Long COVID-related metabolites and/or proteins by a physical separation technique (as described herein above). In some aspects, the kit may be for measurement of the Long COVID-related metabolites and/or proteins by a methodology other than a physical separation method, such as for non-limiting example, a colorimetric, enzymatic, and immunological methodology. The kit may also include one or more appropriate negative and/or positive controls. Kits of the present disclosure may include other reagents such as buffers and solutions needed to perform the tests.

Computer-Implemented System and Method

[00134] In a further embodiment, the metabolite and/or proteome profile measured is based on a profile identified in a previous computer-implemented statistical analysis model that has a predictive value of at least 80%, 85%, 90%, 92%, 94% or 96% and comprises at least one of means comparison, PCA, PLS-DA or recursive SVM data analyses.

[00135] In a further embodiment, the metabolite and/or proteome profile is identified as having the predictive value by classifying the samples into the two groups by a computer-implemented

method based on the predicted metabolites and/or proteins used in the computer model, wherein the data from two groups are sufficiently separated, such as on a scores plot (e.g., see Examples below and Figures 4 and 12 that exemplify separation of a control and COVID cohorts).

[00136] The disclosure is also directed to a computer-implemented method for processing a biological sample of a subject, diagnosing a Long COVID and treating (or causing treatment thereof) of the subject diagnosed with Long COVID. The computer-implemented method may further allow monitoring of Long COVID progression across multiple time points to support a more effective treatment regime.

[00137] The computer-implemented method comprises receiving a biological sample from the subject; processing the sample in a spectroscopy unit directly or wirelessly linked, or may utilize any suitable communication technology, to a processing device, the processing device having memory for storing measurement data from the spectroscopy unit; and in the spectroscopy unit, measuring levels of least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, two, three or all proteins, peptides or fragments thereof are selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5 ; and storing the measurement data in the processor. The processing device comprises one or more data storage devices that may be configured or adapted to store data related to the method. For example, the data storage device may be configured or adapted to store measurement data from the spectroscopy unit. The data storage device may also comprise computer program code stored thereon. The program code of this embodiment may include program code for at least performing the steps of the method aspect upon execution thereof.

[00138] The computer-implemented method further comprises comparing the stored measurement data to a value in the memory representing an Long COVID-negative sample using multivariate statistical analysis; storing on the processing device a result corresponding to at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, two, three or all proteins, peptides or fragments thereof are selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5 from the obtained sample, wherein the result identifies the subject as having Long COVID if the measurement data representing the level of the Long COVID-related metabolite and/or protein is different relative to a concentration value of a reference Long COVID-related metabolite and/or protein from an Long COVID-negative sample; and displaying an Long COVID treatment regime on an electronic display connected directly or wirelessly to the processor for the subject identified as having Long COVID or as having predisposition of developing Long COVID. The displayed treatment regime comprises electronic text, optionally with graphical icons, on a graphical user interface describing one or more of: dietary adjustments, nutritional supplements, behavior training or a combination thereof, to the subject diagnosed as having or predisposed of developing the Long COVID, or adjusting the blood levels of one or more of the Long COVID- related metabolites and/or proteins in the subject diagnosed as having or predisposed of developing the Long COVID until an improvement in the cognitive and/or behavioral performance in the subject is observed; preferably the adjustment of the blood levels of one or more of the Long COVID-related metabolites comprises adjusting the composition of gut microbiota in the subject.

[00139] In one embodiment, a wireless smart device comprising an application receives results from a metabolomic and/or proteomic analysis of the subject. The wireless smart device displays results of the metabolomic and proteomic analysis via the application. The application may have

a dashboard displaying an assigned score for the Long COVID-related metabolites and proteins, and optionally displays a treatment regime based on the assigned scores of the predictive metabolites and proteins. Optionally, the smart device receives data from a database comprising the results of the metabolomic and proteomic analysis. In one embodiment a graphical user interface comprises a dashboard with the graphical icons describing one or more treatment regimes.

EXAMPLES

[00140] The following examples describe some exemplary modes of practicing certain methods that are described herein. It should be understood that the examples are for illustrative purposes only and are not meant to limit the scope of the systems and methods described herein.

[00141] Supervised machine learning is a type of artificial intelligence that can be used to build predictive models for disease prediction. The process uses a large dataset of labeled patient information, which can include demographic information, medical and family history, and clinical results, to train a machine learning algorithm to identify patterns and relationships that are associated with a particular risk of developing that disease. Once the model has been trained, it can be used to make predictions about new patients based on their demographic and clinical information.

[00142] The inventors used this method to classify the molecular profile of long COVID and healthy controls as well as long COVID verses short COVID to develop a machine learning (ML) model that can predict if a new sample belongs to each category. The demographics for the data sets used in the analysis are set forth in Table 1 and Table 2 below. Table 1 shows the demographics for the short COVID samples and the controls, which were healthy age and sex-matched.

Table 1: Short COVID vs. healthy control

summary_stat	case	control	p
n	231	326	
age	43.6 (11.5)	46.0 (12.3)	0.02
female	0.667	0.65	1.0
male	0.333	0.35	1.0

[00143] Table 2 below shows the demographics for the long COVID and the short COVID samples. This comparison was to determine if there were any distinguishing features for patients with long-COVID.

Table 2: Long (case) and short covid (control) data set demographics

No.	summary_stat	case	control	p
0	n	73	125	0.016
1	age	43.1 (11.9)	47.5 (12.5)	0.108
2	bmi	29.2 (7.0)	30.7 (7.7)	0.116
3	height_cm	157.0 (10.3)	160.9 (11.6)	0.361
4	weight_kg	72.0 (19.1)	79.2 (21.4)	0.33
5	female	53	81	0.33
6	male	20	44	0.001
7	exercise_novice	8	13	0.001

8	exercise_intermediate	5	77	0.083
9	exercise_elite	3	15	0.083
10	alcohol_1	11	31	0.083
11	alcohol_2-4	12	37	0.083
12	alcohol_>4	16	19	0.083

Example 1: Student T-test and box plots for short COVID vs. healthy control

Metabolomics

[00144] A means comparison using a student t-test was conducted on transformed metabolomic and proteomic data for short COVID vs. healthy controls. The first 10 rows of the T-test output for the metabolites are displayed below (Table 3). The biomarkers are sorted by the lowest P-value (FDR) first, followed by the highest fold change.

Table 3: Metabolomics T-test results

No.	BIOMARKER	MEAN±SD: CONTROL	MEAN ±SD: CASE	MEAN (IQR): CONTROL	MEDIAN (IQR): CASE	FOLD CHANGE	P	P (FDR)
1	benzoic acid	0.20 ± 0.12	0.31 ± 0.60	0.15 (0.13)	0.22 (0.19)	1.53	<0.001	<0.001
2	lysophosphatidic acid 18:0	23.17 ± 7.18	33.51 ± 10.08	22.29 (8.88)	32.25 (14.43)	1.45	<0.001	<0.001
3	phosphatidylcholine 32:2	3.43 ± 1.22	4.79 ± 1.44	3.26 (1.24)	4.71 (1.82)	1.40	<0.001	<0.001
4	creatine	20.35 ± 9.80	28.13 ± 15.31	17.10 (15.27)	26.10 (19.40)	1.38	<0.001	<0.001

5	lysopc ac16:1	1.93± 0.69	2.58 ± 1.16	1.82 (0.89)	2.31 (1.05)	1.34	<0,001	<0.001
6	sm(OH)c122	11.20 ± 3.95	14.82 ± 3.82	10.01 (5.82)	14.64 (5.55)	1.32	<0.001	<0.001
7	lysopc ac16:0	72.66 ± 19.71	94.34 ± 25.22	69.30 (24.56)	92,89 (33.82)	1.30	<0,001	<0,001
8	glutamic acid	50.47 ± 19.54	62.29 ± 24.06	47.73 (22.92)	58.20 (33.50)	1.23	<0.001	<0.001
9	lysopc a c20:4	4.72 ± 1.83	5.83 ± 1.97	4.371226)	5.49 (2.48)	1.23	<0.001	<0.001
10	pc ae c36:0	1.41 ± 0.54	1.73 ± 0.50	1.28 (0.71)	1.66 (0.62)	1.22	<0.001	<0.001

[00145] The corresponding metabolomic box plots are shown in Figure 1.

Proteomics

[00146] The first 10 rows of the T-test output for the proteins are displayed below (Table 4). As with the metabolites, the proteins are sorted by the lowest P-value (FDR) first, followed by the highest fold change.

Table 4: Proteomics T-test results

No.	BIOMARKER	MEAN±SD: CONTROL	MEAN±SD: CASE	MEAN (IQR): CONTROL	MEDIAN (IQR): CASE	FOLD CHANGE	P
1	fibronectin	694.90 ± 533.86	1324.21 ± 613.68	561.52 (555.36)	1320.35 (917.05)	1.91	<0.001
2	hemoglobin subunit alpha 1	537.41 ± 293.28	964.15 ± 714.65	479.65 (233.79)	807.02 (502.14)	1.79	<0.001
3	Apolipoprotein B	1352.06 ± 491.50	1720.59 ± 893.17	1287.70 (702.72)	1535.55 (1038.25)	1.27	<0.001

4	Vitamin k-dependent protein z	61.46 ± 21.40	75.24 ± 28.69	59.88 (26.50)	70.10 (35.59)	122	<0.001
5	Coagulation factor xiii a chain	96.54 ± 37.85	116.13 ± 41.51	86.66 (36.60)	105.97 (45.18)	1.21	<0.001
6	Fibrinogen beta chain	10424.15 ± 2667.79	12518.04 ± 3411.35	9955.90 (3000.18)	12106.00 (4941.52)	1.20	<0.001
7	apolipoprotein b-100	270.19 ± 76.40	321.04 ± 105.69	273.10 (106.53)	305.62 (148.28)	1.19	<0.001
8	Extracellular matrix protein 1	56.07 ± 12.10	66.70 ± 15.06	55.08 (12.03)	67.03 (18.37)	1.19	<0.001
9	Fibrinogen alpha chain	14232.37 ± 3659.46	16640.05 ± 4756.87	13500.00 (3786.00)	16221.00 (7055.25)	1.17	<0.001
10	Zinc-alpha-2-glycoprotein	339.67 ± 90.56	397.37 ± 81.66	314.93 (131.80)	387.32 (99.51)	1.17	<0.001

[00147] The corresponding proteomics box plots are shown in Figure 2.

Example 2: Student T-test and box plots for long COVID vs. short COVID control

Metabolomics

[00148] A means comparison using a student t-test was conducted on transformed metabolomic and proteomic data for long COVID vs. short COVID controls. The first 10 rows of the T-test output for the metabolites are displayed below (Table 5). The biomarkers are sorted by the lowest P-value (FDR) first, followed by the highest fold change.

Table 5: Metabolomics T-test results

No.	BIOMARKER	MEAN±SD: CONTROL	MEAN±SD: CASE	MEAN (IQR): CONTRO L	MEDIAN (IQR): CASE	FOLD CHANGE	P	P (FDR)
1	lysopc a c18:0	23.16 ± 7.25	34.56 ± 9.89	22.10 (8.80)	33.57 (15.06)	1.49	<0.001	<0.001
2	pc aa c32:2	3.48 ± 1.23	4.76 ± 1.37	3.29 (1.26)	4.71 (1.78)	1.37	<0.001	<0.001
3	lysopc a c16:1	1.92 ± 0.69	2.62 ± 0.96	182 (0.90)	2.53 (1.12)	1.36	<0.001	<0.001
4	lysopc a c16:0	72.53 ± 20.04	95.38 ± 25.25	68.85 (25.19)	93.78 (35.35)	1.32	<0.001	<0.001
5	creatine	20.73 ± 9.90	27.25 ± 12.28	18.00 (15.00)	25.60 (16.60)	1.31	<0.001	<0.001
6	c16:2	0.01 ± 0.00	0.02 ± 0.02	0.01 (0.00)	0.02 (0.01)	1.29	<0.001	<0.001
7	sm (OH) c22:2	11.39 ± 3.97	14.60 ± 3.61	10.19 (5.91)	14.64 (4.79)	1.28	<0.001	<0.001
8	lysopc a c17:0	1.52 ± 0.44	1.89 ± 0.57	1.47 (0.50)	1.76 (0.65)	1.25	<0.001	<0.001
9	pc ae c36:0	1.43 ± 0.54	1.71 ± 0.42	1.29 (0.76)	1.67 (0.49)	1.20	<0.001	<0.001
10	lysopc a c18:1	18.30 ± 6.20	21.99± 6.12	17.00 (8 98)	20.83 (6.18)	1.20	<0.001	<0.001

[00149] The corresponding metabolomics box plots are shown in Figure 3.

Proteomics

[00150] The first 10 rows of the T-test output for the proteins are displayed below (Table 6). As with the metabolites, the proteins are sorted by the lowest P-value (FDR) first, followed by the highest fold change.

Table 6: Proteomics T-test results

No.	BIOMARKER	MEAN±SD: CONTROL	MEAN±SD: CASE	MEAN (IQR): CONTROL	MEDIAN (IQR): CASE	FOLD CHANGE	P
1	fibronectin	671.12 ± 455.38	1290.76 ± 582.92	550.72 (573.64)	1394.90 (951.42)	1.92	<0.001
2	hemoglobin subunit alpha 1	543.90 ± 299.26	967.60 ± 652.29	480.70 (226.12)	834.00 (546.70)	1.78	<0.001
3	Apolipoprotein b-100	268.92 ± 77.37	337.57 ± 93.52	271.29 (105.29)	330.22 (130.92)	1.26	<0.001
4	coagulation factor xii	329.13 ± 120.90	408.50 ± 118.80	323.97 (177.85)	408.55 (164.21)	1.24	<0.001
5	fibrinogen beta chain	10363.62 ± 2466.70	12760.15 ± 3482.62	9955.90 (2962.73)	11576.00 (5427.50)	1.23	<0.001
6	complement factor h	1126.69 ± 243.85	1332.41 ± 294.43	1075.80 (360.74)	1297.00 (417.50)	1.18	<0.001
7	fibrinogen gamma chain	12259.73 ± 3039.92	14470.43 ± 3720.65	11779.50 (3976.95)	14208.00 (5942.00)	1.18	<0.001
8	Complement c2	117.59 ± 21.61	137.15 ± 25.55	114.99 (30.36)	136.13 (35.33)	1.17	<0.001
9	Extracellular matrix protein 1	55.55 ± 11.00	64.82 ± 14.93	55.21 (11.80)	63.59 (17.85)	1.17	<0.001
10	Attractin	104.63 ± 18.04	118.96 ± 17.98	104.75 (34.36)	118.64 (22.12)	1.14	<0.001

[00151] The corresponding metabolomics box plots are shown in Figure 4.

Example 3: Principal component analysis (PCA) of metabolomic and proteomic data for short COVID vs healthy controls and long COVID vs. short COVID controls

[00152] The following describes metabolomic output from principal component analysis (PCA) for short COVID vs. healthy controls.

[00153] A PCA scores plot is a graphical representation of the results of a principal component analysis (PCA) carried out on a dataset. PCA is a statistical technique that is used to reduce the dimensionality of a dataset by identifying and removing redundant or correlated variables and projecting the data onto a smaller number of orthogonal (uncorrelated) dimensions, called principal components.

[00154] A PCA scores plot is a scatterplot that shows the projection of the data onto the first two principal components. Each point on the plot represents a single data point from the original dataset, and the position of the point reflects the values of the data point on the two principal components. The x-axis of the plot represents the first principal component, and the y-axis represents the second principal component.

[00155] The PCA scatter plot for short COVID vs. healthy controls metabolomics data is shown in Figure 5.

[00156] Proteomic output PCA data for short COVID vs. healthy controls is shown in the scatter plot of Figure 6.

[00157] The PCA scatter plot for long COVID vs. short COVID controls metabolomics data is shown in Figure 7.

[00158] Proteomic output PCA data for long COVID vs. short COVID controls is shown in the scatter plot of Figure 8.

Example 4: Partial least squares discriminant analysis (PLS-DA) of metabolomic and proteomic data for short COVID vs healthy controls

Metabolomics

[00159] PLS-DA is a supervised learning technique that combines partial least squares regression (PLS) and linear discriminant analysis (LDA) to classify data into different classes. The analysis is a non-linear technique based on the relationship between predictor and response variables. PLS-DA is used to find latent variables that best discriminate between classes.

[00160] The PCA scatter plot for short COVID vs. healthy controls metabolomics data is shown in Figure 9. The model metrics are shown in Table 7 below:

Table 7: Model metrics for short COVID vs. healthy controls metabolomics PLS-DA data

	R2	MSE	RPD	ACCURACY
1	0.5784752102	0.09754292657	1.542366085	0.9357798165

[00161] The Receiver Operating Curve (ROC) curve for short COVID vs. healthy controls metabolomics data is shown in Figure 10. The CI is between 0.9 and 0.93. ROC is a graphical representation of the performance of a binary classification model. It is plotted on a two-dimensional graph with the true positive rate on the y-axis and the false positive rate on the x-axis. A model with a higher true positive rate and a lower false positive rate will have a better performance. Area under the curve (AUC) is a measure of the separability of the classes in the data. It is calculated as the area under the Receiver Operating Characteristic (ROC) curve, which plots the true positive rate (sensitivity) against the false positive rate (1-specificity) at various threshold settings. AUC is a single value between 0 and 1 that represents the overall performance of the model, regardless of the decision threshold. AUC is insensitive to changes in the class distribution and it does not rely on a specific decision threshold. As shown in Figure 10, the AUC is 0.91.

[00162] Figure 11 shows VIP scores for each metabolite ranked based on average importance. Table 8 below summarizes the top 15 predictive COVID-related metabolites.

Table 8: Top 15 predictive COVID-related metabolites identified by short COVID vs. healthy control data

No.	COVID Metabolite	Higher/Lower than control samples
1	Lysophosphatidylcholine, C18:0 (lysoPC C18:0)	Higher
2	Lysophosphatidylcholine, C16:0 (lysoPC C16:0)	Higher

3	3-Hydroxyoleoylcarnitine (C18:1-OH)	Lower
4	Succinic acid	Lower
5	Hydroxysphingomyelin (SM (OH)) C22:2	Higher
6	Phosphatidylcholine diacyl C32:2 (PC aa C32:2)	Higher
7	Lysophosphatidylcholine, C20:4 (lysoPC C20:4)	Higher
8	Lysophosphatidylcholine C16:1 (lysoPC C16:1)	Higher
9	Hydroxysphingomyelin (SM (OH)) C22:1	Higher
10	Lysophosphatidylcholine A C18:1 (lysoPC C18:1)	Higher
11	Lysophosphatidylcholine A C17:0 (lysoPC C17:0)	Higher
12	Serotonin	Lower
13	Isobutyric acid	Higher
14	Phosphatidylcholine diacyl 38:0 (PC aa C38:0)	Lower
15	Hydroxysphingomyelin (SM (OH)) C24:1	Higher

[00163] The inventors' model identified Lysophosphatidylcholine, C18:0 (lysoPC C18:0) and Lysophosphatidylcholine, C16:0 (lysoPC C16:0) as having the highest average importance among the metabolites tested. Both metabolites were present at higher concentrations in COVID patients relative to the control samples.

[00164] The inventors' model also identified 3-Hydroxyoleoylcarnitine (C18:1-OH) and Succinic acid as having a high average importance among the metabolites tested. Both metabolites were present at lower concentrations in COVID patients relative to the control samples.

Proteomics

[00165] The PCA scatter plot for short COVID vs. healthy controls proteomics data is shown in Figure 12. The model metrics are shown in Table 8 below:

Table 9: Model metrics for short COVID vs. healthy controls proteomics PLS-DA data

R2	MSE	MSE	ACCURACY

0.4252196123	0.1362728295	1.321518109	0.825
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[00166] The ROC curve for short COVID vs. healthy controls proteomics data is shown in Figure 13.

[00167] Figure 14 shows VIP scores for each protein ranked based on average importance. Table 10 below summarizes the top 15 predictive COVID-related proteins.

Table 10: Top 15 predictive proteins/peptides of COVID identified by short COVID vs. healthy control data

No.	COVID highly predictive proteins/peptides	Higher/Lower than control samples
1	Fibronectin	Higher
2	Ficolin-2	Lower
3	Hemoglobin subunit alpha 1	Higher
4	Lumican	Lower
5	Extracellular matrix protein 1	Higher
6	Alpha-1-antitrypsin	Lower
7	Complement C2	Higher
8	Complement factor I	Lower
9	Coagulation factor XIII B chain	Lower
10	Complement factor H	Higher
11	Complement C3	Higher
12	Plasma protease C1 Inhibitor	Lower
13	Coagulation factor X	Higher
14	Plasma serine protease inhibitor	Lower
15	Complement C5	Higher

[00168] The inventors' machine learning model identified Fibronectin as having the highest average importance among the proteins/peptides tested. Fibronectin was present at higher concentrations in COVID patients relative to the control samples.

[00169] The inventors' model also identified Ficolin-2 and Hemoglobin subunit alpha 1 as having a high average importance among the proteins and peptides tested. Ficolin-2 was present at lower concentrations in COVID patients relative to the control samples and Hemoglobin subunit alpha 1 was present at higher concentrations.

Example 5: Partial least squares discriminant analysis (PLS-DA) of metabolomic and proteomic data for long COVID vs short COVID controls

Metabolomics

[00170] The PCA scatter plot for long vs. short COVID metabolomics data is shown in Figure 15. The model metrics are set forth in Table 11 below:

Table 11: Model metrics for long vs short COVID metabolomics PLS-DA data

	R2	MSE	RPD	ACCURACY
1	0.551418988	0.1037765323	1.501416385	0.8333333333

[00171] The ROC curve for long vs. short COVID metabolomics data is shown in Figure 16.

[00172] Figure 17 shows VIP scores for each metabolite ranked based on average importance. Table 12 below summarizes the top 15 predictive COVID-related metabolites based on the long vs. short COVID metabolomics data.

Table 12: Top 15 predictive metabolites of COVID identified by long COVID vs. short COVID control data

No.	COVID Metabolite	Higher/Lower than control samples
1	3-Hydroxyoleoylcarnitine (C18:1-OH)	Lower
2	Lysophosphatidylcholine, C18:0 (lysoPC C18:0)	Higher
3	Beta-alanine	Lower
4	Phosphatidylcholine diacyl C32:2 (PC aa C32:2)	Higher
5	Lysophosphatidylcholine C16:1 (lysoPC C16:1)	Higher
6	Lysophosphatidylcholine, C16:0 (lysoPC C16:0)	Higher
7	Hydroxysphingomyelin (SM (OH)) C22:2	Higher
8	Dodecanedioylcarnitine (C12-DC).	Lower
9	Succinic acid	Lower
10	Propionic acid	Lower
11	Phosphatidylcholine acyl-alkyl C36:0 (PC AE-C36:0)	Higher
12	Hydroxysphingomyelin (SM (OH)) C24:1	Higher
13	Hydroxysphingomyelin (SM (OH)) C22:1	Higher
14	Lysophosphatidylcholine, C17:0 (lysoPC C17:0)	Higher
15	Serotonin	Lower

[00173] The inventors' model identified Lysophosphatidylcholine, C18:0 (lysoPC C18:0) and Lysophosphatidylcholine, C16:0 (lysoPC C16:0) as having high average importance among the metabolites tested. Both metabolites were present at higher concentrations in COVID patients relative to the control samples (Example 4 above) as well as in long vs. short COVID patients (Table 12 above).

[00174] The inventors' model also identified 3-Hydroxyoleoylcarnitine (C18:1-OH) as having a high average importance among the metabolites tested. This metabolite was present at lower concentrations in long vs. short COVID patients (Table 12 above) as well as in short COVID vs. health controls (Example 4).

Proteomics

[00175] The PCA scatter plot for long vs. short COVID proteomics data is shown in Figure 18. The model metrics are shown in Table 13 below:

Table 13: Model metrics for long vs. short COVID controls proteomics PLS-DA data

	R2	MSE	RPD	ACCURACY
1	0.3800201058	0.1404702467	1.274363831	0.8222222222

[00176] The ROC curve for short vs. long COVID proteomics data is shown in Figure 19.

[00177] Figure 20 shows VIP scores for each protein ranked based on average importance. Table 14 below summarizes the top 15 predictive COVID-related proteins based on the long and short COVID data analysis.

Table 14: Top 15 predictive proteins/peptides of COVID identified by long vs short COVID control data

No.	COVID highly predictive proteins/peptides	Higher/Lower than control samples
1	Fibronectin	Higher
2	Lumican	Lower
3	Ficolin-2	Lower
4	Hemoglobin subunit alpha 1	Higher
5	Extracellular matrix protein 1	Higher
6	Complement Component C7	Lower
7	Plasminogen	Lower
8	Attractin	Higher
9	Vitamin K-dependent protein S	Higher
10	Complement factor H	Higher
11	Fibulin 1	Lower

12	Complement C3	Higher
13	Vitamin K-dependent protein Z	Higher
14	C4B-Binding protein alpha chain	Higher
15	Complement C2	Higher

[00178] The inventors' machine learning model identified Fibronectin as having the highest average importance among the proteins/peptides tested. Fibronectin was present at higher concentrations in COVID patients relative to the control samples as well as in long vs. short COVID patients.

[00179] The inventors' model also identified Ficolin-2 and Hemoglobin subunit alpha 1 as having a high average importance among the proteins and peptides tested. Ficolin-2 was present at lower concentrations in long COVID patients relative to the short COVID samples and Hemoglobin subunit alpha 1 was present at higher concentrations in both data sets (both Examples 4 and 5).

[00180] Lumican was also identified as having a high average importance among the proteins and peptides tested in both data sets (Examples 4 and 5).

[00181] In summary, the inventors have identified a surprising and highly predictive combination of metabolites and proteins/peptides for determining whether subjects have long COVID. Such results can be used to accurately predict long COVID in patients using a simple blood test.

[00182] Every document cited herein, including any cross referenced or related patent or application and any patent application or patent to which this application claims priority or benefit thereof, is hereby incorporated herein by reference in its entirety unless expressly excluded or otherwise limited. The citation of any document is not an admission that it is prior art with respect to any disclosure disclosed or claimed herein or that it alone, or in any combination with any other reference or references, teaches, suggests or discloses any such disclosure. Further, to the extent that any meaning or definition of a term in this document conflicts with any meaning or definition of the same term in a document incorporated by reference, the meaning or definition assigned to that term in this document shall govern.

[00183] Headings used throughout the specification should not be construed as limiting to the invention.

[00184] While particular embodiments of the present disclosure have been illustrated and described, it would be obvious to those skilled in the art that various other changes and modifications can be made without departing from the scope of the present disclosure. It is therefore intended to cover in the appended claims all such changes and modifications that are within the scope of this disclosure.

What is claimed is:

1. A method for diagnosing and optionally treating Long COVID in a subject, the method comprising:

- (a) providing a biological sample obtained from the subject;
- (b) measuring concentration levels from the obtained sample, at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin C22:2 (SM (OH) C22:2); Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, at least two, at least three, at least four or at least five Long COVID-related proteins and/or peptides selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5;
- (c) comparing the concentration levels of the Long COVID-related metabolites and/or proteins from the obtained sample to the concentration levels of corresponding reference Long COVID-related metabolites and/or proteins from a Long COVID- negative sample;
- (d) identifying the subject as having Long COVID if the concentration levels of the Long COVID-related metabolites and/or proteins from the obtained sample are different relative to the concentration levels of the reference Long COVID-related metabolites and/or proteins from the Long COVID-negative sample; and
- (e) optionally treating or causing treatment of the subject so identified with a Long COVID treatment regime.

2. The method of claim 1, wherein the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine;

Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); Hydroxysphingomyelin (SM (OH)) C22:2; Dodecanedioylcarnitine (C12-DC); Succinic acid; and Propionic acid.

3. The method of claim 2, wherein the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); and Lysophosphatidylcholine C16:1 (lysoPC C16:1).

4. The method of claim 1, wherein the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; Extracellular matrix protein 1; Complement Component C7; Plasminogen; Attractin; Vitamin K-dependent protein S; and Complement factor H.

5. The method of claim 4, wherein the Long COVID-related proteins are selected from at least at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; and Extracellular matrix protein 1.

6. A method for diagnosing and optionally treating Long COVID in a subject, the method comprising:

- (a) providing a biological sample obtained from the subject;
- (b) measuring from the obtain sample or having measured in a spectroscopy unit the concentration levels of at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from

Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5;

- (c) comparing or having compared concentration levels of the Long COVID-related metabolites and/or proteins as determined in the spectroscopy unit to the concentration levels of reference Long COVID- related metabolites and/or proteins from an Long COVID-negative sample;
- (d) identifying the subject as having Long COVID if the concentration levels of the Long COVID-related metabolites and/or proteins from the obtained sample are different relative to the concentration levels of the reference Long COVID-related metabolites and/or proteins from the Long COVID-negative sample; and
- (e) optionally treating or causing treatment of the subject so identified with a Long COVID treatment regime.

7. The method of claim 6, wherein the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); Hydroxysphingomyelin (SM (OH)) C22:2; Dodecanedioylcarnitine (C12-DC); Succinic acid; and Propionic acid.

8. The method of claim 7, wherein the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); and Lysophosphatidylcholine C16:1 (lysoPC C16:1).

9. The method of claim 1, wherein the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; Extracellular matrix protein 1; Complement Component C7; Plasminogen; Attractin; Vitamin K-dependent protein S; and Complement factor H.

10. The method of claim 9, wherein the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; and Extracellular matrix protein 1.

11. The method according to any one of claims 1 to 10, wherein the Long COVID treatment regime comprises adjusting the blood levels of one or more of the Long COVID-related metabolites and/or proteins in the subject diagnosed as having the Long COVID or predisposed of developing the Long COVID or a combination thereof.

12. The method according to claim 11, wherein the adjustment of the blood levels of one or more of the Long COVID-related metabolites and/or proteins in the subject occurs until an improvement in the Long COVID symptoms in the subject is observed.

13. The method according to claim 12, wherein the adjustment of the blood levels of one or more of the Long COVID-related metabolites comprises adjusting the composition of gut microbiota in the subject.

14. The method according to any one of claims 1 to 13, wherein the identifying step occurs upon determination that the concentration levels of at least one, at least two, at least three, at least four or at least five of the Long COVID-related metabolites and/or proteins from the obtained sample differ by about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, or about 70% or more relative to the concentration levels of the reference Long COVID-related metabolites and/or proteins from the Long COVID-negative sample.

15. The method according to any one of claims 1 to 14, wherein the obtained sample is blood or urine, preferably serum, plasma or urine.

16. The method according to any one of claims 1 to 15, wherein the Long COVID-related metabolites and/or proteins are measured by a spectroscopic technique, wherein the

spectroscopic technique is selected from the group consisting of liquid chromatography, gas chromatography, liquid chromatography mass spectrometry, gas chromatography mass spectrometry, high performance liquid chromatography mass spectrometry, capillary electrophoresis mass spectrometry, nuclear magnetic resonance spectrometry (NMR), raman spectroscopy, and infrared spectroscopy.

17. The method according to any one of claims 1 to 16, wherein the comparison of the concentration levels of the Long COVID-related metabolites and/or proteins from the obtained sample to the concentration levels of the reference Long COVID-related metabolites and/or proteins from the Long COVID-negative sample comprises using multivariate statistical analysis.

18. The method according to claim 17, wherein the multivariate statistical analysis is selected from principal component analysis (PCA), or partial least squares projects to latent structures discriminant analysis (PLS-DA).

19. A method of monitoring Long COVID progression and optionally treating the Long COVID in a subject, the method comprising:

- (a) providing a first biological sample obtained from the subject at a first time;
- (b) assessing a first Long COVID-related metabolite and/or proteomic profile by measuring concentration levels of at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H;

Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5 from the first obtained sample;

- (c) comparing the first Long COVID-related metabolite and/or proteomic profile with a reference Long COVID-related metabolite profile and/or proteomic profile from an Long COVID-negative sample;
- (d) determining that there is a first difference between the first Long COVID-related metabolite and/or proteomic profile and the reference Long COVID-related metabolite and/or proteomic profile from the Long COVID-negative sample, the first difference being indicative of Long COVID;
- (e) providing a second biological sample obtained from the subject at a second time that is after the first time;
- (f) assessing a second Long COVID-related metabolite and/or proteomic profile by measuring concentration levels of the Long COVID-related metabolites and/or proteins from the second obtained sample;
- (g) comparing the second Long COVID-related metabolite and/or proteomic profile with the reference Long COVID-related metabolite and/or proteomic profile from the Long COVID-negative sample;
- (h) determining that there is a second difference between the first Long COVID-related metabolite and/or proteomic profile and the reference Long COVID-related metabolite and/or proteomic profile from the Long COVID-negative sample, the second difference being indicative of Long COVID;
- (i) determining Long COVID progression based on at least in part on the first and second differences; and
- (j) optionally treating the subject as identified with an Long COVID treatment regime.

20. A kit for use in the method of any one of claims 1 to 19 comprising reagents for measuring concentration levels of the Long COVID-related metabolites and/or the Long COVID-related proteins, optionally together with instructions for use.

21. A kit for diagnosis Long COVID comprising:

- (a) a detector configured to detect concentration levels of at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5 in an obtained biological sample;
- (b) a composition comprising the corresponding Long COVID-related metabolites and/or Long COVID-related proteins in control levels corresponding to a control group of Long COVID-negative subjects;
- (c) a multivariate analysis system configured to analyze a difference in the concentration levels of the Long COVID-related metabolites and/or proteins and the control levels, and
- (d) optionally, instruction for an Long COVID diagnosis method; wherein the method comprises measuring, using the detector, the levels of the Long COVID-related metabolites and/or proteins from the obtained biological sample, and comparing the levels of the obtained Long COVID-related metabolites and/or proteins to the control levels of the Long COVID-related metabolites and/or proteins obtained from Long COVID-negative subjects.

22. The kit of claim 21, wherein the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); Hydroxysphingomyelin (SM (OH)) C22:2; Dodecanedioylcarnitine (C12-DC); Succinic acid; and Propionic acid.

23. The kit of claim 22, wherein the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); and Lysophosphatidylcholine C16:1 (lysoPC C16:1).

24. The kit of claim 21, wherein the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; Extracellular matrix protein 1; Complement Component C7; Plasminogen; Attractin; Vitamin K-dependent protein S; and Complement factor H.

25. The kit of claim 24, wherein the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; and Extracellular matrix protein 1.

26. The kit according to any one of claims 21 to 25, wherein the detector comprises a multi-metabolite detector and/or multi-proteomic detector configured to measure the levels of the Long COVID-related metabolites and/or the proteins.

27. A computer-implemented method for processing a biological sample of a subject, diagnosing Long COVID and optionally causing the treating the Long COVID, the computer-implemented method comprising:

- (a) receiving a biological sample obtained from the subject;
- (b) processing the sample in a spectroscopy unit directly or wirelessly linked to a processing device, the processing device having memory for storing measurement data from the spectroscopy unit;
- (c) in the spectroscopy unit, measuring levels of at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites selected from Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC

aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC); and/or at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5 and storing the measurement data in the processor;

(d) comparing the stored measurement data to a value in the memory representing an Long COVID- negative sample using multivariate statistical analysis; and

(e) storing on the processing device a result corresponding to at least one, at least two, at least three, at least four or at least five Long COVID-related metabolites and/or proteins from the obtained sample, wherein the result identifies the subject as having Long COVID if the measurement data representing the levels of the Long COVID-related metabolites and/or proteins are different relative to a concentration levels of reference Long COVID-related metabolites and/or proteins from an Long COVID-negative sample; and

(f) displaying a Long COVID treatment regime on an electronic display connected directly or wirelessly to the processor for the subject identified as having Long COVID or as having predisposition of developing Long COVID, the displayed treatment regime comprising electronic text on a graphical user interface; and

(g) causing the adjusting of blood and/or microbiome levels of one or more of the Long COVID-related metabolites and/or proteins in the subject diagnosed as having or predisposed of developing the Long COVID until an improvement in the cognitive performance in the subject is observed.

28. The method of claim 27, wherein the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine C16:1

(lysoPC C16:1); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); Hydroxysphingomyelin (SM (OH)) C22:2; Dodecanedioylcarnitine (C12-DC); Succinic acid; and Propionic acid.

29. The method of claim 28, wherein the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of

3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); and Lysophosphatidylcholine C16:1 (lysoPC C16:1).

30. The method of claim 27, wherein the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; Extracellular matrix protein 1; Complement Component C7; Plasminogen; Attractin; Vitamin K-dependent protein S; and Complement factor H.

31. The method of claim 30, wherein the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; and Extracellular matrix protein 1.

32. A method for diagnosing and optionally treating Long COVID comprising:

(a) obtaining a signature of metabolites and proteins from a biological sample of a patient, the signature obtained by measuring Long COVID-related metabolites selected from Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2 Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC) and/or at least one, at least two, at least three, at least four or at least five Long COVID-related proteins selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H;

Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5;

(b) classifying the patient into a Long COVID risk class or healthy class using a statistical analysis that has at least 85% predictive value comprising comparing the signature so obtained with a control signature, the classifying comprising a computer-implemented statistical analysis; and

(c) optionally treating or causing the treatment of the patient with a drug effective to treat, ameliorate or reduce the symptoms Long COVID, wherein the treating optionally comprises adjusting the levels of one or more of the metabolites and/or proteins identified in the signature as being present at levels different than the control.

33. The method of claim 32, wherein the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); Hydroxysphingomyelin (SM (OH)) C22:2; Dodecanedioylcarnitine (C12-DC); Succinic acid; and Propionic acid.

34. The method of claim 33, wherein the Long COVID-related metabolites are selected from at least one, at least two, at least three, at least four or at least five of 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); and Lysophosphatidylcholine C16:1 (lysoPC C16:1).

35. The method of claim 32, wherein the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; Extracellular matrix protein 1; Complement Component C7; Plasminogen; Attractin; Vitamin K-dependent protein S; and Complement factor H.

36. The method of claim 35, wherein the Long COVID-related proteins are selected from at least one, at least two, at least three, at least four or at least five of of Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; and Extracellular matrix protein 1.

37. The method of any one of claims 1 to 36, further comprising adjusting or causing the adjustment of the levels of the metabolites and/or proteins comprises nucleic acid therapy.

38. The method of claim 37, wherein the nucleic acid therapy comprises reducing the level of a protein expressed or metabolite produced by siRNA or antisense therapy.

39. The method of claim 37, wherein the nucleic acid therapy comprises increasing the level of a protein expressed or metabolite produced by mRNA therapy.

40. The method of any one of claims 1 to 39, wherein the metabolite and/or proteome profile measured is based on a profile identified in a previous computer-implemented statistical analysis model that has a predictive value of at least 90% and comprises at least one of means comparison, PCA, PLS-DA or recursive SVM data analyses.

41. The method of claim 40, wherein the metabolite and/or proteome profile has been previously identified as having the predictive value by classifying disease and non-disease samples into the two groups by a computer-implemented method based on the predicted metabolites and/or proteins used in the computer model and assessing whether the groups are separated.

42. A method of identifying a subject's risk of developing Long COVID or identifying the subject as having Long COVID comprising:

- (a) obtaining one or more biological samples from the subject;
- (b) conducting a metabolomic and proteomic analysis of the one or more biological samples;
- (c) measuring at least at least one, two or all metabolites selected Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Lysophosphatidylcholine, C16:0 (lysoPC C16:0); 3-Hydroxyoleoylcarnitine (C18:1-OH); Succinic acid; Hydroxysphingomyelin (SM (OH)); C22:2

Phosphatidylcholine diacyl C32:2 (PC aa C32:2); Lysophosphatidylcholine, C20:4 (lysoPC C20:4); Lysophosphatidylcholine C16:1 (lysoPC C16:1); Hydroxysphingomyelin (SM (OH)) C22:1; Beta-alanine; Serotonin; Isobutyric acid; Glutamic acid; and Creatine Dodecanedioylcarnitine (C12-DC) and at least one, two, three or all proteins, peptides or fragments thereof selected from Fibronectin; Ficolin-2; Hemoglobin subunit alpha 1; Lumican; Extracellular matrix protein 1; Alpha-1-antitrypsin; Complement C2; Complement factor I; Coagulation factor XIII B chain; Complement C3; Complement factor H; Plasma protease C1 Inhibitor; Coagulation factor X; Plasma serine protease inhibitor; and Complement C5, optionally wherein the metabolite(s) and/or protein(s) measured have a predictive score of at least 0.8 (80% or more) as determined on a VIP plot; and

(d) identifying the subject as having Long COVID or a risk of development thereof if the levels of the metabolite(s) and/or protein(s) differ from a control reference; and

(e) optionally providing results of the metabolomic and/or proteomic analysis or a treatment regime based on said analysis via an on-line platform.

43. A computer system comprising:

- (a) a high throughput spectroscopy unit for receiving one or more biological samples from a subject, and conducting a metabolomic and proteomic analysis of a panel of at least 25 metabolic and proteomic markers in the one or more biological samples;
- (b) optionally a computer processor for outputting a digital metabolomic and proteomic signature of the subject;
- (c) a computer processor for determining whether at least three Long COVID-related predictive metabolites are present at levels that differ from a control reference, thereby indicative of Long Covid, the predictive metabolites selected from 3-Hydroxyoleoylcarnitine (C18:1-OH); Lysophosphatidylcholine, C18:0 (lysoPC C18:0); Beta-alanine; Phosphatidylcholine diacyl C32:2 (PC aa C32:2); and Lysophosphatidylcholine C16:1 (lysoPC C16:1);
- (d) a computer processor for determining whether at least three Long COVID-related predictive proteins are present at levels that differ from a control reference, thereby indicative of Long Covid, the predictive proteins selected from Fibronectin; Lumican; Ficolin-2; Hemoglobin subunit alpha 1; Extracellular matrix protein 1; Complement Component C7; Plasminogen; Attractin; Vitamin K-dependent protein S; and Complement factor H;

wherein the metabolites and proteins measured in steps (c) and (d) have a predictive score of at least 0.8 (80% or more) as determined on a VIP plot;

optionally, the computer processor compiling results of the step (c) and (d) in a database;

(e) a wireless smart device comprising an application for receiving results from steps (c) and (d), the wireless smart device displaying results of the metabolomic and proteomic analysis via the application, the application having a dashboard displaying an assigned score for the Long COVID-related metabolites and proteins, and optionally displaying a treatment regime based on the assigned scores of the predictive metabolites and proteins, optionally the smart device receiving data from the database; and

(f) optionally a computer processor for displaying a recommended treatment regime for the subject for treating symptoms of the Long Covid.

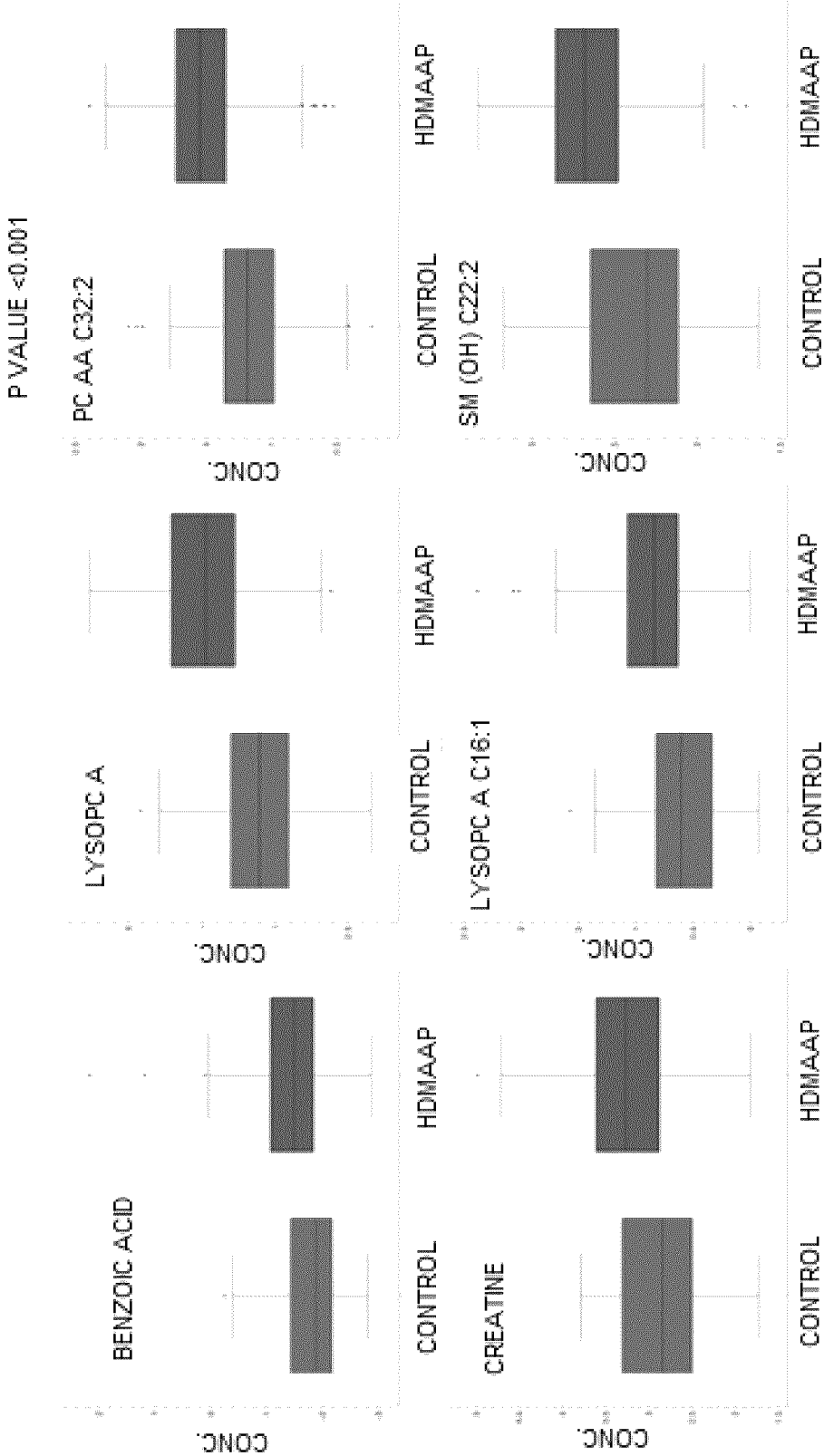


FIG 1A

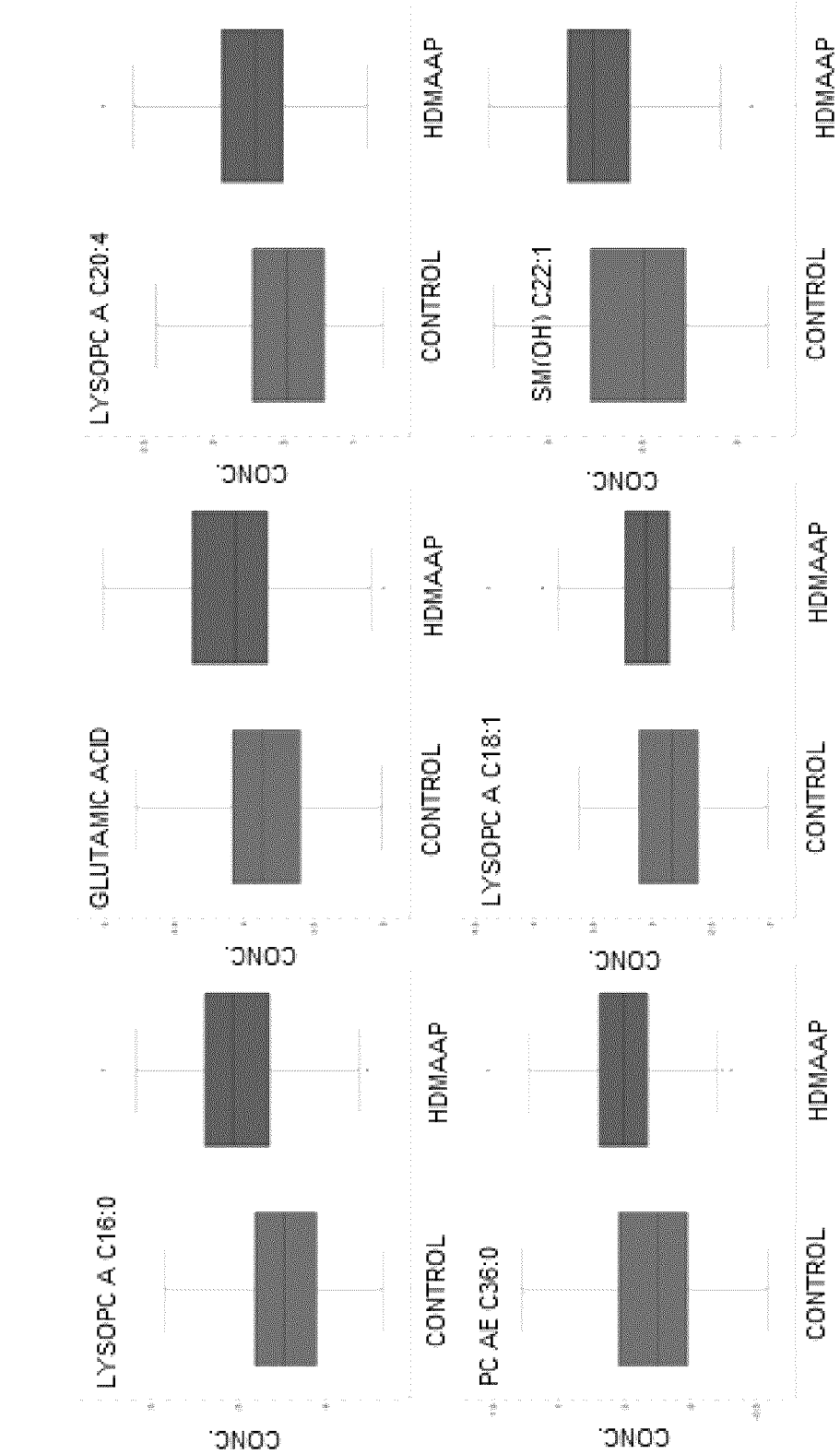


FIG 1B

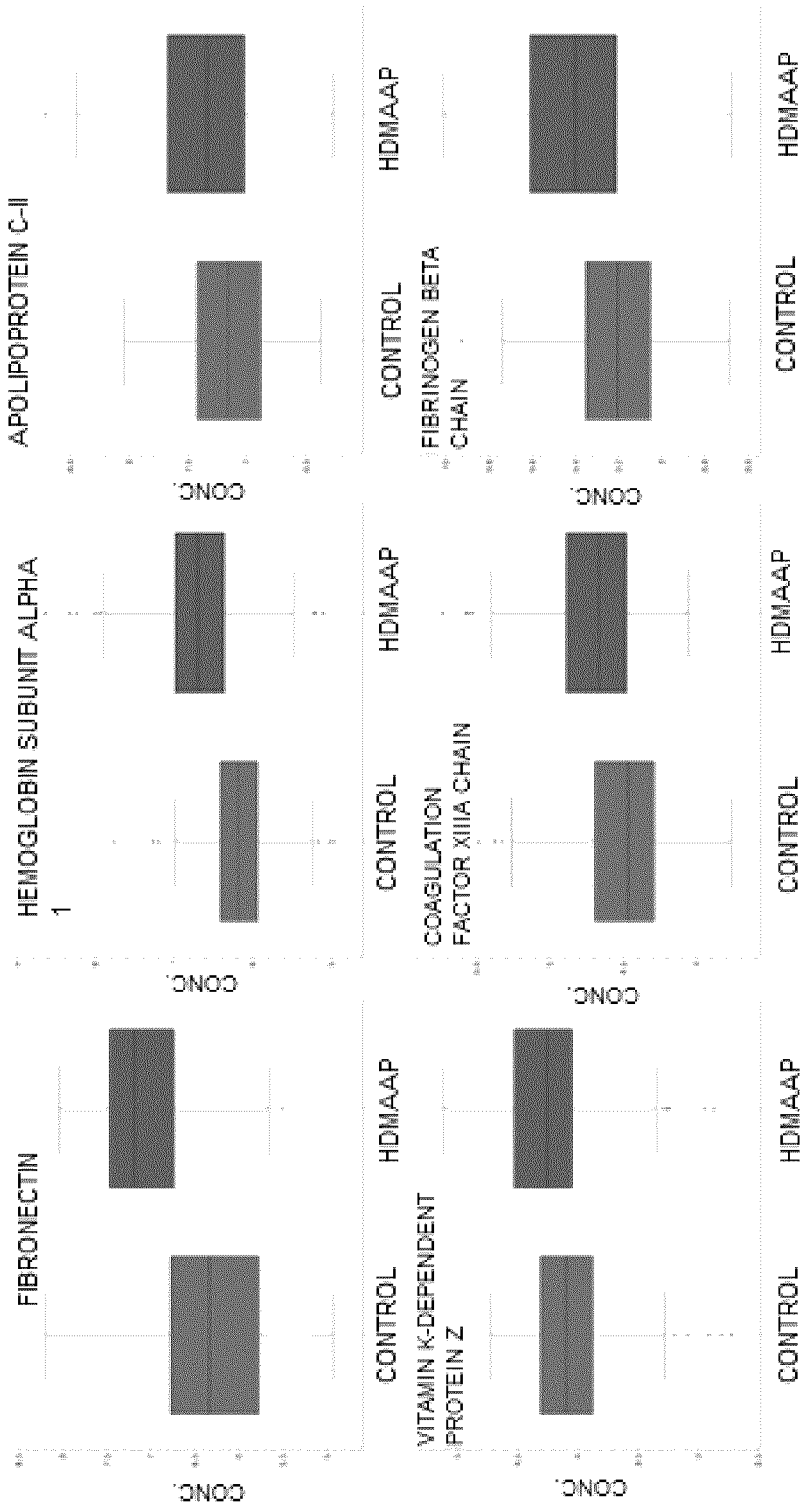


FIG 2A

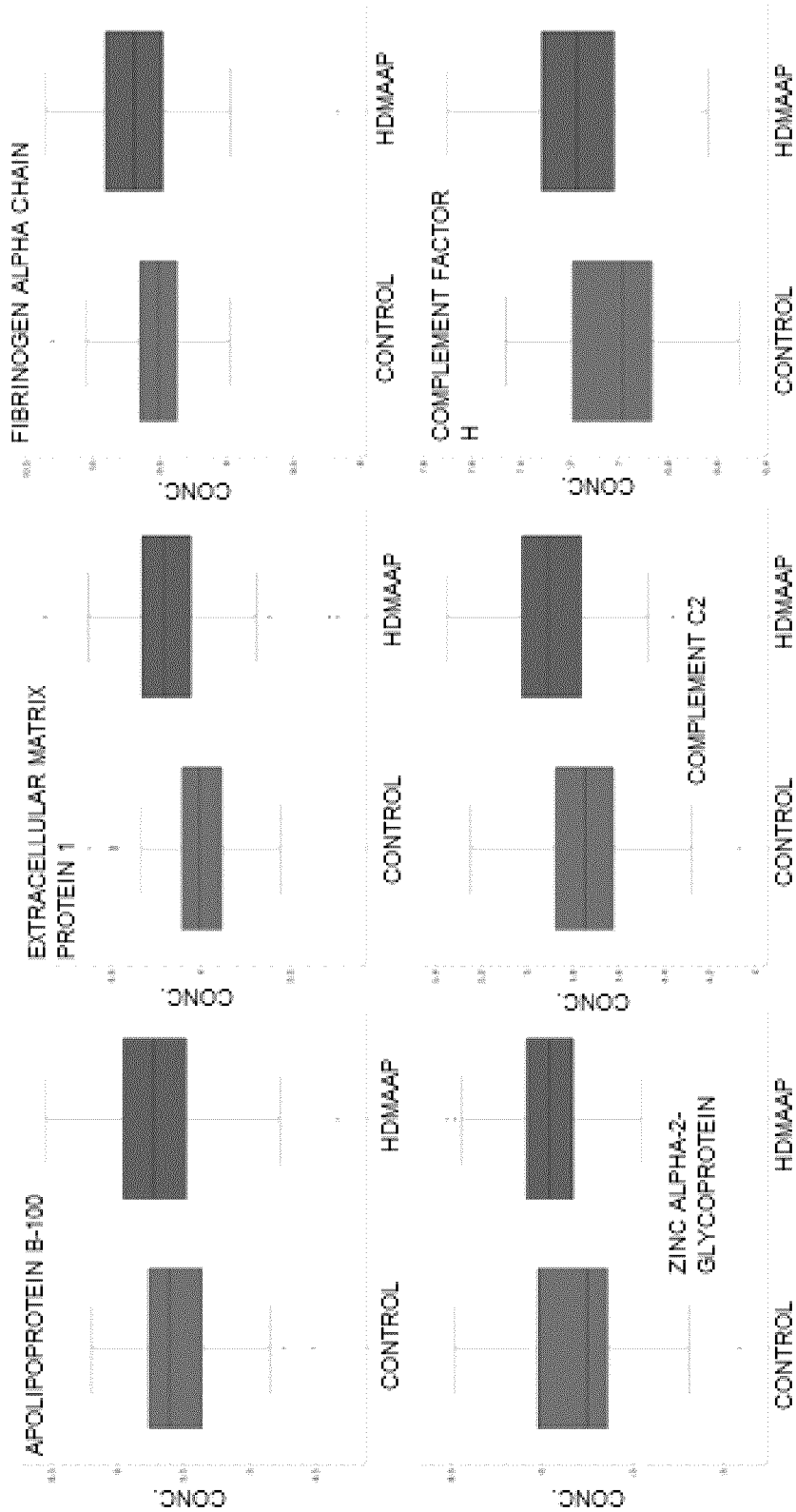


FIG 2B

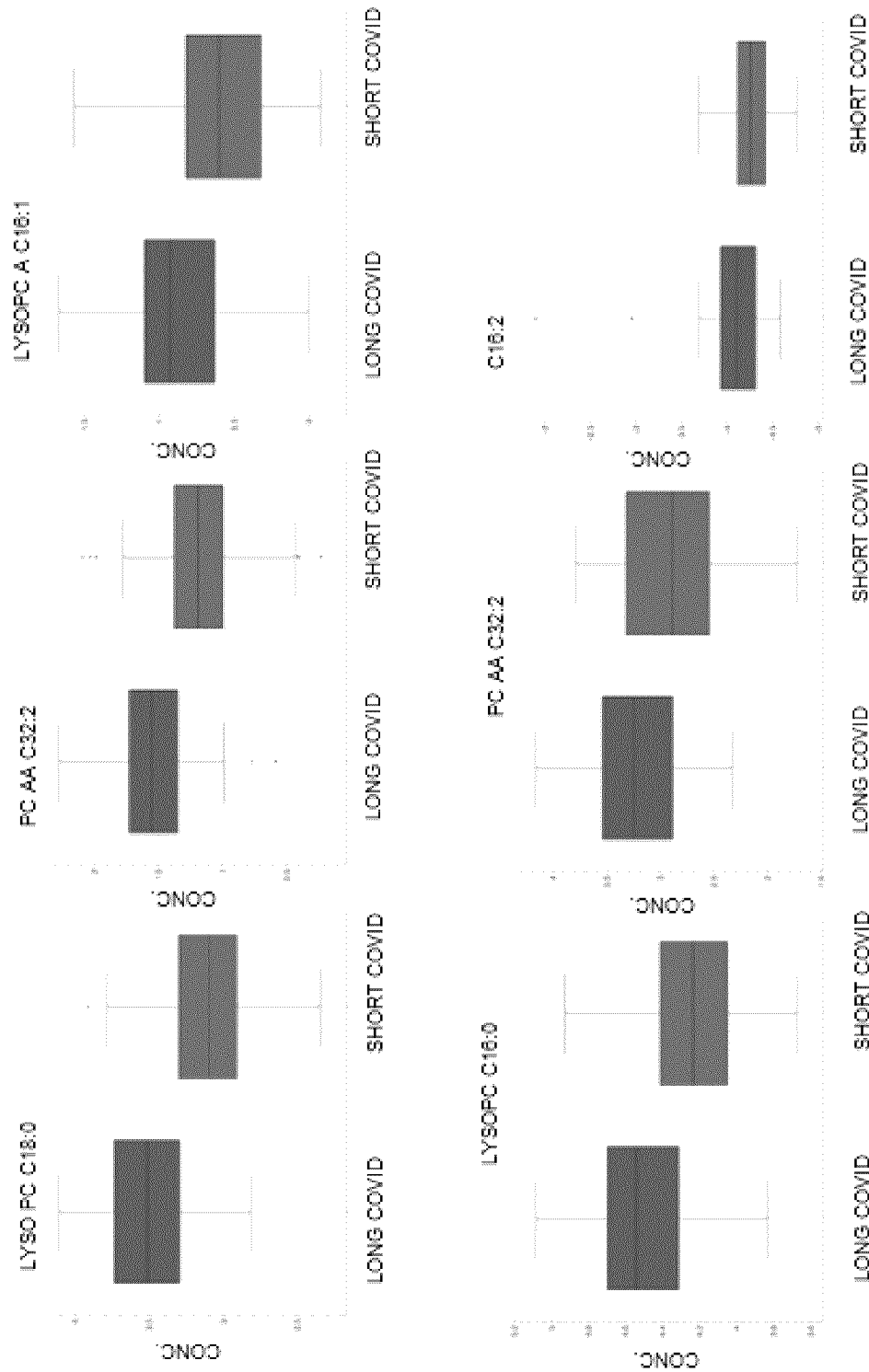


FIG 3A

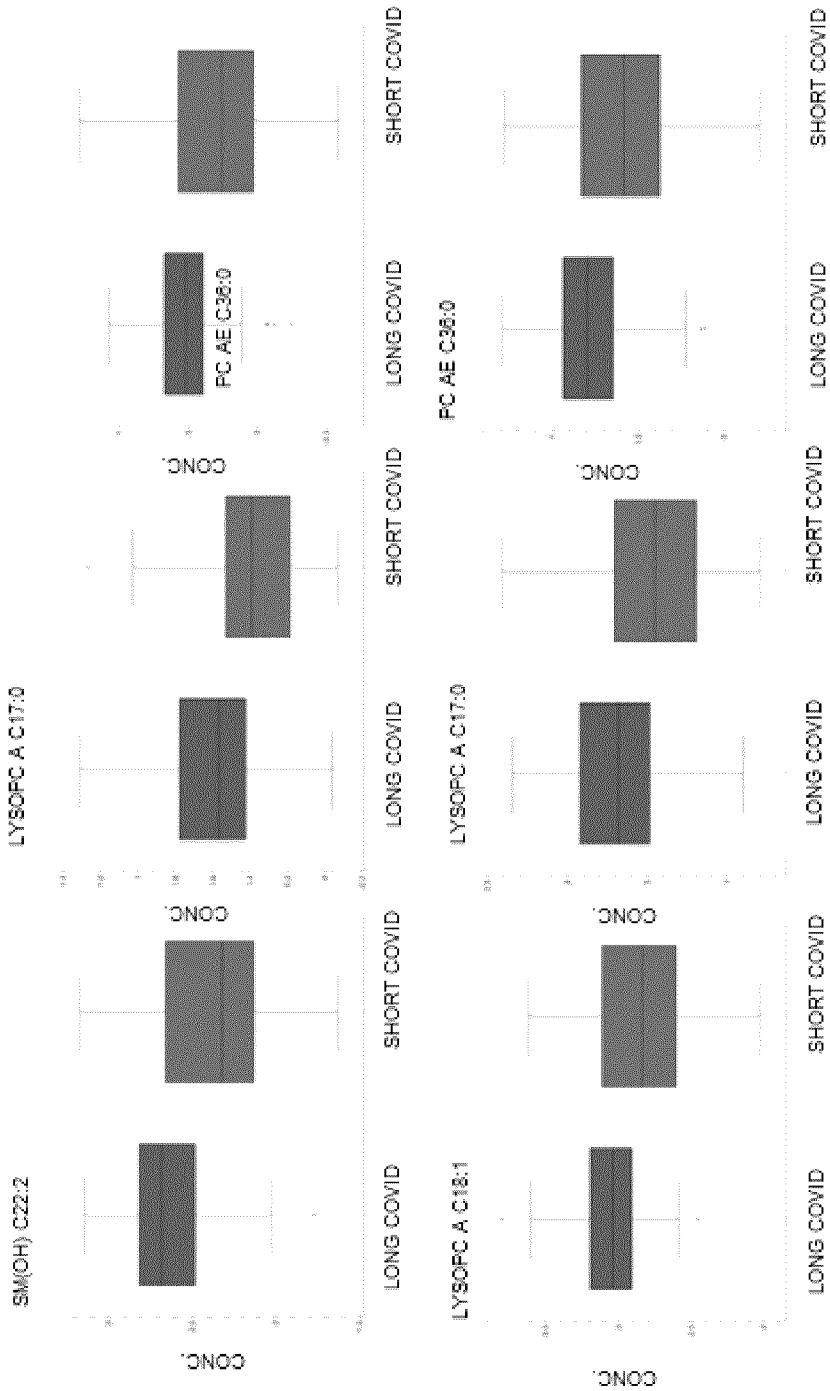


FIG 3B

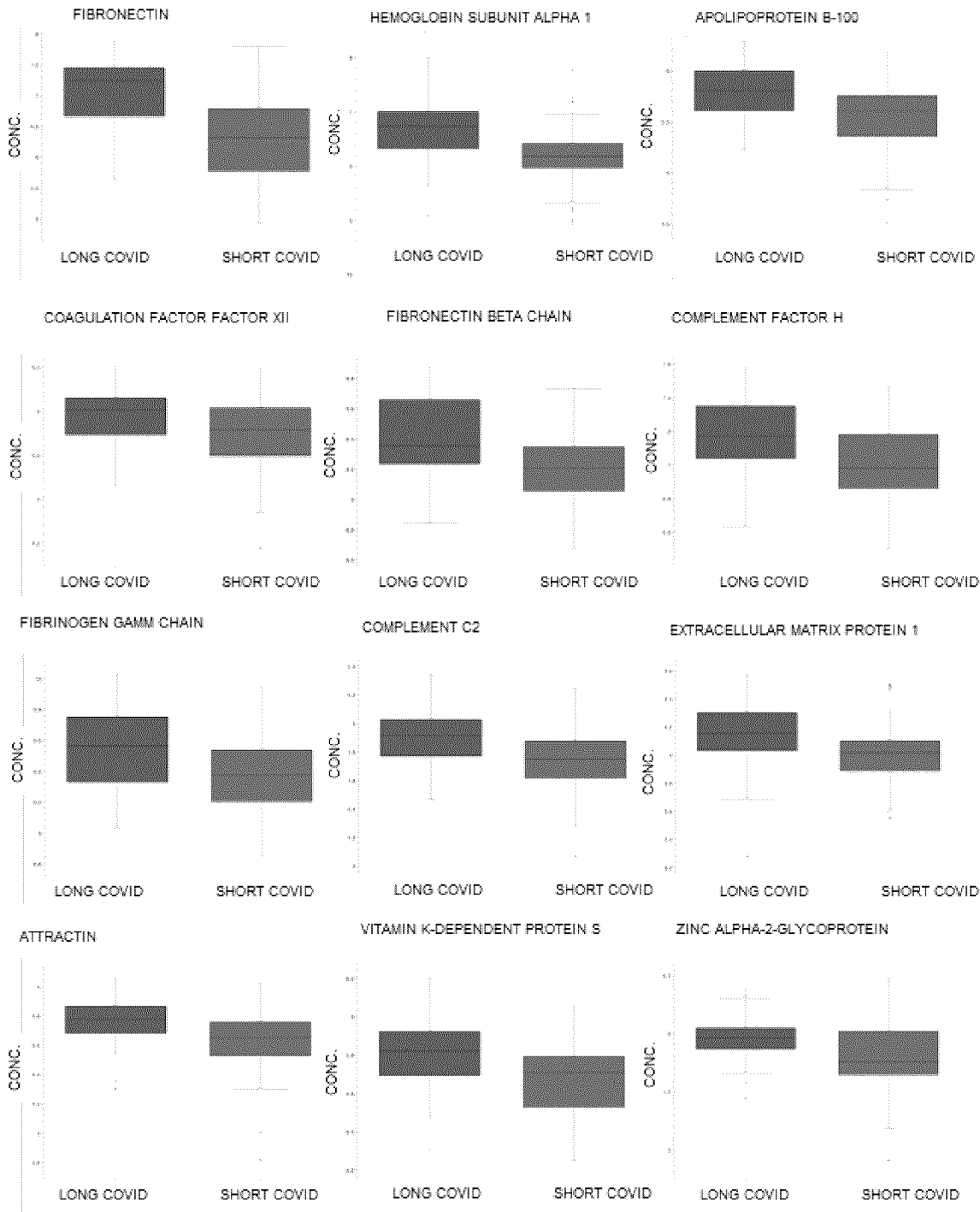


FIG 4

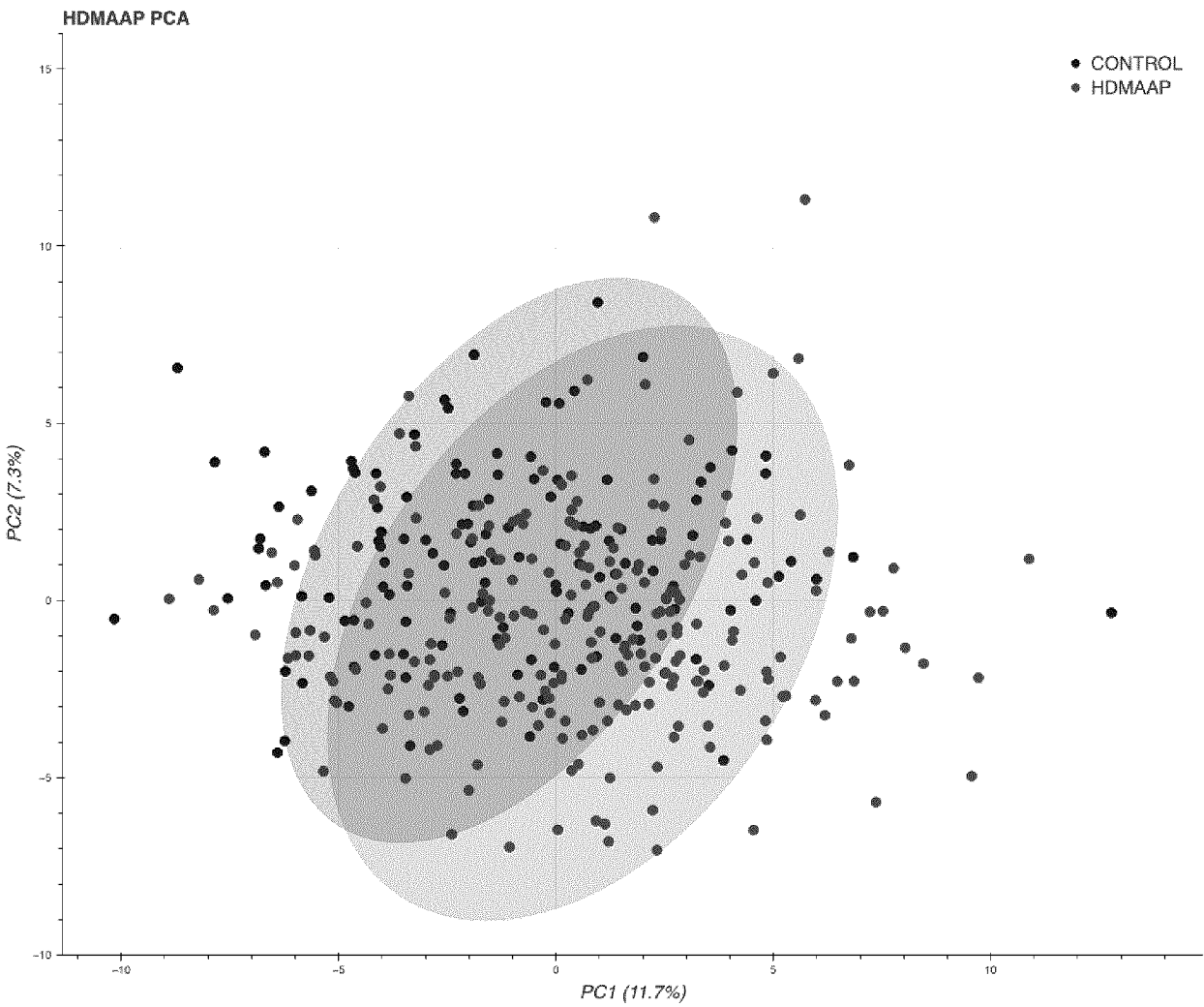


FIG 5

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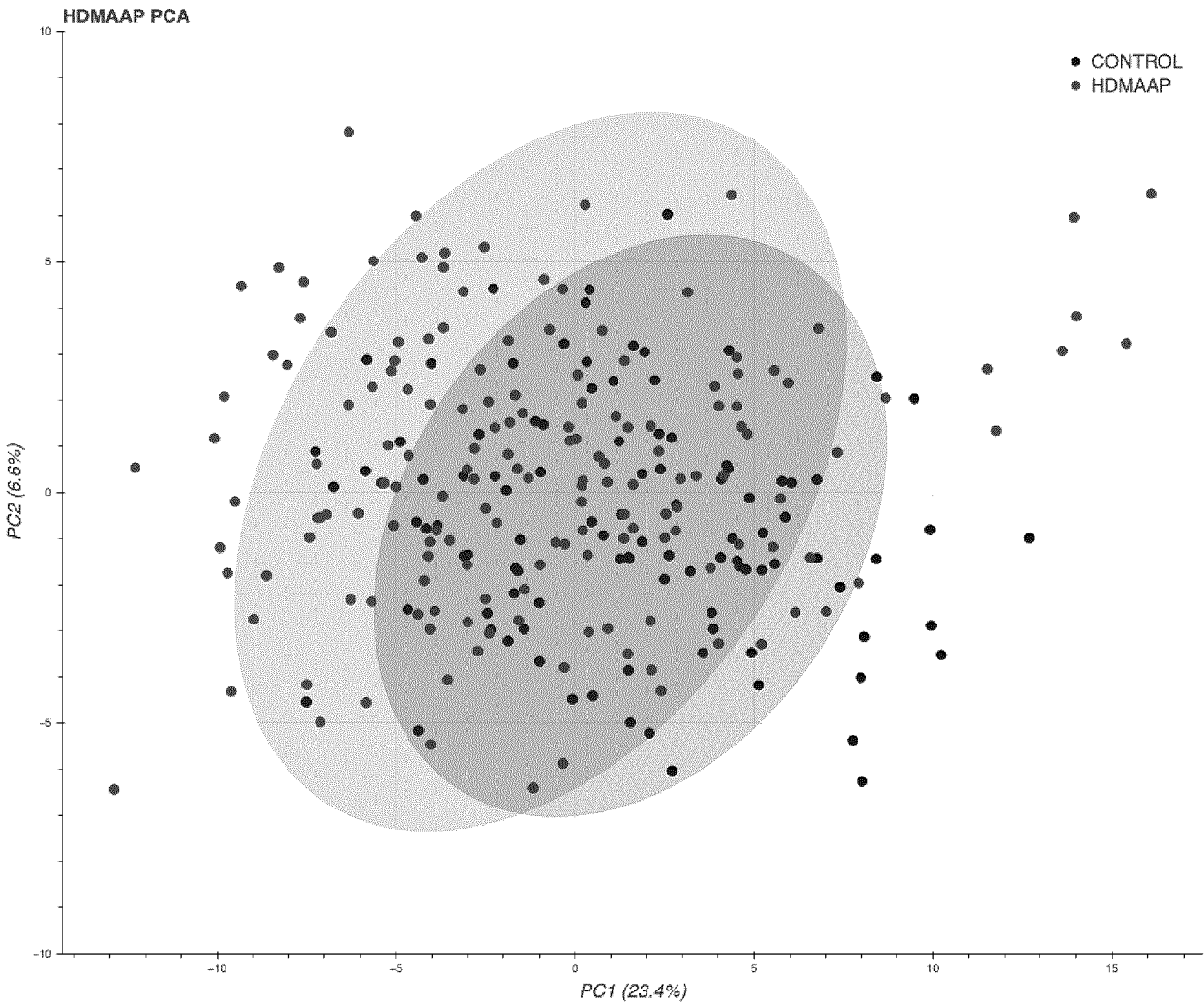


FIG 6

10/23

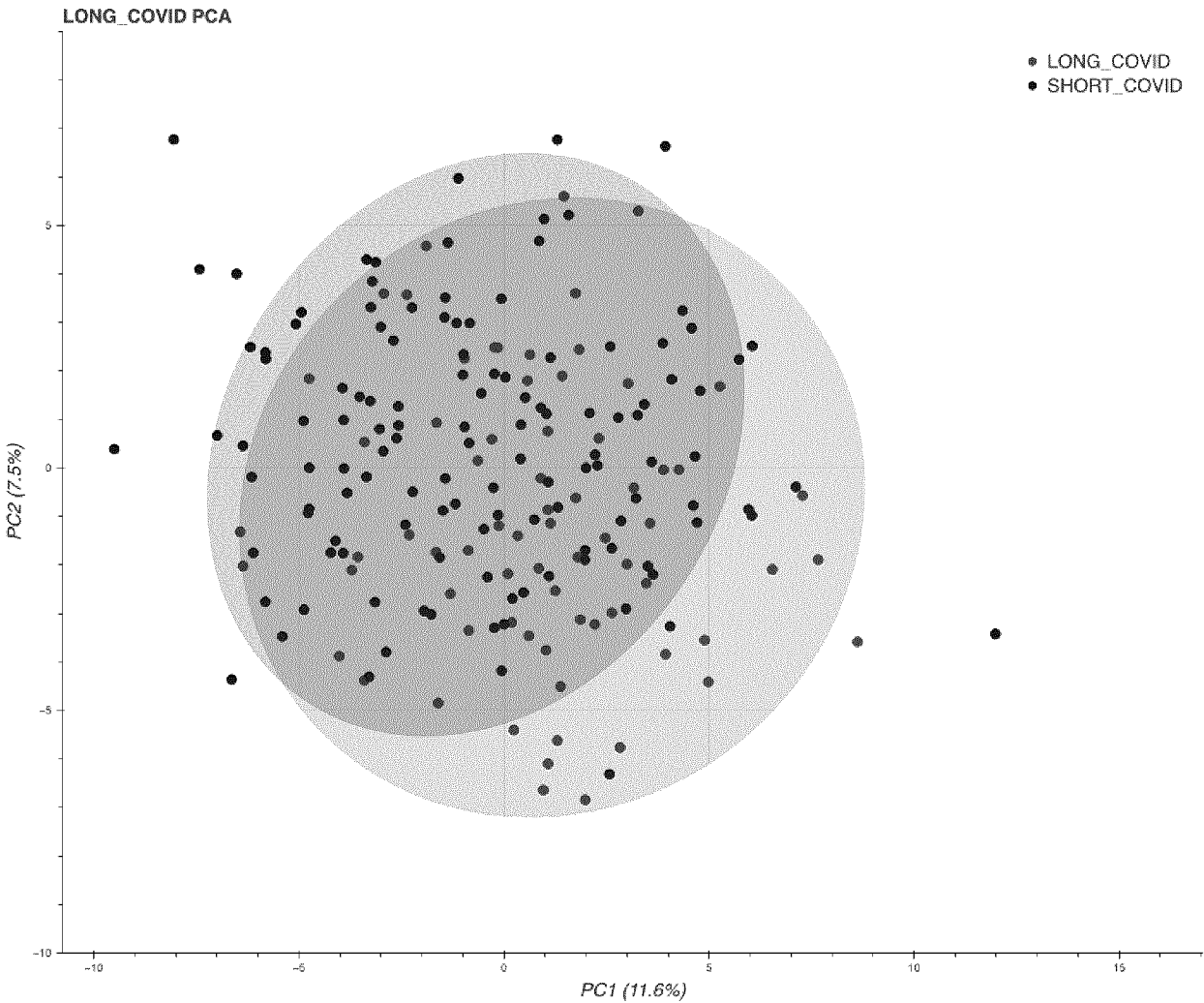


FIG 7

11/23

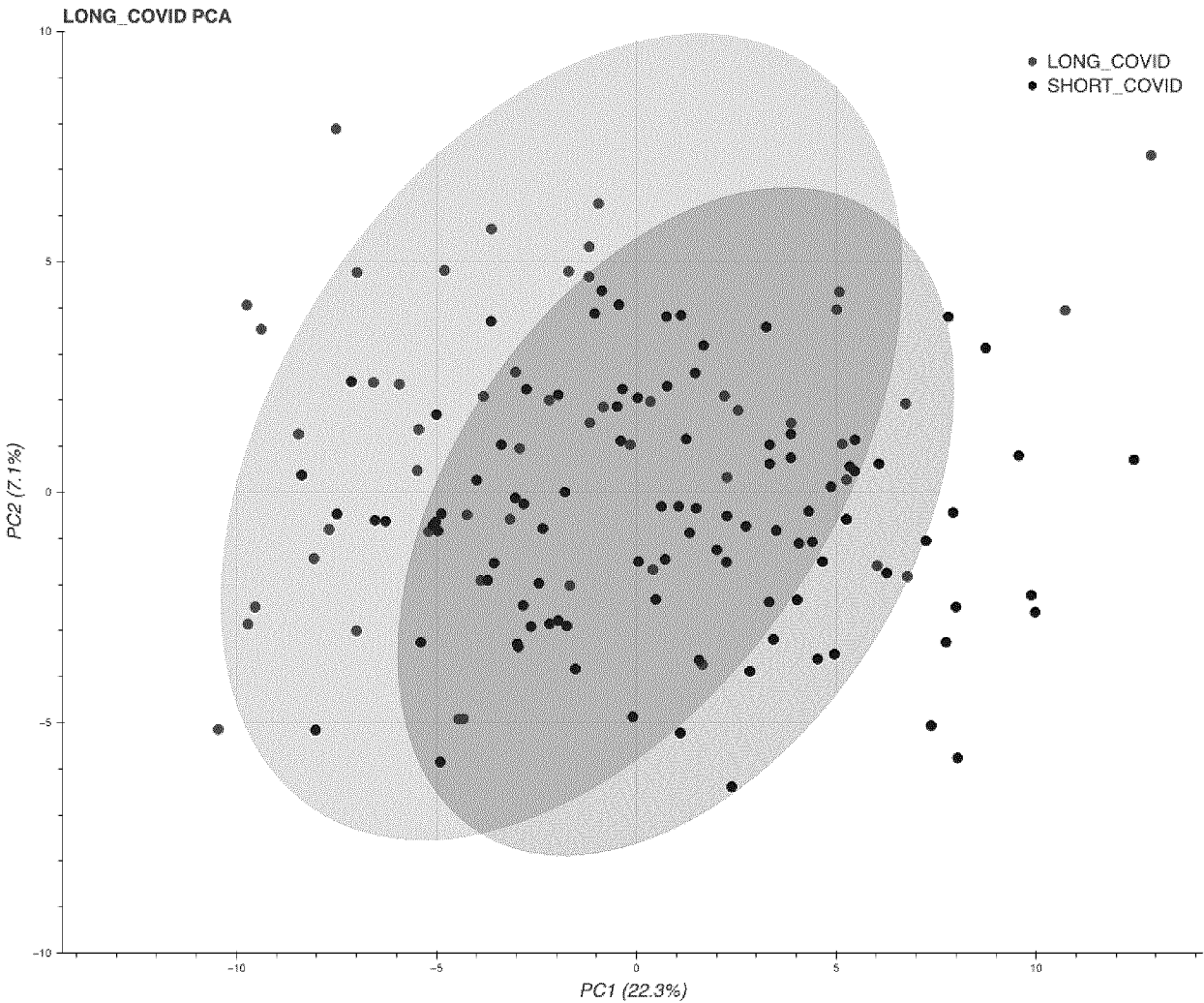


FIG 8

12/23

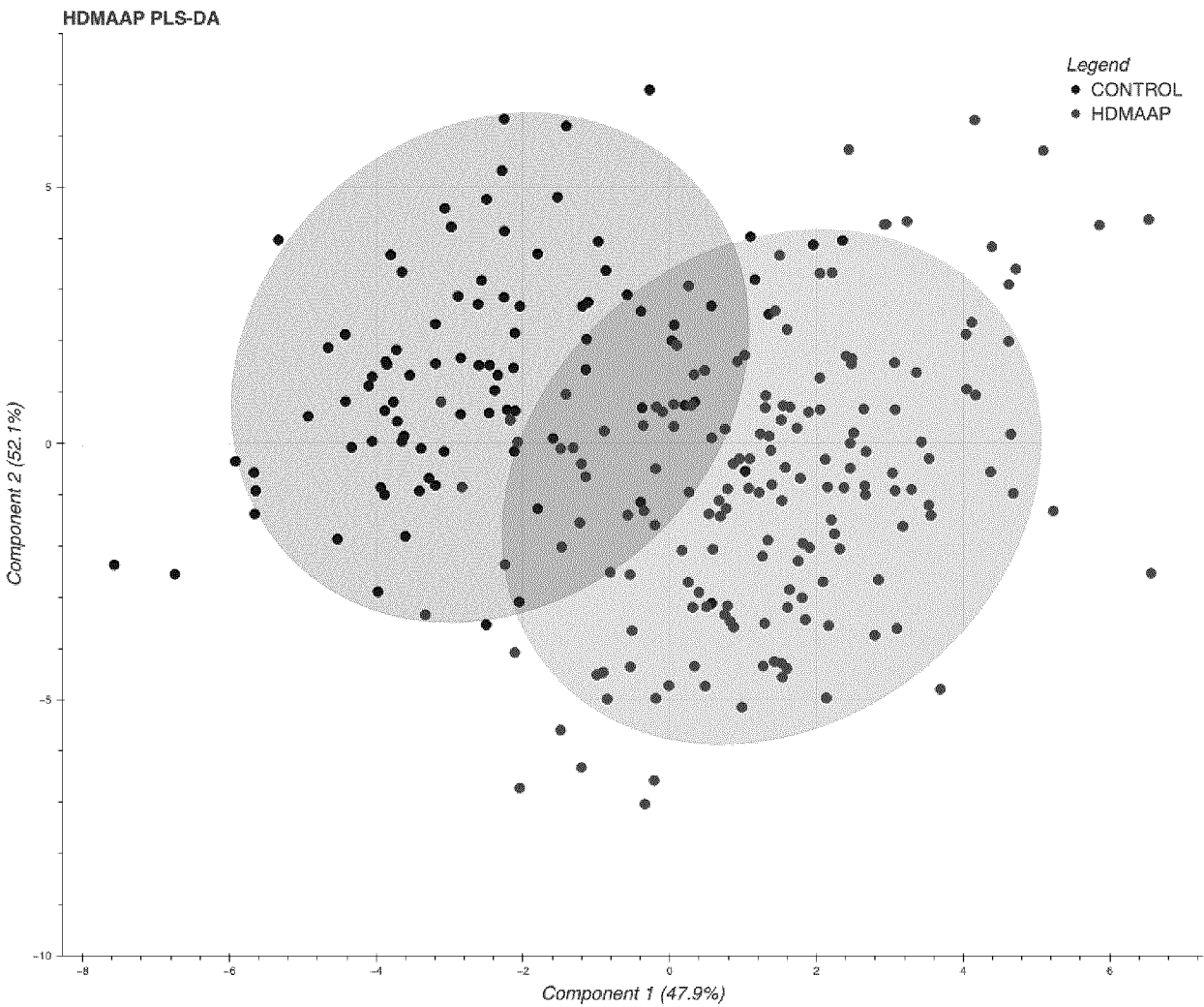


FIG 9

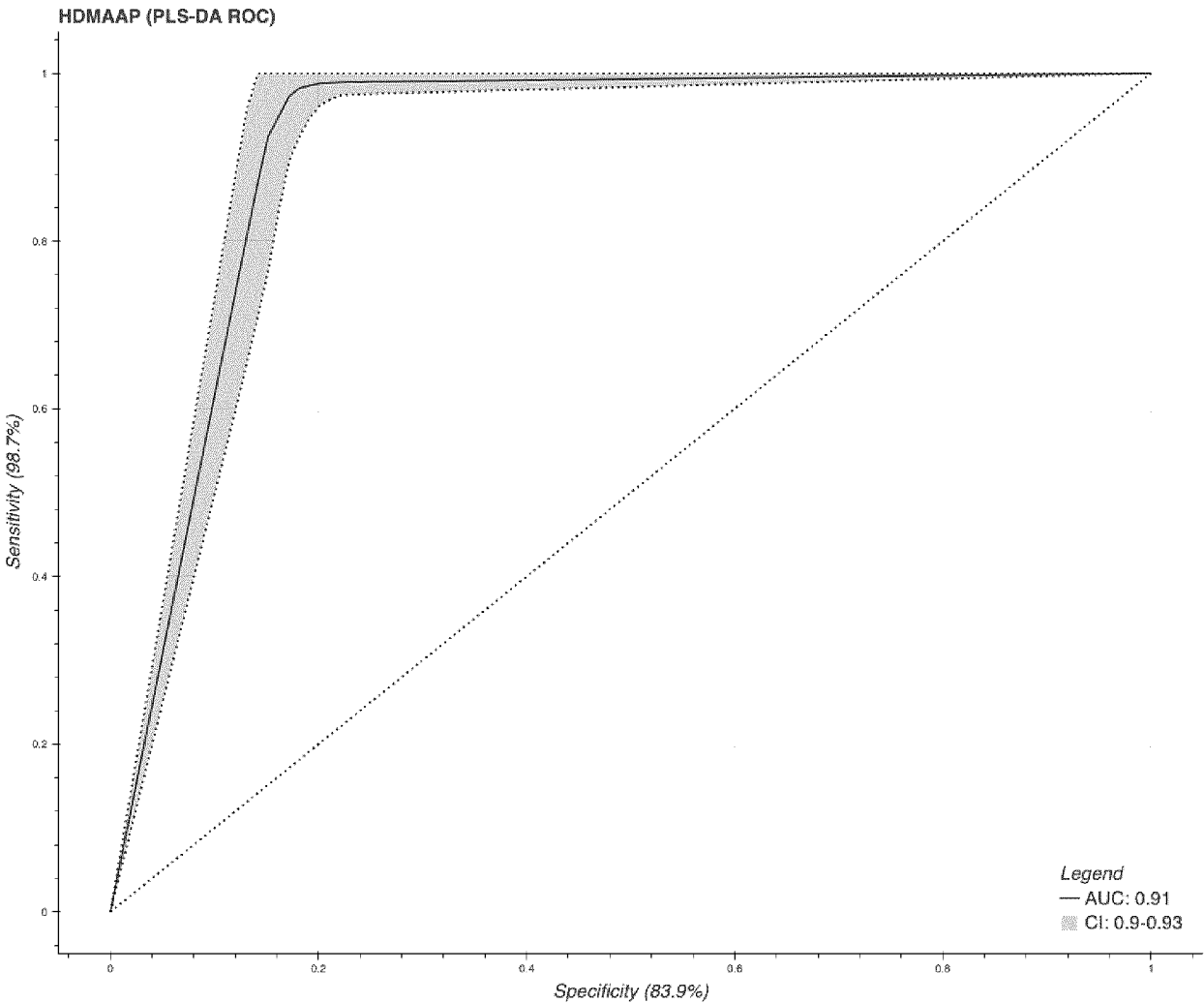


FIG 10

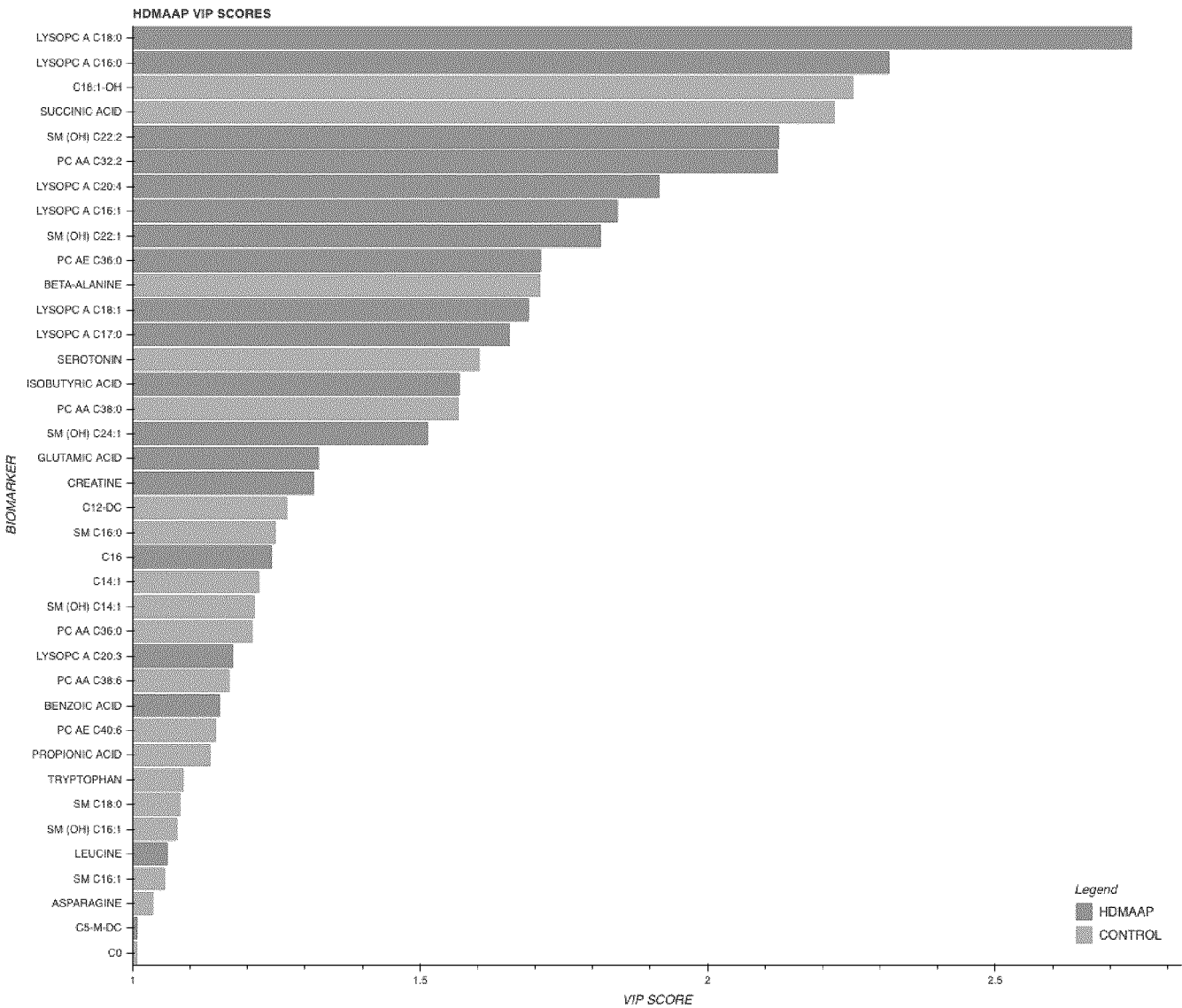


FIG 11

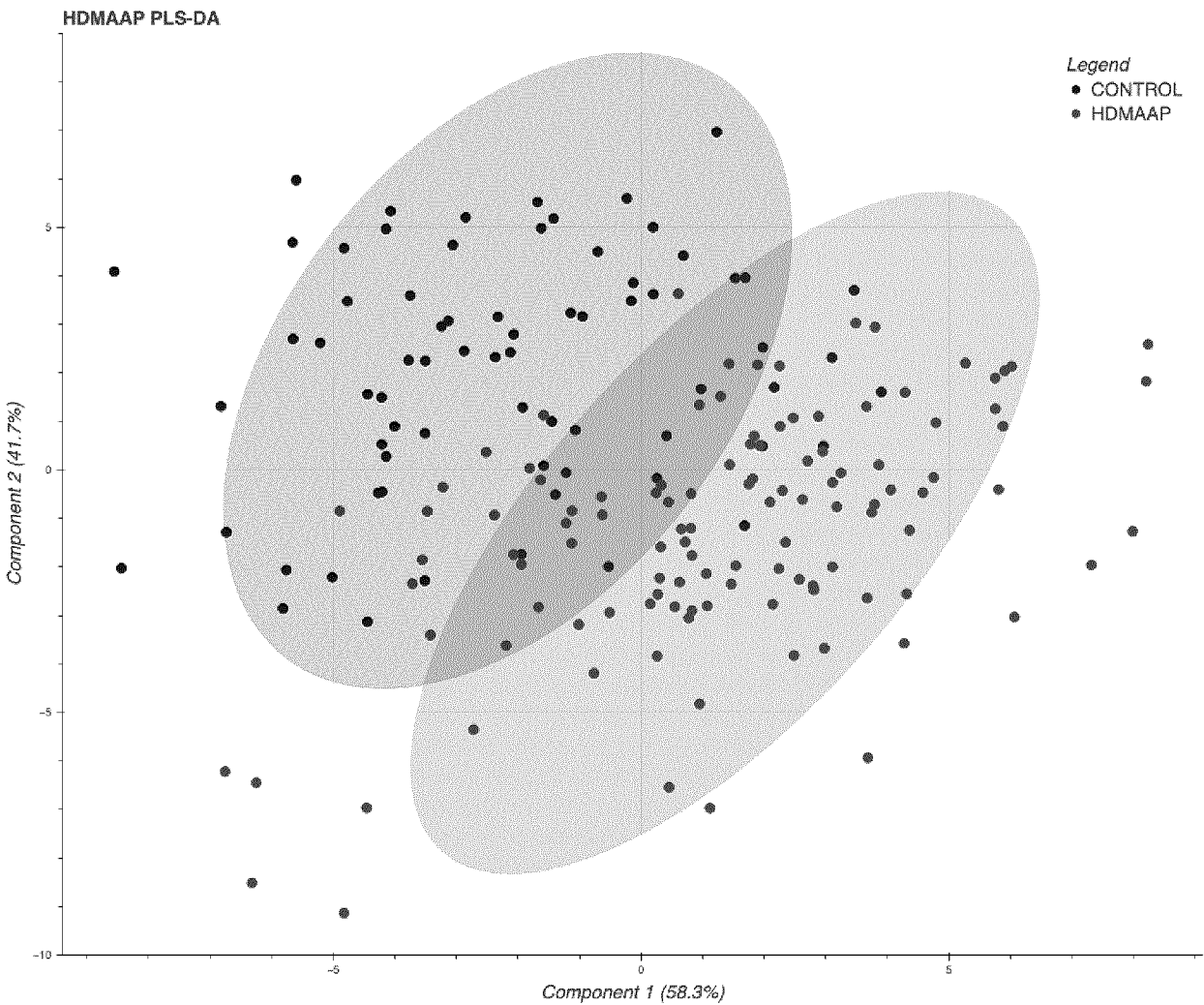


FIG 12

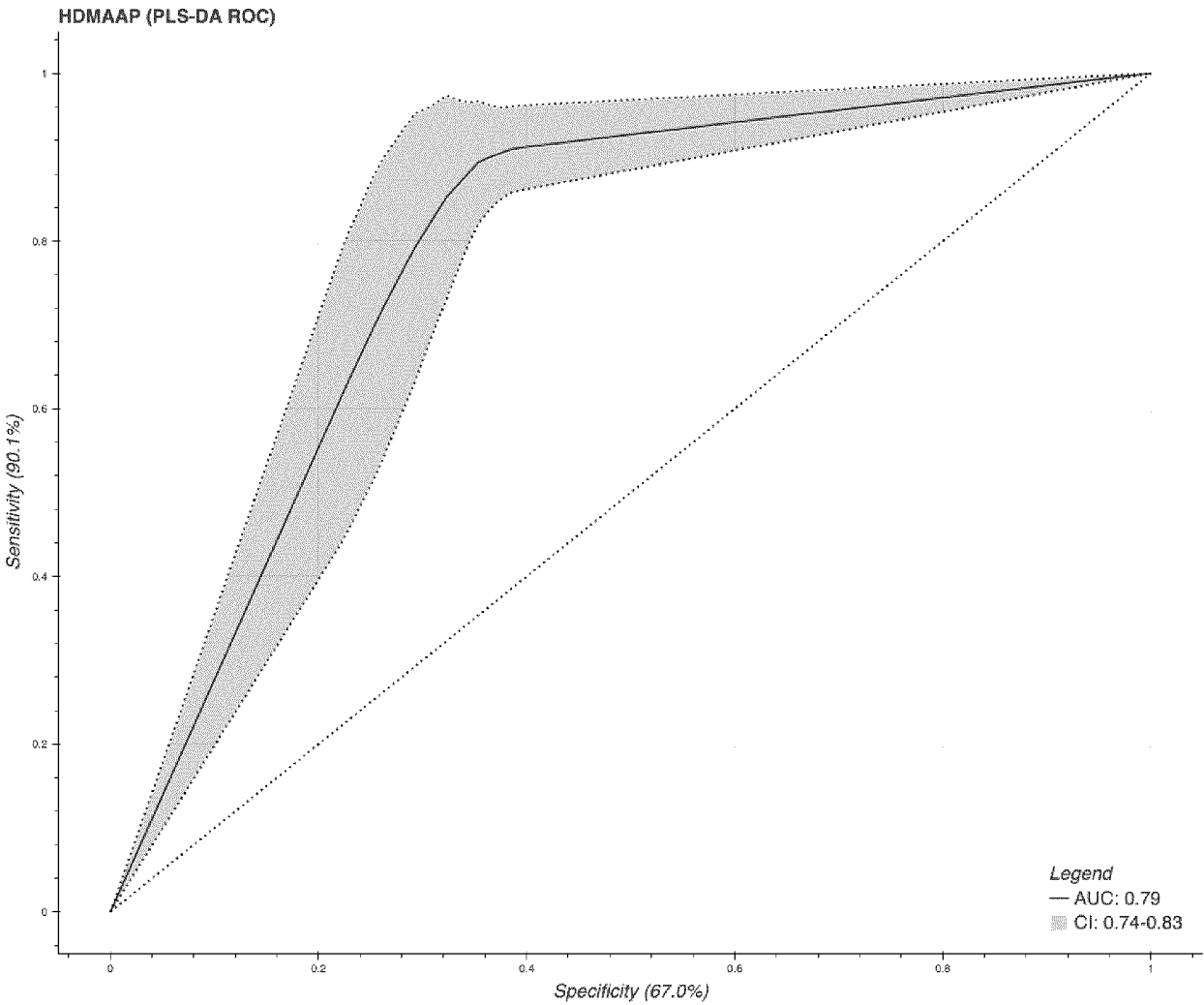


FIG 13

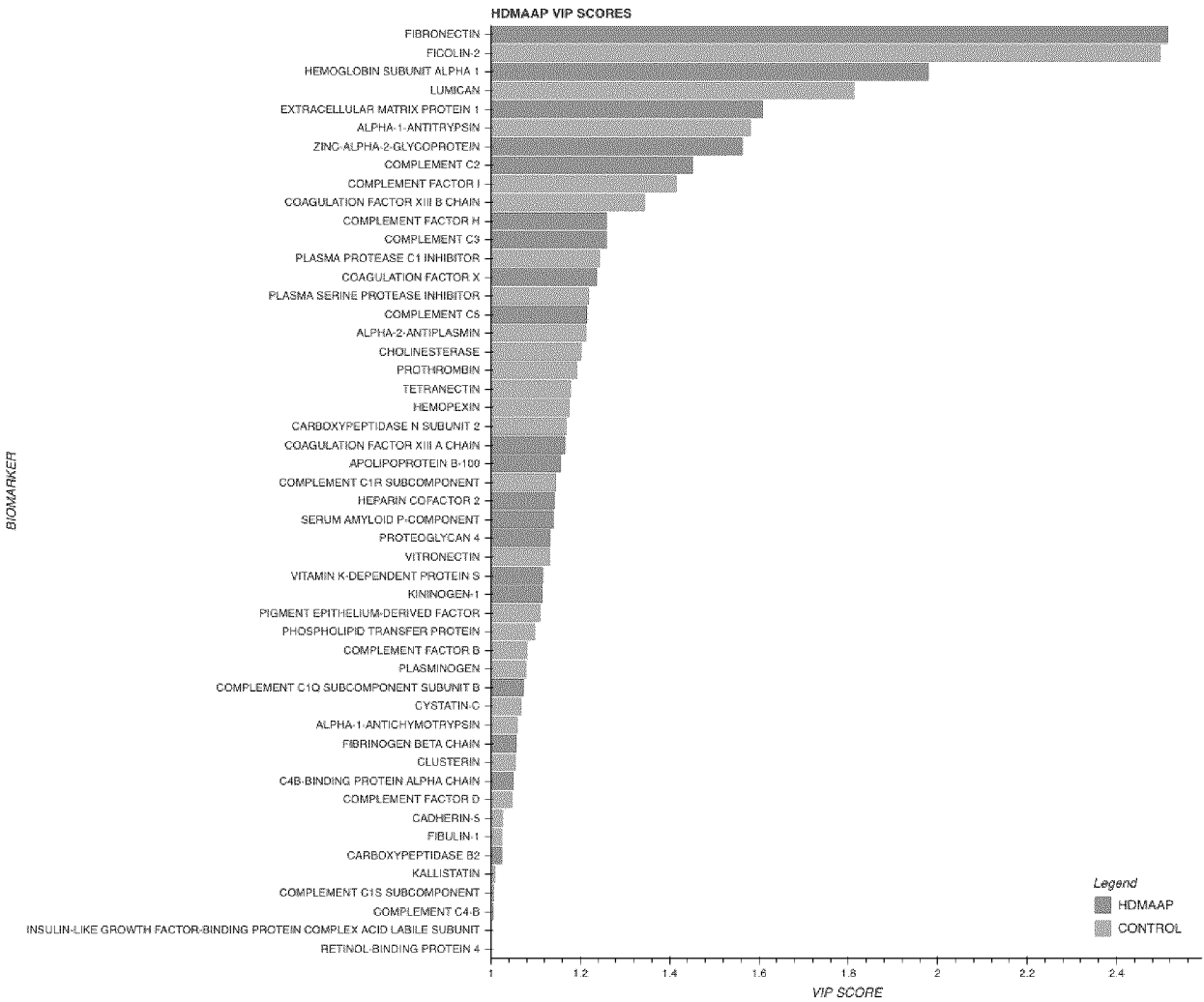


FIG 14

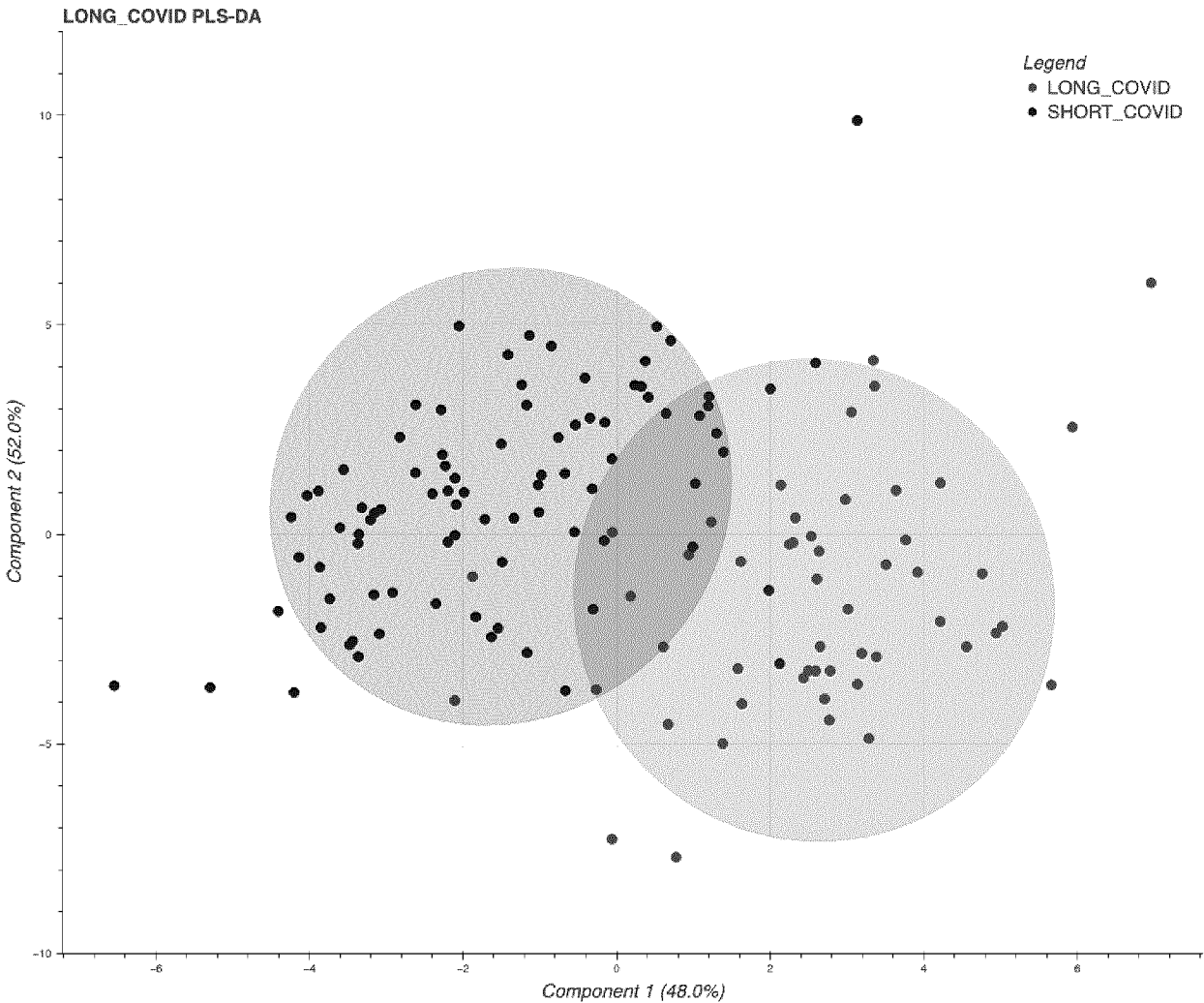


FIG 15

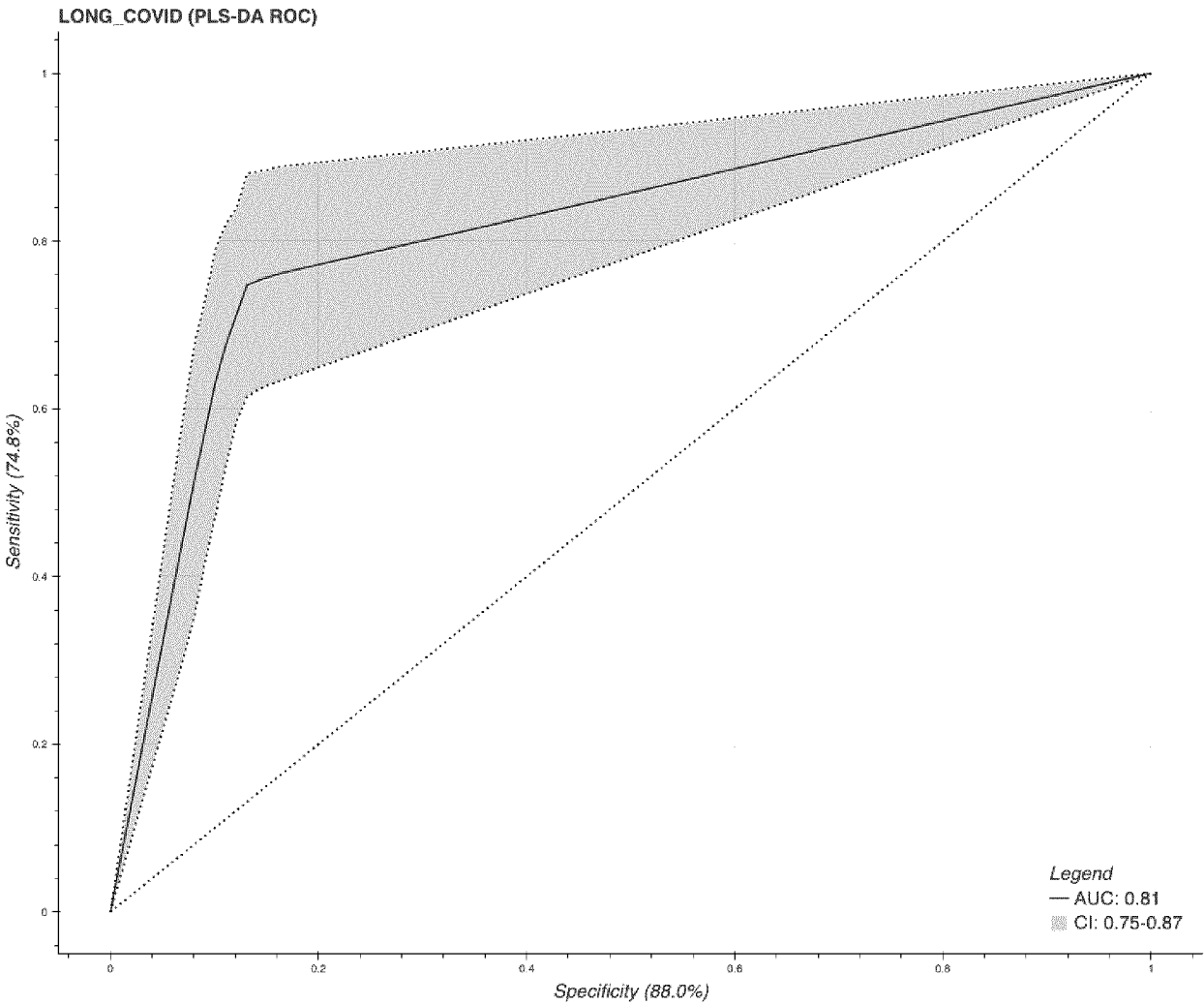


FIG 16

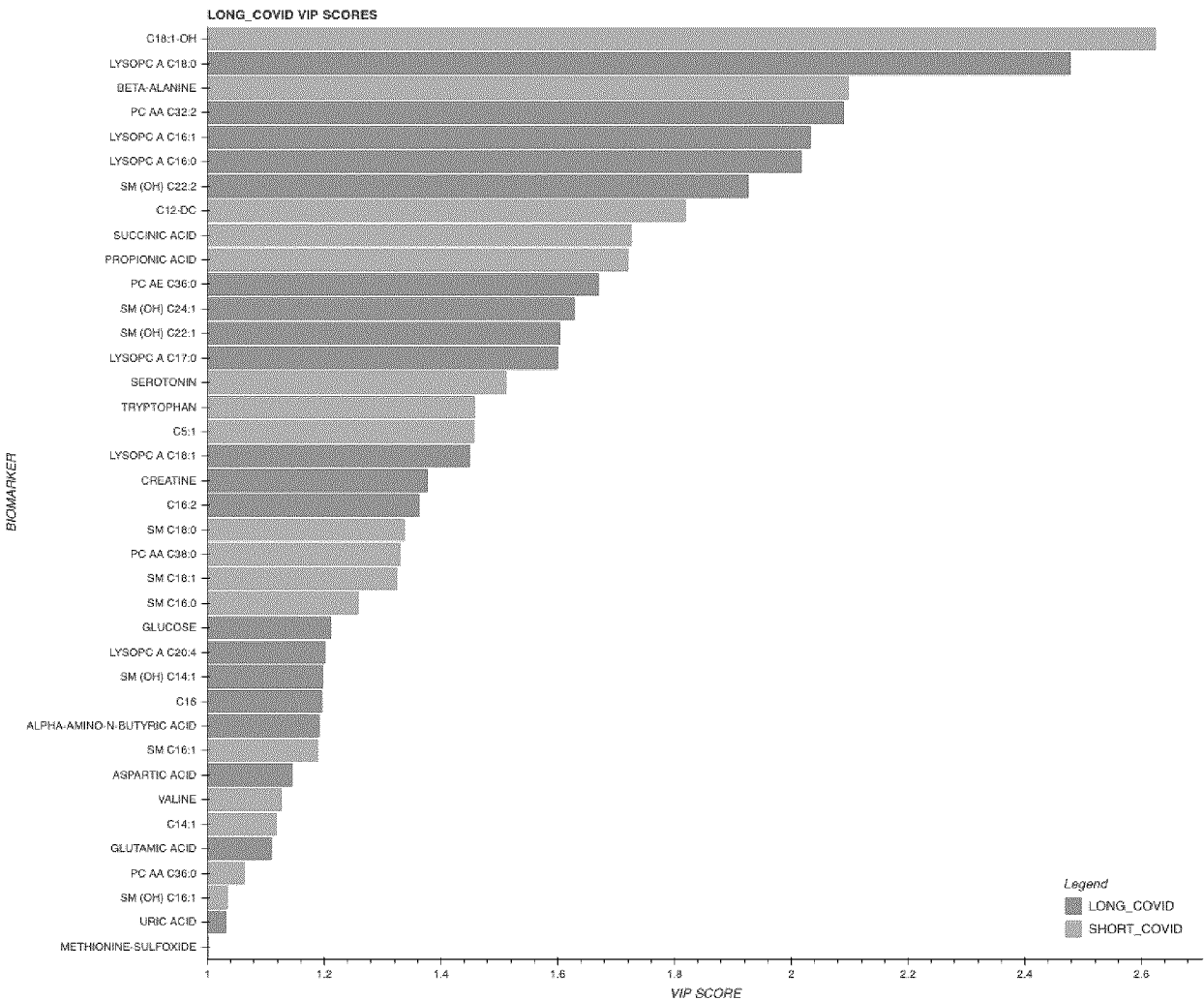


FIG 17

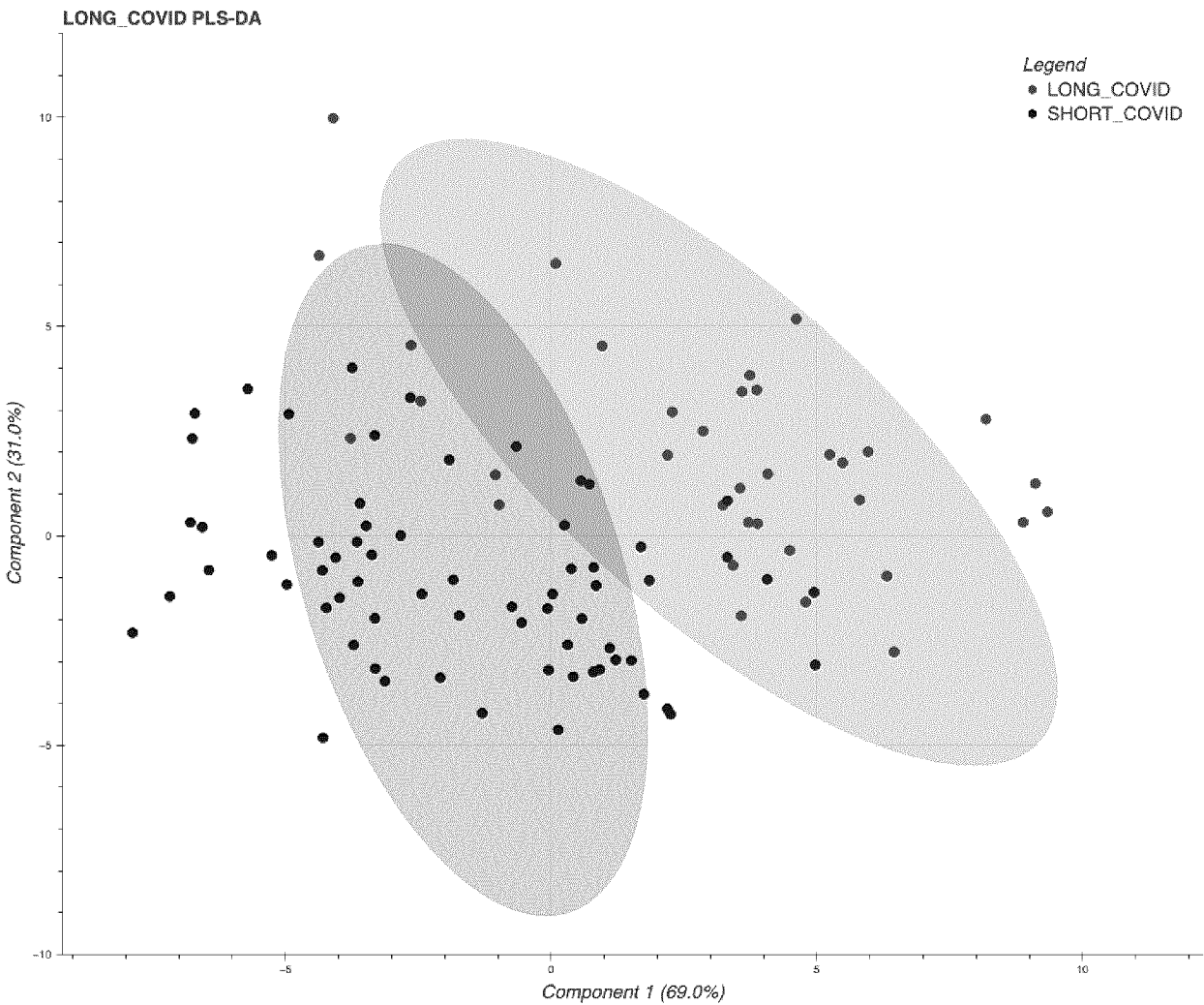


FIG 18

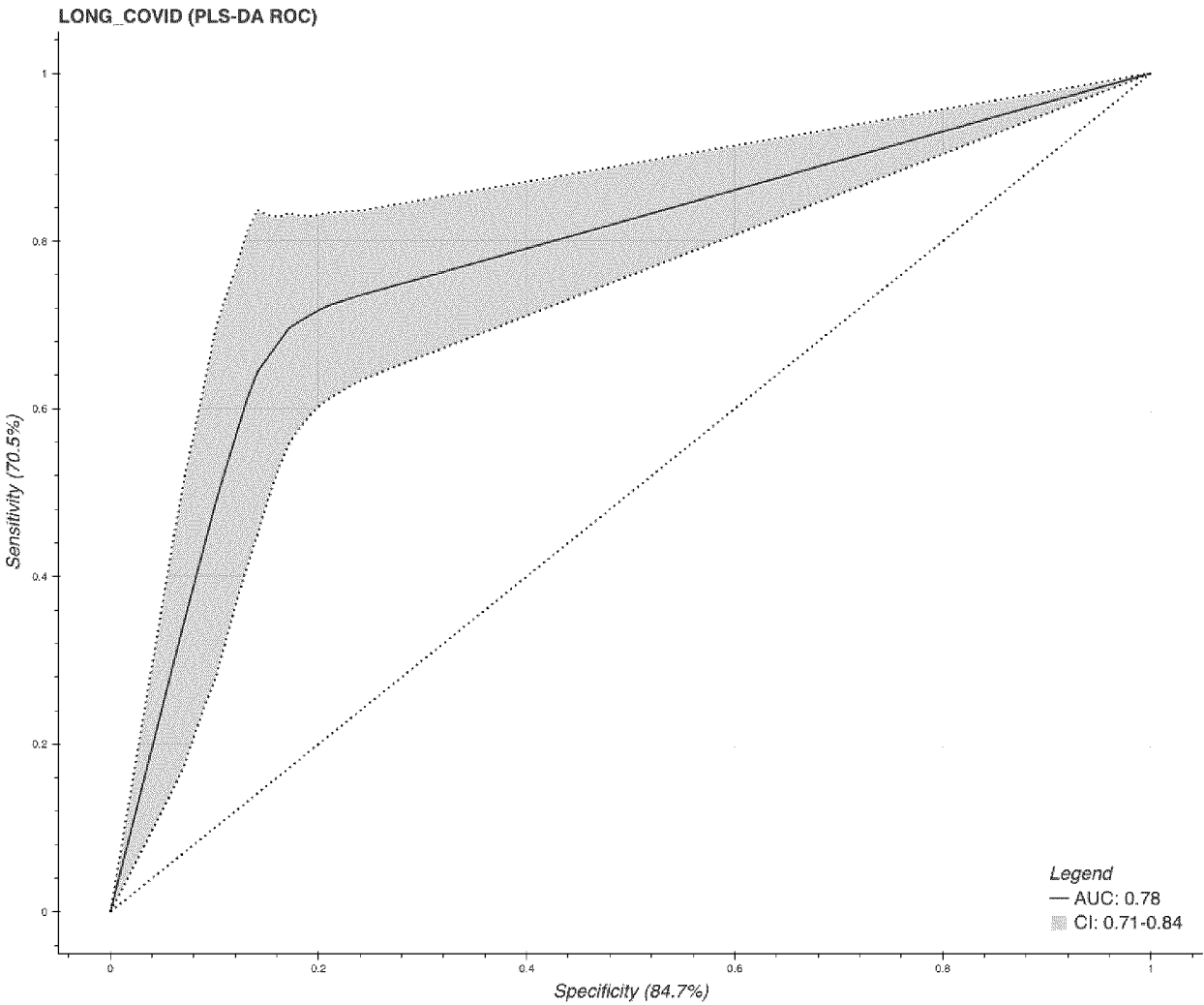


FIG 19

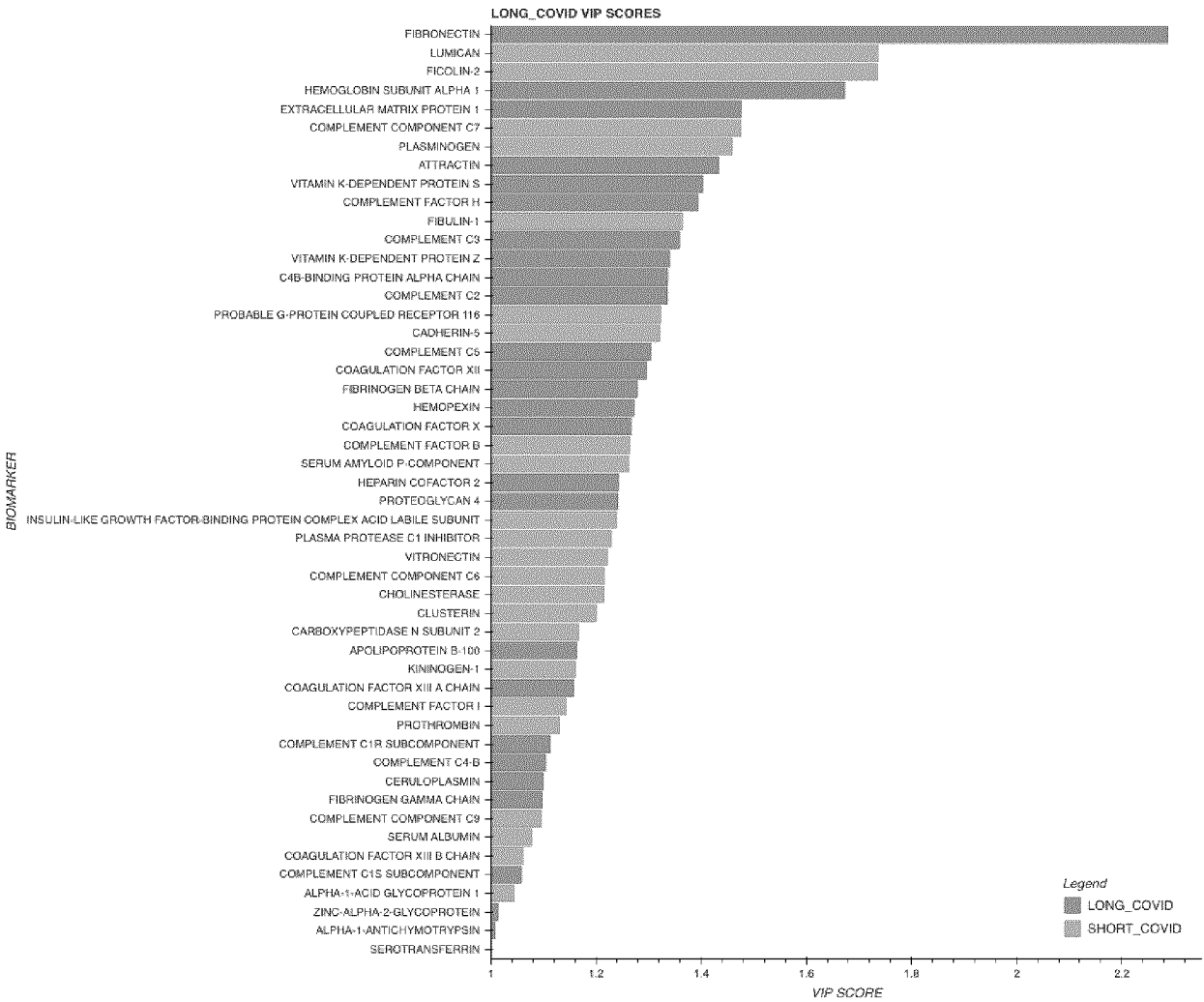


FIG 20

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2024/050833

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **G01N 33/48** (2006.01), **G01N 33/68** (2006.01), **A61K 31/7105** (2006.01)CPC: G01N 33/48 (2019.01), G01N 33/68 (2013.01), G01N 33/6848 (2013.01),
A61K 31/7105 (2013.01), G01N 2800/28 (2013.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: **G01N 33/48** (2006.01), **G01N 33/68** (2006.01), **A61K 31/7105** (2006.01) CPC: G01N 33/48 (2019.01), G01N 33/68 (2013.01),
G01N 33/6848 (2013.01), A61K 31/7105 (2013.01), G01N 2800/28 (2013.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Questel Orbit. Pubmed. Ebscohost. Search terms used: long, covid, lysophosphatidylcholine, lysopce

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO2021209575A2, (MAGNES, C, ZUGNER, E, PRUGGER, E, PRIEBER, T, PRIETL, B., OBERHUBER, M., ELLER, K) 21 October 2021 (21-10-2021). Claims 1, 8, 9.	1-43 (all in part)
A	WO2022185295A1 (ELRAYESS, M., AL-THANI, A., YASSINE, H.) 09 September 2022 (09-09-2022). Whole document	1-43 (all in part)
A	WO2021207858A1, (FRASER, D., CEPINSKAS, G., DALEY, M) 21 October 2021 (21-10-2021). Whole document.	1-43 (all in part)
A	VALDES, A., MORENA, L, RELLO, S., ORDUNA, A., BERNARDO, D., CIFUENTES, A. "Metabolomics study of COVID-19 patients in four different clinical stages" Nature Scientific Reports. (2022) 12:1650. Published 31 January 2022.	1-43 (all in part)

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* "A" "D" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance document cited by the applicant in the international application earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
01 October 2024 (01-10-2024)Date of mailing of the international search report
01 October 2024 (01-10-2024)Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Adam MacKenzie (819) 639-8506

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2024/050833**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Additional Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Group A – Claims 1-43(all in part) are directed to methods (and related kits and computer implemented methods and computer systems) of diagnosing or monitoring long COVID-19 in a patient by performing assays to determine level of one or more biomarkers, and that biomarker is chosen as Lysophosphatidylcholine C18:0;

- Remark on Protest**
- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
 - ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
 - ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2024/050833

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2021209575A2	21 October 2021 (21-10-2021)	WO2021209575A2 WO2021209575A3	21 October 2021 (21-10-2021) 25 November 2021 (25-11-2021)
WO2022185295A1	09 September 2022 (09-09-2022)	WO2022185295A1 US2023043689A1	09 September 2022 (09-09-2022) 09 February 2023 (09-02-2023)
WO2021207858A1	21 October 2021 (21-10-2021)	WO2021207858A1 CA3180489A1 US2023152317A1	21 October 2021 (21-10-2021) 21 October 2021 (21-10-2021) 18 May 2023 (18-05-2023)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2024/050833

The claims are directed to a plurality of inventive concepts as follows:

Group A – Claims 1-43(all in part) are directed to methods (and related kits and computer implemented methods and computer systems) of diagnosing or monitoring long COVID-19 in a patient by performing assays to determine level of one or more biomarkers, and that biomarker is chosen as Lysophosphatidylcholine C18:0;

Group B – Claims 1-43 (all in part) are directed to methods (and related kits and computer implemented methods and computer systems) of diagnosing or monitoring long COVID-19 in a patient by performing assays to determine level of one or more biomarkers, and that biomarker is chosen as Lysophosphatidylcholine C16:0;

Group C – Claims 1-43 (all in part) are directed to methods (and related kits and computer implemented methods and computer systems) of diagnosing or monitoring long COVID-19 in a patient by performing assays to determine level of one or more biomarkers, and that biomarker is chosen as 3-hydroxyoleoylcarnitine (C18:1-OH);

And so on for all recited 2^{28} combinations of one or more of 28 biomarkers.

The claims cannot be considered to be one invention in the sense of being so linked to form a single general inventive concept because each of the groups possess different special technical features and these features do not share any technical relationship.

Each biomarker and combination of biomarkers is its own solution to the problem of providing diagnosis and treatment for long COVID 19 and each requires its own search and opinion.

The claims must be limited to one inventive concept as set out in PCT Rule 13.