Bruker BioSpin GmbH

Analysis Report

Bruker PhenoRisk PACS™ (**P**ost-**A**cute **C**OVID-19 **S**yndrome) RuO

Sample ID:	SID3_NS-32_M1
Measuring Date:	15-Jan-2025 09:49:59
Reporting Date:	15-Jan-2025 12:41:58, 6 page(s)
Quantification Method:	PhenoRisk PACS RuO 1.0.0

Disclaimer

RESEARCH USE ONLY: This is no clinical diagnostic analysis report. Must not be used for clinical (medical or IVD) diagnosis or for patient management! Additional concentration range information (95% range of model) provided numerically or graphically in this report must not be used for clinical diagnostic interpretation. Application of Bruker PhenoRisk PACS RuO requires the use of Bruker's B.I.Methods SOP for plasma and serum.

Results

Risk Marker	Analyte	Value	Unit (*)	95% Range of Model	Graphics
Diabetic	Glucose	4.900	mmol/L	1.730 – 6.080	
Kidney	Creatinine	0.087	mmol/L	0.060 – 0.140	
CVD	TG	41	mg/dL	53 – 490	
CVD	Chol	144	mg/dL	140 – 341	
CVD	LDL-Chol	106	mg/dL	55 – 227	
CVD	HDL-Chol	26	mg/dL	35 – 96	
CVD	LDL-Phos	59	mg/dL	37 – 121	
CVD	HDL-Phos	32	mg/dL	57 – 136	
CVD	Apo-A1	92	mg/dL	112 – 217	
CVD	Apo-B100	67	mg/dL	48 – 160	
CVD	Apo-B100/Apo-A1	0.72	-	0.30 – 1.07	
Inflammatory	GlycA	0.72	p.d.u	0.85 – 1.35	
Inflammatory	GlycB	0.28	p.d.u	0.41 – 0.68	
Inflammatory	Glyc	1.01	p.d.u	1.24 – 2.11	
Inflammatory	SPC	1.01	p.d.u	1.41 – 3.68	
Inflammatory	Glyc/SPC	0.99	-	0.41 – 1.08	

(*) Inflammation markers are reported in procedure defined units (p.d.u). Please see explanation section for details. Yellow color indicates failed quality checks of the underlying NMR data or sample selectively for metabolites marked. Please handle labelled results with caution.

Quality Control

NMR Instrumentation ✓	Experiment ✓	Spectral Quality ✓	Preparation ✓	Matrix ✓	Contamination ✓
Quality control passed.					

Explanations

Intended Use

Bruker PhenoRisk PACS RuO is intended to be used for research use only. The software automatically and quantitatively analyzes NMR spectra obtained from in-vitro NMR measurements of human EDTA plasma and serum samples. Spectral data can be acquired within 20 minutes using the Bruker Avance IVDr platform. The purpose of Bruker PhenoRisk PACS RuO is to provide information related to cardiovascular disorder, pre- or Diabetes Type II, kidney disorder and inflammatory status in the context of post-acute COVID-19 syndrome.

Gray horizontal boxes shown in the results table represent 95% concentration ranges that were derived from the respective model cohorts, whereas black vertical lines represent the sample value. Limits of detection (LOD) are provided where applicable in the NMR quantificaiton section below. A number of quality control checks are performed to ensure compliance with sample preparation and experiment SOPs provided by the B.I.Methods. In case of a failed quality control check, please handle the reported results with caution and contact a Bruker expert.

NMR Quality Control

Bruker PhenoRisk PACS RuO should be used in combination with an AV III HD or AV NEO spectrometer and a minimum TopSpin version of 3.6.2 or 4.1.1, respectively. Compliance of the NMR hardware with the recommended configuration is tested in the NMR Instrumentation panel. Tests related to experimental parameters (Experiment panel) need to be passed successfully in order to document that NMR parameters for 1D-NOESY, 1D-PGPE and 2D-JRES are consistent with the specified B.I.Methods' NMR SOPs.

For more detailed information on the remaining quality checks, please see the Bruker IVDr BioBank QC (B.I.BioBankQC) report.

NMR Quantification

Glucose concentration (LOD = 0.54 mmol/L) was determined by curve fitting of 1D-NOESY data. Creatinine (LOD = 0.01 mmol/L) was quantified from the signal intenstiy in the 2D-JRES spectrum. To assess a wider panel of up to 41 metabolites, please use B.I.QuantPS. Cardiovascular disease (CVD) markers were determined from 1D-NOESY data using a regression model. A complete lipoprotein subclass analysis featuring 112 parameters is provided by B.I.LISA.

Inflammation markers (Glyc and SPC) were quantified from the 1D-PGPE spectrum. In order to ensure full comparability between samples and across different spectrometers, PGPE spectra are quantitatively calibrated based on the spectrometer hardware and experimental parameters. Therefore, the PGPE integral can be interpreted in terms of NOESY concentration equivalents in mmol/L. As the signal intensities of the quantified analytes are subject to multiple spectral editing steps during the experiment, concentrations cannot be directly derived from the integral and are thus reported in procedure defined units (p.d.u).

Background Information

Metabolomic examination detects a complex pattern of disturbance of systemic metabolism caused by a SARS-CoV-2 viral infection associated with multiple organ-specific changes.[1] The distinct metabolic signature in COVID-19 patients was shown at three months after the acute disease, which may indicate a prolonged illness.The breadth of the disturbed pathways indicates a systemic signature involving dyslipidemia, diabetes, and coronary heart disease risk that are consistent with recent reports that COVID-19 is a systemic disease affecting multiple organs and systems.[2] Other studies indicate that diabetes type II is a common but previously overlooked effects of SARS-CoV-2 infection.[3] In addition, it has been shown that beyond the acute phase of COVID-19 illness, survivors of COVID-19 exhibited higher risks of acute kidney injury (AKI), estimated glomerular filtration rate (eGFR) decline, end stage kidney disease (ESKD), major adverse kidney events (MAKE), and steeper longitudinal decline in eGFR.[4]

NMR spectroscopy has played a key role in helping to understand COVID-19 and there is an emerging consensus that an individual's NMR metabolomic signature can provide reliable insight into:

- disease progression during acute infection
- partial or full recovery of the patient
- severity of the disease
- treatment outcome

Recent publications have shown that a SARS-CoV-2 infection causes a complex range of immunologically driven systemic effects in different organ systems, which manifests in multiple biochemical pathway disruptions causing changes in the metabolic signature, a phenomenon named phenoconversion.[5,6] This concept describes consequences when a harmful agent is introduced into the body as a series of rapid localized and systemic effects in metabolism and physiology triggering changes from a normal or healthy state to a disordered pathophysiological state or overt pathology.

Typically, a range of metabolic biomarkers are involved, which are specific to disease state detection and severity measurement giving deep insights into a range of pathophysiological processes. The SARS-CoV-2 infection dysregulates the metabolomic and lipidomic profiles of serum by abnormal concentrations of Apo-A1, Apo-B100, Creatinine, Glucose, GlycA, GlycB and SPC (please see below for details). It was shown that the pattern of disturbed molecular markers persisted in some patients after the virus became undetectable in their blood indicating an incomplete systemic recovery and metabolic phenoreversion in Post-Acute-Phase COVID-19 patients as the parameters mentioned above remained elevated or depleted, or showed only a partial normalization.

These studies have also shown that this panel of phenotypic risk markers identified by NMR indicates a recovery (phenoreversion) that is associated with disease remission.[2] In addition, those metabolic markers distinguish COVID-19 from other intensive care patients and show potential to stratify for disease risk. Metabolomic/lipidomic profiling of COVID-19 may also allow to monitor individual response to drugs as shown for tocilizumab as its use led to at least partial reversion of the metabolic alterations resulting from SARS-CoV-2 infection.

Background Information on Analytes

The following section provides a short summary of the key analytes listed in the Bruker PhenoRisk PACS RuO report. The risk marker panel offered by the property software Bruker PhenoRisk PACS RuO monitors analytes allowing for a deeper understanding of long-term consequences following an acute SARS-CoV-2 infection. Furthermore, it may detect early-stage secondary organ damage and/or risk for secondary disease as the metabolic signature affects clinically known risk markers for inflammation, cardiovascular disease, Diabetes Type II and kidney disorder.

GlycA, GlycB and SPC NMR spectroscopy can be used to quantify a set of composite signals for groups of glycoproteins and phospholipids which were either elevated or reduced in SARS-CoV-2 infected patients compared to controls. The glycoprotein signal (Glyc) constitutes of GlycA and GlycB, which are composite N-acetyl signals from α1-acid glycoprotein and other glyoproteins. Both signals, GlycA and GlycB, depend on glycosylation of circulating acute phase proteins, especially fibrinogen, α1-antichymotrypsin, haptoglobin-1, α1-antitrypsin, complement C3 and α1-acid glycoprotein. Glyc and especially GlycA are significantly increased in patients tested positive for SARS-CoV-2.[2]

The N-acetyl signals of GlycA were originally identified as NMR detectable biomarkers for acute systemic inflammation.[7] In PACS, all Glyc signals are elevated indicating acute inflammation.[2] GlycA is also associated with cardiovascular disease and indicates severity in several inflammatory disorders.[8] Multiple inflammatory associations and correlations have been observed between GlycA and blood triglycerides, lipids, branched chain amino acids, higher IL-6 and C-reactive protein levels as well as future development of Type 2 Diabetes Mellitus. GlycA and GlycB are also associated to insulin resistance.[9]

Another inflammatory biomarker to study COVID-19 pathology is the supramolecular phospholipid composite (SPC), a total composite NMR signal from the choline head groups that are associated with HDL and LDL subfractions, together with lysophosphatidylcholine bound to α1-acid glycoprotein and the glycoprotein N-acetyl composite signals GlycA and GlycB. These markers show excellent discrimination of SARS-CoV-2 positivity from controls or SARS-CoV-2 negative respiratory patients; besides, various ratios of GlycA/GlycB and SPC components are particularly sensitive to the COVID-19 disease presence.[10] In PACS, all SPC signals are lowered indicating a phenoconversion of the metabolome associated with inflammation. Thus, plasma SPC and the Glyc/SPC ratio are proposed as sensitive molecular markers for COVID-19 that could effectively augment current COVID-19 diagnostics and may have value in functional assessment of the disease recovery process in patients with long-term symptoms.[11]

Apo-A1, Apo-B100 Testing of EDTA plasma or serum reveals a complex lipoprotein profile of COVID-19 patients with a redistribution of lipoprotein particle size and composition. Significant metabolic variables are high Apo-B100/Apo-A1 ratio being apparent with much lower levels of major HDL class particles and components.[11] This lipoprotein profiling indicates increased atherogenic risk in COVID-19 patients, indicated by pathogenic Apo-A1, Apo-A2 and Apo-B100 values. Apolipoprotein B (Apo-B100) and Apolipoprotein A1 (Apo-A1) are known risk indicators of coronary heart disease, and their ratio shows promises as potential marker of plasma atherogenicity.[1]

The Apo-B100/Apo-A1 ratio is used clinically to assess cardiovascular disease risk. The higher ratios observed in COVID-19 therefore indicate an increased risk of cardiovascular disease.[12] And indeed, a study showed new onset of atherosclerosis following SARS-CoV-2 infection.[13] Moreover, the pattern is also consistent with recent observations on the NMR measured lipoprotein and metabolic signatures of carotid atherosclerosis and cardiovascular disease and in particular patterns associated with coronary artery calcium levels and carotid intima-media thickness.[14]

Glucose Glucose concentration is significantly raised in SARS-CoV-2 positive individuals consistent with a diabetic or prediabetic trait.[1] Furthermore, the dyslipidemia profile of individuals with diabetes feature reduced HDL cholesterol, a predominance of LDL particles and elevated triglyceride levels as well.[15]

Creatinine The creatinine level is significantly elevated during an acute SARS-CoV-2 infection and PACS thus might be an indicator for renal dysfunction.[2]

References

[1] Lodge, S. et al., "Diffusion and Relaxation Edited Proton NMR Spectroscopy of Plasma Reveals a High-Fidelity Supramolecular Biomarker Signature of SARS-CoV-2 Infection", *Anal. Chem.* **2021**, *93*, 3976–3986.

[2] Kimhofer T. et al., "Integrative Modeling of Quantitative Plasma Lipoprotein, Metabolic, and Amino Acid Data Reveals a Multiorgan Pathological Signature of SARS-CoV-2 Infection", *J. Proteome Res.* **2020**, *19*, 4442–4454.

[3] Chee, Y.J et al., "Diabetic Ketoacidosis Precipitated by Covid-19 in a Patient with Newly Diagnosed Diabetes Mellitus", *Diabetes Res. Clin. Pract.* **2020**, *164*, 108166.

[4] Bowe, B. et al., "Kidney Outcomes in Long COVID", *JASN* **2021**, *32*, 2851–2862.

[5] Masuda R. et al., "Exploration of Human Serum Lipoprotein Supramolecular Phospholipids Using Statistical Heterospectroscopy in *n*-Dimensions (SHY-*n*): Identification of Potential Cardiovascular Risk Biomarkers Related to SARS-CoV-2 Infection", *Anal. Chem.* **2022**, *94*, 4426–4436.

[6] Holmes E. et al., "Incomplete Systemic Recovery and Metabolic Phenoreversion in Post-Acute-Phase Nonhospitalized COVID-19 Patients: Implications for Assessment of Post-Acute COVID-19 Syndrome", *J. Proteome Res.* **2021**, *20*, 3315–3329.

[7] Bell, J.D. et al., "Assignment of resonances for 'acute-phase' glycoproteins in high resolution proton NMR spectra of human blood plasma", *FEBS Lett.* **1987**, *215*, 311–315.

[8] Bruzzone, C. et al., "SARS-CoV-2 Infection Dysregulates the Metabolomic and Lipidomic Profiles of Serum", *iScience* **2020**, *23*, 101645.

[9] Connelly, M.A. et al., "GlycA, a novel biomarker of systemic inflammation and cardiovascular disease risk", *J. Transl. Med.* **2017**, *15*, 219.

[10] Otvos, J.D. et al., "GlycA: A Composite Nuclear Magnetic Resonance Biomarker of Systemic Inflammation", *Clin. Chem.* **2015**, *61*, 714–723.

[11] Kaneva, A.M. et al., "The Apolipoprotein B/Apolipoprotein A-I Ratio as a Potential Marker of Plasma Atherogenicity", *Dis. Markers* **2015**, 591454.

[12] Wallidus G. et al., "Apolipoprotein B and apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-modifying therapy", *J. Intern. Med.* **2004**, *255*, 188–205.

[13] Liu, Y. et al., "Vigilance on New-Onset Atherosclerosis Following SARS-CoV-2 Infection", *Front. Med.* **2021**, *7*, 629413.

[14] Tzoulaki, I. et al., "Serum metabolic signatures of coronary and carotid atherosclerosis and subsequent cardiovascular disease", *Eur. Heart J.* **2019**, *40*, 2883–2896.

[15] Liu, X. et al., "High plasma glutamate and low glutamine-to-glutamate ratio are associated with type 2 diabetes: Case-cohort study within the PREDIMED trial", *Nutr. Metab. Cardiovasc. Dis.* **2019**, *29*, 1040–1049.