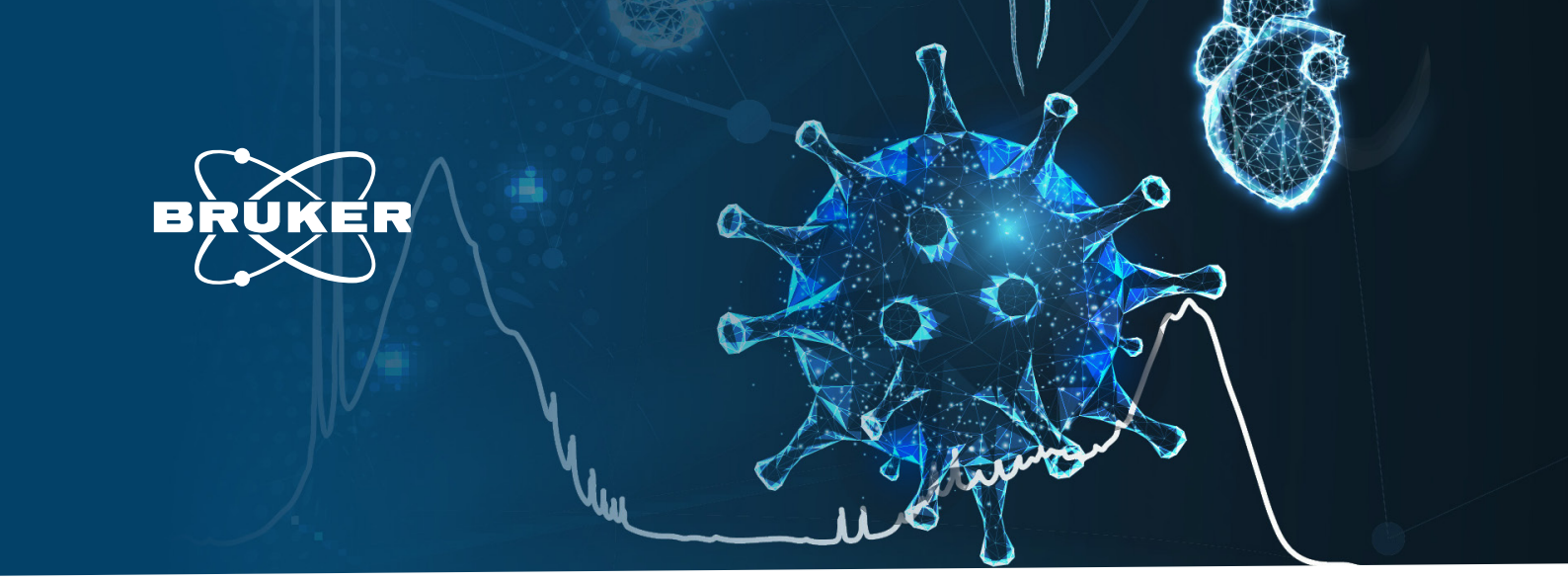


CLINICAL RESEARCH - WHITE PAPER

Contributing to Researchers' Unlocking our clinical understanding of Post-Acute COVID Syndrome (PACS), patient recovery, and risk of subsequent disease

The growing body of evidence from members of the NMR International COVID-19 Research Network suggests new NMR* markers can provide vital insight as patients recover from acute SARS-CoV-2 infection.

Innovation with Integrity

Introduction

Following the first COVID-19 cases—recorded in Wuhan, China on 31 December 2019 the clinical research community had an immediate response by establishing a series of international cooperation, and global scientific networks were rapidly formed. These included the NMR International COVID-19 Research Network, which was initiated by Bruker, under the guidance of the Australian National Phenome Center (ANPC) at Murdoch University, supervised by Professor Jeremy Nicholson, along with other expert institutes. The primary goal was to establish a worldwide research network that would allow institutions to work together, using standardized Nuclear Magnetic Resonance (NMR) spectroscopy procedures, to produce spectral data from COVID-19 positive patients that could be exchanged and integrated across the network.

The research undertaken in the months immediately after the onset of the pandemic and subsequently published in August 2020[1], provided early insights into the biomolecular basis for what clinicians were observing on the ground with COVID-19 patients, i.e. it was a virus that not only affected the respiratory system but also had a significant systemic impact – on the heart and vascular system, for example.

***Bruker NMR Instruments are for Research Use Only. Not for Use in Clinical Diagnostic Procedures**

In this white paper, we review how, beginning with the first publication mentioned above, NMR spectroscopy has played a key role in helping to understand COVID-19 and how, almost two years later, there is a strong consensus that an individual's NMR 'biosignature', and a series of biomarkers that are only accessible by NMR technology have the potential to help researchers revealing reliable insights into:

- disease severity
- progress towards recovery
- risk of developing Post-Acute COVID-19 Syndrome (PACS), or 'Long COVID'
- early-stage secondary organ damage
- risk of developing secondary disease.

Scientific publications from Prof. Nicholson group at the ANPC, responsible for the first one, are featured here, along with key papers from other members of the NMR International COVID-19 Research Network, namely: CIC bioGUNE, Spain, the University of Lubeck, Germany, and the Magnetic Resonance Centre (CERM) at the University of Florence, Italy. (**Figure 1**),

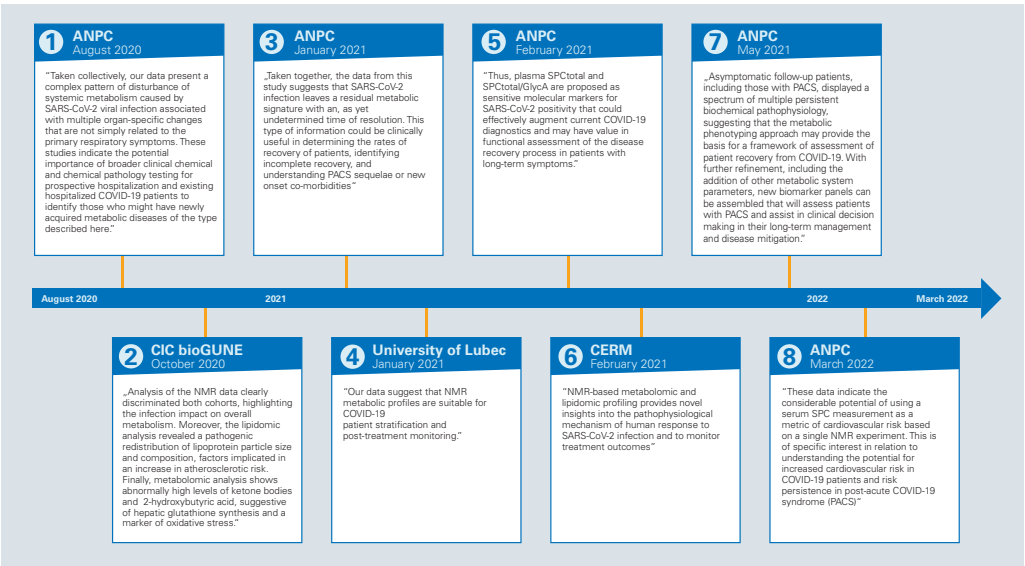


Figure 1: Publication under review in the white paper
A timeline of the publications under review in this white paper, from August 2020 to March 2022.

Post-Acute COVID-19 Syndrome (PACS)

It is well established that COVID-19 is a complex respiratory and systemic disease, caused by infection with the SARS-CoV-2 coronavirus, that results in damage to multiple organ systems. Now, it is also clear that a large number of patients do not fully recover from an acute infection, but rather experience persistent and highly variable symptoms. This seems to be true for symptomatic patients, those who test COVID-19 positive but remain asymptomatic, and those who only experience mild disease following infection.

The term Post-Acute COVID-19 Syndrome or 'PACS' has been coined to describe these sequelae of acute infection. It is also known as 'Long COVID'.

To put PACS into context:

- British population data suggest up to 38% of people with acute infection will have at least one symptom of PACS.[2]
- In the US, more than 1 million people have been out of the workforce at any given time because of PACS.[3]
- An analysis performed at Harvard Medical School estimated that US$2.6 trillion of cost could be attributed to PACS.[4]

Against this background, PACS research is focused on finding metabolic markers that inform clinical practice for individual patients, and illuminate possible public healthcare 'time-bombs' for future medico-economic burdens that may be faced around the world as a result of continuing and widespread exposure of populations to SARS-CoV-2.

Phenoconversion and phenoreversion - its role in COVID-19, and its assessment by NMR spectroscopy

The concept of phenoconversion is well known in the field of drug development, where exposure to a specific drug changes the phenotype of the organism by inducing a specific Cytochrome P450 enzyme that alters the subsequent metabolism of the drug on further administration.

Prof. Nicholson at ANPC had previously worked on this concept in drug metabolism[5], and he and his group understood that, in a broad sense, when any harmful agent (chemical or biological) is introduced into the body, there is a series of rapid localized and systemic effects in metabolism and physiology which is also a process of phenoconversion—the change from a normal or healthy state to a disordered pathophysiological state or overt pathology.

Typically, a range of metabolic phenoconversion biomarkers that are effectively specific to disease state detection and severity can be identified and subsequently analyzed[6].
For COVID-19 patients, biomarkers such as lipoproteins, glycoproteins, amino acids, lipids, and other metabolites, as measured by NMR spectroscopy, have been identified, with such SARS-CoV-2 positive 'metabotypes' shown to be distinct from both healthy controls and SARS-CoV-2 negative respiratory patients[7].

Importantly, from a theoretical point of view, a process of 'phenoreversion' would involve the reversal of these metabolic and physiological effects. It has been postulated that such a process could be partial or complete, reflecting the degree of systemic recovery from COVID-19 infection, and that it could be assessed with the same series of biomarkers.

The ANPC research group hypothesized that the SARS-CoV-2 virus's pathological effects could be understood in terms of systemic phenoconversion, and that recovery could be characterized by phenoreversion, with both being expressed in the metabolic profile of blood plasma. They set out to test and develop this hypothesis further by applying quantitative NMR spectroscopy to measure multiparametric plasma lipoprotein profiles, inflammation markers and a set of low-molecular-weight metabolites.

The power of biofluids measurement by NMR*

NMR spectroscopy is a powerful analytical technique that allows non-invasive, non-destructive, and quantitative investigations into molecular structures and dynamic processes at the atomic level. Initially used in analytical chemistry, NMR applications subsequently expanded to include analysis of biological molecules, and the technique has become a key tool for clinical research. Bruker is the leading industry in manufacturing unique high-performance magnetic resonance instruments. The Bruker Avance IVDr platform, currently for research use only, is a complete, proven, and standardized NMR platform for clinical research, as well as screening and translational research.

It features high sensitivity and information-rich output at 600 MHz proton-NMR frequency and incorporates advanced hardware, software, automation, standard operating procedures (SOPs) and consumables for high-performance biofluid assays. Benefits include:

- Minimal sample preparation
- Full automation in high throughput screening mode
- Highest reproducibility and data quality from sample to sample and instrument to instrument
- Direct and absolute quantification of large sets of metabolites in urine and plasma/serum with automated reporting
- Direct and absolute quantification of main and subclasses lipoprotein in plasma/serum
- Classification and discrimination using statistical tools with automated reporting
- Low cost per sample and lowest cost per parameter

*Bruker NMR is for research use only. Not for use in clinical diagnostic procedures.

A timeline to understanding

The research papers presented below, in chronological order of publication, illustrates how our understanding of a representative molecular signature for SARS-CoV-2 infection began with the first work at ANPC[1], and how a further study of a large cohort of patients corroborated the initial findings[8].

Next, it was shown that the pattern of disturbed molecular markers persisted in some patients after the virus became undetectable in their blood. It was also reported that the metabolic markers could differentiate COVID-19 patients from other patients in intensive care[9].

As knowledge increased, researchers were able to confirm that (i) some patients were suffering from PACS and the metabolic markers could help clinicians manage these individuals as the disease progressed[10], and (ii) markers could also show how an individual was responding to therapeutics[11]. Furthermore, the research team was able to conclude that the clinical status of PACS patients could be accurately assessed using a panel of metabolic markers in plasma/serum[12].

In the final paper discussed here, published in March 2022, a plama/serum supramolecular phospholipid composite (SPC), previously noted as a marker of inflammation, was investigated as a possible metric of cardiovascular risk based on a single NMR experiment. It was found to have the potential to predict increased cardiovascular risk in COVID-19 patients, and risk persistence in PACS[13].

The following chapter will describe the key papers more in detail.

1

Publication Date: 17 August 2020

Integrative Modelling of Quantitative Plasma Lipoprotein, Metabolic, and Amino Acid Data Reveals a Multiorgan Pathological Signature of SARS-CoV-2 Infection

Torben Kimhofer, Samantha Lodge, Luke Whiley, Nicola Gray, Ruey Leng Loo, Nathan G. Lawler, Philipp Nitschke, Sze-How Bong, David L. Morrison, Sofina Begum, Toby Richards, Bu B. Yeap, Chris Smith, Kenneth G. C. Smith, Elaine Holmes, and Jeremy K. Nicholson
J. Proteome Res. 2020, 19, 11, 4442–4454

https://doi.org/10.1021/acs.jproteome.0c00519

Study description

Plasma samples were collected from adults who presented with COVID-19 disease symptoms and subsequently tested positive for SARS-CoV-2 infection from upper and/lower respiratory tract swabs by RT-PCR and healthy controls recruited from the population who did not and had not exhibited any COVID-19 disease symptoms and were serologically tested negative with respect to IgA/IgG antibodies. SARS-CoV-2 RNA positive-tested patients presented with symptoms including fever, cough, shortness of breath, and fatigue.

All NMR analysis was completed on Bruker Avance In Vitro Diagnostics research (IVDr) platform. All experiments were completed using the Bruker IVDr methods and common statistical techniques, namely: principal component analysis (PCA) and an orthogonal-projections to latent structures (OPLS) method. These were used to construct an exceptionally strong (AUROC = 1) model that enabled detailed metabolic discrimination between patient groups and their biochemical relationships.

Results, conclusions, and clinical significance

The results, both from PCA analysis, and the OPLS-DA model, clearly indicated significant systematic metabolic differences in the plasma of SARS-CoV-2 positive patients, when compared with healthy controls (**Figure 2**).

The multiorgan pathological signature of SARS-CoV-2 Infection seen in the study population included, as a key discriminant, metabolite markers of inflammation, including elevated Glyc A. There was also an abnormal lipoprotein and glucose consistent with diabetes and coronary artery disease (low total and HDL Apolipoprotein A1, low HDL triglycerides, high LDL and VLDL triglycerides).

The breadth of the disturbed pathways indicates a systemic signature of SARS-CoV-2 positivity that includes elements of dyslipidemia, diabetes, and coronary heart disease risk – all consistent with reports that COVID-19 is a systemic disease affecting multiple organs and systems. The dyslipidemia profile of individuals with diabetes feature reduced HDL cholesterol, a predominance of LDL particles, and elevated triglyceride levels.

The study data indicates large systematic metabolic differences in the plasma of SARS-CoV-2 positive when compared with healthy controls. The Nicholson group has suggested previously that the use of orthogonal phenoconversion tests for COVID-19 detection might be considered to augment polymerase chain reaction (PCR) based diagnostics based on systemic disease effects.[7] Such diagnostics can also be extended to clinical trial monitoring[9,10] and long-term disease recovery studies.

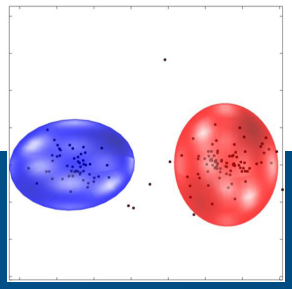
	healthy control (n = 25)	SARS-CoV-2 positive (n=17)	p-value[a]
Glyc A (rel. intensity)	1.99×10^5	3.3×10^5	2.13×10^{-7}
	[1.66×10^5]	[1.96×10^5 to 4.02×10^5]	
Glyc B (rel. intensity)	3.31×10^5	5.19×10^5	2.74×10^{-7}
	[1.90×10^5 to 4.62×10^5]	[3.57×10^5 to 7.49×10^5]	
Glyc A + Glyc B (rel. intensity)	2.36×10^5	3.86×10^5	2.93×10^{-9}
	[1.86×10^5 to 3.14×10^5]	[2.41×10^5 to 4.77×10^5]	
Glyc A/Glyc B ratio	5.95	6.05	0.69
	[4.89–9.38]	[4.30–8.91]	
glucose (mmol/L)	5.70	7.40	2.86×10^{-4}
	[3.90–8.10]	[4.40–11.00]	

[a]Statistical group comparisons of SARS-CoV-2 patients versus controls were performed with the Kruskal–Wallis rank sum test.

Figure 2:
Diagnostic Indices relating to α-1-Acid Glycoproteins Glyc A and Glyc B

In summary, the authors were able to make a strong conclusion:

"Taken collectively, our data present a complex pattern of disturbance of systemic metabolism caused by SARS-CoV-2 viral infection associated with multiple organ-specific changes that are not simply related to the primary respiratory symptoms. These studies indicate the potential importance of broader clinical chemical and chemical pathology testing for prospective hospitalization and existing hospitalized COVID-19 patients to identify those who might have newly acquired metabolic diseases of the type described here."



Data analysis explained

Throughout this paper, two common statistical approaches to data analysis are mentioned, namely principal component analysis (PCA) and an orthogonal-projections to latent structures (OPLS) method.

PCA is a dimensionality-reduction method that is often used to reduce the dimensionality of large data sets, by transforming a large set of variables into a smaller one that still contains most of the information in the large set.

Smaller data sets are easier to explore and visualize, making analysis more straightforward and faster for machine learning algorithms.

OPLS is a discriminant analysis and at the same time a dimensionality-reduction method. It removes variation from the X dimension (descriptor variables) that is not correlated to the Y dimension (property variables). Applying O-PLS results in reduced data complexity whilst preserving prediction ability.

2

Publication Date: 23 October 2020

SARS-CoV-2 Infection Dysregulates the Metabolomic and Lipidomic Profiles of Serum

Bruzzone, C., Bizkarguenaga, M., Gil-Redondo, R., et al. iScience, 2020; 23(10): 101645.

https://pubmed.ncbi.nlm.nih.gov/33043283/

Researchers in Spain, led by the group from The Precision Medicine and Metabolism Laboratory at the CIC bioGUNE research and technology alliance, investigated a large cohort of COVID-19 patients using NMR spectroscopy.[7]

This work, published two months after the first ANPC paper, highlights the impact that COVID-19 disease / SARS-CoV-2 infection has on overall metabolism. The metabolomic and lipidomic serum profiles from 263 (training cohort) + 135 (validation cohort) symptomatic patients hospitalized after positive PCR testing for SARS-CoV-2 infection were measured by NMR spectroscopy. The profiles of a further 280 subjects (collected before the coronavirus pandemic started) were also reported.

» Analysis of the NMR data clearly discriminated both cohorts, highlighting the infection impact on overall metabolism. Moreover, the lipidomic analysis revealed a pathogenic redistribution of lipoprotein particle size and composition, factors implicated in an increase in atherosclerotic risk.
Finally, metabolomic analysis shows abnormally high levels of ketone bodies and 2-hydroxybutyric acid, suggestive of hepatic glutathione synthesis and a marker of oxidative stress.

Taken together, these results are consistent with the findings from the initial work at ANPC, and highlight that, in the population studied, SARS-CoV-2 infection induces liver damage associated with dyslipidemia and oxidative stress.

3

Publication Date: 11 January 2021

NMR Spectroscopic Windows on the Systemic Effects of SARS-CoV-2 Infection on Plasma Lipoproteins and Metabolites in Relation to Circulating Cytokines

Samantha Lodge, Philipp Nitschke, Torben Kimhofer, Jerome D. Coudert, Sofina Begum, Sze-How Bong, Toby Richards, Dale Edgar, Edward Raby, Manfred Spraul, Hartmut Schaefer, John C. Lindon, Ruey Leng Loo, Elaine Holmes, and Jeremy K. Nicholson
J. Proteome Res. 2021, 20, 2, 1382–1396

https://doi.org/10.1021/acs.jproteome.0c00876

Study description

Three groups of participants were recruited for this study: (i) 15 patients who presented COVID-19 disease symptoms and subsequently tested positive for SARS-CoV-2 infection from upper and/or lower respiratory tract swabs by PCR, (ii) 34 healthy controls who had not exhibited COVID-19 disease symptoms, and (iii) 35 patients with COVID-19 disease symptoms and who tested negative. The SARS-CoV-2 negative participants were further classified as those who required hospitalization versus those who were recruited from a COVID-19 clinic but did not require hospitalization.

All NMR analysis was completed on the Bruker Avance IVDr platform. All experiments were completed using the Bruker in vitro Diagnostics research (IVDr) methods, lipoproteins and subclasses and small molecules have been automatically calculated and common statistical techniques, namely: PCA, which was used to assess the main sources of structured variation within each dataset, and OPLS-DA modeling, which allowed the identification of the lipoproteins and metabolites differentiating SARS-CoV-2 positive participants from healthy controls.

Results, conclusions, and clinical significance

This paper provides further evidence that biomarkers of the systemic metabolic effects of SARS-CoV-2 infection can be elucidated by NMR spectroscopy.[8] In addition, the authors observed, for the first time, "…that some patients in the respiratory recovery phase and testing virus-free were still metabolically highly abnormal…"

They suggest the potential importance of these biomarkers in assessing recovery and the long-term effects of the infection.

The plasma from SARS-CoV-2 positive participants was clearly biochemically distinct from that of either the healthy controls* or the SARS-CoV-2 negative participants **(Figure 3)**.

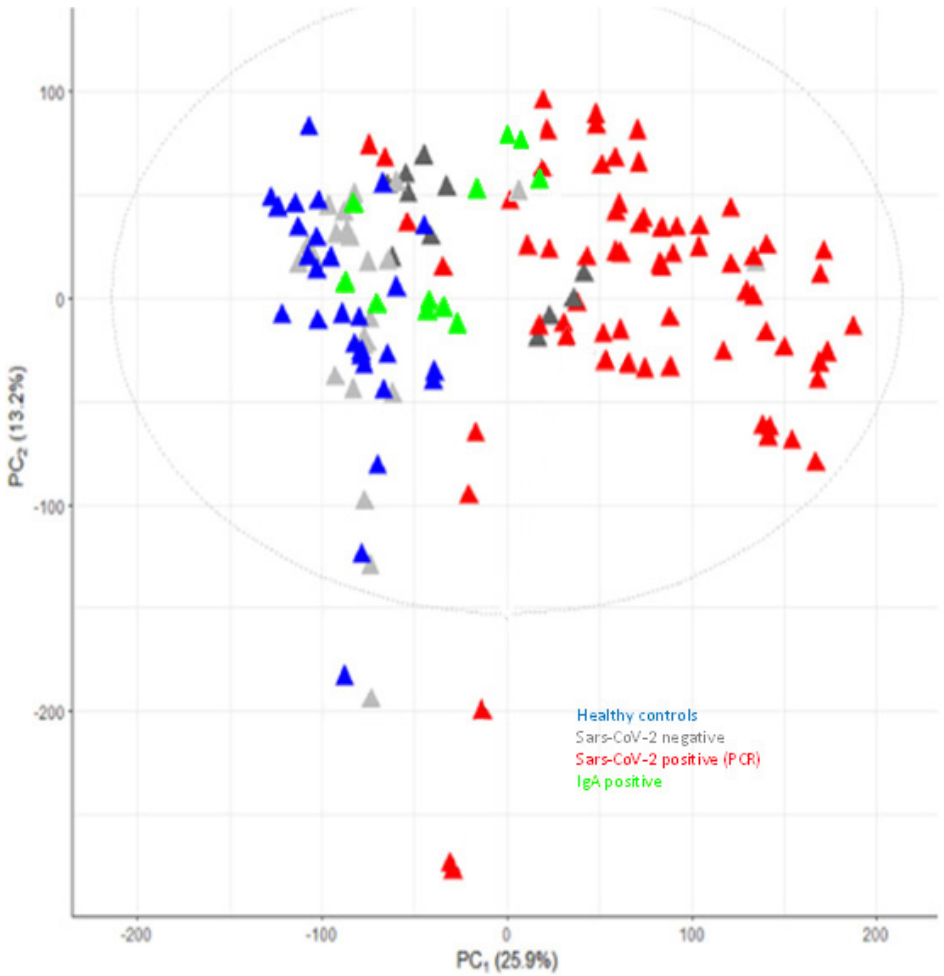


Figure 3: PCA of 1D NMR spectral data
PCA score plot showing clustering patterns for the healthy controls (blue), SARS-CoV-2 positive (red), non-hospitalized SARS-CoV-2 negative (light grey), hospitalized SARS-CoV-2 negative (dark grey) samples, and control participants who tested positive for IgA (green). Reproduced from reference 8 under Creative Commons Attribution License 4.0.

*Note: To ensure that only truly healthy control data was included in the analysis, the healthy control group underwent additional testing, and individuals that had a positive result on a serological COVID-19 IgA/IgG test were reported separately (green data points in **Figure 3** above).

In addition, there were strong indications of the underlying systemic disease in the NMR data, indicative of diabetes, liver dysfunction, and cardiovascular abnormalities. Long-term cardiovascular problems appear to be common for COVID-19 patients, and these may be reflected in abnormal lipoproteins after the acute respiratory symptoms have subsided, indicating a potential role for these measurements in the assessment of systemic patient recovery.

The NMR results indicated that the SARS-CoV-2 patients in the study were dominated by signals from GlycA, GlycB, glucose, and lactate, a finding consistent with previous observations that diabetes and acute inflammation are associated with COVID-19 disease. Importantly, GlycA has been shown to be a robust marker of inflammatory and proinflammatory conditions. It has been found to be a better predictor of inflammatory conditions than either C reactive protein (CRP) or interleukin-6 (IL-6), and is independent of both.

» Taken together, the data from this study suggests that SARS-CoV-2 infection leaves a residual metabolic signature with an, as yet, undetermined time of resolution. This type of information could be clinically useful in determining the rates of recovery of patients, identifying incomplete recovery, and understanding PACS sequelae or new onset co-morbidities.

4

Publication Date: 16 January 2021

Metabolic markers distinguish COVID-19 from other intensive care patients and show potential to stratify for disease risk

Schmelter, F., Foeh, B., Mallagaray, A., et al. MedRxiv, 2021.

https://www.medrxiv.org/content/10.1101/2021.01.13.21249645v1

Researchers from the University of Luebeck, and other institutes in Germany, focused their efforts on proving that the metabolic signature, as measured by NMR (on a Bruker Avance IVDr platform), was able to distinguish COVID-19 patients from other intensive care patients.[9] This work, reported in Jan 2021, revealed that not only did the COVID-19 patients show a distinct metabolic serum profile and a deeply altered metabolic status compared to healthy controls, but also a similarly perturbed profile was apparent, even when compared to other Intensive Care Unit (ICU) patients suffering from cardiogenic shock.

In this study, 276 serum samples from 92 individuals were analyzed using NMR metabolomics, including longitudinally collected samples from 5 COVID-19 and 11 cardiogenic shock intensive care patients, 18 SARS-CoV-2 antibody-positive individuals, and 58 healthy controls.

» **In conclusion, the authors suggest that clear clinical value can be extracted from their work:**

"Our data suggest that NMR metabolic profiles are suitable for COVID-19 patient stratification and post-treatment monitoring."

5

Publication Date: 12 February 2021

Diffusion and Relaxation Edited Proton NMR Spectroscopy of Plasma Reveals a High-Fidelity Supramolecular Biomarker Signature of SARS-CoV-2 Infection

Samantha Lodge, Philipp Nitschke, Torben Kimhofer, Julien Wist, Sze-How Bong, Ruey Leng Loo, Reika Masuda, Sofina Begum, Toby Richards, John C. Lindon, Wolfgang Bermel, Tony Reinsperger, Hartmut Schaefer, Manfred Spraul, Elaine Holmes, and Jeremy K. Nicholson
Anal. Chem. 2021, 93, 8, 3976–3986

https://doi.org/10.1021/acs.analchem.0c04952

Study description

Published just one month after their previous paper, this work from ANPC provides detailed evidence for a useful combined set of molecular markers (defined below) which could have practical application in managing patients as they recover from COVID-19.[10]

As previously reported, NMR spectroscopy was the central analytical technique; in this case, it was 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. Five groups of participants were recruited for this study: (i) 17 patients who presented with COVID-19 disease symptoms and subsequently tested positive for SARS-CoV-2 infection from upper and/or lower respiratory tract swabs by RT–PCR (sampled at various times); (ii) 26 healthy controls who had not exhibited COVID-19 disease symptoms; (iii) 23 individuals with respiratory disease symptoms who tested negative for SARS-CoV-2 and were non-hospitalized; (iv) 11 hospitalized SARS-CoV-2 negative respiratory patients; (v) 6 individuals who were serologically IgA positive for SARS-CoV-2.

All NMR analysis was performed on the Bruker Avance IVDr platform using the Bruker in vitro diagnostics research (IVDr) methods. A range of different experiments can be performed in order to assess all the relevant parameters in a single plasma/serum sample. 1D NMR spectra usually contain thousands of peaks, however, dedicated NMR methods such as Diffusional and Relaxation Editing (DIRE) pulse sequence have been optimized to edit out unwanted signals, leaving only those from a flexible domain of macromolecules such as proteins and large phospholipid complexes. The NMR spectra produced show unique biomarker signal combinations of GlycA and GlycB, composite N-acetyl (-NCOCH3) signals from α-1-acid glycoprotein and other glycoproteins, and the newly identified Supramolecular Phospholipid Composite (SPC) signals from the $-N^{+}-(CH_3)_3$ choline headgroups that are associated with HDL and LDL subfractions.

Results, conclusions, and clinical significance

Both GlycA and GlycB biomarkers, which are only accessible with NMR, have been shown to correlate with C-reactive protein (CRP) levels in plasma, and it has been suggested that they may be superior biomarkers of systemic inflammation over CRP, currently the main clinical chemistry marker of inflammation.

In this study population, the normal healthy and SARS-CoV-2 positive samples were significantly different based on their DIRE NMR biosignatures, with increased intensity of the GlycA and GlycB signals and decreased intensity of SPC.

The team at ANPC have previously shown the complex relationships between the lipoprotein patterns and other metabolic and cytokine data from COVID-19 patients, noting the inflammatory driven connections to COVID-19 dyslipidemia (elevated VLDL and LDL and elevated apolipoprotein B100/A1 and their possible implications in new onset diabetes and cardiovascular/atherosclerotic risk). In this subsequent work, the lipoprotein data was used to establish a structural and compartmental connectivity to the novel SPC data and the SPC/glycoprotein ratios. A strong pattern of correlation emerges between the SPC and total plasma and the total HDL apolipoprotein A1 and A2 levels.

In summary, the authors point out how their findings can translate directly into clinical practice:

"Thus, plasma SPCtotal and SPCtotal/GlycA are proposed as sensitive molecular markers for SARS-CoV-2 positivity 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."

6

Publication Date: 1 February 2021

Metabolomic/lipidomic profiling of COVID-19 and individual response to tocilizumab

Meoni, G., Ghini, V., Maggi, L., et al. PLOS Pathogens, 2021.

https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1009243

The group from the Magnetic Resonance Center (CERM) at the University of Florence, together with colleagues from other leading facilities in Italy, were among the first researchers to broaden the use of the NMR analytical toolbox to include an assessment of drug treatment success on a population of COVID-19 patients.[11]

In this study, also published in Feb 2021, the effect of tocilizumab administration was evaluated in a subset of subjects from a group of 30 patients, assessed by NMR metabolic profiling analysis and compared with 30 age- and sex-matched controls.

It was observed that tocilizumab treatment led to at least partial reversion of the metabolic alterations resulting from SARS-CoV-2 infection **(Figure 4)**.

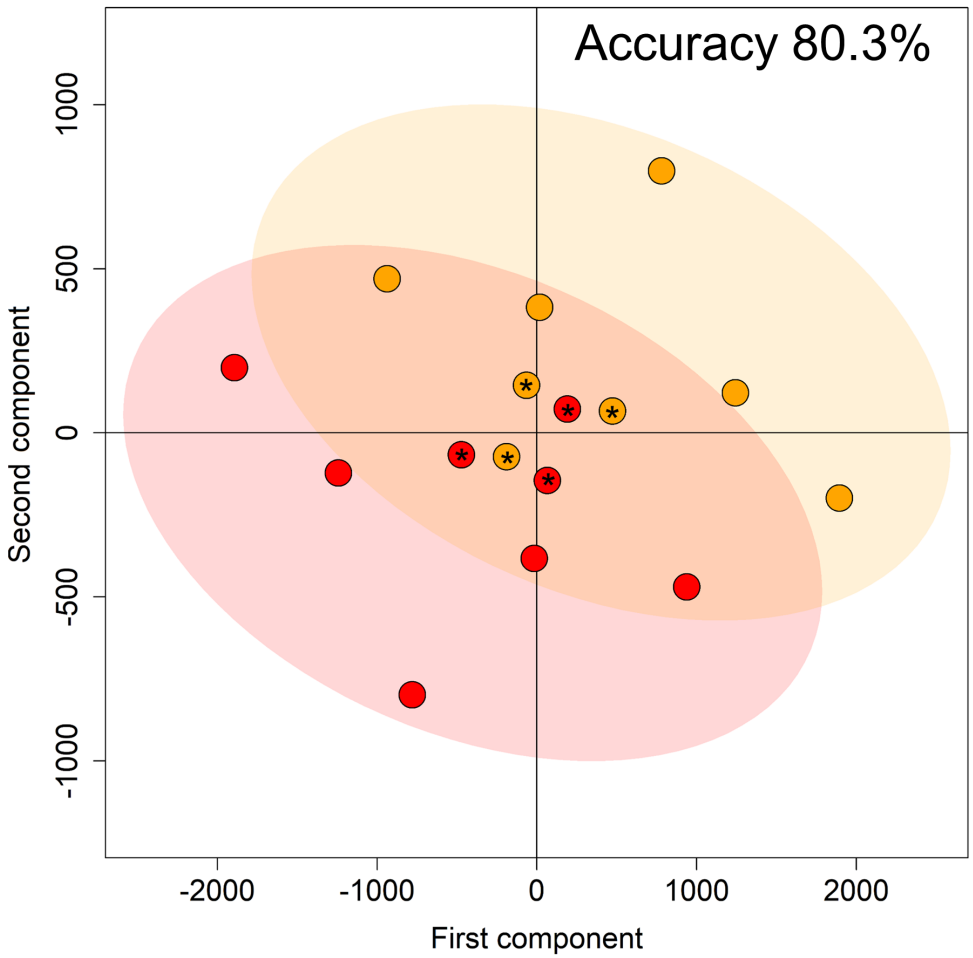


Figure 4. Tocilizumab treatment reverts metabolomic/lipidomic alterations in COVID-19 patients.

Score plot (of the first two principal components) and accuracy of the mPLS-DA model discriminating COVID-19 patients at pre- (red dots) and post- (orange dots) tocilizumab treatment using the 21 quantified metabolites (as quantified by the Bruker IVDr system).

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In conclusion, the authors note:

"NMR-based metabolomic and lipidomic profiling provides novel insights into the pathophysiological mechanism of human response to SARS-CoV-2 infection and to monitor treatment outcomes. "

7

Publication Date: 19 May 2021

Incomplete Systemic Recovery and Metabolic Phenoreversion in Post-Acute-Phase Nonhospitalized COVID-19 Patients: Implications for Assessment of Post-Acute COVID-19 Syndrome

Elaine Holmes, Julien Wist, Reika Masuda, Samantha Lodge, Philipp Nitschke, Torben Kimhofer, Ruey Leng Loo, Sofina Begum, Berin Boughton, Rongchang Yang, Aude-Claire Morillon, Sung-Tong Chin, Drew Hall, Monique Ryan, Sze-How Bong, Melvin Gay, Dale W. Edgar, John C. Lindon, Toby Richards, Bu B. Yeap, Sven Pettersson, Manfred Spraul, Hartmut Schaefer, Nathan G. Lawler, Nicola Gray, Luke Whiley, and Jeremy K. Nicholson
J. Proteome Res. 2021, 20, 6, 3315–3329

https://doi.org/10.1021/acs.jproteome.1c00224

Study description

Continuing the theme of searching for and validating practical tools to help clinicians assess and treat patients infected with the SARS-CoV-2 virus, this seminal paper presents a multivariate metabolic phenotyping approach applied to the long-term impacts of the infection—what has become known as PACS or 'Long COVID'.[12]

Metabolic data from three cohorts were examined: (i) a healthy control group of 41 patients, (ii) a hospitalized group of 18 patients that provided samples during the acute infection phase, and (iii) a recovery cohort consisting of 27 non-hospitalized patients that provided samples 3 months after the acute phase of the disease and still experienced symptoms 6 months post-SARS-CoV-2 infection.
All NMR analysis was completed on a Bruker Avance IVDr platform. For each sample, data were processed using industry standard computational and statistical techniques
– PCA and OPLS-DA.

Results, conclusions, and clinical significance

For several metabolic and lipoprotein parameters characteristically altered during SARS-CoV-2 infection, a normalization to concentrations within the healthy range occurred for the majority of participants. Parameters that were substantially normalized included GlycA, GlycB, total plasma cholesterol (TPCH), and SPC/GlycA signal ratios, but in all cases, there were patient outliers that had not normalized underscoring the heterogeneity of response to SARS-CoV-2 infection. However, other parameters remained elevated or depleted, or showed only a partial normalization. Some 57% of the patients had one or more persistent symptoms, including respiratory-related symptoms like cough, dyspnea, and rhinorrhea or other non-respiratory symptoms including chronic fatigue, anosmia, myalgia, or joint pain.

Diabetes is a known risk factor for early progression to severe COVID-19 disease, and new-onset diabetes has also been reported in COVID-19 patients. Plasma glucose, a well-known marker of type 2 diabetes, was elevated in active-phase patients, and high blood glucose on hospital admission has been associated with poor prognosis. The 3 month follow-up patients appear to have a glucose distribution similar to that of the control patients (none of whom were diabetic), although there were several elevated plasma glucose outliers in both groups.

Metabolic phenotyping appears to be a highly effective tool for assessing phenoreversion and functional systemic recovery of patients following COVID-19 and the evaluation of post-acute COVID-19 syndrome. There are clear metabolic patterns associated with persistent inflammatory and metabolic derangements in a majority of non-hospitalized 3-month-post-COVID- 19 patients.

A series of six individual metabolic parameter comparisons is shown in **Figure 5**. For each parameter, results from plasma samples from healthy, SARS-CoV-2 positive, and post-acute COVID-19 patients are presented.

Heterogeneous metabotype patterns were observed for the PACS patient spectrum, and if reflective of the clinical picture, these could enable personalized interpretation of the persisting inflammatory/metabolic abnormalities and give insights into strategies for long-term disease mitigation in affected individuals.

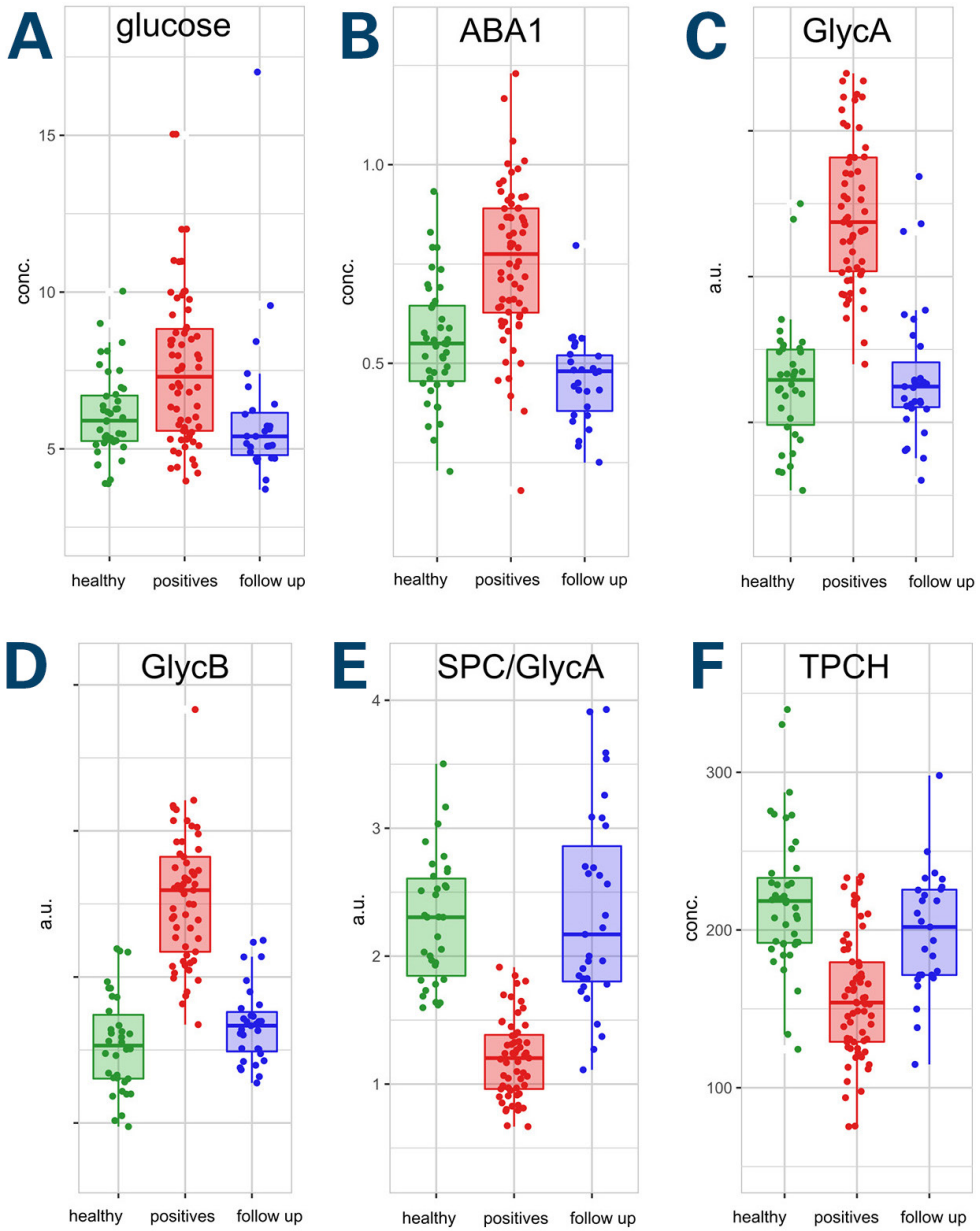

Figure 5. Six exemplar univariate plasma metabolic parameters in healthy, SARS-CoV-2-positive, and follow-up patients showing a range of individual and group phenoreversion and nonrecovery patterns.

(A) glucose, (B) lipoprotein ABA1 fraction, (C) glycoprotein A integral, (D) glycoprotein B integral, (E) SPCtotal/glycoprotein A ratio, and (F) lipoprotein total plasma cholesterol (TPCH). Reproduced from reference 12 under Creative Commons Attribution License 4.0 conditions.

In summary, the authors conclude:

Asymptomatic follow-up patients, including those with PACS, displayed a spectrum of multiple persistent biochemical pathophysiology, suggesting that the metabolic phenotyping approach may provide the basis for a framework of assessment of patient recovery from COVID-19. With further refinement, including the addition of other metabolic system parameters, new biomarker panels can be assembled that will assess patients with PACS and assist in clinical decision making in their long-term management and disease mitigation.

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Publication Date: 1 March 2022

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

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Study description

At the time of writing (June 2022), this latest paper from the group at ANPC(ref) further advances the understanding that the use of NMR can measure the increased cardiovascular risk in COVID-19 and post-acute COVID-19 syndrome (PACS) patients in a research setting, and that this new approach shows great potential for translation into clinical practice.

The newly identified NMR-only biomarkers described in previous work - supramolecular phospholipid composites were subjected to a Statistical Heterospectroscopy in n-dimensions (SHY-n) approach that integrates several orthogonal methods, in order to assess how well they correlated with the apolipoprotein B100/A1 ratio, a well-established marker of cardiovascular disease risk.

Blood serum samples were collected from adult individuals, and the study cohort consisted of control participants and 32 participants who tested positive for SARS-CoV-2 infection from RT–PCR on nasopharyngeal swab samples.

All NMR analysis was completed on a Bruker Avance IVDr platform. For each sample, data were processed using industry standard computational and statistical techniques.

Results, conclusions, and clinical significance

For each serum sample, the ANPC team integrated and correlated both the paired SPC signal data with phospholipidomic data and lipoprotein and subclasses data with quantified phospholipid subfraction data and apolipoprotein B100, A1, and A2 data (obtained using B.I.LISA).

The relationship between the SPC signals and the apolipoprotein B100/A1 ratio for both the 99 control participants and SARS-CoV-2 positive cohorts is shown in **Figure 6**.

These data strengthen understanding of the location, composition, and potential diagnostic value of the SPC peaks, particularly in relation to cardiovascular disease risk, both in general and in COVID-19 related cardiovascular complications.

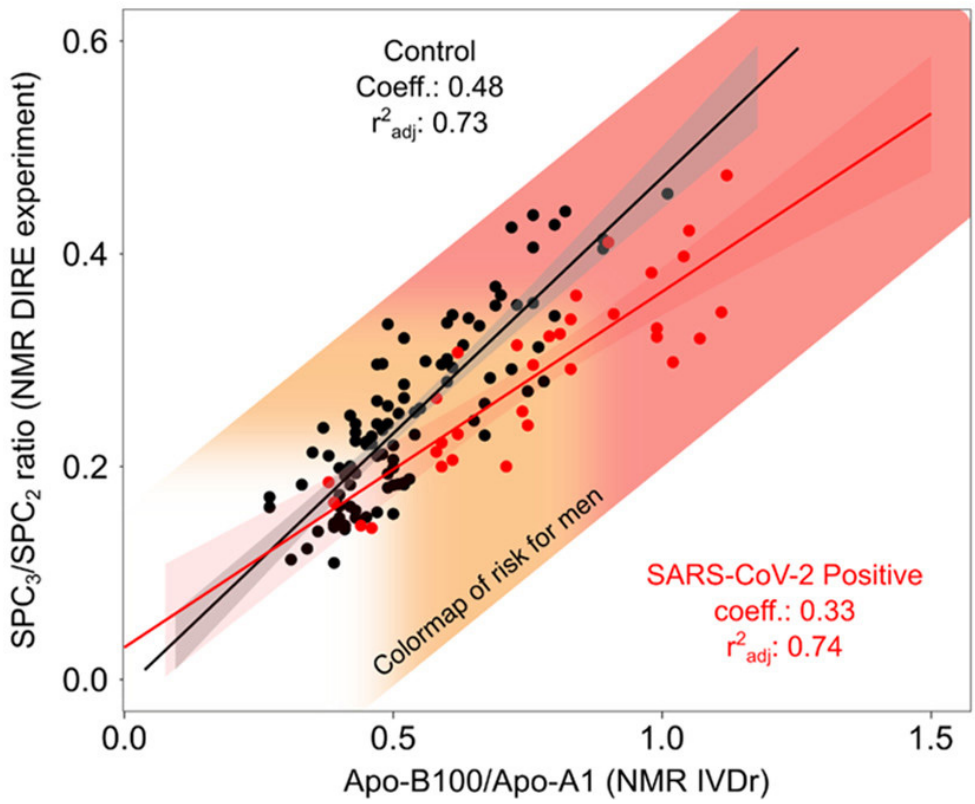

Figure 6: Relationships between SPC signal intensities and cardiovascular disease risk markers: Regression of the DIRE-derived SPC3/SPC2 ratio against the apolipoprotein B100/A1 ratio for control (black) and SARS-CoV-2 positive (red) individuals.

The authors made the following conclusion from this latest work:

"These data indicate the considerable potential of using a serum SPC measurement as a metric of cardiovascular risk based on a single NMR experiment. This is of specific interest in relation to understanding the potential for increased cardiovascular risk in COVID-19 patients and risk persistence in post-acute COVID-19 syndrome (PACS)."

Interviews with members of the NMR International COVID-19 Research Network, as well as a link to an
on-demand webinar that discuss the approach, methodology, and outcomes of the collaboration, are hosted at:

https://www.bruker.com/en/products-and-solutions/mr/make-mr-more-relevant/covid19-nmr-international-research-network-at-work.html

Conclusion

This white paper presents selected publications – from August 2020 to March 2022 – a period of intensive clinical research into COVID-19 infection and its long-term sequelae.

The detailed research has elucidated the impact that the virus has on human metabolism and how it affects a multitude of organs, not just the respiratory system.

Importantly, the data suggests strongly that a new combined series of powerful biomarkers, only accessible by NMR spectroscopy, have the potential to guide clinical practice, monitor disease recovery, and predict risk of PACS and secondary disease development.

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