Current Research in Immunology 3 (2022) 199–214

Contents lists available at ScienceDirect

Current Research in Immunology

journal homepage: www.sciencedirect.com/journal/current-research-in-immunology

NMR spectroscopy spotlighting immunogenicity induced by COVID-19 vaccination to mitigate future health concerns

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ARTICLE INFO

Keywords:
COVID-19
Immunogenicity
Vaccination
Nuclear magnetic resonance spectroscopy
Chemometrics

ABSTRACT

In this review, the disease and immunogenicity affected by COVID-19 vaccination at the metabolic level are described considering the use of nuclear magnetic resonance (NMR) spectroscopy for the analysis of different biological samples. Consistently, we explain how different biomarkers can be examined in the saliva, blood plasma/serum, bronchoalveolar-lavage fluid (BALF), semen, feces, urine, cerebrospinal fluid (CSF) and breast milk. For example, the proposed approach for the given samples can allow one to detect molecular biomarkers that can be relevant to disease and/or vaccine interference in a system metabolome. The analysis of the given biomaterials by NMR often produces complex chemical data which can be elucidated by multivariate statistical tools, such as PCA and PLS-DA/OPLS-DA methods. Moreover, this approach may aid to improve strategies that can be helpful in disease control and treatment management in the future.

1. Introduction

Coronavirus disease–2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is a worldwide health concern. In addition to COVID-19 in humans, SARS-CoV-2 can impact other organisms (Shi et al., 1979). COVID-19 has risked the population regardless of age, gender and health conditions. Those with health conditions (diabetes, hypertension, cardiovascular disease, etc.) are more vulnerable, contributing to mortalities (Yang et al., 2020). Beyond comorbidities and older age, several signs and symptoms of this disease are fatigue, fever, nasal congestion, sore throat, chest pain and organs failure, among others (Jutzeler et al., 2020). The detection of SARS-CoV-2 has been based on nucleic acid amplification tests (NAATs–PCR), rapid antigen tests, antibody tests and blood biomarkers assays (Jacofsky et al., 2020; Kahn et al., 2021). As of November 17, 2021, about 255, 356, 599 confirmed cases with 5,134,319 deaths from 223 countries were documented by the World Health Organization (WHO). A high magnitude of COVID pandemic regarding mortality, morbidity and economic loss has accelerated the development of safer vaccines. Besides development, numerous vaccines have been settled and proposed for emergency use. Some of these vaccines are reported for unusual thrombosis, immune thrombocytopenia (ITP), vaccine-induced

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https://doi.org/10.1016/j.crimmu.2022.08.006
Received 28 June 2022; Accepted 1 August 2022
Available online 22 August 2022

thrombocytopenia (VITT), intracranial hemorrhage, myocarditis and pericarditis issues (Das et al., 2021; Lee et al., 2021; Rose and McCullough, 2021). So, immunogenicity evaluation is an urgent need to understand the performance and type of immune response produced by COVID-19 vaccination, and to adapt better strategies in future.

Immunological complications are challenging, so their coverage could be highly appreciable. There is always a need for approaches capable of seeking rational solutions to mitigate current and future problems concerned to the immunological context. Standard methods used in immunology encompass an array of tools (ELISA, ELISPOT, immunoblotting, immunohistochemistry, etc.) that are specific for the detection of antibodies, antigens and immune cells. Some of these methods use a specific antibody to tag or label particular molecule(s) for detection. These methods have been supplemented by modern methodologies that aim at assaying molecular biomarkers in a non-selective way– e.g., the -omics approaches. Across the available tools, the use of nuclear magnetic resonance (NMR) spectroscopy-based omics is limited in the field of immunology. Similar to characterizing diseases and pathogenic events (Costa dos Santos Junior et al., 2020; Whiley et al., 2021; Park et al., 2020), the use of NMR could be promising in immunological investigation including the interaction of vaccine (Polack et al., 2020; Speiser and Bachmann, 2020; Rambe et al., 2015) in a biological system. NMR-based omics data allied to such interference are of high complexity that oblige proper simplification via supplementary tools. The simplification of the NMR data has driven mostly by the principal component analysis (PCA) (Bro and Smilde, 2014; Bayne and Kramer, 1999), partial least squares (PLS) (Amante et al., 2019; Brereton and Lloyd, 2018), orthogonal partial least squares (O-PLS) or combining them with discriminant tools (PLS/OPLS-DA). These tools have a central and supplementary role in simplifying the NMR dataset that led to comprehending mechanisms involved in a disease with a recovery pathway, such as COVID-19 (Kimhofer et al., 2020).

Besides disease, the immunogenicity and metabolic pathways induced by COVID-19 vaccination are the main interests of this review. This paper focused on vaccines that have shown adverse effects, and therefore, we have considered certain metabolic pathways that could be linked to the problems in question.

2. Nuclear magnetic resonance (NMR) spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is an analytical tool that deals with the atomic nuclei when exposed to a static magnetic field (B_0) to reveal information on chemical composition of samples in qualitative and quantitative fashions (Santos et al., 2018; Claridge, 2016). Then, this chemical profile can be used to explain disease and immunogenicity of a particular system. In addition to providing unique information on the elucidation of chemical structures, NMR is nondestructive and reproducible, allowing the acquisition of high-quality data from samples in any state (liquid, semi-solid, and solid) (Khattri et al., 2021; Crook and Powers, 2020; Skorupa et al., 2021). NMR requires minimal sample preparation and is suitable for "*in vivo*" and "*in vitro*" molecular screening. NMR can detect several classes of substances such as amino acids, organic acids, lipids, carbohydrates, proteins, lipoproteins and the compounds involved in the metabolism and can reveal aspects related to diseases and immunogenicity (Crook and Powers, 2020; Loo et al., 2020; Lodge et al., 2021a).

Low sensitivity, high-cost of NMR spectrometers and signals overlap– mainly in complex matrices– are major drawbacks associated with NMR spectroscopy. NMR data acquisitions of complex biological samples are mostly performed by 1D– e.g., ^{1}H CPMG and ^{1}H NOESY experiments. The structural details present in ^{1}H NMR spectra are usually supported by the use of 2D J-resolved (J-RES), 2D total correlation spectroscopy (TOCSY) and 2D heteronuclear single quantum correlation (HSQC) NMR. The problem of signal overlap is balanced by the application of multidimensional experiments (nD). Numerous one – nD (homo- or heteronuclear) NMR experiments such as ^{1}H, ^{1}H–^{1}H, ^{1}H–^{13}C, ^{1}H–^{15}N, etc. have successfully shown high throughput chemical data (Table 1). In turn, NMR sensitivity has been improved through the progress in hardware, magnetic field strength, probes design and pulse sequence programs (Serkova et al., 2019).

Up to date, NMR spectroscopy alone or in integration with other analytical tools (Awuchi et al., 2022; Sturm et al., 2021; Ocampos et al., 2017) has been a powerful tool. NMR has a stand-alone contribution in uncovering the genetic variants that cause changes in phenotype and metabolism (Rueedi et al., 2017). NMR applications have been extended from plant (Ali et al., 2020, 2021; Dutra et al., 2020) to human diseases (Tayanloo-Beik et al., 2020; Fuertes-Martín and VallvéAmigó, 2020), viral events (Hinkov et al., 2020; Sharma and Varani, 2020; Jaiswal et al., 2020), COVID-19 phenotyping (Kimhofer et al., 2020), immunometabolism and metabolic pathways depiction. Based on its potential, NMR has large-scale applications in SARS-CoV-2, thus Table 1 depicts a few examples of how NMR has been applied in COVID-19 pandemic.

2.1. Severe acute respiratory syndrome coronavirus 2 (SARS-COV-2)

Human coronaviruses are classified in the realm *Riboviria*, order *Nidovirales*, suborder *Cornidovirineae* and family *Coronaviridae* (Gorbalenya et al., 2020). Amongst alpha, beta, gamma and delta variant of coronavirus (Hulswit et al., 2016), the first two variants are known to cause infections in mammalian (including humans), while rest of them are reported in wild birds (Karpiński et al., 2021). SARS-CoV-2 is a beta-coronavirus (as do SARS-CoV, MERS-CoV, HKU1, etc.) (Karpiński et al., 2021). This virus has 88% genetic similarity to bat-derived SARS-like coronaviruses (bat-SL-CoVZC45 and bat-SL-CoVZXC21) and 50%–79% when comparing human MERS-CoV and SARS-CoV (Lu et al., 2020). SARS-CoV-2 is a positive-sense RNA (+ssRNA) virus with genome size of 29,903 nucleotides (NCBI nucleotide database; reference sequence: NC 045512.2). Its genomic structure encodes 16 non-structural proteins (Nsps) and four major structural proteins; spike (S), membrane (M), envelope (E) and nucleocapsid (N) (Arya et al., 2021) (Fig. 1).

SARS-CoV-2 utilizes S glycoprotein to bind to the angiotensin-converting enzyme 2 (ACE2) via S1 subunit's receptor-binding domain, followed by fusing S2 subunit to the cell membrane and enter the host cell (Naqvi et al., 2020). ACE2 receptors are widely allocated in mouth, lung, adipose tissue, intestine, kidney, cardiovascular system, central nervous system and reproductive system (Gheblawi et al., 2020; Trypsteen et al., 2020). ACE2 density is higher in adults than in children that can explain usually more chronic acute respiratory distress syndrome and deaths in adults (Karpiński et al., 2021). The M protein is a transmembrane glycoprotein with three domains that binds to the nucleocapsid and plays a critical role in virus assembly (Siu et al., 2008). The E protein is a small membrane protein that forms protein lipid bilayer pores with ion channeling activity (Mandala et al., 2020). Nucleocapsid N protein is useful in viral genome packaging, capsid formation (Gorbalenya et al., 2020), RNA replication, and transcriptional mechanism (Cong et al., 2019).

Open reading frames (ORFs) are capable to encode nonstructural proteins (Nsp1-16), essentially prohibit mRNA translation and virus dependent signaling pathways, with cellular antiviral defense mechanisms that rely on the host's factor expression comprising interferon response. SARS-CoV-2 in severe cases can deregulate immunity (V'kovski et al., 2021) and reduce interferons (I–III) and highly expressed interleukin (IL-6) (Blanco-Melo et al., 2020). Throughout Nsps, Nsp5 is cysteine major protease that cleaves viral polyproteins, 96% of which have a similar sequence to SARS-CoV (Arya et al., 2021). Nsp6 limits autophagosome expansion, while Nsp7 stimulates Nsp8-dependent RNA binding and, together with Nsp8, displays a functional complex with RNA polymerase activity (te Velthuis et al., 2012). Nsp9 strongly interacts with Nsp8, and studies suggest that this complex behaves like a membrane anchor for viral replication and transcription (Sutton et al., 2004). Nsp10 acts as an allosteric regulator

Table 1
NMR spectroscopic applications in covid-19 pandemic.

Application	Matrix/ target	^{1}H NMR (Sol. state)	NMR Experiment	Solvent	Refs.
Metabolic phenotyping, biomarkers, diagnostic modeling	Blood plasma	600 MHz	^{1}H CPMG and 2D J-resolved	D2O	Lodge et al. (2021b)
SARS-CoV-2 biomarkers	Blood plasma/ serum	600 MHz	^{1}H CPMG and 2D J-resolved	D2O	Lodge et al. (2021a)
Metabolomics, identification and quantification of biomarkers	Blood plasma	700 MHz	^{1}H NOESY	D2O	Fraser et al. (2020)
Quantification of lipoprotein, metabolic profile and evaluation of protocol for investigation to SARS-CoV-2	Plasma/serum	600 MHz	^{1}H CPMG and 2D J-resolved	D2O	Loo et al. (2020)
Metabolomic and lipidomic, metabolites quantification	Serum	600 MHz	^{1}H CPMG, ^{1}H NOESY, 2D J-resolved and ^{1}H–^{1}H TOCSY	90% H2O: 10% D2O, MeOD	Bruzzone et al. (2020)
Metabolic phenotyping and metabolites quantification	Human blood plasma	600 MHz	^{1}H CPMG and 2D J-resolved	–	Kimhofer et al. (2020)
Metabolomic and lipidomic	Plasma	600 MHz	^{1}H CPMG and ^{1}H NOESY	–	Meoni et al. (2021)
Structural identification	Nsp3	700 MHz	2D ^{1}H–^{15}N HSQC, 2D ^{1}H–^{15}N TROSY, 3D HN(CO)CA, 3D HNCA, 3D TROSY HN(CO)CACB, 3D TROSY HNCACB, 3D HN(CA)CO, 3D HNCO, 3D HNHA, 3D HBHA(CO)NH, 3D (H)CCH TOCSY, ^{1}H–^{13}C HSQC and 3D ^{1}5N-edited NOESY	90% D2O: 10% D2O	Gallo et al. (2021a)
Structural identification	Nucleocapsid protein dimeric N-CTD SARS-CoV-2 N-CTD	600–950 MHz	^{1}H–^{15}N HSQC, ^{1}H–^{15}N TROSY, HNCACB, HN(CO) CACB, 15N-NOESY-HSQC and ^{1}H–^{15}N NOESY	5% D2O	Korn et al. (2021)
Structural assignment and molecular dynamic	nsp10	600–950 MHz	^{1}H–^{15}N BEST-TROSY, BEST-TROSY-HN(CO)CACB, BEST-TROSY-HNCACB, BEST-TROSY-HN(CA)CO, BEST-TROSY-HNCO, 15NR1/15NR2/^{15}N-NOE and TRACT	95% H2O: 5% D2O	Kubatova et al. (2020)
Structural assignment	Nsp7	600 MHz	^{1}H–^{15}N HSQC, HNCACB, CBCA(CO)NH, HNCO, HN (CA)CO, ^{1}H–^{13}C HSQC, C(CO)NH, HBHA(CO)NH, H (CCO)NH, H(C)CH-TOCSY, NOESY ^{1}H–^{13}C HSQC and (HB)CB(CGCD)HE	7% D2O	Tonelli et al. (2021)
Characterization and Structural assignment	Stem-loop 5a (SL5a) and 5′-untranslated region (5′-UTR) genome	600, 700, 800 or 950 MHz	^{1}H–^{13}C HSQC, ^{1}H–^{1}H-NOESY, ^{1}H–^{13}C CT-HSQC, (H)C (CCN)H, 3D ^{13}C–CNC, ^{1}H–^{1}H TOCSY, 3D ^{13}C-NOESY-HSQC, 3D TROSY–HCCH–COSY, 3D (H)CCH-TOCSY, 3D H(C)CH-TOCSY, BEST-TROSY–HNN–COSY, H(N) CO and (H)C(CCN)H	–	Schnieders et al. (2021)
Structural assignment	N-terminal domain of nsp3	850 MHz	^{1}H–^{15}N HSQC, BT-HNCO, BT-HN(CA)CO, BEST-HNCA, BEST-HN(CO)CA, BT-iHNCACB and BT-HN(CO)CACB	50 mM Na-phosphate, pH 6.5, 150 mM NaCl	Salvi et al. (2021)
Structural assignment	N-NTD in HKU1–CoV	800 MHz	^{1}H–^{15}N HSQC, HNCO, HN(CA)CO, HNCA, CBCA(CO) NH, HNCACB, HBHA(CO)NH, ^{13}C-HSQC, (H)CCH-TOCSY, HCCH-TOCSY, ^{15}N/^{13}C-NOESYHSQC and ^{15}N-NOESY	5% D2O	de Luna Marques et al. (2021)
Structural assignment	SARS-CoV-2 macro domain	600 or 850 MHz	2D ^{1}H–^{15}N HSQC, HNCACB, CBCA(CO)NH, HNCA, HNCO, HN(CA) CO, ^{13}C-HCCHTOCSY and ^{13}C–(H) CCH–TOCSY	–	Lin et al. (2021)
Structural assignment	Nsp3b macrodomain and Nsp3b macrodomain with ADP-ribose	700 MHz or 1.2 GHz	^{1}H–^{15}N HSQC, ^{1}H–^{15}N best-TROSY, Best-TROSY-HN (CO)CACB, Best-TROSY-HNCACB, Best-TROSY-HN (CA)CO, Best-TROSY-HNCO, ^{15}NR1/^{15}NR2/15N-NOE, Relaxation experiments (^{15}N, T1, T2 and ^{1}H–^{15}N NOE) and TROSY pseudo3D pulse sequences	5% D2O	Cantini et al. (2020)
Structural assignment	Nsp9	600 MHz	2D ^{1}H–^{15}N HSQC, ^{1}H–^{13}C HSQC, 3D HNCA, HNCOCA, HNCACB-(^{13}Cβ), CBCA(CO)NH, HCC-TOCSY-NNH, CC-TOCSY-NNH, HNCO, HNCACO, 3D ^{15}N-edited TOCSY-HSQC, NOESYHSQC, ^{13}C-edited NOESY-HSQC and 2D-HBCBCGCDHD	100 mM NaCl, 20 mM Tris, 1.0 mM dithiothreitol, pH 7.0	Buchko et al. (2021)
Structural assignment	ORF8 in SARS-CoV-2 in tobacco BY-2 cells	–	^{1}H–^{15}N HSQC and ^{1}H–^{13}C HSQC	–	Imamura et al. (2021)
Structural assignment	SARS-CoV-2 nsP3c SUD-M and SUD-C	700 MHz	2D ^{1}H–^{15}N HSQC, 2D ^{1}H–^{15}N TROSY, 3D HN(CO)CA, 3D HNCA, 3D TROSY HN(CO)CACB, 3D TROSY HNCACB, 3D HN(CA)CO, 3D HNCO, 3D HNHA, 3D HBHA(CO)NH, 3D (H)CCH TOCSY, CBCA(CO)NH, 2D ^{1}H–^{13}C HSQC and 3D ^{15}N-edited NOESY	10% D2O	Gallo et al. (2021b)
Secondary structure determination	Conserved SARS-CoV-2 RNA elements	600, 700, 800, 900 and 950 MHz or 1 GHz	^{1}H–^{1}H NOESY, ^{1}H–^{15}N BEST-TROSY, ^{1}H/^{15}N HSQC, ^{1}H–^{15}N CPMG-NOESY, 2D-BEST-TROSY HNN–COSY, ^{1}H–^{15}N sfHMQC, Hadamard-encoded NOESY, ^{13}C–^{15}N-filter NOESY with WATERGATE, ^{1}H–^{1}H TOCSY with	95% D2O: 5% D2O	Wacker et al. (2020)

(*continued on next page*)

Table 1 (*continued*)

Application	Matrix/ target	^{1}H NMR (Sol. state)	NMR Experiment	Solvent	Refs.
Structural characterization and interaction	GRL0617 and SARS-CoV-2 PLpro protein	600 MHz	Excitation sculpting water suppression, TOCSY mixing time, BEST-long range HNN–COSY and ^{1}H–^{13}C sfHMQC ^{1}H–^{15}N HSQC	10% D2O	Fu et al. (2021)

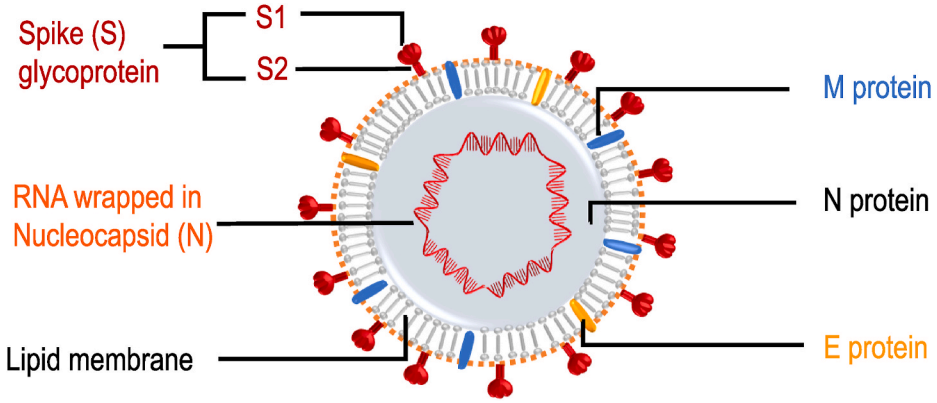

Fig. 1. Basic structure of SARS-CoV-2.

of Nsp16, and both form methyltransferases involved in RNA capping, whereas Nsp10 also interacts with Nsp14, to form a functional endoribonuclease complex (Bouvet et al., 2012). Furthermore, Nsp10 and Nsp16 interact with Nsp14 by targeting the guanine residue in the structural cap (Bouvet et al., 2012). Nsp11 is critical for viral replication, while Nsp12 displays RNA polymerase (replicase) activity (te Velthuis et al., 2009). Nsp13 has helicase and RNA triphosphatase activity, while Nsp15 is nidoviral RNA uridylate-specific endoribonuclease (Kim et al., 2020).

NMR has proven key structural assignments (^{1}H, ^{13}C, ^{15}N) included SARS Unique Domains; SUD-M, SUD-C (Gallo et al., 2021b) and Nsp3 (Korn et al., 2020). The C-terminal dimerization domain of SARS-CoV-2 nucleocapsid protein provides a basis for downstream purposes, particularly site-resolved drug binding. Along with Nsps, NMR has also revealed structural assignments of the ORF8 (Imamura et al., 2021; Korn et al., 2020). A secondary structure of 15 conserved RNA elements at the 5′ end with ribosomal frameshift and 3-untranslated region (3-UTR) of SARS-CoV-2 genome was successfully established (Schnieders et al., 2021). Likewise, GRL0617 inhibitor and papain-like protease (PLpro) was monitored by NMR indicating that GRL0617 blocks binding of the C-terminus of interferon-stimulated gene 15 (ISG15) to PLpro (Fu et al., 2021).

The use of NMR has got the attention to cover metabolic details that are relevant in clinical context for therapeutic development. This entails phenotype investigation, however to explain disease state and vaccination influence at molecular level. Consistent with SARS-CoV-2, it interacts with host metabolome and generates variation in the metabolic phenotype. The virus on pre-replication takeovers and reprograms metabolism in a local microbiome in order to accede replication. Once established this relationship, host's immune system and immunometabolism is stimulated. Similar to interactive SARS-CoV-2, vaccination may also impact immunity and immunometabolism. NMR profiling of the biological compartments can portray these mechanisms providing an insight into the associated metabolic pathways at molecular level.

2.2. *Multi-samples analysis directing disease and therapy examination*

In omics, the metabolomics, lipidomic, proteomics and pharmacometabolomic deal with assessing different molecules in a targeted sample. When assessed, these molecules are used to depict biological functions of organisms against various stimuli; infections/diseases, therapeutic input and intrusion in a cellular environment. These and further stimuli (lifestyle, food and genetics/genetic modulation) affect local phenotype and the metabolites level in cells, tissues and organs of a host under study. As described in next sections, NMR analysis of different bio-samples can track these affects and the chemical signatures of allied metabolic pathways that involve in disease, immunological and metabolism disorders. However, selection and analysis of multiple samples is necessary because they can share manifold chemical data tied to similar mechanism(s). This strategy can be beneficial, when it has a lower analyte concentration and improperly controlled sample.

Omics approach can also be improved by analyzing small to large weight molecular components in each biospecimen. For example, blood plasma/serum generally represents amino acids, sugars, nucleotides, lipids, proteins and lipoproteins that are detected in metabolomics, lipidomic and proteomic studies. Some of parallel molecules are detectable in saliva, bronchoalveolar-lavage fluid (BALF), cerebrospinal fluid (CSF), breast milk and feces, when compared to urine comprising creatinine, ammonia, organic acids, toxins, urea and metabolites from AA metabolism, etc. The coverage of these molecular contents is important, since most of them are proficient indicators not only of diseases but can highlight the status of immunity (immunometabolism).

2.2.1. *Saliva*

After the gut microbiota, the oral cavity is a renowned second largest microbiome comprising about 700 bacterial species. Of this biome, saliva recruits nutrients digestion, taste order, molecular exchange and safeguarding oral mucosa. Rather than water and electrolytes, saliva can contain immune and epithelial cells, immunoglobulin A (IgA), lysozymes, protein and many small metabolites. As per report, α/β-defensins, agglutinin, lactoferrin, cathelicidin, carbohydrates, fatty acids and amino acids, etc. can be spotted in the saliva, which are suitable in oral defense and disease control (Costa dos Santos Junior et al., 2020). Chemoattractant cytokines along lactate hydrogenase, malic acid, succinate and guanosine monophosphate are determinable in saliva, which aid cellular respiration, modulation and regulation of

saliva. Main signatures of saliva, steroids, interleukin (IL), tumor necrosis factor-α (TNF-α) and matrix metalloproteinase are known to be correlated with several disorders (Bellagambi et al., 2020).

The glucose and immunity biomarker IgA are known targets and are damaged by viruses – e.g., SARS-CoV-2 and HIV. SARS-CoV-2 rely on glycosylated mucin rich protein, because it supports virus entry into the host's cell (Tian et al., 2021). Saliva is beneficial, in terms of its easy-going manifold collections without the need for dedicated skills. Once saliva is easily obtainable, scientific interest is increased in disease identification (Chen et al., 2019). Saliva analysis has become a vital diagnostic tool in assessing periodontal disease, oral cancer, genetic diseases, ancestry problems, heart disease and infections such as COVID-19. Oral mucosa preserves abundant ACE2 that are main routes for SARS-CoV-2 (Xu et al., 2020). Frequently, COVID-19 exposition has been completed by tracing viruses, antibodies and antigens, using swabs based on polymerase chain reaction (PCR), and others (Jacofsky et al., 2020; Kahn et al., 2021). In contrast, salivary assay at molecular level can underscore not only a single disease but can expose interesting insights.

In this sense, NMR can track chemical composition in a non-invasive way, where more than sixty molecules were determined in saliva (Silwood et al., 2002). Suggesting that most of these molecules are key indicators of oral health, diet and medication of individuals. This implies that salivary metabolome evaluation by NMR is an indispensable approach to describe disease and immunological concerns (Costa dos Santos Junior et al., 2020; Gardner et al., 2020). Not surprisingly, SARS-CoV-2 can disarray taste ordering receptors that are known mediators of molecular sensation. Such aberration in taste could be made evident by NMR. NMR has explored overexpressed proline, lysine and pyruvate in more sensitive participants, when compared with acetate, (iso)leucine and butyrate in less sensitive ones (Mounayar et al., 2014). Albeit in poor concentration (~1%) numerous metabolic contents of oral fluid can be found abundantly in other bio-samples. Therefore, saliva is stated to be challenging and require powerful tools for its investigation, yet pre-standardized methods are strongly recommended (Costa dos Santos Junior et al., 2020; Bellagambi et al., 2020; Sapkota et al., 2020). NMR in this context can be useful, once available pre-handled samples, well-suited pulse sequences, possibly higher magnetic fields (>400 MHz) with sensitive NMR probes (cryoprobes) and good quality NMR tubes could assure a rich analysis (Costa dos Santos Junior et al., 2020).

2.2.2. Blood (serum/plasma)

Blood is a major matrix, analyzed mostly in "omics" (Meoni et al., 2021), biomonitoring (Heitland and Köster, 2021), therapeutics and COVID-19 investigations (Lee and Choi, 2021). In addition to erythrocytes and WBCs, plasma contains water, proteins, lipids, hormones and small metabolites. In WBCs, the lymphocytes, neutrophils and monocytes/macrophages are largely distributed in white blood that make part of the immune system and immunometabolism. Numerous diseases, infections such as COVID-19, therapeutic effects, and immunometabolism could be assessed through blood analysis. The detection of virus, antibody and/or metabolic irregularities caused by virus can be explored in blood plasma/serum (Shen et al., 2020; Thomas et al., 2020; Wu et al., 2020).

The evaluation of molecular variabilities is useful in understanding immunologic problems, inflammation, hepatic dysfunction, diabetic and heart disorders. NMR has been used to profile manifold such metabolic changes that occurred in plasma/serum metabolome (Kimhofer et al., 2020; Bruzzone et al., 2020). NMR has also revealed that SARS-CoV-2 disrupts plasma metabolome, inferring disease-triggered phenoconversion and immune response (Lodge et al., 2021b). Along this disruption, a central effect was found for the lipo- and glycoprotein, glucose, acetate, citrate, format and (iso)leucine in the plasma (Meoni et al., 2021). Besides kynurenine and arginine that were found the diagnostic/prognostic biomarkers, an altered level of sarcosine, lysophosphatidylcholine, creatinine and creatine was examined in the COVID candidates (Fraser et al., 2020). Such destructive propensity can be found also for amino acid (AA) metabolism showing an aberrated level of alanine, phenylalanine, lactate and pyruvate. Also, a decreased level of 1-methylhistidine was observed. Thus, the cytokines, chemokines and lipoproteins have been classified to be immunity biomarkers indicating the recovery of the patients (Lodge et al., 2021b). The participation of AA metabolism has a supporting role, when the liver is injured by SARS-CoV-2. Within an array of metabolites, numerous phospholipids, ketone bodies, cholesterol and diverse apolipoproteins have been caused by SARS-CoV-2 (Bruzzone et al., 2020). Similar changes in the lipids, cysteine, alanine, aspartic acid, succinic acid and further metabolites profile have been reported (Wu et al., 2017). Additionally, SARS-CoV-2 can damage macrophages and platelets by imposing a crucial effect on systemic pathways (Shen et al., 2020; Wu et al., 2020). Damage in macrophages can lead to an uncontrolled release of mediators which furthermore affect tissues and produce chronic disorders. While, degranulation of platelets can cause the release of growth factors by evolving thrombotic complications.

On pre-analysis, basic handling (storage, preparations) and proper experimental arrangements are imperative, which can better asset high throughput chemical data from blood plasma/serum. In this regard, some practical considerations have been suggested for NMR analysis of blood sample (Loo et al., 2020). As shown, blood sample storage at 4 °C or −80 °C for 48h has negligible effect on molecular quantity, when surpassed storage duration (4 °C/168h) or heated (56 °C/30 min), the lipoproteins components were critically damaged with increased metabolic modification (Loo et al., 2020). This indicates system perturbation outside of its physical limits or the conditions suitable for the quantitative pattern. Another NMR study allowed quantifiable 112 metabolites in the serum, however certain technical issues related to the size of NMR tubes (3–5 mm) were addressed. The size of the NMR tube has a remarkable effect on the data resolution that correlates with the experimental parameters set such as scan numbers (NS), magnetic field stability and acquisition time. In statistical perspective, the low-resolution data with 3-mm NMR tube were reliable by preserving sufficient metabolic details (Lodge et al., 2021a).

2.2.3. Bronchoalveolar-lavage fluid (BALF)

Bronchoalveolar-lavage fluid (BALF) relates to a lower respiratory system, which can be analyzed to describe diseases and viral infections. Similar to saliva and blood, analysis of BALF has revealed viral conditions in the lungs (Davidson et al., 2020; Ciaramelli et al., 2017). This indicates the respiratory system is more disposed to aggravated viral infections that belong to etiological studies. Together with pulmonary complaints, BALF is a prioritized specimen that can prove valued evidence without the need for patient's previous records (e.g., physical, clinical, etc.) (Davidson et al., 2020). It has a larger chemical similarity with blood plasma. More than 2000 metabolites are detectable in BALF, and most of them including lipids (>1000) can be measured in the blood plasma (Cruickshank-Quinn et al., 2017).

BALF analysis proved high shedding rates of coronaviruses– e.g., HCoV-NL63 and HcoV-HKUI in contrast to lower rates of rhinovirus, parainfluenza, and numerous other viruses (Garbino et al., 2009). It has also emphasized that the viral infections prompt bacterial contagions and asthma growth (Garbino et al., 2009). BALF is mainly studied by bronchoscopy; endobronchial or transbronchial biopsies, transbronchial needle aspiration, bronchial brushings and endobronchial ultrasound-guided needle aspirations (Davidson et al., 2020). Nevertheless, the cell counts, cytopathology, molecular culturing and immunologic diagnosis are also reported (Davidson et al., 2020). BALF in conjunction with sputum, blood, feces and urine is prominently used to expose viral RNA. In comparison to sputum, blood or urine, rRT-PCR examination revealed 93% virus occurrence in BALF (Wang et al., 2020). Other than RNA and immunological tests, BALF has a significant number of chemical compounds that can be valuable to delineate

infection and vaccine-induced interference in the body, and can better explain immunometabolism.

The presence of a variety of chemical compounds suggests a decent placement and analysis of BALF in the context of "omics". However, this biospecimen represents an array of immunomodulatory lipids (Cruickshank-Quinn et al., 2017), volatile aromatics, ketones, alcohols, organosulfur, organonitrogen, esters and acetate compounds (Ciaramelli et al., 2017; Nasir et al., 2018). Non-volatile nitrogenous and sulfurous metabolites, prostanoids, leukotrienes, cytokines and many more compounds that are similarly reported in the breath exhalate (Rahimpour et al., 2018), can be detected in the BALF. Interestingly, most of these compounds are essential to examine infection, diseases, immunogenicity and immunometabolism.

Except for clinical, genetic or immunologic diagnosis, presently, there is a gap in studies evaluating chemical biomarkers of COVID-19, principally by NMR tool in the BALF samples. However, the study of this matrix can be promising, because it can properly display respiratory problems, such as acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) studied in the last decades (Rai et al., 2013). NMR tool has exposed manifold metabolic variations caused by the virus that led ARDS or ALI in the patients, suggesting the potential of NMR tool in relevant contexts. In addition, such investigation can support the characterization of any influence caused by viruses, bacteria, etc. and can benefit the understanding of immunometabolism. As a remarkable advantage, when swabs are negative but there are still doubts about infections, BALF can be used as an ultimate source in probing the targeted details (Barberi et al., 2021).

2.2.4. Semen

Semen contains post-translationally modified proteins and itself is a complex substance (Sun et al., 2020). This fluid has known contents; organic acids, sialic acid, amino acid, ascorbic acid, pyruvate, creatine, citrate, flavins, phosphorylcholine, lipids, enzymes, proteins, elements (Ca, Mg, etc.), glucose and fructose (Owen and Katz, 2005). Among them, the monosaccharide fructose is a major representative involved in pathogenicity, and can be a main biomarker for viral infections – e.g., HIV-1 (Johnson et al., 2020). Similar to other organs, genital organs harbor ACE2 receptors, and based on this fact, semen can be a promising site to study SARS-CoV-2 and the phenotype alteration this virus causes.

According to the studies, about 27 viral species have been observed in semen (Salam and Horby, 2017). Although, limited probability is noticed for SARS-CoV-2 with controversial findings. In terms of viral detection, reports have highlighted semen analysis could be a best tool, while others assumed it a poor source of SARS-CoV-2. For instance, the detection of SARS-CoV-2 in the semen from six out of 38 COVID male subjects has been based on RT-PCR represented positivity (Li et al., 2020). In accordance with Paoli et al., SARS-CoV-2 positivity has been shown for the semen in conjunction with urine (Paoli et al., 2020). However, in contrast, the qRT-PCR analysis revealed no viral indication in semen, while authors have mentioned high luteinizing hormones with lowered testosterone per case (Ma et al., 2021). Further studies performed for 34 candidates with COVID-19, concluded lack of virus in semen, but sperm quality was damaged (Holtmann et al., 2020). The presence of ACE2, fluctuating hormones and sperm quality degradation represent a strong impact of the virus in seminal metabolome, demanding further investigations. Also, it is worth noting that seminal fluid has exclusively screened for genetic material while missing for molecular notion at a high-throughput level.

Up to date, typically small sized semen samples have been measured. Thus, this fact suggests to evaluate possibly large number of samples with a nontargeted molecular characterization approach. However, the controversy noted, appeared also to be due to the inadequate protocol, improper harvest and detection timing, disease severity and virus shedding intervals, among other factors. Keeping these worthy points in mind, proper sampling and handling to preparation and semen analysis through NMR metabolomics has been addressed (Lombó et al., 2021). Taking in account, NMR analysis of semen can be performed to diagnose diseases and infection guided by pathogens, and moreover to comprehend immunologic complaints.

2.2.5. Feces

Feces are a semisolid waste of the digestive system, and a key metabolic network within the human body. Symbiotic link of this system has reflective function in metabolism, immunity in conjunction with disorders and therapeutic interference. Similar to other bio-samples, feces analysis offers key advantages to describe disease and therapeutic events in the body. Such a biomatrix is a complex composition of undigested fibrous supplements, intestinal secretions, nonabsorbable toxins, water, microbial particles, proteins, carbohydrates, nitrogenous, fats and lipids, and the majority of more metabolites. Feces are known to be the major transporters of infectious viruses and harmful bacteria that cause diseases. Analyzing fecal metabolome is important to identify health issues such as fat metabolism, digestive ulcer, cancer, calprotectin, obesity, neurodegeneration, inflammation (Chen et al., 2019), infections such as COVID-19 that belongs to SARS-CoV-2 and immunity concerns.

Similar to the respiratory system, gastrointestinal tract (GIT) represents higher ACE2 expression. Thus, SARS-CoV-2 is more susceptible and therefore has exhibited damaged GIT and gut microbiome, reflecting disordered metabolic levels (Ma et al., 2020). Feces analysis can be better for detecting SARS-CoV-2 in any of the (a)symptomatic individuals with COVID-19. Of note, fecal screening can investigate virus and the influence it causes, even after a long period of disease recovery (Zhang et al., 2021; Chen et al., 2020). Moreover, anal swab has also been introduced by successfully monitoring COVID-19. Anal swab has distinguished lymphocytopenia, higher lactate hydrogenase and high-sensitivity C-reactive protein (Gan et al., 2020), indicating an improved infection and tissue damage associated with more risks. A low oxygen environment in lungs, could trigger anaerobic metabolism and result in increased lactate production. Except for other issues, the exacerbated influence of SARS-CoV-2 has not yet been met by NMR-based fecal analysis. As a major benefit, this approach can explore fecal phenotype alteration generated by exogenous stimuli and can discover biomarkers relevant to disease, infection, immunogenicity and immunometabolism.

SARS-CoV-2 interaction with the fecal metabolome can affect metabolism, metabolic pathways and can cause changes in the chemical state. These variabilities in feces could be tracked through NMR-based metabolic profiling. High throughput metabolic profiling necessitates proper experimental setup, sampling and handlings of the fecal matrix. In line with the NMR-based fecal investigation, certain settings should be considered, which are reported (Karu et al., 2018; Gratton et al., 2016). NMR assay of fecal metabolome can allow health and fitness coverage based on biochemical compositions. Amongst the metabolites in the fecal metabolome such as amino acids, fatty acids, lipoproteins, chemical markers of fitness, many metabolites were observed also in the blood plasma (Cui et al., 2021). In order to track the influence of SARS-CoV-2, fecal metabolome was analyzed by gas chromatography coupled with mass spectrometry (GC-MS) exploring 205 metabolites. Through them, COVID individuals indicated an enlarged level of sucrose, 2-palmitoyl-glycerol, diet-related 1,5-anhydroglucitol, D-pinitol and toxic oxalate in the feces. However, emphasizing that these metabolites were correlated with the blood neutrophils, serum's metabolites (e.g., oxalate) and gut microbes (e.g., *Ruminococcaceae*, *Actinomyces*, *Sphingomonas* and *Aspergillus*) (Lv et al., 2021). Also, purine (deoxyinosine and hypoxanthine), fatty acids (behenic acid), D-allose, D-arabinose and microbe related 2,4-di-tert-butylphenol were found to be depleted in the feces. Providing key benefits and untargeted nature, NMR can screen molecules without derivatization and purification steps, therefore its use could be promising in context.

Analysis of feces by NMR can describe changes occurred in phenotype by discovering biomarkers essential to diseases and immunity.

Similar to NMR based diagnosis of cancer, diabetes, malnutrition, viral, bacterial and more pathogenic infections that cause gut microbiome, its application can be highly promising to monitor vaccination effect on the immunometabolism, respectively.

2.2.6. Further biological samples

Human body is a natural reservoir, providing massive details that are measurable in the bio-samples mentioned, including cerebrospinal fluid (CSF), breast milk and urine. In line with COVID-19 and vaccination effect as well as immunogenicity, these bio-fluids may be of great interest to analyze them by NMR. However, the influence of SARS-CoV-2 could be tracked in CSF, breast milk and urine analysis by NMR. Through them, urine is a complex microbiome with an array of molecules. In accordance with "Human Urine Metabolome Database," about 3100 metabolites are detectable in urine. Urine is a known carrier for metabolic products of food, drugs, drinks, endogenous waste metabolites, environmental chemicals and bacteria. It can show creatinine, ammonia, organic acids, toxins, urea and several metabolites from AA metabolism. The analysis of urine may be a prominent field, screening several biomarkers and detecting subtle metabolic change caused in response to diseases and therapeutics. SARS-CoV-2 has an uplifting capacity in acute kidney injury (AKI). Kidneys can be more vulnerable to viruses. Therefore, renal compartments, particularly, tubules and glomerular cells have often been affected by viruses. As seen, SARS-CoV-2 has influenced kidney's function and renal metabolism that underlines mechanism by which this virus induces AKI and worsen health state (Andrade Silva et al., 2021). In renal problems, the metabolic syndrome was manifested by NMR (Bruzzone et al., 2021). Urine has proved with a prominent viral load, suggesting SARS-CoV-2 positivity (de Souza et al., 2021). Besides disease, urine analysis can allow the examination of therapeutics and prognostic outcomes by permitting insight into the biochemical process that could be linked with health status (Boulange et al., 2019) and immunometabolism upon vaccination.

2.2.7. Pharmacometabolomic

As the onset of infections cause lipids, amino acids (tryptophan, serine and threonine), so vaccination impact carbohydrates and bile acids (Diray-Arce et al., 2020), but proteins could also be involved. An increased level of threonine has known correlation with stress/trauma and is a major activator of the immune system to produce antibodies in severe situations. Metabolic processes and metabolites are mutually tied with the immunologic functions, reflecting responses against irregular conditions. As of great interest, "omics" has central role in exposing infection and vaccine immunogenicity at metabolites level, covered mostly by spectrometry, chromatography and NMR spectroscopy. NMR can non-selectively cover high throughput chemical data from samples exclusive of fractionation and derivatization as do in other tools (Mussap et al., 2020).

Pharmacometabolomic is a subdiscipline in omics, that has to be done with the pharmacological influence of any therapeutics in a biological system. Pharmacometabolomic provides a comprehensive readout of metabolites present in system metabolome, in question. Pharmacological outcome, based on pharmacokinetic (PK) and pharmacodynamic (PD) are profiled at baseline, during and post-operating therapeutics. Between these, PK importantly informs adsorption, distribution, metabolism and excretion of therapeutics, whereas PD captures pharmacological or toxicological response (Palleria et al., 2013). The main benefit of this approach is that it does not entail prior knowledge of the metabolites that carry PK/PD properties (Mussap et al., 2020), although they generate along the variation in phenotype instigated by therapeutics. Overall, it combines features based on response molecular entities of a precise metabolic pathway and individual's capability in treatment (Kaddurah-Daouk and Weinshilboum, 2014). In this regard, NMR spectroscopy may provide the opportunity to evaluate the influence of any therapeutics used in health conditions. For instance, a life-threatening sepsis condition alongside L-carnitine treatment was examined by NMR pharmacometabolomic, determining a set of metabolites. In line, 38 metabolites were traced in the serum, observing variation in the ketone bodies– e.g., 3-hydroxybutyrate, acetoacetic acid and 3-hydroxyisovalerate, assigning an effect of the treatment (Puskarich et al., 2015). As stated, therapeutics or vaccines contribute to metabolic pathways, knowingly amino acids (tryptophan, glutamine, serine, etc.), carbohydrates, glycolysis, glucose, propanoate, nucleotides and lipids (Arts et al., 2016; O'Neill et al., 2016), although age, genetics, gut biota and further trends can also contribute to these mechanisms. Whereas, some of these metabolic pathways have central role in immunometabolism.

Metabolic pathways can be tracked based on intermediate or end products– e.g., metabolites. Recalling the metabolomics and lipidomic, which have been pivotal in revealing the biochemistry and events relevant to an infested metabolome. Whereas this combinative approach over the blood plasma/serum driven by NMR with chemometrics tool has depicted the influence generated by tocilizumab administration against COVID-19. According to the studies, a very low-density lipoprotein (VLDL) was found to be higher than the apolipoproteins (A1, A2), although a stronger effect captured was for the cholesterol– e.g., HDL–3/4 and LDL–4/5 (Meoni et al., 2021). Correlation pattern proven mannose and phenylalanine, thus anticorrelated citrate with alveolar-arterial O_2 gradient– e.g., oxygen saturation with inspired oxygen fraction (SaO_2/FiO_2) was verified by direct correlation. An increased level of pyruvate and 3-hydroxybutyrate against decreased level of citrate and free amino acids (alanine, glycine, glutamine and histidine) was found to be deleterious to energy metabolism (Meoni et al., 2021). This data suggested a restored metabolism both at baseline and post-tocilizumab application. Once combined the experimental outcome with the clinical data, some critical biomarkers were observed suitable for disease examination (Meoni et al., 2021). It indicates that pharmacometabolomic can be a useful way to monitor vaccine's reactogenicity and immunogenicity that will benefit to understand the vaccines-induced metabolic pathways and metabolism which furthermore result in adverse reactions (ARs). However, vaccine-induced effects or pathway inhibitor substrates on host metabolism should be critically analyzed at any stage of vaccination, in order to realize suitable outcomes. NMR-based pharmacometabolomic of vaccines is necessary, in order to accelerate their progress and mitigate the chances for expected ARs by vaccination in the future.

3. COVID-19 vaccines, reactogenicity and immunogenicity

In general, vaccines are the biological formulations that support immunity against viral infections by reducing mortality and hospitalization rate. The virus is inactivated, attenuated or rendered to be non-infectious, and allowed to trigger only a better immune response, but not an unwelcomed condition.

In regard to the COVID-19, a variety of vaccines; Sinopharm (Xia et al., 2020) and Sinovac (Zhang et al., 2020) have proved results in preclinical analyses, and patients treated with them represented improved titers of neutralizing antibodies (NAbs). Vaccines that contain nucleic acid (e.g., DNA and mRNA) have been used to code targeted protein. Consistently, vaccines from Pfizer/BioNTech (BNT162b1/2) have successfully encoded S-protein and analysis on post-vaccination indicated maximized T helper type 1 (TH1)-biased cluster of differentiation-4 ($CD4^+$) T cell response (Mulligan et al., 2020; Jackson et al., 2020). A similar response has been shown for mRNA-1273 vaccine by Moderna/NIAID (Jackson et al., 2020). Some vector vaccines; adenovirus type-5 (CanSino Biological Inc.), ChadOx1 nCoV-19 (AstraZeneca/Oxford University) and Ad26COVS1 (Janssen Pharmaceuticals), etc. have also presented increased NAbs in phase-I/II (Dong et al., 2020). All or some of these formulations have been settled for emergency use, and meanwhile more companies have also developed vaccines with minimal cost and safety concerns and with maximum protection against the virus. Besides better performance, certain vaccines shown mild to

critical ARs that are briefly explained in this manuscript.

Vaccines that use mRNA (S protein of SARS-CoV-2) encased in lipid nanoparticles delivery system, such as BNT162b1/BNT162b2 and mRNA-1273 (Khurana et al., 2021). They are useful immunogen to encode viral protein and adjuvant to promote vaccine performance. Recipients vaccinated with BNT162b2 (efficacy 95%) have revealed reemerged COVID-19 (nine in placebo vs. one in BNT162b2 group) with no deaths without mild to moderate ARs (Polack et al., 2020). Except dose-dependent antibody response, fatigue, chills, headache, myalgias or injection site pain, the individuals vaccinated with mRNA-1273 have shown critical ARs. Amongst ARs, more prominent was the low platelet counts leading to thrombocytopenia within two weeks post-vaccination (Lee et al., 2021). Emphasizing that mRNA vaccines may be correlated to the development of immune thrombocytopenia (ITP) which is poorly studied for critical ARs at the metabolic level. In addition to ITP, skin's round spots (petechiae), nosebleeds, gingival and vaginal bleeding between 1 and 23 days of vaccination are underlined (Lee et al., 2021). A high mortality, intracranial hemorrhage and 69% cases with myocarditis/pericarditis have been determined, suggesting that the latter case is recoverable within days to weeks (Das et al., 2021). Another data from "Vaccine Adverse Events Reporting System–VAERS," concluded 19-fold higher vaccine-induced myocarditis– e.g., 80% male adolescents (12–15 years old) (Rose and McCullough, 2021). Till to date, thrombocytopenia/thrombosis or vaccine-induced thrombotic thrombocytopenia (VITT) with increased rate of myocarditis and death has been caused by more vaccines. These incorporate– e.g., viral vector ChAdOx1-nCoV-19/AZD1222 (efficacy 70.4%), adenovirus-26/5 (rAd26/rAd5, 91.6% efficacy) and Janssen/Johnson & Johnson (Rose and McCullough, 2021; Pai et al., 2021; Schultz et al., 2021; Pottegård et al., 2021). Among ARs, the rate of VITT has was shown to be due to the activated platelets antibodies against platelet factor-4 (Smith et al., 2021).

To the best of our knowledge, these hostile effects may represent either the earlier clinical conditions, individual's sensitivity to vaccine, immunologic disorder and/or disturbed metabolic pathways that are not yet or completely studied at a high throughput molecular level. There may also be additional reasons for these ARs, such as the thrombocytopenia which could be due to the thrombopoietin (TPO) hormone production and probably vaccine may come in contact to cause mechanistic aberration of TPO hormone. With respect to the interferon malsignaling, death receptor and protein ubiquitination pathways may also be linked to ITP. Moreover, VITT and myocarditis conditions may be occurred due to some damage to mitochondria which has a central role in platelet cells (Fuentes et al., 2019). However, immunogenicity evaluation is necessary to understand the performance and type of immune response generated by vaccination. This paper has focused only on some vaccines for which some critical ARs are reported, and therefore, by underlining some metabolic pathways could be linked to immunogenicity induced by vaccination. Aiming to consider these conditions, we intended to bring NMR spectroscopy for the analysis of site-specific bio-samples which can allow a non-selective discrimination of the metabolites that could be related with COVID-19 and vaccine-induced immunogenicity and reactogenicity. To better understand, first we show how vaccines interfere in metabolome and then how they could be linked with metabolites and metabolic pathways.

3.1. Vaccine interaction

Habitually, upon vaccination, the vaccine's carrier materials (mRNA/DNA) enter the host's cell and start to mediate spikes protein production. Spike proteins recruit the immune system to begin generating antibodies for viral neutralization in the cell, this mechanism could be stated as immunogenicity.

In-depth, this occurs in cytoplasmic membrane-bounded vesicles, the so-called early to late endosomes, but intracellular surface and compartments like cytosol also makes part of the innate immunity. Preserving toll-like receptors (TLRs), these organelles capably recognize and bind pathogen-associated molecular patterns that comprise lipids, protein, lipoprotein and nucleotides. Taking benefit of binding, TLR captures vaccine's mRNA in endosome and cytosol by resulting cellular immune activation and the release of signaling molecules; cytokines (interferons Iα, chemokines, etc.).

Some of the COVID-19 vaccines comprise ssRNA of modified nucleotides to reduce binding to TLR that restrict excessive production of interferon and its inhibitory function on cellular translation. When injected, lipid nanoparticles (LNPs) of vaccine are trapped in dendritic cells (DCs), by presenting antigens to T cells activating the adaptive immune response via MHC proteins. Amongst vaccines, the adenovirus vector (AdV) interferes with DCs, and alongside DCs, AdV interacts with the macrophages by stimulating innate immunity response to induce interferon discharge. DCs and vaccine-derived nucleic acid deliver antigen and inflammatory signals to T cells in lymph nodes. Which, beyond activating S-protein specific T cells, mobilizes adaptive immunity against SARS-CoV-2. Vaccines with mRNA or AdV, encourage the innate immune response by priming $CD4^+$ and $CD8^+$ T cells to differentiate into effector and memory subsets. Also, vaccine-driven interferons promote to differentiate $CD4^+$ and $CD8^+$ effector T cells producing inflammatory and cytotoxic mediators, and $CD4^+$ T follicular helper (TFH) cells that promote B cells differentiation into the antibody secreting plasma cells (Teijaro and Farber, 2021) (Fig. 2).

However, besides general interactions the underlined immunometabolism and certain metabolic pathways are decisively involved to drive and make part of COVID-19 vaccination mechanisms.

3.2. Immunometabolism and the metabolic pathways

Cellular immune phenotype has a unique link with biochemical reactions, metabolic states and cellular metabolism that tends to alter through vaccination. Vaccination has a guiding role in immunity (Diray-Arce et al., 2020), shown by omics based on immune response biomarkers that led vaccination schedules (Borriello et al., 2018). In context, molecular profiles usually involve many types of molecules that could be equally influenced by disease and immunologic activity along vaccine administration. Therefore, immunometabolism illustrates the refined variations that happen in the metabolic pathways in immune cells, in turn to stimulate their effector function. Generally, immunometabolism is accompanied by glycolysis, amino acid (AA) metabolism, tricarboxylic acid (TCA) cycle, fatty acid oxidation, fatty acid synthesis and pentose phosphate pathway, and irregularities in metabolites profile in these pathways could signify and configure the cellular immune response.

Amongst these pathways, the glycolysis is useful in various immune activities– e.g., lipopolysaccharide (LPS) activates macrophages, while dendritic cells (DCs) often stimulate NKs, effector T cells, B cells, T helper cells (1, 2, and 17) and $CD8^+$ T cells. Activated T lymphocytes have a high rate of glycolysis, oxidative phosphorylation (OxPhos) and metabolic glucose to lactate. Memory T lymphocytes rely on the lipids and mitochondrial products– e.g., citrate. Being core component, lipids are consumed to produce triglycerides or triacylglycerides that are transferred into subset fatty acids (FAs) and glycerol via β-oxidation by supporting OxPhos through the production of acetyl-CoA and ketone bodies. Regulatory T cells support β-oxidation and OxPhos through exogenously derived FAs (Arts et al., 2016). FAs help the performance of CD4 and CD8 T cells, while they can be affected by extra- and intracellular FAs. Several T cell surface receptors, the so-called G protein-coupled receptors (GPCR40, 41, 43, 84, and 120) are specific to subclasses of FAs. Between them, GPCR84 is critical to the medium FAs and known to be expressed by CD4 and CD8 T cells. Considering the immune response in shingles (herpes infection), the Zostavax vaccine has shown a strong correlation with the lipid metabolic pathways; glycosphingolipid, linoleate, glycerophospholipid and arachidonic acid (Li et al., 2017). Fatty acid oxidation (FAO) pathway can control innate and

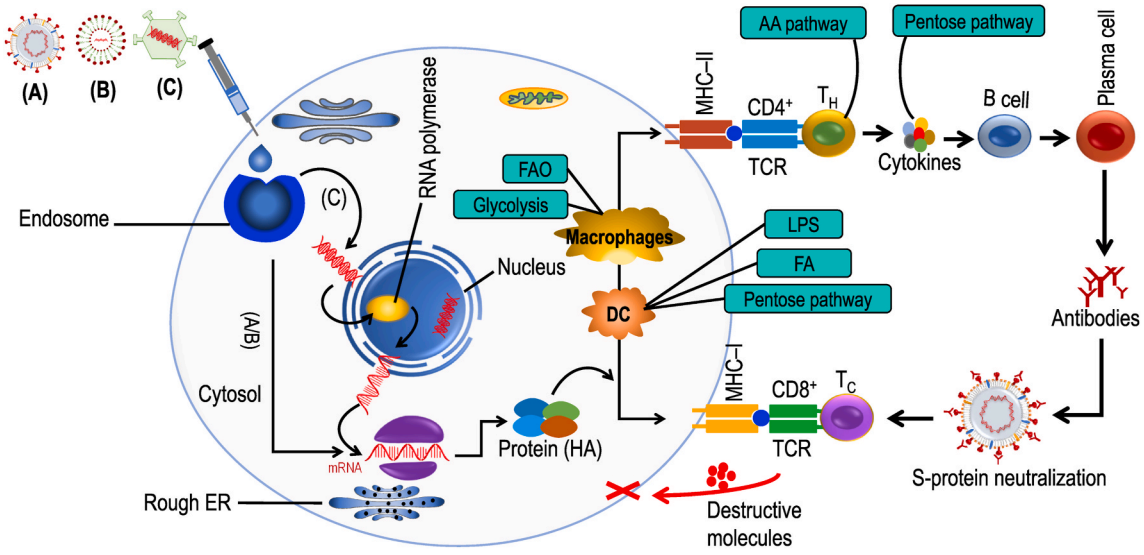

Fig. 2. A generalized mechanistic overview of candidate vaccines: (A) represents inactivated vaccines that uses whole virion of SARS-coV-2 as immunogen (S-protein), directly contacting with ribosomal units for protein (HA) translation; (B) shows m-RNA based vaccines, encapsulated in lipid nanoparticle, and after injecting, the lipid-nanoparticle remains outside of the plasma membrane of human cell and m-RNA penetrates the plasma membrane to cytoplasm and then to ribosomal subunits for protein translation (antigen); (C) is a viral vector vaccine or vector-based vaccine different from A and B, and is a DNA based vaccine that used adeno-virus as vector and post injection, the DNA penetrates plasma membrane following contact with cell nucleus, however, without integrating with DNA, converting to m-RNA via enzyme (RNA polymerase), and transported to cytoplasm for protein synthesis. The immune response generated by vaccines: MHC genes expressed to produce surface antigens – e.g., MHC-II expresses on antigen presenting cells to activate T_H, and linked through TCRs and cluster of differentiation-4 (CD4) T cells will be activated and release cytokines further activate B-cells to proliferate and differentiate to form plasma cells that result antibodies, directing against S-proteins and start neutralization. Similarly, MHC-I protein present endogenous antigens after neutralization of SARS-CoV-2 the cell will destroy. Infected cell by activating Tc cells via CD8 cells create pores in the infected cells via proteins called "perforins." FAO represents fatty acid oxidation pathway; it aids modulating macrophage's inflammatory function, and crucially controls the innate and adaptive immune systems. AA reveals amino acid pathway; a signaling pathway triggering inflammatory responses via T_H cell by secreting cytokines. Pentose pathway; signaling pathway support LPS-induced cytokines secretion which then help in B-cell activation and proliferation.

adaptive immune systems. FAO usefully modulates macrophage's inflammatory function. In adaptive immune system, it can regulate immune response of T cells. In contrast to FAO, FA promotes regulation of pro-inflammatory cells in innate and adaptive immunity. Similarly, triggering inflammatory stimuli like cytokines and LPS can be due to FAs in the macrophages. FA could sponsor inflammatory responses. FA may also be involved in linking adaptive and innate immune systems by DCs regulation that encourage Tc cells. FA can also facilitate the functions of T- and B cells, respectively.

Pentose phosphate pathway has a function to proliferating cells, nucleotide and nicotinamide adenine dinucleotide phosphate (NADPH) production. NADPH has to do with the activation of macrophages and neutrophils to smash down hazardous pathogens. This pathway has triggering role in DCs to mimic endoplasmic reticulum and cytokines production. TCA cycle activates T cells subset, especially the memory CD8 T cells. In addition, TCA cycle differentiates M1 and M2 macrophage that leads metabolites in the mitochondrial matrix to promote immune response. TCA together with the urea cycle has found to be distorted by SARS-CoV-2 (Wu et al., 2020).

Furthermore, AA metabolism is involved in the immune response against various infectious agents. Major metabolites from this pathway are glutamine, arginine and tryptophan. Arginine, tryptophan and asparagine are competitive epicenters that form association between pathogen and host (Ren et al., 2018). Glutamine is consumed by the immune system to differentiate and proliferate macrophages and T lymphocytes (TH1, TH17) (Koeken et al., 2019; Ren et al., 2014). Glutamine is an immunomodulatory supplement that serves host's cells in infection and exerts therapeutic effect (Karinch et al., 2001). Notably, TH17 cells assist tissue-resided memory cells elicited by mucosal immunization to enhance host's immunity. AA performs a mediating role in innate and adaptive immunity. In line with the macrophages, arginine and glutamine metabolism help in cytokines and nitric oxide (NO) production, while tryptophan subdues the activity of the adaptive immune (T cells) system. In regard to the production of T cells driven by tryptophan, the glutamine and arginine metabolism functionally stimulates TCRs and cytokines production. Also, AA metabolism is useful in the maintenance of cell physiology, immunity, reproduction and growth. Several metabolites from AA provide energy and regulating function for cell signaling in metabolism (Li et al., 2007; Wu, 2009).

The optimal expansion and activation of T cells is carried out by serine metabolite in the presence of adequate glucose concentrations, which improves the production of purine and pyrimidine (Ma et al., 2017; Eisenreich et al., 2013). Several of such metabolic pathways may be linked with genes specific signaling pathways, including major histocompatibility complex-toll like receptor 7/8 (MHC-TLR7/8), antigen presentation, myeloid DC activation and B cell biomarkers (Li et al., 2017). The Zostavax vaccination has shown a major connection between transcriptional and metabolomics signatures. Purine and lysine metabolism are showed to combine with transcriptomics at baseline and post-vaccination. Then, this relationship vanishes over time (one week), suggesting the pharmacokinetics of vaccine-induced metabolic remodeling may be gene expressed (Li et al., 2017), which is beyond the scope of this paper.

An inactivated hantavirus vaccine (Hantavax) in its preclinical report, has exposed dose-dependent variations in metabolic pathways (Khan et al., 2019). Hantavax-based induced immunity biomarkers included cellular immunity through folate biosynthesis and immune regulation through AA pathway (Oh et al., 2010), phagocytosis with T cell differentiation (Manzetti et al., 2014) and activation as well as pyrimidine metabolism (Sharma et al., 2014). Studies disclosed a mutual correlation of phenylalanine, cholesteryl nitrolinoleate, arginine, cholesteryl and octanoylcarnitine metabolites with the host's immune response. Higher level of cholesteryl nitrolinoleate may indicate increased inducible nitric oxide synthase (iNOS) and macrophage

activation–e.g., INF-α and LPS that upgrade the production of this metabolite (Ferreira et al., 2009). In light of metabolomics, arginine and phenylalanine after activating macrophages augment nitric acid production to imposed antiviral function, as seen in herpes simplex (Naito, 2009). Phenylalanine is related also with colon microenvironment, which is converted to phenyl acetate and displaced to derivatized glutamine in the liver.

The study of influenza immunization via metabolomics has outlined vaccine-based metabolic deviations. Amongst the perturbations, bile acid metabolism in microbiota mostly occurred by directing antibiotics post-influenza vaccination, triggering antibodies (immunoglobulin–IgG1) response along with FA metabolism. Whereas it is suggested that a deviated microbiome could impact metabolites and in turn fluctuate immune response with respect to the metabolic profile of vaccine (Hagan et al., 2019). Somehow, linking the overall description with COVID-19 vaccination, certain metabolic pathways may distress in response to immunoglobulin (IgA and IgG) and nucleotide metabolism. All or some of such mechanisms may be similar in COVID-19 vaccination that mimic cellular metabolism, chemical contents and metabolic pathways, as commonly reported carbohydrate and bile acid pathways (Diray-Arce et al., 2020).

Many metabolic pathways stated above, somehow, have direct association with the powerhouse, known as cellular mitochondrion. As largely distributed among various cell types, mitochondrion is one of the organelles that takes control of multiple activities within a cellular environment. Once damaged, however, mitochondrion can lead to thrombosis and cardiovascular abnormalities. Blood's anucleate platelets are known inflammatory cells involved in the innate and adaptive immunity. These cells have distinctive metabolic phenotype and preserve a strong relation with a mitochondrion and the chemical products–e.g., ATP. Nevertheless, glycolysis and OxPhos promote platelets activation. While highly activated platelets through procoagulant caused by collagen, thrombin and hyperglycemia provoke mitochondrial dysfunction that cause thrombosis and related conditions (Fuentes et al., 2019). Activated platelets also emit mitochondria that aid some bacterial species sponsoring inflammatory mediators– e.g., lysophospholipids, fatty acids, and the mitochondrial DNA responsibly induces platelet activation. Mitochondrial DNA methylation has been known to be involved in cardiovascular diseases (Fuentes et al., 2019), which can turn an atypical myocarditis condition spotted in the COVID-19 vaccination (Rose and McCullough, 2021). Also, the activated platelet has thrombo-inflammatory function linking coagulation to immune responses in infections guided by the virus. COVID-19 vaccine may stimulate thromboinflamatory response in candidates observed with thrombocytopenia/thrombosis and thrombotic thrombocytopenia.

In this way, platelet metabolic profiling may be an efficient approach, exposing these and further important information. The analysis of platelets by NMR has distinguished modified metabolism and the metabolic pathways caused by critical conditions such as brain cancer. NMR over platelets undertaken from the patients with brain tumors has revealed altered level of lactate, acetate, glutamine, glutamate, succinate, alanine and pyruvate. This indicates homeostatic change of reduced pyruvate, lactate and alanine in glycolysis and downgraded TCA cycle activity showed a reduced level of glutamate, glutamine and succinate in the platelets from patient with brain cancer, when compared to healthy. It was reported that the turnover of the ATP in diseased platelets was comparatively higher, indicating a high dependence on energy metabolism for platelet (Pudakalakatti et al., 2021). Hence, NMR-based omics may open up ways to snapshot these variations in order to better understand immunological conditions.

4. Chemometrics approach

The analysis in metabolomics or other omics approaches provide multidimensional chemical data of multivariate nature that potentially cover important information. According to the standard metabolic reporting structures (SMRS), omics analysis usually depends on powerful analytical tools– e.g., NMR spectroscopy and MS spectrometry. These tools generate complex spectral data per sample, where using chemometrics becomes essential to simply guide the results and make them useful (Lindon et al., 2005; Holmes et al., 2008). Chemometrics, in this regard benefit to differentiate samples based on their (bio)chemical relationships by outlining patterns of metabolites that are allied with the aims– e.g., disease status and immunological conditions.

Post-analyses, the details tracked in chemometrics are coordinated with the process involved in the system metabolome. Such details could be, for example, the biochemical (dis)similarity and metabolites pattern that portray health status and immunity activation. Over the past few decades, chemometrics analysis if the omics data has significantly supported medicine, public health (Holmes et al., 2008), biomedical, pharmacological and toxicological efforts (Gowda et al., 2008; Bujak et al., 2015). Multivariate and artificial intelligence are generally used to simplify NMR dataset for proper examination of disease and tracking their recovery pathway (Kimhofer et al., 2020). NMR spectra in context of "omics" are driven mostly by linear projection methods; principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and orthogonal partial least squares discriminant analysis (OPLS-DA).

As an unsupervised exploratory tool, principal component analysis (PCA) is used to reduce dimensions and process the data (Bro and Smilde, 2014)– e.g., NMR spectral dataset. Post-processing, PCA distributes original dataset into a new orthogonal space of variables – e.g., principal components, PCs (Bayne and Kramer, 1999) and represents the original data into scores (T) and loadings (P) matrices. The T matrix allocates each sample spectrum with a score value along PCs (Ciecka, 1982). In omics, such distribution can be interpreted as same (bio) chemical features that grouped together, while unreliable samples as outliers appear apart in the PC's space. The P matrix is defined by the relevance of each variable that explains (non)grouping samples exposed by the T matrix (Vandeginste et al., 1998)– e.g., the chemical shifts of the NMR spectra responsible to cluster similar samples. The non-modeled data are stored as residual matrix (E), expressing an unexplained detail by PCs system. With respect to the NMR dataset, usually the E matrix is attributed to noise, imperfect pulse, spectral artifacts, improper sample amount, volume and concentration, or additional factors that allied with data acquisition, but E matrix is still important to distinguish deviated sample(s) (Mark and Workman, 2007). PCA provides a quick overview for pattern recognition, sample selection or anomaly detection (Fonville et al., 2010) in NMR data. Lodge et al., studied the influence of sample's volume to predict SARS-CoV-2 by NMR spectroscopy (Lodge et al., 2021a). In this study, they analyzed different volumes of the blood plasma, using 3-mm NMR tubes (100 μL) and standard 5-mm NMR tubes (300 μL). This association of varied volumes of the samples were found to be identical by PCA, suggesting the presence of adequate metabolic information. The details covered with PCA could then be extended to supervised modelling, using PLS/OPLS and/or PLS-DA/OPLS-DA, respectively.

In supervised tools, partial least squares (PLS) differ from PCA by the addition of an independent variable (matrix Y) in data operations, summarization and decomposition (Brereton and Lloyd, 2018). Data decomposition is performed to a highest possible correlation between the spectra and the independent variable "Y". Using this correlation, predictive models can be built for attributes of interest that can either be a quantitative response or a class-based feature (Amante et al., 2019). If response data is a class, then the use of PLS is imperative for samples discrimination. Therefore, partial least squares discriminant analysis (PLS-DA) is a method to separate classes of knowledge.

The use of PLS regression and DA in omics studies has become increasingly popular. Further advances have been made in PLS, such as adding the concept of orthogonal partial least squares (OPLS) introduced by Johann Trygg and Svante Wold in 2001. (Wold, 1976; Marini, 2020; Snee, 1985). The use of OPLS is essential, when systematic

variation constitutes a large part of the spectra under investigation or the analyte of interest provides a small part of the spectral range (Wold et al., 1998). The idea is to remove systematic information in the X matrix that does not match Y modeling (Fonville et al., 2010; Marini, 2020; Wold et al., 1998) by improving its correlation during data decomposition. The implementation of OPLS serves to minimize confounding physical and the biochemical variations in metabolomics (Fonville et al., 2010). Loo et al., evaluated mishandling for the samples that could occur during NMR-based virus investigation (Loo et al., 2020). This study showed exploratory performance of PCA followed by OPLS-DA to distinguish viable conditions for viral samples characterization. In summary, for tested conditions, sample heating for virus deactivation had revealed pivotal effects on the NMR data (e.g., quantifiable lipoprotein).

Consistent with COVID-19, NMR dataset by chemometrics have disclosed abnormalities based on characteristic metabolic phenotypes (Kimhofer et al., 2020; Loo et al., 2020; Lodge et al., 2021a). All over the metabolic phenotypes, COVID-19 had a strong correlation with the lipoproteins, glycoproteins, amino acids, lipids and other chemical biomarkers (Kimhofer et al., 2020; Loo et al., 2020; Lodge et al., 2021a, 2021b). The underlined metabolic phenotypes of SARS-CoV-2 positivity were characterized by OPLS-DA applied to the NMR dataset (Kimhofer et al., 2020). This study examined (non)healthy groups; where plasma samples were collected from 17 adults with confirmed COVID-19 and 25 healthy controls. The authors pointed out the need for a larger sample set and rigorous validation to build a robust model for disease prediction. Therefore, exploratory analyses were performed aiming at observing group clusters based on OPLS-DA to investigate phenoconversion models for SARS-CoV-2 detection.

Beyond PCA and discriminant analysis, various machine learning methods; support vector machine (SVM), random forests (RF), extreme learning machine (ELM), and deep learning convolutional neural networks (CNN) have become more popular in the recent years. Generally, these methods require more computational power, but they represent more, once it can be applied non-linear computation to deal with situations for complex data, like -omics. Adding these statistical tools to the NMR spectra can contribute to recognize pattern and classification of disease evolution or suppression, immunometabolism and the metabolic pathways induced by vaccination.

NMR-based omics integrated with advanced multivariate tools are preferred, determining phenotypes of disease and treatment by assaying metabolic composition in the stated biological samples. The multivariate tools, in this regard, have better explained the analyzed system studied in the last years (Loo et al., 2020; Misra et al., 2019). Adding this approach and related information could support an in-depth understanding of disease, COVID-19 vaccination, immunometabolism and the metabolic pathways. Integrating these tools with NMR spectroscopy can open up ways to strategic planning; the control of diseases, interventions checkup and improved precision medicine in the future.

5. Conclusion

As a worldwide health concern, COVID-19 has somehow been controlled by large-scale vaccination. Providing better efficacies, some of the COVID-19 vaccines have shown critical adverse reactions as discussed. However, such reactions might occur due to some disturbance in the metabolic pathways caused by vaccination, therefore immunogenicity evaluation is of high importance. With interest to assess disease course and immunogenicity at molecular level, NMR-based omics approach has been proposed for the analysis of various bio-samples. We have provided ample aspects and importance of each bio-samples with their analysis by NMR integrated with statistical tools that could guide understanding any disease, and in particular the immunogenicity in immunology discipline. NMR, in a non-targeting way, can assess many molecules in complex biological mixtures without fractionation and derivatization. Nevertheless, this tool can perform metabolomics, lipidomic, proteomic and pharmacometabolomic profiling that can capably discriminate the targeted mechanisms in question. Next, we focused on different bio-samples, because they share manifold important molecular signatures. This strategy is useful, because there might be less available samples (e.g., CSF, etc.), lower analyte concentration and or improperly controlled sample. A surplus benefit is, when one sample is incapable to prove adequate details, so they can be proxied through alternative samples by increasing sensitivity and reducing false positive/negative results. By this review, we encourage people involved in the field of omics, human diseases and infections, therapies, immunology and NMR spectroscopy struggling to mitigate health concerns in the present and in the future.

Funding

This work in part was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant number 2022/03952-1) and The World Academy of Sciences-National Council for Scientific and Technological (TWAS-CNPq, grant number 190735/2015-5).

Data availability

The data used to support the finding of this study are included within the article.

CRediT authorship contribution statement

Sher Ali: Conceptualization, Investigation, Project administration, Writing – original draft, Writing – review & editing. **Štěpánka Nedvědová:** Writing – original draft. **Gul Badshah:** Writing – original draft. **Muhammad S. Afridi:** Writing – original draft. **Abdullah:** Writing – original draft. **Lívia M. Dutra:** Writing – original draft. **Umar Ali:** Writing – original draft. **Samara G. Faria:** Writing – original draft. **Frederico L.F. Soares:** Data curation, Writing – review & editing. **Rafi U. Rahman:** Writing – original draft. **Fernando A.C.Q. Cançado:** Writing – original draft. **Micheli M.C.C. Aoyanagi:** Writing – original draft. **Lucas G.D. Freire:** Writing – original draft. **Alan D.C. Santos:** Data curation, Writing – review & editing. **Andersson Barison:** Conceptualization, Funding acquisition, Investigation, Project administration, Validation, Writing – review & editing. **Carlos A.F. Oliveira:** Conceptualization, Funding acquisition, Investigation, Project administration, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

All authors thank Dr. Kelly Mara Seronato and Dr. Anwar Shamim for critical reading and revision of the manuscript.

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