



High-throughput approaches of diagnosis and therapies for COVID-19: antibody panels, proteomics and metabolomics

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The urgent need for diagnostics and therapeutics against the COVID-19 pandemic has shown the great potential of antibodies, proteomics and metabolomics in this direction. Several clinical trials are underway using antibodies from COVID-19 patients that show very specific and strong binding to viral proteins leading to neutralization. On the other hand, proteomic and metabolomic profiles of COVID-19 patients present novel diagnostic biomarkers to predict patient outcomes and enable the development of personalized therapeutics to target the dysregulated pathways, as revealed by those profiles. Here, we discuss how studies based on antibodies, proteomics and metabolomics contribute to the development of diagnostics and therapeutics against COVID-19. The elegant technology can extend to high-throughput, rapid and reliable drug discovery strategies of the future.

Lay abstract: In order to prevent and treat the ongoing COVID-19 pandemic, several groups around the world are focused on a detailed understanding of the biology of SARS-CoV-2 infection, the biological events occurring inside the patients and the response of the patients to the infection. SARS-CoV-2 is the coronavirus that causes COVID-19. Some of the approaches to combat the pandemic have provided important results that can help toward therapeutic developments against COVID-19. This review discusses three such areas – antibody treatment to prevent COVID-19 infection, analysis of changes in protein profiles of COVID-19 patients, and analysis of metabolism or energy-related changes in COVID-19 patients. Antibodies are molecules produced in the host's body as a defense response to infection. Antibodies extracted from patients who recovered from COVID-19 have been used to successfully manufacture large amounts of antibodies to treat COVID-19 patients. The analysis of protein and metabolism profiles of COVID-19 patients has shown that several proteins and metabolism-related entities in the body are either upregulated or downregulated in COVID-19. These abnormal levels can either be attenuated by medical intervention or can be monitored as indicators of COVID-19 diagnosis. Overall, antibodies, protein and metabolism profiles are three important tools, among several others, that are helpful in combating the COVID-19 pandemic.

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Overview of SARS-CoV-2 infection & antibody-based therapies

COVID-19 is caused by SARS-CoV-2, which belongs to the family of coronaviruses and has 79.5% identity to SARS-CoV [1]. The virus contains spike (S) proteins on its surface, whose S1 subunit contains a receptor binding domain (RBD), that binds to the host cell receptor ACE2 for infection. Following entry into the host cell, the virus releases its genome, which is composed of RNA, that undergoes replication and transcription to produce multiple viruses that exit the cell to infect other cells [2–4]. The binding of S protein to ACE2 is a crucial process of the infection, hence it is targeted by various therapies to prevent COVID-19. One of the therapeutic approaches is the

Table 1. Diverse approaches toward vaccine and therapeutic developments against COVID-19.

NCT identifier	Approach	Sponsors	Ref.
NCT04368728	Lipid nanoparticle-formulated mRNA vaccine, candidates: BNT162b1 – encodes a secreted trimerized SARS-CoV-2 receptor-binding domain; BNT162b2 – encodes membrane-anchored SARS-CoV-2 full-length spike, stabilized prefusion	Pfizer, BioNTech SE	[5]
NCT04380701	Anti-viral RNA vaccines BNT162a1, BNT162b1, BNT162b2 and BNT162c2 which elicit TH1T cell responses	BioNTech RNA Pharma	[6]
NCT04405076	mRNA vaccine, candidate: mRNA-1273 – encodes stabilized prefusion SARS-CoV-2 spike protein (S-2P)	Moderna Inc. (MA, USA)	[7]
NCT04582201	Agent-797 – unmodified, allogeneic invariant natural killer T (iNKT) cells therapy	AgenTus Therapeutics (MA, USA)	
NCT04453852	Recombinant S protein antigen formulated with adjuvant to drive potent T cell and neutralizing antibody response against target virus	Vaxine (SA, Australia)	
NCT04542993	Utilizing resveratrol as zinc transporter to minimize viral load and severity of COVID-19	Swedish Medical Center (WA, USA)	
NCT04546841	Multipptide vaccination of investigational medicinal product (IMP) of SARS-CoV-2 HLA-DR (human leukocyte antigen – DR isotype) peptides	University Hospital Tuebingen (Germany)	[8]

NCT: National Clinical Trial.

use of antibodies that bind specifically to viral S protein and RBD, leading to neutralization so that they can no longer interact with ACE2.

Although the next section of this review focuses mostly on neutralization of RBD and S protein, it is important to note that neutralization of ACE2 receptors affects several downstream cellular processes involving ACE2. Hence, alternative strategies for therapeutic developments against COVID-19 also need consideration. Some of the alternative strategies in clinical trials are listed in Table 1 with corresponding National Clinical Trial (NCT) identifier numbers. The mRNA vaccine from Pfizer (NY, USA) and BioNTech (Mainz, Germany) has shown 90% efficacy and has been granted fast track designation by the US FDA (MD, USA).

In addition to those mentioned in Table 1, several other approaches to prevent and treat SARS-CoV-2 infection are being pursued, among which the neutralization of ACE2 receptors is one of the most prevalent approaches. Hence, the following section focuses on antibody-based neutralization of ACE2 to combat COVID-19. Several groups around the world have developed panels of full human antibodies with neutralizing activities against SARS-CoV-2 [9–16].

Therapies based on antibodies are currently in clinical trials, with one of the public–private partnership trials from National Institutes of Health (MD, USA) in Phase III (NCT04501978). This is an adaptive clinical trial titled '*ACTIV-3: Therapeutics for Inpatients With COVID-19 (TICO)*' and sponsored by the National Institute of Allergy and Infectious Diseases (NIAID; MD, USA). In ACTIV-3, an adaptive two-stage Phase III protocol design is used to study an investigational monoclonal antibody LY-CoV555 identified in a blood sample from a recovered COVID-19 patient. A large number of participants with SARS-CoV-2 infections will be intravenously infused with LY-CoV555 or saline placebo. Additionally, the antiviral remdesivir and standard care for COVID-19 will also be administered to the participants. Patient response and need for oxygen, ventilation or supportive care will be monitored over time. Abcellera Biologics (BC, Canada), NIAID's Vaccine Research Center and Eli Lilly and Company (IN, USA) have collaborated for the venture. Regarding the use of antibodies against COVID-19, several workflows around the world successfully generated antibody panels to neutralize SARS-CoV-2, one of which is briefly discussed below.

Potential of anti-COVID-19 antibody panels

Isolation & identification

The following methodology (Figure 1) was adopted by Wan *et al.* leading to high-throughput and timely development of human recombinant antibodies against SARS-CoV-2 components [12]. The methodology from Wan *et al.* is described here only as one of the several representative successful procedures to clone, express and characterize human recombinant antibodies from patients who recovered from COVID-19. Globally, it was one of the first studies that prepared an antibody panel from recovered COVID-19 patients. Although, two of the 26 authors of Wan *et al.* share organizational affiliation with the author of this review, the discussion on Wan *et al.* is solely focused on the scientific aspect of the study and not presented as a superior or only method to generate anti-COVID-19

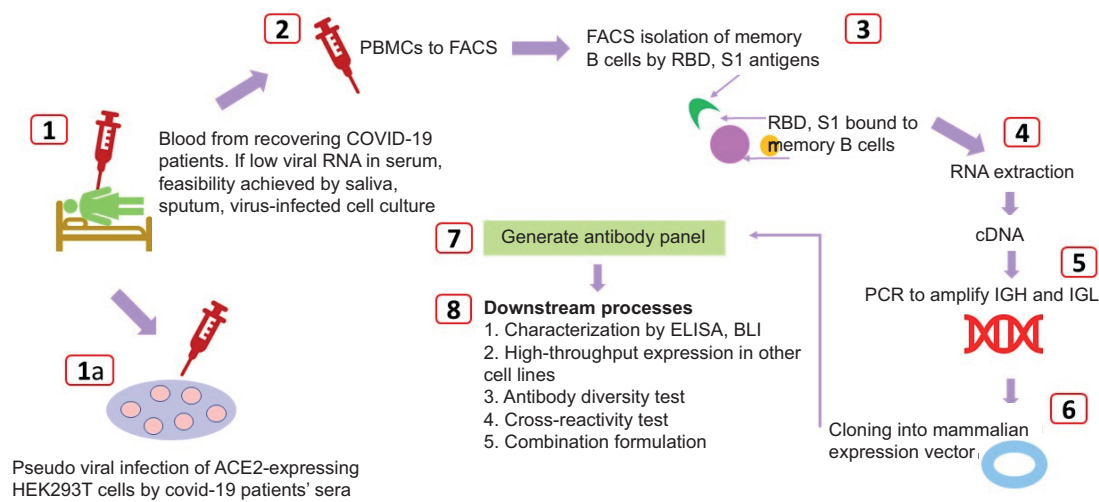


Figure 1. Workflow to generate antibody panel against SARS-CoV-2 component. Methodology is described in section titled 'Successful procedures to generate anti-COVID-19 antibody panels'.

FACS: Fluorescence-activated cell sorting; IGH: Immunoglobulin heavy chain; IGL: Immunoglobulin light chains; PBMC: Peripheral blood mononuclear cell.

antibodies. The discussion aims to inform researchers developing antibodies against COVID-19 and other viral diseases, and is not intended for promoting the organization of affiliation. The review also mentions other corporate organizations involved in anti-COVID-19 therapeutics to which the author is not affiliated.

Wan *et al.* obtained sera from patients who recovered from COVID-19 and their neutralization activity was tested against SARS-CoV-2 pseudoviral infection of human cells (HEK293T line) where ACE2 was expressed. Neutralization was observed against SARS-CoV-2 RBD and S proteins. The successful binding and neutralization capacity of the antibodies render them as potential candidates for anti-COVID-19 therapies. To identify the antibodies, peripheral blood mononuclear cells from the patients were subjected to fluorescence-activated cell sorting using recombinant RBD and S1 antigens to isolate memory B cells on which RBD and S1 are bound. RNA was extracted from the isolated B cells and converted to cDNA, and sequences which encode immunoglobulin heavy (IGH) and light chains (IGL) were amplified and cloned into mammalian expression vectors. The study resulted in large-scale generation of naturally paired IGH and IGL clones that were catalogued [12].

Characterization

Further characterization was performed by ELISA where the antibodies were tested against viral S extracellular domain and RBD. Antibodies with strong affinity were identified based on EC₅₀ values. ELISA was also used to map the different RBD epitopes. Binding affinity was cross-validated by bio-layer interferometry assay, which analyzes the interference pattern of light reflected from protein immobilized on biosensors to measure biomolecular interactions. Hence, multiple techniques were employed to analyze the antibody binding.

Therapeutic promise, antibody diversity, cross-reactivity & combination therapy

To translate the antibodies into therapeutic development, their expression was tested in Chinese hamster ovary (CHO) cells. Since the antibodies were simple to express and the yield was high, they are potential candidates for therapeutic developments.

Antibody-based therapies are significantly affected by antibody diversity hence, the study further analyzed the complementarity-determining region 3 of the antibody, which is most important for its diversity. Alignment of complementarity-determining region 3 sequences of the heavy and light chains of viral neutralizing antibodies revealed nine unique candidates. However, cross-reactivity of these antibodies to proteins from related virus species will require further optimizations. Nonetheless, the antibodies generated by the above methods show promise as therapeutic candidates, if multiple antibodies are used in combination. Anti-COVID-19 therapies involving a combination of antibodies are under active investigation [15,17,18]. It also needs to be considered that the study

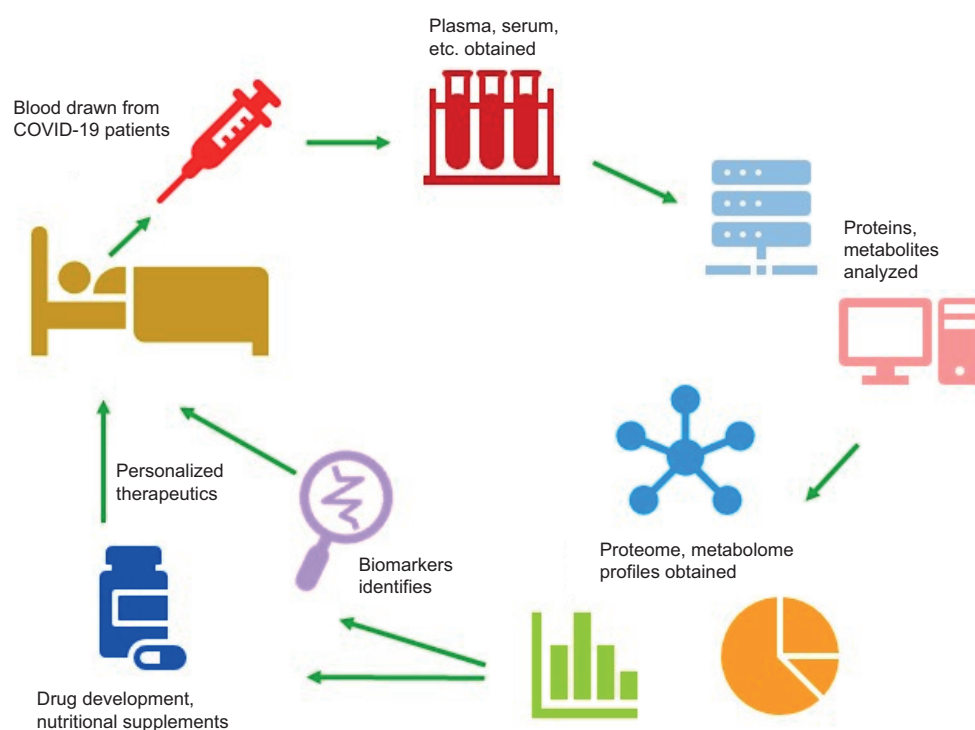


Figure 2. Proteomics and metabolomics profiles of COVID-19 patients show promise as biomarker diagnostic resources and potential therapeutic strategies.

by Wan *et al.* is just one approach among various other methods that researchers have been pursuing to develop therapeutics against COVID-19, which have also been successful in their respective applications.

Therapeutic potential of the REGN-COV-2 monoclonal antibodies

REGN-COV-2 is a cocktail of two potent neutralizing monoclonal antibodies (REGN10987/imdevimab+REGN10933/casirivimab) that target nonoverlapping epitopes of the SARS-CoV-2 S protein and aimed to prevent mutational escape or evolved resistance [15,16,19]. The cocktail is administered to COVID-19 patients grouped into various cohorts based on disease severity in multiple clinical trials sponsored by Regeneron Pharmaceuticals (NY, USA). The disease severity ranges from not requiring supplemental oxygen to requiring mechanical ventilation (NCT04426695), symptomatic versus asymptomatic (NCT04425629), adolescents and asymptomatic household contacts (NCT04452318), having chronic medical conditions and comorbidities (NCT04519437). REGN-COV-2 significantly lowers viral load in lower and upper airways and pathological sequelae in rhesus macaques, lowers lung titers, pneumonia and limits weight loss in hamsters, which proves its therapeutic capability [19].

Analysis of translome & proteome for COVID-19 therapies

Proteome and metabolome of COVID-19 patients provide important insights for diagnostic and therapeutic developments against COVID-19 (Figure 2). An optimistic approach to combat COVID-19 has been recently published by Bojkova *et al.* where high-throughput analysis of translome and proteome enabled the inhibition of cellular pathways by small-molecules to prevent SARS-CoV-2 replication in cultured human cells [20]. Key proteomics-based findings from the study are summarized below.

Establishment of a model system to study translome & proteome upon SARS-CoV-2 infection

A highly permissive SARS-CoV-2 cell-culture model was established using human colon epithelial carcinoma cell line Caco-2. SARS-CoV-2 was administered at a multiplicity of infection of one in order to infect most of the cells

but to prevent multiple infections, which resulted in a fast progression of viral infection with cytopathogenic effects observed after 24 h of infection. A continuous increase in SARS-CoV-2 RNA quantity and the presence of viral protein in most cells guaranteed the establishment of a functional SARS-CoV-2 cell-culture model [20].

Analyses of transcriptome & proteome following SARS-CoV-2 infection

The transcriptome and proteome changes were quantified by a new high-resolution, high-sensitive method called multiplexed enhanced protein dynamics (mePROD) [21]. mePROD offers unbiased analysis of cellular response to viral infection which is free of perturbations, with negligible background noise. This is because mePROD does not affect cellular behavior since translational changes are quantified by stable isotope labelling by amino acids in cell culture. mePROD measures relative translation at the level of nascent chain, and its advantages as mentioned below. mePROD includes quantification of heavy label incorporation following very brief labeling times while maintaining depth or accuracy. mePROD has a booster channel which elevates the signal of interest in a multiplexed and dynamic stable isotope labeling by amino acids in cell culture-labeled sample at low stoichiometry, accurately measures global translation rates and allows for comparison of samples from different runs. mePROD was developed to overcome challenges like lack of tools to study the correlation of transcriptome and proteome, and lack of transcriptome data. Current methods to study the transcriptome are labor-intensive, biased, expensive and require large amounts of starting material. mePROD overcomes the above issues because it is simple, sensitive, lowly priced, unbiased and requires less than 100,000 cells. It offers direct results on nascent and novel-synthesized proteins which convey another layer of translational data. Hence, limited clinical samples and primary cells can be assayed with mePROD [21].

Following infection, global translation rates of five viral proteins were detected, which showed rising translation rates with time. Next, host proteins whose translation kinetics correlated with viral proteins were screened to identify pathways that could be potential regulators of SARS-CoV-2 replication. Pathway analyses of the network of screened proteins revealed an upregulation of translation machinery, splicing and nucleobase synthesis of the host. Hence, these protein processing pathways likely participate in SARS-CoV-2 replication. Tables 2 & 3 show reactome analysis of proteins which increased and decreased, respectively, after infection.

To test if SARS-CoV-2 replication is affected by interfering with translation, translation inhibitors, such as cycloheximide and emetine were administered at nontoxic concentrations to Caco-2 cells. Cycloheximide inhibits translation elongation, while emetine inhibits 40S ribosomal protein S14; both caused significant inhibition of SARS-CoV-2 replication [20].

Proteomics analyses identified two main clusters of proteins upon SARS-CoV-2 infection

Analysis of the proteome of Caco-2 cells after 24 h of infection showed two main clusters of differentially regulated proteins by hierarchical clustering. In the first cluster, proteins were reduced upon infection, and included proteins for cholesterol metabolism and transport like APOE, APOA, etc. In contrast, the second cluster consisted of proteins that were increased upon infection and were enriched for RNA-modifying proteins, like spliceosome components and carbon metabolism [20].

To test if inhibition of the spliceosome affects viral replication, the authors administered pladienolide B which is a spliceosome inhibitor against the splicing factor SF3B1 [22]. The drug inhibited SARS-CoV-2 replication at nontoxic concentrations for Caco-2 cells, indicating that spliceosome contributes to SARS-CoV-2 replication and, hence, can be therapeutically targeted [20].

In the second cluster, the following proteins were annotated to carbon metabolism: ENO1, CS, GAPDH, MDH2, GOT2, IDH3G, IDH3B, IDH2, PDHB, HK2, PKM and HKDC1. Consistently, the authors saw that 2-deoxy-d-Glucose, which inhibits hexokinase that is the rate-limiting enzyme in glycolysis, impedes SARS-CoV-2 replication. The observation indicated that glycolysis can be pharmacologically targeted to prevent SARS-CoV-2 infection.

Proteomics analysis developed a protein interaction database & identified additional clusters

To monitor individual protein levels over time across different organelles, the authors developed a STRING network which is a biological database of established and predicted protein–protein interactions. The network was generated by filtering of proteins based on significant changes in levels of individual proteins in the organelles using circle-heat maps. It also serves to compare SARS-CoV-2 with other coronaviruses.

Table 2. Reactome pathway analysis of genes increased in protein level during infection.

Eukaryotic translation elongation (R)
p38 signaling mediated by MAPKAP kinases (N)
Mitochondrial calcium ion transport (R)
Deubiquitination (R)
Antigen processing and presentation (K)
Neutrophil degranulation (R)
Translocation of SLC2A4 (GLUT4) to the plasma membrane (R)
SUMOylation (R)
TGF-beta receptor signaling (N)
MAPK6/MAPK4 signaling (R)
Proteasome (K)
Protein folding (R)
DNA-PK pathway in nonhomologous end joining (N)
Parkinson disease (P)
Regulation of actin cytoskeleton (K)
Glucose metabolism (R)
Validated targets of C-MYC transcriptional activation (N)
Biosynthesis of amino acids (K)
a6b1 and a6b4 Integrin signaling (N)
Adherens junction (K)
Protein processing in endoplasmic reticulum (K)
2-Oxocarboxylic acid metabolism (K)
Citrate cycle (TCA cycle) (K)
Signaling by ROBO receptors (R)
HIF-1 signaling pathway (K)
Pathogenic <i>Escherichia coli</i> infection (K)
Carbon metabolism (K)
The citric acid (TCA) cycle and respiratory electron transport (R)
IL-12 family signaling (R)
Regulation of mRNA stability by proteins that bind AU-rich elements (R)
Spliceosome (K)
Processing of capped intron-containing pre-mRNA (R)
Pathways ranked according to false discovery rate values from highest to lowest. Data taken from [20].

Proteomics analysis of abundance trajectories and ontology network analysis identified a major cluster of metabolic pathways consisting of diverse nucleic acid metabolism sub-pathways which serves to present more proteomic targets for inhibiting SARS-CoV-2 replication. Drawing from the analysis, the authors prevented SARS-CoV-2 replication using ribavirin, which inhibits guanosine nucleotide synthesis.

Proteomics analysis revealed that proteostasis is affected upon SARS-CoV-2 infection

Proteomics analysis of SARS-CoV-2 infected cells showed that proteins involved in proteostasis, or protein homeostasis, pathways behaved similar to viral proteins. The observation was attributed to elevated translation of viral proteins due to increased folding load caused by disruption of host cell proteostasis by SARS-CoV-2 infection.

The cells were treated with NMS-873 which is a small-molecule inhibitor of a key component of proteostasis called p97, to test its effect on SARS-CoV-2 replication. p97 is an AAA ATPase p97 that regulates protein degradation, membrane fusion, vesicular trafficking and stress granule disassembly [23]. Replication of SARS-CoV-2 was seen to be inhibited by NMS-873 at nanomolar amounts [20]. Hence, SARS-CoV-2 infects the host cells leading to drastic perturbations in its proteome through interruptions of cellular functions, and the study showed that compounds that modulate those functions can interfere with SARS-CoV-2 replication in human cells.

Table 3. Reactome pathway analysis from second cluster of proteins that decreased following SARS-CoV-2 infection of Caco-2 cells.

Mitochondrial calcium ion transport (R)
Eukaryotic translation elongation (R)
Transferrin transport
Viral process
High-density lipoprotein particle assembly
Hepatocyte growth factor receptor signaling pathway
Cellular oxidant detoxification
Phospholipid efflux
Regulation of translation in response to stress
Pyrimidine nucleobase biosynthetic process
Negative regulation of apoptotic process
'De novo' CTP biosynthetic process
Response to reactive oxygen species
Membrane organization
High-density lipoprotein particle clearance
Regulation of lipid metabolic process
Regulation of stress-activated MAPK cascade
Triglyceride catabolic process
Regulation of cholesterol transport
Cholesterol efflux
Negative regulation of endopeptidase activity
Lipoprotein metabolic process
Cholesterol metabolic process
Positive regulation of hydrolase activity
Lipoprotein biosynthetic process
Low-density lipoprotein particle remodeling
Chylomicron assembly
Positive regulation of cholesterol esterification
Chylomicron remodeling
Cellular protein metabolic process
Retinoid metabolic process
Post-translational protein modification
Pathways ranked according to false discovery rate values from highest to lowest. Data taken from [20].

Overall, the proteomics analyses revealed several novel features about SARS-CoV-2 infection that can be harnessed for developing therapeutics against COVID-19. First, a human cell culture system was successfully established to study SARS-CoV-2 infection. Second, an improved method for proteomics analysis called mePROD was developed that can apply to other proteomics-based studies on limited clinical infectious samples. Third, translation, spliceosome and nucleobase synthesis, which are crucial for in protein synthesis, were revealed as pharmacological targets to prevent SARS-CoV-2 replication. Fourth, pharmacological targeting of glycolysis and proteostasis were found to inhibit SARS-CoV-2 replication. Hence, proteomics analyses identify novel pharmacological targets for therapeutic developments against COVID-19. Fifth, development of STRING network reveals changes in proteins over infection time across various organelles and their interaction partners which is large reservoir of information to compare with other viruses and identifying therapeutic targets.

Metabolomics profiles provide COVID-19 diagnostic benefits with potential contributions to therapeutic developments

Another approach of developing therapeutics and predicting their efficacy against COVID-19 is through an understanding of the effects of SARS-CoV-2 infection on the metabolome. This is because a group of drugs with

Table 4. Differentially regulated metabolites identified from metabolomic analysis of COVID-19 patients.

Study (year)	n of patients	Upregulated entities	Downregulated entities	Ref.
Shen B <i>et al.</i> (2020)	28 severe, control groups of 28 healthy, 25 non-severe, 25 negative for SARS-CoV-2 but with similar clinical characteristics as COVID-19 patients	21-hydroxypregnenolone, kynurenate, kynurenine, 8-methoxykynurenate, phosphocholine	Sphingolipids, glycerophospholipids, choline and its derivatives, glutamate, arginine, N-(l-arginino) – succinate, citrulline, ornithine, glutamine, 2-oxoglutarate, N-acetyl-L-glutamate, urea, fumarate, argininate, asymmetric dimethylarginine, symmetric dimethylarginine, homoarginine, N-acetyl-arginine	[25]
Thomas <i>et al.</i> (2020)	33 COVID-19 patients	Kynurenine, kynurenic acid, picolinic and nicotinic acid, arginine, methionine sulfoxide, cystine, creatine, creatinine, spermidine, acetyl-spermidine, glycolysis and pentose phosphate intermediates	Tryptophan, serotonin, indole pyruvate, alanine, glycine, serine, glutamine, histidine, cysteine, taurine, ornithine, citrulline	[29]
Wu D <i>et al.</i> (2020)	34 COVID-19 patients	Diglycerides, free fatty acids, triglycerides	Sarcosine, L-aspartic acid, malic acid	[30]

antiviral activity against SARS-CoV-2 that lower the viral burden in patients require intracellular ATP-dependent activation depending on the bioenergetic profile of the patient. Specifically, nucleoside-based drugs including remdesivir, ribavirin and favipiravir, which are currently in under focus as potential therapies for SARS-CoV-2, need to be converted to active triphosphate forms by host enzymes that use endogenous ATP. The conversion is necessary for these drugs to become functionally active [24].

The relationship between COVID-19 and metabolomics have been revealed by metabolomics studies on COVID-19 patients with acute respiratory distress who show plasma metabolomic signatures resembling sepsis syndrome [20,24,25]. Sepsis patients with poor outcomes have an acute crisis in their bioenergetics profile, and hence the metabolomic signatures in COVID-19 patients suggest metabolic suppression, platelet degranulation and dysregulated macrophage function [26,27].

The adverse effect of SARS-CoV-2 infection on the metabolome of patients not only affects their physiology but also the efficacy of anti-COVID-19 drugs that are dependent on endogenous cellular energy for their functional activation. For example, a low bioenergetics profile leads to reduction in energy-rich metabolites like ATP and phosphoribosyl pyrophosphate [28], that impairs functional activation of drugs, such as remdesivir, which depend on cellular energy for functional activation. The conversion of remdesivir to the triphosphate form renders it as a substrate for viral replicase-transcriptase, leading to its integration into the propagating viral RNA chain. The integration prevents complete viral replication [3]. Hence, deficiency in cellular energy-rich metabolites may explain why some nucleoside-based ATP-dependent medications that target the viral replicase-transcriptase system do not succeed. Table 4 lists the differentially regulated metabolites from various studies described below.

Methodology to study metabolomic profile

The metabolomic analysis of COVID-19 patients have been described in detail [25]. A cohort of 28 severe COVID-19 patients were studied, along with control groups consisting of 28 healthy individuals, 25 patients with non-severe COVID-19 and 25 patients who are negative for SARS-CoV-2 nucleic acid test but have similar clinical characteristics as COVID-19 patients. Serum samples were obtained from the individuals for proteomic and metabolomic analysis. The study resulted in the identification and quantification of 894 proteins, 941 metabolites, 36 drugs and their metabolites [25].

The metabolomic study encompassed various endogenous biochemical categories by using positive and negative ionization to study both hydrophilic and hydrophobic molecules. A random forest machine learning model was built using omics data resulting in the prioritization of 29 important variables including seven metabolites. It was found that 373 metabolites in COVID-19 patients were significantly altered, and variations in 204 metabolites correlated with severity of COVID-19 as measured by mFuzz, a software for soft clustering of bioinformatics data where each data point may belong to multiple clusters. Proteomics analysis showed a significant change in 80 metabolites that are involved in three biological processes.

Metabolomic analyses show abnormal levels of several metabolites, lipids & amino acids in COVID-19 patients

COVID-19 patients show a dysregulation in metabolites related to lipid metabolism, where increased levels of 11 steroid hormones are predicted to result in macrophage modulation [25]. The study showed an enrichment in metabolites of corticosterone synthesis and kynurenine pathway for NAD⁺ synthesis, which indicate the roles of these two pathways in COVID-19 response [31]. On the other hand, a decrease was observed in: sphingolipids, which regulate macrophage activation and migration to inflammation sites; apoptosis; glycerophospholipids, which regulate phagocytosis and platelet degranulation; and choline and its derivatives, which regulate cytokine secretion [25]. Metabolites required for amino acid metabolism and amino acid derivatives were also reduced which indicate toward a dysfunction of the hepatic system, because previous studies have also shown that viral infection caused interferons to interfere with the urea cycle [32] and arginine metabolism is reduced upon viral infection [33].

Overall, the metabolomics profile of the study showed over 100 lipids reduced in the sera of COVID-19 patients. Disrupted lipidome can have direct consequences on the coronavirus infection cycle because of the following reasons: early enveloped-virus development is partially regulated by glycerophospholipid, sphingolipids and fatty acids [34]; blocking cholesterol synthesis by methyl- β -cyclodextrin (MbCD) inhibits SARS-CoV release in infected Vero E6 cells [35]; and lipid synthesis inhibition by drugs like statin can be administered against hepatitis C virus [36] and COVID-19 [37]. Hence, the variations in metabolome and lipidome reported here can be used for therapeutic developments against COVID-19.

Metabolomics analysis of COVID-19 patients using ultra-high-pressure LC-MS & mapping to Kyoto Encyclopedia of Genes & Genomes or KEGG pathway

In another study on 33 COVID-19 patients, sera of the patients were subjected to targeted metabolomics analyses by ultra-high-pressure LC-MS to reveal differences in metabolic phenotypes [29]. Hierarchical clustering analysis show links between COVID-19, IL-6 levels, and amino acid metabolism, purines, acylcarnitines and fatty acids. Analysis of volcano plots for negative and positive ion modes revealed 3034 and 2484 differential metabolites in COVID-19-positive versus controls.

Metabolism pathways for five amino acids were found to be significantly affected by COVID-19 using metabolite set enrichment analysis of merged targeted and untargeted metabolomics data. The highest hits were mapped to the KEGG pathway map hsa01100, where hsa denotes *Homo sapiens*. The study further presents targeted absolute quantitative measurements analyzed by stable isotope-labeled internal standards to identify subsets of metabolites belonging to the pathways that underwent maximum perturbation.

Metabolomic analysis show dysregulation of tryptophan metabolism, among other amino acids, metabolites & pathways

In the above study, receiver operating characteristic curves depicting absolute quantitation of tryptophan and kynurenine show that tryptophan is reduced, which is inversely proportional to IL-6 concentration, and kynurenine is elevated during COVID-19 [29]. By-products of collagen metabolism and proteolysis were also observed. From a renal perspective, COVID-19 patients show a decrease in ornithine and citrulline which are urea cycle metabolic intermediates. In contrast, COVID-19 patients with moderate-to-high levels of IL-6 show an elevation in creatine, creatinine, polyamines spermidine and acetyl-spermidine. Collectively, the observations indicate renal dysfunction which was supported by clinical quantitation of creatinine and blood urea nitrogen. The study further showed that in COVID-19 patients, there is hyperglycemia, elevation in glycolysis metabolic intermediates and pentose phosphate pathways. The observations indicate toward potential hemolysis or rupturing of red blood cells. Changes in levels of lactate and α -ketoglutarate were also observed. Overall, the observations indicated toward variations in transamination homeostasis, which corresponds to perturbed nitrogen balance. The metabolomic analysis corresponds to markers of inflammation and renal function that are clinically assessed in the laboratory.

Metabolomics analysis of COVID-19 patients using a LC-ESI-MS/MS system

Analysis of hydrophilic and hydrophobic metabolites from blood plasma of 34 COVID-19 patients led to the identification and quantification of 431 metabolites and 698 lipids, revealing significant changes in the plasma metabolome and lipidome [30]. The analysis was done using a LC-ESI-MS/MS system, followed by developing a database that helps with identification of metabolites, their retention time and ion pairs. Metabolomic profiles of

COVID-19 patients versus controls were distinguished using orthogonal partial least-squares discriminant analysis. Functional relevance of the differentially abundant metabolites was annotated using KEGG.

Metabolomic analyses of COVID-19 patients reveal perturbations in pathways linked to metabolism of sugars, thyroid hormone & lipids

The study further revealed an enrichment of metabolites involved in pathways associated with thyroid hormone synthesis and signaling, as well as metabolism of purine, autoimmune thyroid, pyrimidine, carbon, fructose and mannose. The observation indicates that perturbations in the above pathways are connected to advancement and detrimental effects of COVID-19. Differential presence of lipids were observed that are linked to retrograde endocannabinoid signaling, pathogenic *Escherichia coli* infection, Kaposi sarcoma-associated herpesvirus infection, glycosyl phosphatidyl inositol-anchor biosynthesis, glycerophospholipid metabolism and autophagy. Area under the curve values for some of the metabolites including malic acid, D-xylulose 5-phosphate and glycerol 3-phosphate belonged to the ideal range for diagnosis of disease in patients, indicative of their potential as COVID-19 biomarkers [30].

Discussion & conclusion

The studies discussed above establish that therapies and diagnosis based on antibodies, proteomics and metabolomics have the potential for therapeutic developments against COVID-19. Although promising, the cost, time and effort in producing efficient antibody-based therapies continue to be a challenge [38]. Despite the challenges, the results are convincing enough to encourage the pursuit of this line of therapy because it has culminated into numerous clinical trials at various phases across the world. In this direction, administrative support at the local, national and international level is crucial to ensure that as soon as an antibody-based therapy is launched, it can be made accessible to all populations across various geographical and economic strata.

Similar approaches need to be implemented for proteomics- and metabolomics-based assays and diagnostic tests. The altered metabolomic and lipidomic profiles can be used to identify diagnostic metabolic markers of SARS-CoV-2 infection. COVID-19 patients show perturbations in energy metabolism processes with abnormalities in ATP and *de novo* NAD production, endogenous precursors to NAD, mitochondrial function, purine and pyrimidine nucleobases and nucleosides. Patients also exhibit accumulated levels of unprocessed tricarboxylic acid cycle metabolites and carnitine esters. These markers of drastic deterioration of metabolism, mitochondrial function and bioenergetic crisis were observed during a long period before death, hence they could predict mortality in COVID-19 patients [26,27,39].

Due to the additional complications associated with a bioenergetic crisis in cellular metabolism that affects patient mortality, antiviral drugs may behave unpredictably in severe metabolically dysfunctional patients [24]. During those situations, targeted metabolic approaches can attenuate the perturbations in energy metabolism by replenishing NAD and ATP to improve immune and repair mechanisms for preventing multiorgan damage [24]. Nutritional supplements can be administered to attenuate the lipidome and metabolome perturbations as well. Attenuation of the bioenergetic crisis is also essential for optimization of pharmaceutical interventions. One of the challenges for proteomics- and metabolomics-based assays can be shortage of starting sample quantity. This issue can be addressed by recent advances in methodology where proteomics and metabolomics analyses can be performed on the same sample [40].

Overall, metabolomic phenotyping is important for developing personalized medicine for COVID-19 patients [24]. Metabolic phenotyping will improve the effectiveness of ATP-dependent replicase-transcriptase inhibitors which are undergoing under clinical testing against COVID-19. In this direction, therapeutics which heavily rely on ATP for activation may not benefit patients with severe metabolic dysfunction [24]. Metabolomic phenotyping will enable the co-administration of required metabolic and nutritional approaches in the overall treatment plan. Hence, outcomes of COVID-19 patients can be improved by addressing the perturbations in their metabolome through a risk-stratified and personalized method. Proteomics and metabolomics profiles are useful in clinical and epidemiological studies on pandemics where risk factors from various underlying conditions are addressed, like the implications of renal cancer on COVID-19 [41].

Harnessing high-throughput datasets to obtain metabolomic, transcriptomic and proteomic signatures for incorporation into adaptive trial designs will have a great potential in developing therapeutics against COVID-19. This is because collective observations indicate that vascular dysfunction could influence the pathogenesis of ARDS induced by COVID-19, implying that COVID-ARDS is likely a distinct vascular endotype or distinctly defined

subtype, of ARDS [42,43]. Risk factors for severe sickness and demise have been identified in COVID-19 patients in Europe and Asia, among which some risk factors are overrepresented in COVID-ARDS patients, that indicate toward a distinct ARDS endotype. While diabetes is a COVID-ARDS risk factor, it is a negative predictor of Lung Injury Prediction Score during non-COVID ARDS [43,44]. The importance of underlying vascular dysfunction in critically ill patients of COVID-19 ARDS is indicated by the prevalence of cardiovascular diseases including hyperlipidemia, hypertension, etc., in these individuals [43]. However, it should also be noted that further research is necessary to establish COVID-19-induced ARDS as an endotype of ARDS [45].

The integration of proteomics-, metabolomics- and antibody-based approaches are needed for optimal drug development because several clinical trials are ongoing successfully but the final success against COVID-19 is still awaited. In this direction, repurposing of antiviral drugs which inhibit RNA polymerase have shown promise in treating COVID-19 patients [46]. Combination therapy has proven to be promising and needs to be further developed. For example, the combination of ribavirin, a nucleoside analog, with two non-nucleosidic antivirals used against HIV has shown promise in treating COVID-19 patients with mild-to-moderate sickness [47]. The nucleoside-based antiviral favipiravir [48] has also shown therapeutic potential against COVID-19. Remdesivir (GS-441524) has established itself as the drug with the most potential so far, with the FDA granting its emergency use access for COVID-19 treatment [49]. With the expansion of anti-COVID-19 drugs that will be administered by oral ingestion or transdermal methods, new drug delivery methodology will need to be optimized, including various considerations like heat exposure [50]. Additionally, cell-based therapies offer another method of combating COVID-19 [51].

It will be a collective responsibility for pharmaceutical industries, health insurance providers and all the governments to ensure efficient distribution of medication to combat the COVID-19 pandemic. Overall, diagnosis and therapies using antibodies, proteomics and metabolomics are significant steps toward future drug discovery to combat infectious diseases.

Future perspective

Three important topics in the context of therapeutic developments against COVID-19 are discussed here – antibodies, proteomics profiles and metabolomics profiles of COVID-19 patients. While antibodies extracted from patients who recovered from COVID-19 show strong and specific binding to SARS-CoV-2 S and other proteins to prevent viral infection, the proteomics and metabolomics profiles reveal new targets that can be pharmacologically targeted to inhibit viral infection. These three areas need to be further developed because currently there is still no cure for COVID-19, although recent developments in mRNA-based vaccines show >90% efficacy against COVID-19. To develop effective therapies, further research is needed to extract COVID-19 antibodies and manufacture them quicker and with less expense on a large scale. The antibodies need to overcome the disadvantages of the current ones. Regarding proteomics and metabolomics profiles, data from more patients with varying disease severity and underlying conditions need to be obtained. Advanced methodologies need to be developed to interpret and analyze the data in a quick and accurate manner. In addition, the methodologies need to be applied to treat other infectious diseases as well so that there is no threat from other pandemics in the future. Further, the methodologies need to be adapted to prepare for mutant strains of SARS-CoV-2. Finally, the methodologies need to be assessed for administration as combination therapies against COVID-19.

Executive summary

- The COVID-19 pandemic has killed more than 247,220 people and infected more than 11 million people in the USA alone, causing more than 1,333,230 deaths worldwide.
- Some vaccines developed by different organizations are proposed to be highly effective, but a cure is still being investigated.
- In this direction, generation of panels of recombinant antibodies from recovered patients of COVID-19 show excellent neutralizing and strong binding activity to SARS-CoV-2 spike and other proteins, thus preventing viral replication.
- Several proteins and metabolite levels become abnormally high or low after SARS-CoV-2 infection. While some of them can be therapeutically targeted to resume normal levels to mitigate the effects of COVID-19, abnormal levels of others can designate their use as biomarkers against COVID-19.
- Development of superior methodology and bioinformatics analysis procedures have helped to harness data from the above processes to understand the pathophysiology of COVID-19 for therapeutic developments.

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The author is employed by Active Motif, Inc., in USA. The citation Wan *et al.* Cell Reports 2020 which have been majorly discussed in section 1, have two authors out of 26 who are affiliated to Active Motif, China. However, this manuscript has not presented the work of Wan *et al.* for promoting Active Motif's portfolio as a company. This manuscript discussed the study of Wan *et al.* only as a step toward developing therapeutics against COVID-19 to present new scientific methodology, and acknowledges that the work of Wan *et al.* is one among various other methods that have been successful in contributing to therapeutic developments against COVID-19. The manuscript is not intending to bias the readers by projecting the methods and results of Wan *et al.* as the only antibody-based approach against COVID-19, which is also clarified at the end of section 1. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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