Metabolomics to Predict Antiviral Drug Efficacy in COVID-19

Infection with the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can lead to severe pneumonia, lung function impairment, and multiple organ failure that can be fatal (1). There are currently no U.S. Food and Drug Administration–approved therapies across the spectrum for patients affected with coronavirus disease (COVID-19). However, several experimental approaches, including repurposing of the RNA polymerase–inhibiting antiviral agents, have improved the health outcomes among patients with COVID-19 (2). In Southeast Asia, a combination therapy of ribavirin, a nucleoside analog, together with two nonnucleosidic antivirals used to treat the human immunodeficiency virus (HIV) has shown some promise in mild-to-moderately ill patients (3), as did a study employing another nucleoside-based antiviral agent, favipiravir (4). In the United States, the most promising drug therapy thus far has been remdesivir (GS-441524). A multisite trial indicated that treatment with remdesivir was associated with speedy recovery among hospitalized patients infected with SARS-CoV-2, which prompted the U.S. Food and Drug Administration to allow emergency use of access of the drug for COVID-19 treatment on May 1, 2020 (5). Despite these promising recent developments, strategies that could help clinicians predict which patients are most likely to respond effectively to a given therapeutic regimen remain perfunctory. Patient prioritization and treatment matching should be paramount in ensuring optimization of therapeutics to thwarting this pandemic.

Along these lines, we reported that patients who die from sepsis syndrome and acute respiratory failure initially present in the emergency department and the medical intensive care unit with a conspicuous metabolomic profile (6–9). Among the most striking changes were the increases in metabolites related to the de novo production of nicotinamide adenine dinucleotide (NAD; a key cofactor central to metabolism), mitochondrial function, and production of ATP as summarized in Table 1. In these patients, the normal endogenous precursors to NAD, as well as purine and pyrimidine nucleobases and nucleosides, were rerouted from their normal biosynthetic pathways. Furthermore, patients with poor outcomes presented with metabolic dysfunction that appears to be irreversible as evidenced by the accumulation of unprocessed tricarboxylic acid cycle metabolites and carnitine esters. Together, these markers not only predict mortality but also suggest that nonsurvivors have an acute bioenergetic crisis likely attributable to severe decrements in mitochondrial function and metabolism that we have observed several days prior to death (6–9).

Recent metabolomics and proteomics studies on patients with COVID-19 with associated severe respiratory distress demonstrated plasma metabolomic signatures similar to those described above for sepsis syndrome (10, 11). The results implicated dysregulation of macrophage function, platelet degranulation and complement system pathways, and metabolic suppression, similar to the acute bioenergetic crisis profile we previously observed in patients with sepsis with poor outcomes (6, 8).

Here, we posit that success in reducing the viral burden in patients with SARS-CoV-2 using antiviral drugs that first require intracellular ATP-dependent activation will be contingent on the overall bioenergetic phenotype of the patient. All the nucleoside-based drugs currently considered for SARS-CoV-2 treatment (e.g., remdesivir, ribavirin, and favipiravir) require functional activation by host enzymes that employ endogenous ATP for their conversion to the active triphosphate species. For instance, remdesivir must be converted to its triphosphate form to become a substrate for the viral replicase-transcriptase and get integrated into the growing viral RNA chain to prevent the full replication of the virus (12). Ribavirin also needs ATP for activation, whereas favipiravir, a nucleobase analog, requires initial conversion to its nucleotide form via a mechanism that requires phosphoribosyltransferases.

Table 1. Changes in the Abundance of Selected Metabolites in Patients with Sepsis with Poor Outcomes, Altogether Markers of Metabolic Imbalance

| Pathway        | Metabolite                  | Change in Nonsurvivors |
|----------------|-----------------------------|------------------------|
| NAD metabolism| N-acetyl-tryptophan          | ↑↑↑                    |
|                | Tryptophan                  | ↑↑                     |
|                | Kynurenine                  | ↑↑                     |
|                | 3-Hydroxy-kynurenine        | ↑↑                      |
|                | Kynuremate                  | ↑↑                     |
|                | Picolinate                  | ↑                      |
|                | 2-Hydroxyadipate            | ↑                      |
|                | Quinate                     | ↑                      |
|                | Quinolinate                 | ↑                      |
|                | 1-Methyl-nicotinamide       | ↑                      |
| TCA β-oxidation| Succinate                   | ↑                      |
|                | Succinylcarnitine           | ↑                      |
|                | Acetyl carnitine            | ↑                      |
|                | Glutaryl carnitine          | ↑                      |
|                | 2-Methylbutyryl carnitine   | ↑                      |
| Nucleobases    | 1,3-Dimethylurate           | ↑                      |
|                | 1-Methylxanthine            | ↑                      |
|                | Adenine                     | ↑                      |
|                | Cytidine                    | ↑                      |
|                | Thymine                     | ↑                      |
|                | Uracil                      | ↑                      |
| Nucleosides    | N2,N2-Dimethylguanosine     | ↑↑                     |
|                | N1-Methylguanosine          | ↑                      |
|                | N1-Methyladenosine          | ↑                      |
|                | N2-Methylguanosine          | ↑                      |
|                | N6-Succinyladenosine        | ↑                      |
|                | Uridine                     | ↓                      |
| Lipids         | 1-Arachidonoyl-GPC          | ↓↓                     |
|                | 1-Palmitoyl-GPC             | ↓↓                     |

Definition of abbreviations: GPC = glycerophosphocholine; NAD = nicotinamide adenine dinucleotide; TCA = tricarboxylic acid.
One could also consider the severity of the bioenergetic crisis in the context of cellular metabolism and its relationship to patient outcomes and survival. For example, antiviral agents may be infective in patients presenting with an advanced metabolic dysfunction. This may explain why remdesivir can improve duration of symptoms but has no statistical benefit on patient survival (11, 15). In such cases, targeted metabolic strategies or nutritional supplementation that include remediation of the NAD and ATP pools could be implemented to reduce the impact of the acute bioenergetic crisis on dysregulated immune and repair responses that lead to multiorgan failure. Moreover, correction of these nutritional deficiencies may be necessary to optimize drug responses.

In conclusion, metabolomic phenotyping may represent an important step toward personalized therapeutics in patients infected with COVID-19. First, it will help enhance the therapeutic efficacy of ATP-dependent replicase-transcriptase inhibitors currently under clinical investigations against COVID-19. Drugs with significant dependence on ATP to achieve functionality against the viral target might be less effective in patients presenting with advanced metabolic dysfunction. Second, this metabolomic phenotyping will also inform the need to integrate balanced metabolic and nutritional strategies within the treatment regimen to optimize patient recovery. Defining and modulating the bioenergetic state in a risk-stratified and personalized approach could have long-term impact in improving patient outcomes to SARS-CoV-2 infections.

**Figure 1.** Response to viral drug therapies in patients with severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2) may be dependent on the metabolic status of the patient. A patient’s metabolomic phenotype can predict patient outcomes as well as the status of cellular metabolism. In particular, the function of nicotinamide adenine dinucleotide (NAD) is critical for cellular metabolism as well as energy production such as ATP. Because most viral transcriptase inhibitors are dependent on ATP for activation and incorporation with viral RNAs, cellular metabolism and energy production can critically affect the efficacy of certain antivirals. Monitoring the metabolomic phenotype in clinical trials that use antiviral drugs will be critical for optimization of drug efficacy.

Pyrophosphate, another high-energy intracellular biomolecule (13), these activation processes and their dependence on ATP levels may explain the limited success of some of the nucleoside-derived drugs targeted at the viral replicase-transcriptase. An impaired “energy status” of a patient, characterized by the decrement of high-energy metabolites such as ATP and phosphoribosyl pyrophosphate (14), may impede effective drug conversion and thus decrease efficacy against viral replication.

The implications of the present considerations (as outlined in Figure 1) offer opportunities for stratification of patients with COVID-19 based on their metabolic phenotype to maximize drug efficacy. Monitoring patients’ bioenergetics status might help rationalize why a given replicase-transcriptase inhibitor is successful in some patients and not in others. With this perspective, drugs with significant dependence on ATP will be less effective in patients presenting with advanced metabolic dysfunction. Therefore, we propose that ribavirin or favipiravir, drugs that require multiple-stage functionalization, would have a better chance of success in patients presenting with a near-normal metabolic profile. However, patients that present with a metabolomic phenotype of an acute bioenergetic crisis could be treated with drugs that require less energy, such as remdesivir, as it requires low ATP commitment for drug activation.

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Marie Migaud, Ph.D.
University of South Alabama
Mobile, Alabama

Sheetal Gandotra, M.D.
University of Alabama Birmingham
Birmingham, Alabama

Hitendra S. Chand, Ph.D.
Florida International University
Miami, Florida

Mark N. Gillespie, Ph.D.
University of South Alabama
Mobile, Alabama

Victor J. Thannickal, M.D.*
University of Alabama Birmingham
Birmingham, Alabama

Raymond J. Langley, Ph.D.‡
University of South Alabama
Mobile, Alabama

ORCID IDs: 0000-0002-9626-2405 (M.M.); 0000-0002-0574-9307 (H.S.C.); 0000-0001-8849-9325 (R.J.L.).

*V.J.T. is Associate Editor of AJRCMB. His participation complies with American Thoracic Society requirements for recusal from review and decisions for authored works.

‡Corresponding author (e-mail: rlangley@southalabama.edu).
To the Editor

Protein C

Cholesterol Metabolism in the Absence of Surfactant

Spontaneous Fibrotic Remodeling and Impaired

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Comments on “Air Space Distension Precedes Spontaneous Fibrotic Remodeling and Impaired Cholesterol Metabolism in the Absence of Surfactant Protein C”

To the Editor

We read with great interest the recent original research article by Ruwisch and colleagues (1) and the accompanying editorial (2) published in the Journal. We would like to raise a few points about the interpretation the authors and editors provided in relation to the electron-dense crystals found in alveolar macrophages.

We are writing to call for caution when interpreting these “novel findings using EM,” because it is important to take into consideration the large body of scientific literature on the subject. Without devaluing their work, that literature can address the discussion by providing a different view regarding the molecular and cellular aspects involved in lung remodeling.

In particular, the electron-dense crystals in the macrophage cytoplasm reported in the paper were attributed to altered cholesterol metabolism in macrophages and have been linked to a “dramatic reduction in the expression of Abca1 and Abcg1 which leads to cholesterol accumulation in alveolar macrophages.” After citing a series of published papers (their References 40, 56–59 [1]), the authors postulate that the crystallloid inclusions present in their mouse macrophages are similar to cholesterol crystals seen in these papers and are thus due to an accumulation of cholesterol.

Unfortunately, this is not a correct interpretation, as insoluble lipid materials present in alveolar macrophages of patients with alveolar proteinosis (3), crystals formed in peritoneal macrophages incubated with oxidized LDL (low-density lipoprotein) (4), cholesterol monohydrate crystals in macrophage-derived foam cells (5), and lamellar bodies found in macrophages of Abcg1lox/lox (flexed ATP-binding cassette subfamily G member 1) and Abcg1lox/ko (ATP-binding cassette subfamily G member 1 transgenic knockout) mice (6) are completely different from and not consistent with—by ultrastructural appearance and characteristics—those observed by Ruwisch and coworkers. In addition, as reported by Glasser and coworkers in sp-c-SFTPC gene–targeted mice characterized by an extensive loss of alveoli (7), macrophages containing crystallloid inclusions may be seen in association with another distinct population of macrophages containing abundant surfactant components, including lamellar bodies and tubular myelin, which are extracellular forms of pulmonary surfactant. The latter population of macrophages can also be found in several strains of mice exposed to cigarette smoke at various time intervals from the start of the treatment, together with a distinct population of macrophages containing crystallloid in variable quantities. Thus, in our opinion, the conclusion that crystallloid inclusions are due to cholesterol accumulation represents speculation that is not supported by the literature (3–7).

The nature of intracytoplasmic crystallloid inclusions observed in macrophages from connective tissue undergoing rapid remodeling (8, 9) and the type described by Ruwisch and coworkers has been a subject of debate (10). Using EM and immunogold labeling, we identified these inclusions as collagen-derived products, and they have been related to the intracellular route of collagen degradation that, in the lung, takes place during septal destruction (11). In other organs or tissues undergoing rapid collagen turnover (e.g., uterus during postpartum involution, periodontal gingiva, periodontal ligament, and periosteum), the intracellular pathway of collagen degradation takes place under physiological conditions (12). In the lung, collagen are subject to continuous remodeling and turnover (13); however, the intracellular pathway of collagen degradation is unusual under steady-state conditions. Using an immunogold technique, the crystallloid inclusions identified in alveolar macrophages as collagen-derived products and related to collagen phagocytosis...