Metabolomics to Predict Antiviral Drug Efficacy in COVID-19

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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 FDA-approved therapies across the spectrum for patients affected with the coronavirus-induced disease of 2019 or COVID-19. However, several experimental approaches including repurposing of the RNA polymerase inhibiting antiviral agents have improved the health outcomes among COVID-19 patients (2). In Southeast Asia, a combination therapy of ribavirin, a nucleoside analog, along with two non-nucleosidic antivirals used to treat 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 US, the most promising drug therapy thus far has been remdesivir (GS-441524). A multi-site trial indicated that the treatment with remdesivir was associated with speedy recovery among hospitalized patients infected with SARS-CoV-2, which prompted the FDA to allow emergency use access of the drug for COVID-19 treatment on May 1st, 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 metabolomic dysfunction that appears to be irreversible as evidenced by the accumulation of unprocessed tricarboxylic

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acid (TCA) cycle metabolites and carnitine esters. Together, these markers not only predict mortality, but they also suggest that non-survivors 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 COVID-19 patients with associated severe respiratory distress demonstrated plasma metabolomic signatures similar to those described above for the 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 sepsis patients with poor outcomes (6, 8).

Here, we posit that success in in reducing the viral burden in SARS-CoV-2 patients 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, while favipiravir, a nucleobase analog, requires initial conversion to its nucleotide form via a mechanism that requires phosphoribosyl pyrophosphate (PRPP), 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 like ATP and PRPP (14), may impede effective drug conversion and thus decrease efficacy against viral replication.

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The implications of the present considerations (as outlined in Figure 1) offer opportunities for COVID-19 patient stratification based on their metabolic phenotype to maximize drug efficacy. Monitoring patients' bioenergetics status might help rationalize why a given replicase/trancriptase inhibitor (rTI) 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.

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 which 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 multi-organ failure. Moreover, correction of these nutritional deficiencies may be necessary to optimize drug responses.

In conclusion, metabolomic phenotyping may represent an important step towards personalized therapeutics in patients infected with COVID-19. First, by enhancing the therapeutic efficacy of ATP-dependent rTIs 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. Secondly, this metabolomic phenotyping will also inform the need to integrate balanced metabolic and nutritional strategies

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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.

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Figure 1: Response to viral drug therapies in SARS-CoV2 patients may be dependent upon 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 NAD is critical for cellular metabolism as well as energy production such as ATP. Since most viral transcriptase inhibitors are dependent upon 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.

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Table 1: Changes in the abundance of selected metabolites in sepsis patients with poor outcomes, together markers of metabolic imbalance.

Pathway	Metabolite	Change in nonsurvivors
NAD metabolism	n-acetyl-tryptophan	↑↑↑
	tryptophan	↓
	kynurenine	↑↑
	3-hydroxy-kynurenine	↑↑
	kynurenate	↑↑
	picolinate	↑↑
	2-hydroxyadipate	↑↑
	quinate	↑
	quinolinate	↑↑
	1-methyl-nicotinamide	↑↑
TCA β-oxidation	succinate	↑
	succinylcarnitine	↑↑
	acetylcarnitine	↑↑
	glutarylcarnitine	↑↑
	2-methylbutyrylcarnitine	↑↑
nucleobases	1,3-dimethylurate	↑↑
	1-methylxanthine	↑↑
	adenine	↑
	cytidine	↑
	thymine	↑↑
	uracil	↑↑
nucleosides	N2, N2 - dimethylguanosine	↑↑
	N1-methylguanosine	↑↑
	N1-methyladenosine	↑↑
	N2-methylguanosine	↑↑
	N6-succinyladenosine	↑↑
	uridine	↓
	5-methyluridine	↓
Lipids	1-arachidonoyl-GPC	↓↓
	1-palmitoyl-GPC	↓↓

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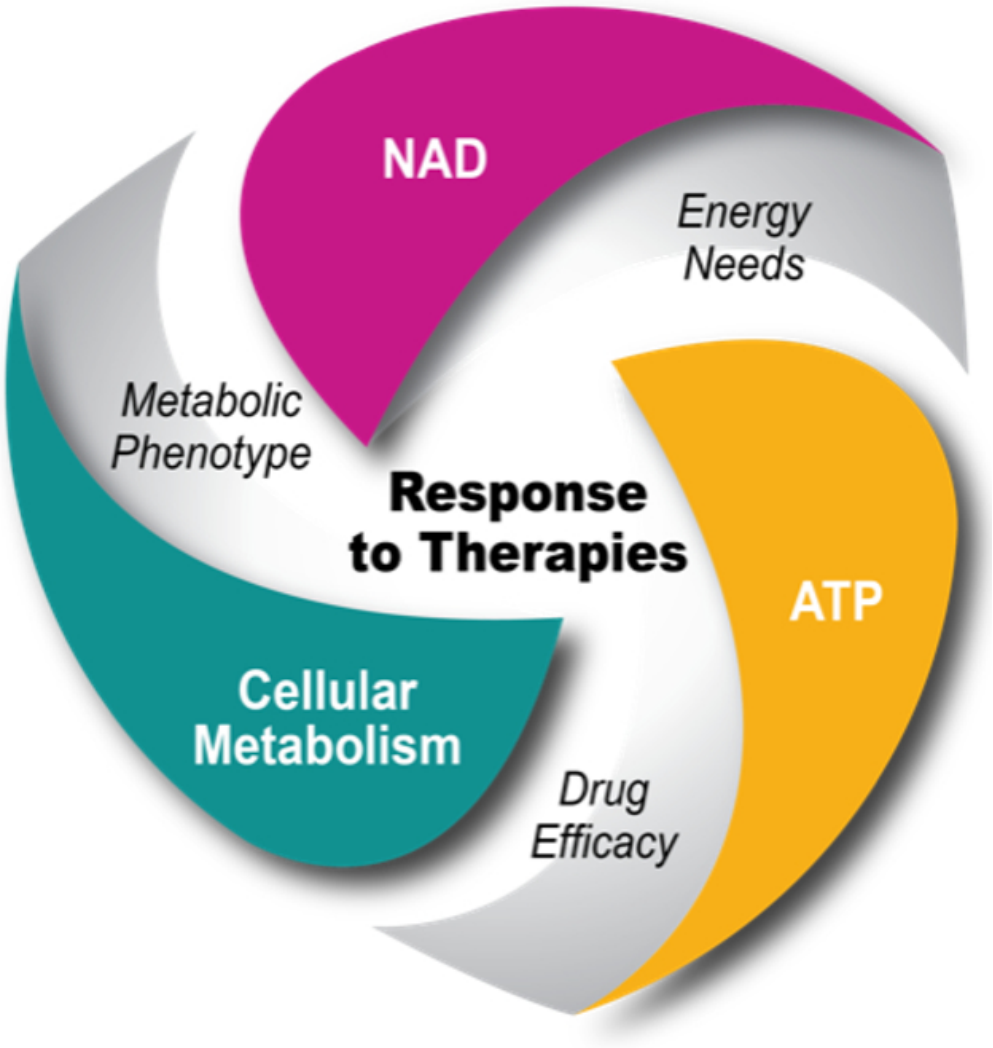

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