



Early View

Editorial

Does acute and persistent metabolic dysregulation in COVID19 point to novel biomarkers and future therapeutic strategies?

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Does acute and persistent metabolic dysregulation in COVID19 point to novel biomarkers and future therapeutic strategies?

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Take home message:

Metabolomics changes in COVID19 predict acute patient outcomes and suggest a pivotal role for a bioenergetic crisis driven by viral hijacking of the mitochondria to escape the innate immune response. Thus, metabolomics changes in COVID 19 may serve not only as a biomarker, but provide insight into pathogenic mechanisms and pharmacologic targets.

When the COVID19 pandemic first appeared in December of 2019, the pathophysiologic underpinnings of the disease were largely unknown. Scientists, physicians and government institutions from around the globe took an “all-hands on deck” approach with the hope of identifying potential therapies to treat as well as understand the pathophysiology of the disease [1]. Currently, more than 4800 clinical trials listed on clinicaltrials.gov have been performed or proposed around the world, many with subjects from vastly different ethnic and racial backgrounds, as well as different standard-of-care strategies [2]. Despite this effort, apart from monoclonal antibodies, few therapies have emerged as effective treatments of COVID-19; vaccines remain the best approach to control and mitigate the pandemic [3].

Despite the lack of therapeutic successes, we have gained unprecedented insight into the progression of the disease [4]. Among the vast amount of clinical and biological data published over the course of the pandemic, some of the most consistent and exciting results have come from metabolomics profiling of patient serum samples [5]. Metabolomics, a rapidly developing field of research in which metabolites present in tissue or fluids are comprehensively analyzed, has deepened our understanding of the pathobiology of multiple disorders, identified predictive biomarkers, and highlighted potential novel therapeutic strategies. Throughout 2020 and 2021, several metabolomics papers focusing on COVID-19 have been published. The earliest reports identified changes in the metabolic pathways for processing lipids, amino acids, and carbohydrates in severely ill COVID-19 patients. More recent studies have begun to associate metabolomics changes with symptoms representing specific organ system failures – for example, the acute delirium and post-recovery mental health issues of the nervous system and disruptions of the digestive system [6, 7]. Dysregulation of the kynurenine pathway has been among the most consistent findings as reported in

numerous, independent studies [5]. Of potential significance is that many of the metabolomics abnormalities in COVID-19 are similar to those found in sepsis and acute respiratory failure, suggesting a common mechanism leading to an acute bioenergetic crisis [8, 9].

Along these lines, the paper *Metabolomic analyses reveals new stage-specific features of COVID-19* by Jia et al. reports an in-depth, well-designed metabolomics analysis of confirmed COVID-19 patients [10]. The discovery patients (n=63) were subdivided into mild, severe, and recovery groups and independently validated in a second cohort with an additional 90 patients along with 41 non-infected controls. The investigators utilized both broad-spectrum, semi-quantitative mass spectrometry analysis as well as targeted mass spectrometry analysis. The authors found consistent disruptions in glucose metabolism and dysregulation in the TCA and urea cycles which were identified as potential targets for therapeutic intervention (Figure 1a). These metabolomics changes are consistent with metabolomics studies in other contexts and point to an underlying bioenergetic crisis as a key pathogenic feature of COVID-19 [5].

Furthermore, the authors were able to correlate changes in IL-1 β , TNF- α and IL-6 with metabolomics profiles. Not surprisingly, all three cytokines increased with the severity of disease. Although the levels fell during recovery, they were persistently elevated relative to non-infected control patients. Importantly, cytokine levels positively correlated with TCA cycle-related metabolites, including aspartate, creatinine, malate, and 2-oxoglutarate. There was also positive correlation with arginine, a key component of the uric acid cycle, which can be converted to either ornithine or nitric oxide and citrulline. The authors posited that the decrease in arginine and increase in ornithine suggests that arginine is metabolized via arginase and the urea cycle as opposed to the nitric oxide cycle. Nonetheless, since nitric oxide could reduce viral RNA production by affecting the spike protein and its primary target angiotensin converting enzyme 2 (ACE2), clinical trials of the therapeutic gas in COVID-19 are currently underway [11].

The metabolomic findings by Jia and coworkers, like similar studies in sepsis and acute respiratory failure (ARF) [9, 12-15], point to a pivotal role for mitochondrial dysfunction as a driver of COVID-19 outcomes [16]. In this context, the abundance of mitochondrial DNA damage-associated molecular patterns (mtDNA DAMPs) – a category of DAMP known to promote cytokine production by both immune and non-immune cells [17, 18] – in the plasma of COVID-19 patients is an early predictor of ICU admission, need for intubation, and mortality in COVID-19 [19]. Experimental studies have identified multiple pathways by which SARS-CoV-2 evoke mitochondrial dysfunction leading to pathophysiologic effects of COVID-19 (Figure 1b). For example, SARS-CoV-2 impairs oxidative metabolism and promotes a transition to a glycolytic phenotype in peripheral blood mononuclear cells from COVID-19 patients [20]. This effect may be mediated by ACE2, widely known as the receptor mediating SARS-CoV-2 entry into the cell, which is believed to directly alter mitochondrial function leading to decreased ATP production and activation of NADPH oxidase (NOX) 4 [21, 22]. Increased ROS generation associated with NOX4 activation could exert multiple deleterious events, including damaging the mitochondrial genome leading to its fracture into proinflammatory mtDNA DAMPs [23, 24] as well as activating PARP1 causing NAD⁺ depletion with attendant reduced interferon production, enhanced viral

replication, and decreased mitophagy [25]. Along with ACE2, proteins encoded by SARS-CoV-2 also may perturb mitochondrial functions. Here, studies on open reading frames ORF-9b, ORF-8a, and ORF-7a of SARS show that these proteins localize to mitochondria. ORF9b can inactivate the retinoic acid-inducible gene I-mitochondrial antiviral signaling protein (RIG1-MAVS)-dependent interferon signaling pathway by disrupting K63-linked polyubiquitination of nuclear factor κ B (NF- κ B) essential modulator (NEMO) [26]. ORF-7a and ORF-8a promote viral replication, while ORF-8a can activate caspase-3 mediated apoptosis [22, 27]. The release of the virus as well as proinflammatory cytokines and mtDNA DAMPS induce a hyper-inflammatory response [22, 24, 27-29]. Determining how these bioenergetic metabolomic changes relate to mitochondrial dysfunction could project to new therapies that aim to return the metabolomic profiles back to homeostasis.

One of the more surprising findings was that the greatest number of metabolomics differences between control subjects and Covid-19 patients were in the recovery group, with 98 of 240 metabolites reported as significantly different. This finding may be of particular significance to so-called “Long COVID”, which bears certain similarities to the long-term reduction in quality-of-life (QOL) noted in survivors of other forms of severe illness [30, 31]. It is estimated that 50% of patients admitted to the ICU requiring mechanical ventilation go on to develop post-intensive care syndrome, and up to 80% of survivors of critical illness are readmitted to a nursing home, rehabilitation center or ICU within two years after their initial illness. Half of survivors suffer long-term cognitive decline. Each additional day in the ICU can lead to an 11% loss in muscle mass even after two year follow-up. With many of these patients never returning to work, the health care system and society will be dealing with these issues many years after the pandemic is abated. Understanding how the metabolomics changes relate to QOL outcomes could potentially identify therapeutic strategies to improve longterm QOL in COVID19 survivors.

Final strengths of this study were that many of the metabolites identified can predict patient outcomes, were validated in the independent cohort, and had strong overlap with other studies. A large meta-analysis with predictive modeling split between discovery and validation cohorts could provide valuable biomarkers, not only for prediction for SARS-CoV-2 infection, but also may determine whether acute and chronic outcomes in sepsis and ARF display pathways in common with COVID-19.

There were still some limitations in this report. As the authors note, their cohorts were not age-matched due to the fact that the severe cases were primarily observed in the elderly. Larger cohorts would provide further confidence in these results.

Considering the consistent metabolic derangements seen in multiple studies, as well as substantial overlap with metabolomics changes in sepsis and ARF, these results suggest that metabolic biomarkers should be regularly monitored to determine time-dependent changes during evolution of COVID-19. Future therapies could potentially consider how targeted nutraceutical interventions may return the metabolomics profiles back to homeostasis [32]. For example, Jia et al. suggest further investigation into whether type I interferon regulates the urea cycle in infected epithelial cells and whether COVID-19 infection switches the metabolic pathway of glucose metabolism to the urea cycle by reducing nitric oxide production, thereby protecting viral replication. Other therapies that target the kynurenine pathway and the NAD⁺/NADH ratio may mitigate

the bioenergetic crisis and NAD-regulated immune responses [8, 25]. Finally, strategies to suppress mitochondrial oxidant stress or repair oxidative mtDNA damage also have the potential to emerge as therapeutic strategies guided by targeted metabolomics monitoring [23, 33, 34].

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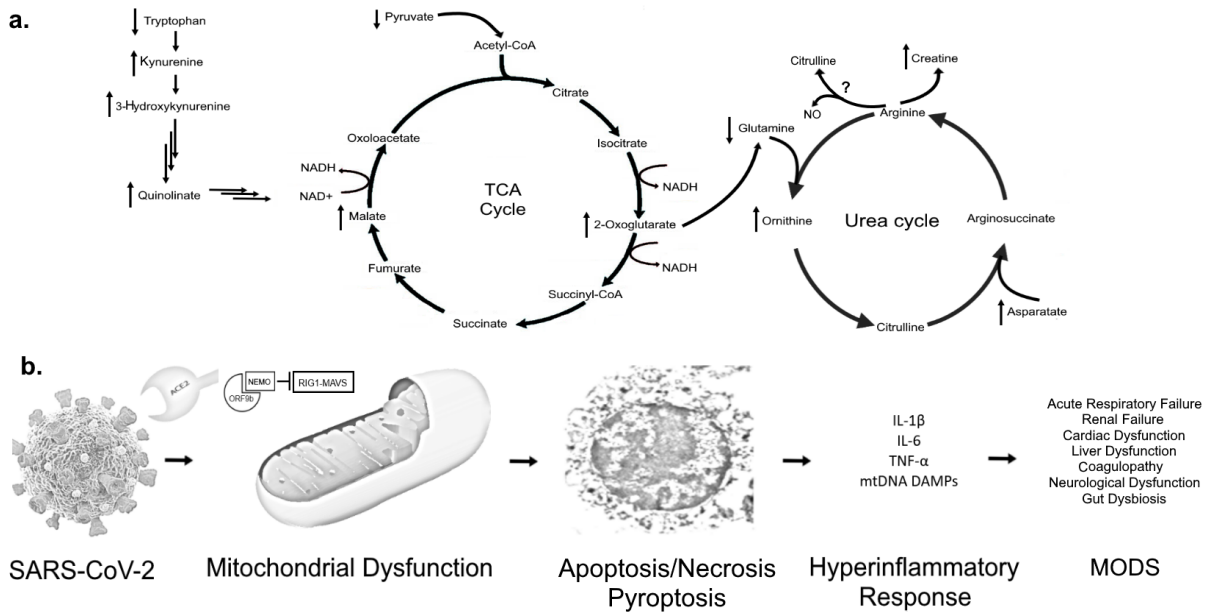


Figure 1: Metabolomics changes due to SARS-CoV-2 are predictors of patient acute and long-term outcomes and reflect a bioenergetic crisis likely due to viral hijacking of the mitochondria. a). Metabolomics changes found due to SARS-CoV-2 infections commonly lead to disruption of the kynurenine pathway, TCA cycle and the urea cycle. It is unknown if arginine is converted to citrulline and NO, however, due to the increase in ornithine and creatine Jia et al., speculate that the urea cycle metabolizes arginine instead of the NO producing pathway. b). SAR-CoV-2 is internalized into the cell via the ACE2 receptor. Many viral proteins including ORF7a, ORF8a and ORF9b locate within the mitochondria. These proteins can inhibit RIG1-MAVS dependent interferon signaling, enhance viral replication and disrupt mitochondrial function. This can ultimately lead to cell death via apoptosis, necrosis or pyroptosis, the release of proinflammatory cytokines as well as mitochondrial DAMPs and ultimately cause multiple organ dysfunction syndrome (MODS). Jia et al, also demonstrate that many of the predictive metabolites altered due to SARS-CoV-2 strongly correlate with proinflammatory cytokines.

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