Multi-Dimensional Outcome Prediction Algorithm for Hospitalized COVID-19 Patients

SPECIFIC AIMS

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-mediated coronavirus disease (COVID-19) is an evolutionarily unprecedented natural experiment that causes major changes to the host immune system. While it is too soon to fully understand the impact of COVID-19 on overall health and well-being, there are already several reports of significant sequelae, including neurologic, cardiac, respiratory and inflammatory, which appear to correlate with disease severity. There is a clear and urgent need to develop prediction tests for adverse short- and long-term outcomes for COVID-19 in order to accurately assess prognosis and to more effectively deliver appropriate care during and after acute infection.

We hypothesize that integrating genomics, transcriptomics and proteomics data into a combinatorial biomarker model along with clinical data will provide accurate predictors of COVID-19 short- and long-term health outcomes. A single parameter provides limited information and is incapable of adequately characterizing the complex biological responses in symptomatic COVID-19 to predict outcome. Since they were designed for other illnesses, it is unlikely that existing clinical tools, such as respiratory, cardiovascular, and other organ function assessment scores, will precisely assess the long-term prognosis of this novel disease. Our extensive experience in biomarker development suggests that integrating molecular and clinical data increases prediction accuracy of long-term outcomes. **We propose that complementary multi-dimensional information gathered near the time of symptom onset can be used to predict new onset or worsening frailty, organ dysfunction and death within one year after COVID-19 onset.**

We will test our hypothesis in a mixed US population that will include hospitalized patients from a civilian population in the country's most populous county (Los Angeles) and a representative National Veteran's population from Los Angeles and three other major metropolitan areas: Atlanta, Georgia, Bronx, New York, and Houston, Texas. This consortium of sites is both demographically and geographically diverse.

The main goal of the proposed work is to develop a prediction test that performs well in this hospitalized patient group that broadly reflecting US demographics. We anticipate this test will: 1) demonstrate precision prediction in the general US population, 2) meaningfully guide triaging and treatment decisions and, therefore, 3) reduce morbidity and mortality rates, enhance patient quality of life, and improve healthcare cost-effectiveness. More accurate prognostic information will also assist clinicians in goals of care discussions in situations of likelihood of futility and assist patients and families in this decision-making process. Finally, it will provide a logical means for allocating resources in short supply, such as ventilators or therapeutics with limited availability. A secondary goal of this research is to identify and better understand expression of genes and their biological role in patients with COVID-19.

SPECIFIC AIM 1: CORRELATE MULTI-OMIC DATA WITH OUTCOMES

We hypothesize that various clinical COVID-19 disease outcome phenotypes correlate with molecular data and clinical patterns and are associated with immunologic pathways.

Aim 1.1: Single-omic identification of candidate genomic, transcriptomic, proteomic and clinical parameters that correlate with outcomes. Our objective is to identify circulating blood-based single-omic parameters (genomic, transcriptomic, proteomic) and clinical data that correlate with COVID-19 outcomes.

Aim 1.2: Multi-omics correlation analysis. Our objective is to establish a correlation pattern between the multi-omics data and the outcome data in symptomatic COVID-19 patients.

Aim 1.3: Weighted Gene Co-Expression Network Analysis (WGCNA) and pathway analysis. To better understand co-expression of genes and their biological role in patients with symptomatic COVID-19, we will perform WGCNA and pathway analyses.

SPECIFIC AIM 2: TRAIN AND VALIDATE ALGORITHM FOR OUTCOME PREDICTION

We hypothesize that our algorithm will predict COVID-19 short- and long-term outcomes of new onset or worsening frailty, organ dysfunction and death within one year after COVID-19 onset more accurately than clinical parameters alone.

Aim 2.1: Train algorithm for outcome prediction. Our objective is to train our algorithm in a mixed hospitalized COVID-19 population.

Aim 2.2: Independently validate predictive algorithm for outcome prediction. Our objective is to validate the algorithm in an independent COVID-19 population that was not used for algorithm development.

RESARCH STRATEGY

SIGNIFICANCE

Epidemiology. SARS-CoV-2-mediated coronavirus disease (COVID-19) causes major changes to the host immune system and can lead to injury and dysfunction in virtually every organ(1, 2). Several high risk COVID-19 populations have been identified. A systematic review of over 10,900 COVID-19 cases found that preexisting chronic conditions and co-morbidities(3, 4), such as hypertension (20%), diabetes (10%), cardiovascular disease (8%), and chronic pulmonary disease (3%) were strongly correlated with disease severity and admission into the intensive care unit(5). Older persons, individuals with immunocompromised systems, non-Hispanic blacks, Latinos or Hispanics, and those with obesity and chronic kidney disease are at higher risk for hospitalization and mortality from COVID-19(6, 7). Some reports have found a significantly higher mortality rate in men versus women with COVID-19(8, 9). Recent information suggests that not only elderly persons with comorbidities are vulnerable to negative health outcomes. There have been several reports of younger COVID-19 patients without underlying diseases that have developed stroke, multisystem inflammatory syndrome and other complications(10-14). Among various US-high risk COVID-19 populations, Veterans have experienced a higher percentage of COVID-19-related deaths than the general population. As of October 23, 2020, the crude fatality rate within the Veterans Affairs (VA) Healthcare System was 5.4% versus 2.7% in the overall US population(15, 16). Roughly half of all Veterans are over 65 years old and ~87% are male(17). Compared to the overall US population, Veterans have a greater incidence of chronic diseases and mental illnesses, as well as high rates of homelessness, which contribute to their elevated risk of developing severe COVID-19(18-28). Compared to infection with influenza, Veterans with COVID-19 have increased risk of several complications, including pneumonia, respiratory failure, acute respiratory distress syndrome (ARDS), pneumothorax, cardiogenic shock, myocarditis, deep vein thrombosis, pulmonary embolism, disseminated intravascular coagulation, cerebral ischemia or infarction, intracranial hemorrhage, acute kidney failure, dialysis initiation, acute hepatitis or liver failure, sepsis, bacteremia, and pressure ulcers (29). **The sites of the consortium that will be participating in this project are both demographically and geographically diverse and will provide representation from these various populations discussed above. We anticipate that this will result in a predictive algorithm that is generalizable to the wider US population.**

Long-term outcomes. It is too soon in the experience with this novel disease to have a comprehensive understanding of long-term outcomes in patients recovering from COVID-19. As just described, COVID-19 is associated with high rates of complications that may continue to incur morbidity, disability, and delayed mortality in survivors. Nearly 20% of young adults aged 18-34 years with no chronic medical conditions diagnosed with COVID and not requiring hospitalization, had not returned to their usual state of health 21 days after testing(30). In an observational cohort, 78 of 100 patients who had recovered from COVID-19 had abnormal findings on cardiovascular MRI (median of 71 days after diagnosis) and 36 of those reported dyspnea and unusual fatigue(31). In a small cohort (n=174) of recovered COVID-19 patients, approximately half required medium to long-term specialist follow-up, and significant persistent physical, psychological, and cognitive impairments were identified despite clinical resolution of the infection(32). In the prior severe acute respiratory syndrome (SARS) epidemic of 2003, survivors were noted to have long-term impact on pulmonary function, functional capacity and quality of life(33). Functional disability was thought to be out of proportion to the degree of lung function impairment and may have been related to additional factors such as muscle deconditioning and steroid myopathy(33, 34). This will be an important consideration when looking at long-term outcomes in COVID-19 due to routine use of corticosteroids in severe disease. A significant proportion of SARS survivors at 2 years showed persistent impairment of exercise capacity and health status(35); 30% of healthcare workers (HCW) and 7% of non-HCW had not returned to work. A post-SARS syndrome of chronic fatigue, pain, weakness, depression and sleep disturbance, which overlaps with the clinical and sleep features of fibromyalgia and chronic fatigue syndrome has been reported(36). Over one-third of patients hospitalized with SARS had moderate to severe depression and anxiety one year after physical recovery(37). A constellation of physical, cognitive, and psychological disabilities has been described in those surviving critical illness(38). Patients experiencing post-intensive care syndrome (PICS) often report cognitive and physical dysfunction, which can persist long-term(39)[. PICS can also lead to disability and moderate or severe pain(40). The physical and psychological difficulties experienced by critical care survivors result in frequent readmissions to hospital and subsequent healthcare resource utilization. Taken together, these studies suggest that COVID-19 will have significant long-term impact on many patients (41-43). Therefore, being able to predict these events with precision would be greatly beneficial in their management.

Inflammatory Responses. **COVID-19 and Clinical Organ Dysfunction.** SARS-CoV-2 infection causes major changes to the host immune system. Although inflammation is necessary to fight infection, exaggerated inflammatory responses in symptomatic COVID-19 may lead to unfavorable short- and long-term outcomes, such as new onset or worsening of frailty, organ dysfunction and death. Like other viral respiratory tract infections, SARS-CoV-2 can cause pneumonia and acute respiratory distress syndrome(44, 45), but also injury and *clinical level dysfunction in virtually every organ* system, including the cardiovascular(46-49), neurological(10, 50-52), renal(53, 54), hepatic(55-57), gastro-intestinal(58, 59), clotting(10) and immune systems(58).

Organ dysfunction and Cytokine Storm Syndrome. Organ dysfunction is linked to severity of sepsis(60). A subset of patients with SARS-CoV-2 infection develop profound inflammation and multi-organ dysfunction(61-63). Symptomatic COVID-19 may result in "*cytokine storm*"(64, 65), which is characterized by excessive release of pro-inflammatory cytokines and may accelerate multi-organ failure(65-71). Interleukin (IL) 1β and macrophage colony-stimulating factor (M-CSF) have been identified as candidate target genes for inflammatory storm(72). IL-6, IL-12, and C-reactive protein are important modulators of inflammation and may play key roles in the progression of COVID-19(61, 73). Elevated levels of pro-inflammatory ferritin and IL-6 have been identified as potential markers for fatality in COVID-19 patients(65, 67).

Circulating Cell-Free DNA (cfDNA). Release of mitochondrial DNA via organ-failure-related cell necrosis into the circulation may result in severe immune consequences, such as hyper-inflammation(74, 75). The spleen, liver and kidneys are responsible for eliminating circulating DNA and are often damaged in critical illness conditions, such as severe COVID-19(75-77). Thus, inflammation may be triggered not only by viral infection, but also by elevated cfDNA. During infection with SARS-CoV-2, high levels of cfDNA, composed of nuclear and mitochondrial DNA, can be produced and may induce cytokine storm and vascular abnormalities(70). cfDNA levels are associated with COVID-19 progression and severity including intubation and death (78, 79).

Understanding Cytokine Storm Syndrome through Multi-Omic Systems Biology. Infection with SARS-CoV-2 and other viruses may lead to significant alterations in the host transcriptome and modulated immune response(80, 81). In PBMCs of COVID-19 patients, more than 1,000 genes were reported to be differentially expressed compared to controls. Some of these genes are directly involved in immunity, such as humoral responses, lymphocyte-mediated immunity and complement activation. Thus, studying transcriptomic changes provides a high resolution understanding of SARS-CoV-2-induced immune activity in PMBCs. Up- and downregulated PBMC genes are also involved in other biological processes, such as protein translation(64). *Immunophenotyping* generates systemic and tissue specific immune profiles. Patients with severe COVID-19 often present with profound lymphopenia, develop neutrophilia, and high hemoglobin levels(72, 82, 83). Immune cell ratios and activation, as well as inflammatory mediators, are associated with disease state and prognosis(46, 56, 82, 84-86). Immunophenotyping analysis demonstrated decreased levels of lymphocytes, including NK, T, B, and Treg cells, as well as monocytes(55, 68, 72). A heterogeneous interferon-stimulated gene signature, downregulation of HLA class II, and a novel B cell-derived granulocyte population have also been identified(87). A recent multi-omic analysis by RNA-seq and high-resolution mass spectrometry on 128 blood samples from COVID-19-positive and -negative patients with diverse disease severities mapped 219 molecular features with high significance to COVID-19 status and severity involved in complement activation, dysregulated lipid transport, and neutrophil activation (88). While presenting the most comprehensive evidence of predictive potential of COVID-19-related outcomes based on multi-omic analysis, the authors conclude that limitations include the single-center restricted demographics of the population not representing "replicate factors related to, among others, geography or population socioeconomic status" and the fact that the "study was not powered to demonstrate the association of omic data with survival, which is the most impactful patient-centered outcome measure" (88). *We intend to address both of these limitations in our study.*

Utility of Prediction Tests. There is a high priority to develop prediction tests for adverse short- and long-term outcomes. Adverse outcomes include frailty, single organ dysfunction (heart, lung, liver, gut, coagulation, kidney, immune and brain systems), multi-organ dysfunction, and death. Clinical tools alone, such as respiratory diagnostics, cardiovascular parameters, organ function assessment scores and co-morbidity scores are not informative enough to assess the prognosis of this novel disease (88). One systematic review identified 10 clinical models of predictive outcomes. However, these models were "poorly reported and at high risk of bias, raising concerns that their prediction could be unreliable when applied in daily practice"(89). These models did not utilize molecular data, which we have shown can significantly increase prediction precision(90).

Genomics has been widely employed in prediction algorithms with great success. In 2004, Oncotype DX was launched as the first assay that both quantifies the likelihood of breast cancer recurrence and predicts the magnitude of chemotherapy benefit(91). Low scores allow doctors to recommend patients to forego chemo-

and/or radiation-therapy, and the associated side effects. Dr. Deng co-led the effort to develop the AlloMap™ test to rule-out heart transplant allograft rejection without invasive endomyocardial biopsy(92). Today, the test is part of the transplantation medicine protocol in >90% of US heart transplant centers(93, 94) OncotypeDX™, AlloMap™, and other genomic assays provide evidence that molecular tests add critical information to the shared decision-making process, improve quality of healthcare, and enhance cost-effectiveness(95). We have successfully developed and validated an algorithm that predicts mortality in advanced heart failure patients (see preliminary data section). Combining preoperative transcriptomic (mRNA sequencing) and clinical data into one model increased prediction accuracy compared to using clinical tools alone(90). *Given our experience developing predictive tools for heart failure and transplant rejection, we hypothesize that incorporating molecular data into our proposed test will facilitate COVID-19 outcome prediction* (96).

Conclusion. We postulate that a good prediction tool that is integrated in clinical settings can enhance the decision-making process, decrease morbidity and mortality, and improve healthcare cost-effectiveness. COVID-19-related organ injury can modulate cfDNA patterns, which may regulate changes in PBMC gene expression(22) and release of cytokines(23-26). These molecules are highly dynamic and interactive. A single parameter alone provides limited information and is incapable of adequately characterize biological response in symptomatic COVID-19. We hypothesize that integrating genomics, transcriptomics and proteomics data into a combinatorial biomarker model along with clinical data will provide accurate predictors of COVID-19 short- and long-term health outcomes. We propose that these data, gathered near the time of symptom onset, can be used to predict frailty, organ dysfunction and death within one year of COVID-19 onset. While different acute treatment options (e.g., antiviral therapy, anti-inflammatory therapy, ventilator support, etc.) are initiated based on clinical data, our test would help optimize short- and long-term management plans. Event prediction may also assist in decision-making in goals of care discussions due to likelihood of futility or for allocation of limited resources.

INNOVATION

The goal of this project is to develop a test that accurately predicts short- and long-term (within one-year) outcomes in a hospitalized COVID-19 population using both clinical and molecular data. Our overall proposition is that new onset or worsening frailty, organ dysfunction and death from COVID-19 within one year after diagnosis can be most accurately predicted by an algorithm that incorporates genomic, transcriptomic and proteomic data in addition to clinical data. We hypothesize that the immunological profile of a patient at the time of COVID-19 onset contains information about their ability to recover from SARS-CoV-2 infection. The *potential to recover* from disease is likely related to the balance between innate and adaptive immunity(97) and reflects the effects of the primary immunological stressor (i.e., viral infection), secondary organ dysfunction, co-morbidities, frailty, disabilities and age(98). Characterizing the comprehensive changes to cfDNA, PBMC transcriptome, and cytokines in patients presenting with COVID-19 will provide the framework for establishing a model for outcome prediction.

We have chosen to test our hypothesis in a mixed hospitalized COVID-19 population that is at increased risk of adverse outcomes from COVID-19. To do so, we propose a dual strategy, combing a civilian population in Los Angeles County, one of the country's largest metropolitan areas, and a representative National Veteran's population who are at increased risk of adverse outcomes from COVID-19(19, 99-101). We anticipate that a prediction test that performs well in this mixed cohort will: 1) demonstrate precision prediction in the general population, 2) meaningfully guide triaging and treatment decisions and, therefore, 3) reduce morbidity and mortality rates, enhance patient quality of life, and improve healthcare cost-effectiveness. More accurate prognostic information will also assist clinicians in goals of care discussions in situations of likelihood of futility and assist patients and families in this decision-making process. Finally, it will provide a logical means for allocating resources in short supply, such as ventilators or therapeutics with limited availability.

Patients with multiple diagnoses, chronic conditions, and lower health status require more intensive resource utilization upon hospital admission(102). Cost analyses suggest that a hospitalized COVID-19 patient may incur an average of $73,000. The pandemic is projected to cost $360 billion to $1.4 trillion, depending on the assumed incidence rate(103). Implementation of the proposed testing strategy will likely improve healthcare cost-effectiveness.

RESEARCH PLAN

Study Design. We propose to conduct an observational, non-randomized study to predict new onset or worsening frailty, single organ dysfunction, multi-organ dysfunction and survival within one year following diagnosis in Veterans and civilians with symptomatic COVID-19 **(Figures 1 and 2).**

Multi-Center Organization. We are initiating a Multiple-PI study between UCLA (Contact-PI Dr. Mario Deng) and VA Greater Los Angeles Healthcare System (Co-PI Dr. David Beenhouwer). We will work in collaboration with Harbor-UCLA Medical Center (Co-Investigator Dr. Tim Hatlen) and Olive View-UCLA Medical Center (Co-Investigator Glenn Mathisen) as well as VA Medical Centers in Atlanta (Co-Investigator Dr. Vincent Marconi), Bronx (Co-Investigator Dr. Sheldon Brown) and Houston (Co-Investigator Dr. Maria Rodriguez-Barradas). Each of the four participating VA centers will recruit 75 VA patients over the 42-month recruitment period (n=300 Veteran patients). UCLA Westwood/Santa Monica, Harbor-UCLA Medical Center and Olive View-UCLA Medical Center together will recruit 300 civilian patients over the 42-month recruitment period.

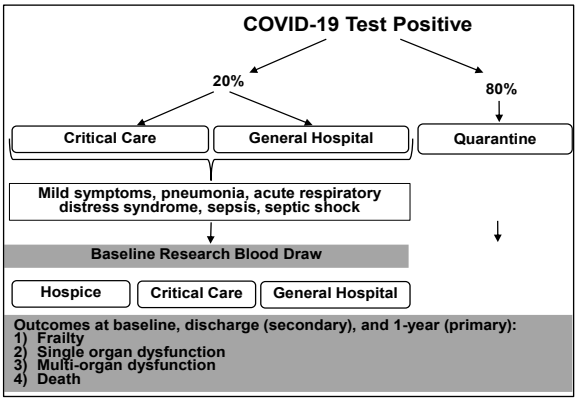

Figure 1. COVID-19 clinical decision making, study population and primary endpoint [modified after(96)].

Patient Population. We plan to complete 1-year follow-up in 500 patients from a large US-metropolitan area (LA-County) (n=250) and four national metropolitan VA centers (n=250). To account for a dropout rate of ~17%, we will recruit a total of 600 patients. LA-County is one of the largest metropolitan areas in the US. As per October 30, 2020, there were 290,278 laboratory confirmed cases, 24,670 hospitalized patients and 6,999 deaths(104). Since the first reported case of COVID-19 in the US, the four VA sites in Atlanta, Bronx, Houston and Los Angeles, have admitted 847 Veterans. While these sites vary in their COVID-19 experiences, combined they appear to be representative of the National VA population (**Table 1, re-submission Introduction**). These four sites belong to the SUPERNOVA consortium, which is a CDC-funded study ongoing for over 5 years that performs active surveillance for acute gastroenteritis and respiratory infection, including COVID-19 (28). The local site investigators are infectious disease physicians and have study staff trained in enrolling and following patients with infections. Study staff are well versed in identifying patients admitted with COVID-19, using a combination of admission log screening and TheraDoc (clinical surveillance software that integrates with the electronic medical record). Coordinators at the sites are experienced with abstraction of clinical data into REDCap and obtaining, processing, storing and shipping biological specimens. ***Inclusion criteria.*** Adult COVID-19 patients admitted to the hospital with any symptoms, including pneumonia, ARDS, sepsis, and septic shock(44) will be approached for consent (UCLA Informed Consent Form 20-000763, PI Dr. Deng). Since a majority of COVID-19-related deaths and hospitalizations are in male patients, we expect to enroll more men than women (9). ***Exclusion criteria.*** 1) Lack of consent by either patient or legally authorized representative, 2) <18 years old, 3) COVID-19 re-infection.

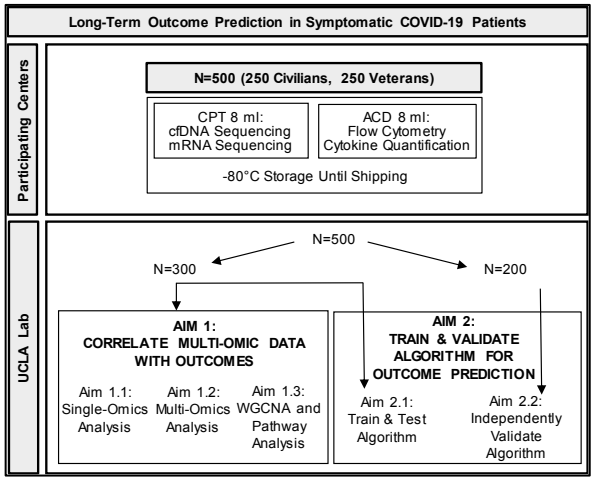

Figure 2. Study design.

Hematologic/immunologic	**Renal**
White blood cell count	Blood urea nitrogen
Total lymphocyte count	Creatinine
Absolute neutrophil count	
Hematocrit	**Hepatic**
Platelets	Bilirubin
	Aspartate transaminase
SARS-CoV-2 antibody	Alanine aminotransferase
	Lactate dehydrogenase
Inflammatory	
C-reactive protein	**Coagulation**
Erythrocyte sedimentation rate	Prothrombin time
Procalcitonin	Partial thromboplastin time
Interleukin 6	International normalized ratio
Ferritin	D-dimer
Cardiac	**Chemistries**
Troponin	Sodium
Creatine kinase MB	Glucose
Brain natriuretic peptide	Lactate

Table 1. Routine laboratory values to be abstracted from electronic medical record.

Endpoint Definition. Clinical composite endpoints will include new onset or worsening frailty, single organ dysfunction, multi-organ dysfunction, and death within one year (primary endpoint) and at time of discharge (secondary endpoint).

Data Collection. The data collection method is simple, cost-effective, and minimally invasive, requiring *only one research blood draw at enrollment*, abstraction of clinical parameters from the electronic medical record, and a brief interview at enrollment, at discharge, and at one year. ***Clinical Data.*** Demographics, laboratory values, organ function parameters, frailty parameters, comorbidities, disability data as well as COVID-19 treatment data will be collected. **Table 1** summarizes the *routine clinical laboratory values* to be collected. Validated *organ function scores*, such as SOFA score, MELD-XI score, the Seattle Heart Failure Model will be calculated(105-107). *Comorbidities* will be assessed using Charlson and Elixhauser scoring systems(108, 109). Risk of alcohol use disorders will be assessed with AUDIT-C (110). *National Health Service Frailty Scale scores* will be assessed(111). All clinical data will be collected at baseline, at discharge and at one year after COVID-19 enrollment, abstracted into REDCap, and later analyzed using R (Vienna, AU). All patients will be interviewed in person (if not available, then by televisit) at discharge and one year after enrollment. *The National Death Index* will be searched for subjects who are uncontactable(112). **Blood Collection and Processing.** Research blood will be collected within 72h of admission and COVID-19 diagnosis confirmation and processed within 2h of sampling, into one 8 ml CPT tube (BD Biosciences) for PBMC transcriptomic analysis and cfDNA analysis and one 8 ml ACD tube (BD Biosciences) for immunophenotyping and cytokine concentration analysis. Each study site will isolate plasma and PBMC using standard procedures, aliquot these samples in duplicates and store samples at -80°C. Samples will be shipped in batches to UCLA on dry ice by express delivery. ***cfDNA Sequencing.*** The Illumina NovaSeq platform will be used to sequence paired-end reads of cfDNA for 2x150 bp with 1% Phix control using S4 flow cell. For cfDNA concentration quantification, we will use Invitrogen Qubit™ dsDNA HS Assay Kit and Invitrogen Qubit 2.0 Fluorometer. Then, cfDNA sequencing will be done on an Illumina HiSeq 3000. ***RNA Sequencing.*** Total RNA will be isolated from PBMC(25)(90). Purified RNA quality will be verified on an Agilent 2100 Bioanalyzer. RNA concentrations will be determined using a NanoDrop ND-1000 spectrophotometer. The mRNA library will be prepared with Illumina TruSeq RNA kit. The cDNA libraries will be quantitated using Qubit. Total mRNA will be amplified and sequenced on the whole-genome Illumina HiSeq 3000. ***Flow Cytometry.*** Immunophenotyping of PBMCs will be performed with 12-color monoclonal antibody panels to identify the subpopulations listed in **Table 2**. Cell fluorescence will be acquired on an LSR Fortessa™ cell analyzer, using standard acquisition templates. ***Cytokine Quantification.*** We will quantify plasma cytokine levels using a 38 multiplex Luminex array. Fluorescence intensity is acquired using a Luminex 200 instrument and xPONENT software.

Immune Cell Subsets	
Cell Population	**Phenotype**
T cells	CD3+CD4+ or CD3+CD8+
Naive	CCR7+CD45RA+
Effector Memory	CCR7-CD45RA-
Central Memory	CCR7+CD45RA-
TEMRA	CCR7-CD45RA+
Activated Effector	CD25++CD127hi
Regulatory	CD4+CD25++CD127lo
Antigen-experienced	KLRG1+
Limited capacity memory	CD38+
Chronically activated	CD57+
Activated suppressive	PD1+
Natural Killer Cells	CD3-CD56+
Cytotoxic	CD56dimCD16+
Cytokine producing	CD56brightCD16+
B Cells	CD19+
Transitional	CD38++CD24+
Mature Naïve	CD27-IgD+
Memory	CD27+
Non-Switched memory	CD27+IgD+
Switched Memory	CD27+IgD-
Plasmablasts	CD27+CD38++
Regulatory	CD5+CD24+

Table 2. Cell subsets discerned through use of multi-parameter panels. Our panels allow extensive peripheral blood leukocyte population analysis as demonstrated by the list above (details see text).

Sample Size Requirements and Power Calculations. Current available literature suggests that at least 20% of the symptomatic COVID-19 infected population may develop frailty, organ dysfunction or death within one year(46, 47, 52, 56, 57, 61, 62, 113-116). Based on these assumptions, we will perform power calculations on the following numbers (250 civilians, 250 Veterans) (**Figure 2**): Aim 1.1 single-omic identification (n=100, 80 endpoint negative/20 endpoint positive) and confirmation (n=200, 160 endpoint negative/40 endpoint positive) of candidate genomic, transcriptomic, proteomic and clinical parameters that correlate with outcomes; Aim 1.2 multi-omics correlation analysis (n=300 from Aim 1.1, 240 endpoint negative/60 endpoint positive); Aim 1.3 WGCNA and pathway analysis (n=300 from Aim 1.1); Aim 2.1 training/testing of the predictive algorithm (n=300 from Aim 1.1); Aim 2.2 independent validation of predictive algorithm (independent n=200). Samples will be processed in 5 batches, approximately 120 samples per year within 5 years. Statistical analysis will begin after one-year outcomes of the first 100 patients have been collected. Patients will be stratified by outcomes, Veteran/civilian status, age, gender, ethnicity, comorbidities, frailty and other parameters that appear to be significant such as alcoholism.

Timeline. The timeline of the entire project is summarized in **Table 3**. We anticipate that: 1) recruitment of 600 COVID-19 patients will be begin 3 months after funding start, be completed within 42 months, 2) the 12-month follow-up period will be completed by month 57, and 3) the analysis will be completed by month 60.

PROJECT ACTIVITIES	YEAR 1				YEAR 2				YEAR 3				YEAR 4				YEAR 5			
	QUARTERS																			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Finalize IRB approval, study protocol, staff training, and REDCAP																				
Recruit participants for Aim 1.1 (n=100)																				
Recruit participants for Aims 1.2, 1.3, and 2.1 (n=200)																				
Recruit participants for Aim 2.2 (n=200)																				
Collection of patient clinical variables from EMR																				
Data checks and quality assurance																				
Single-omics identification and confirmation of correlates (Aim 1.1)																				
Multi-omics analysis (Aim 1.2)																				
WGCNA and pathway analysis (Aim 1.3)																				
Train & test predictive algorithm (Aim 2.1)																				
Independent validation of predictive algorithm (Aim 2.2)																				
Data reporting and publication of study results																				

Table 3. Overall Study Timeline.

PRELIMINARY DATA. We have developed and validated a survival outcome prediction algorithm for patients evaluated for advanced heart failure therapies using preoperative clinical and transcriptomic data(90, 98). This algorithm accurately predicted both short-term recovery and long-term survival (Study 1). In a separate study, we characterized the temporal dynamics of the immune system under standardized exercise and resting conditions in both patients with heart failure and healthy volunteers. We performed integrated analysis of genomic, transcriptomic, proteomic, and clinical data from the multivariate cardiopulmonary exercise panel. Our results showed a difference in each of the parameters of healthy volunteers and heart failure patients (Study 2). We propose to expand on our previous work to develop a *COVID-19 test to predict adverse short- and long-term outcomes*. We hypothesize that transcriptomic and clinical biomarkers will accurately predict the short- and long-term (within one year) outcome following SARS-CoV-2 infection. Integration of additional parameters, such as cfDNA, immunophenotyping and cytokine concentrations may increase prediction accuracy compared to using transcriptomic and clinical data alone.

Development and Validation of a Survival Outcome Prediction Algorithm for Patients Evaluated for Advanced Heart Failure Therapies (Study 1). Clinical Prediction Model Development (n=96). First, we aimed to understand the relationship of clinical information with patients' post-surgical outcomes. A retrospective study was conducted in 96 advanced heart failure patients undergoing mechanical circulatory support (MCS) surgery from 2012 to 2016 in a tertiary academic medical center. Clinical data was collected one day before and one year after MCS implantation. Twelve clinically established prognostic variables(107, 117-131) were selected, including serum bilirubin, serum creatinine, blood glucose, white blood cell count, alveolar oxygen pressure, fraction of inspired oxygen, systolic and diastolic blood pressures, International Normalized Ratio, heart rate, respiratory rate, temperature, and the Glasgow Coma Scale. We calculated Sequential Organ Failure Assessment (SOFA)(105) and Model for End Stage Liver Disease Except-INR (MELD-XI) scores(106). We also included chronological age as an important demographic parameter, which has been shown to correlate with immune system function, and overall health and survival(132-134). Of 12 variables included in our clinical prediction model, age showed the highest correlation with patients' outcome after surgery, followed by respiratory rate, white blood cell count, and minimum diastolic blood pressure (**Table 4**)(135-137). **Gene Discovery on Single-Intervention (MCS) Cohort (n=29).** Next, we conducted a study with 29 patients undergoing MCS surgery from 2012 to 2014. PBMC samples were collected one day before surgery (day -1). Clinical data was collected on day -1, day 8 and one year postoperatively. Patients were classified by SOFA and MELD-XI scores into improving (both scores improved from day -1 to day 8, n=17) and not improving (either one or both scores did not improve from day -1

Parameter	Estimate	Std. Error	t value	Pr (>|t|)
Age	-0.0068	0.0027	-2.540	0.012
SOFA Score	-0.0141	0.0189	-0.744	0.4578
GCS	0.1874	0.3366	0.557	0.5785
Temperature	0.0037	0.0610	0.061	0.9514
Resp. Rate	-0.2517	0.1277	-1.972	0.0503
Bilirubin	-0.0608	0.0398	-1.527	0.1286
WBC	-0.1456	0.0726	-2.005	0.0467
Glucose	0.0011	0.0009	1.137	0.257
Heart Rate	0.0016	0.0020	0.815	0.4165
Max. Systolic	-0.0003	0.0022	-0.128	0.8984
Min. Diastolic	0.0046	0.0027	1.732	0.0851
INR	0.0561	0.0478	1.173	0.2426

Table 4. Twelve clinically established prognostic variables. Gray indicates four parameters; p-value <0.10 used for model development.

to day 8, n=12). RNA sequencing was performed on purified mRNA and analyzed using Next Generation Sequencing (NGS) Strand. Differentially expressed genes were identified by Mann-Whitney test with Benjamini-Hochberg false discovery rate (FDR) correction. Preoperative differentially expressed genes were used to construct a support vector machine algorithm to predict improving vs. non-improving membership. Out of 28 patients alive 8 days postoperatively, one-year survival was 88% in the improving group and 27% in the non-improving group. We identified 28 preoperative differentially expressed genes between groups, with an average 93% prediction accuracy of group membership. Out of 105 genes identified preoperatively between one-year survivors and non-survivors, 12 genes overlapped with the 28 predictive genes (*AGRN, SAP25, ANKRD22, BATF2, FRMD6, HEXA-AS1, DNM1P46, NAPSA, KIR2DL4, RHBDD3, TIMP3*, and *BCORP1*). These predictive genes are all implicated in inflammation and immune system regulation (138-162). The 12 genes and 4 clinical parameters are summarized in **Table 5**. The 12 genes demonstrated similar gene expression

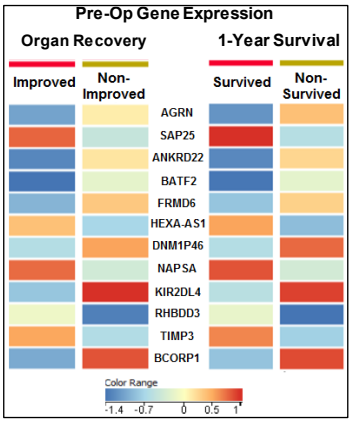

Figure 3. Average gene expression profiles of 12 within short-term recovery and one-year survivor groups and were consistently differentially expressed between both groups. Average gene expression profiles are displayed in **Figure 3**. We concluded that in advanced heart failure patients following MCS implantation, preoperative gene expression profiling predicts early changes in organ function scores and correlates with long- term outcomes and gene expression lends itself to outcome prediction(90). The 12, 28, and 105 gene lists have been patented by UCLA. **Algorithm Prototype Development.** Based on these data, we constructed a prototype algorithm to convert gene expression values of the 12 genes and 4 clinical parameters into a numerical survival prediction estimate. The algorithm is written as follows: $P = \frac{e^{(\beta + \alpha_1 X_1 + \ldots \alpha_n X_n)}}{1+e^{(\beta + \alpha_1 X_1 + \ldots \alpha_n X_n)}}$, β and α estimates were

	Parameter	Average of Survivors (n=18)	Average of Non-Survivors (n=11)	Description
Transcriptomic Data	BCORP1	-0.22±2.25	0.64±1.61	BCORP1 is a pseudogene. Although not fully functional, pseudogenes may be functional, similar to other kinds of noncoding DNA, which can perform regulatory functions.
	BATF2	-1.24±1.41	-0.19±0.86	BATF2 belongs to a class of transcription factors that regulate various immunological functions and control the development and differentiation of immune cells. BATF2 controls Th2 cell functions and lineage development of T lymphocytes. Following infection, BATF2 participates in the development of and differentiation of CD8+ thymic conventional dendritic cells. BATF2 is involved in IFN signaling and positive regulation of immune responses by altering expression of cytokines and chemokines.
	FRMD6	-0.81±1.10	-0.81±1.10	FRMD6 has been linked to various complex diseases, such as asthma, Alzheimer's disease, lung cancer, and colorectal cancer. It regulates cell proliferation and apoptosis and is thought to have tumor suppressor properties. FRMD6 mediates Vitamin D inhibition of immune cell proliferation.
	KIR2DL4	-0.63±1.73	0.62±1.13	KIR2DL4 codes for transmembrane glycoproteins expressed by natural killer (NK) cells and subsets of T cells. KIR2DL4 inhibits the NK cell activity and reduces T cell death in Sezary syndrome. KIR2DL4 recognizes human leukocyte antigen G and has been suggested as a useful diagnostic biomarker of neoplastic NK-cell proliferations.
	HEXA-AS1	0.46±0.85	-0.75±1.21	HEXA-AS1 is an antisense RNA Gene and is affiliated with the lncRNA class. Diseases associated with HEXA-AS1 include Tay-Sachs Disease.
	DNM1P46	-1.25±1.09	0.58±1.37	DNM1P46 is a pseudogene associated with diagnosing, treating, and monitoring chronic inflammatory response syndrome. DNM1P46 is a long noncoding RNA that is mediated by p53, a gene that codes for a protein that regulates the cell cycle and hence functions as a tumor suppression. p53 was activated in DNM1P46 during apoptotic pathways.
	RHBDD3	0.24±1.28	-1.43±1.19	RHBDD3 is a member of the rhomboid family of proteases that suppresses the activation of dendritic cells (DCs) and production of interleukin 6 (IL-6) triggered by Toll-like receptors. The rhomboid proteins are involved in signaling via the receptor for epidermal growth factor, mitochondrial homeostasis and parasite invasio. RHBDD3 negatively controls the activation of DCs and maintains the balance of regulatory T cells and TH17 cells by inhibiting the production of IL-6 by DCs, thus contributing to the prevention of autoimmune diseases.
	SAP25	0.79 ± 1.05	-0.59±1.13	SAP25 is a member of the nucleocytoplasmic shuttling proteins that are located in promyelocytic leukemia nuclear bodies. Promyelocytic leukemia nuclear bodies are implicated in diverse cellular functions, such as gene regulation, apoptosis, senescence, DNA repair, and antiviral response.
	NAPSA	0.51±1.16	-0.35±1.06	NAPSA is a pronapsin gene, which may have a considerable diagnostic value as a marker for primary lung cancer. NAPSA was detected in a subset of poorly differentiated papillary thyroid carcinomas and anaplastic carcinomas.
	TIMP3	0.58±1.40	-0.60±1.01	TIMP3 is an extracellular matrix-bound protein, which regulates matrix composition and affects tumor growth. TIMP3 suppresses tumor inactivation in cancer by mechanisms of invasion and angiogenesis. TIMP-3 downregulation is associated with aggressive non-small cell lung cancer and hepatocarcinoma cells, as compared with less invasive or normal lung and liver cells. It mediates VEGF by blocking the binding of VEGF to VEGF receptor-2, inhibiting downstream signaling, and prevents angiogenesis. These inhibitive properties seem to be independent of its matrix metalloproteinases-inhibitory activity.
	AGRN	-1.81±1.52	0.17±0.60	AGRIN is evolutionarily conserved in the extracellular matrix. Its intracellular processes include proliferation, apoptosis, migration, motility, autophagy, angiogenesis, tumorigenesis, and immunological responses. AGRIN interacts with the α/β-dystroglycan receptor in the formation of immunological synapses with lymphocytes and aids in activation and maintainence of monocyte cell survival downstream in an α-dystroglycan dependent manner. The AGRIN LG3 domain has been used as a biomarker for detection of prematurely ruptured fetal membranes.
	ANKRD22	-0.81±1.87	0.17±0.49	ANKR22, involved in protein lipid modification, has been patented as a possible biomarker for several types of cancers to identify patient responses to cancer immunotherapy.
Clinical Data	Age	54±15	61±14	Age is associated with immune system function, overall health and survival.
	Min. DBP	49±8	51±13	Minimum diastolic blood pressure has been shown to have an inverse relationship with mortality rates in patients ages ≥85 years old.
	WBC	11.19±4.21	11.01±4.40	Total white blood cell count is a prognostic indicator of long-term survival, reflects immune system inflammatory activation, and may indicate progressive multi organ dysfunction.
	Resp. Rate	20±4	23±5	Respiratory rate serves as a key marker in patients experiencing sepsis or systemic inflammation.

Table 5. 12 genes and 4 clinical parameters used in preliminary model development.

calculated using a LASSO logistic regression model(163). Youden's index was used to establish a cutoff point at 0.749, with values >0.749 predicting survivor status and ≤0.749 predicting non-survivor status. **Predictive Power on Independent Cohort Undergoing Various Advanced Heart Failure Therapies (n=48).** Next, we validated the predictive accuracy of the prototype algorithm that contains the previously identified 12 genes and 4 clinical parameters, in independent samples from advanced heart failure patients undergoing various therapies (n=48, including guidelines-directed medical therapy, heart transplant, MCS, and transcatheter aortic valve replacement). Performance characteristics for the algorithm on the independent cohort are summarized in **Table 6.** On this larger, more heterogeneous cohort, our test had 71% sensitivity, 90% specificity, 56% positive predictive value, and 95% negative predictive value. **Test Performance on Stage 1 Proof-of-Concept Pooled Cohort (n=77).** Next, we pooled the 29 patients used for gene selection with the 48 patients undergoing various advanced heart failure therapies to perform the following analysis (**Table 7**). One-year survival was 77% (59/77) in this combined cohort. ComBat was used for batch effect correction(164-166). Univariate analysis between 1-year survival status and the 12, 28, and 105 gene lists confirmed that all significantly differentially expressed genes in this combined cohort exhibited the same directionality as in the original cohort used for initial gene discovery(90). Various logistic regression models were constructed using the LASSO method(163) and measures of predictive performance such as AUCs are summarized in **Table 8**. Prediction of 1-year survival using 4 clinical parameters alone (age, white blood cell count, respiratory rate, and minimum diastolic blood pressure) achieved an AUC=0.69. Adding the 12-gene candidates to the clinical model without ComBat correction improved the 1-year survival prediction to AUC=0.90. The 12-gene list with ComBat correction and clinical data led to an observed AUC=0.86. We concluded that: 1) the direction of gene expression in the pooled 77 patient cohort was similar to that of the original 29 patients used for gene discovery, 2) batch effects played a minor role in data performance and 3) the addition of 12 genes meaningfully improved 1-year survival prediction of outcomes in patients undergoing diverse advanced heart failure therapies compared to the clinical model alone. **Prototype Score Construction (n=77).** Next, from the prototype raw numerical survival prediction algorithm score, we calculated a prototype clinical score for each of the 77 patients using non-batch adjusted data and risk stratified into 5 quintiles (**Table 9**). Note that, consistent with the UCLA patent application language guided by the intended clinical use, higher prototype raw scores translate into higher clinical scores. Higher deciles indicated binning of higher clinical scores and a higher likelihood of 1-year survival post-advanced heart failure therapy. The pooled survival rate for the top 6 deciles was 0.96 (47/49), and the survival rate for the pooled lower 4 deciles was 0.43 (12/28).

Cut Off 0.749 (Youden's Index)	29 Patients (Gene Discovery)	48 Patients (Independent Validation)
Sensitivity	100%	71%
Specificity	72%	90%
Positive Pred. Value	69%	56%
Negative Pred. Value	100%	95%

Table 6. Performance characteristics of prototype test.

	Total (n=77)	1-Year Survivors (n=59)	Non-Survivors (n=18)	P-value
Age	56 (16)	54 (17)	63 (12)	0.037
SOFA Score	9.3 (3.4)	9.2 (3.2)	9.7 (4.0)	0.567
Male	64 (83.1%)	49 (83%)	15 (83.3%)	0.978
Procedure				0.124
GDMT	4 (5%)	3 (5%)	1 (6%)	
HTx	31 (40%)	28 (47%)	3 (17%)	
MCS	38 (49%)	25 (42%)	13 (72%)	
TAVR	4 (5%)	3 (5%)	1 (6%)	
WBC count	9.3 (3.7)	9.2 (3.8)	9.8 (3.4)	0.502
Resp. Rate	19.7 (5.8)	19.5 (5.9)	20.3 (5.5)	0.550
MELD-XI Score	13.9 (6.5)	13.5 (6.6)	15.3 (6.2)	0.300
Min. DBP	54.2 (12.5)	54.8 (12.0)	52.3 (14.1)	0.473

Table 7. Demographic data of 77 patients in Proof-of-Concept Study.

Model	AUC	% Increase in Accurate Survival Prediction
Clinical (age, WBC, RR, MDBP)	0.69	--
12 genes (raw) + clinical	0.90	68%
12 genes (COMBAT) + clinical	0.86	55%

Table 8. Modeling and test algorithm predictive performance on combined cohort

	Outcome				
Quintile	Survivor	Non-Survivor	Total	Survivor rate	Prototype Algorithm Score (1-10)
5	15	0	15	1.00	10-9
4	15	1	16	0.94	8-7
3	14	1	15	0.93	6-5
2	12	4	16	0.75	4-3
1	3	12	15	0.20	2-1
Total	59	18	77	0.77	

Table 9. Predictive performance of risk-stratified deciles.

Multi-Omics Analysis of Exercise-Induced Changes in Health and Heart Failure (Study 2). In order to assess the relationship between cf-mtDNA and the PBMC transcriptome, PBMC immunophenotypes, cytokines and clinical exercise physiology parameters in health and disease, we characterized the temporal dynamics of the immune system under exercise and resting conditions in healthy volunteers (n=4) and patients with heart failure (n=16). We performed integrated analysis of genomic, transcriptomic, and proteomic data, as well as

clinical data from the multivariate cardiopulmonary exercise panel. All participants underwent exercise testing. Blood samples were collected at 3 time-points: before (TP1), during (TP2), and 1-hour after peak oxygen uptake (VO2) (TP3). **Genome Data.** To understand the cfDNA dynamics during exercise testing, we performed total cfDNA as well as cf-mtDNA fragment quantification correlation analysis with VO2 max and percent predicted of VO2 max for all 20 participants at 3 timepoints (**Figure 4**). We observed a positive correlation between cardiorespiratory performance (as measured by peak VO2 and percent predicted of peak VO2) and cf-mtDNA at TP2. A negative correlation was found between cardiorespiratory performance and cf-mtDNA, beginning at the time of peak VO2 and more pronounced 1 hour after peak VO2. Numbers of cf-mtDNA fragments decreased after peak VO2 in healthy volunteers and increased in heart failure. Heart failure patients had pronounced cf-mtDNA fragments one hour after peak VO2. Numbers of cf-mtDNA fragments decreased after peak VO2 in healthy volunteers and increased in heart failure. **Transcriptome Data.** In both groups, a differential relationship was observed between cf-mtDNA fragment number and expression of 9 PBMC transcripts associated with reactive oxygen species (**Figure 5**). **Proteome Data.** A differential relationship was found between the reactive oxygen species related PBMC transcripts and pro-inflammatory cytokines of both groups (**Figure 6**). These preliminary data suggest that temporal dynamics of cf-mtDNA correlates with gene expression and cytokine production and that these patterns differ in healthy individuals and those with chronic disease.

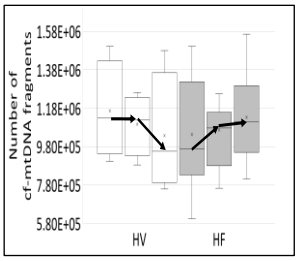

Figure 4. Trends in VO2 max, % predicted VO2 max, and genomic data. Median number of cf-mtDNA fragments in healthy volunteers (HV, white boxplots) and heart failure (HF, grey boxplots).

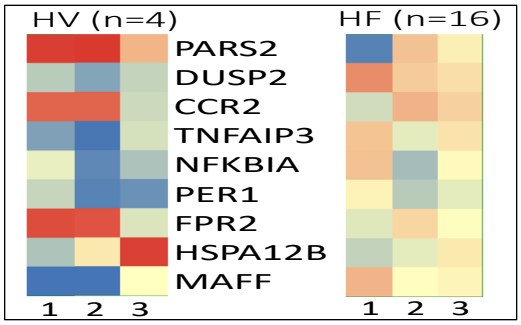

Figure 5. Heat map of Pearson correlation of 9 differentially expressed genes involved in oxidative stress signalling by reactive oxygen species and fragment number of cell free mitochondrial DNA at timepoint 1, 2, and 3 in healthy volunteers (HV) and heart failure patients (HF).

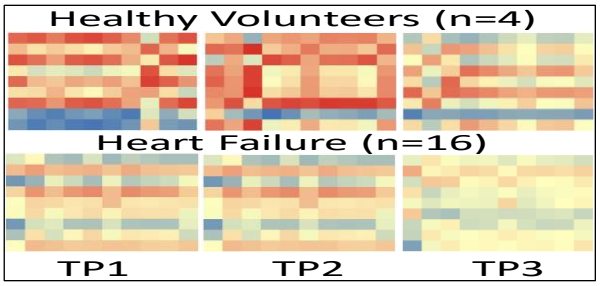

Figure 6. Heat map of Pearson correlation of 9 differentially expressed genes involved in oxidative stress signaling by reactive oxygen species (from top to bottom: PARS2, DUSP2, CCR2, TNFAIP3, NFKBIA, PER1, FPR2, HSPA12B, and MAFF) and 10 differentially expressed cytokines (from left to right: FGF-2, G-CSF, fractalkine, MCP-3, IL-13, IL-17A, IL-1a, IL-4, IL-6, TNFb, and VEGF) at timepoint (TP) 1, 2, and 3 in healthy volunteers and heart failure patients.

SPECIFIC AIMS

AIM 1: CORRELATE MULTIOMIC DATA WITH OUTCOMES. Hypothesis and Objectives. We hypothesize that various clinical COVID-19 disease outcome phenotypes correlate with molecular data and clinical patterns and are associated with immunologic pathways. Our first objective is to identify and confirm mono-dimensional genomic, transcriptomic, proteomic and clinical parameters that correlate with outcomes (Aim 1.1). Outcomes are defined as new onset or worsening frailty, single organ dysfunction, multiorgan dysfunction, and death within one year (primary endpoint) and at time of discharge (secondary endpoint). Since genomics, transcriptomics and proteomics often have complementary roles, our second objective is to perform multi-omics correlation analysis (Aim 1.2). Our third objective is to perform Weighted Gene Co-Expression Network Analysis (WGCNA) and pathway analysis to understand the role of the genes involved in the inflammatory response during SARS-CoV-2 infection (Aim 1.3).

Aim 1.1: Single-omic identification of candidate genomic, transcriptomic, proteomic and clinical parameters that correlate with outcomes. Objective. Our objective is to identify single-omic parameters (genomic, transcriptomic, proteomic) and clinical data that correlate with COVID-19 outcomes. We will perform exploratory analyses on the first 100 samples and confirm these parameters on the following 200 samples (**Figure 2**). **Genomic Analysis.** Raw cfDNA sequences will be aligned to HG38 and cf-mtDNA to HG38_MT_only (Strand NGS 3.3.1). ComBat will be used for batch effect correction(164-166). We will calculate the number of cf-mtDNA fragments in each sample. **Transcriptomic Analysis.** Raw transcriptome data will be aligned (HG19) and normalized (DESeq), batch effect corrected by ComBat(164-166) and filtered by expression (20^{th} to 100^{th} percentile) (Strand NGS (Avadis)). Differentially expressed genes between positive and negative endpoint groups will be identified by non-parametric statistics (Mann-Whitney test with Benjamini-Hochberg correction). We will use a more relaxed criteria of FDR=0.1 and fold change of at least 2.0 to identify significant genes as previously described by our group(90, 167, 168). **Immunophenotyping Analysis.** We will identify COVID-19

antigen exposed PBMC subsets for comparative analysis to genome, transcriptome and cytokine data. Analyses will be performed using the FCS Express V4 analysis software. **Cytokine Analysis.** The median fluorescent intensity data will be processed using a 5-parameter logistic fitting method to calculate cytokine concentrations by Milliplex analyst 3 software. We expect to achieve test results with 3-4% intra-plate coefficient of variation and less than 15% inter-plate coefficient of variation. **Interpretation of Results.** We expect COVID-19 patients with new onset or worsening frailty, organ dysfunction and death within one year to have more pronouncedly altered inflammatory profiles than patients who recover. We expect to find differentially expressed genes and differences in cf-mtDNA patterns, leukocyte subpopulations and cytokine production between patients with positive and negative endpoint outcomes.

Aim 1.2: Multi-omics correlation analysis. Objective. Genomics, transcriptomics and proteomics often have complementary roles to jointly perform a certain biological function and create a specific observable phenotype. Our objective in Aim 1.2 is to establish a correlation pattern between the multi-omics data and the outcome data in symptomatic COVID-19 patients. **Bioinformatics.** Multi-omics analyses will be performed on the first 300 samples to correlate outcome with cfDNA patterns, PBMC coding and non-coding mRNA expression, PBMC subpopulations and cytokine profiles (**Figure 2**). We will construct mixed effects regression models for each candidate feature. For continuous markers (cytokines) we will construct linear models. All logistic regression models will be stratified by primary composite outcome (single organ dysfunction, multiorgan dysfunction, and death). For count markers (sequencing), we will run negative binomial models. We will use the Benjamini-Hochberg procedure to control the FDR at alpha 0.05. If significant interaction effects are found after FDR adjustment, post hoc comparisons will be estimated using model contrasts and summarized with 95% confidence intervals estimated from the model. **Interpretation of Results.** Multi-omics correlations will add essential information in addition to the single omics analysis. We expect that the candidate genomic, transcriptomic, proteomic and clinical predictors will have distinct correlations within the two endpoint groups. This information can help characterize the biology of the immune response to COVID-19 and identify the most promising members for the prediction algorithm.

Aim 1.3: WGCNA and pathway analysis. Objective. The role of the genes involved in the inflammatory response during SARS-CoV-2 infection is unclear. To better understand co-expression of genes and their biological role in patients with symptomatic COVID-19, we will perform WGCNA and pathway analysis. To dissect the pro- and anti-inflammatory responses during SARS-CoV-2 infection with an non-supervised strategy, we will use WGCNA(170). WGCNA provides a non-supervised systematic method to identify co-expressed genes and pathways linked to the primary outcome. **Bioinformatics.** Normalized transcriptome data from the first 300 samples will be stratified by composite endpoint, batch effect corrected by ComBat and filtered by variance and entropy criteria to remove uninformative transcripts (**Figure 2**)(164-166, 169). This approach offers a powerful way to reduce dimensionality while enhancing biological interpretability. The co-expressed modules and their respective eigen-genes will be interpreted in their relationship to clinical and biological features. The clinical relevance of the modules will be inferred using a linear mixed effect model. The biological relevance of the modules will be deduced using gene ontology (GOSim(171, 172)) and pathway analysis (Strand NGS (Avadis)). **Interpretation of Results.** The results from WGCNA will offer a useful framework for relational and mechanistic reasoning. Our model will provide an integrative representation of leukocyte biology and related pathways during the COVID-19 inflammatory response. We anticipate that this strategy will enhance identification of candidate entities for the development of the prediction algorithm for adverse COVID-19 short- and long-term outcome events in Aim 2.

AIM 2: TRAIN AND VALIDATE ALGORITHM FOR OUTCOME PREDICTION

Hypothesis and Objectives. We hypothesize that our algorithm will predict COVID-19 short- and long-term outcomes more accurately than clinical parameters alone. Our objectives are to train the algorithm in a mixed US-high risk COVID-19 population (Aim 2.1) and validate the predictive algorithm on an independent mixed US-high risk COVID-19 population (Aim 2.2).

Aim 2.1: Train algorithm for outcome prediction. Objective. Our objective is to train and test our algorithm in a mixed US COVID-19 population, which will improve prediction of adverse short- and long-term outcomes(92, 173). **Bioinformatics.** ***Parameter Selection.*** Model development will largely follow the modeling reported in the preliminary data section and will be constructed using significant entities identified in the marker screening phase (Aim 1). Since VA and civilian patients may have different co-morbidity and risk profiles, these models will also include a term for whether the patient is from the VA or civilian setting. This term, if significant, will allow flexibility in our models to make different predictions based upon known characteristic differences between VA and civilian patient populations. ***Power calculations.*** The first 300 samples (240 endpoint negative and 60 endpoint positive)

will be used for model building (**Figure 2**). The overall sample size will support the following: 1) This sample size will be sufficient to include approximately 10 clinical features based on conventional rules of thumb suggesting that we should have 10-15 subjects in the less frequent outcome category per variable in a logistic regression model; 2) This will allow us to estimate the AUC for the model with a precision of approximately 0.032 (based on the 95% confidence interval for the AUC and an overall AUC of at least 0.8); 3) Provide 80% power for detecting delta AUCs between pairs of models (see below) of 0.1 assuming that the models will have a correlation of 0.5 and AUCs of ~0.8. ***Logistic regression modeling. Logistic regression modeling.*** We will assess the algorithm by constructing three separate logistic regression models, one using clinical factors alone as our baseline model, one using clinical factors plus the significant omics markers from the screening phase (genomics, transcriptomics, and proteomics parameters), and one using clinical factors plus a simplified list of significant omics features (e.g. bedside, rapid test, easy to obtain). Each of the three models will have variable selection and coefficients estimated by the LASSO method and validation results and comparisons between the three models performance (AUC) will be validated in Aim 2.2. ***Model building optimization by cross-validation.*** Ten-fold cross-validation will be used to estimate model performance in terms of AUC. The AUCs will be compared between models with DeLong's test for paired AUCs. **Interpretation of Results.** We expect to derive a multi-omics algorithm that will predict outcomes of COVID-19 patients more accurately than clinical data alone.

Aim 2.2: Independently validate predictive algorithm for outcome prediction. Objective. Our objective is to validate the algorithm in an independent randomly selected mixed US COVID-19 population who were not used for algorithm development(173). **Bioinformatics.** We will validate the performance of the prototype test algorithm to predict the composite endpoints in an independent cohort of 200 patients (**Figure 2**). Rigorous testing of the analytical performance of the test will identify and quantify the technical variations of the test performed on the patient samples and will be done in a blinded fashion, without knowledge of the clinical status or treatment outcomes of the patients whose data was obtained. For model testing, the sample size of 200 (160 endpoint negative and 40 endpoint positive) will provide precision of +/- 0.066 for the testing AUC as well as an 80% power to detect differences in AUCs between models of 0.12, when comparing models based on clinical features alone and models combining clinical and molecular features. Finally, with this sample size we will be able to estimate the precision of the sensitivity and specificity (based on the width of the 95% confidence intervals) between 0.0545 to 0.11 depending on the observed values of those performance characteristics. *We plan to make the raw data and the algorithm publically available as a web-based free tool for further scientific development by the international scientific community.* **Interpretation of Results.** We expect that the algorithm will confirm the desired clinical test performance, i.e. increase accuracy of one-year primary endpoint (new onset or worsening frailty, organ dysfunction or death) prediction of at least AUC 0.12.

POTENTIAL PROBLEM AREAS & ALTERNATIVE METHODS/APPROACHES

Pitfall #1: The arrival of evidence-based antiviral therapies (e.g., remdesivir) may alter the clinical course of the disease(174), *however this has not yet been established and while some studies suggest remdesivir provides a modest benefit, interim analysis of the large WHO SOLIDARITY trial suggest no mortality benefit (175)*. ***Alternative*:** We will model clinical trajectories after initiation of anti-viral therapy by dichotomizing the study cohort into two groups: short versus long recovery time. By comparing these groups, we expect to identify an effective prediction algorithm from our baseline dataset for higher (shorter time to recovery) versus lower functional recovery potential (longer time to recovery). It will be important to analyze for corticosteroid treatment, which has become routine treatment for severe COVID-19(34), and was recognized as a potential contributor (e.g., steroid myopathy) to long-term sequelae in the SARS epidemic(176).

Pitfall #2: The implementation of effective vaccine strategies may alter the recruitment modeling for our study. ***Alternative*:** Rapid vaccine implementation is a major initiative in the fight against COVID-19 and there are several parallel efforts(177-181). Thus, it is quite likely that an effective vaccine against COVID-19 will be implemented during the study period. While it is difficult to predict with precision when vaccination levels will provide effective herd immunity and thus lead to a significant reduction in cases, it is unlikely that this will happen in less than 1.5 years(178). It is also unlikely that a vaccine strategy will be so effective that it will prevent all disease or eliminate the disease entirely. The latter has happened with only a single human pathogen to date, and the worldwide effort to eliminate smallpox took well over two decades of sustained effort. It is currently not thought to be possible to effectively eradicate an infection that has an animal reservoir (like SARS-CoV-2). Our goal is to enroll 3.3 patients/week from 7 sites over 42 months (July 1, 2021 through December 31, 2024). Given the experience of the sites involved and the location of these sites in major metropolitan areas prone to high rates of COVID-19, we expect that the majority of subjects will be enrolled relatively quickly during the first half

of the enrollment period. Thus, we expect to be able to complete the study as designed even if there is rapid implementation of an effective vaccine strategy that affects our ability to recruit subjects in the latter years of the project.

Pitfall #3: Our predictive algorithm does not perform well on certain subgroups. ***Alternative*:** We think this is unlikely because the biological responses (or lack thereof) that lead to poor outcomes are likely to be similar among people with differences in demographic and underlying diseases (182, 183). However, in the validation process, we will look at important subgroups to see if there are issues with performance. While we have guarded against a sub-group imbalance by proposing a dual strategy, combining a civilian population in one of the country's largest metropolitan areas and a representative National Veteran's population, it is possible that our algorithm may not be as accurate in predicting outcome in subgroups (e.g., women versus men). If this is the case, we will train the algorithm on the specific subgroup only and determine whether we identify different parameters. Using this approach, we may also uncover clues pointing to underlying pathogenic mechanisms in SARS-CoV-2 infection.

Pitfall #4: Currently, there is not enough data available to develop a clinical phenotype scale for COVID-19. ***Alternative:*** We will have a comprehensive outcome list and will be able to explore a more detailed way of characterizing COVID-19 outcomes by the end of our study. In order to better define clinical phenotypes, we will correlate molecular and clinical data from our 500 patients to a tentative 10-point COVID-19 Functional Recovery Scale, modified from the WHO 8-point ordinal scale (**Figure 7**)(44, 174). Since the COVID-19 landscape is continually changing, we will modify this scale as necessary. The COVID-19 Functional Recovery Scale scores will be assessed by tele-medicine or in-person visits at baseline, discharge (secondary endpoint) and one-year (primary endpoint). An ordinal scale of continuous phenotypes may provide better power for outcome prediction than a scale that only detects presence or absence of adverse outcome.

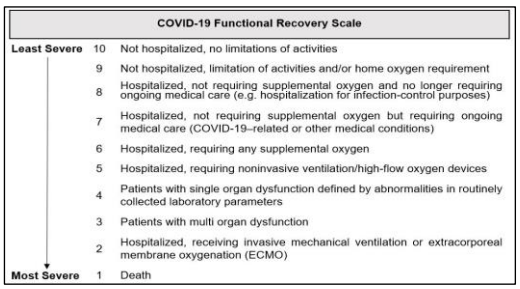

Figure 7 Tentative Ordinal COVID-19 Functional Recovery Scale.

Pitfall #5: SARS-CoV-2 genetic drift may affect outcomes. ***Alternative:*** As SARS-CoV-2 spread geographically and temporally, its genetic diversity increased (184, 185), and several genetic subgroups have already been defined. Certain mutations have been associated with higher mortality (186, 187), while others demonstrate improved binding to the respiratory tract (188, 189). It is currently not clear how viral evolution has affected the development or prevalence of sequelae and outcomes in general. To examine this crudely, we will monitor trends over the study period (i.e., early versus late). However, other time-specific effects could confound this analysis. The SUPERNOVA study is collecting respiratory samples for SARS-CoV-2 genome analysis. By integrating this information, we could look for differences based on genetic lineages.

Pitfall #6: Impact of influenza or other seasonal respiratory viruses on COVID-19. ***Alternative:*** While co-infection with SARS-CoV-2 and influenza virus have been described (190, 191), it remains unclear how influenza or other seasonal respiratory viruses will impact COVID-19. Additional studies are needed to establish whether viral co-infections in SARS-CoV-2 patients could potentially drive viral interference or otherwise influence disease outcomes (192, 193). It is possible that steps taken to mitigate spread of SARS-CoV-2, may significantly reduce influenza rates (194), as appears to have happened in Australia (195). However, if both viruses are prevalent, co-infection is certainly possible and may be common. All patients admitted to the enrolling sites with suspecting respiratory viral infection, will be tested for both SARS-CoV-2 and influenza. We will include co-infected patients in our analyses. If we enroll enough patients with infection with SARS-CoV-2 and influenza or another seasonal respiratory virus, we will further analyze this subset.

SUMMARY

We propose to develop a tool that predicts adverse short- and long-term (within one year) outcomes for hospitalized COVID-19 patients. We hypothesize that new onset or worsening frailty, organ dysfunction and death from COVID-19 within one year after COVID-19 diagnosis can be accurately predicted by our algorithm that incorporates baseline genomic, transcriptomic, proteomic and clinical data. We will test our hypothesis in a mixed US COVID-19 population. We anticipate that a prediction test that performs well in the demographically and geographically diverse patient population that we will recruit will also demonstrate powerful prediction precision in the general population. Event prediction will assist clinicians in making tailored treatment plans. Future implementation of our tool into clinical settings may decrease morbidity and mortality, enhance patient quality of life and improve healthcare cost-effectiveness.

REFERENCES

1. Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, et al. Extrapulmonary manifestations of COVID-19. Nat Med. 2020;26(7):1017-32.
2. Wu T, Zuo Z, Kang S, Jiang L, Luo X, Xia Z, et al. Multi-organ Dysfunction in Patients with COVID-19: A Systematic Review and Meta-analysis. Aging Dis. 2020;11(4):874-94.
3. Iaccarino G, Grassi G, Borghi C, Ferri C, Salvetti M, Volpe M, et al. Age and Multimorbidity Predict Death Among COVID-19 Patients: Results of the SARS-RAS Study of the Italian Society of Hypertension. Hypertension. 2020;76(2):366-72.
4. Emami A, Javanmardi F, Pirbonyeh N, Akbari A. Prevalence of Underlying Diseases in Hospitalized Patients with COVID-19: a Systematic Review and Meta-Analysis. Arch Acad Emerg Med. 2020;8(1):e35.
5. Liu H, Chen S, Liu M, Nie H, Lu H. Comorbid Chronic Diseases are Strongly Correlated with Disease Severity among COVID-19 Patients: A Systematic Review and Meta-Analysis. Aging Dis. 2020;11(3):668-78.
6. Prevention CfDCa. Groups at Higher Risk for Severe Illness. Online; 2020.
7. Ko JY, Danielson ML, Town M, Derado G, Greenlund KJ, Daily Kirley P, et al. Risk Factors for COVID-19-associated hospitalization: COVID-19-Associated Hospitalization Surveillance Network and Behavioral Risk Factor Surveillance System. Clin Infect Dis. 2020.
8. Kim L, Garg S, O'Halloran A, Whitaker M, Pham H, Anderson EJ, et al. Interim Analysis of Risk Factors for Severe Outcomes among a Cohort of Hospitalized Adults Identified through the U.S. Coronavirus Disease 2019 (COVID-19)-Associated Hospitalization Surveillance Network (COVID-NET). Clinical Infectious Diseases. 2020.
9. Gadi N, Wu SC, Spihlman AP, Moulton VR. What's Sex Got to Do With COVID-19? Gender-Based Differences in the Host Immune Response to Coronaviruses. Front Immunol. 2020;11:2147.
10. Oxley TJ, Mocco J, Majidi S, Kellner CP, Shoirah H, Singh IP, et al. Large-Vessel Stroke as a Presenting Feature of Covid-19 in the Young. N Engl J Med. 2020.
11. Viner RM, Whittaker E. Kawasaki-like disease: emerging complication during the COVID-19 pandemic. The Lancet.
12. DeBiasi RL, Song X, Delaney M, Bell M, Smith K, Pershad J, et al. Severe COVID-19 in Children and Young Adults in the Washington, DC Metropolitan Region. J Pediatr. 2020.
13. Riphagen S, Gomez X, Gonzalez-Martinez C, Wilkinson N, Theocharis P. Hyperinflammatory shock in children during COVID-19 pandemic. Lancet. 2020;395(10237):1607-8.
14. Belhadjer Z, Méot M, Bajolle F, Khraiche D, Legendre A, Abakka S, et al. Acute heart failure in multisystem inflammatory syndrome in children (MIS-C) in the context of global SARS-CoV-2 pandemic. Circulation. 2020.
15. Department of Veterans Affairs COVID-19 National Summary [Internet]. 2020 [cited May 7, 2020]. Available from: https://www.accesstocare.va.gov/Healthcare/COVID19NationalSummary.
16. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU) [Internet]. Johns Hopkins University of Medicine Coronavirus Resource Center. 2020 [cited May 7, 2020]. Available from: https://coronavirus.jhu.edu/map.html.
17. 2018: ACS 1-Year Estimates Subject Tables [Internet]. United States Census Buraeu. 2018 [cited March 27, 2020]. Available from: https://data.census.gov/cedsci/table?q=veterans&hidePreview=false&tid=ACSST1Y2018.S2101&t=Veterans&vintage=2018.
18. Tsai J, Wilson M. COVID-19: a potential public health problem for homeless populations. Lancet Public Health. 2020;5(4):e186-e7.
19. Prevention CfDCa. Interim Guidance on Unsheltered Homelessness and Coronavirus Disease 2019 (COVID-19) for Homeless Service Providers and Local Officials: Interim Guidance. Online; 2020.
20. Fargo J, Metraux S, Byrne T, Munley E, Montgomery AE, Jones H, et al. Prevalence and risk of homelessness among US veterans. Prev Chronic Dis. 2012;9:E45.
21. Olenick M, Flowers M, Diaz VJ. US veterans and their unique issues: enhancing health care professional awareness. Adv Med Educ Pract. 2015;6:635-9.
22. Prevention CfDCa. Heart Disease and Mental Health Disorders. Online; 2020.
23. De Hert M, Detraux J, Vancampfort D. The intriguing relationship between coronary heart disease and mental disorders. Dialogues Clin Neurosci. 2018;20(1):31-40.
24. Chaddha A, Robinson EA, Kline-Rogers E, Alexandris-Souphis T, Rubenfire M. Mental Health and Cardiovascular Disease. The American Journal of Medicine. 2016;129(11):1145-8.

25. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19). JAMA Cardiol. 2020.
26. Vance MC, Wiitala WL, Sussman JB, Pfeiffer P, Hayward RA. Increased Cardiovascular Disease Risk in Veterans With Mental Illness. Circulation Cardiovascular quality and outcomes. 2019;12(10):e005563.
27. Ramchand R, Harrell MC, Berglass N, Lauck M. Veterans and COVID-19: Projecting the Economic, Social, and Mental Health Needs of America's Veterans. Online: Bob Woodruff Foundation; 2020.
28. Cristina Cardemil RD, Mila Prill, Sheldon Brown, Adrienne Perea, Vincent Marconi, LaSara Bell, Maria Rodriguez-Barradas, Gilberto Rivera- Dominguez, David Beenhouwer, Aleksandra Poteshkina, Mark Holodniy, Cynthia Lucero-Obusan, Neha Balachandran, Aron Hall, Lindsay Kim, Gayle Langley. COVID-19-associated hospitalization rates and severe outcomes among Veterans from multi-site surveillance, February 27-July 17, 2020. JMIR Public Health and Surveillance. 2020.
29. Cates J, Lucero-Obusan C, Dahl RM, Schirmer P, Garg S, Oda G, et al. Risk for In-Hospital Complications Associated with COVID-19 and Influenza - Veterans Health Administration, United States, October 1, 2018-May 31, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(42):1528-34.
30. Tenforde MW, Kim SS, Lindsell CJ, Billig Rose E, Shapiro NI, Files DC, et al. Symptom Duration and Risk Factors for Delayed Return to Usual Health Among Outpatients with COVID-19 in a Multistate Health Care Systems Network - United States, March-June 2020. MMWR Morb Mortal Wkly Rep. 2020;69(30):993-8.
31. Puntmann VO, Carerj ML, Wieters I, Fahim M, Arendt C, Hoffmann J, et al. Outcomes of Cardiovascular Magnetic Resonance Imaging in Patients Recently Recovered From Coronavirus Disease 2019 (COVID-19). JAMA Cardiol. 2020.
32. O'Brien H, Tracey MJ, Ottewill C, O'Brien ME, Morgan RK, Costello RW, et al. An integrated multidisciplinary model of COVID-19 recovery care. Ir J Med Sci. 2020.
33. Hui DS, Wong KT, Ko FW, Tam LS, Chan DP, Woo J, et al. The 1-year impact of severe acute respiratory syndrome on pulmonary function, exercise capacity, and quality of life in a cohort of survivors. Chest. 2005;128(4):2247-61.
34. Prescott HC, Rice TW. Corticosteroids in COVID-19 ARDS: Evidence and Hope During the Pandemic. JAMA. 2020;324(13):1292-5.
35. Ngai JC, Ko FW, Ng SS, To KW, Tong M, Hui DS. The long-term impact of severe acute respiratory syndrome on pulmonary function, exercise capacity and health status. Respirology. 2010;15(3):543-50.
36. Moldofsky H, Patcai J. Chronic widespread musculoskeletal pain, fatigue, depression and disordered sleep in chronic post-SARS syndrome; a case-controlled study. BMC Neurol. 2011;11:37.
37. Lee AM, Wong JG, McAlonan GM, Cheung V, Cheung C, Sham PC, et al. Stress and psychological distress among SARS survivors 1 year after the outbreak. Can J Psychiatry. 2007;52(4):233-40.
38. Ohtake PJ, Coffey Scott J, Hinman RS, Lee AC, Smith JM. Impairments, activity limitations and participation restrictions experienced in the first year following a critical illness: protocol for a systematic review. BMJ Open. 2017;7(1):e013847.
39. Pandharipande PP, Girard TD, Jackson JC, Morandi A, Thompson JL, Pun BT, et al. Long-term cognitive impairment after critical illness. N Engl J Med. 2013;369(14):1306-16.
40. Griffiths J, Hatch RA, Bishop J, Morgan K, Jenkinson C, Cuthbertson BH, et al. An exploration of social and economic outcome and associated health-related quality of life after critical illness in general intensive care unit survivors: a 12-month follow-up study. Crit Care. 2013;17(3):R100.
41. Unroe M, Kahn JM, Carson SS, Govert JA, Martinu T, Sathy SJ, et al. One-year trajectories of care and resource utilization for recipients of prolonged mechanical ventilation: a cohort study. Ann Intern Med. 2010;153(3):167-75.
42. Prescott HC, Langa KM, Iwashyna TJ. Readmission diagnoses after hospitalization for severe sepsis and other acute medical conditions. JAMA. 2015;313(10):1055-7.
43. Ruhl AP, Huang M, Colantuoni E, Karmarkar T, Dinglas VD, Hopkins RO, et al. Healthcare utilization and costs in ARDS survivors: a 1-year longitudinal national US multicenter study. Intensive Care Med. 2017;43(7):980-91.
44. Organization WH. Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected: interim guidance. In: Communications Do, editor. Interim guidance ed. who.int2020.
45. Pan F, Ye T, Sun P, Gui S, Liang B, Li L, et al. Time Course of Lung Changes On Chest CT During Recovery From 2019 Novel Coronavirus (COVID-19) Pneumonia. Radiology. 2020:200370.
46. Zheng Y-Y, Ma Y-T, Zhang J-Y, Xie X. COVID-19 and the cardiovascular system. Nature Reviews Cardiology. 2020.

47. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China. JAMA Cardiology. 2020.
48. Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, et al. Coronavirus Disease 2019 (COVID-19) and Cardiovascular Disease. Circulation. 2020.
49. Xiong TY, Redwood S, Prendergast B, Chen M. Coronaviruses and the cardiovascular system: acute and long-term implications. European heart journal. 2020.
50. Poyiadji N, Shahin G, Noujaim D, Stone M, Patel S, Griffith B. COVID-19-associated Acute Hemorrhagic Necrotizing Encephalopathy: CT and MRI Features. Radiology. 2020:201187.
51. Li YC, Bai WZ, Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. J Med Virol. 2020.
52. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. JAMA Intern Med. 2020.
53. Akalin E, Azzi Y, Bartash R, Seethamraju H, Parides M, Hemmige V, et al. Covid-19 and Kidney Transplantation. N Engl J Med. 2020.
54. Pan XW, Xu D, Zhang H, Zhou W, Wang LH, Cui XG. Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single-cell transcriptome analysis. Intensive Care Med. 2020.
55. Fan Z, Chen L, Li J, Cheng X, Yang J, Tian C, et al. Clinical Features of COVID-19-Related Liver Damage. Clin Gastroenterol Hepatol. 2020.
56. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clinical Infectious Diseases. 2020.
57. Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. Lancet Gastroenterol Hepatol. 2020;5(5):428-30.
58. Neurath MF. Covid-19 and immunomodulation in IBD. Gut. 2020.
59. Mao R, Liang J, Shen J, Ghosh S, Zhu LR, Yang H, et al. Implications of COVID-19 for patients with pre-existing digestive diseases. Lancet Gastroenterol Hepatol. 2020;5(5):426-8.
60. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801-10.
61. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunologic features in severe and moderate Coronavirus Disease 2019. J Clin Invest. 2020.
62. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395(10223):507-13.
63. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ. 2020;368:m1091.
64. Xiong Y, Liu Y, Cao L, Wang D, Guo M, Jiang A, et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerging Microbes & Infections. 2020;9(1):761-70.
65. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Medicine. 2020;46(5):846-8.
66. Huang JF, Zheng KI, George J, Gao HN, Wei RN, Yan HD, et al. Fatal outcome in a liver transplant recipient with COVID-19. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2020.
67. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. The Lancet. 2020;395(10229):1033-4.
68. Wang H, Ma S. The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome. Am J Emerg Med. 2008;26(6):711-5.
69. Teijaro JR, Walsh KB, Rice S, Rosen H, Oldstone MB. Mapping the innate signaling cascade essential for cytokine storm during influenza virus infection. Proc Natl Acad Sci U S A. 2014;111(10):3799-804.
70. Liu B. Free DNA, a reason for severe COVID-19 infection? Med Hypotheses. 2020;142:109812.
71. Cron RQ, Behrens EM. Cytokine Storm Syndrome: Springer; 2019.
72. Wen W, Su W, Tang H, Le W, Zhang X, Zheng Y, et al. Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. Cell Discov. 2020;6:31.
73. Ridker PM, MacFadyen JG, Glynn RJ, Bradwin G, Hasan AA, Rifai N. Comparison of interleukin-6, C-reactive protein, and low-density lipoprotein cholesterol as biomarkers of residual risk in contemporary

practice: secondary analyses from the Cardiovascular Inflammation Reduction Trial. European heart journal. 2020.
74. Riley JS, Tait SW. Mitochondrial DNA in inflammation and immunity. EMBO Rep. 2020;21(4):e49799.
75. Zhang R, Nakahira K, Guo X, Choi AM, Gu Z. Very Short Mitochondrial DNA Fragments and Heteroplasmy in Human Plasma. Sci Rep. 2016;6:36097.
76. Lam NY, Rainer TH, Chan LY, Joynt GM, Lo YM. Time course of early and late changes in plasma DNA in trauma patients. Clin Chem. 2003;49(8):1286-91.
77. Duvvuri B, Lood C. Cell-Free DNA as a Biomarker in Autoimmune Rheumatic Diseases. Front Immunol. 2019;10:502.
78. Cheng AP, Cheng MP, Gu W, Lenz JS, Hsu E, Schurr E, et al. Cell-Free DNA in Blood Reveals Significant Cell, Tissue and Organ Specific injury and Predicts COVID-19 Severity. medRxiv. 2020.
79. Scozzi D, Cano M, Ma L, Zhou D, Zhu JH, O'Halloran JA, et al. Circulating Mitochondrial DNA is an Early Indicator of Severe Illness and Mortality from COVID-19. bioRxiv. 2020.
80. Channappanavar R, Fehr AR, Vijay R, Mack M, Zhao J, Meyerholz DK, et al. Dysregulated Type I Interferon and Inflammatory Monocyte-Macrophage Responses Cause Lethal Pneumonia in SARS-CoV-Infected Mice. Cell host & microbe. 2016;19(2):181-93.
81. Thaker SK, Ch'ng J, Christofk HR. Viral hijacking of cellular metabolism. BMC Biology. 2019;17(1):59.
82. Cossarizza A, De Biasi S, Guaraldi G, Girardis M, Mussini C, for the Modena Covid-19 Working G. SARS-CoV-2, the Virus that Causes COVID-19: Cytometry and the New Challenge for Global Health. Cytometry Part A. 2020;97(4):340-3.
83. Wang Z, Du Z, Zhu F. Glycosylated hemoglobin is associated with systemic inflammation, hypercoagulability, and prognosis of COVID-19 patients. Diabetes Res Clin Pract. 2020;164:108214.
84. Thevarajan I, Nguyen THO, Koutsakos M, Druce J, Caly L, van de Sandt CE, et al. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. Nature medicine. 2020;26(4):453-5.
85. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nature medicine. 2020.
86. Shetty AK. Mesenchymal Stem Cell Infusion Shows Promise for Combating Coronavirus (COVID-19)-Induced Pneumonia. Aging and disease. 2020;11(2):462.
87. Wilk A, Rustagi A, Zhao N, Roque J, Martinez-Colon G, McKechnie J, et al. A single-cell atlas of the peripheral immune response to severe COVID-19. medRxiv; 2020.
88. Overmyer KA, Shishkova E, Miller IJ, Balnis J, Bernstein MN, Peters-Clarke TM, et al. Large-scale Multi-omic Analysis of COVID-19 Severity. medRxiv. 2020.
89. Wynants L, Van Calster B, Bonten MMJ, Collins GS, Debray TPA, De Vos M, et al. Prediction models for diagnosis and prognosis of covid-19 infection: systematic review and critical appraisal. BMJ. 2020;369:m1328.
90. Bondar G, Togashi R, Cadeiras M, Schaenman J, Cheng RK, Masukawa L, et al. Association between preoperative peripheral blood mononuclear cell gene expression profiles, early postoperative organ function recovery potential and long-term survival in advanced heart failure patients undergoing mechanical circulatory support. PloS one. 2017;12(12):e0189420.
91. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med. 2004;351(27):2817-26.
92. Deng MC, Eisen HJ, Mehra MR, Billingham M, Marboe CC, Berry G, et al. Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2006;6(1):150-60.
93. Costanzo MR, Dipchand A, Starling R, Anderson A, Chan M, Desai S, et al. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. The Journal of Heart and Lung Transplantation. 2010;29(8):914 - 56.
94. Deng MC. The evolution of patient-specific precision biomarkers to guide personalized heart-transplant care. Expert Review of Precision Medicine and Drug Development. 2020:1-13.
95. Evans RW, Williams GE, Baron HM, Deng MC, Eisen HJ, Hunt SA, et al. The economic implications of noninvasive molecular testing for cardiac allograft rejection. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2005;5(6):1553-8.

96. Deng MC. Multi-dimensional COVID-19 short-and long-term outcome prediction algorithm. Expert Review of Precision Medicine and Drug Development. 2020.
97. Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat Rev Cardiol. 2018;15(9):505-22.
98. Deng MC. A peripheral blood transcriptome biomarker test to diagnose functional recovery potential in advanced heart failure. Biomark Med. 2018;12(6):619-35.
99. Team CC-R. Severe Outcomes Among Patients with Coronavirus Disease 2019 (COVID-19) - United States, February 12-March 16, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(12):343-6.
100. Mahajan UV, Larkins-Pettigrew M. Racial demographics and COVID-19 confirmed cases and deaths: a correlational analysis of 2886 US counties. J Public Health (Oxf). 2020.
101. Fouad MN, Ruffin J, Vickers SM. COVID-19 is Out of Proportion in African Americans. This Will Come as No Surprise…. Am J Med. 2020.
102. Randall M, Kilpatrick KE, Pendergast JF, Jones KR, Vogel WB. Differences in Patient Characteristics between Veterans Administration and Community Hospitals: Implications for VA Planning. Medical Care. 1987;25(11):1099-104.
103. Health F. The Projected Economic Impact of the COVID-19 Pandemic on the US Healthcare System. Online; 2020.
104. Los Angeles County COVID-19 Data Information [Internet].2020 [cited October 30,2020]. Available from: http://publichealth.lacounty.gov/media/coronavirus/locations.htm.
105. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med. 1996;22(7):707-10.
106. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, et al. A model to predict survival in patients with end-stage liver disease. Hepatology (Baltimore, Md). 2001;33(2):464-70.
107. Levy WC, Mozaffarian D, Linker DT, Sutradhar SC, Anker SD, Cropp AB, et al. The Seattle Heart Failure Model: prediction of survival in heart failure. Circulation. 2006;113(11):1424-33.
108. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. Journal of chronic diseases. 1987;40(5):373-83.
109. Elixhauser A, Steiner C, Harris DR, Coffey RM. Comorbidity Measures for Use with Administrative Data. Medical Care. 1998;36(1).
110. Bush K, Kivlahan DR, McDonell MB, Fihn SD, Bradley KA. The AUDIT alcohol consumption questions (AUDIT-C): an effective brief screening test for problem drinking. Ambulatory Care Quality Improvement Project (ACQUIP). Alcohol Use Disorders Identification Test. Arch Intern Med. 1998;158(16):1789-95.
111. Excellence NIfHaC. COVID-19 rapid guideline: critical care in adults. Online; 2020.
112. Prevention CfDCa. National Death Index. Online; 2020.
113. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020;395(10223):497-506.
114. Yang R, Gui X, Zhang Y, Xiong Y. The role of essential organ-based comorbidities in the prognosis of COVID-19 infection patients. Expert Rev Respir Med. 2020:1-4.
115. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The Lancet. 2020;395(10229):1054-62.
116. Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD. Renin-Angiotensin-Aldosterone System Inhibitors in Patients with Covid-19. N Engl J Med. 2020.
117. Troughton RW, Frampton CM, Yandle TG, Espine EA, Nicholls MG, Richards AM. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. The Lancet. 2000;355(9210):1126-30.
118. Doust JA, Pietrzak E, Dobson A, Glasziou P. How well does B-type natriuretic peptide predict death and cardiac events in patients with heart failure: systematic review. BMJ. 2005;330(7492):625.
119. Aaronson KD, Schwartz JS, Chen TM, Wong KL, Goin JE, Mancini DM. Development and prospective validation of a clinical index to predict survival in ambulatory patients referred for cardiac transplant evaluation. Circulation. 1997;95(12):2660-7.
120. Ketchum ES, Moorman AJ, Fishbein DP, Mokadam NA, Verrier ED, Aldea GS, et al. Predictive value of the Seattle Heart Failure Model in patients undergoing left ventricular assist device placement. J Heart Lung Transplant. 2010;29(9):1021-5.

121. Sartipy U, Dahlstrom U, Edner M, Lund LH. Predicting survival in heart failure: validation of the MAGGIC heart failure risk score in 51,043 patients from the Swedish heart failure registry. Eur J Heart Fail. 2014;16(2):173-9.
122. Sartipy U, Goda A, Mancini DM, Lund LH. Assessment of a University of California, Los Angeles 4-variable risk score for advanced heart failure. J Am Heart Assoc. 2014;3(3):e000998.
123. Sartipy U, Goda A, Yuzefpolskaya M, Mancini DM, Lund LH. Utility of the Seattle Heart Failure Model in patients with cardiac resynchronization therapy and implantable cardioverter defibrillator referred for heart transplantation. Am Heart J. 2014;168(3):325-31.
124. Martínez-Sellés M, Teresa Vidán M, López-Palop R, Rexach L, Sánchez E, Datino T, et al. End-Stage Heart Disease in the Elderly. Revista Española de Cardiología (English Edition). 2009;62(4):409-21.
125. Flint KM, Matlock DD, Lindenfeld J, Allen LA. Frailty and the selection of patients for destination therapy left ventricular assist device. Circ Heart Fail. 2012;5(2):286-93.
126. Smits JM, de Vries E, De Pauw M, Zuckermann A, Rahmel A, Meiser B, et al. Is it time for a cardiac allocation score? First results from the Eurotransplant pilot study on a survival benefit-based heart allocation. J Heart Lung Transplant. 2013;32(9):873-80.
127. Kirklin JK, Naftel DC, Pagani FD, Kormos RL, Stevenson LW, Blume ED, et al. Sixth INTERMACS annual report: a 10,000-patient database. J Heart Lung Transplant. 2014;33(6):555-64.
128. Chyu J, Fonarow GC, Tseng CH, Horwich TB. Four-variable risk model in men and women with heart failure. Circ Heart Fail. 2014;7(1):88-95.
129. Cowger J, Sundareswaran K, Rogers JG, Park SJ, Pagani FD, Bhat G, et al. Predicting survival in patients receiving continuous flow left ventricular assist devices: the HeartMate II risk score. J Am Coll Cardiol. 2013;61(3):313-21.
130. Kormos RL, Teuteberg JJ, Pagani FD, Russell SD, John R, Miller LW, et al. Right ventricular failure in patients with the HeartMate II continuous-flow left ventricular assist device: incidence, risk factors, and effect on outcomes. J Thorac Cardiovasc Surg. 2010;139(5):1316-24.
131. Gardner RS, Ozalp F, Murday AJ, Robb SD, McDonagh TA. N-terminal pro-brain natriuretic peptide. A new gold standard in predicting mortality in patients with advanced heart failure. European heart journal. 2003;24(19):1735-43.
132. Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, et al. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature. 2011;479(7372):232-6.
133. Yoshikawa TT. Epidemiology and unique aspects of aging and infectious diseases. Clin Infect Dis. 2000;30(6):931-3.
134. Marioni RE, Shah S, McRae AF, Chen BH, Colicino E, Harris SE, et al. DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol. 2015;16:25.
135. Nilsson G, Hedberg P, Ohrvik J. White blood cell count in elderly is clinically useful in predicting long-term survival. J Aging Res. 2014;2014:475093.
136. Mochizuki K, Shintani R, Mori K, Sato T, Sakaguchi O, Takeshige K, et al. Importance of respiratory rate for the prediction of clinical deterioration after emergency department discharge: a single-center, case-control study. Acute Med Surg. 2017;4(2):172-8.
137. Boshuizen HC, Izaks GJ, van Buuren S, Ligthart GJ. Blood pressure and mortality in elderly people aged 85 and older: community based study. BMJ. 1998;316(7147):1780-4.
138. Guler R, Roy S, Suzuki H, Brombacher F. Targeting Batf2 for infectious diseases and cancer. Oncotarget. 2015;6(29):26575-82.
139. Roy S, Guler R, Parihar SP, Schmeier S, Kaczkowski B, Nishimura H, et al. Batf2/Irf1 induces inflammatory responses in classically activated macrophages, lipopolysaccharides, and mycobacterial infection. Journal of immunology. 2015;194(12):6035-44.
140. Neill T, Schaefer L, Iozzo RV. Decoding the Matrix: Instructive Roles of Proteoglycan Receptors. Biochemistry. 2015;54(30):4583-98.
141. Iozzo RV. Basement membrane proteoglycans: from cellar to ceiling. Nat Rev Mol Cell Biol. 2005;6(8):646-56.
142. Iozzo RV, Schaefer L. Proteoglycan form and function: A comprehensive nomenclature of proteoglycans. Matrix Biol. 2015;42:11-55.
143. Zhang J, Wang Y, Chu Y, Su L, Gong Y, Zhang R, et al. Agrin is involved in lymphocytes activation that is mediated by alpha-dystroglycan. FASEB J. 2006;20(1):50-8.
144. Mazzon C, Anselmo A, Soldani C, Cibella J, Ploia C, Moalli F, et al. Agrin is required for survival and function of monocytic cells. Blood. 2012;119(23):5502-11.

145. Chakraborty S, Lakshmanan M, Swa HL, Chen J, Zhang X, Ong YS, et al. An oncogenic role of Agrin in regulating focal adhesion integrity in hepatocellular carcinoma. Nat Commun. 2015;6:6184.
146. Fujiwara Y, Kondo HX, Shirota M, Kobayashi M, Takeshita K, Nakagawa A, et al. Structural basis for the membrane association of ankyrinG via palmitoylation. Sci Rep. 2016;6:23981.
147. Caba O, Prados J, Ortiz R, Jiménez-Luna C, Melguizo C, Alvarez PJ, et al. Transcriptional profiling of peripheral blood in pancreatic adenocarcinoma patients identifies diagnostic biomarkers. Dig Dis Sci. 2014;59(11):2714-20.
148. Baine MJ, Chakraborty S, Smith LM, Mallya K, Sasson AR, Brand RE, et al. Transcriptional profiling of peripheral blood mononuclear cells in pancreatic cancer patients identifies novel genes with potential diagnostic utility. PloS one. 2011;6(2):e17014.
149. Kariuki SN, Maranville JC, Baxter SS, Jeong C, Nakagome S, Hrusch CL, et al. Mapping Variation in Cellular and Transcriptional Response to 1,25-Dihydroxyvitamin D3 in Peripheral Blood Mononuclear Cells. PloS one. 2016;11(7):e0159779.
150. Abdul Aziz NA, Mokhtar NM, Harun R, Mollah MM, Mohamed Rose I, Sagap I, et al. A 19-Gene expression signature as a predictor of survival in colorectal cancer. BMC Med Genomics. 2016;9(1):58.
151. Thonnart N, Caudron A, Legaz I, Bagot M, Bensussan A, Marie-Cardine A. KIR3DL2 is a coinhibitory receptor on Sézary syndrome malignant T cells that promotes resistance to activation-induced cell death. Blood. 2014;124(22):3330-2.
152. Rajagopalan S, Long EO. KIR2DL4 (CD158d): An activation receptor for HLA-G. Front Immunol. 2012;3:258.
153. Rajagopalan S, Moyle MW, Joosten I, Long EO. DNA-PKcs controls an endosomal signaling pathway for a proinflammatory response by natural killer cells. Sci Signal. 2010;3(110):ra14.
154. Küçük C, Hu X, Gong Q, Jiang B, Cornish A, Gaulard P, et al. Diagnostic and Biological Significance of KIR Expression Profile Determined by RNA-Seq in Natural Killer/T-Cell Lymphoma. Am J Pathol. 2016;186(6):1435-41.
155. Ng SB, Selvarajan V, Huang G, Zhou J, Feldman AL, Law M, et al. Activated oncogenic pathways and therapeutic targets in extranodal nasal-type NK/T cell lymphoma revealed by gene expression profiling. J Pathol. 2011;223(4):496-510.
156. Shiio Y, Rose DW, Aur R, Donohoe S, Aebersold R, Eisenman RN. Identification and characterization of SAP25, a novel component of the mSin3 corepressor complex. Mol Cell Biol. 2006;26(4):1386-97.
157. Garofalo M, Di Leva G, Romano G, Nuovo G, Suh SS, Ngankeu A, et al. miR-221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. Cancer Cell. 2009;16(6):498-509.
158. Lui EL, Loo WT, Zhu L, Cheung MN, Chow LW. DNA hypermethylation of TIMP3 gene in invasive breast ductal carcinoma. Biomed Pharmacother. 2005;59 Suppl 2:S363-5.
159. Urban S, Dickey SW. The rhomboid protease family: a decade of progress on function and mechanism. Genome Biol. 2011;12(10):231.
160. Mann DL. Inflammatory mediators and the failing heart: past, present, and the foreseeable future. Circulation research. 2002;91(11):988-98.
161. Shoemaker R, Ryan J, inventorsMethods for diagnosing, treating, and monitoring chronic inflammatory response syndrome. USA2017.
162. Beer L, Nemec L, Wagner T, Ristl R, Altenburger L, Ankersmit H, et al. Ionizing radiation regulates long non-coding RNAs in human peripheral blood mononuclear cells. Journal of radiation research. 2016;58.
163. Tibshirani R. Regression shrinkage and selection via the lasso. Journal of the Royal Statistical Society: Series B (Methodological). 1996;58(1):267-88.
164. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics (Oxford, England). 2012;28(6):882-3.
165. Leek JT, Scharpf RB, Bravo HC, Simcha D, Langmead B, Johnson WE, et al. Tackling the widespread and critical impact of batch effects in high-throughput data. Nat Rev Genet. 2010;11(10):733-9.
166. Leek JT, Storey JD. Capturing Heterogeneity in Gene Expression Studies by Surrogate Variable Analysis. PLOS Genetics. 2007;3(9):e161.
167. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B (Methodological). 1995;57(1):289-300.
168. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A. 2003;100(16):9440-5.

169. Wisniewski N, Bondar G, Rau C, Chittoor J, Chang E, Esmaeili A, et al. Integrative model of leukocyte genomics and organ dysfunction in heart failure patients requiring mechanical circulatory support: a prospective observational study. BMC Med Genomics. 2017;10(1):52.
170. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9:559.
171. Fröhlich H. The GOSim package. 2015.
172. Fröhlich H, Speer N, Poustka A, Beißbarth T. GOSim – an R-package for computation of information theoretic GO similarities between terms and gene products. BMC Bioinformatics. 2007;8(1):166.
173. Committee on the Review of Omics-Based Tests for Predicting Patient Outcomes in Clinical Trials BoHCS, Board on Health Sciences Policy, Institute of Medicine. Evolution of Translational Omics: Lessons Learned and the Path Forward. In: Micheel CM, Nass SJ, Omenn GS, editors. Evolution of Translational Omics: Lessons Learned and the Path Forward. Washington (DC): National Academies Press (US); 2012. p. 33-63.
174. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the Treatment of Covid-19 - Preliminary Report. N Engl J Med. 2020.
175. WHO Solidarity Trial Consortium HP, Richard Peto, Quarraisha Abdool Karim, Marissa Alejandria, Ana Maria Henao Restrepo, Cesar Hernandez Garcia, Marie Paule Kieny, Reza Malekzadeh, Srinivas Murthy, Marie-Pierre Preziosi, Srinath Reddy, Mirta Roses, Vasee Sathiyamoorthy, John-Arne Rottingen, Soumya Swaminathan. Repurposed antiviral drugs for COVID-19 –interim WHO SOLIDARITY trial results. 2020.
176. Hui DS, Joynt GM, Wong KT, Gomersall CD, Li TS, Antonio G, et al. Impact of severe acute respiratory syndrome (SARS) on pulmonary function, functional capacity and quality of life in a cohort of survivors. Thorax. 2005;60(5):401-9.
177. Yang L, Tian D, Liu W. Strategies for vaccine development of COVID-19. Sheng Wu Gong Cheng Xue Bao. 2020;36(4):593-604.
178. Thanh Le T, Andreadakis Z, Kumar A, Gómez Román R, Tollefsen S, Saville M, et al. The COVID-19 vaccine development landscape. Nat Rev Drug Discov. 2020;19(5):305-6.
179. Ahmed SF, Quadeer AA, McKay MR. Preliminary Identification of Potential Vaccine Targets for the COVID-19 Coronavirus (SARS-CoV-2) Based on SARS-CoV Immunological Studies. Viruses. 2020;12(3).
180. Prompetchara E, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol. 2020;38(1):1-9.
181. Services USDoHaH. HHS Accelerates Clinical Trials, Prepares for Manufacturing of COVID-19 Vaccines. 2020.
182. Justice AC, Modur SP, Tate JP, Althoff KN, Jacobson LP, Gebo KA, et al. Predictive accuracy of the Veterans Aging Cohort Study index for mortality with HIV infection: a North American cross cohort analysis. J Acquir Immune Defic Syndr. 2013;62(2):149-63.
183. Justice AC, Tate JP. Strengths and Limitations of the Veterans Aging Cohort Study Index as a Measure of Physiologic Frailty. AIDS Res Hum Retroviruses. 2019;35(11-12):1023-33.
184. Hahn G, Lee S, Weiss ST, Lange C. Unsupervised cluster analysis of SARS-CoV-2 genomes reflects its geographic progression and identifies distinct genetic subgroups of SARS-CoV-2 virus. bioRxiv. 2020.
185. Gomez-Carballa A, Bello X, Pardo-Seco J, Perez Del Molino ML, Martinon-Torres F, Salas A. Phylogeography of SARS-CoV-2 pandemic in Spain: a story of multiple introductions, micro-geographic stratification, founder effects, and super-spreaders. Zool Res. 2020:1-16.
186. Becerra-Flores M, Cardozo T. SARS-CoV-2 viral spike G614 mutation exhibits higher case fatality rate. Int J Clin Pract. 2020:e13525.
187. Majumdar P, Niyogi S. ORF3a mutation associated higher mortality rate in SARS-CoV-2 infection. Epidemiol Infect. 2020:1-16.
188. Paris KA, Santiago U, Camacho CJ. On the molecular mechanism of SARS-CoV-2 retention in the upper respiratory tract. bioRxiv. 2020.
189. Dehury B, Raina V, Misra N, Suar M. Effect of mutation on structure, function and dynamics of receptor binding domain of human SARS-CoV-2 with host cell receptor ACE2: a molecular dynamics simulations study. J Biomol Struct Dyn. 2020:1-15.
190. Konala VM, Adapa S, Naramala S, Chenna A, Lamichhane S, Garlapati PR, et al. A Case Series of Patients Coinfected With Influenza and COVID-19. J Investig Med High Impact Case Rep. 2020;8:2324709620934674.
191. Kondo Y, Miyazaki S, Yamashita R, Ikeda T. Coinfection with SARS-CoV-2 and influenza A virus. BMJ Case Rep. 2020;13(7).

192. Ehrlich H, Boneva D, Elkbuli A. The intersection of viral illnesses: A seasonal influenza epidemic amidst the COVID-19 pandemic. Ann Med Surg (Lond). 2020;60:41-3.
193. Pinky L, Dobrovolny HM. SARS-CoV-2 coinfections: Could influenza and the common cold be beneficial? J Med Virol. 2020.
194. Antony SJ, Almaghlouth NK, Heydemann EL. Are coinfections with COVID-19 and influenza low or underreported? An observational study examining current published literature including three new unpublished cases. J Med Virol. 2020.
195. Olsen SJ, Azziz-Baumgartner E, Budd AP, Brammer L, Sullivan S, Pineda RF, et al. Decreased Influenza Activity During the COVID-19 Pandemic - United States, Australia, Chile, and South Africa, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(37):1305-9.