Update on the Virology & Immunology of COVID/SARS-CoV-2

D.E. Bramblett, PhD
and
Gabor Szalai, PhD

OBJECTIVES

1. **Compare and contrast SARS-COV2 variants of concern (VOCs) that are circulating in the United States impact of VOC mutations on vaccine effectiveness and diagnostic success/failure**
2. **Describe the immunopathology of SARS-COV2 infections.**
3. **Describe what is currently known about the lasting symptoms and long-term impacts of COVID19.**

COVID-19 TIMELINE

2019

SARS-Cov2 emerged in December 2019 in China and spread across the globe

Vaccine Development

2020

- February EUA for SARS-CoV2 diagnostic test
- March 11 WHO declares pandemic
- March 17 first trial of Moderna Vaccine
- April 10 US surpasses Italy in COVID deaths
- Illumination of racial disparities: African American and Navajo Nation
- April 30 Operation Warp Speed
- May 28 US Death-toll 100,000
- September 22 Death toll over 200,000
- December 14, First vaccine employed outside of a clinical trial
- December 14 more than 1millian people in US vaccinated

59 long-COVID publications in Pubmed

2021

- January 18 Death toll over 400,000
- Brazil variant in Minnesota
- March US surpasses 100 million vaccinations
- April 21 200 million vaccinations
- June 1 Delta variant becomes dominate strain in US
- July Mask mandates due to up swing in cases
- November 26 Omicron variant reported in S. Africa
- November 29 , booster shot recommended for all over 18 YO

998 Long-COVID publications in Pubmed

2022

- Omicron VOC is the primary circulating variant

Globally 546, 074,816 cases
6, 333,727 deaths
US 87,335,820 cases
1,0170155 deaths

- Recognition of Long-COVID as a major public health concern

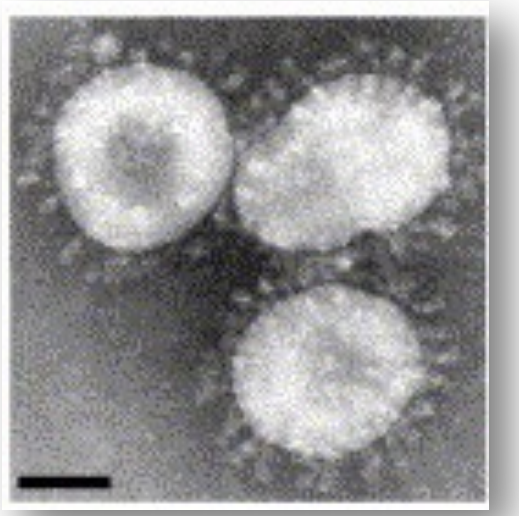
1760 long-COVID publications in Pubmed so far

US unemployment rate (14.7%) highest since the great depression

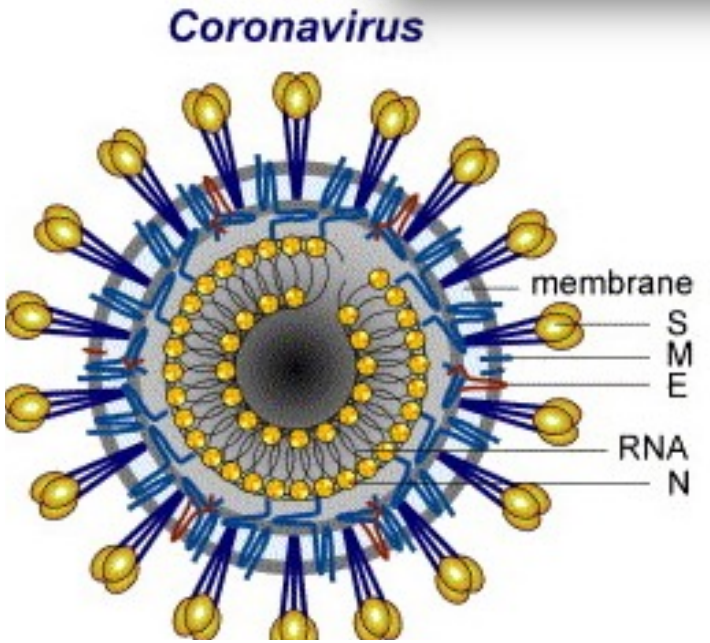
COVID VARIANT OF CONCERN

- VOCs: Alpha (B1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.17.2), Epsilon (B.1.427), Omicron (B.1.1.529)
- Omicron was declared a variant of concern November 30, 2021, after it was detected in South Africa and then the UK.
- Omicron is the predominate variant in the United States at this time and has been detected in every state and territory.
  - Concerning spike protein substitutions
    - Reduced susceptibility to available monoclonal antibody therapeutics
    - Reduced neutralization by convalescent and vaccine sera
    - Prone to immune escape and break through infections
    - Potential increased transmissibility compared to the Delta variant
    - Omicron infection generally causes less severe disease than infection with prior variants

CORONAVIRUS STRUCTURE



- Spherical virion (125nm) and a helical nucleocapsid
- Most notable common feature is a fringe of widely spaced, club-shaped spikes that project from the surface.
  - Described as giving the viral particle a solar corona
- Genome composition: ss (+) RNA of ~30kb
- Four main structural proteins:
  - **S** – spike protein, mediates cell entry
  - M- membrane protein
  - E- Envelope
  - N-nucleocapsid



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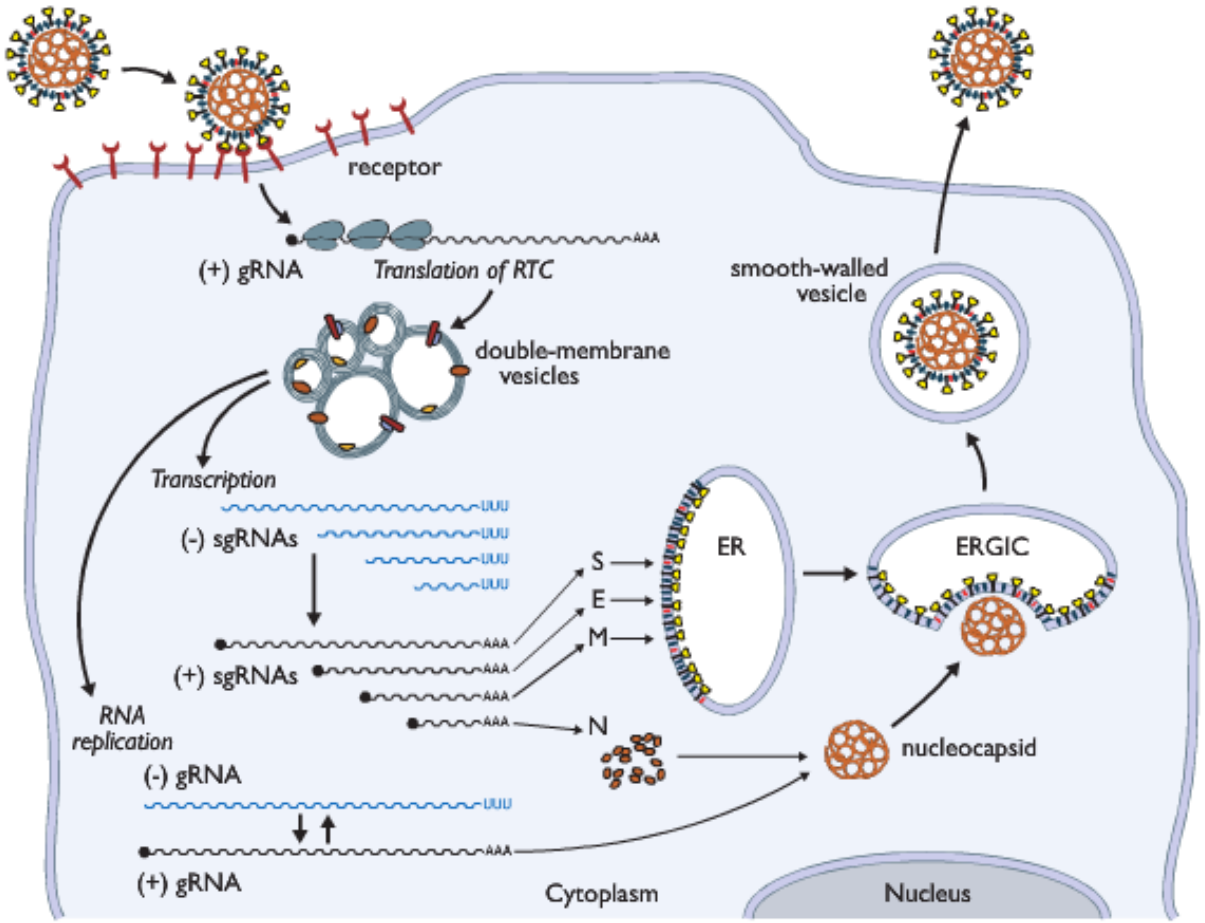
CORONAVIRUS REPLICATION

1. S- spike protein binds to angiotensin-converting enzyme 2 (ACE2) receptor on the cell surface.
2. The Coronavirus RNA genome is translated into a large polyprotein which is proteolytically cleaved to produce non-structural proteins important for replication- RNA dependent RNA polymerase (**L**)
3. Transcription of mRNAs and replication of the genome by **L** occurs in the cytoplasm associated with membrane vesicles.
4. Genome is bound by N protein to form the spherical nucleocapsid.
5. The nucleocapsid associates with rough ER membrane and buds into the lumen of the ER where it acquires envelop proteins and S spike protein.
6. The virus is assembled in the ER and then exported through the Golgi apparatus to the cell membrane where exocytosis occurs.



Fields Virology Sixth Edition Volume 1 Chapter 28,

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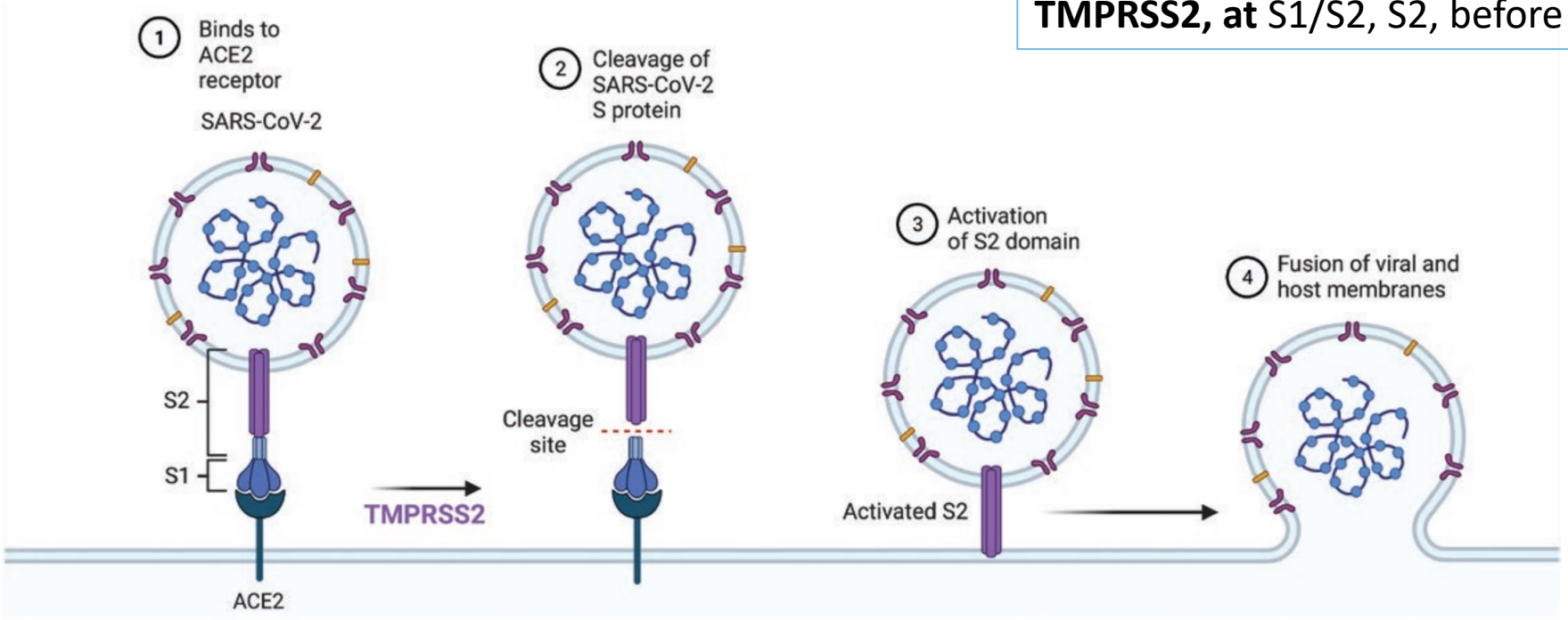
- Spike glycoprotein **S** facilitates entry into the cell by binding angiotensin-converting enzyme 2 (ACE2) receptor on the cell surface
- The S protein consists of two subunits
  - S1 binds to ACE2 and
  - S2 facilitates membrane-fusion

S is processed by a cellular plasma membrane–associated type II transmembrane serine protease, **TMPRSS2, at** S1/S2, S2, before membrane fusion.



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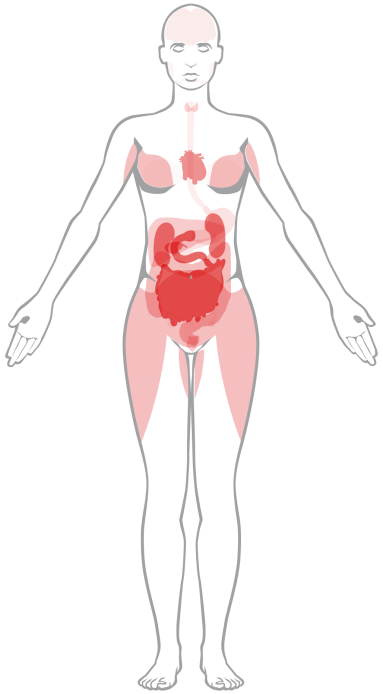
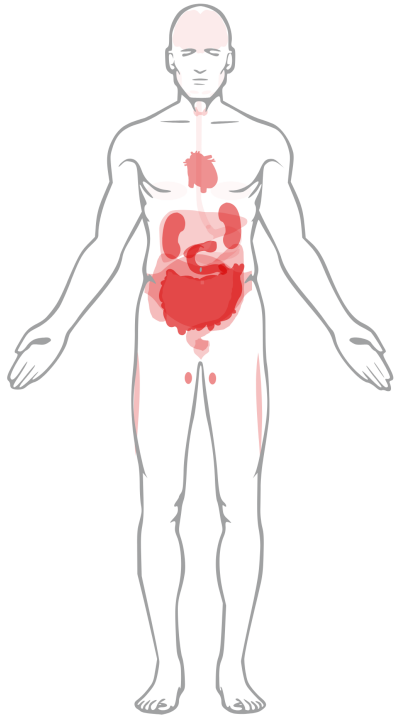
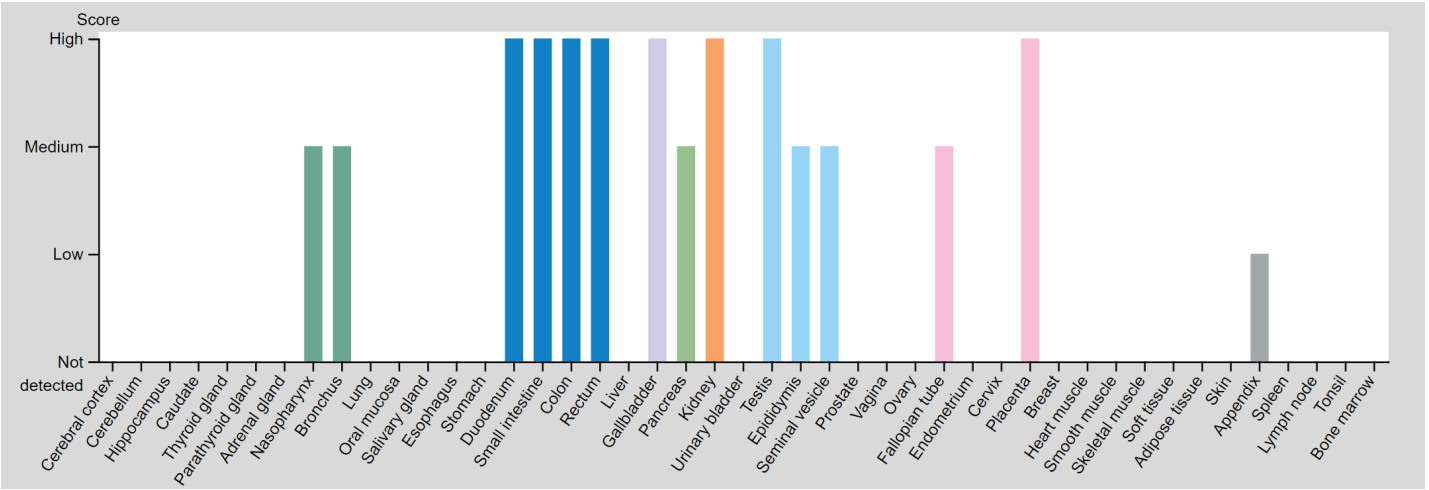
ACE2 EXPRESSION IN THE BODY







Adipocytes

Breast adipocytes

Enterocytes of GI tract

Cardiomyocytes

Hepatocytes

Alveolar cells type 2

Stomach

Spermatogonia

Sertoli cells

Leydig cells

Proximal tubular cells of the kidney

Macrophage of many tissues

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CURRENT DEFINITION OF LONG COVID

- **Long COVID**- the presence of symptoms far longer than it would be expected following recovery from SARS-Cov2
- **"Long hauler"**- a person suffering from long COVID
- Proposed timeline:
  1. Potentially infection related symptoms 4-5 weeks after symptoms onset
  2. **Acute post-COVID** symptoms week 5 to weeks 12 after
  3. **Long post-COVID** symptoms lasting from weeks 12 to 24 weeks after
  4. **Persistent post-COIVD** lasting more than 24 weeks after

} Chronic post COVID

- Importantly there is a need for more studies of acute post –COVID and Long post-COVID manifestations in patients with asymptomatic or mild COVID-19 disease.

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FREQUENCY

- According to meta-analyses 31-69% of COVID-19 survivors will develop at least one post-COVID symptom.
- More common in female patients and patients of advanced age.
- Increasing evidence indicated that long-COVID can develop regardless of the severity of the original symptoms.
- Vaccination status may influence frequency

GS

POST-INFECTION SYNDROMES ARE NOT NEW

Acute infections that promote uncontrolled inflammation may lead to chronic consequences

- Post infective fatigue syndrome is defined as fatigue, musculoskeletal pain and neurocognitive disturbance during 12 months after acute infection by **Epstein Barr (EBV)**, **Ross river virus** and **Q-fever** *(C . burnetti).*
- Post-**Ebola** syndrome-symptoms that develop or persist after discharge: musculoskeletal pain, headache, ocular problems.
- Guillain-Barré (ascending polyneuropathy) has been associated with **CMV** post transplant, **EBV**, **Zika** virus and *Campylobacter jejuni*
- Post infectious irritable bowel syndrome following resolution of bacteria and viral infections including **Norovirus.**
- Neurological complications following infections with **Influenza A (H1N1)** including Guillain-Barré , polyneuropathy and seizures.
- **Chikungunya virus**- Chronic inflammatory Rheumatism occurs three months after the acute illness: Polyarthralgia, Fibromyalgia, Adhesive capsulitis and plantar fasciitis.

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LATENT VIRAL ACTIVATION DURING COVID AND AFTER

- Reactivations of Herpes Simplex-1 (HSV-1), Varicella Zoster, EBV, CMV and HHV-6 strongly associated with COVID-19
- HSV-1 reactivation in critically ill COVID-19 patients was associated with increased mortality
- Case reports of Acute Retinal Necrosis in patient who had recovered from SARS-CoV-2 infection due to reactivation of herpes simplex virus.
- Significant number of long COVID-19 patients have been reported to have EBV reactivation.

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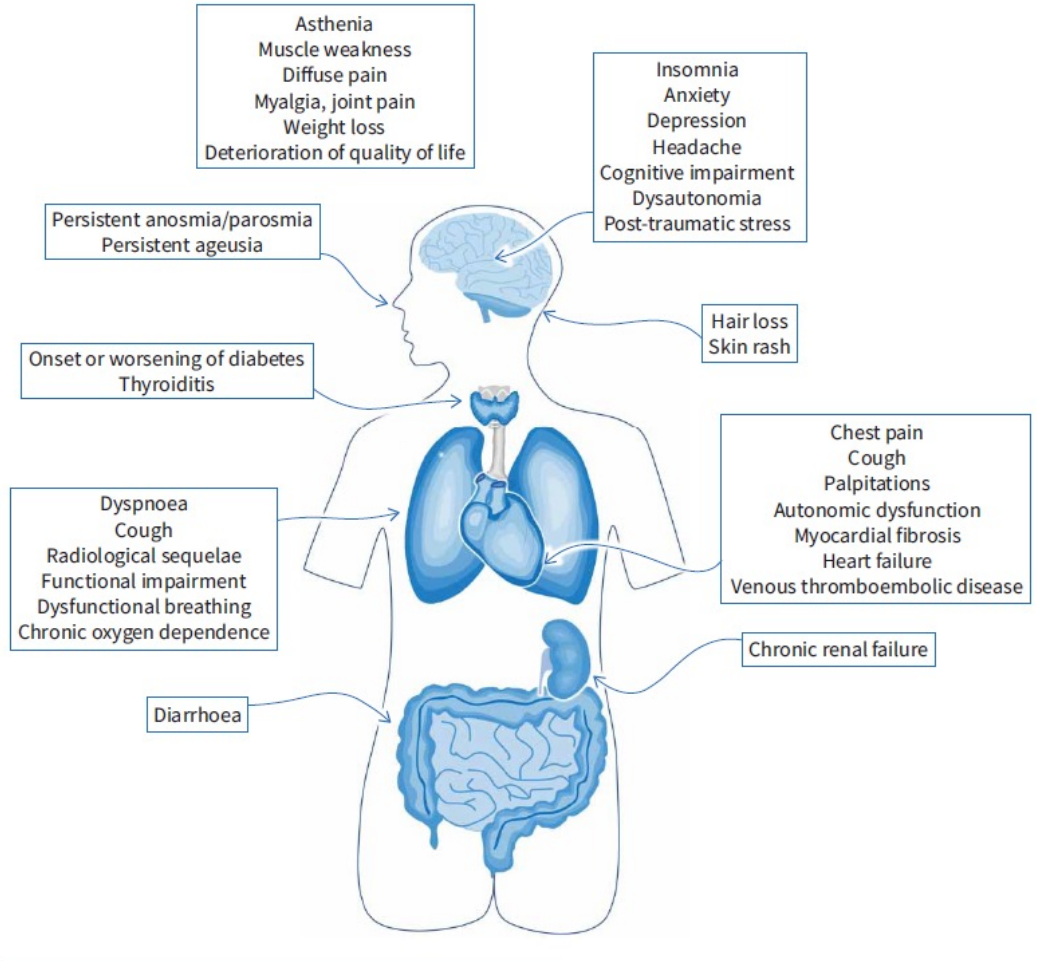
POST-COVID SYMPTOMOLOGY

- **Multisystem:** hear, brain, kidney, pancreas and digestive system.
- Post acute sequalae of COVID:
  - Neurological (25%)(Memory loss (brain fog) or cognitive impairments
  - Gastrointestinal (GI) distress,(9%)
  - Respiratory symptoms (42%), including shortness of breath, dyspnea, cough(25%)
  - Anosmia/dysgeusia (18%)
  - Endocrine
- Other Clinical signs can include fatigue, myalgia, diffuse pain, head aches, anxiety/depression.
- Long COVID symptoms can be a diagnostic challenge for rheumatologist given the similar symptoms with systemic autoimmune rheumatic diseases (SARDs)



EUROPEAN RESPIRATORY REVIEW
COVID-19 REVIEW
D. MONTANI ET AL.

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PREDISPOSITION FOR POST ACUTE SEQUALAE OF COVID?

- Both severe and mild cases are associated with PASC
- There are significant correlations of PASC with certain factors measured at the time of diagnosis
  - type 2 diabetes (Cough, Resp viral, Fatigue)
  - female patients with COPD (Shortness of breath and Neurological)
  - SARS-COV2 RNAemia
  - Epstein-Barr viremia (EBV)
  - Specific Auto-antibodies
  - HLA subtypes

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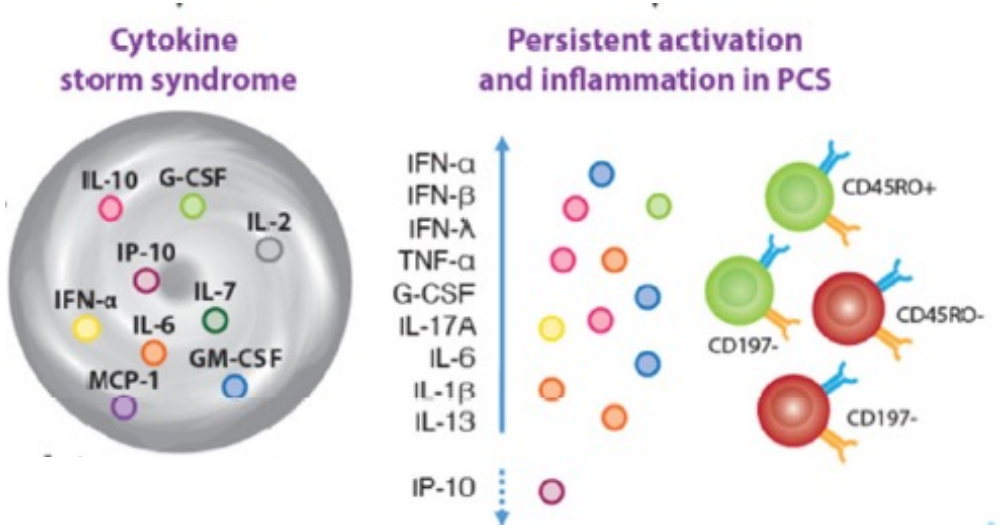
PLASMA MARKERS THAT POTENTIALLY ANTICIPATE PASC

- Low cortisol levels
- Increase in proteins associated with circadian sleep/wake cycle negative regulation
- Reactivation of latent EBV
- SARS-CoV-2 RNAemia
- Auto antibodies
- Sustained levels of inflammatory cytokines
- Sustained levels of CD8+ memory T-cells
- Plasma protein CTSL which is also predicative of patient mortality in certain cohorts.



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AUTO ANTIBODIES CORRELATED WITH CERTAIN PASC

- Auto antibodies (44%) likely predating SARS-CoV infection with subclinical conditions
  - **Anti-IFN-α2**, five different anti-nuclear autoAb (ANAs) associated with systemic-lupus erythematosus (SLE)
    - Ro/SS-A , La/SS-B. U1-snRNP, Jo-1 and P1
- Auto antibodies (Anti-IFN-α2 and anti-nuclear) may be correlated with SARS-CoV-1 IgGs
- Certain PASC correlated with specific autoAbs
  - Patients with neurological PASC exhibited higher levels of antiSARS-CoV-2 nucleocapsid protein IgG
  - Patients with GI- PASC and Patients with sputum production is associated with several autoAbs .
  - Anti-IFN-α2 is strongly associated with respiratory-viral PASC
- In summary AutoAb levels may be anticipatory biomarkers for certain PASC

Noteworthy, such autoantibodies became negative after **treatment with intravenous immunoglobulin (IVIG),** Although the mechanisms for latent autoimmunity emergence in this syndrome are unclear, a recent report suggested that excessive inflammation drives tissue destruction and **exposure to cryptic epitopes**, which may explain the emergence of several autoantibodies with diverse specificities in this condition. **Anti-apolipoprotein** A-1 IgG autoantibodies have been found in 93% of patients at one month and declined to 15% at 12 months after COVID-19. Their presence was associated with PASC.

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GASTROINTESTINAL PASC OR LONG-COVID

- High expression of the ACE2 receptor on brush border of the small intestinal mucosa likely leads to GI symptoms in 15-50% of patients during acute COVID-19.
- PASC Involves bystander activation of CMV-specific T cells
- Non-specific T cell activation may also contribute to GI PASC
- Long-COVID patients report a range of symptoms including loss of appetite, abdominal pain, nausea, wight loss, altered bowel motility patterns and new or exacerbated irritable bowel syndrome

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IMPACT ON THE ENDOCRINE SYSTEM

- Ace2 is expressed by pancreatic islet cells and SARS-CoV2 has been found to cause islet cell injury resulting in acute type 1 diabetes mellitus.
  - New onset diabetes and ketoacidosis have been reported in patients with acute COVID-19
- Persistent glycemic abnormalities have been found is a significant number of patients for at least 2 months after COVID-19.
- Other endocrine abnormalities that have been reported after the resolution of the SARS-coV2 infection:
  - Subacute thyrotoxicosis,
  - Hashimoto's thyroiditis,
  - Graves disease.

Certain amino acids of SARS-CoV-2 mimic the host adrenocorticotropic hormone (ACTH), leading to cross-reactivity probably by molecular mimicry. Consequently, antibodies directed toward the virus will ultimately destroy circulating ACTH. Therefore, patients with severe COVID-19 could be more prone to develop critical illness-related corticosteroid insufficiency. The hypothalamus-pituitary axis might be affected by the SARS-CoV-2 infection.

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COVID IMPACT ON THE NEUROLOGICAL FUNCTION

- Acute SARS-CoV2 infection can include a broad range of neurological and neuropsychiatric problems :
  - olfactory and gustatory impairments
  - encephalopathy and delirium, stroke,
  - neuromuscular complications stress reactions and psychoses.
- Complex pathophysiology of SARS-CoV2 on the brain
  - Direct viral neuronal damage,
  - Neuroinflammation
  - Rupture of blood brain barrier
  - Microvasculitis
  - Hypoxia

In general population studies, high levels of circulating inflammatory markers such as C-reactive protein (CRP), IL-6, and TNF-α have been linked to depressive symptoms. IL-6, in particular, has been linked to central and peripheral nervous system disorders. Tiredness, sleeping troubles, sadness, and anxiety may be caused by persistent IL-6 dysregulation in PASC.

A comprehensive analysis of major depressive disorder biomarkers in PASC found elevated IL-6, IL-1β, TNF-α, IFN-α, IL-10, IL-2, CRP, MCP-1, serum amyloid A, and kynurenine pathway metabolite.

DB/GS

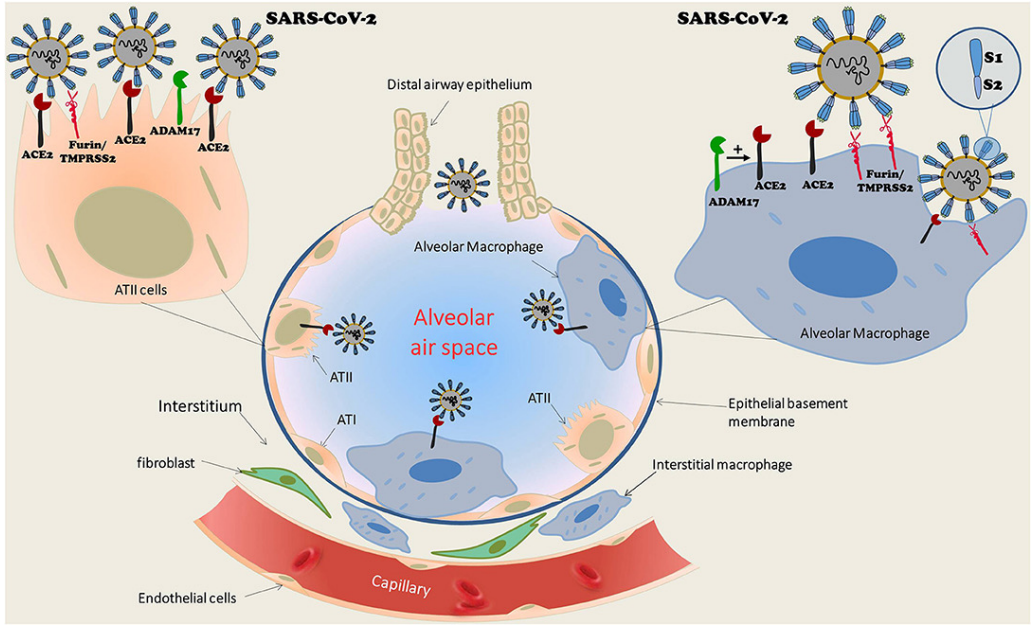
NEUROLOGICAL LONG-COVID

- Long-COVID is particularly associated with neurological , neurodegenerative, psychiatric and cognitive problems, especially brain fog.
  - Chronic malaise, sleep abnormalities, Chronic headache and olfactory and gustatory impairments
  - First manifestations of neurodegenerative disorders, psychiatric disorders , depression , anxiety and post-traumatic stress disorder are reported in patients with Post-COVID syndrome
- Long-COVID and the brain Cognitive Complaints (brain fog) and dysfunction of the **cingulate cortex**
  - Demonstrated by Fluorodeoxyglucose (FDG) Positron Emission Tomography (PET) which showed hypometabolic regions of the cingulate cortex
  - Anterior and posterior cingulate cortex are implicated in emotions, memory, depression and decision of action.

DB

RESPIRATORY LONG-COVID

- Long term pulmonary complications include: dyspnea, ventilator dependence, Oxygen dependence, Pulmonary function test (PFT) abnormalities and fibrotic lung .
- Most of the common manifestations of acute COVID resolve within a few days of discharge but in some patients lung changes evolve to pulmonary fibrosis detected by CXR after 3 months.
  - Direct Viral entry leading to cell death, causes increase in proinflammatory cytokines, recruitment of lymphocytes, macrophage and neutrophils which recruit fibroblast resulting in fibrosis.
  - Tissue-resident Alveolar macrophage have key roles in clearance of surfactant and damages cells (homeostatic fuction) and are crucial to maintenance of lung tissue integrity. They depend on production of CSF-1 by alveolar type II lung epithelial cells (AT2).
  - AT2 cells express ACE2 and are key targets of SARS-CoV-2. So, it is possible that damage to AT2 cells contributes to early reduction of the homeostatic function of tissue –resident macrophages in the lung.
  - ACE2 is also expressed in alveolar macrophage and while macrophages play an important role in antiviral defense mechanisms, in the case of SARS-CoV. they may also serve as a Trojan horse
  - The fibrotic state is probably drive by transforming growth factor-β and epithelium derived IL-6



Abassi Zaid, Knaney Yara, Karram Tony, Heyman Samuel N. he Lung Macrophage in SARS-CoV-2 Infection: A Friend or a Foe? Frontiers in Immunology VOL 11 2020 https://www.frontiersin.org/article/10.3389/fimmu.2020.01312

DB

FOUR POSSIBLE IMMUNE –ENDOTYPES THAT EVOLVE DURING POST-COVID MIGHT BE PREDICTABLE AT THE ONSET OF DISEASE

- Type 1- enriched with Th1-like signature in CD4+ T cells, M1-like proinflammatory signatures in monocytes and memory B-cells.
- Type2- enriched for Th2-like CD4+ T cells M2-like (anti-inflammatory) monocyte signatures and plasma B cells signatures
- Intermediate-transitional immune status between types 1 and type 2
- Naïve – naive like T and B cell signature and resting NK cell signatures

Group	
Type 1	More PASC
Type 2	More PASC; Highest Hospitalization
Intermediate	More PASC: EBV reactivation and Anti-IFN-α2 autoAb
Naive	Less PASC ; Anosmia/Dysgeusia

DB/GS

PASC-ANTICIPATING BIOLOGICAL FACTORS CAN NOT ONLY BE ANTICIPATORY BUT ALSO DEVELOPING TREATMENTS

- Antivirals given early may not only treat COVID-19 but also reduce later PASC
- Cortisol replacement in patients with respiratory-viral PASC
- Therapies controlling hyperinflammation in the acute stage may influence PASC based on PASC-anticipating AutoAbs
- Correlation between SARS-CoV-2 IgG and AutoAbs may suggest that patients with elevated autoAb levels are more susceptible to break though infections.

DB

AUTOIMMUNITY VERSUS AUTOINFLAMMATION

The term “autoinflammatory” was first derived in 1999 to describe episodes of recurrent inflammation characterized by fever without evidence of infectious conditions. These were the familial Mediterranean fever and TNF receptor-associated periodic syndrome

The main mechanisms is overactivation of inflammation (primarily related to type I IFNs) and loss of inhibition in some signaling pathways, including the NF-κB.

Autoimmune diseases: T and B cells are autoreactive against specific antigens

Autoinflammatory diseases: there is no specific autoreactivity. The predominance of the immune response is determined by the activation of signaling pathways mainly related to the production of IL-1β and IFN-α.

Currently, there is evidence supporting PASC lying between the two conditions.

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VACCINE PROTECTION AGAINST POST-COVID

In the first 12 weeks after vaccination, the odds of reporting PASC decreased by 12.8%,

Analyzing large national healthcare databases, vaccination protection from PASC was about 15% six months after infection

Most pronounced in recipients of BNT162b2 and mRNA-1273 vaccines compared to Ad26.COV2, probably due to differential adjuvant effects.

Because vaccination only partly reduces the risk of PASCS, relying on it as the sole strategy to reduce the risk of long-term health consequences could be inefficient

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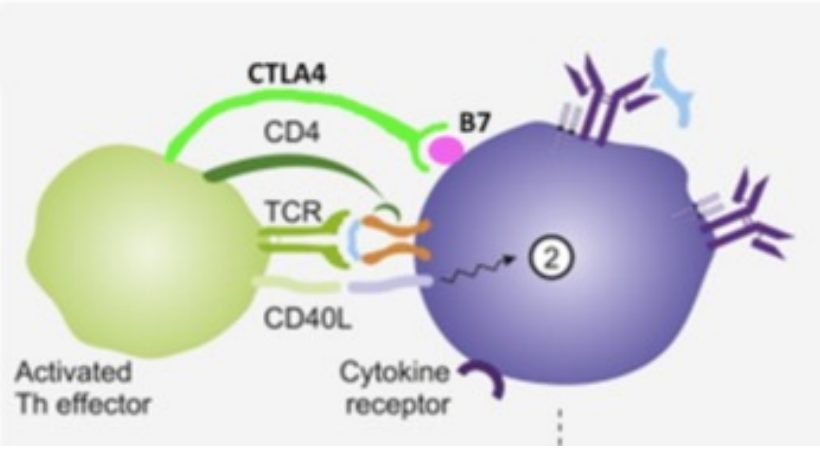
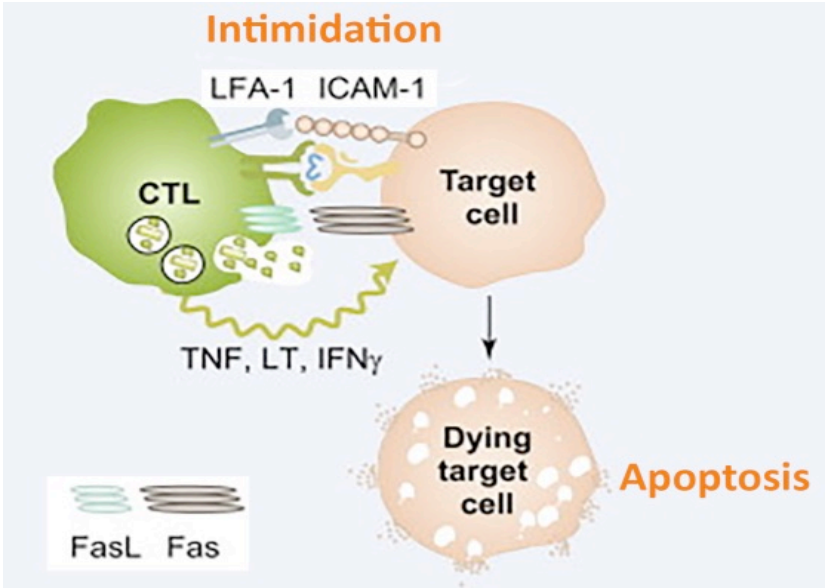
GENETIC ASSOCIATION WITH MHC ALLELES



Some HLA-DRB1 alleles are associated with enhanced or muted post natural infection and vaccination T-cell responses.



HLA A*02, B*35, and C*04 alleles were found to be associated with PASC

T cell activation via superantigen-like motif of SARS-CoV-2 spike glycoprotein

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POSSIBLE PREVENTION POST COVID BY EARLY MANAGEMENT

- Cortisol
- Blockade of cytokines such as IL6, TNFα,
- Immunomodulatory molecules like CD20 (B cell marker)
- IVIG to lessen the acute disease severity - latent autoimmunity may be treatable and reversible after the acute onset of the disease

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DATA IS LACKING

- Patient data before 2019
- Is COVID just an exacerbation of previous sub clinical diseases

Therapies for Long COVID in non-hospitalised individuals: from symptoms, patient-reported outcomes and immunology to targeted therapies (The TLC Study)

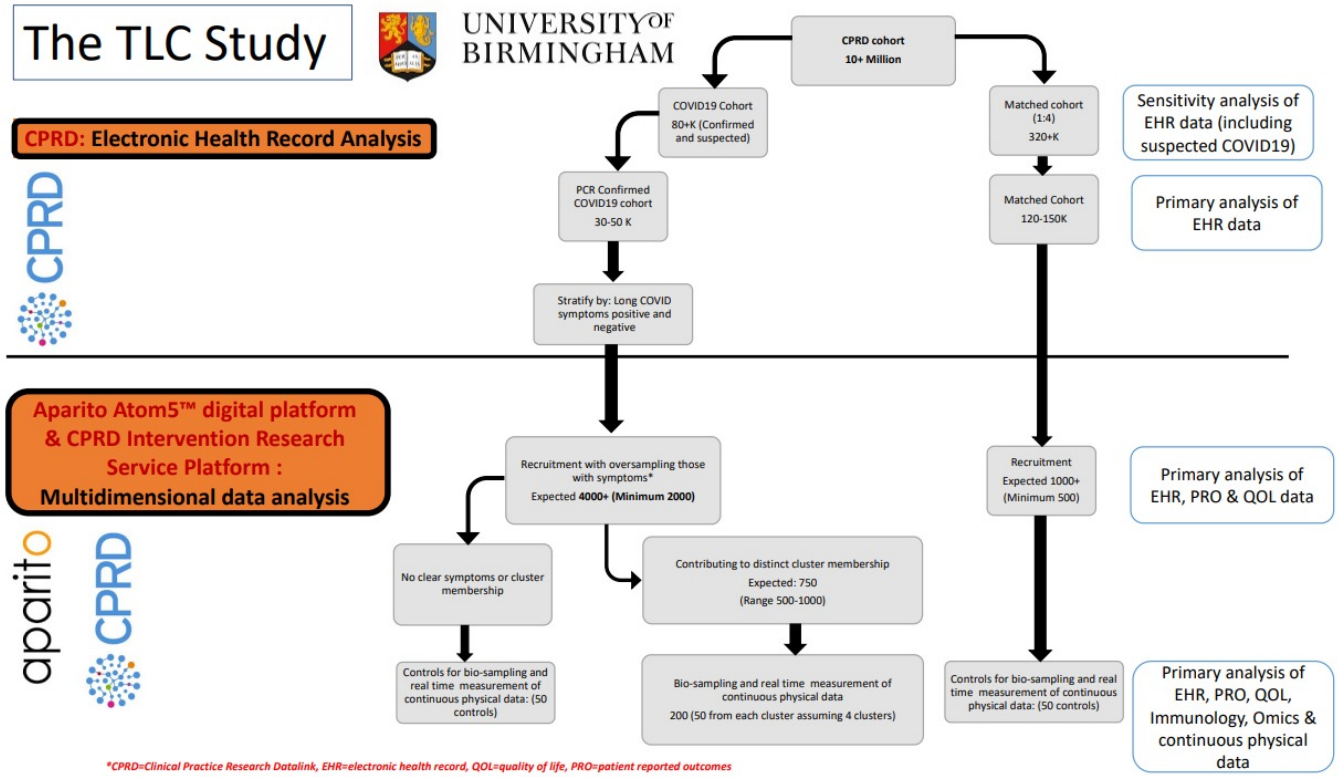

Figure 2 Study flow chart. CPRD, Clinical Practice Research Datalink; EHR, electronic health record; QOL, quality of life; PRO, patient-reported outcome.

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Thank You

Questions?

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