Pathogenesis of Long COVID: Organicity and Mechanisms

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Financial Relationships With Ineligible Companies (Formerly Described as Commercial Interests by the ACCME) Within the Last 2 Years

Dr Paredes served on advisory boards for Gilead Sciences, Inc, Pfizer, Inc, Roche Therapeutics, MSD, GSK, ViiV Healthcare, Eli Lilly and Company, and Atea Pharmaceuticals, Inc. He has had research grants paid to his institution from MSD, ViiV Healthcare, Gilead Sciences, and PharmaMar. (Updated June 8, 2023)

Slide 2

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Learning Objectives

After attending this presentation, learners will be able to:

- Understand the important and persistent damage on multiple body organs and tissues associated with Long COVID
- Review the current understanding of the pathogenesis of the Long COVID syndrome
- Identify current knowledge gaps and research priorities in Long COVID pathogenesis

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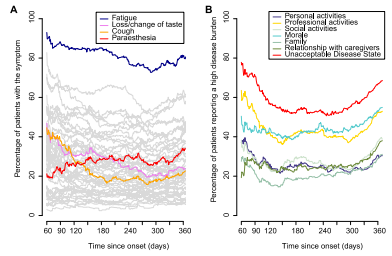
Long COVID is here to stay



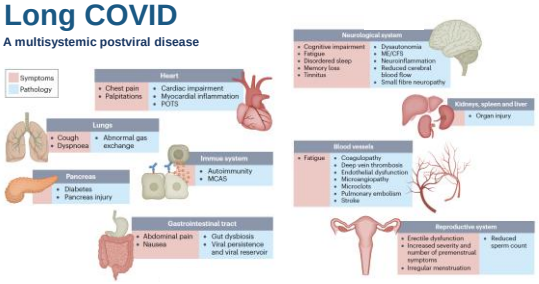
Tran V-H et al. Course of post COVID-19 disease symptoms over time in the ComPaRe long COVID prospective e-cohort. Nature Comms 2022

Slide 4

4

Long COVID

A multisystemic postviral disease



Davis HE et al. Long COVID: major findings, mechanisms and recommendations. Nature Microb Rev 2023

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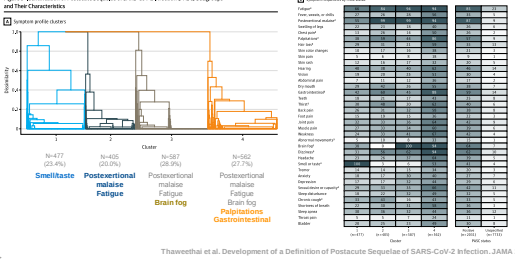
PACS subsyndromes

RECOVER Study

Figure 3. Identification of Postacute Sequelae of SARS-CoV-2 Infection (PASC) Subgroups and Their Characteristics



Thaweethai et al. Development of a Definition of Postacute Sequelae of SARS-CoV-2 Infection. JAMA 2023

Slide 6

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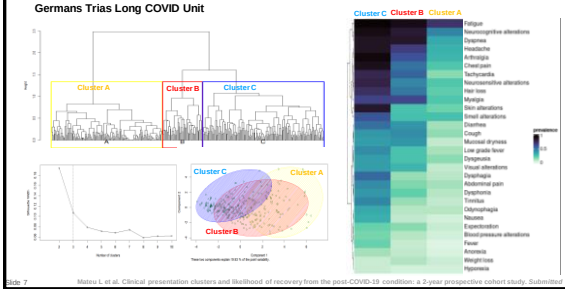
Long COVID subsyndromes

Germans Trias Long COVID Unit

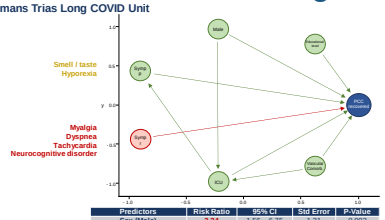


Mateu L et al. Clinical presentation clusters and likelihood of recovery from the post-COVID-19 condition: a 2-year prospective cohort study. *Submitted*

7

Who recovers from Long COVID?

Germans Trias Long COVID Unit



Predictors	Risk Ratio	95% CI	Std Error	P-Value
Sex (Male)	3.24	1.56 – 6.75	1.21	0.002
ICU admission	8.3	2.84 – 24.27	4.54	<0.001
Educational level	2.34	1.08 – 5.09	0.93	0.032
Vascular comorbidities	1.99	1.01 – 3.92	0.69	0.048
Symptoms_p (Protective)*	2.53	1.16 – 5.51	1.01	0.02
Symptoms_r (Risk)*	0.28	0.14 – 0.57	0.1	<0.001

Only **7.6%** recover over 2 years

All except 2 subjects, from

Mateu L et al. Clinical presentation clusters and likelihood of recovery from the post-COVID-19 condition: a 2-year prospective cohort study. *Submitted*

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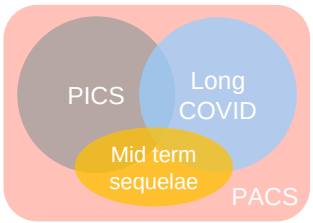
PACS, PICS, Sequelae … and Long COVID

- Well known
- Virus not needed
- Always post ICU
- Direct insult of MV, ECMO, immobilization, shock, etc
- Slowly recovers with possible long-term sequelae



- New syndrome
- Post viral
- Even in mild COVID-19
- Complex immunopathogenesis
- Lasts for years
- No cure (so far)
- No biomarkers

- Smell/taste, thirst, anxiety, etc
- Clear pathogenesis or highly unspecific
- Eventually resolve

Mateu L and Paredes R, personal communication

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Organ damage

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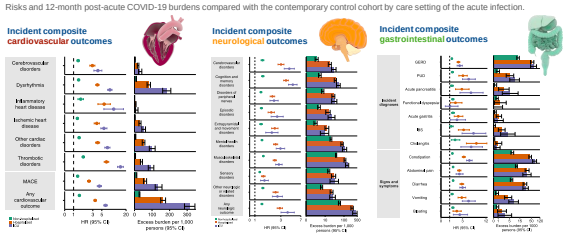
Long-term outcomes of COVID-19

Risks and 12-month post-acute COVID-19 burdens compared with the contemporary control cohort by care setting of the acute infection.



Xie et al. Long-term cardiovascular outcomes of COVID-19 . Nature Medicine 2022. Xu et al. Long-term neurologic outcomes of COVID-19 . Nature Medicine 2022. Xu et al. Long-term neurologic outcomes of COVID-19 . Nature Communications 2023

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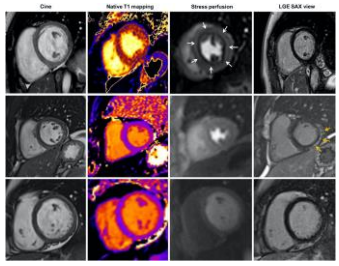
Chest pain in Long COVID

Germans Trias Long COVID Unit

186 patients prospectively evaluated
51 (27%) had **persistent chest pain**
→ First 10 shown here



Subendocardial ischaemia with normal coronary arteries **(n=5)**

Myocarditis-like late gadolinium enhancement **(n=2)**

Normal CMR findings **(n=3)**

Vallejo et al European Heart Journal , 2021; Vallejo et al. Rev Esp Card 2021

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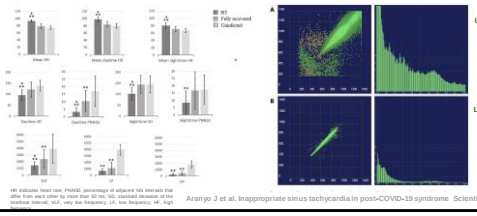
Inappropriate synus tachicardia

Germans Trias Long COVID Unit

- In **40/200 (20%)** prospective Long COVID patients
- No underlying structural heart disease, pro-inflammatory state, myocyte injury, or hypoxia
- Decrease in most heart rate variability parameters, especially those related to cardiovagal tone
- **Suggests cardiac autonomic nervous system imbalance with decreased parasympathetic activity**



HR indicates heart rate; PNN50, percentage of adjacent NN intervals that differ from each other by more than 50 ms; SD, standard deviation of the beat-to-beat interval; VLF, very low frequency; LF, low frequency; HF, high.

Aranyo J et al. Inappropriate sinus tachycardia in post-COVID-19 syndrome Scientific Reports 2022

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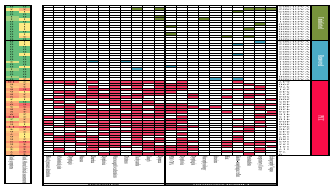
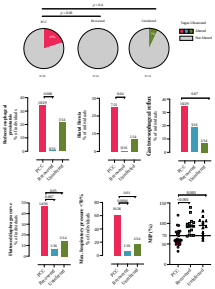
Vagus nerve dysfunction

Germans Trias Long COVID Unit

30 **Long COVID**, 16 COVID-19-**recovered**, 14 **uninfected**





Llados G et al. ECCMID 2022

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Mechanisms

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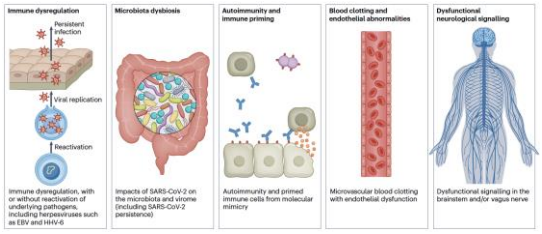
Long COVID

Hypothesized mechanisms



Davis HE et al. Long COVID: major findings, mechanisms and recommendations. Nature Microb Rev 2023

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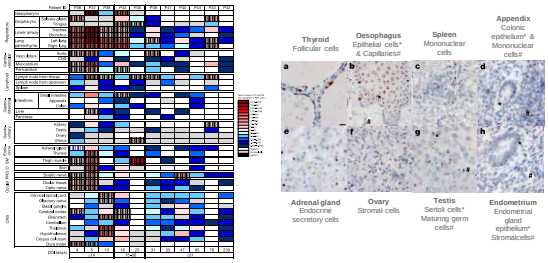
Persistent SARS-CoV-2 at autopsy



Stein SR et al. SARS-CoV-2 infection and persistence in the human body and brain at autopsy. Nature 2022

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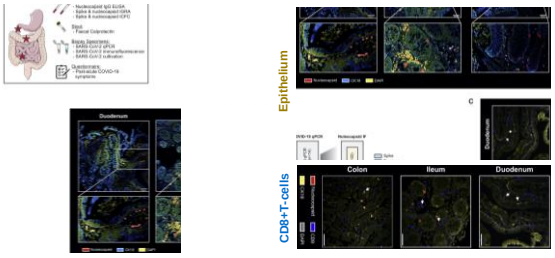
SARS-CoV-2 gut invasion & persistence (7 mo)



Zollner et al. Postacute COVID-19 is Characterized by Gut Viral Antigen Persistence in Inflammatory Bowel Diseases. Gastroenterology 2022

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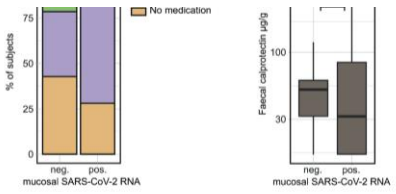
SARS-CoV-2 gut invasion & persistence



Zollner et al. Postacute COVID-19 is Characterized by Gut Viral Antigen Persistence in Inflammatory Bowel Diseases. Gastroenterology 2022

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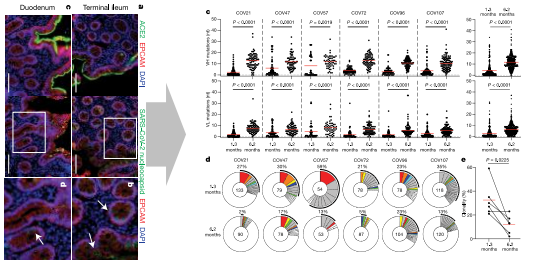
Evolution of antibody immunity to SARS-CoV-2



Gaebler et al. Evolution of antibody immunity to SARS-CoV-2. Nature 2021

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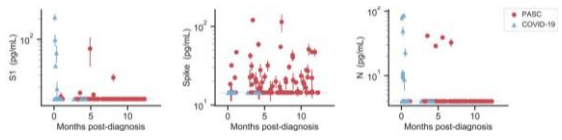
Persistent circulating SARS-CoV-2 Spike

63 adults with
- Acute **COVID-19** without PACS (n=32):
- **With PACS** (n=31):

samples up to 5 months — more often men, hospitalized
samples up to 12 months — more often women, not hospitalized



- N may be preferentially hydrolyzed, spike may be more efficiently transported into the bloodstream, evading degradation.
- Circulating anti-N antibodies may be more effective at clearing N compared with the anti-spike antibodies produced.
- Both S1 epitopes may not be accessible depending on the conformation of circulating spike.
- Assay calibrated with the spike ectodomain, which is missing the TM domain → S1 assay may not bind spike in its natural conformation.

Swank et al. Persistent Circulating Severe Acute Respiratory Syndrome Coronavirus 2 Spike Is Associated With Post-acute Coronavirus Disease 2019 Sequelae. CID 2023

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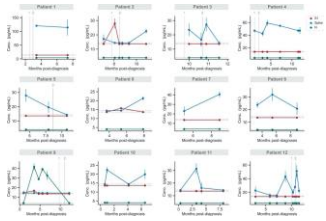
Persistent circulating SARS-CoV-2 Spike



Antigen detection more frequent if:

- Ongoing cardiovascular, systemic, head-eye-ear-nose-throat, and musculoskeletal symptoms. (>70% positive)
- Acute gastrointestinal and neuropsychiatric symptoms.
- The greater the number of organ systems involved

- **No differences in IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-22, and TNF-α**

Swank et al. Persistent Circulating Severe Acute Respiratory Syndrome Coronavirus 2 Spike Is Associated With Post-acute Coronavirus Disease 2019 Sequelae. CID 2023

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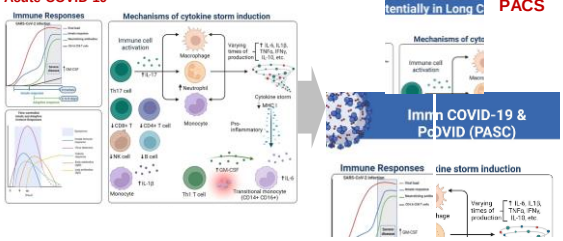
Immune mechanisms

Acute COVID-19

PACS



Mohandas S et al. Immune mechanisms underlying COVID-19 pathology and post-acute sequelae of SARS-CoV-2 infection (PASC). eLife 2023

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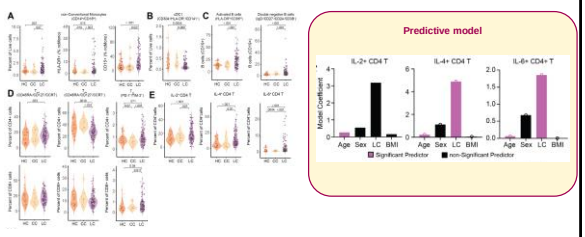
Cellular markers



Klein et al. Distinguishing features of Long COVID identified through immune profiling. medRxiv 2022. https://doi.org/10.1101/2022.08.09.22278592

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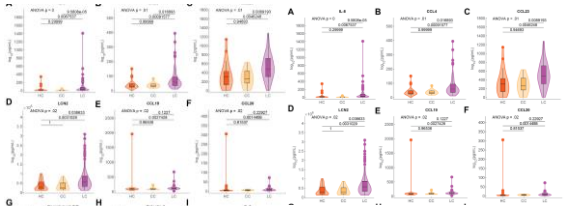
Soluble markers

Compared with healthy controls Long COVID has:

- **Higher IL-8, CCL4, CCL23, C4b, Galectin-1, IL-6, LCN2, CCL20, TGF-beta1**
- **Lower ADAMTS13**



Klein et al. Distinguishing features of Long COVID identified through immune profiling. medRxiv 2022. https://doi.org/10.1101/2022.08.09.22278592

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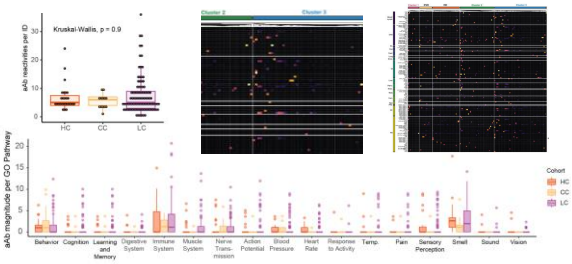
Autoimmunity



Klein et al. Distinguishing features of Long COVID identified through immune profiling. medRxiv 2022. https://doi.org/10.1101/2022.08.09.22278592

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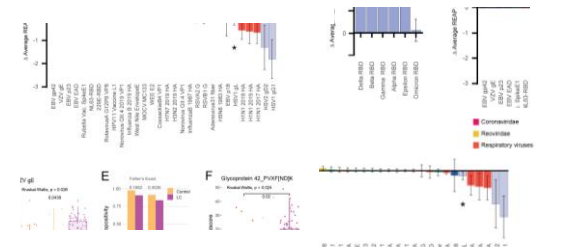
Herpesvirus reactivation



Klein et al. Distinguishing features of Long COVID identified through immune profiling. medRxiv 2022. https://doi.org/10.1101/2022.08.09.22278592

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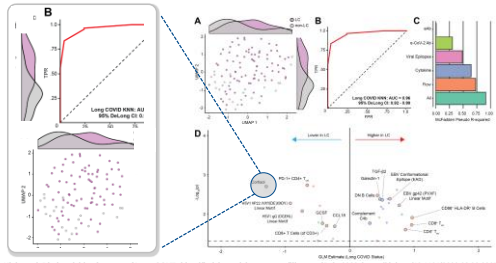
Biochemistry



Klein et al. Distinguishing features of Long COVID identified through immune profiling. medRxiv 2022. https://doi.org/10.1101/2022.08.09.22278592

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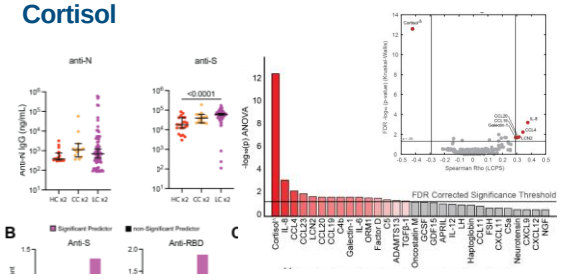
Cortisol



Klein et al. Distinguishing features of Long COVID identified through immune profiling. medRxiv 2022. https://doi.org/10.1101/2022.08.09.22278592

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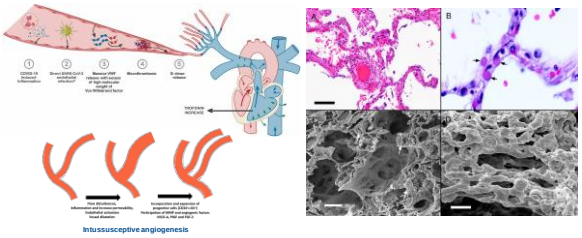
Systemic endotheliopathy



Smadja DM, et al. COVID-19 is a systemic vascular hemopathy: insight for mechanistic and clinical aspects. Angiogenesis 2021

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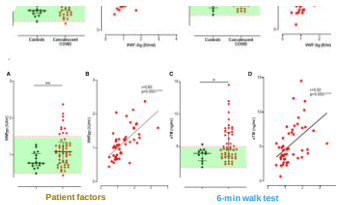
Persistent endotheliopathy

50 patients, median of 68 days following SARS-CoV-2 infection

Prothrombotic changes
- Shorter lag times in thrombin generation assays
- Increased endogenous thrombin potential
- Increased peak thrombin

Elevated endothelial cell biomarkers:
- von Willebrand factor antigen (VWF:Ag), VWF propeptide (VWFpp), and factor VIII.

Elevated plasma soluble thrombomodulin levels
- Independent of ongoing acute phase response or active NETosis.



Fogarty H, el al. Persistent endotheliopathy in the pathogenesis of long COVID syndrome. J Thromb Haemost. 2021

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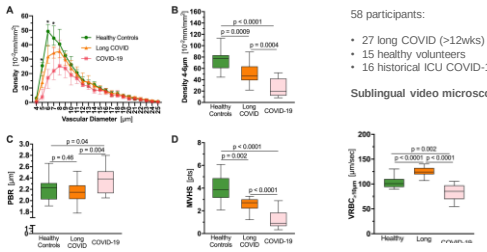
Persistent capillary rarefaction



58 participants:
- 27 long COVID (>12wks)
- 15 healthy volunteers
- 16 historical ICU COVID-19

Sublingual video microscopy

PBR perfused boundary region, *MVHS*™ micro-vascular health score

Osiaevi C, el al. Persistent capillary rarefication in long COVID sindrome. Angiogenesis 2023

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Neurobiology

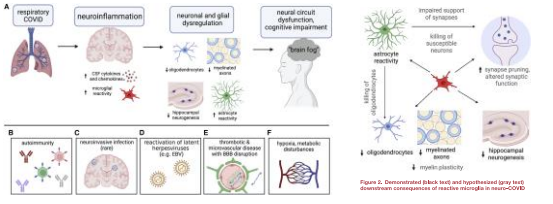

Figure 2. Demonstrated (black text) and hypothesized (grey text) downstream consequences of reactive microglia in neuroCOVID

Monje M, Iwasaki A. The neurobiology of long COVID. Neuron 2023

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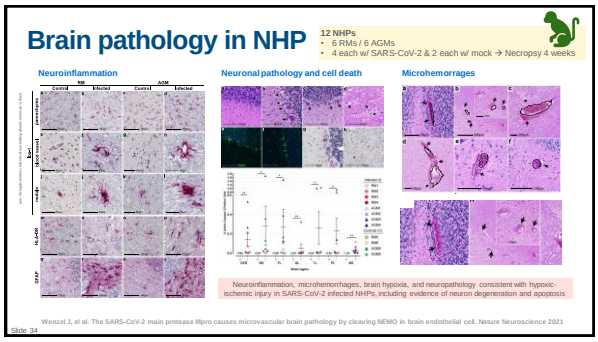
Brain pathology in NHP
12 NHPs
6 RMs / 6 AGMs
4 each w/ SARS-CoV-2 & 2 each w/ mock → Necropsy 4 weeks
Neuroinflammation
Neuronal pathology and cell death
Microhemorrages
Neuroinflammation, microhemorrhages, brain hypoxia, and neuropathology consistent with hypoxic-ischemic injury in SARS-CoV-2 infected NHPs, including evidence of neuron degeneration and apoptosis
Wenzel J, et al. The SARS-CoV-2 main protease Mpro causes microvascular brain pathology by cleaving NEMO in brain endothelial cell. Nature Neuroscience 2021
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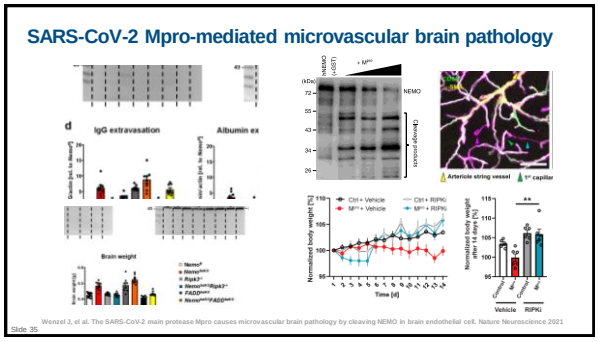
SARS-CoV-2 Mpro-mediated microvascular brain pathology
IgG extravasation
Albumin ex
Brain weight
NEMO
Cleavage-resistant
Arteriole string vessel
5th capillar
Ctrl + Vehicle
Mpro + Vehicle
Ctrl + RIPKi
Mpro + RIPKi
Time [d]
Vehicle
RIPKi
Wenzel J, et al. The SARS-CoV-2 main protease Mpro causes microvascular brain pathology by cleaving NEMO in brain endothelial cell. Nature Neuroscience 2021
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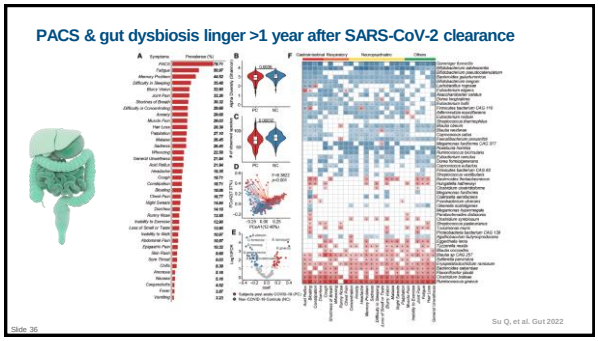
PACS & gut dysbiosis linger >1 year after SARS-CoV-2 clearance
Su Q, et al. Gut 2022
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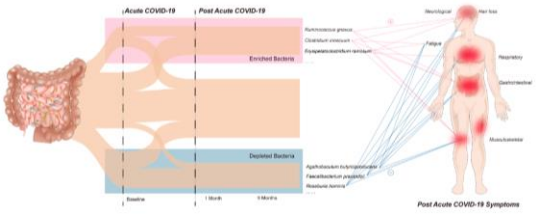
The microbiome in Long COVID



Liu Q, et al. Gut 2022

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Conclusions

What ([the heck]) is Long COVID?

- Long COVID is a **real** postviral disease with **clear organic involvement** and various simultaneous pathophysiological alterations
- Different long COVID **subphenotypes** can be identified, but:
  - **Symptoms overlap** and **accumulate** across clusters, rather than separate into exclusive syndromic patterns
  - It is uncertain if subphenotypes reflect independent pathogenic mechanisms or, more likely, **additive severity** of a multisystemic, multifaceted post-viral disease
  - Subphenotypes may be useful to establish **prognosis**: Long COVID resolution is rare during the first 2 years, and almost exclusively occurs in the less severe subphenotypes
- Long COVID **overlaps,** but **is different** from PICS and from shorter term sequelae like smell/taste alterations. **PACS** comprises everything, so might include different pathogenic entities

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Conclusions

Simultaneous, multifaceted pathogenic mechanisms

- **Viral persistence** → In any tissue expressing ACE2, including brain. Spike Ag detectable in serum up to 12 months only in subjects with PACS→ 1st clinically-actionable **PACS biomarker**?
- **Immune dysregulation**
  - **Peripheral blood** → Consistent inflammatory and immune activation profiles in soluble markers, but high overlap with controls and small magnitudes of change, often within normal limits. Subtle changes in peripheral immune cell repertoirs
  - **Tissues** → Clear picture with inflammation, necrosis, apoptosis, microthrombosis endotheliopathy and resident cell damage
- **Autoimmunity** → Simultaneous polyreactivity against various tissular antigens → Higher polyreactivity correlates with PACS → Not a simple autoimmunity biomarker

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Conclusions

Simultaneous, multifaceted pathogenic mechanisms

- **Endocrine** → Lower cortisolemia in Long COVID → Adrenal syndrome? Clear biomarker, but variation between groups within normal clinical limits. Requires validation
- **Endotheliopathy** → Systemic and persistent → Prothrombosis and intussusceptive angiogenesis → Mediated by systemic inflammation & direct SARS-CoV-2 effects (Mpro)
- **Gut dysbiosis**
  - Gut SARS-CoV-2 invasion & persistence associated with PASC
  - The gut microbiome changes during acute COVID-19, continues to evolve thereafter, and acquires a specific Long COVID-19 profile
- **Vagus nerve dysfunction** → Larynx, pharynx, lungs, heart and gastrointestinal tract → Controls heart rate and digestive rhythm, and modulates systemic inflammation

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Gràcies

The Patients

Lourdes Mateu, MD, PhD
Head, Long COVID Unit
Department of Infectious Diseases
Hospital germans Trias I Pujol

Germans Trias i Pujol

Fundació Lluita contra les Infeccions

Nosaltres nos COVID19 PERSISTENT

Marta Massanella, PhD
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Our Team

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