Condición POST COVID-19
Un largo camino hacia la recuperación

UnidadCovidPersistente

Importancia de las pruebas cardiológicas en la medición de disautonomía. Prevalencia de autoanticuerpos GPCRs en la condición post COVID-19.

Barcelona. Jueves 21 de Abril de 2022.

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Conflict of interest

- Angelini Pharma Spain - consulting fees
- Berlin Cures GmbH – Advisory Board fees
- GlaxoSmithKline (Spain) - speaking fees and meetings grants
- Pharma Mar SA (Spain) – speaking, consulting & Advisory Board fees

Are we in front of a new clinical entity?

- Observational and epidemiological studies
- People with asymptomatic or mild symptoms infection
- Variety of mental or somatic symptoms, lasting at least 12 weeks or more after recovering from infection

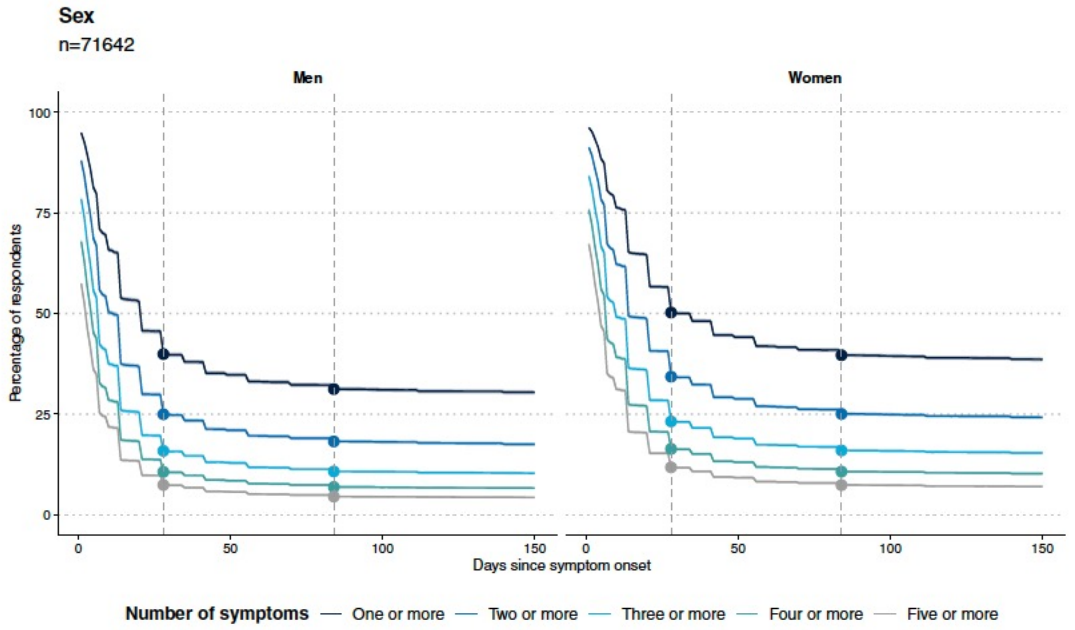


Fig. 2 Persistence of symptoms over time. Plots showing persistence of symptoms as a proportion of those who reported symptoms at any time, among $n = 71,642$ respondents for whom we had 150 days' observation time. Women have higher rates of persistent symptoms; a slower decline in symptom prevalence is observed after 12 weeks in both sexes. The vertical dashed lines show 4 and 12 weeks post symptom onset, respectively.

Whitaker et al. Persistent COVID-19 symptoms in a community study of 606,434 people in England. *Nat Commun* 13, 1957 (2022).

Roessler M et al. Post COVID-19 in children, adolescents, and adults: results of a matched cohort study including more than 150,000 individuals with COVID-19. medRxiv 2021; Available from Oct 22;2021.10.21.21265133. Preprint.

Are we in front of a new clinical entity?

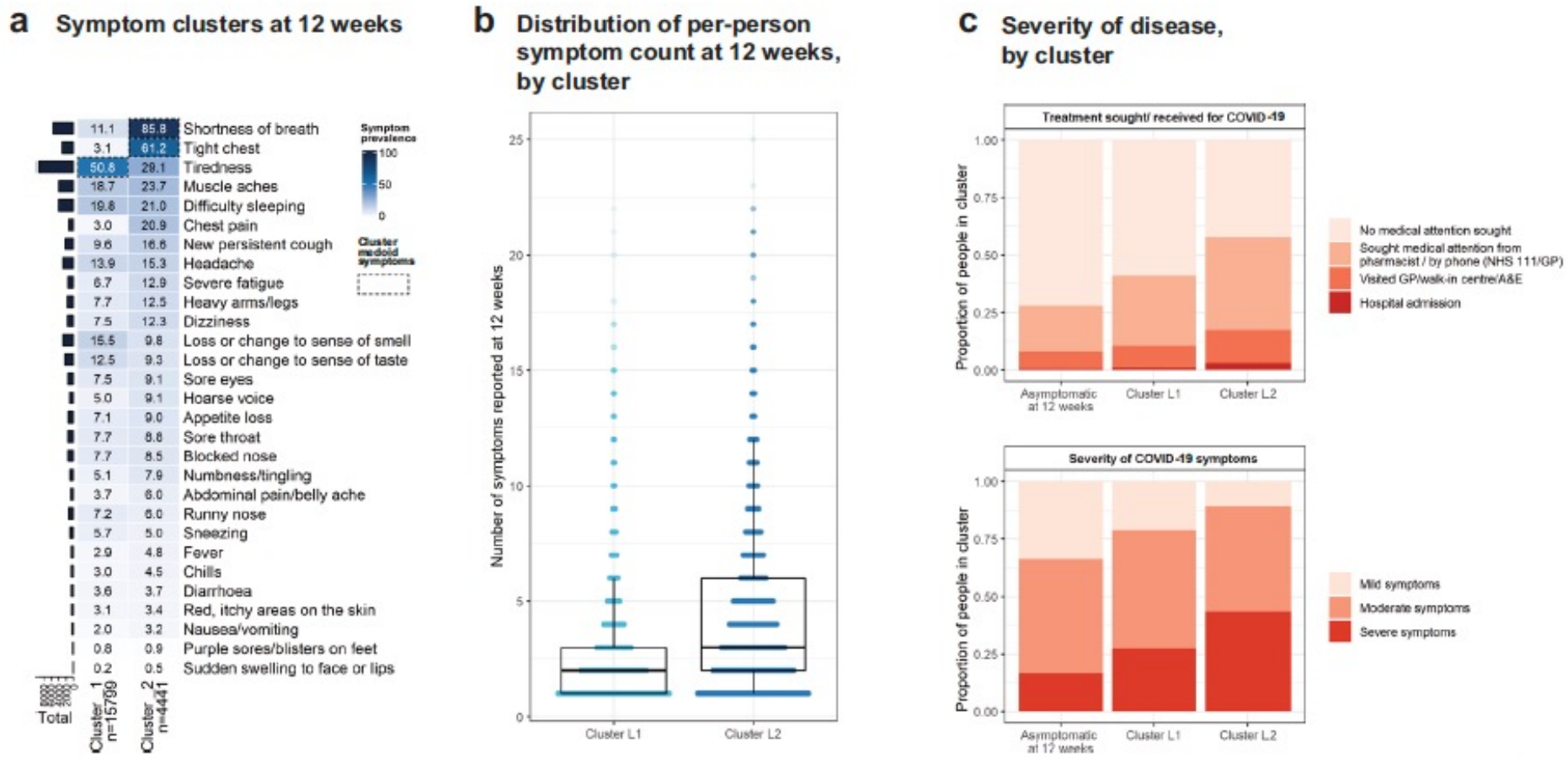


Fig. 5 Results of clustering on symptom profile at 12 weeks. Clustering was conducted using CLARA (partitioning around medoids) algorithm. Two stable

Whitaker et al. Persistent COVID-19 symptoms in a community study of 606,434 people in England. *Nat Commun* 13, 1957 (2022).

Are we in front of a new clinical entity?

Post-infectious syndromes

- Symptoms share some similarities with those in chronic fatigue syndrome/multiple encephalomyelitis (CFS/ME)
- Post-intensive care syndrome (PICS)
- Depending on their initial severity

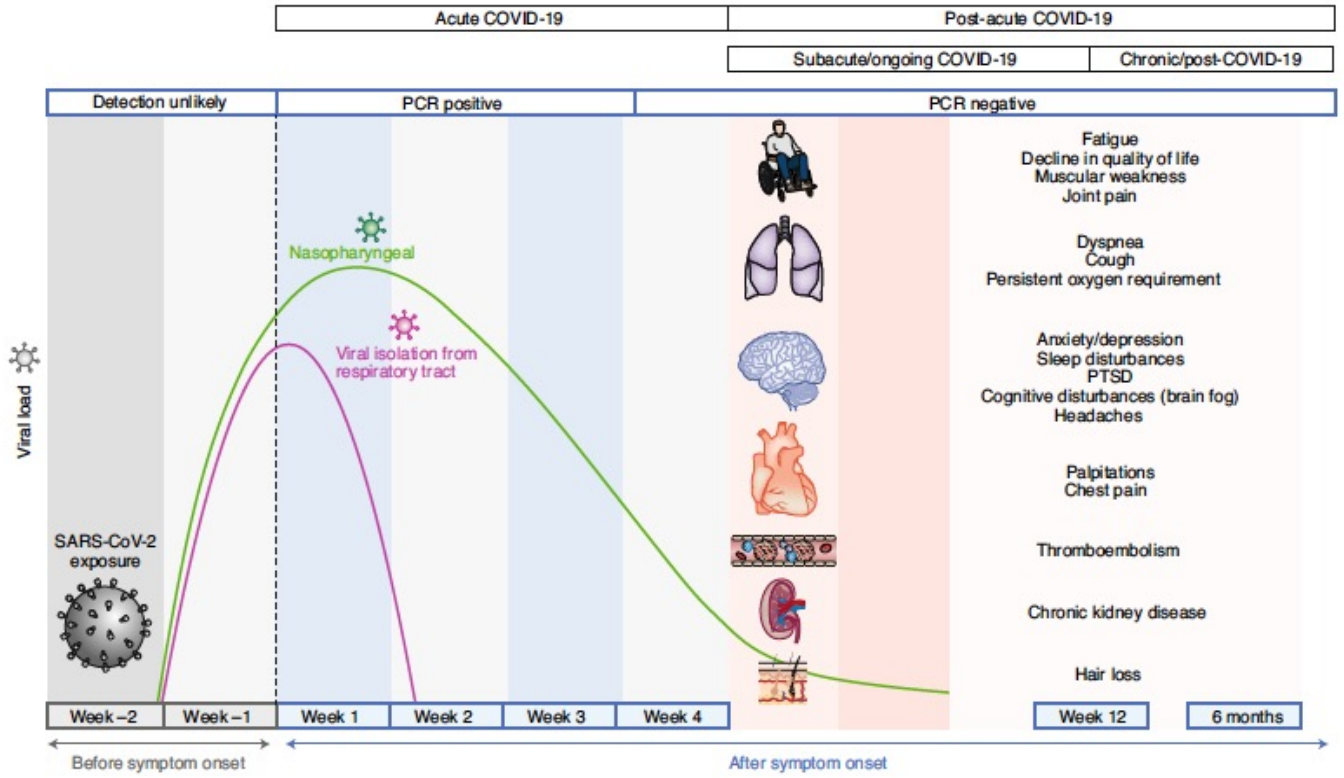


Fig. 1 | Timeline of post-acute COVID-19. Acute COVID-19 usually lasts until 4 weeks from the onset of symptoms, beyond which replication-competent SARS-CoV-2 has not been isolated. Post-acute COVID-19 is defined as persistent symptoms and/or delayed or long-term complications beyond 4 weeks from the onset of symptoms. The common symptoms observed in post-acute COVID-19 are summarized.

Nalbandian A et al. Post-acute COVID syndrome. Nat Med 2021 Apr;27(4):601-605.

‘Long COVID’ and post-COVID syndrome

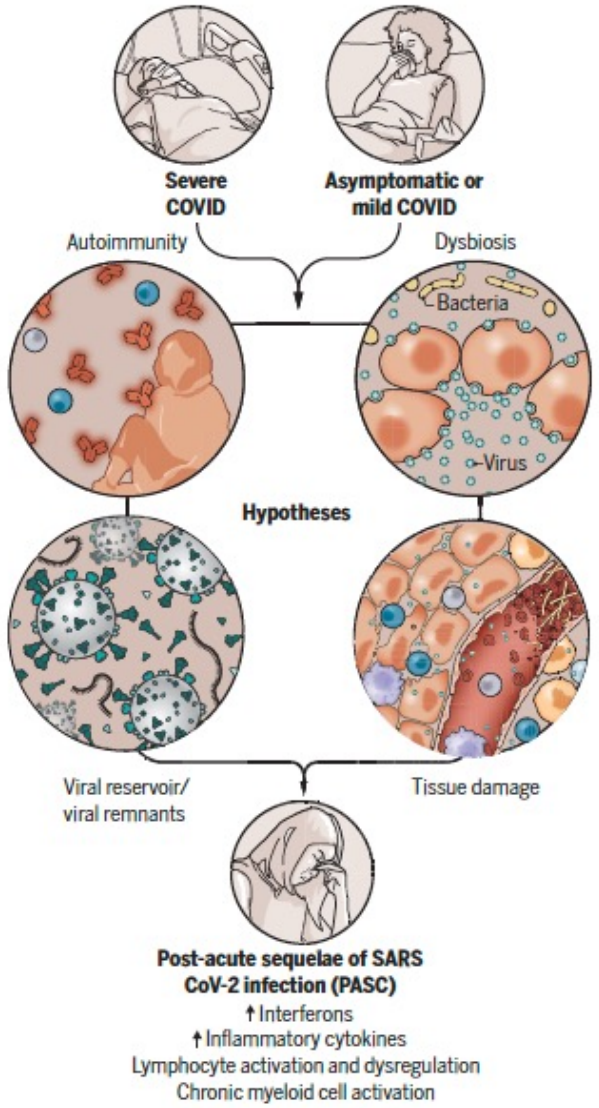
Post-acute sequelae of SARS-CoV-2 infection (PASC)

- ‘long COVID’ named by people living with the syndrome, belongs to a wider concept: post-infectious disease syndromes.
- Broad of signs and symptoms reported, such as weakness, post-exertional malaise, fatigue, palpitations, concentration impairment, breathlessness
- Reduce quality of life, impact on working activity
- Affect to different domains or body systems.

Rogers-Brown J et al. Outcomes among patients referred to outpatient rehabilitation clinics after COVID-19 diagnosis - United States, January 2020-March 2021. MMWR Morb Mortal Wkly Rep 2021 Jul 9; 70 (27):967-971.

Davies HE et al. Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. EClinicalMedicine 2021 Aug; 38:101019.

Immunopathology under post-COVID syndrome



Merad M et al. The immunology and immunopathology of COVID19. Science 2022; 375(6385):1122-1127.

The immunobiology of PASC is currently under investigation

- There is no causal link between viral proteins and RNA and elevated cytokines
- Studies have shown that some inflammatory cytokines—including IL-6, IL-8, TNF-a, and IL-1b— are elevated in PASC patients. IFN-b and
- Some molecules of IFN family remain elevated 8 months after infection
- Complex combinations of cytokines, no widely available in daily clinical practice only have associated 78.5 - 81.6% accuracy for PASC.
- AutoAbs (Aab) were anticorrelated with anti–SARS-CoV-2 Abs

Merad M et al. The immunology and immunopathology of COVID19. Science 2022; 375(6385):1122-1127.

‘long COVID’ vs prolonged viral replication (SARS-CoV-2)

- Rather confusion among patients and social media. PLACEBO effect!
- The disease is characterized by flare-remission.
- We don’t know exactly the evolution in time
- Scarce (if any) reliable data in immunocompetent population.
- We are NOW already taking about a complete different clinical entity.

New clinical entity: interstitial bilateral pneumonia due to prolonged viral replication (SARS-CoV-2)

- New paradigm
- Largest cohort of persistent symptomatic COVID-19 infection in patients with lymphoid malignancies
- Particularly high in those affected of B-cell lymphomas
- Active therapy and diminished T-cells counts
- B-cell depletion as the key immunologic driver of persistent infection
- B-cell depletion therapies – active or former –anti-CD20 monoclonal antibodies
- Intrahost viral evolution – specially impaired $CD8^+$ T cells.
- No reinfection or co-infection

Lee CY et al. Prolonged SARS-CoV-2 infection in patients with lymphoid malignancies. Cancer Discov (2022) 12 (1): 62–73.

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New clinical entity: interstitial bilateral pneumonia due to prolonged viral replication (SARS-CoV-2)

- UK Coronavirus Cancer Monitoring Project (UKCCMP)
- Solid tumours and haematological malignancies.
- N=2,515. 38% overall mortality. 49% severe COVID19. Only 5% in intensive care.
- High-mortality in patients with hematological malignant neoplasms, particularly myelodysplastic syndrome, myeloma or plasmacytoma
- Lung cancer was also significantly associated with higher COVID-19–related mortality.
- No association between higher mortality and receiving chemotherapy in the 4 weeks before COVID-19 diagnosis

Várnai C et al. Mortality among adults with cancer undergoing chemotherapy or immunotherapy and infected with COVID-19. JAMA Network Open. 2022;5(2):e220130

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A pilot program for its compassionate use Plitidepsin

- Increasing population of patients with high mortality risk.
- Preclude no/low answer to vaccines.
- Immunosuppression: haematological malignancies, autoimmune diseases, solid tumours, solid organ transplant.
- ISARIC score: high-risk populations with other risk factors.
- A compassionate use approved.
- Spanish Agency for Medicinal Products (AEMPS) (AUT334100148189/21).

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Compassionate use program of plitidepsin: single center experience

n=25. 11 Mar. 2022

A) Active/inactive chemotherapy before

B) Patients outcome (mortality) after plitipdesin infusion

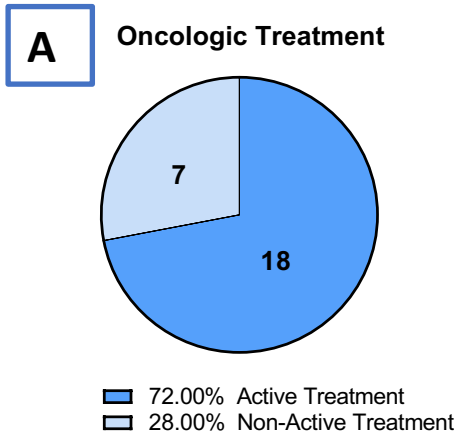
C) Diseases of patients treated with plitidepsin

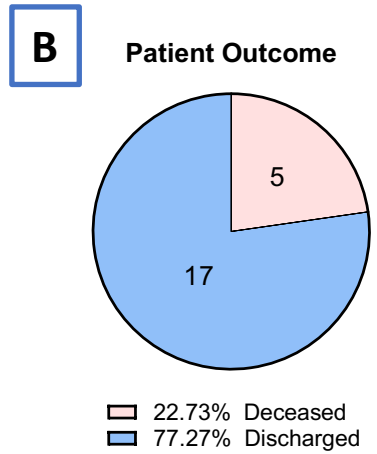
PD: 3 cases did not complete the 3 days scheduling. All death.

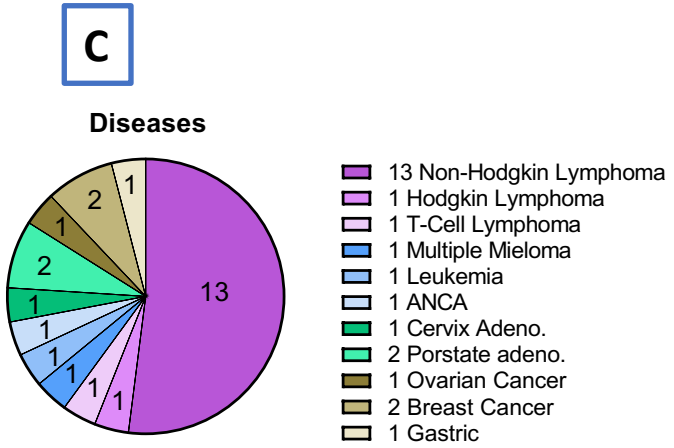
1 admitted to IC at day 1

2 transfer to another hospital at day 2

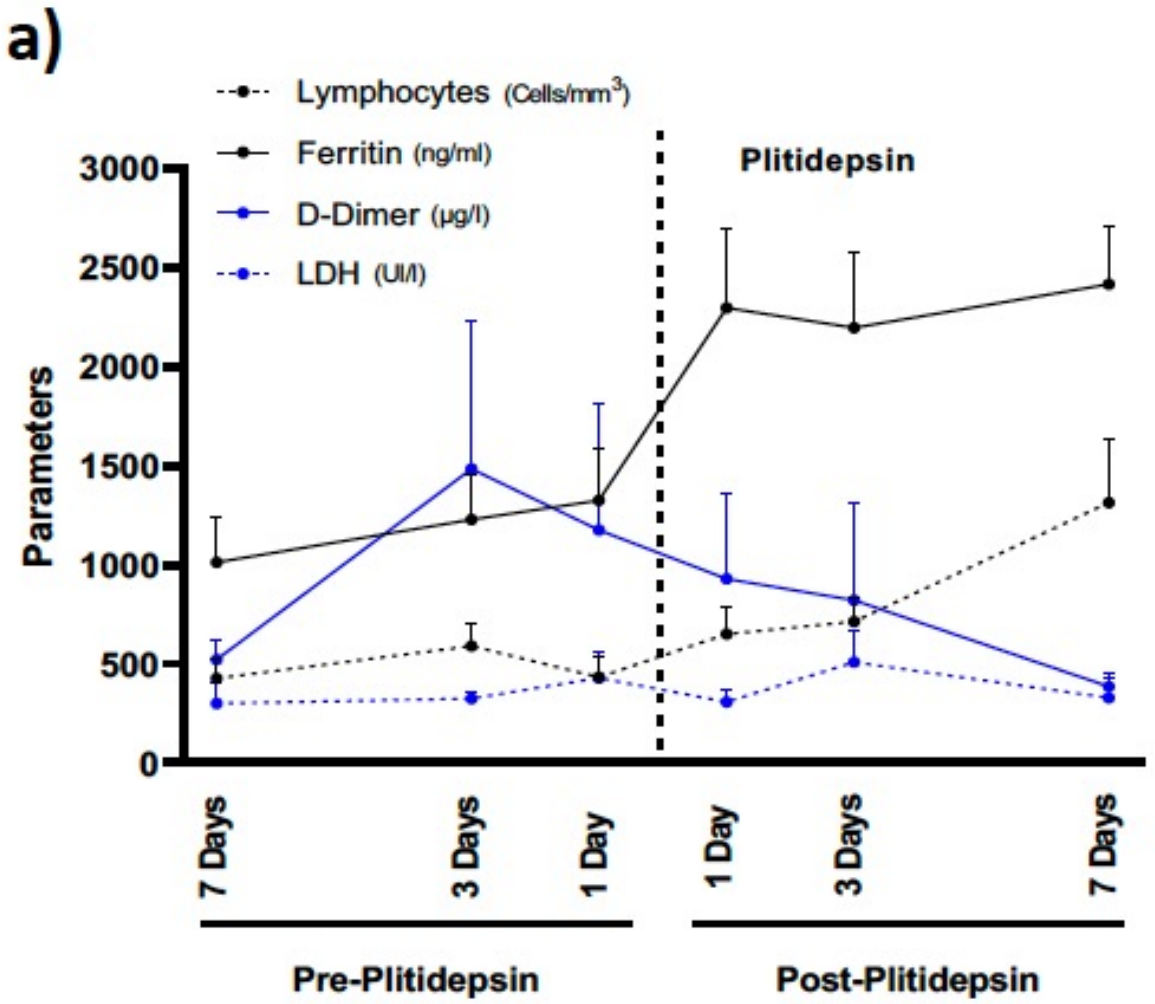
Data not included.





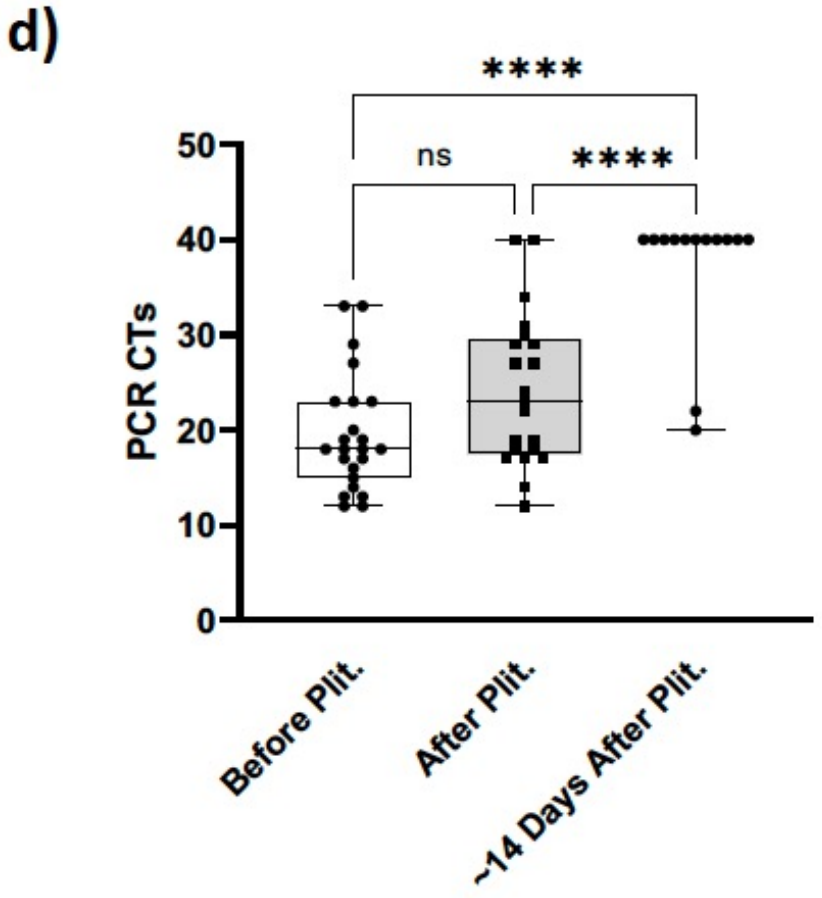


Compassionate use program



Change in laboratory parameters in patients treated with plitidepsin – completed 3 days course. Data were collected at days 1,3,7 after and before plitidepsin infusion.

Compassionate use program



Change in PCR RNA SARS-CoV-2 Ct values in patients treated with plitidepsin – completed 3 days course. Data w.ere collected 24 h before and after plitidepsin infusion, and bwt 14 days of last infusion.

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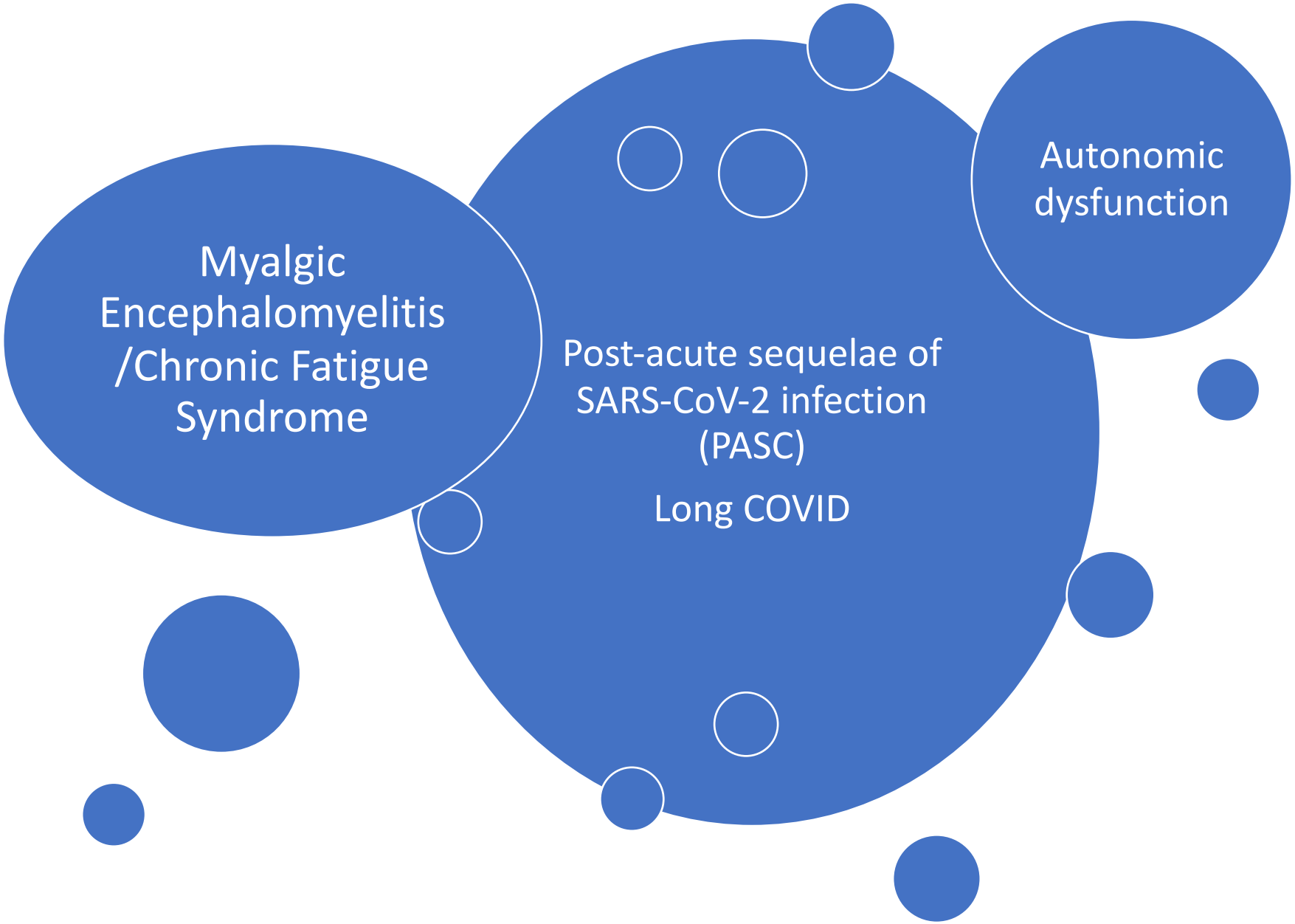
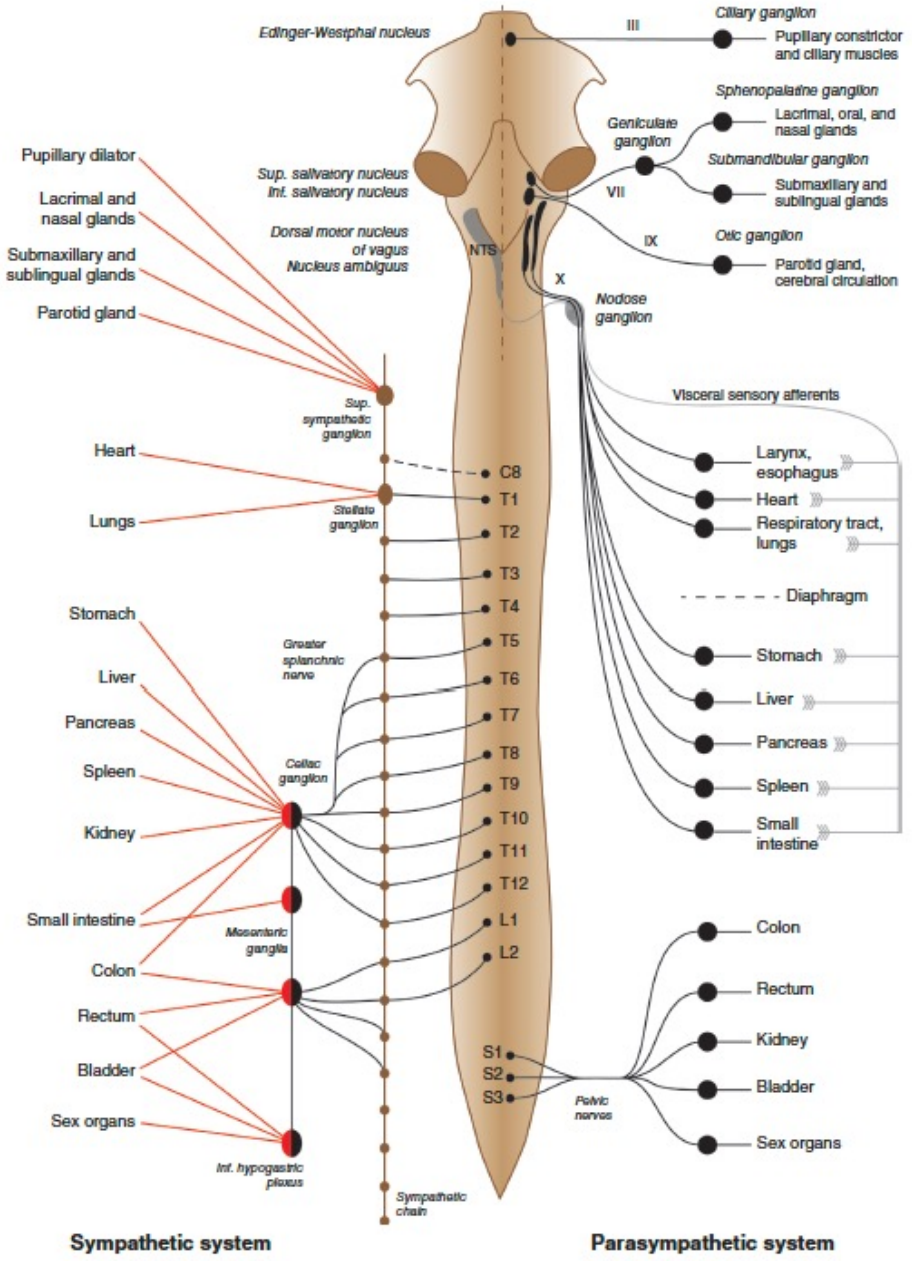


Sotzny F et al. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome - Evidence for an autoimmune disease. Autoimmun Rev 2018; 17(6):601-609

ME/CFS, post-infectious syndromes & dysautonomia

- In up to 95% of those with ME/CFS, an immobile, upright position (eg, prolonged standing or sitting) will cause or worsen symptoms such as lightheadedness, nausea, fatigue, palpitations, and cognitive impairment.
- Assumption of a sitting or supine position can alleviate symptoms.
- This phenomenon is called orthostatic intolerance and includes orthostatic hypotension, postural orthostatic tachycardia syndrome and neurally mediated hypotension

Bateman L et al. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: essentials of diagnosis and management. Mayo Clin Proc 2021; 96(11):2861-2878



The autonomic nervous system is virtually connected with everywhere

Every system, function or organ domain

Wehrwein EA et al. Overview of the anatomy, physiology, and pharmacology of the autonomic nervous system. Compr Physiol 2016; 6(13)1239-78.

Autonomic dysfunction (AD) & post–SARS-CoV-2 infection

- Affects primarily female patients without a clear history of pre-existing conditions.
- Heart rate variability (HRV) during a change in position is commonly measured to diagnose autonomic dysregulation
- Complex methods not widely available
- Input should be easy to interpretate
- “extended ANS” (EAS)
  - 3 components—the sympathetic nervous system (SNS), the parasympathetic nervous system (PNS), and the enteric nervous system (ENS).
  - Expansion in 2 ways—neuroendocrine and neuroimmune.

Barizien N et al. Clinical characterization of dysautonomia in long COVID-19 patients. Sci Rep 2021 Jul 7; 11(1):14042.
Goldstein DS. The possible association between COVID-19 and postural orthostatic tachycardia syndrome. Heart Rhythm 2021 Apr; 18(4):508-509.

Autonomic dysfunction (AD) & post–SARS-CoV-2 infection

- Sera from Long-COVID patients (n=31) contained functionally active autoantibodies which target G-protein coupled receptors (GPCRs AAb).
- The list included pathogenic AAb targeting: nociception receptor, β2- and α1-adrenoceptors, angiotensin II AT1-, muscarinic M2-, MAS-, and ETA-receptors
- Included syndromes were of neurological and cardiological origin (or both)
- This type of pathogenic AAb and specific AAb pattern.
- Adding a chronotropic effect in neonatal rat cardiomyocytes model
- 2 symptoms free, 50% cardiovascular symptoms

Wallukat G et al. Functional autoantibodies against G-protein coupled receptors in patients with persistent long-COVID-19 symptoms. J Transl Autoimmun 2021; 4:100100.

Hypothetical mechanisms in POTS

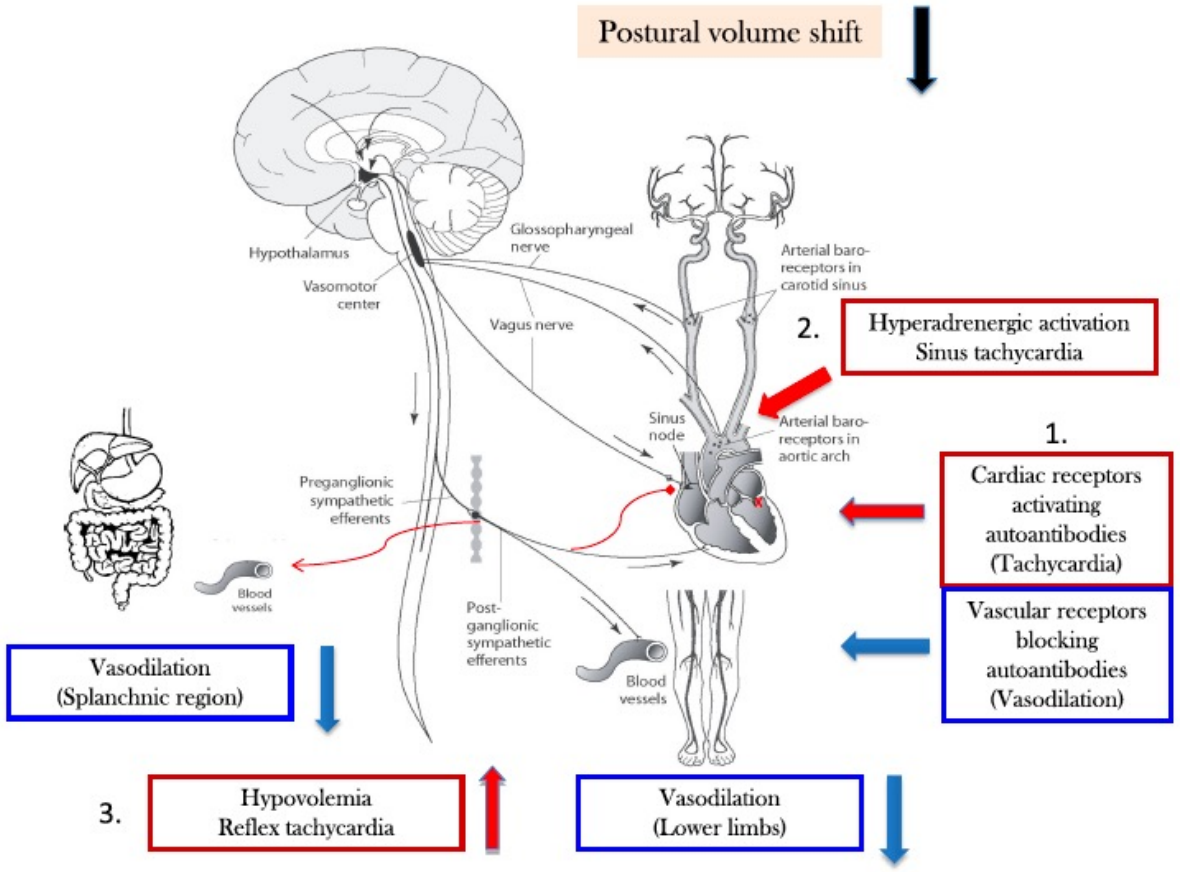


Fig. 1 *Postulated mechanisms of orthostatic intolerance and tachycardia in POTS. Direct action of hypothetical autoantibodies on adrenergic and other cardiovascular receptors. Abnormally increased sympathetic activity and circulating catecholamine excess. Peripheral sympathetic denervation, venous pooling and central hypovolaemia leading to reflex sinus tachycardia.*

Fedorowski A. Postural orthostatic tachycardia syndrome: clinical presentation, aetiology and management J Intern Med 2019; 285: 352–366.

Our experience…

- We designed observational, cross-sectional, retrospective, single-center study
- Outpatient office of the internal medicine department in the hospital universitario quironsalud Madrid (Pozuelo de Alarcón, Spain).
- End of April 2020 till January 2021 (cohort is active and ongoing)
- It is a tertiary care, academic medical center, serving approximately 2,500,000 citizens.
- The Research Ethics Committee of the Fundación Jiménez Diaz approved the study protocol.

Inclusion criteria and WHO

- Age > 18 years, confirmed SARS-CoV-2 assessing epidemiology, RT-PCR from NP swab, serostatus or antigen tests, causing or not to hospital admission due to COVID-19 since March 2020 till June 2021.
- Mostly had asymptomatic or mild symptomatic SARS-CoV-2 – we did select some cases with moderate COVID-19.
- We did not include cases of severe/critical COVID19 for the current research.
- Post-COVID-19 condition, or long COVID, by WHO.

Soriano JB et al. WHO clinical case clinical definition working group on post COVID-19 condition. Lancet Infect Dis 2022 Apr; 22(4):e102-e107.

POTS criteria and related clinical entities

- All patients had (POTS): sustained increase in heart rate, ≥30 beats per minute, in adults from supine position to upright within 10 minutes of standing
- Absence of orthostatic hypotension (decrease in systolic blood pressure (BP) > 20 mm Hg or diastolic BP > 10 mm Hg, or a decrease in systolic BP to < 90 mmHg).
- Patients should suffer from typical orthostatic intolerance symptoms, such as lightheadedness, orthostatic palpitations (“heart racing”), atypical chest discomfort.
- All the cases should have persistence fatigue, exercise intolerance & deconditioning.
- We recorded other symptoms related to different domains
- If patients in the long COVID cohort do not fulfill the POTS criteria, then myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)

Raj SR et al. Can J Cardiol 2020 Mar; 36(3):357-372. Brignole M et al. Eur Heart J 2018 Jun 1;39(21):1883-1948
Carruthers BM et al. J Intern Med 2011 Oct; 270(4):327-38

Control cohorts

We selected 2 control cohorts.

- A historical POTS cohort, according to same criteria and considering a period of at least 6 months or longer of symptoms. All the cases had SARS-CoV-2 infection during following up in the outpatient office and suffered from worsening of their symptoms lasting more than 4 weeks.

- Exclusion criteria: pregnancy, previous type 1 diabetes mellitus with microvascular damage, neurological or gastrointestinal complications; primary neurodegenerative diseases which could cause dysautonomia, active viral infection (HCV, HBV, HIV) causing peripheral neuropathy, inflammatory bowel disease, or prolonged bed immobilization for any different reasons.

Control cohorts

- HCW cohort: the negative control of GPCR Aab.
- Selecting exclusion criteria of any symptom's suggestion CFS or features of POTS in previous four weeks (it includes any working inactivity due to susception of symptoms).
- We collected previous SARS-CoV-2 infection (or not), vaccination status (and data of 2nd dose and boosters, if applicable)
- Absence any known neurodegenerative disease (including multiple sclerosis), long-standing type 1 diabetes mellitus, and any former autoimmune systemic disease.
- Pending blood sampling processing.

Results

- Most of the cases were mild or asymptomatic.
- 12.5% were admitted to hospital in a general ward.
- 2 cases had organ damage not related to POTS (one interstitial lung changes after COVID-19 pneumonia, one unilateral subsegmental pulmonary embolism).
- 68.75% fulfilled the POTS criteria, and 31.25% had EM/CFS, in the moment of the clinical assessment and GPCR AAb measurement.

Results

	Dysautonomia before SARS-CoV-2 infection N=18	Dysautonomia onset after SARS-CoV-2 infection N=16
General Demographics		
Age, years (median, IQR)	42 (28-52)	43.5 (23-57)
Gender (n, %)		
Male	1 (5.6%)	6 (37,5%)
Female	17 (94.4%)	10 (62.5%)
SARS-CoV-2 diagnosis (n, %)		
PCR RNA/antigen (+) - onset	4 (22.22)	4 (22.5)
Serology (+) – follow up	9 (50)	12 (75)
Epidemiology (+) – onset	14 (77.78)	12 (75)
COVID-19 (n, %)		
Mild/asymptomatic	15 (83.33)	14 (87.5)
Moderate	3 (16.67)	2 (12.5)
Severe/critical	-	-
Working status (n, %)		
Active	13 (72.22)	6 (37.5)
Inactive	5 (27.78)	10 (62.5)
POTS criteria (n, %)	18 (100)	11 (68.75)
EM/CFS criteria	-	5 (31.25)

Time to autoantibodies measurements and diagnosis (long-covid symptoms)		
Symptom's onset to 1st visit (days)	79 (15-373)	121 (66-283)
Symptom's onset to autoantibodies determination (days)	86 (43-434)	237 (68-351)
Symptom's onset to last follow up (days)	265.5 (159-434)	296 (88-431)

What are we looking for?

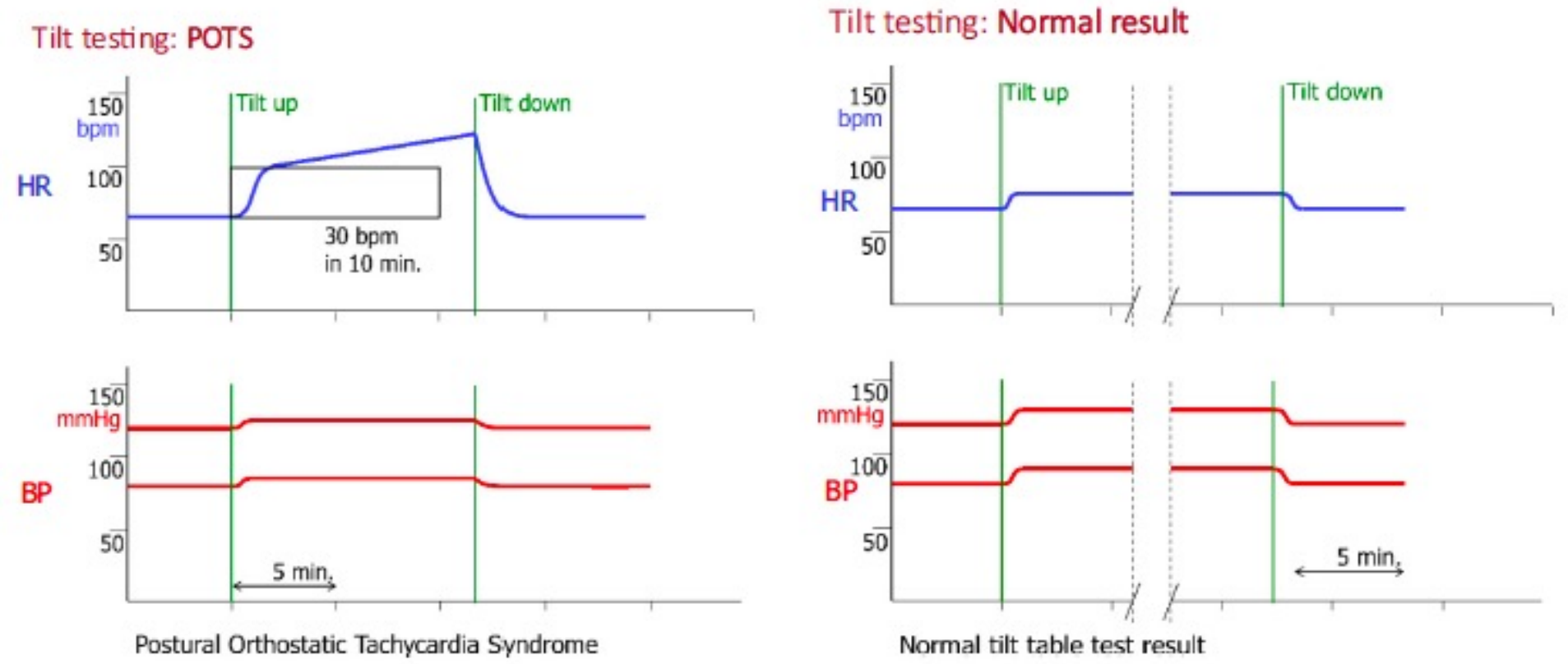


Fig. 2 *The characteristic pattern of orthostatic tachycardia during head-up tilt testing in patient with POTS (left panel) compared with normal haemodynamic response (right panel). (from: Brignole M, Moya A, de Lange FJ,* et al. *Practical Instructions for the 2018 ESC Guidelines for the diagnosis and management of syncope.* Eur Heart J *2018;* ***39:*** *e43-e80).*

Fedorowski A. Postural orthostatic tachycardia syndrome: clinical presentation, aetiology and management J Intern Med 2019; 285: 352–366.

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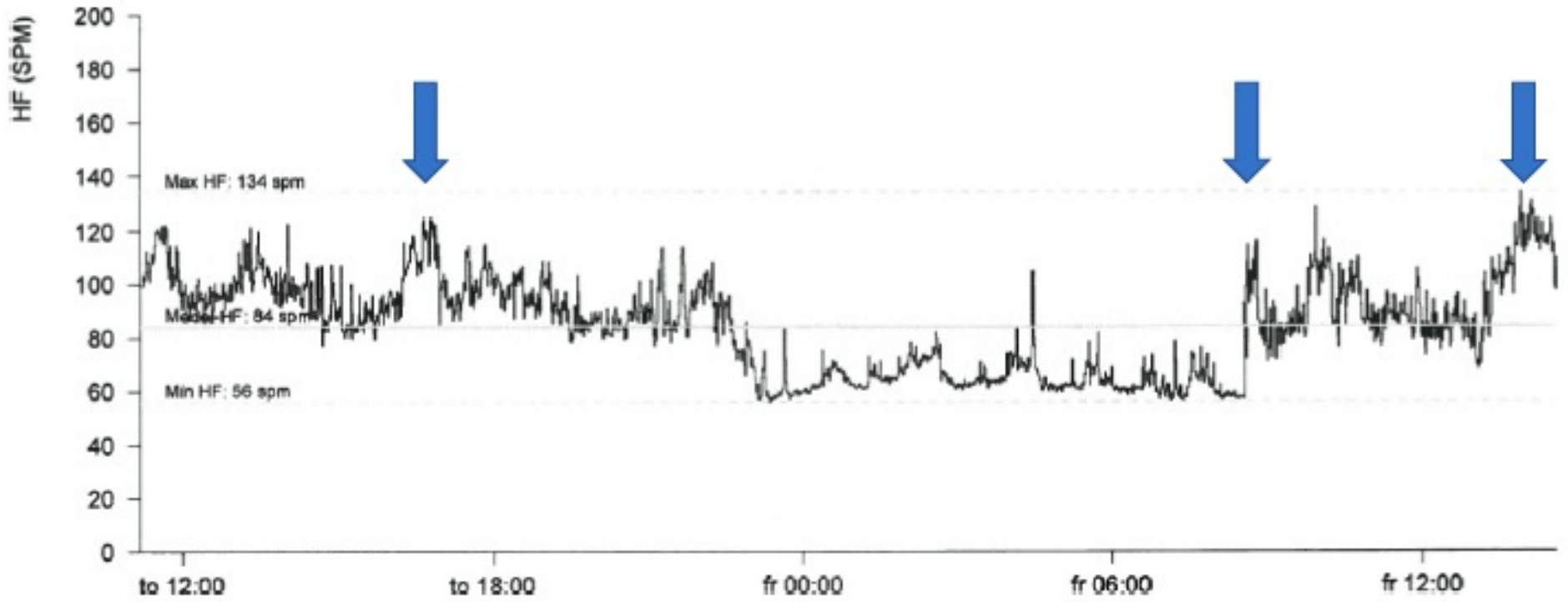


Fig. 3 *The characteristic pattern of sinus tachycardia in patient with POTS during daily activities and after awakening (woman, 26 years old). Note that heart rate is distinctly lower (56 bpm) compared with awaken time (above 85 bpm). Arrows mark significant heart rate increase.*

Fedorowski A. Postural orthostatic tachycardia syndrome: clinical presentation, aetiology and management J Intern Med 2019; 285: 352–366.

AAb GPCRs plasma levels at diagnosis

- 5 patients had positivity for ANA and/or low plasma levels of complement C3 (25%).
- Median values of AAb GPCR were conditioned by the individuals finally tested for each AAb.
- Blood samples sent to CellTrend Gmbh (Berlin, Germany).
- Most frequent positive autoantibodies were anti α-1 and α-2 adrenergic receptors Ab.
- We obtained intermediates values for anti-ETA, anti-AT1, β-2 adrenergic, muscarinic 3 and 4 receptors autoantibodies (AAb).
- We found a significative difference comparing to former dysautonomia (POTS) in AT1, β-1 and β-2 adrenergic, and muscarinic 1 receptors AAb.

Considering the whole sample, we observed a positive correlation using a Spearman's test between the alpha-1, β-1 and β-2 adrenergic and muscarinic 3 receptors AAb.

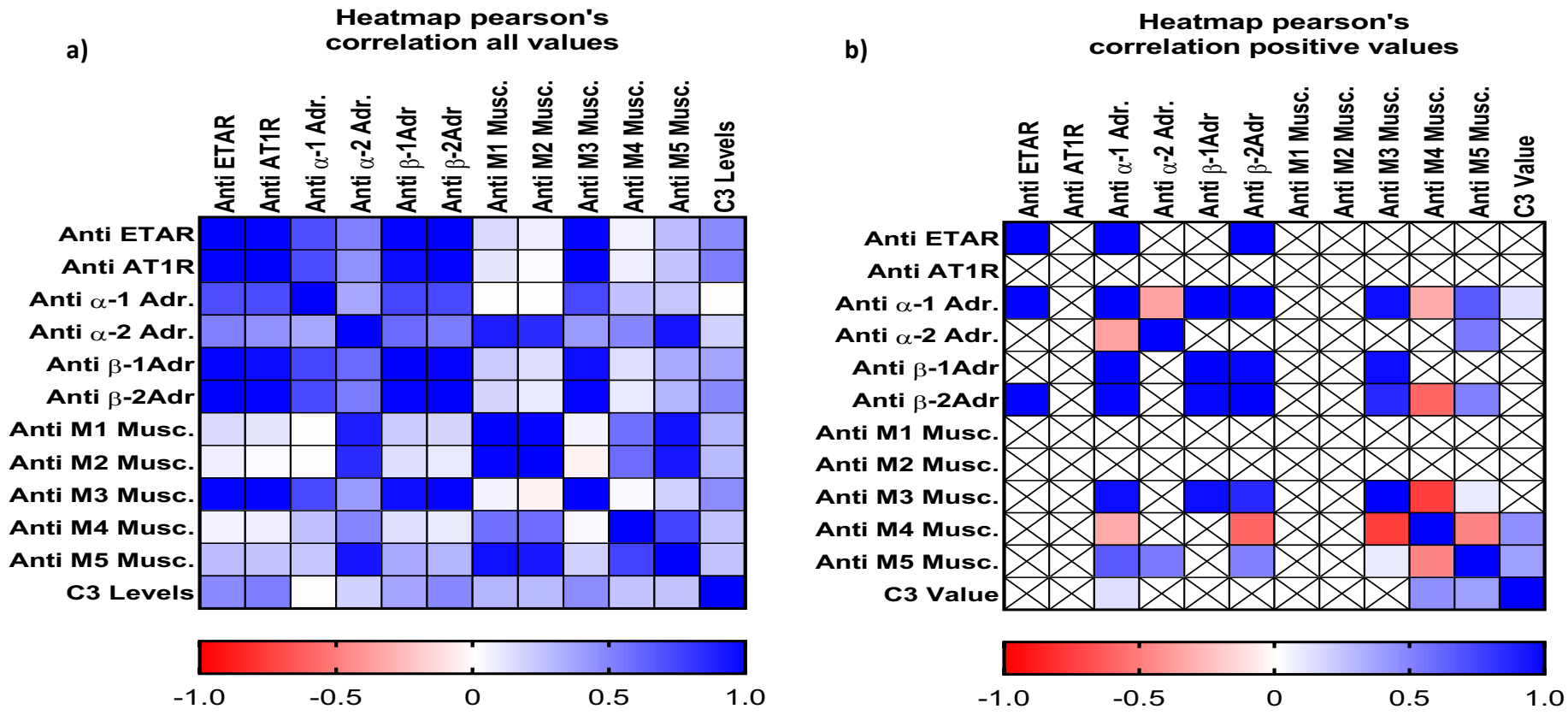
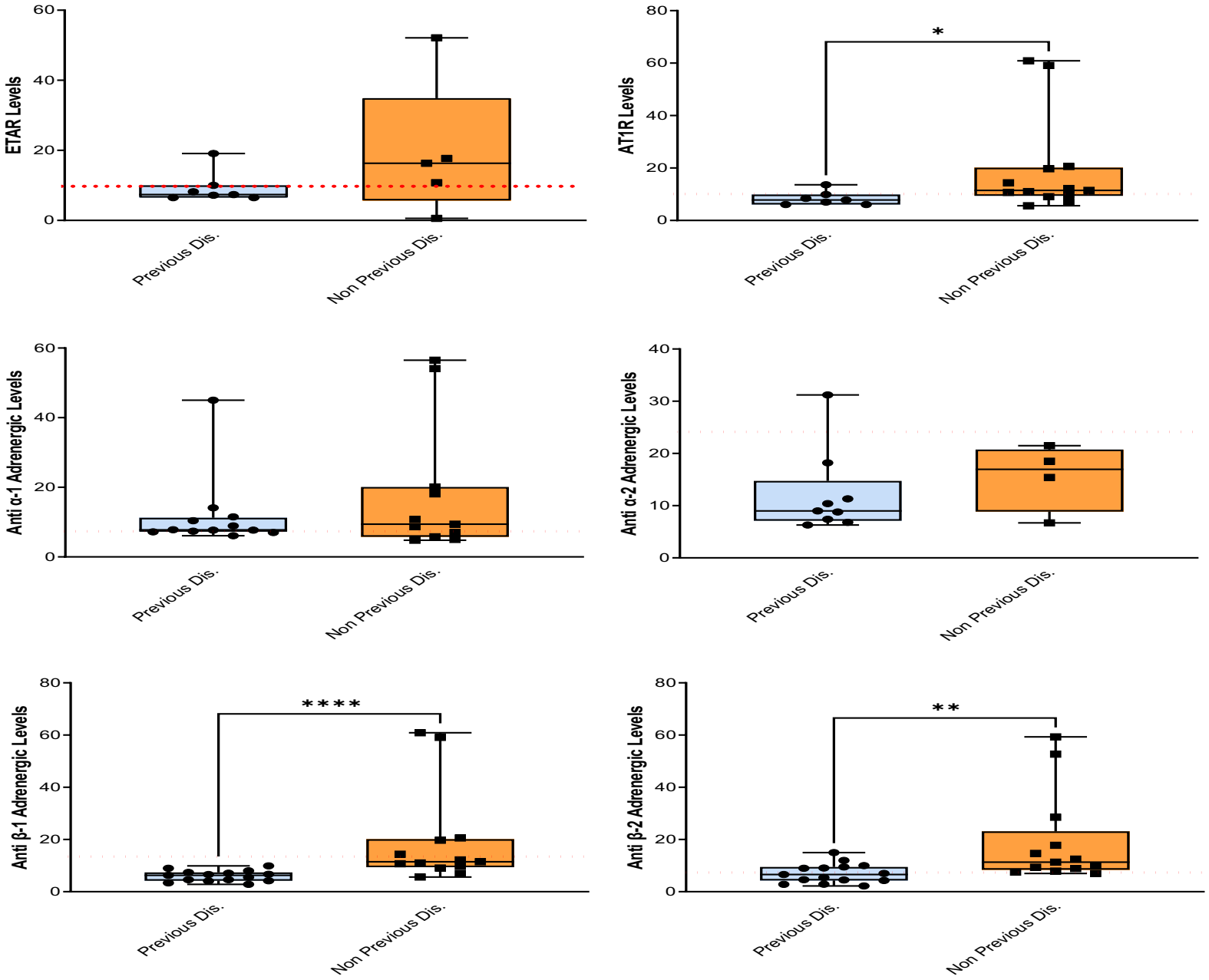
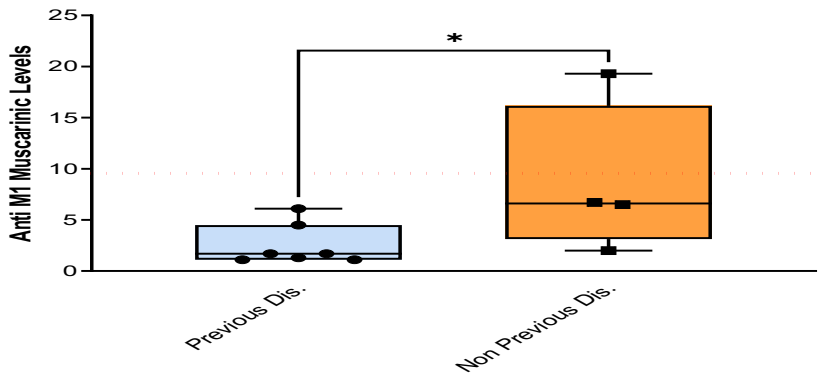
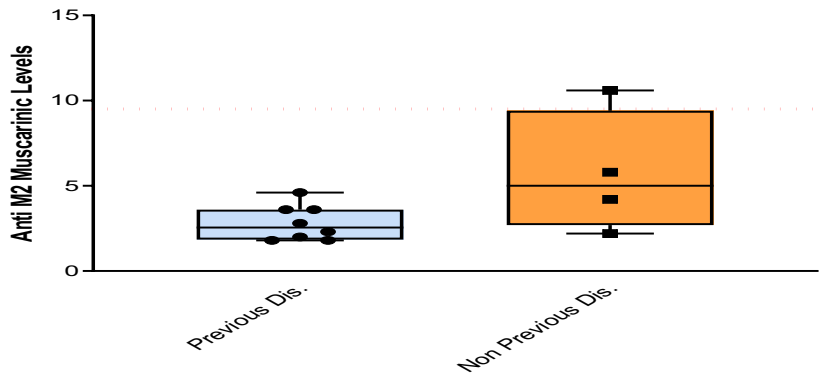


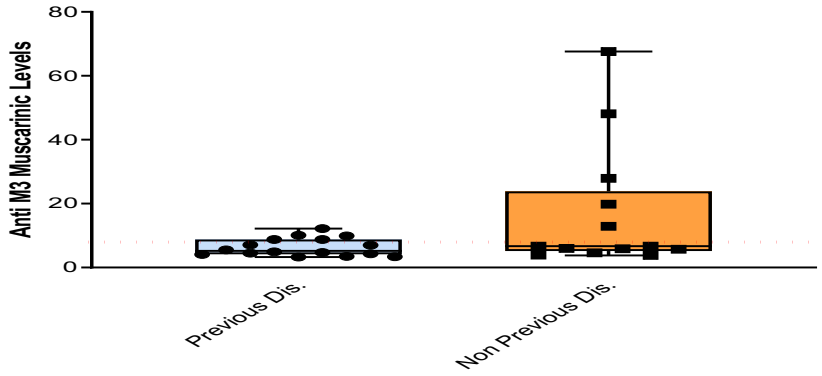
Fig.: R^2 heatmap of Pearson's correlation between levels of: Anti-ETAR; Anti-AT1R; Anti-α-1 Adrenergic; Anti-α-2 Adrenergic; Anti-β-1 Adrenergic; Anti-β-2 Adrenergic; Anti-M1 Muscarinic; Anti-M2 Muscarinic; Anti-M3 Muscarinic; Anti-M4 Muscarinic, Anti-M5 Muscarinic. and C3 complement levels. **a)** Represents Pearson's correlation in positive and negative autoantibodies measurements, **b)** represents correlation only between positive values (C3 complement low levels <90 mg/dl). Autoantibodies parameters.

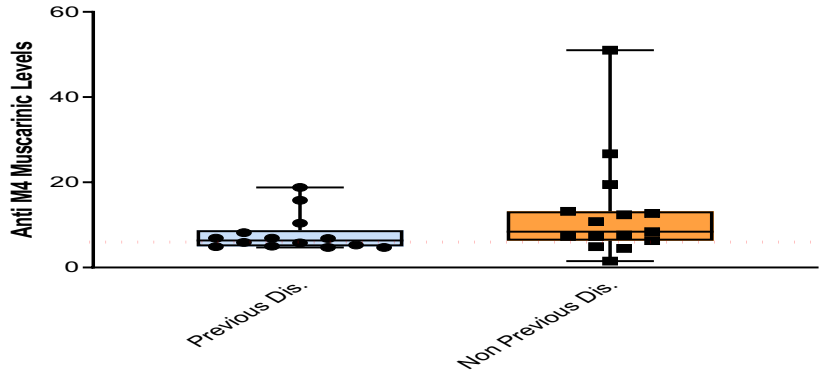
Fig. Red dotted lines indicate positive threshold of each autoantibody. Statistics: Mann-Whitney test * p-value <0,05; ** p-value <0,001; **** p-value <0,0001.

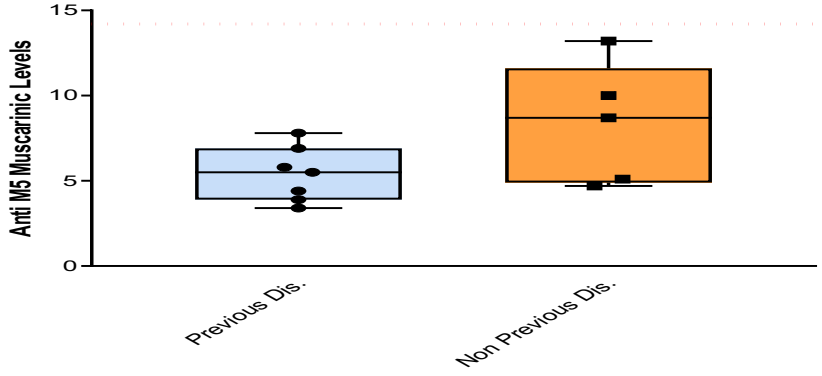



Anti M1 Muscarinic Levels
25
20
15
10
5
0
*
Previous Dis.
Non Previous Dis.


Anti M2 Muscarinic Levels
15
10
5
0
Previous Dis.
Non Previous Dis.


Anti M3 Muscarinic Levels
80
60
40
20
0
Previous Dis.
Non Previous Dis.


Anti M4 Muscarinic Levels
60
40
20
0
Previous Dis.
Non Previous Dis.


Anti M5 Muscarinic Levels
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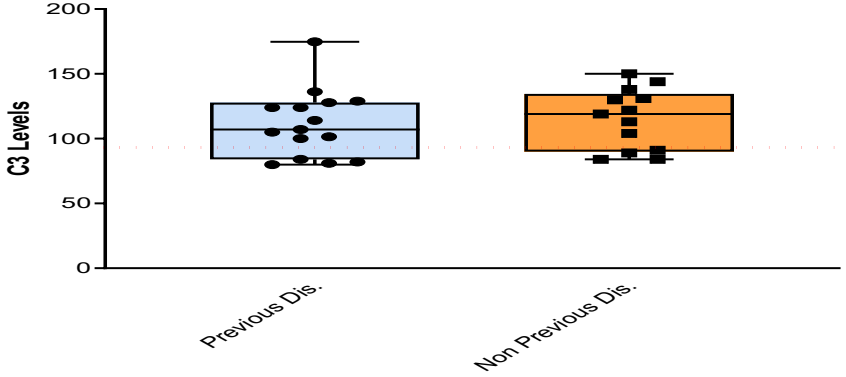
10
5
0
Previous Dis.
Non Previous Dis.


C3 Levels
200
150
100
50
0
Previous Dis.
Non Previous Dis.

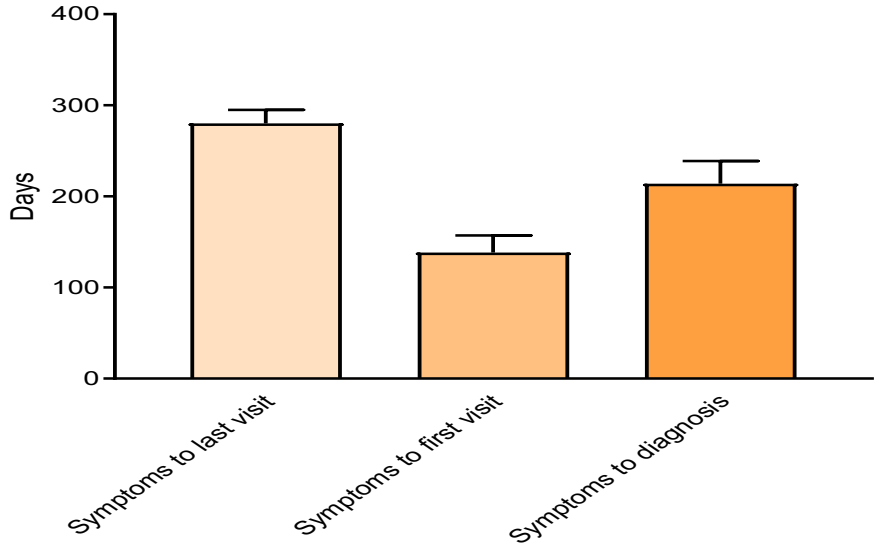


Fig.: Time of follow in days since symptom onset (to June' 21); Time in days since symptom onset to the first visit (excluded patients with previous autoimmune dysautonomia) and time in days since symptom onset to autoimmune dysautonomia diagnosis.

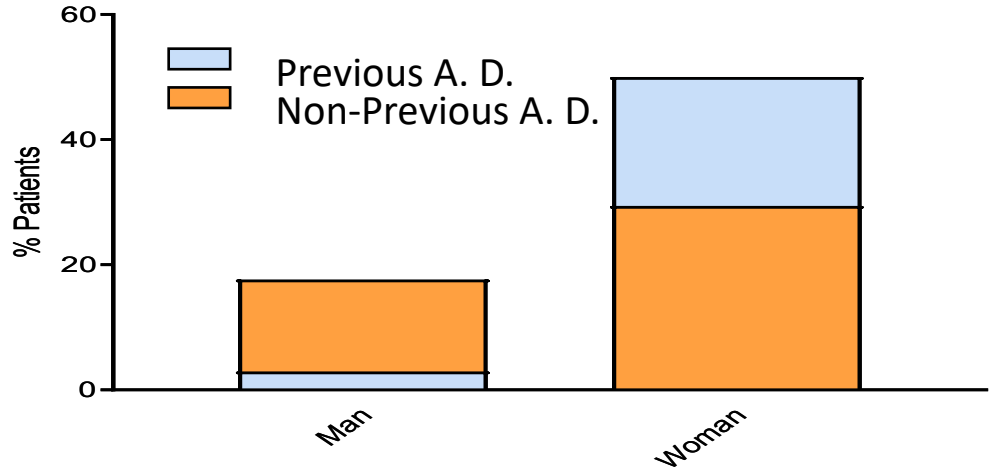


Fig. Percentage of patients classified by gender with previous or non-previous autoimmune dysautonomia to Covid-19 diagnosis.

Open questions

- Retrospective design: time and some other bias confounding.
- Some correlations with symptoms are not significative or might not be assessed because of the small sample size.
- Unmet need of networking between autonomic clinics and centers increasingly focusing on long-COVID, and in a broader range, in dysautonomia.
- Not able to assess 2 samples per patient.
- Changes during following up time in AAb serum levels.
- We hypotheses a strong elevation in serum some weeks after and then, a decline in some of the AAb to a low positivity value.
- These AAb against GPCRs and relation with innate immune system, B and T-cells.

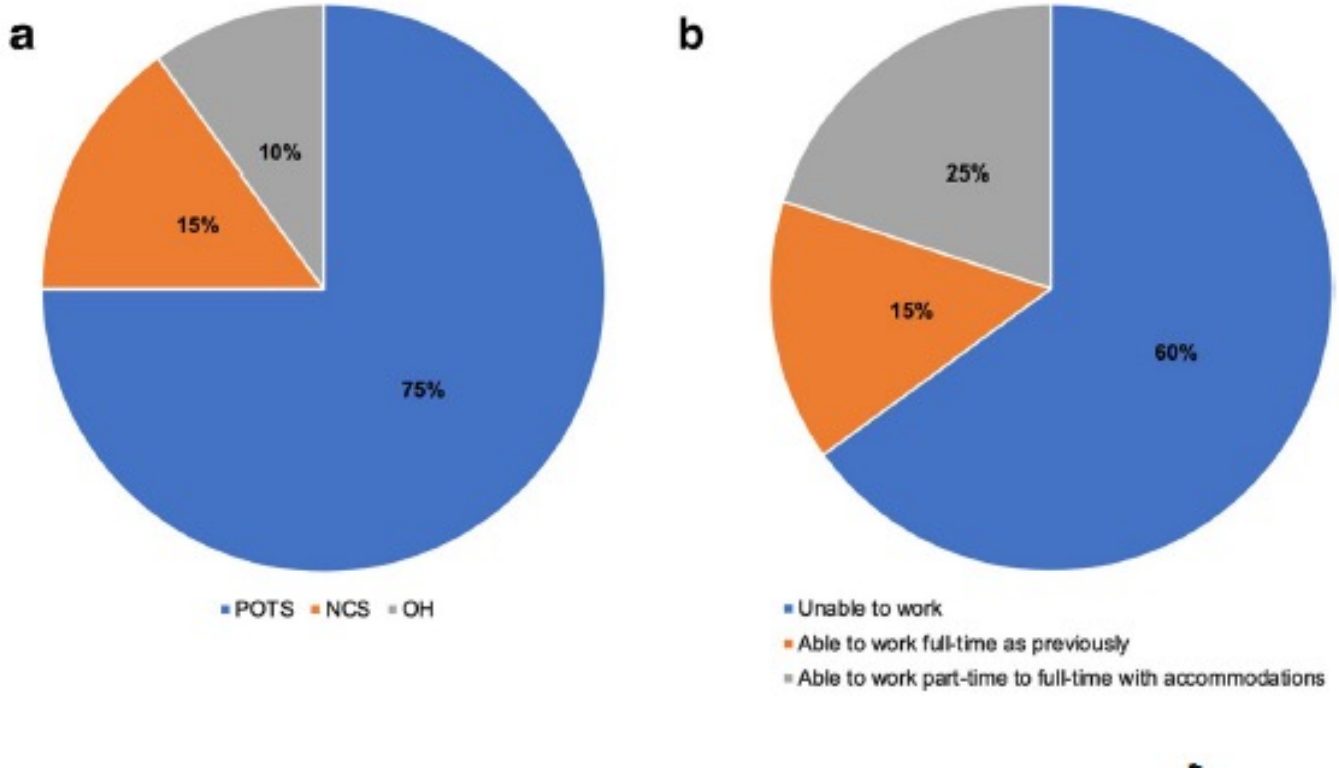
But similar observations

- Prospective evaluation, head-up tilt table (HUTT).
- PACS with poor exertional tolerance, tachycardia with minimal activity or positional change, and palpitations.
- Similar exclusion criteria.
- Mean of 5.8 ± 3.5 months after symptom onset.
- 23/24 had orthostatic intolerance:
  - 4 POTS
  - 15 provoked orthostatic intolerance (POI) after nitroglycerin
  - 3 neurocardiogenic syncope
  - 1 orthostatic hypotension.

Sameer M et al. Prospective Evaluation of Autonomic Dysfunction in Post-Acute Sequela of COVID-19. J Am Coll Cardiol 2022 Mar 28;S0375-1097(22)04581-8.

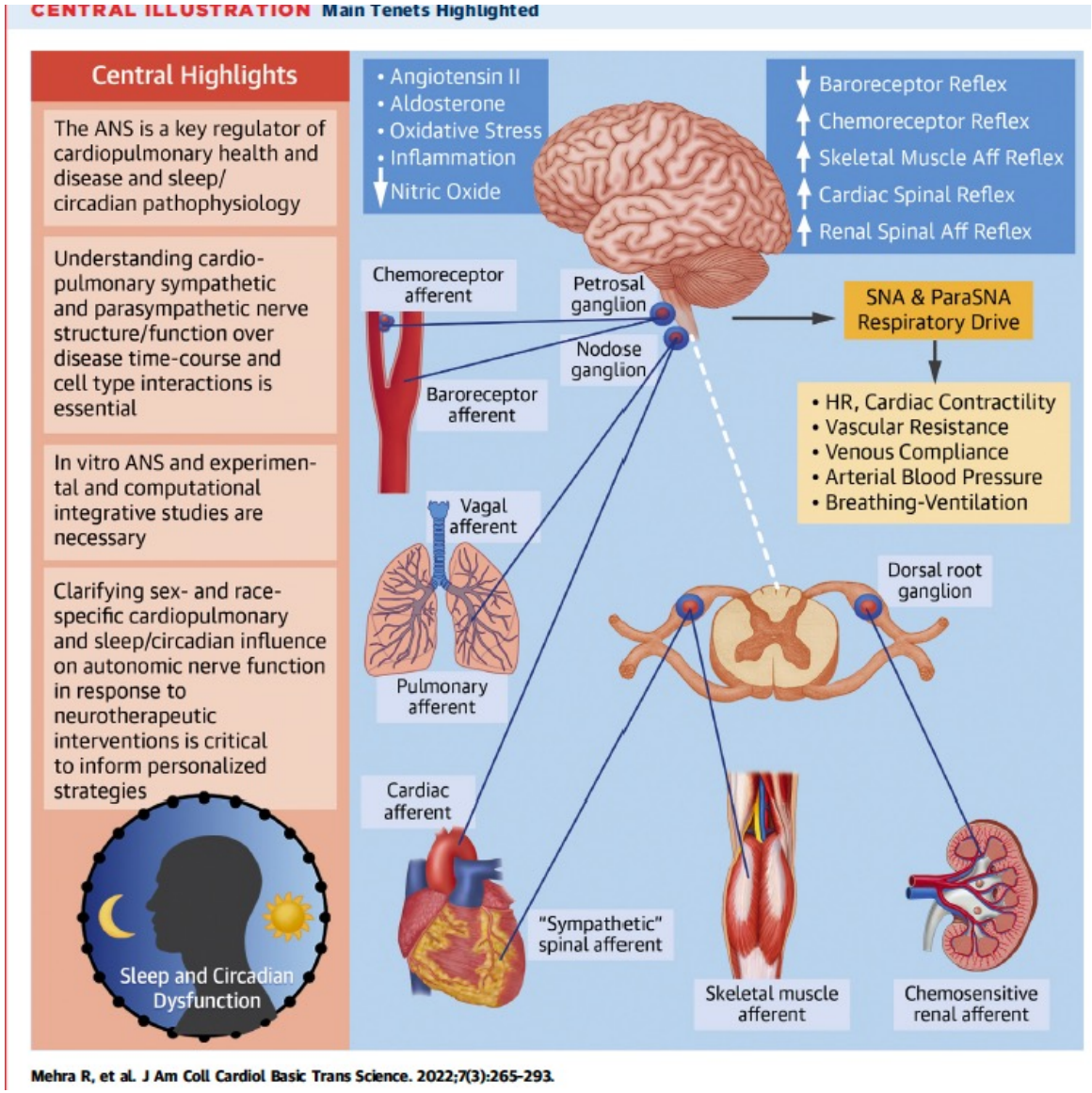
But similar observations

Fig. 1 **a** Autonomic disorders and **b** Patient outcomes 6–8 months after COVID-19. *POTS, postural orthostatic tachycardia syndrome; NCS, neurocardiogenic syncope; OH, orthostatic hypotension*



Blitshteyn S, Whitelaw S. Postural orthostatic tachycardia syndrome (POTS) and other autonomic disorders after COVID19 infection: a case series of 20 patients. Immunol Res 2021; 69:205-211.

Chances to improve our knowledge



Mehra R et al. J Am Coll Cardiol Basic Trans Science 2022 Mar, 7(3_Part_1) 265-293.

Future directions
Clinical trials: the unmet need

- Randomized and control with PLACEBO.
- Those with pulmonary fibrosis/interstitial disease with clear impaired pulmonary respiratory test might a distinct population.
- PICS vs PASC. And the grey zone.
- Nevertheless, NEW TECHNOLOGY might is need to solve the problem.

Future directions
Clinical trials: the unmet need

- Randomized and control with PLACEBO
- BC007.
  - Oligonucleotide agent for Long Covid – case reports.
  - Non modified 15mer ssDNA oligonucleotide (aptamer)
  - Single iv administration
  - Previous phase 1 clinical trial, phase 2 on going in elderly with heart failure
- Immunoglobulins.
  - A chance to clarify their role. POTS and related.
  - Phase 2 running in US (not long-COVID)
  - Previous mixed experience in EM/CFS in late 90s last century

Becker et al. 2020, Clin Drug Investig 2020, 40(5):433-447

▲Thanks to the Team!

▲¡Gracias, equipo!

▲Vielen Dank, Team!

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