

Post-Acute Infection Syndromes (PAIS) and ME/CFS: Bridging Clinical Phenotypes and Immune Dysregulation

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No conflict of interest

Co-Director: National Reference Center for post-viral syndromes: BMSGPK

PI, third party-funded research projects: Horizon Europe, WE&ME Foundation, WWTF



WE&ME FOUNDATION



Funded by
the European Union

Topics of today's talk

Relevance of patients' subgrouping in PAIS and ME/CFS

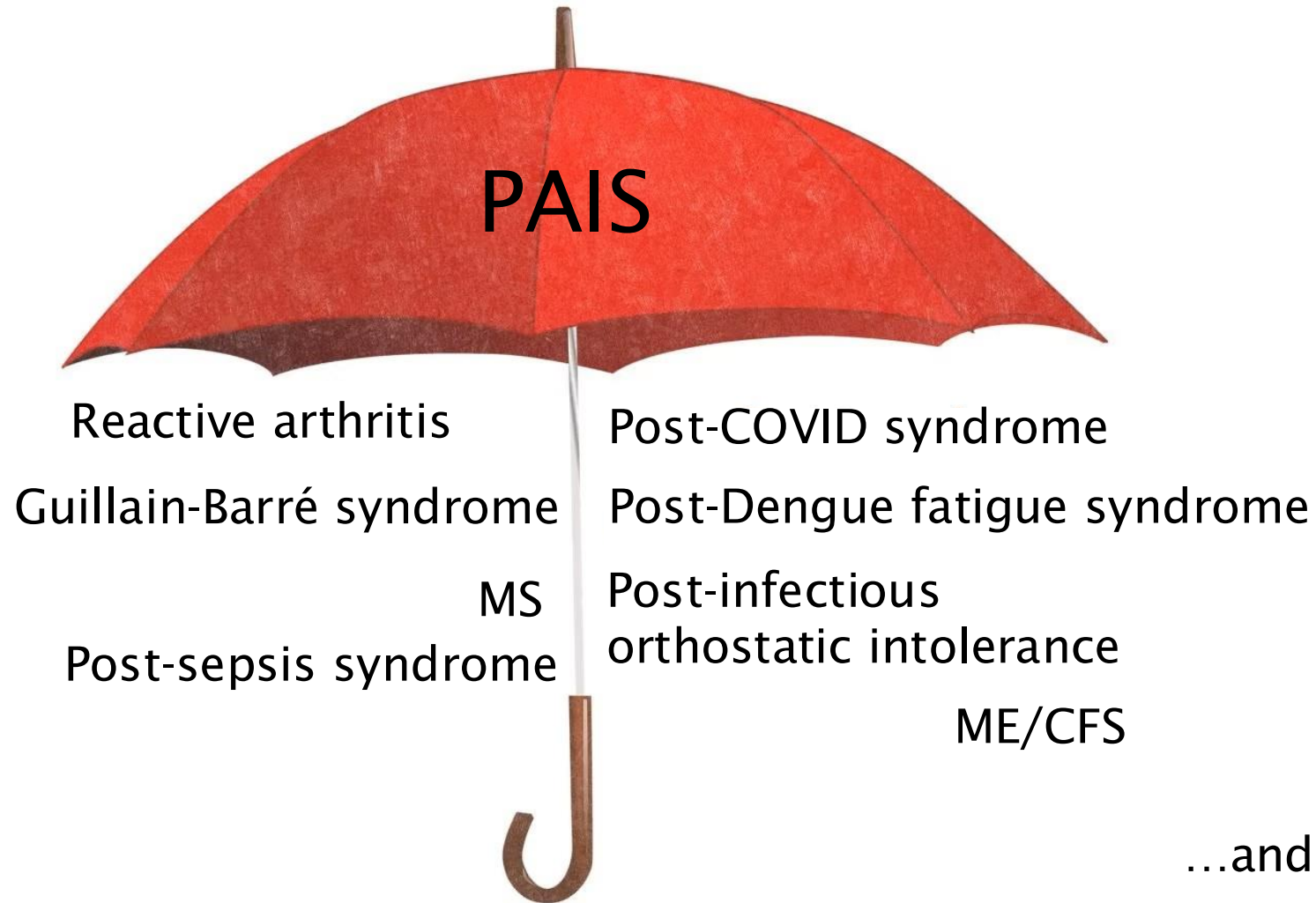
Clinical phenotypes and underlying immunological pathomechanisms

Immunodeficiencies, autoimmunity and mast cell activation in Post-COVID syndrome and ME/CFS



<https://freepngimg.com/icon/101-45-minutes>

Post-acute infection syndrome (PAIS): an umbrella term



Various triggers of post-acute infection syndromes

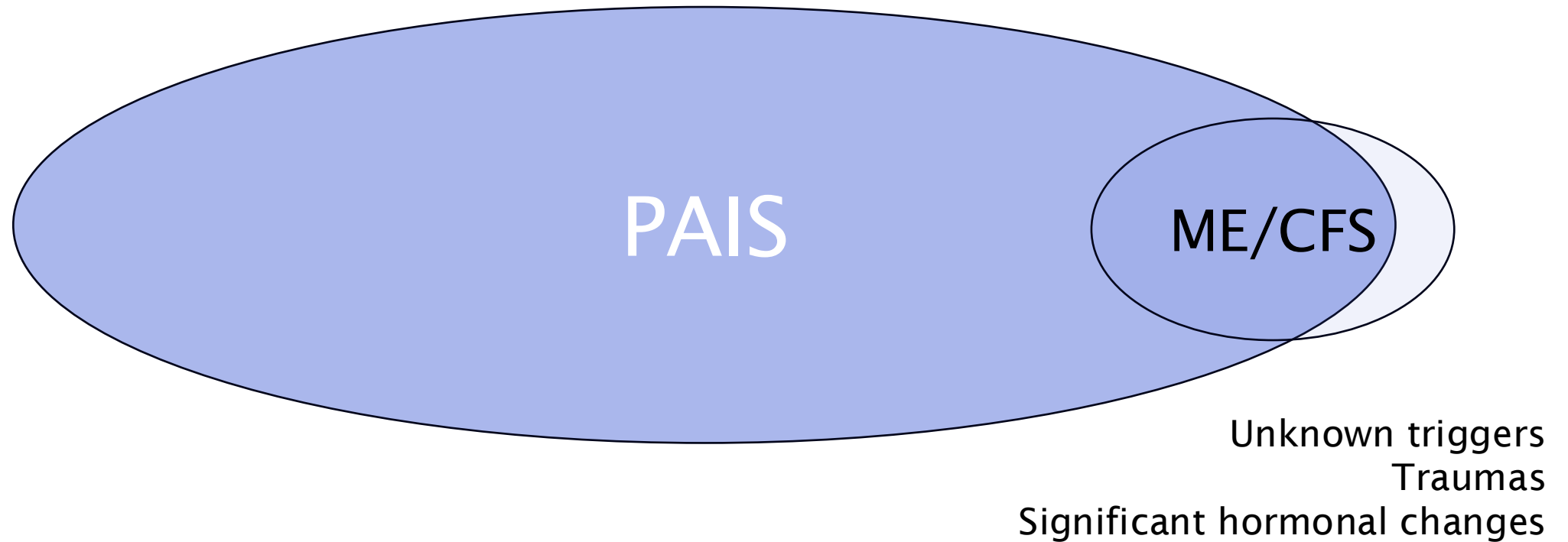
Pathogen	Name of PAIS
Viral pathogens	
SARS-CoV-2	Post-acute sequelae of SARS-CoV-2 infection (PASC) Post-acute COVID-19 syndrome (PACS) Long COVID
Ebola	Post-Ebola syndrome (PES) Post-Ebola virus disease syndrome (PEVDS)
Dengue	Post-dengue fatigue syndrome (PDFS)
Polio	Post-polio syndrome (PPS)
SARS	Post-SARS syndrome (PSS)
Chikungunya	Post-chikungunya chronic inflammatory rheumatism (pCHIK-CIR) Post-chikungunya disease
EBV	No name
West Nile virus	No name
Ross River virus ^a	No name
Coxsackie B ^a	No name
H1N1/09 influenza ^{a,b}	No name
VZV ^{a,b}	No name

Choutka J et al. Nature Medicine 2022

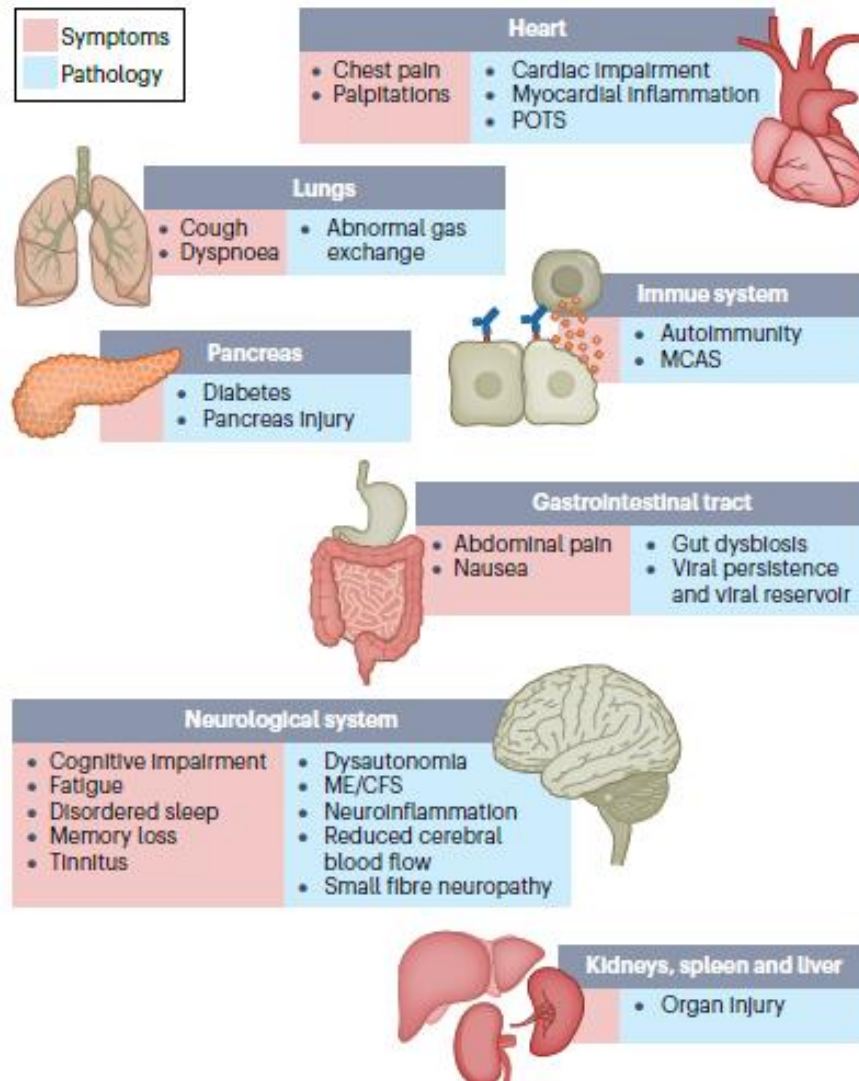
Pathogen	Name of PAIS
Non-viral pathogens	
<i>Coxiella burnetii</i>	Q fever fatigue syndrome (QFS)
<i>Borrelia</i> ^c	Post-treatment Lyme disease syndrome (PTLDS)
<i>Giardia lamblia</i> ^{a,d}	No name
^a Limited or very limited evidence base. ^b Association with increased use of ME/CFS diagnosis in health registry. ^c Contradicting or unclear evidence base. ^d Supporting evidence derives from a single outbreak in Norway.	

Following 5–30% of infections (e.g. 20% in Q fever fatigue)

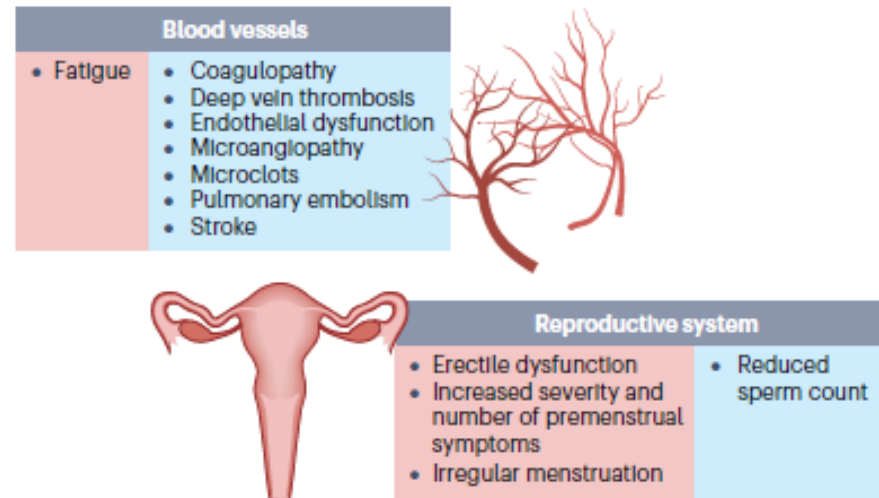
ME/CFS: a subgroup of post-acute infection syndromes (PAIS)



Post-COVID Syndrome: A wide variety of symptoms

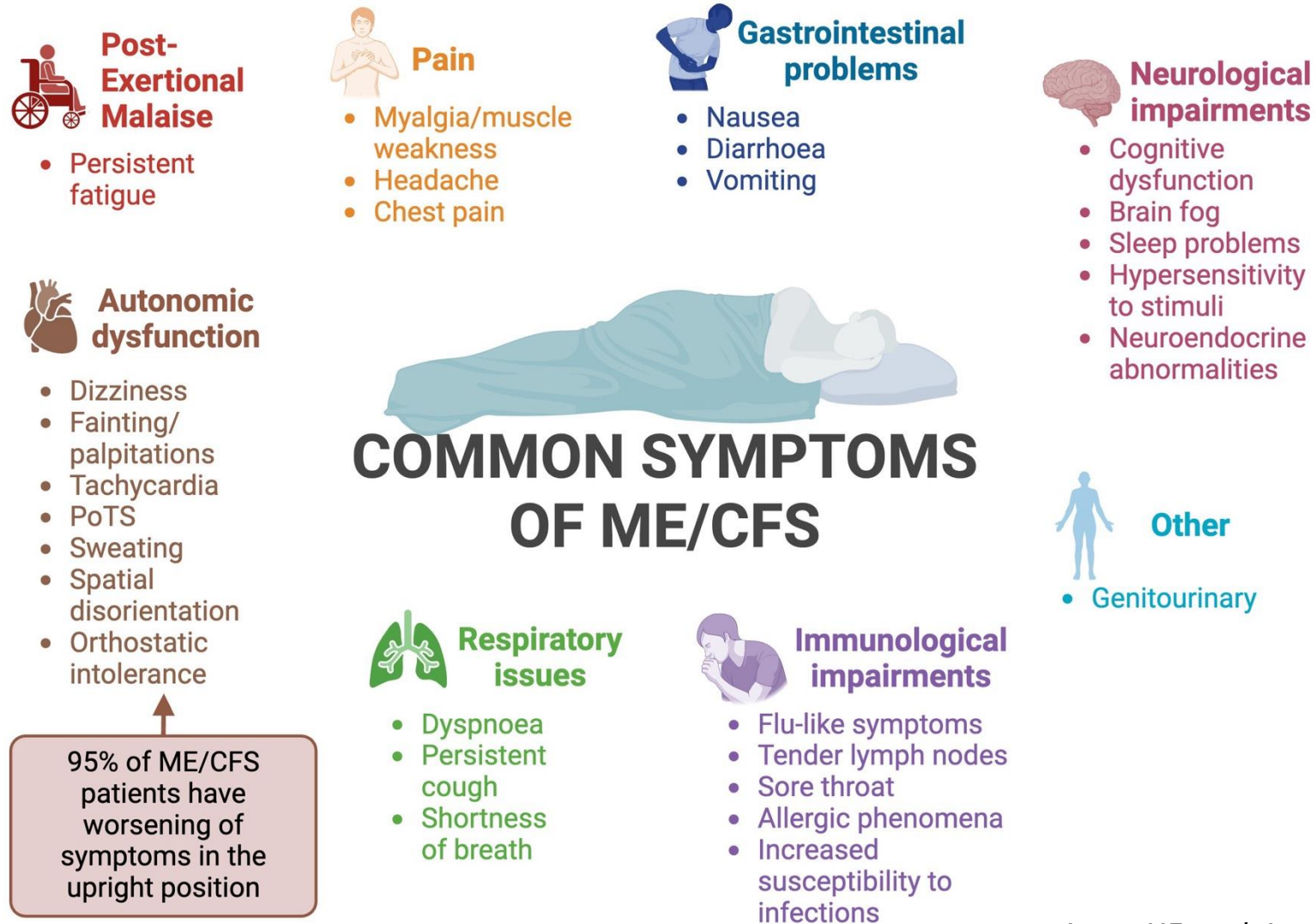


- Various organ systems affected
- **More than 200 symptoms** associated with Post-COVID syndrome
- Difference in disease presentation in different patients



Davis HE et al. Nature Rev Microbiol 2023

Various organ systems affected in ME/CFS



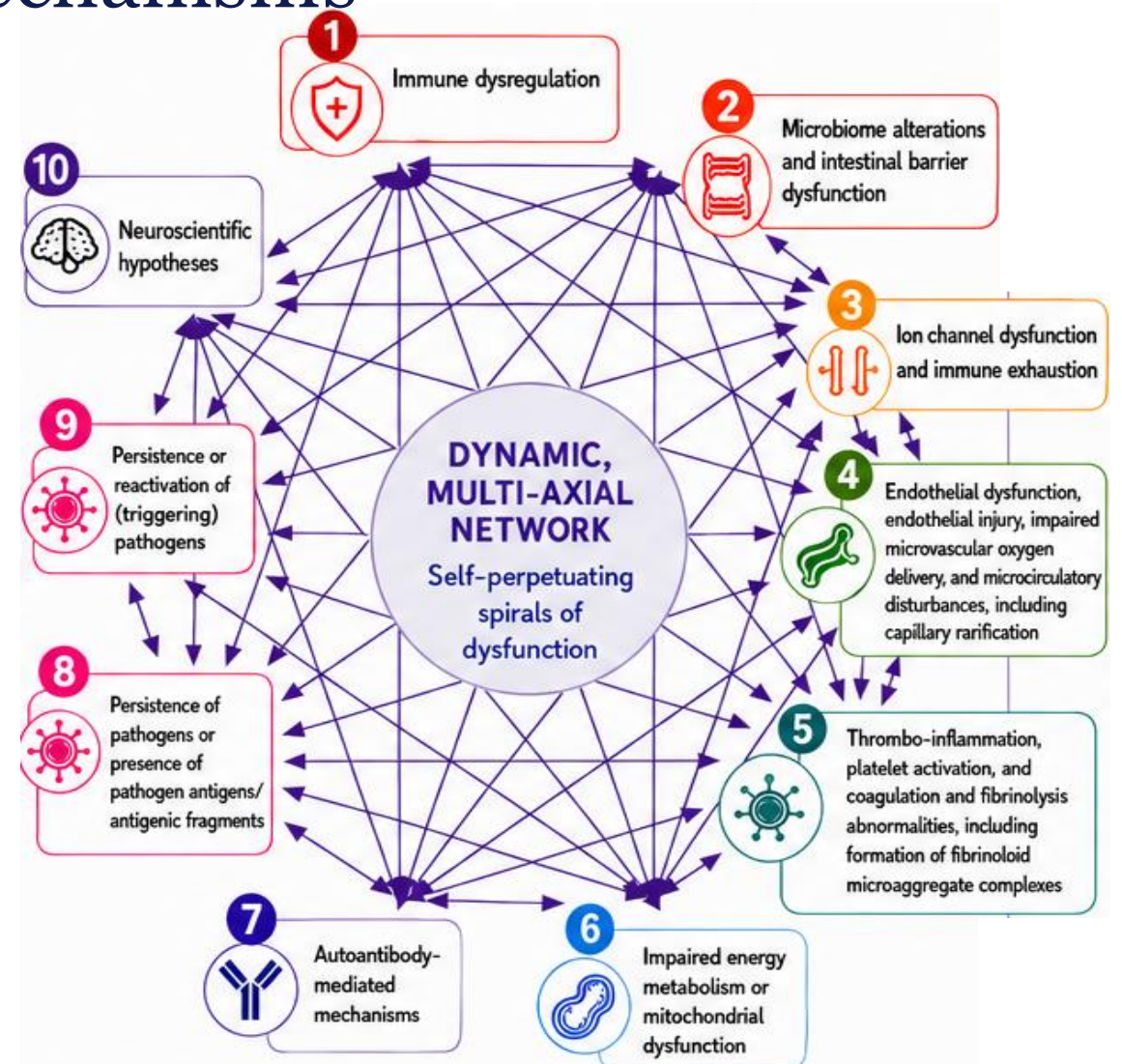
Arron HE et al. Immunol Front 2024

Interconnected PAIS pathomechanisms

Pathophysiological mechanisms (endotypes) and modulators interact → complex biological network → shape the diverse PAIS phenotypes

Influences in individual patients on:

- disease severity
- specific manifestation
- combination of PAIS diseases, syndromes, and domains



Hoffmann K et al. Ms submitted

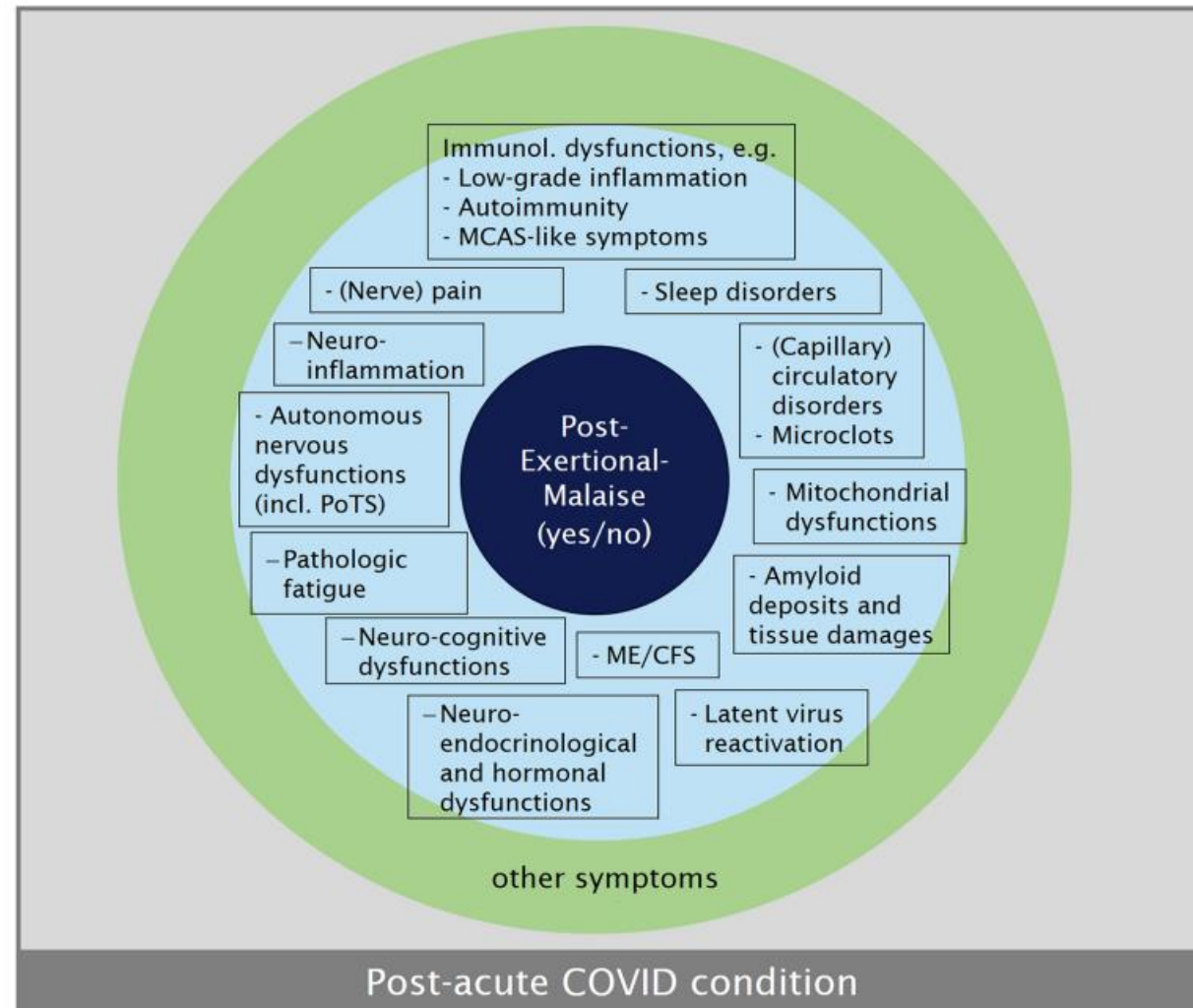
Studies with non-stratified PAIS cohorts



<http://www.teaching-learning.eu/aktuelles/einzelsicht/article/ects-grades-hilfsmittel-zur-besseren-vergleichbarkeit-von-noten.html>

Considerations for patient stratification in PAIS

Post-exertional malaise: relevance for patient stratification

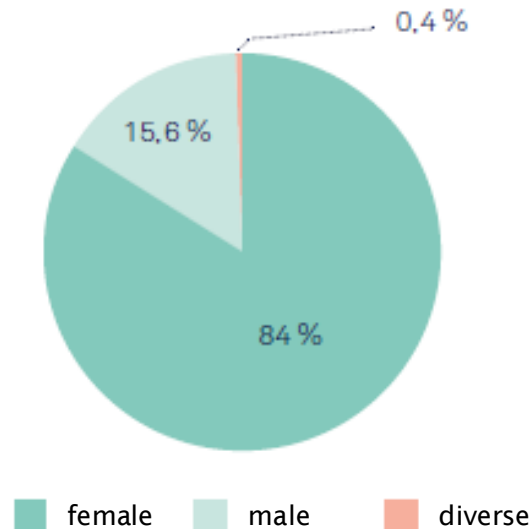


Hoffmann K et al. Infect Dis Ther 2024

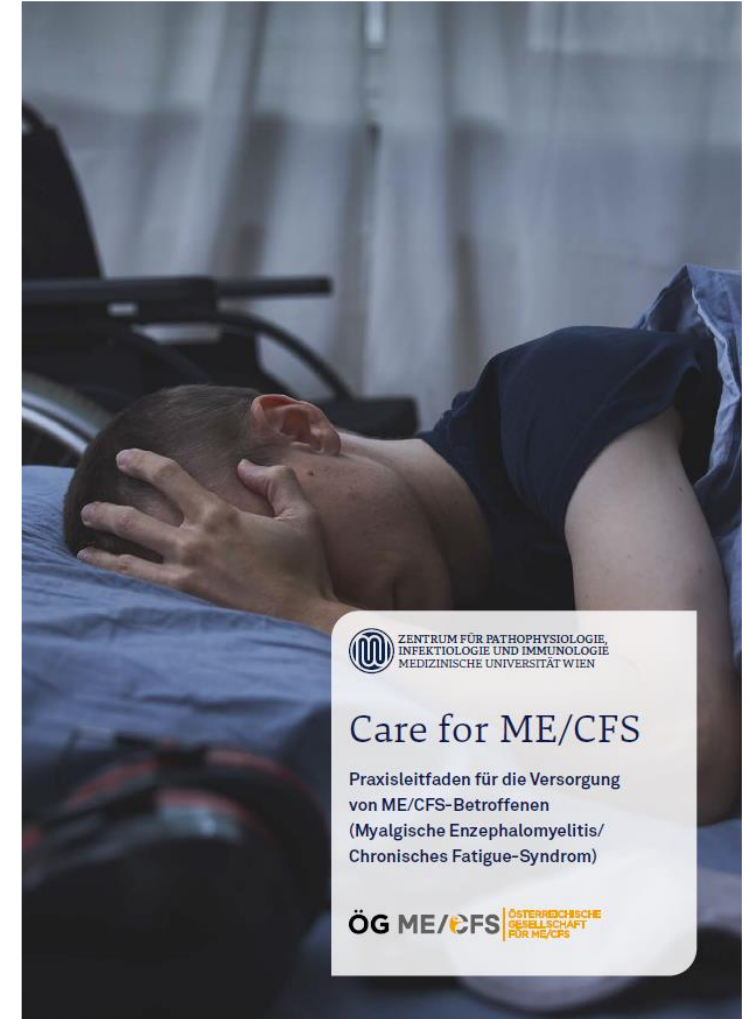
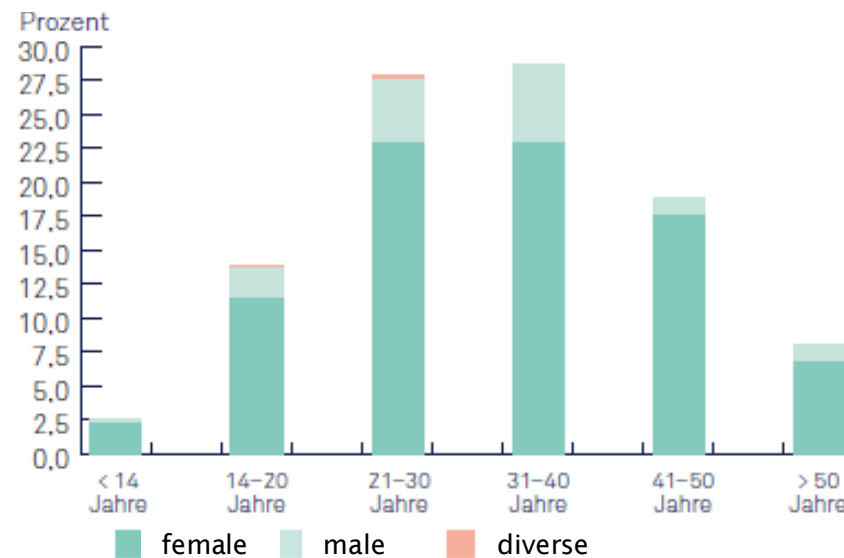
Gender and age distribution in ME/CFS

Data from **687 ME/CFS patients** from Austria, Germany, Northern Italy and Switzerland

Sex distribution



Disease onset (sex distribution)

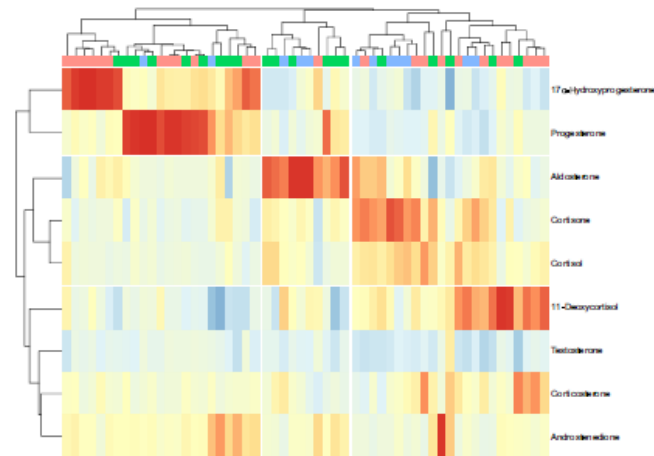


Hainzl A., Rohrhofer J., Schweighardt J. et al. 2024
DOI: 10.5281/zenodo.12091631

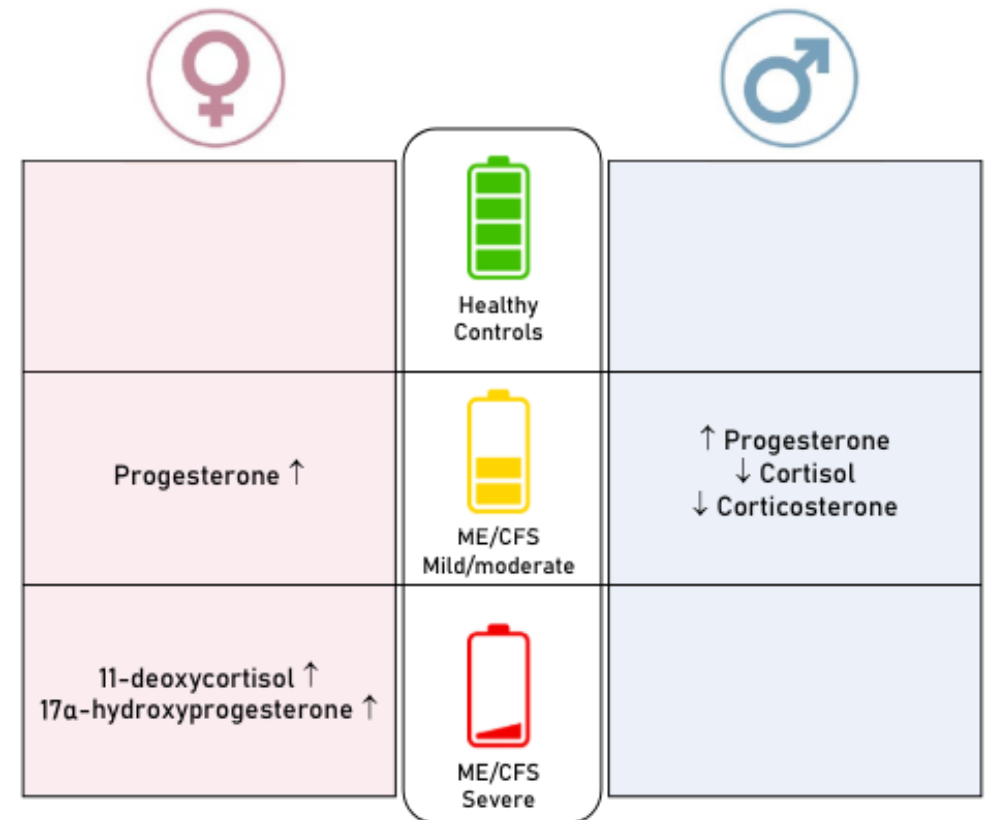
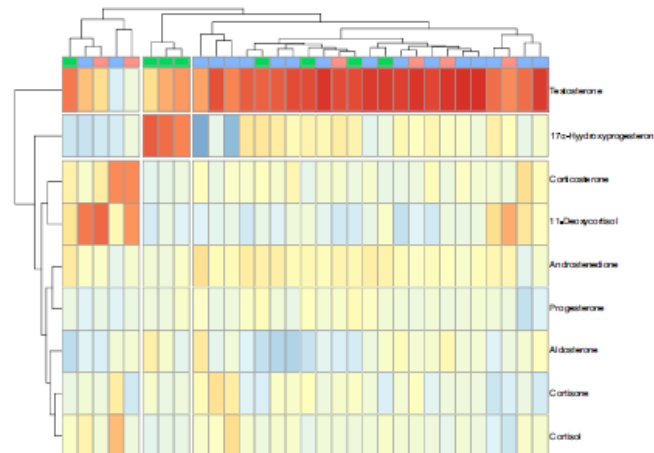
The relevance of sex and disease severity for patient stratification in ME/CFS

Hierarchical cluster analysis of plasma steroid hormone levels

Female cohort



Male cohort

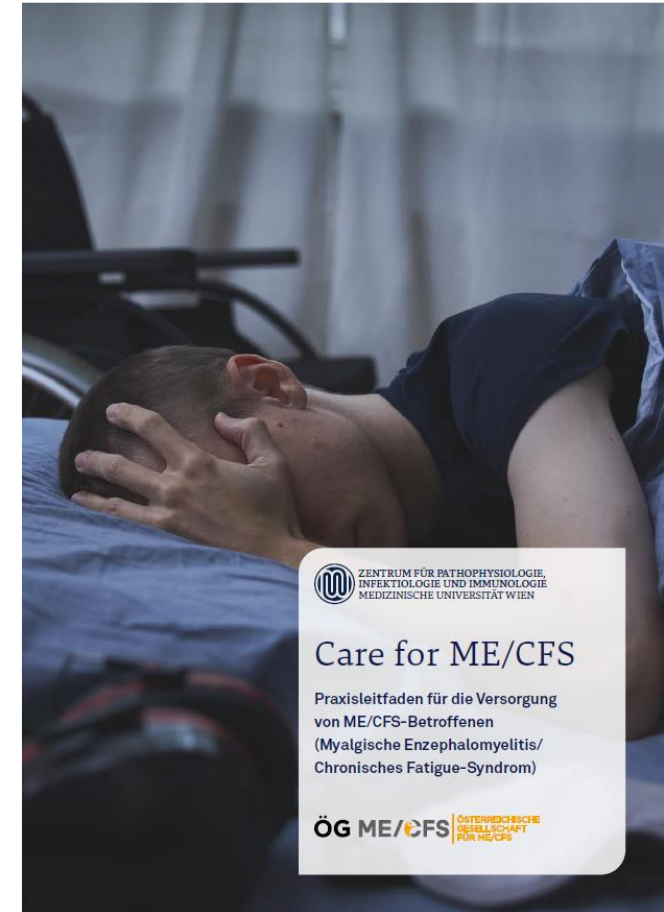
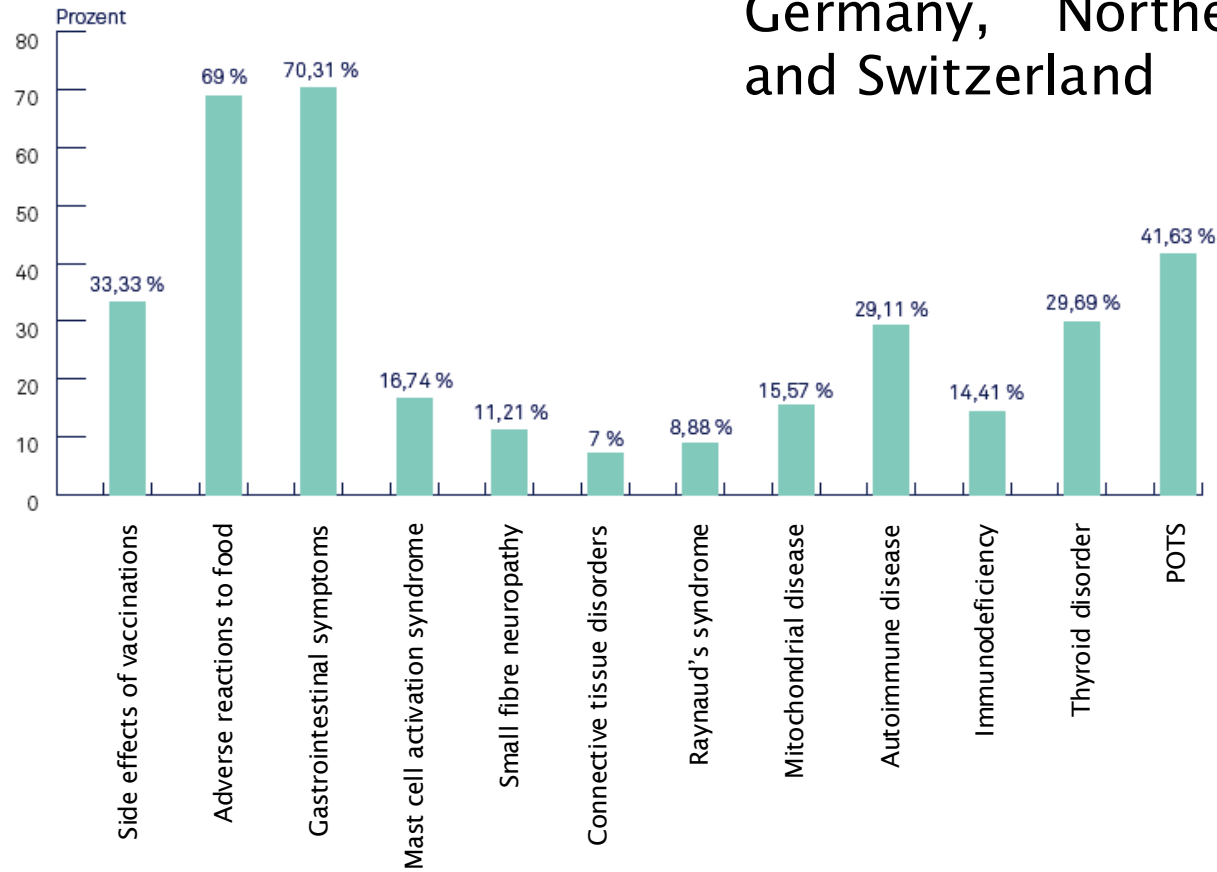


Pipper C et al. J Endocrinol Invest. 2024

Comorbidities and new symptoms in ME/CFS patients

Comorbidities/symptoms
(newly diagnosed/acquired
following ME/CFS diagnosis)

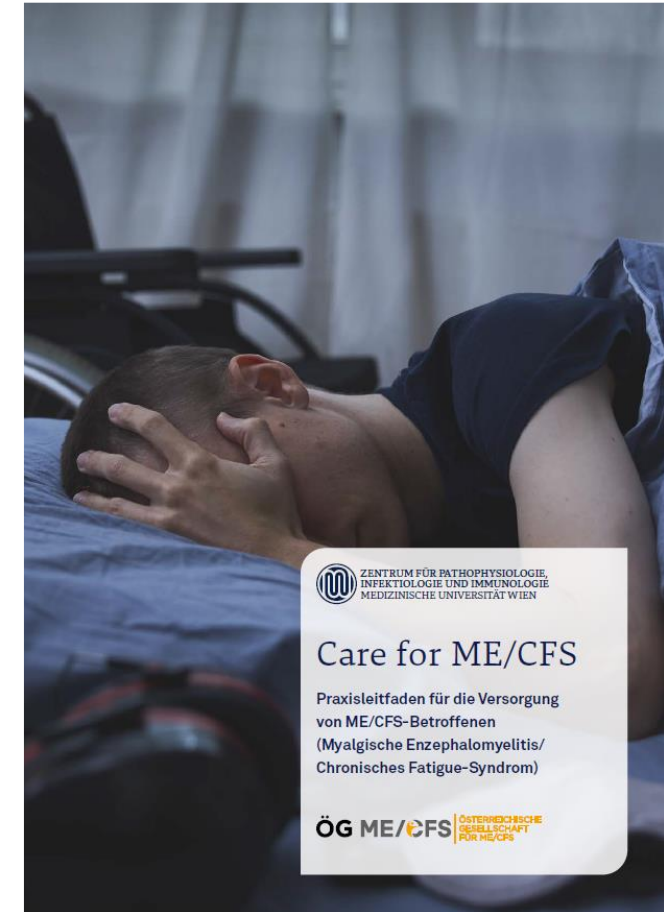
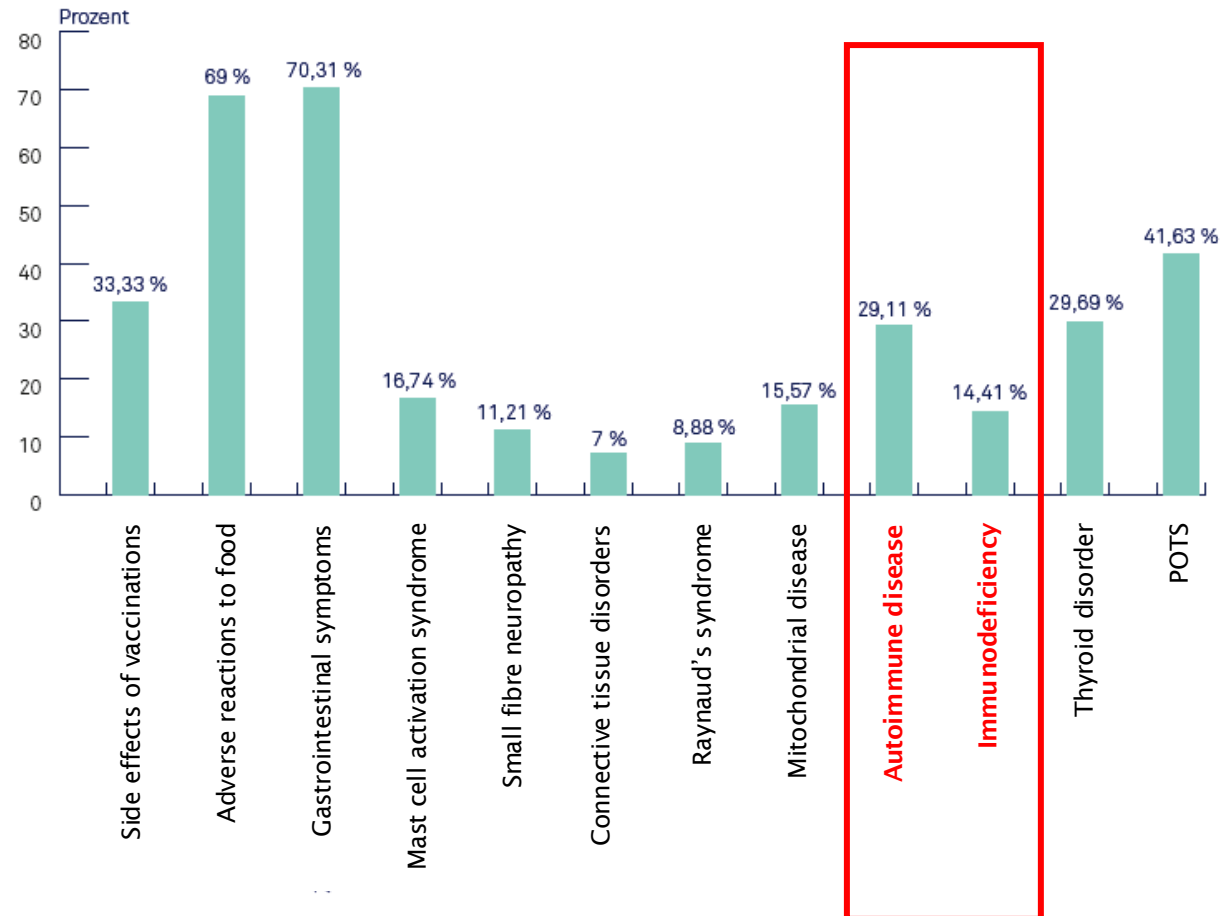
Data from **687 ME/CFS patients** from Austria, Germany, Northern Italy and Switzerland



Hainzl A., Rohrhofer J., Schweighardt J. et al. (2024)
DOI: 10.5281/zenodo.12091631

Immunological comorbidities in ME/CFS patients

Comorbidities/symptoms
(newly diagnosed/acquired
following ME/CFS diagnosis)



Hainzl A., Rohrhofer J., Schweighardt J. et al. (2024)
DOI: 10.5281/zenodo.12091631

Infection-related symptoms in Post-COVID or ME/CFS

Recommendations from guidelines

Immunological assessment via

- a detailed medical history
- lymphocyte subtyping
- evaluation of the humoral immune component
- functional immune cell analysis

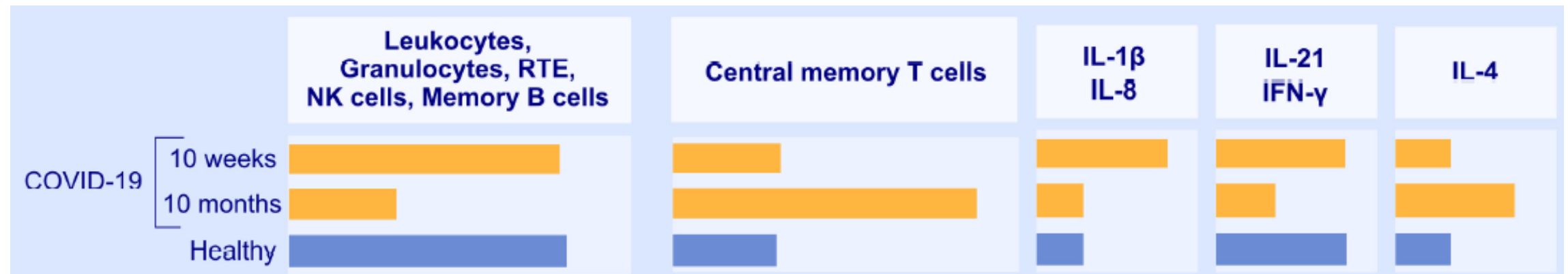


Immunological changes following SARS-CoV-2 infection

Case-control study

133 COVID-19 patients (rtPCR-confirmed (n = 116) and/or SARS-CoV-2 antibody-confirmed)
98 uninfected control subjects who had been asymptomatic for the past 10 weeks, with negative SARS-CoV-2 antibody test results

Cellular and humoral changes in the immune system up to 10 months after infection



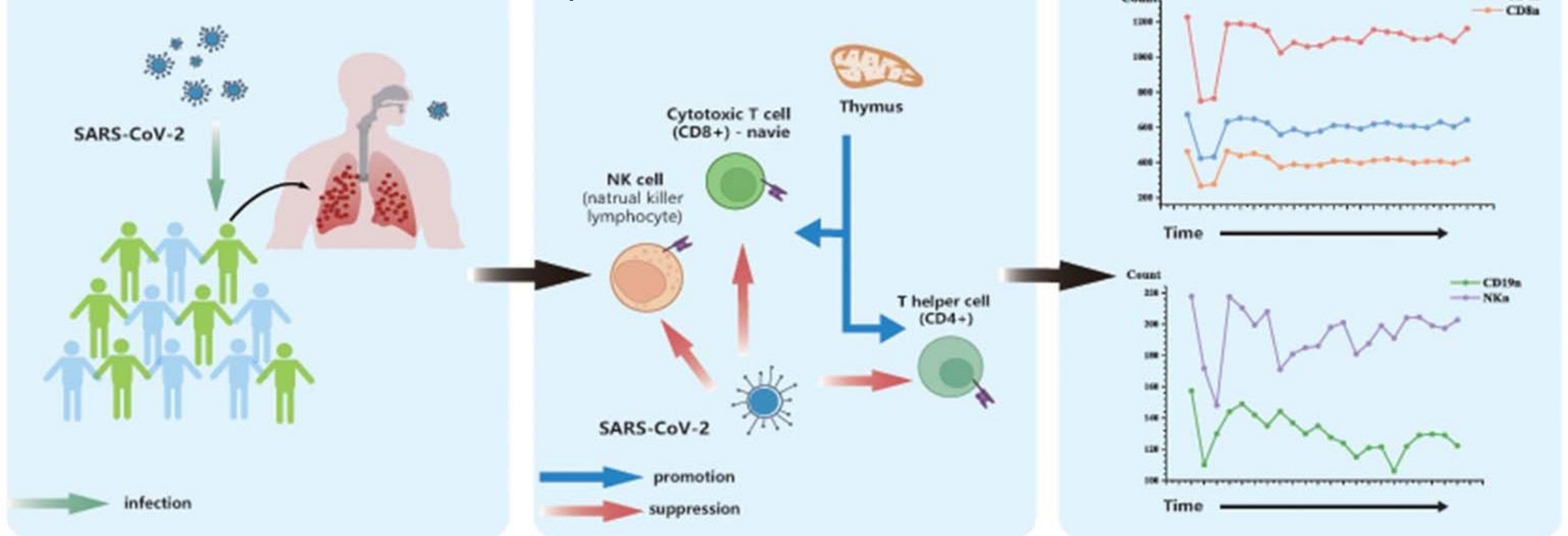
Kratzer B et al. Allergy 2024

Long-term immunological changes following SARS-CoV-2 infection

40,537 patients analysed

Changes to the immune system

Lymphocyte dysregulation



Jiang Z et al. IJID 2025

20 months after infection

ME/CFS: Recurrent infections in a group of patients

Studies show a link between changes in the immune system and the development of ME/CFS

Skowera A et al. Clin Exp Immunol 2004
Guenther S et al. Hum Immunol 2015
Sotzny F et al. Autoimmune Rev 2018

CCCFS study: Questionnaire-based study involving people with ME/CFS (n=687)

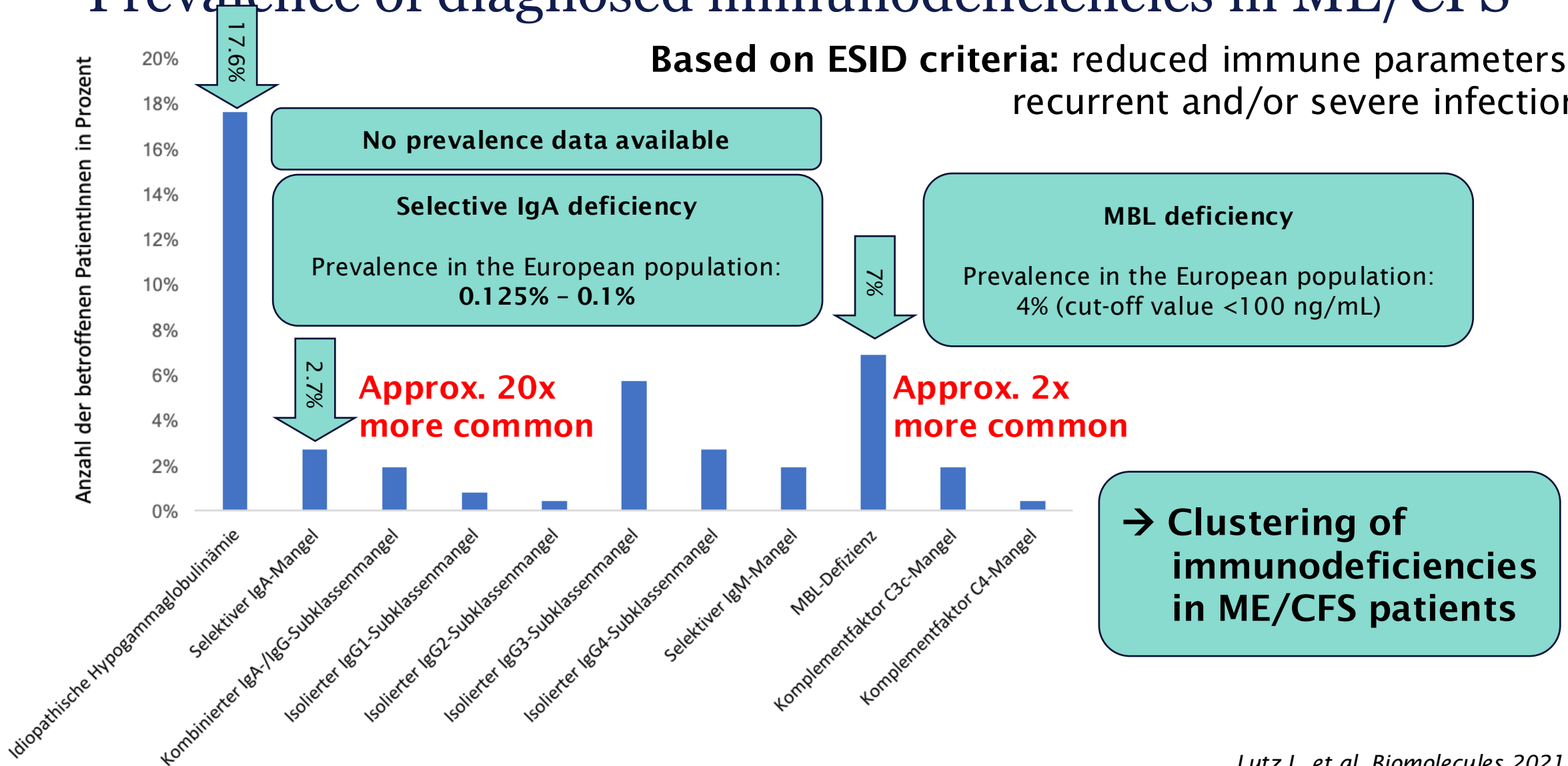
Recurrent infections prior to ME/CFS onset	Affected patients
Influenza	73.7
Other viral illnesses	60.8
Bacterial/parasitic diseases	42.2
Respiratory tract infections	66.7
Gastrointestinal infections	55.3
Urogenital infections	43.1

Some patients developed ME/CFS following COVID

Rohrhofer J, Milicic M et al. Manuscript in preparation

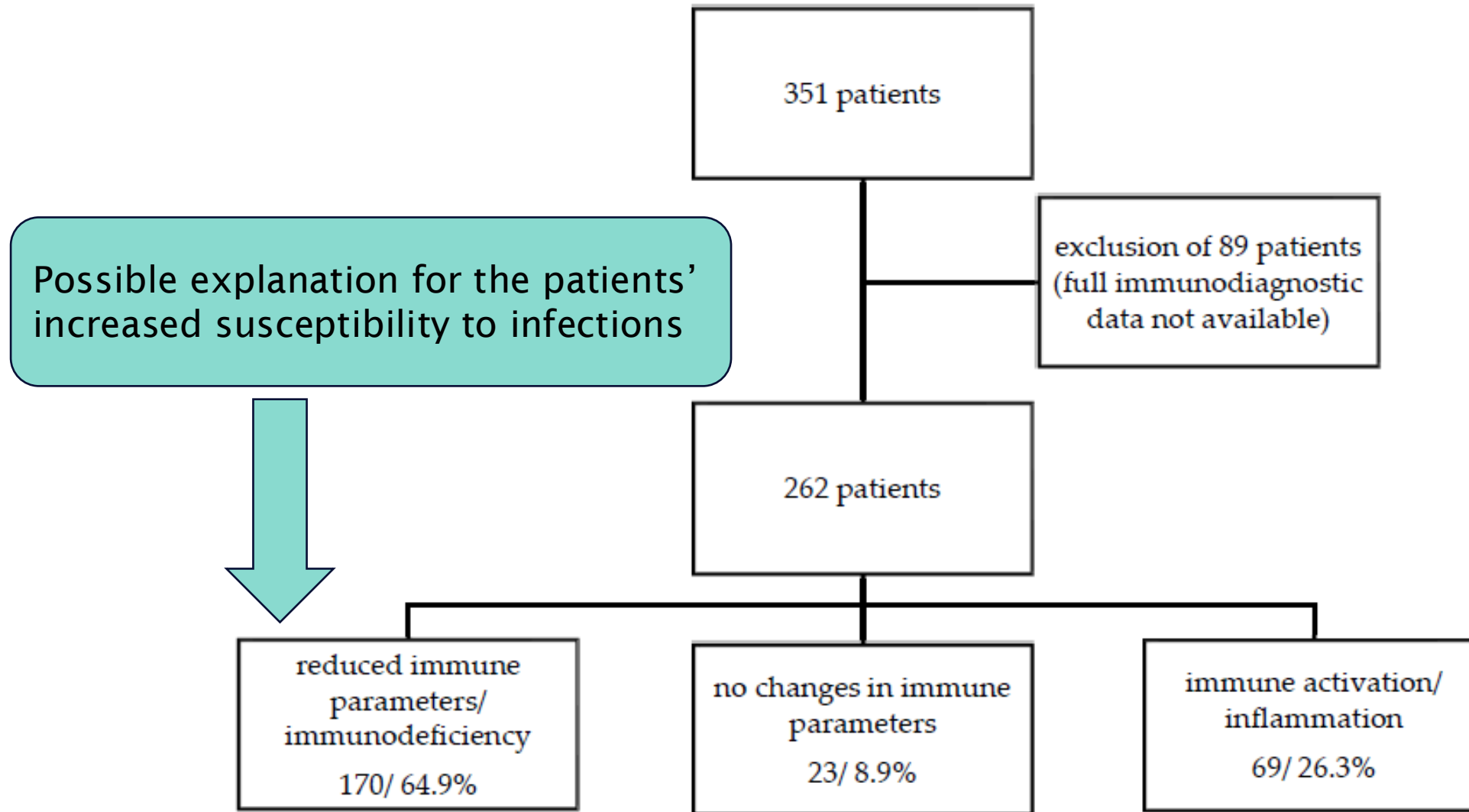
Prevalence of diagnosed immunodeficiencies in ME/CFS

Based on ESID criteria: reduced immune parameters + recurrent and/or severe infections



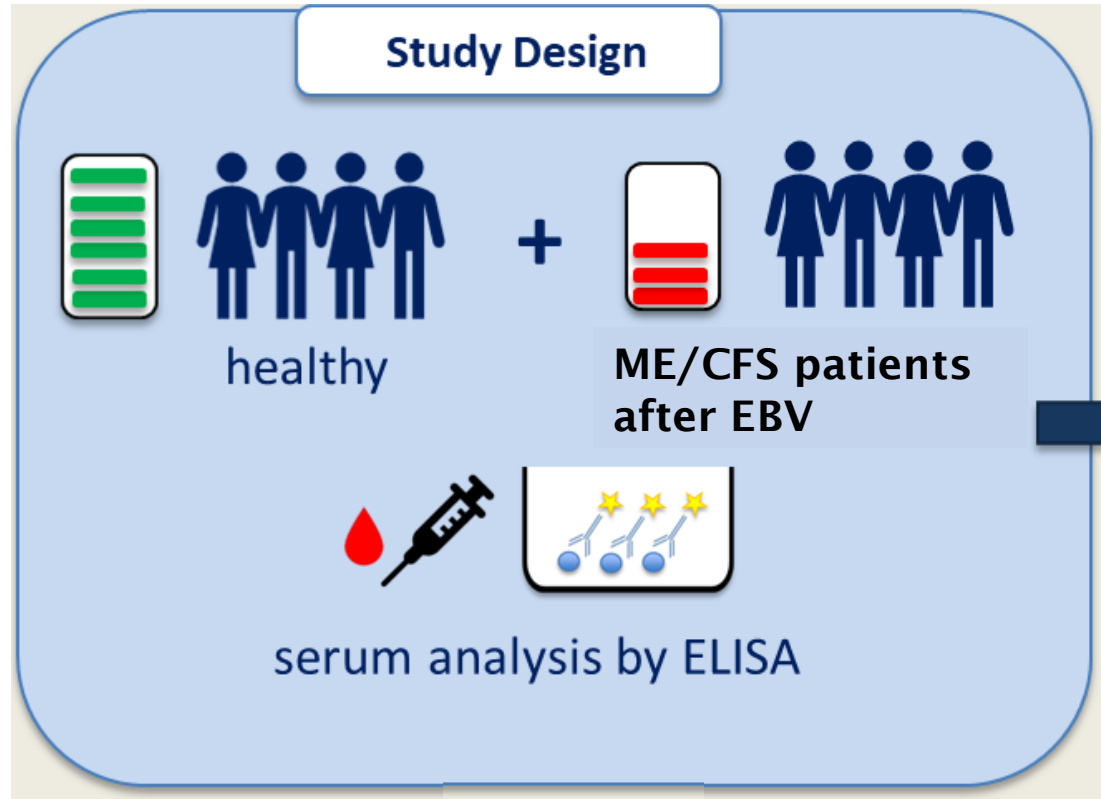
Lutz L. et al. Biomolecules 2021

Results – reduced immune parameters/immune deficiencies



Lutz L. et al. Biomolecules 2021

Immunological patient stratification is relevant for post-infectious diseases



→ Different disease mechanisms and potential biomarkers

Rohrhofer J et al. JCM 2024

Meta-analysis of NK cell function in ME/CFS



OPEN ACCESS

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identifier CRD42024542140.

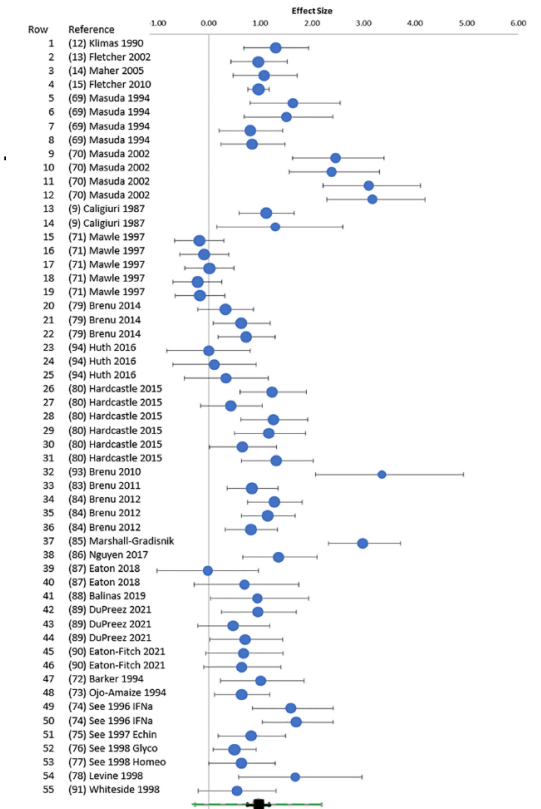
Meta-analysis of natural killer cell cytotoxicity in myalgic encephalomyelitis/chronic fatigue syndrome

James N. Baraniuk^{1,2*}, Natalie Eaton-Fitch²
and Sonya Marshall-Gradisnik²

¹Department of Medicine, Georgetown University, Washington, DC, United States, ²National Centre for Neuroimmunology and Emerging Diseases, Griffith University, Gold Coast, QLD, Australia

28 studies and
55 data points:
effector-to-target
cell ratio (E:T)

Significant
reduction in
NK cytotoxicity
in ME/CFS to
approximately
half the levels
seen in the
healthy control
group



The outcomes
confirm reproducible NK cell dysfunction in ME/CFS and will guide studies using
the NK cell model system for pathomechanistic investigations.

Autoimmunity and ME/CFS

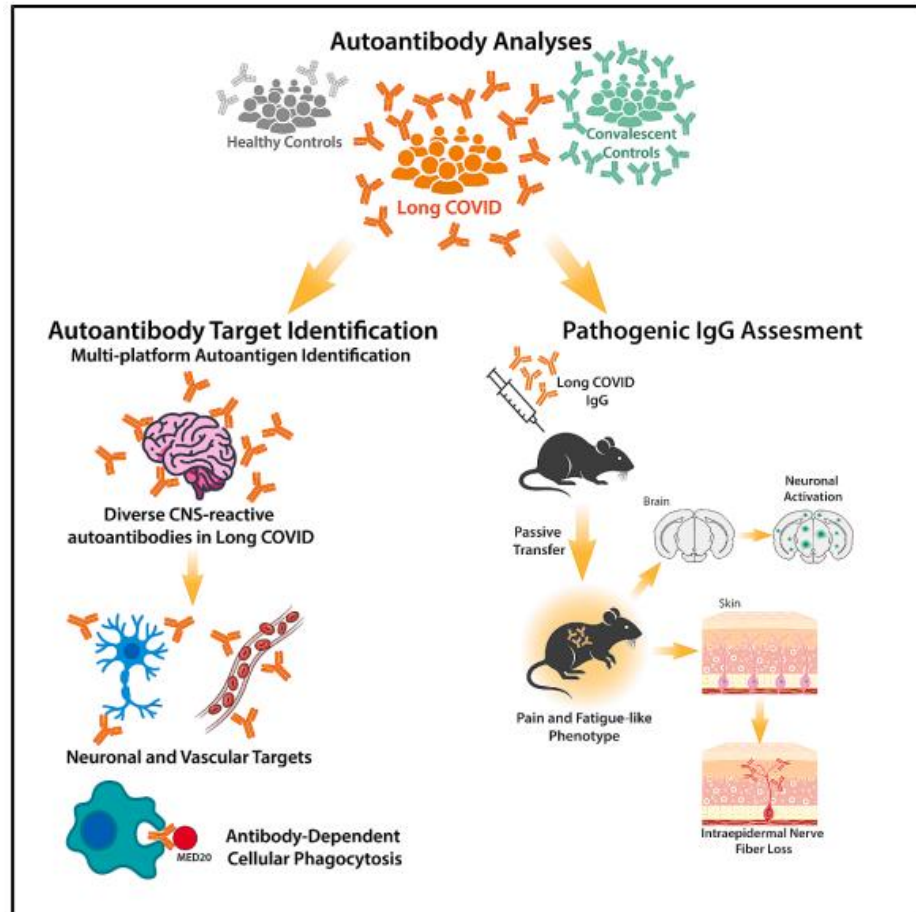
CCCFS Study: Analysis of 687 questionnaires completed by ME/CFS patients

	Pre-existing conditions	After ME/CFS diagnosis
Autoimmune disease	15.7%	29.1%

Rohrhofer J, Milicic M et al. Manuscript in preparation

Autoantibodies in post-COVID patients

Purified IgG → reaction with human locus coeruleus, thalamus, adrenal gland, and thyroid
→ cross-reacted with mouse sciatic nerve and meninges



CNS-reactive AABs correlated with several neurological symptoms.

Transfer of total IgG purified from post-COVID patients: into mice:

- Induction of fatigue-like behavior, loss of balance/coordination, thermal hyperalgesia, small fiber nerve damage, and increased pain-related neuronal activity, recapitulating patients' symptoms.

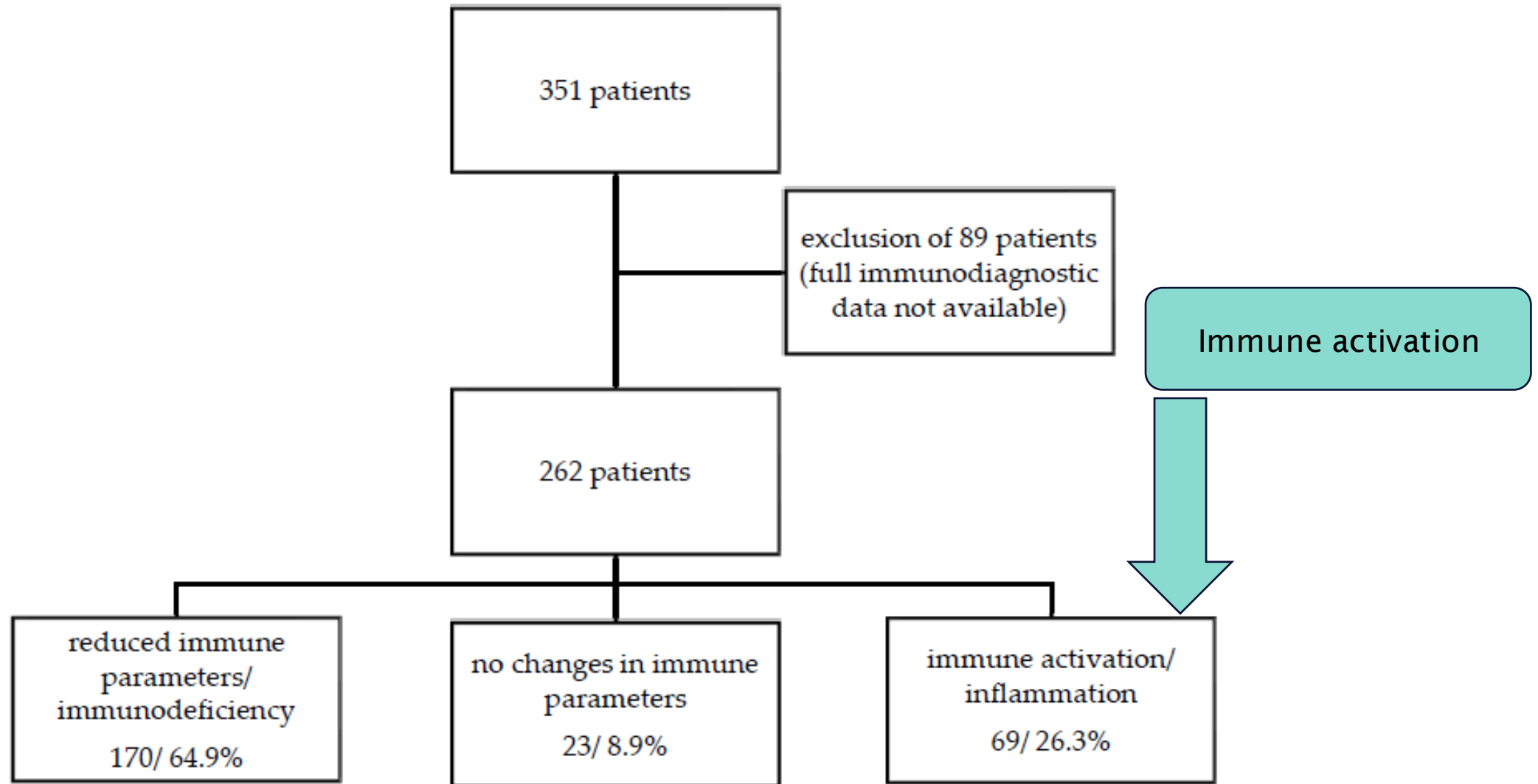
Defining criteria for autoimmune diseases (Witebsky's postulates revisited)

Noel R. Rose and Constantin Bona

With new knowledge gained from molecular biology and hybridoma technology, as well as the original Witebsky postulates, we propose that three types of evidence can be marshalled to establish that a human disease is autoimmune in origin. They include direct evidence from transfer of pathogenic antibody or pathogenic T cells; indirect evidence based on reproduction of the autoimmune disease in experimental animals; and circumstantial evidence from clinical clues.

Santos Guedes de Sa K et al. Cell 2026

Results – Immune activation/inflammation



Lutz L. et al. *Biomolecules* 2021

Immunological associations with PAIS

Immunological associations in post-infective fatigue syndromes including Long COVID—a systematic review and meta-analysis



Ruud P. H. Rajmakers,^{a,*} Lise Lund Berven,^{b,c} Stephan P. Keijmel,^{d,e} Chaturaka Rodrigo,^f Vegard B. B. Wyller,^{c,g} Ben Z. Katz,^{h,i} Dedra Buchwald,^j Rachael A. Evans,^k Patrick Gérardin,^l Hans Knoop,^{m,n,o} Maria Prins,^{p,q} Knut Stavem,^{g,r,s} Tonje Stiansen-Sonerud,^c Renee Taylor,^t Braulio Mark Valencia Arroyo,^u Knut-Arne Wensaas,^v Joel Pradeepkumar Selvakumar,^{c,g} Cees van den Wijngaard,^w Andrew R. Lloyd,^x and Carolina X. Sandler^y



14,985 studies were screened; **30 articles were included** (n = 5,102 participants; 833 PIFS/PIFS-like cases, n = 4,269 recovered control participants)

Meta-analysis (11 studies; n = 413 PIFS cases, analysed using random-effects models): PIFS cases showed elevated levels of: white blood cell count after 3–6 months, and circulating levels of RANTES and TNF α after 6–12 months, compared with control participants who had recovered from the same exposure.

Interpretation These findings provide cautious **support for persistent immune activation in PIFS,** but warrant further replication. Future studies should include better documentation of acute infection and PIFS case characterisation.

Key take home message

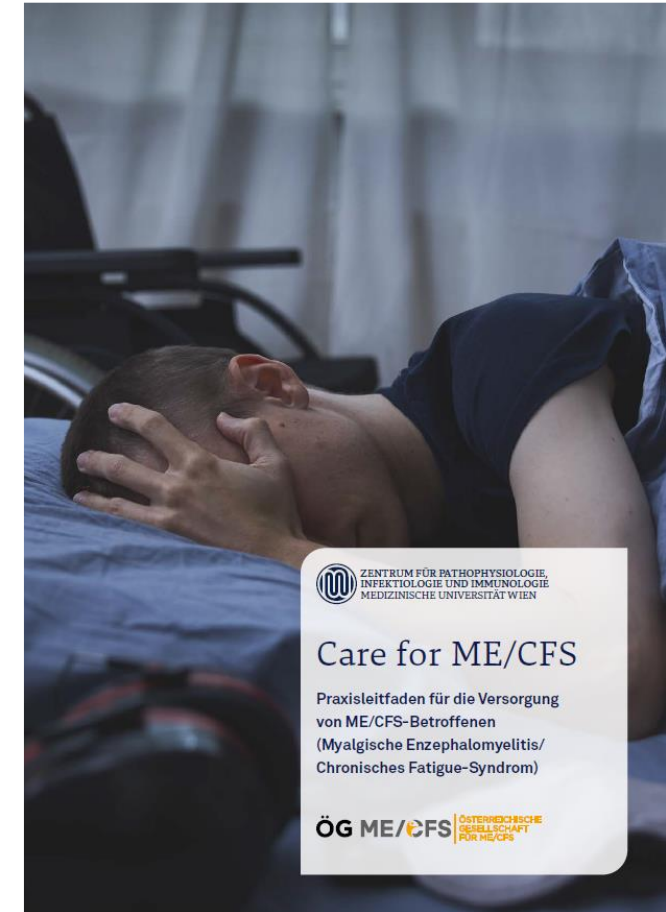
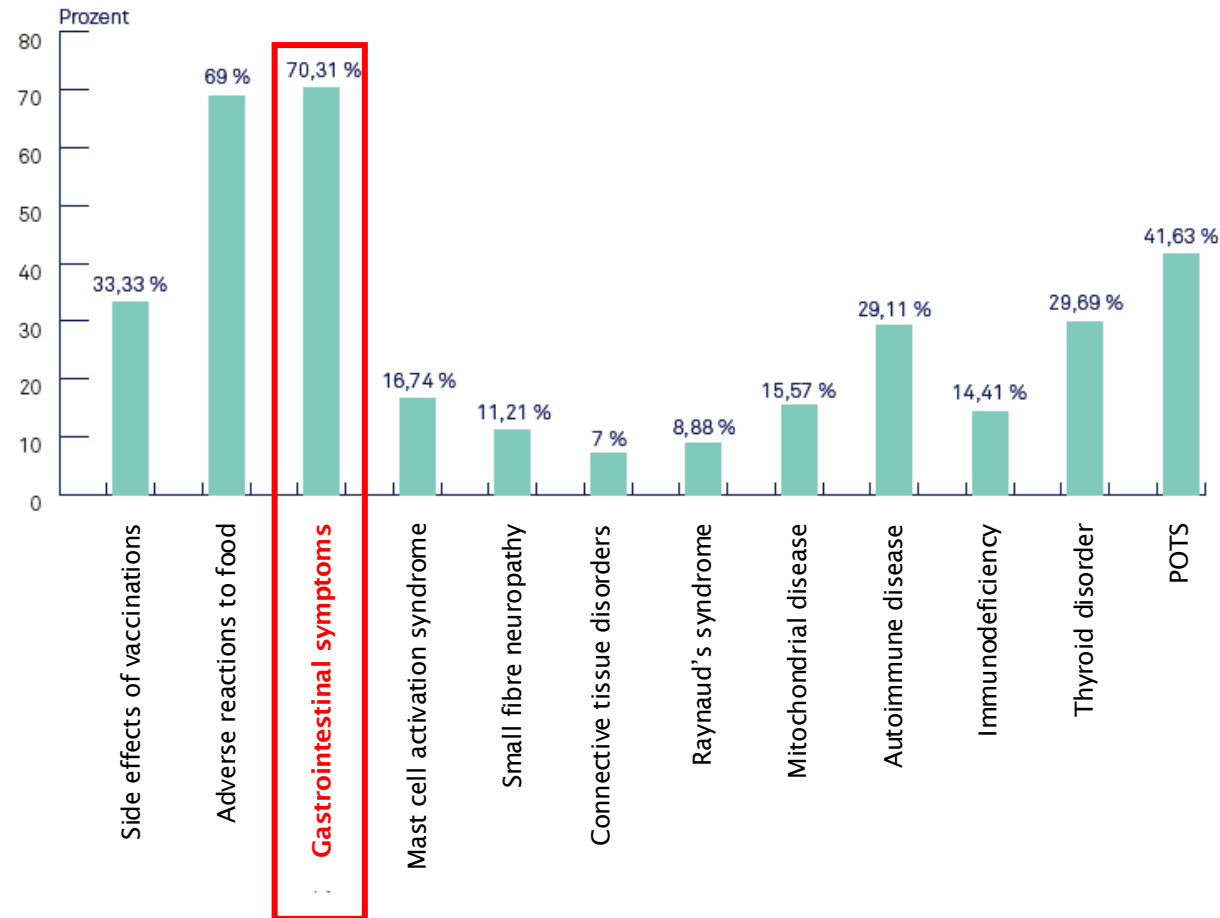
Immunological mechanisms might play a role in PAIS onset and progression

Immune diagnostics are relevant for PAIS patients with recurrent infections and infect-associated symptoms

Relevance of autoimmunity in patients' subgroups

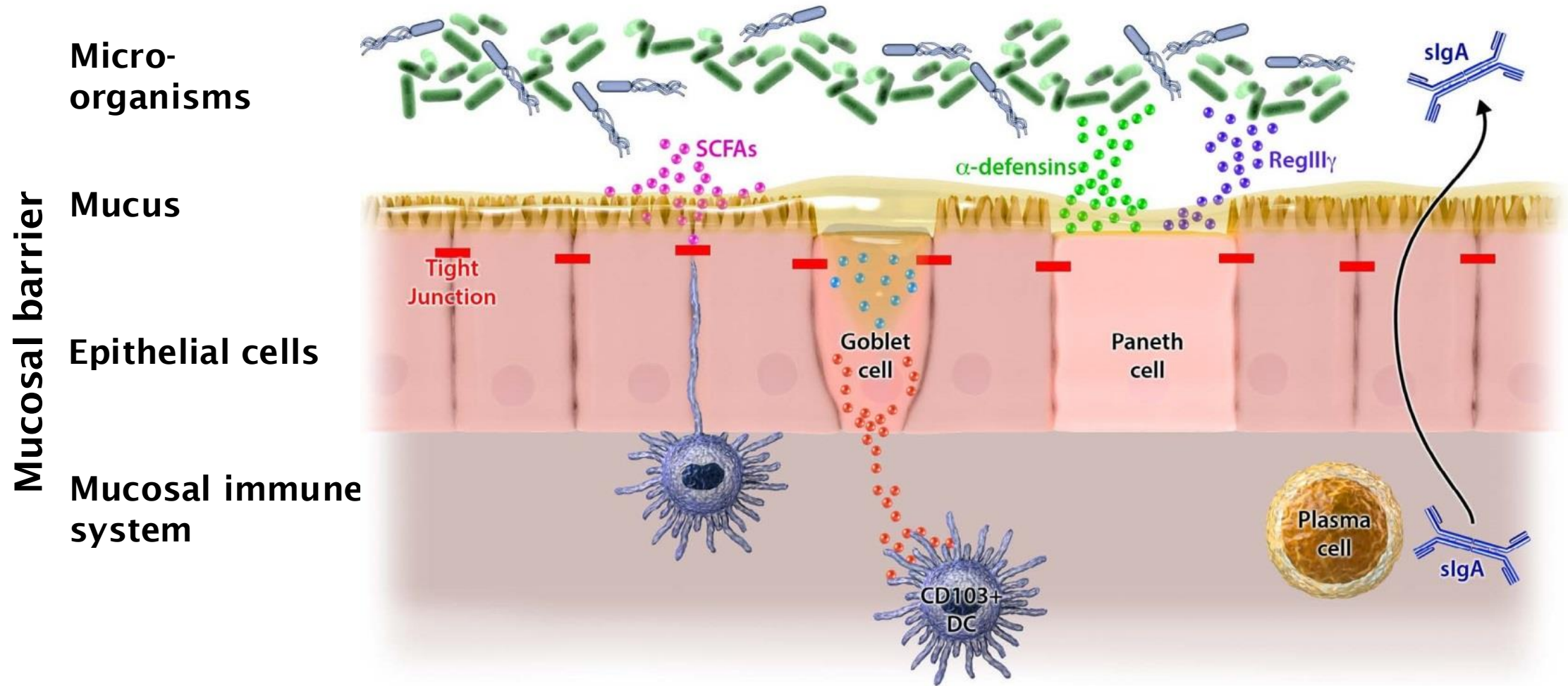
Immunological comorbidities in ME/CFS patients

Comorbidities/symptoms
(newly diagnosed/acquired
following ME/CFS diagnosis)



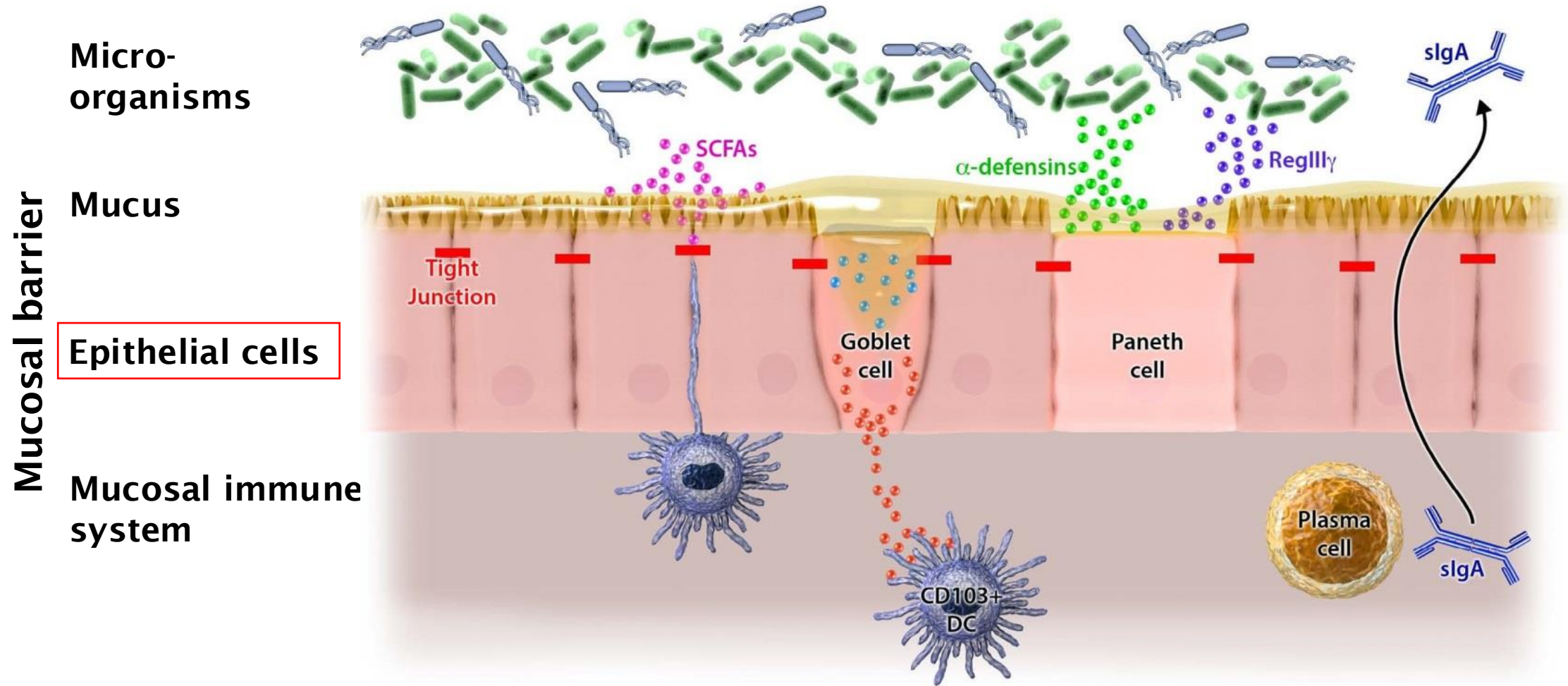
Hainzl A., Rohrhofer J., Schweighardt J. et al. (2024)
DOI: 10.5281/zenodo.12091631

The intestinal barrier and Post-COVID syndrome



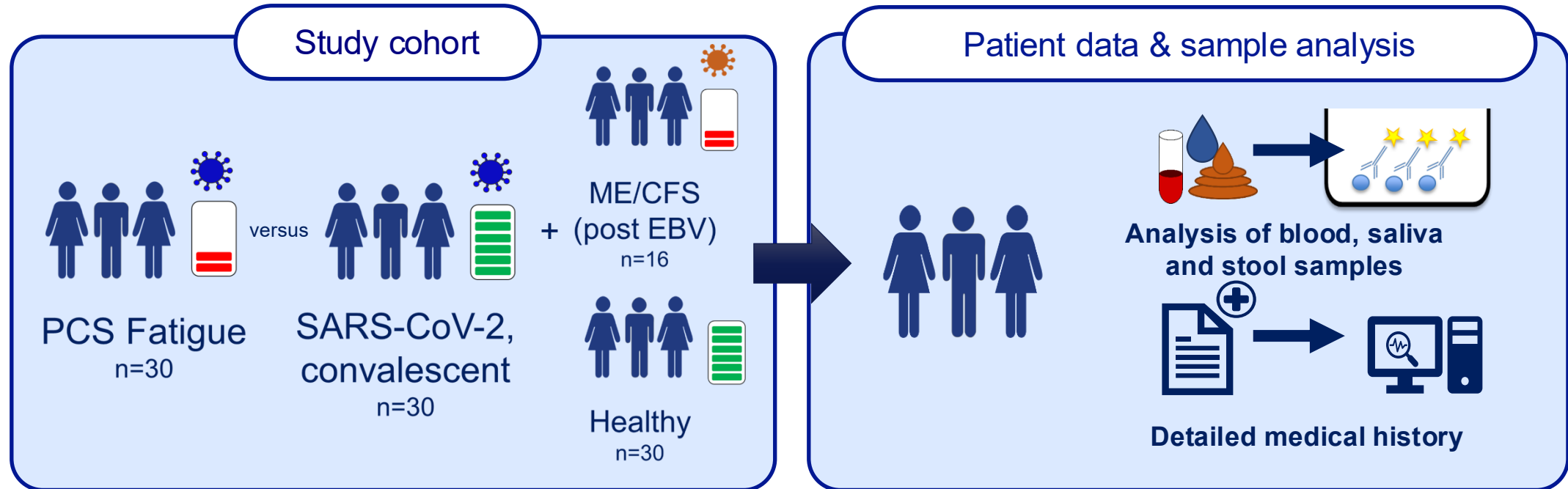
Samadi N et al. Ann Allergy Asthma Immunol 2018

The intestinal barrier and Post-COVID syndrome



Samadi N et al. Ann Allergy Asthma Immunol 2018

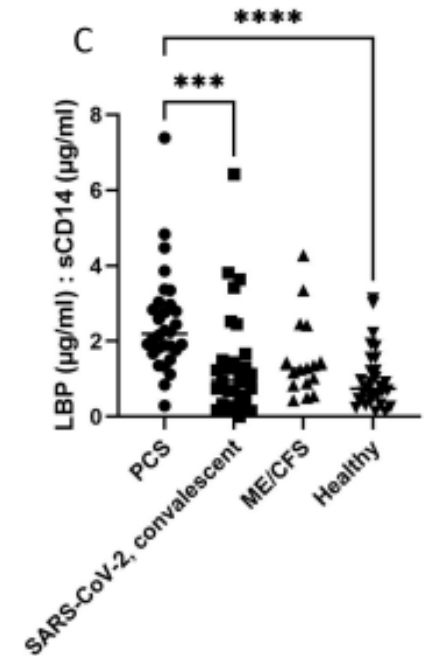
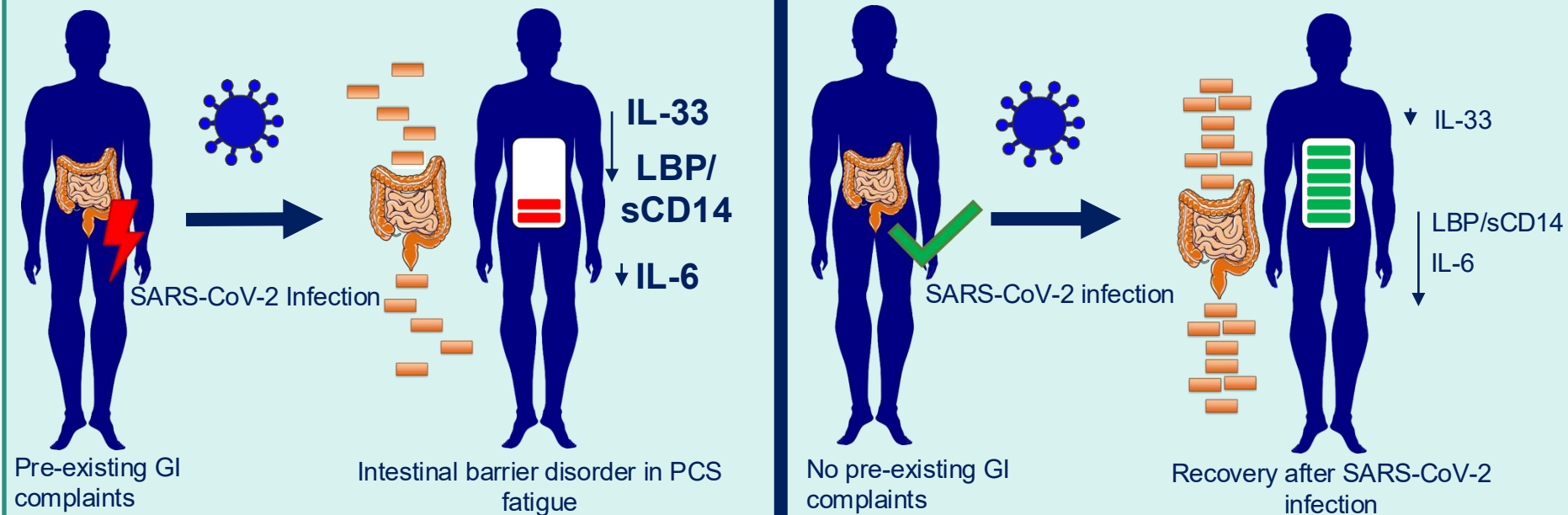
Gut health and Post-COVID Fatigue



Rohrhofer J et al. Allergy 2025

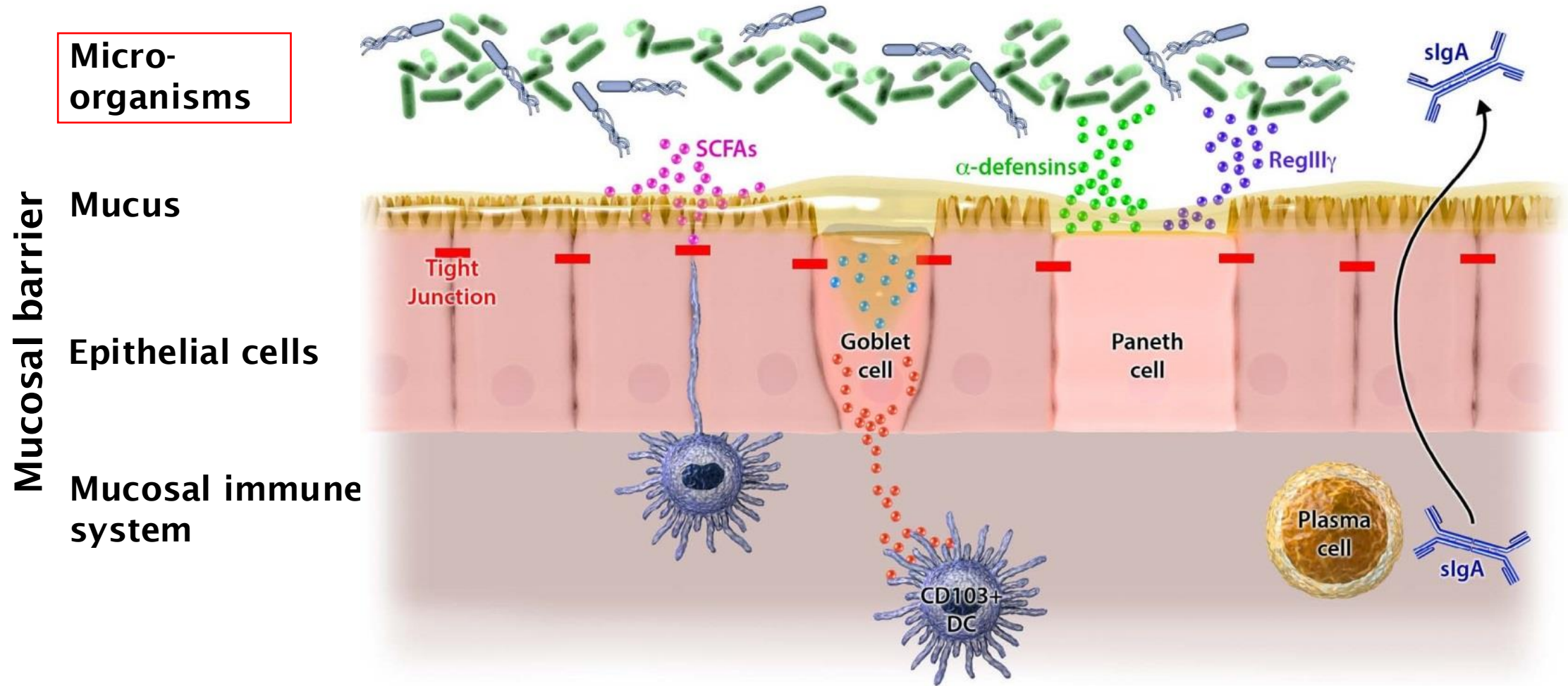
Intestinal barrier disorder in Long COVID

Intestinal barrier dysfunction is associated with PCS fatigue



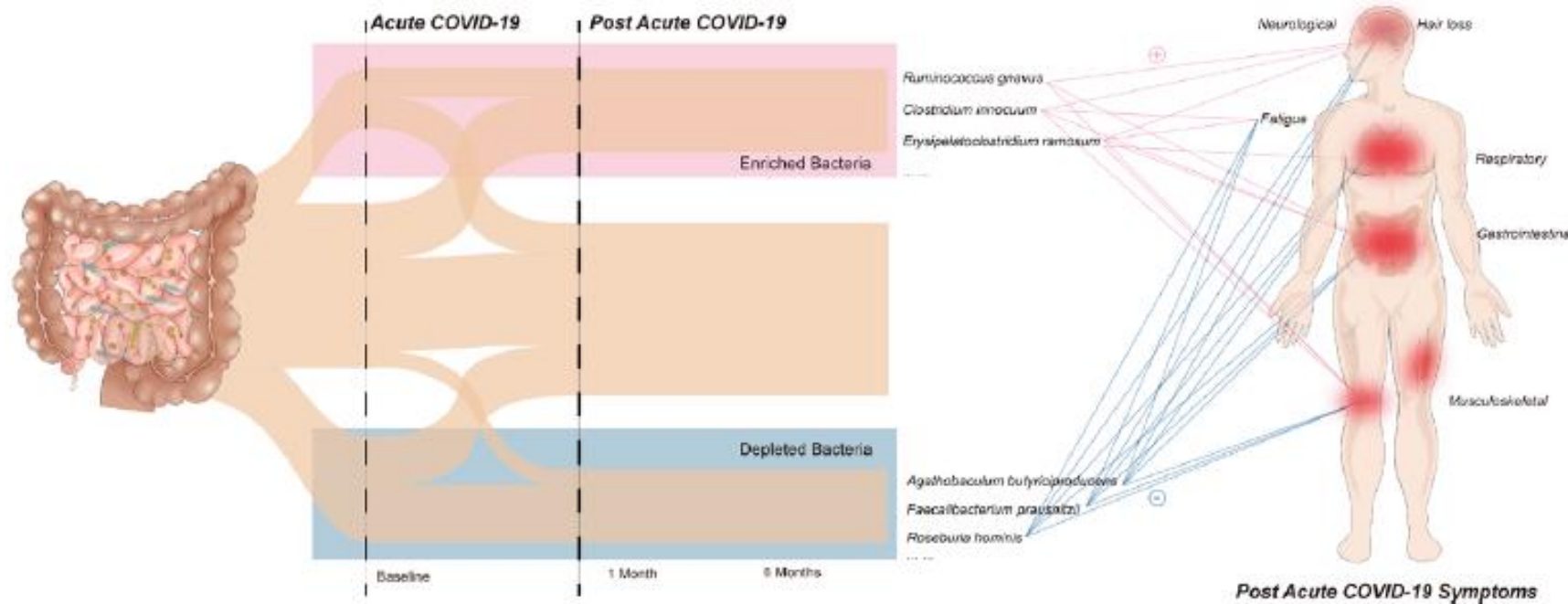
Rohrhofer J et al. Allergy 2025

The intestinal barrier and Post-COVID syndrome



Samadi N et al. Ann Allergy Asthma Immunol 2018

Specific microbial patterns and long COVID



Patients without Long-COVID: microbiota recovers after 6 months (comparable composition to non-COVID infected controls)

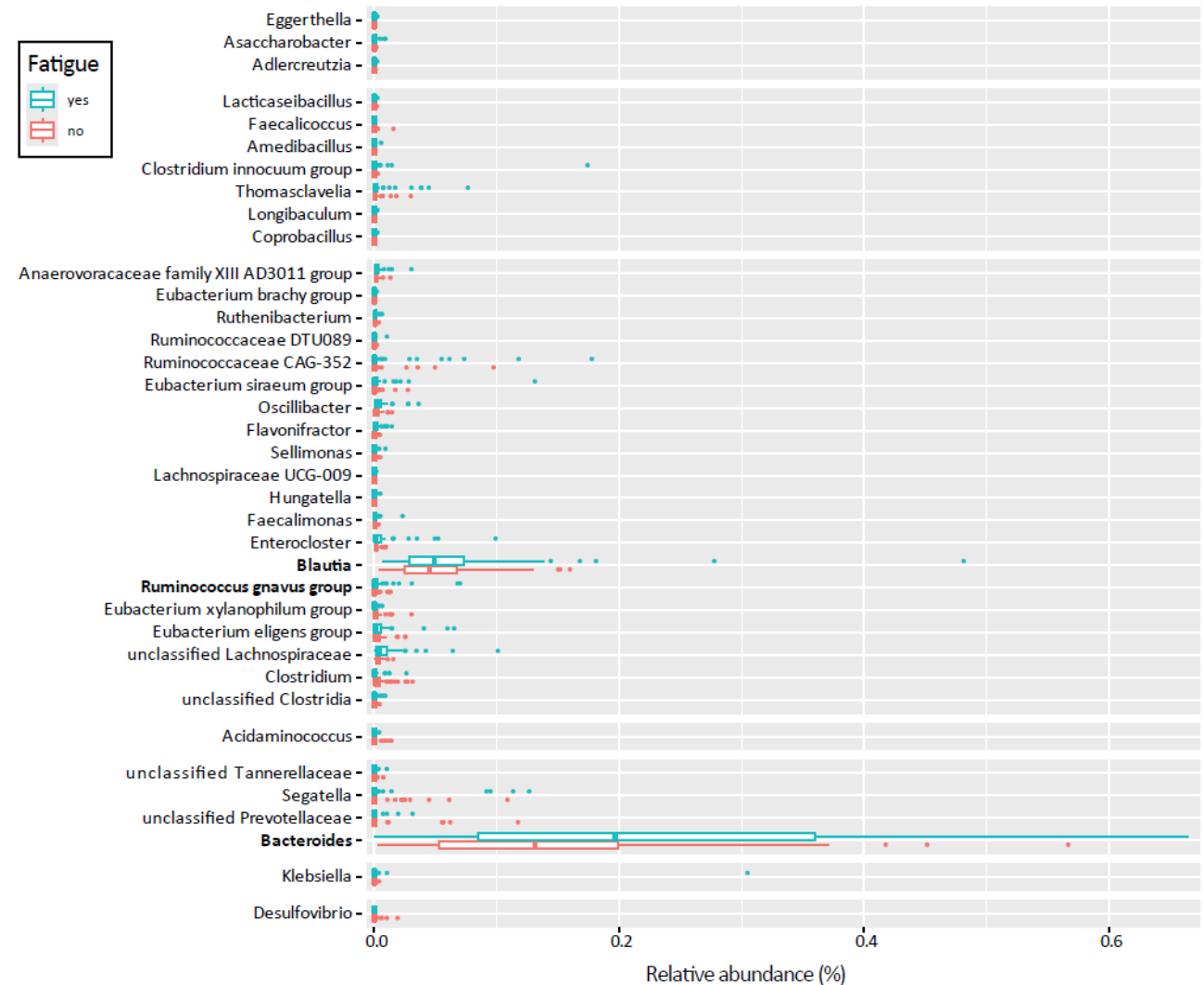
Association between gut microbiome composition at baseline and long-term COVID:
Long-COVID patients: higher levels of *Ruminococcus gnavus*, *Bacteroides vulgatus*, less *Faecalibacterium prausnitzii*. Inverse correlation between butyrate-producing bacteria (*Bifidobacterium pseudocatenulatum*, *Faecalibacterium prausnitzii* and PASC after 6 mo)

Liu Q et al. Gut2022

Microbial changes in Post-COVID and ME/CFS patients

Genera *Blautia* and *Bacteroides* were more relatively abundant in patients with fatigue

Rohrhofer J et al. Allergy 2025



Gut microbiome signatures: predictive markers?

RESEARCH ARTICLE

 OPEN ACCESS

 Check for updates

Gut microbiome signatures during acute infection are associated with long COVID

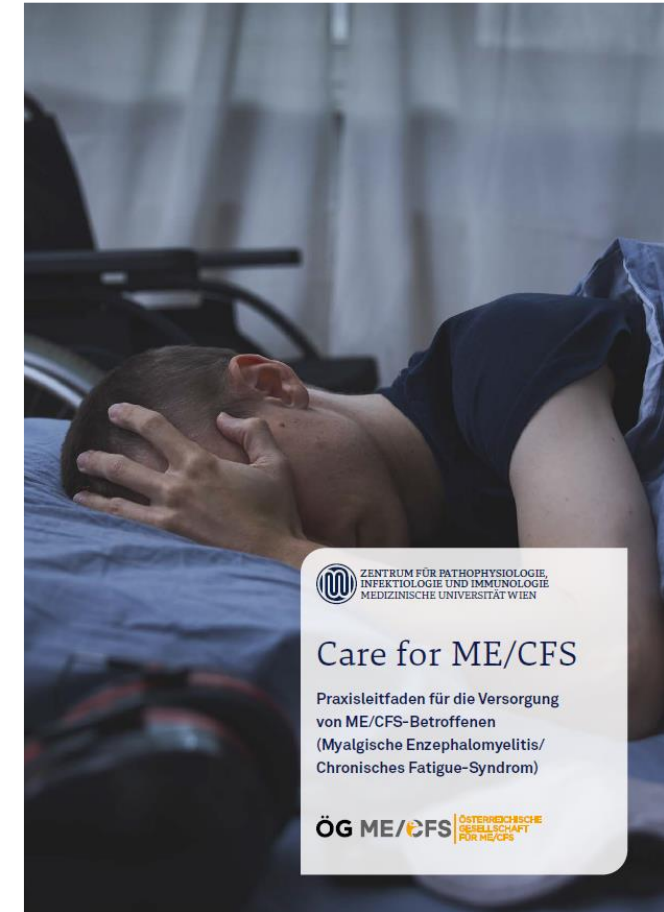
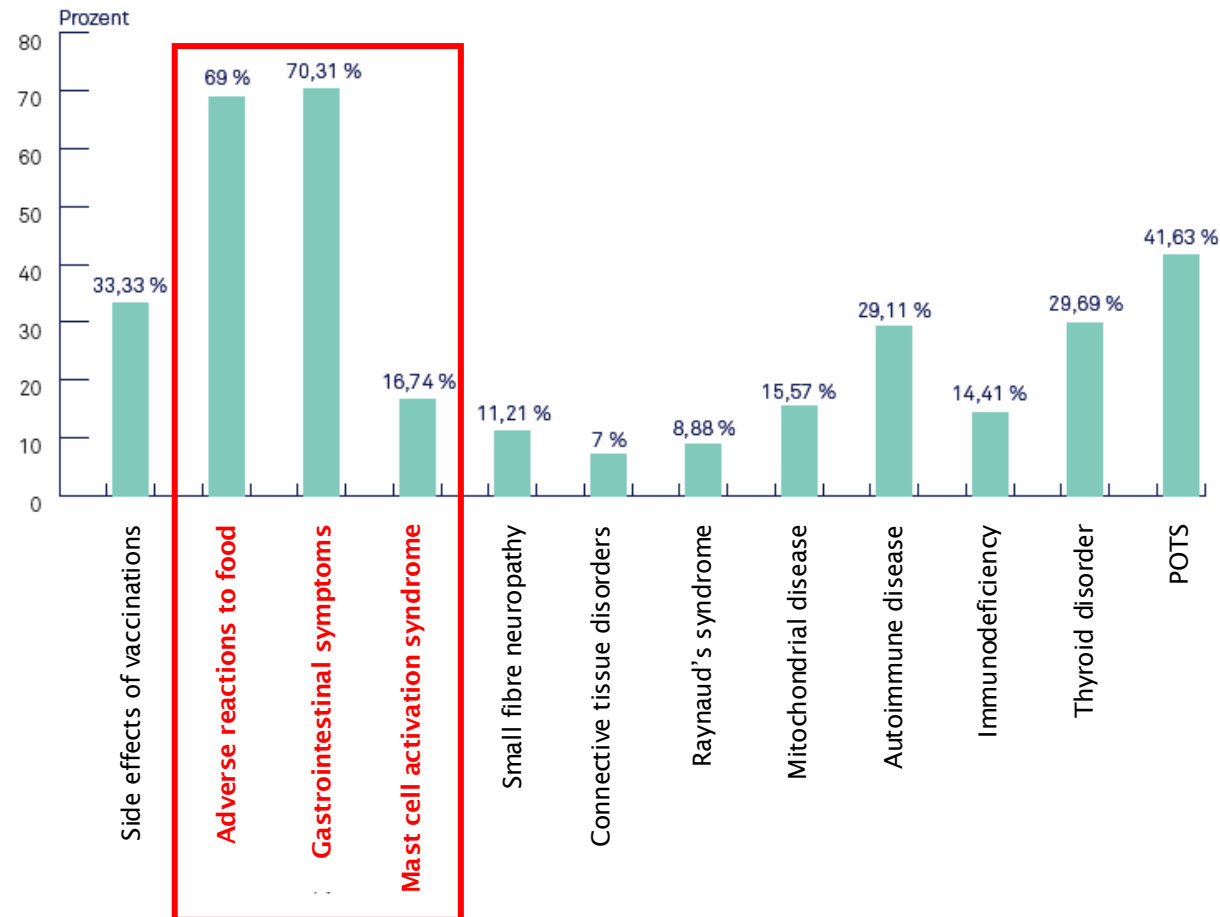
Isin Y. Comba^a, Ruben A. T. Mars^{b,c}, Lu Yang^{c,d}, Mitchell Dumais^e, Jun Chen^{c,d}, Trena M. Van Gorp^c, Jonathan J. Harrington^c, Jason P. Sinnwell^d, Stephen Johnson^d, LaRinda A. Holland^f, Adam K. Khan^f, Efrem S. Lim^f, Christopher Aakre^e, Arjun P. Athreya^g, Georg K. Gerber^{h,i,j}, Jack C. O'Horo^{a,k}, Konstantinos N. Lazaridis^b  and Purna C. Kashyap^{b,l,1} 

^aDivision of Public Health, Infectious Diseases, and Occupational Medicine, Mayo Clinic, Rochester, MN, US; ^bDivision of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA; ^cCenter for Individualized Medicine, Mayo Clinic, Rochester, MN, USA; ^dDepartment of Quantitative Health Sciences, Mayo Clinic, Rochester, MN, USA; ^eDepartment of Medicine, Mayo Clinic, Rochester, MN, USA; ^fCenter for Fundamental and Applied Microbiomics, the Biodesign Institute, Tempe, Arizona, USA; ^gDepartment of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, Minnesota, USA; ^hDivision of Computational Pathology, Brigham and Women's Hospital, Boston, MA, USA; ⁱHealth Sciences and Technology, Harvard University and MIT, Cambridge, MA, USA; ^jHarvard Medical School, Boston, MA, USA; ^kDivision of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN, USA; ^lDepartment of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA

Comba IY et al. Gut microbes 2026

Immunological comorbidities in ME/CFS patients

Comorbidities/symptoms
(newly diagnosed/acquired
following ME/CFS diagnosis)



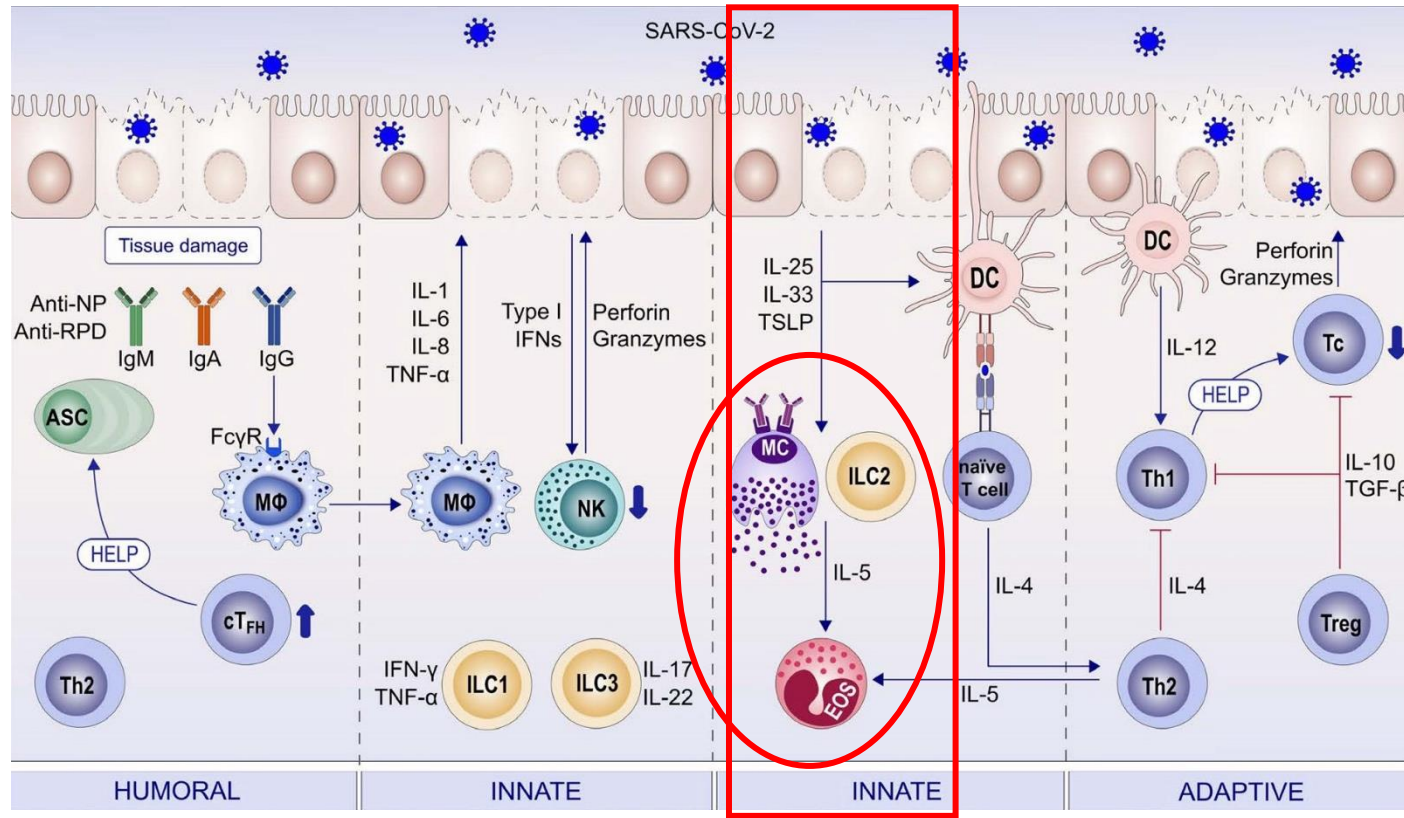
Hainzl A., Rohrhofer J., Schweighardt J. et al. (2024)
DOI: 10.5281/zenodo.12091631

Allergy-like symptoms increasingly reported after COVID



*Openclipart.org: Stomach
pain (#2) by oksmith*

Immune response after SARS CoV2 infection



3-9 months after hospital discharge following severe COVID → significantly increased **Th2 and Th17 markers:**

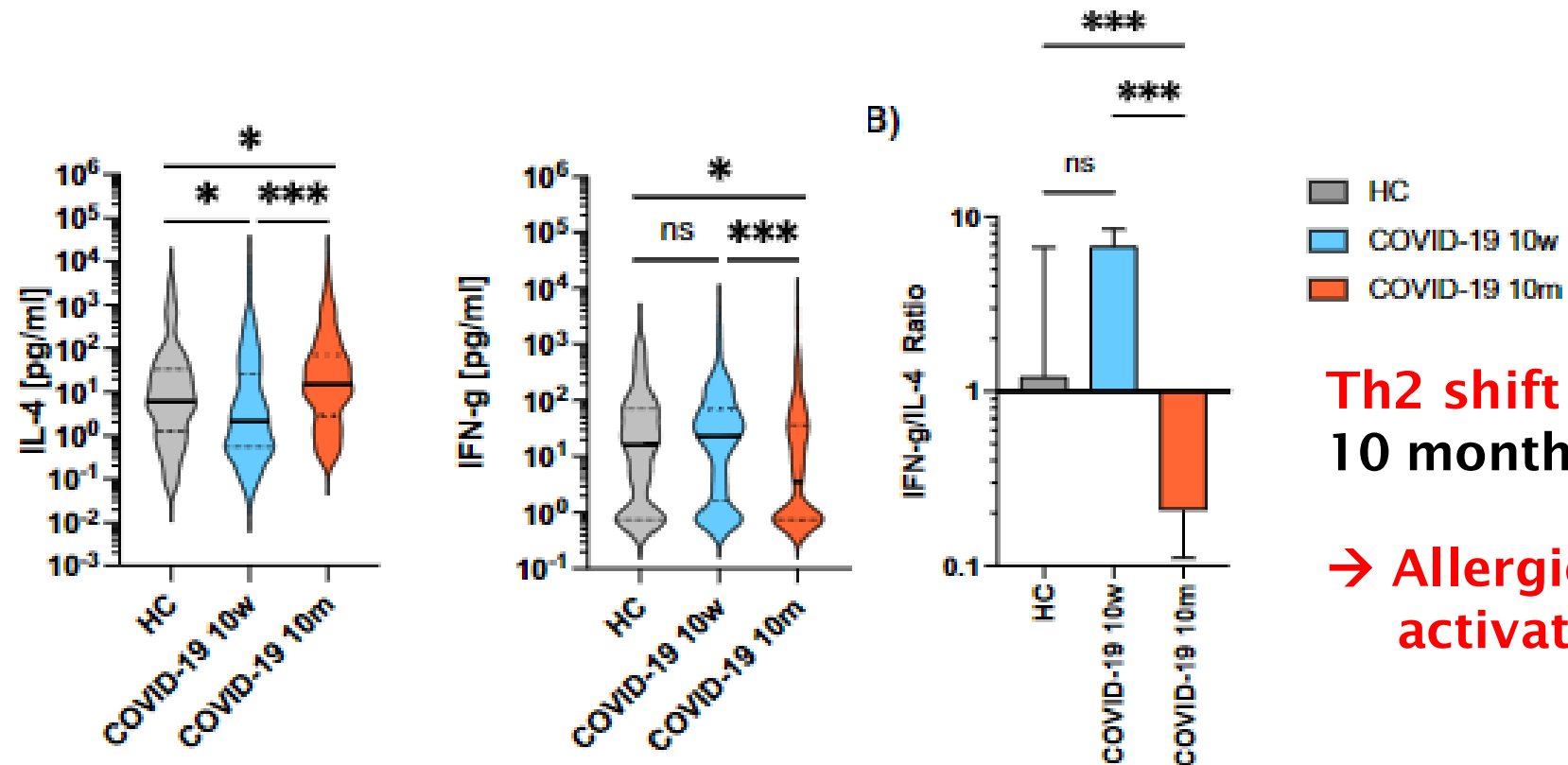
- IL-17A
- IL-4
- MIP-3α
- MDC
- IL12/23p40
- TSLP

Ahearn-Ford S et al. Allergy 2021

Inflammation and cell destruction → Release of *danger* signals and alarmins (IL-33, IL-25, TSLP) → **Th2 cells and ILC2s activation**
→ **Activation of additional effector cells**

Vultaggio A et al. Allergy 2020

Dysregulation of the immune system after COVID

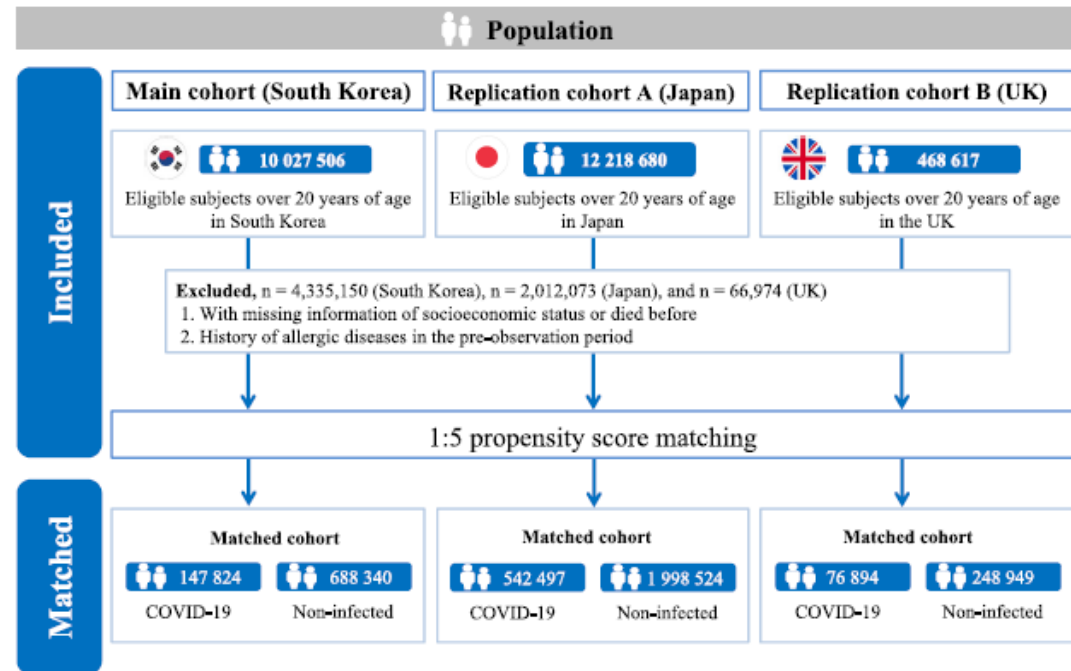


Th2 shift
10 months after COVID

→ **Allergies and mast cell activation**

Kratzer B et al. Allergy 2024

Are allergies becoming more common after COVID?



Factors	Group	Exposure	Events/total number (%)	HR (95% CI)	
				Model 1 ^a	Model 2 ^a
COVID-19 severity	Total	Non-infected control	5633/688,340 (0.82)	1.00 (reference)	1.00 (reference)
		Mild COVID-19	1125/127,641 (0.88)	1.13 (1.06–1.20)	1.14 (1.07–1.21)
		Moderate to severe COVID-19	293/20,183 (1.45)	1.55 (1.38–1.74)	1.48 (1.31–1.66)
Strain type (original)	Total	Non-infected control at the same index date ^a	4174/220,904 (1.89)	1.00 (reference)	1.00 (reference)
		Original SARS-CoV-2 infection	1050/46,900 (2.24)	1.20 (1.12–1.29)	1.20 (1.12–1.29)
		Delta SARS-CoV-2 infection	368/100,924 (0.36)	1.18 (1.05–1.32)	1.18 (1.05–1.32)
Strain type (delta)	Total	Non-infected control at the same index date ^a	1459/467,436 (0.31)	1.00 (reference)	1.00 (reference)
		Delta SARS-CoV-2 infection	368/100,924 (0.36)	1.18 (1.05–1.32)	1.18 (1.05–1.32)
		Without vaccination	1133/68,456 (1.66)	1.24 (1.16–1.32)	1.24 (1.16–1.32)
Number of SARS-CoV-2 vaccinations	Patients with COVID-19	Non-infected control	5633/688,340 (0.82)	1.00 (reference)	1.00 (reference)
		Vaccination 1 time	148/14,125 (1.05)	1.43 (1.22–1.69)	1.44 (1.22–1.69)
		Vaccination ≥2 times	137/65,243 (0.21)	0.81 (0.68–0.96)	0.81 (0.68–0.96)

Increased risk of **developing allergies**
 (HR, 1.20; 95% CI, 1.13–1.27)
 espec. asthma (HR, 2.25; 95% CI, 1.80–2.83)
 and allergic rhinitis (HR, 1.23; 95% CI, 1.15–1.32)
 Risk of atopic dermatitis and NM allergy after COVID increased only in cohort A

Increased risk of allergies after

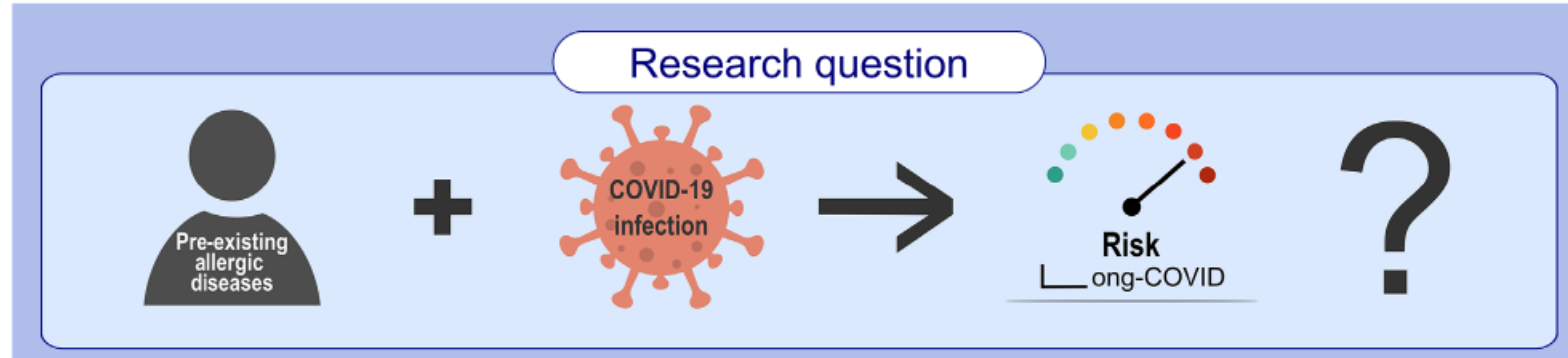
- severe COVID
- For certain SARS-CoV-2 strains

Risk of allergies reduced by

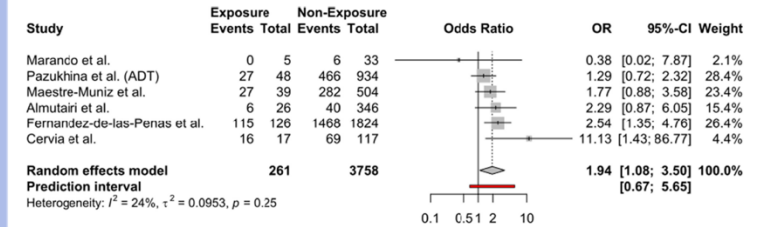
- vaccinations

Oh J et al. Nature Comm 2024

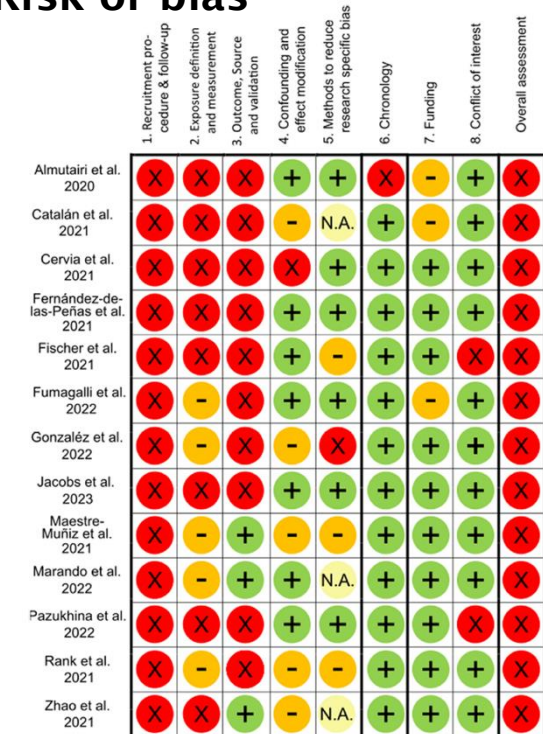
Allergies: predisposing for Long COVID?



LC increased risk in allergies



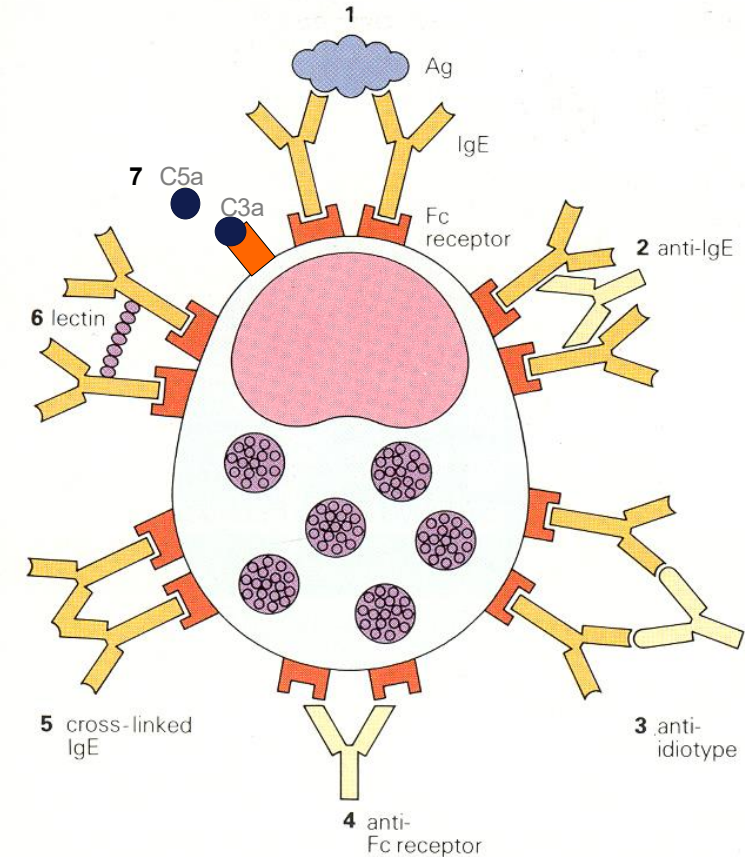
Risk of bias



Wolff D et al. Clin Trans Allergy 2023

Immunological text book knowledge: Mechanisms of mast cell activation

- **AG cross-linking of surface IgE = allergy**
- Divalent antibodies against IgE Fc region
- Anti-idiotypic antibodies
- Anti-Fc receptor antibodies
- Covalently cross-linked IgE
- Lectins
- Complement (C3a, C5a) via C3aR, C5aR (CD88)
- Nerve growth factor
- IgG via FcγRI
- TLR ligands (e.g. TLR3 dsDNA → IFN-γ production in mast cells)



Chales A. Janeway. Immunology

Mast cell activation syndrome: Consensus protocols

AAAAI Work Group Report



AAAAI Mast Cell Disorders Committee Work Group Report: Mast cell activation syndrome (MCAS) diagnosis and management



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Rochester, Minn; Boston, Mass; Ann Arbor, Mich; Chicago, Ill; Cincinnati and Columbus, Ohio; Verona, Italy; Bethesda, Md; New York and Rochester, NY; Edmonton, Alberta, Canada; and Richmond, Va

Weiler CR. et al JACI 2019

3 diagnostic criteria:

- Severe recurrent systemic symptoms (affecting at least 2 organ systems), often anaphylaxis
- Laboratory diagnostics
- Therapeutic response

Special Article

Proposed Diagnostic Algorithm for Patients with Suspected Mast Cell Activation Syndrome



Peter Valent, MD,^a Cem Akin, MD, PhD^b, Patrizia Bonadonna, MD^c, Karin Hartmann, MD^d, Knut Brockow, MD^e, Marek Nideszytko, MD, PhD^f, Boguslaw Nideszytko, PhD^g, Frank Siebenhaar, MD^h, Wolfgang R. Sperr, MD^a, Joanna N.G. Oude Elberink, MD, PhDⁱ, Joseph H. Butterfield, MD^j, Ivan Alvarez-Twose, MD, PhD^k, Karl Sotlar, MD^l, Andreas Reiter, MD^m, Hanneke C. Kluin-Nelemans, MD, PhDⁿ, Olivier Hermine, MD, PhD^o, Jason Gotlib, MD, MS^p, Sigurd Broesby-Olsen, MD, PhD^q, Alberto Orfao, MD, PhD^r, Hans-Peter Horny, MD^s, Massimo Triggiani, MD, PhD^t, Michel Arock, PharmD, PhD^u, Lawrence B. Schwartz, MD, PhD^v, and Dean D. Metcalfe, MD, PhD^w
Vienna and Salzburg, Austria; Verona and Salerno, Italy; Luebeck, Munich, Berlin, and Mannheim, Germany; Gdansk, Poland; Groningen, The Netherlands; Toledo and Salamanca, Spain; Paris, France; Odense, Denmark; and Ann Arbor, Mich; Rochester, Minn; Stanford, Calif; Richmond, Va; and Bethesda, Md

Valent P. et al JACI in Pract. 2019

4 relevant organ systems:

- Cardiovascular system
- Skin
- Respiratory tract
- Gastrointestinal tract

Food and/or drug intolerances
Anaphylactic reactions

Mast cell activation syndrome: Most recent consensus document

Workshop summary

Mast cell activation syndrome: Current understanding and research needs



Mariana Castells, MD, PhD,^{a,f} Matthew P. Giannetti, MD,^{a,f} Matthew J. Hamilton, MD,^{b,f} Peter Novak, MD, PhD,^{c,f} Olga Pozdnyakova, MD, PhD,^{d,f} Jennifer Nicoloro-SantaBarbara, PhD,^{e,f} Susan V. Jennings, PhD,^g Clair Francomano, MD,^h Brian Kim, MD,ⁱ Sarah C. Glover, DO,^j Stephen J. Galli, MD,^k Anne Maitland, MD, PhD,^{l,w} Andrew White, MD,^m J. Pablo Abonia, MD,ⁿ Valerie Slee, RN, BSN,^g Peter Valent, MD,^{o,p} Joseph H. Butterfield, MD,^q Melody Carter, MD,^r Dean D. Metcalfe, MD,^r Cem Akin, MD, PhD,^s Jonathan J. Lyons, MD,^{t,u} Alkis Togias, MD,^r Lisa Wheatley, MD,^r and Joshua D. Milner, MD^v
Ann Arbor, Mich; Bethesda, Md; Boston and Sterling, Mass; Cincinnati, Ohio; Indianapolis, Ind; La Jolla, San Diego, and Stanford, Calif; New Orleans, La; New York, NY; Rochester, Minn; Salt Lake City, Utah; and Vienna, Austria

Mast cell activation syndrome (MCAS) is a term applied to several clinical entities that have gained increased attention from patients and medical providers. Although several descriptive publications about MCAS exist, there are many gaps in knowledge, resulting in confusion about this clinical syndrome. Whether MCAS is a primary syndrome or exists as a constellation of symptoms in the context of known inflammatory, allergic, or clonal disorders associated with systemic mast cell activation is not well understood. More importantly, the underlying mechanisms and pathways that lead to mast cell activation in MCAS patients remain to be elucidated. Here we summarize the known literature, identify gaps

in knowledge, and highlight research needs. Covered topics include contextualization of MCAS and MCAS-like endotypes and related diagnostic evaluations; mechanistic research; management of typical and refractory symptoms; and MCAS-specific education for patients and health care providers. (*J Allergy Clin Immunol* 2024;154:255-63.)

Key words: Mast cell activation syndrome, Mast cell, mastocytosis, hereditary alpha-tryptasemia, tryptase, mast cell activation-related disorders

Castells M. et al JACI 2024

Mast cell activation syndromes (MCASs) are characterized by recurrent episodes of systemic symptoms associated with the release of mast cell (MC)-derived mediators (Fig 1).^{1,2} The consensus diagnostic criteria of MCAS, updated in 2022, consists of the following (Table I):²⁻⁵

1. Recurrent severe systemic symptoms in at least 2 organ systems consistent with MC mediator-related symptoms.
2. Significant transient increases in serum tryptase level (20% over baseline plus 2 ng/mL) or other MC-derived mediators, such as urinary histamine/*N*-methyl histamine, leukotriene E₄, or prostaglandin D₂/2,3-dinor-11β-prostaglandin F₂ alpha (PGF₂α) metabolite over baseline during a period of increased symptoms.
3. A significant response of clinical symptoms to medications that counteract MC mediator effects (eg, histamine H₁ and H₂ receptor blockers, leukotriene, and prostaglandin blockers), and/or suppress MC activation (MCA) (eg, sodium cromolyn, ketotifen, and anti-IgE).

Symptoms associated with mast cell mediator release

Neuropsychiatric:

brain fog, depression, anxiety, short memory span, inability to concentrate, small fiber neuropathy.

Skin:

urticaria, flushing, pruritus, angioedema

Gastrointestinal:

nausea, abdominal pain, bloating, cramping, diarrhea.

Upper Respiratory:

sinonasal pruritus, nasal stuffiness, conjunctival infection

Lower Respiratory:

throat/chest tightness, dyspnea, wheezing

Cardiovascular:

hypotension, tachycardia, palpitations, chest pain

Musculoskeletal:

pain

Systemic:

anaphylaxis, fatigue, dysautonomia.

- Skin: flushing, urticaria, angioedema.
- GI: abdominal bloating/cramping/pain, nausea, vomiting, diarrhea.
- Respiratory: wheezing, chest tightness.
- Nasocular: pruritus, nasal stuffiness conjunctival injection.
- Cardiovascular: hypotension, tachycardia, chest pain/tightness.
- Brain: brain fog, headache.
- Systemic: fatigue, anaphylaxis.

Castells M. et al JACI 2024

Mast cell activation: diagnosis and treatment

Diagnosis: Laboratory markers are diagnostic criteria!

Mast cell mediators (note preanalytics!)

- Tryptase (increase to patient-specific baseline: $20\% + 2 \text{ ng/ml}$) increase detectable within 3 hours in acute cases
- Histamine (and histamine degradation products)
- Leukotrienes, prostaglandins

Allergy diagnostics, intolerance assessment

Therapy: Response to adequate therapy is a diagnostic criterion!

- Antihistamines
- Mast cell stabilisers
- Blockade of mast cell products

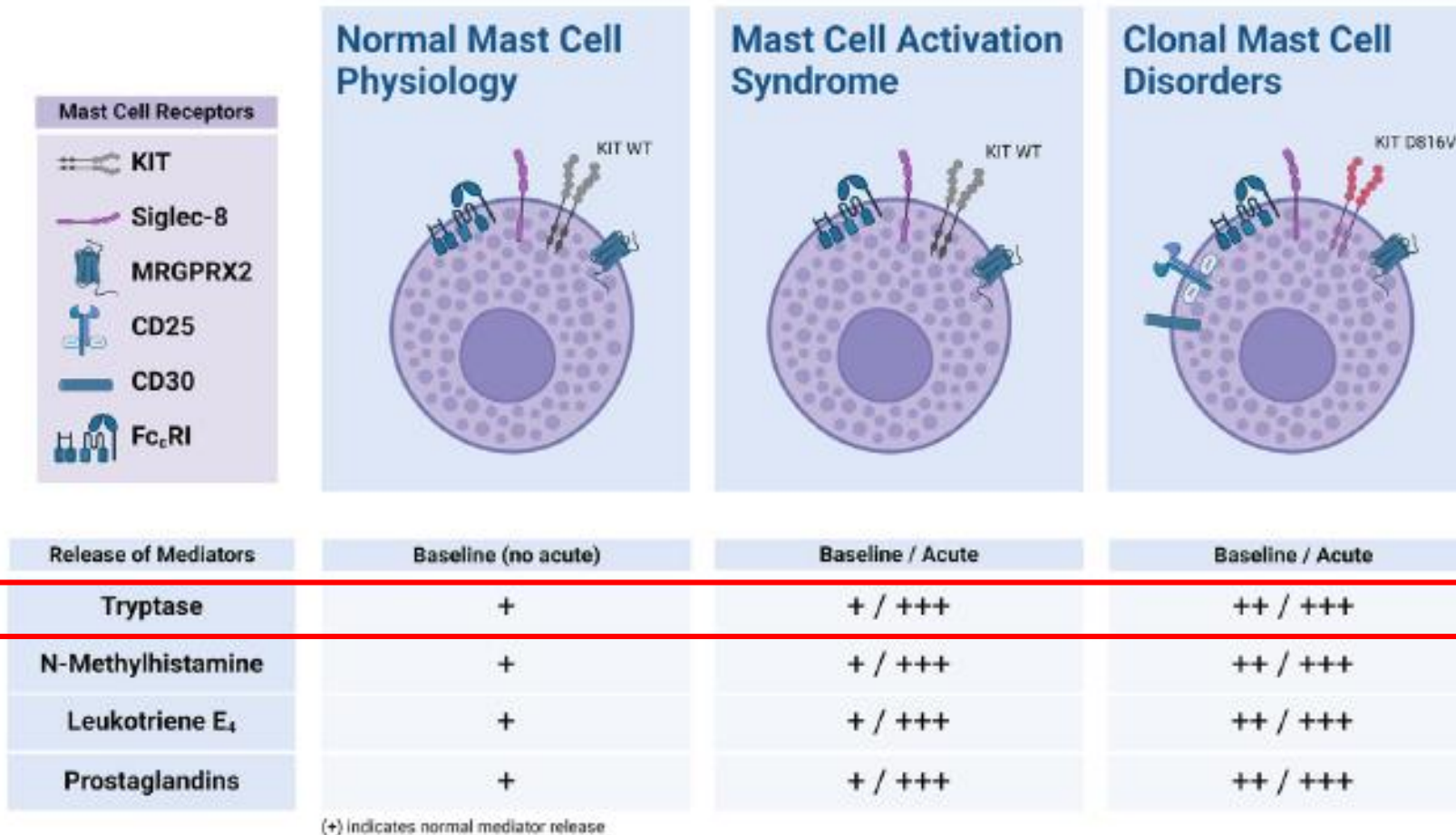
≠ **Mastocytosis:** clonal proliferation of mast cells

Genetic testing not necessary in every case (note tryptase levels!)

TABLE III. Tryptase algorithm for diagnosing systemic anaphylaxis^{1,11,93-95}: $sAT > (1.2 \cdot sBT) + 2$

1. Neither an sBT nor an sAT by itself has sufficient sensitivity to assess an MC activation event, regardless of whether it is outside of or within the normal range.
2. Sensitivity increases with clinical severity, primarily correlating with hypotension.
3. The optimal time to collect an acute blood sample based on experimental insect sting-triggered anaphylaxis is 30 to 120 minutes after onset of symptoms; sensitivity diminishes outside of this range.
4. The optimal time to collect a baseline blood sample is either before the event or at least 24 hours after all signs and symptoms have resolved.
5. This test has high specificity (>90%), whereas sensitivity varies with time of collection, clinical severity, and trigger.

Differences between mast cell activation syndrome and mastocytosis



Castells M. et al JACI 2024

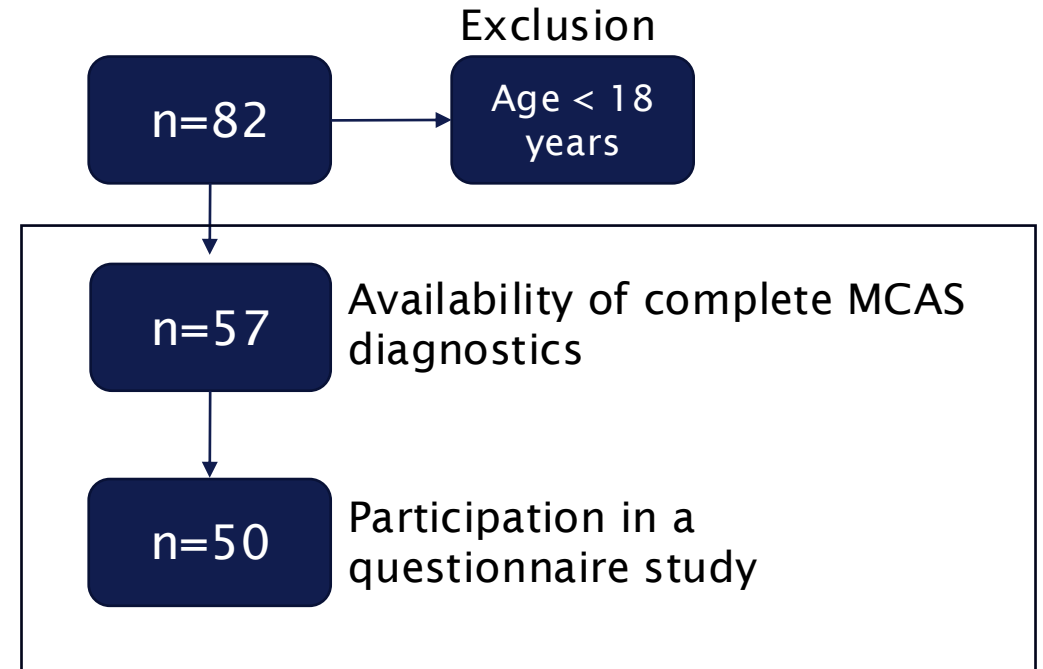
MCAS in PCS patients – *real world data*

82 patients treated for PCS

Aged between 18 and 100 (**average age 35.9 years**)

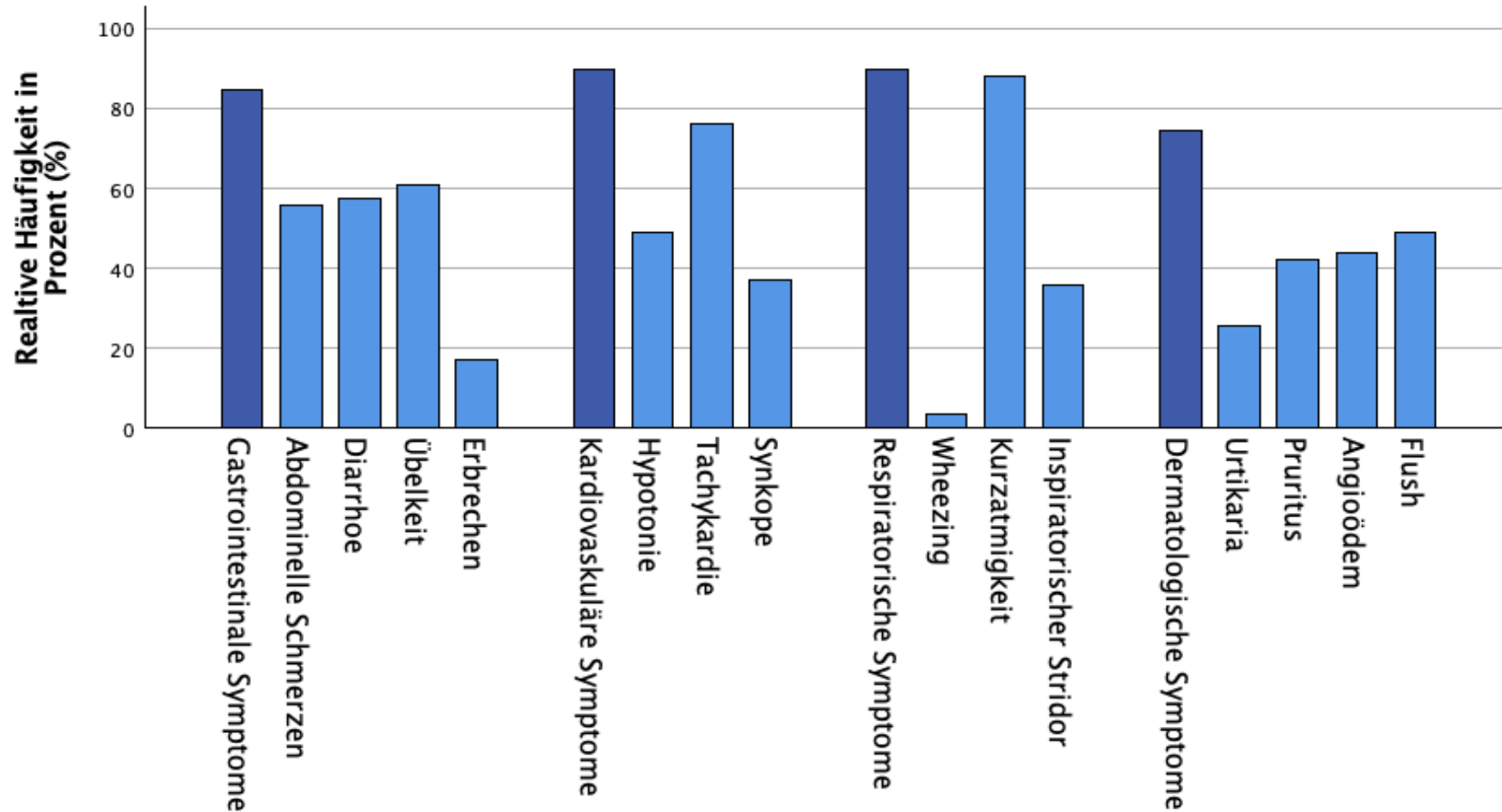
69 women (89.6%)

8 men (8.9%)



Kinnebrock P et al. Ms. in preparation

MCAS-associated symptoms in PCS patients

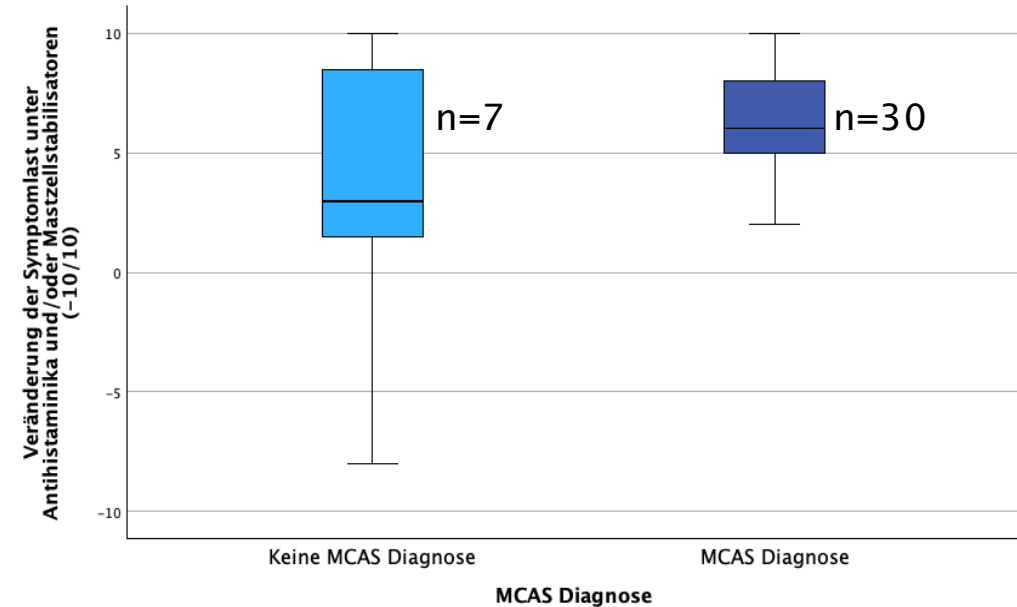


Kinnebrock P et al. Ms. in preparation

Frequency of MCAS diagnosis in PCS patients

33 patients were diagnosed with MCAS
(57.9% of 57 patients)

Questionnaire data: significant improvement of symptom burden ($p=0.004$) through MCAS therapy (histamine receptor antagonists/mast cell stabilizers)

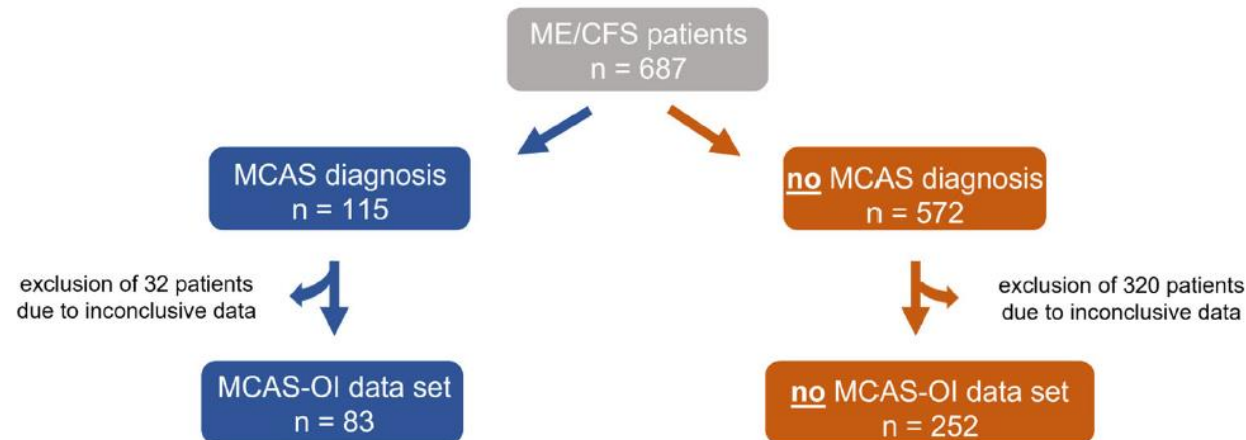


Kinnebrock P. et al Ms. in preparation

Clinical relevance of MCAS in ME/CFS

ME/CFS Data Sets	ME/CFS (Total)
Participants	687 (100%)
Sex (f/m/d)	84%/15.6%/0.4%
Age, median (IQR)	41 (33.0–50.0)

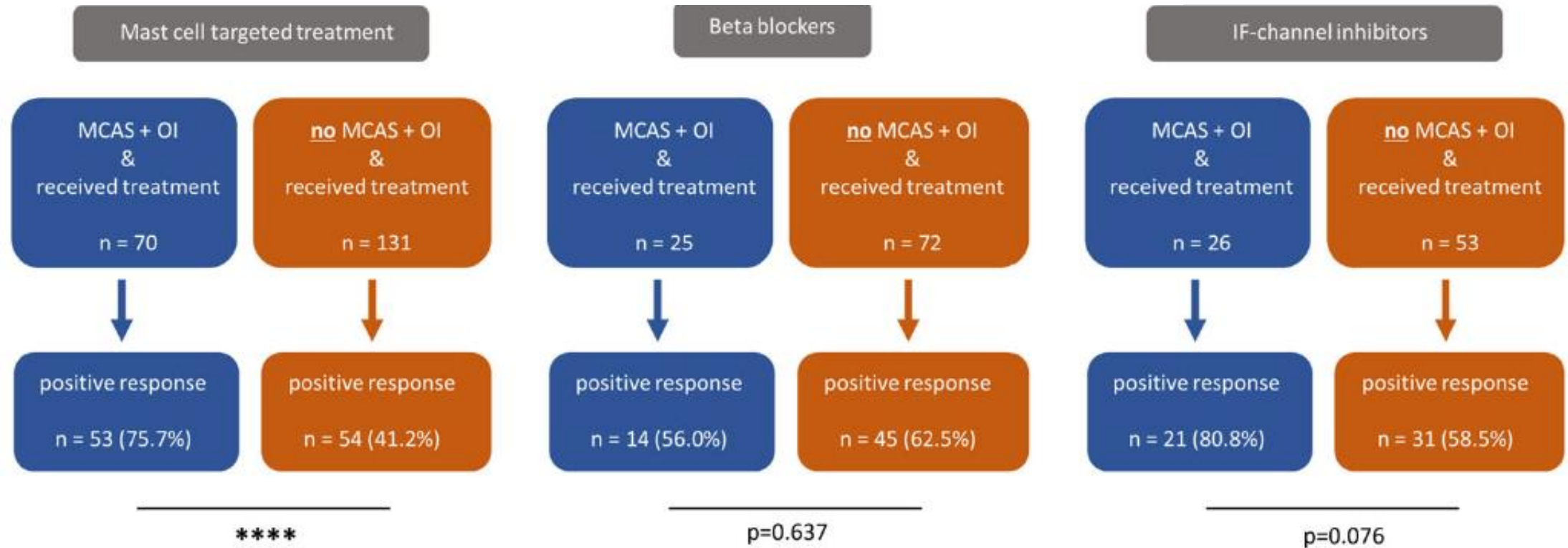
2.8% of the entire cohort was diagnosed with MCAS before the onset of ME/CFS.



Rohrhofer J. et al *Diagnostics* 2025

Therapeutic relevance of MCAS in ME/CFS

ME/CFS Data Sets	ME/CFS (Total)	MCAS Diagnosis	No MCAS Diagnosis
Participants	687 (100%)	115 (16.7%)	572 (83.3%)
Sex (f/m/d)	84%/15.6%/0.4%	87.0%/11.3%/1.7%	83.4%/16.4%/0.2%
Age, median (IQR)	41 (33.0–50.0)	39 (32.0–48.5)	41 (33.0–50.0)



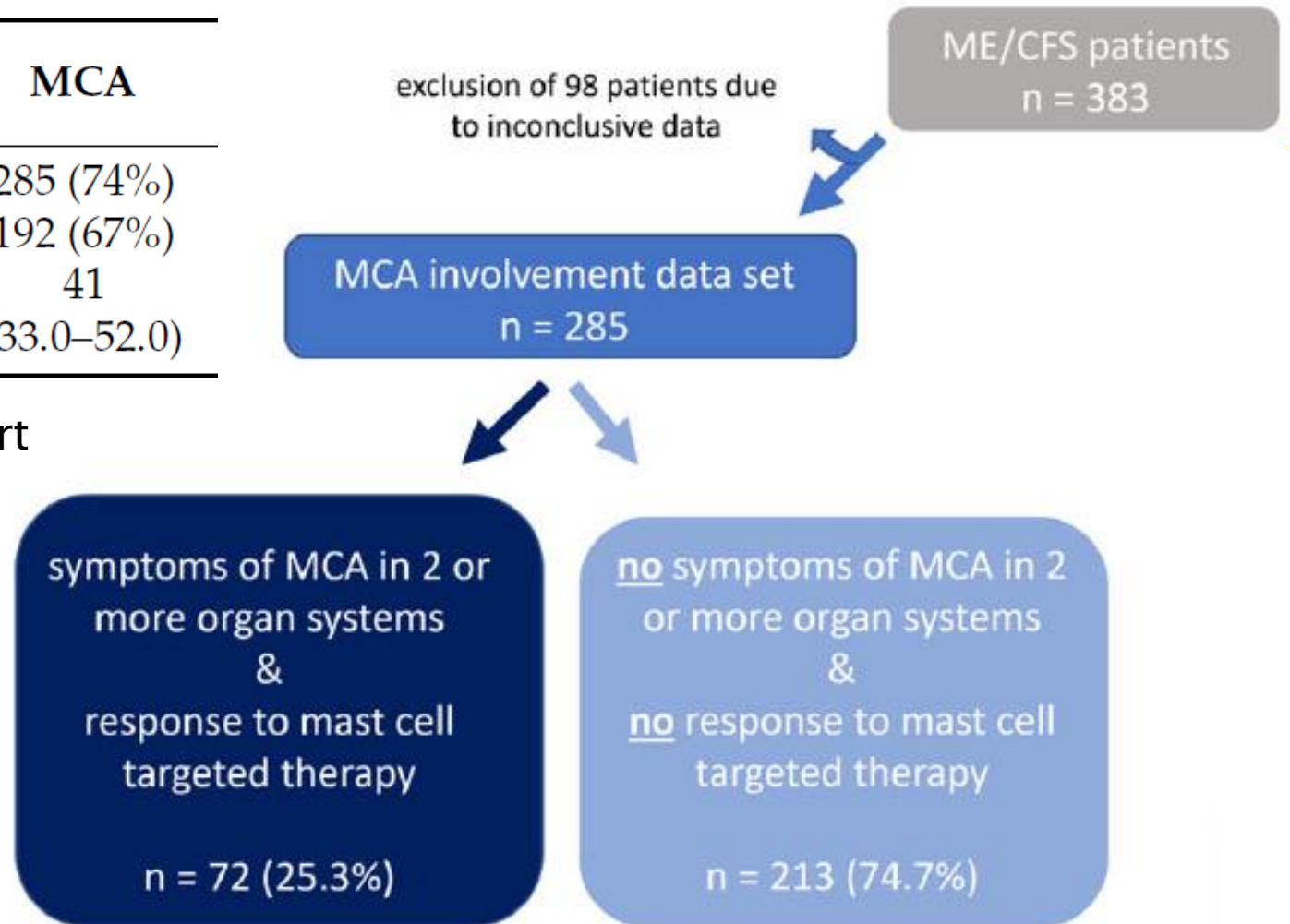
Rohrhofer J. et al Diagnostics 2025

Clinical relevance of MCA in ME/CFS

ME/CFS Data Sets	ME/CFS (Total)	MCA
Participants	383 (100%)	285 (74%)
Sex, female (%)	257 (67%)	192 (67%)
Age, median (IQR)	42 (33.0–52.5)	41 (33.0–52.0)

Unselected Austrian ME/CFS cohort

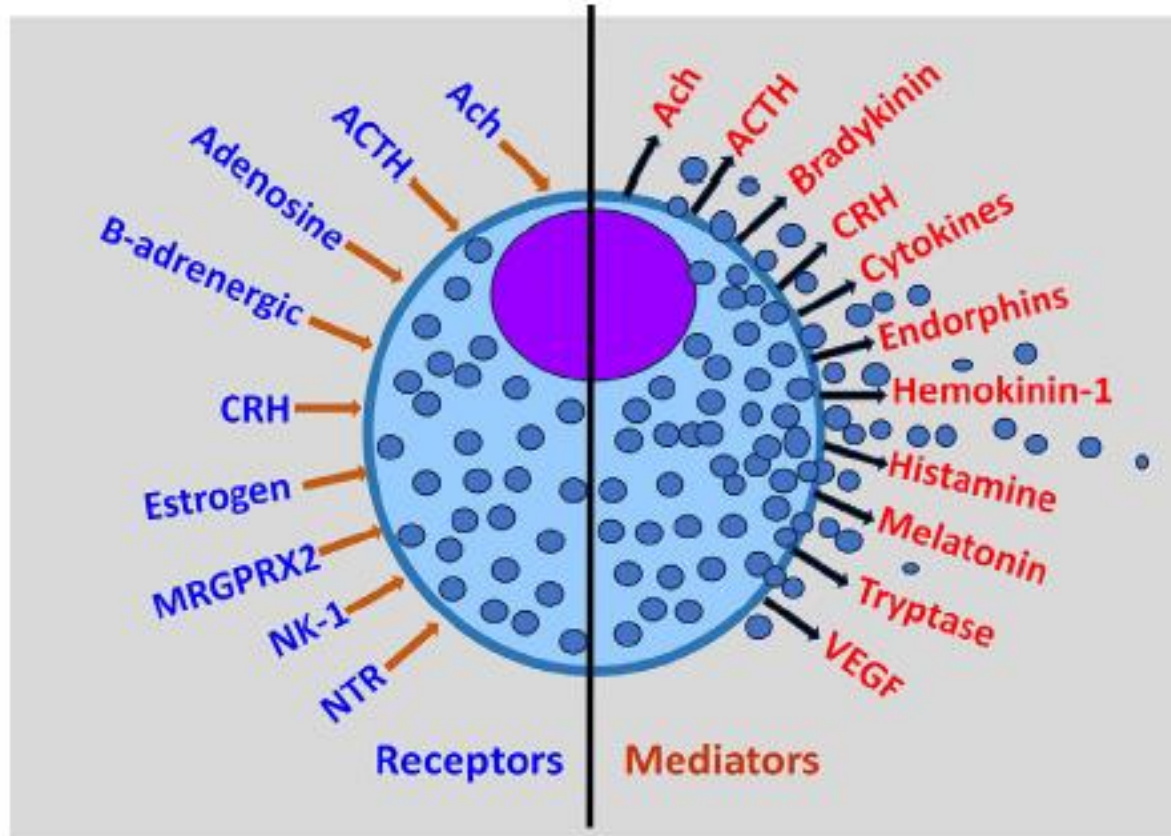
Orthostatic intolerance
significantly more common



Rohrhofer J. et al Diagnostics 2025

Connection between ANS and mast cells

MCs may participate in autonomic function and dysfunction



ANS-associated mast cell actions:

- Activation of microglia and astrocytes
- BBB disruption
- Cardiac pacemaking
- Coronary artery constriction
- HPA axis regulation
- Neurinflammation
- Neurosensitization
- Vascular tone control

Theoharides TC. et al Ann Allergy Asthma Immunol 2024

Degranulate-ME: Mechanisms of mast cell activation in PAIS

Study cohort:

	Median age	IQR 1	IQR 3	Sex, f (n)	Sex, f (%)	MCA*
A (n=20)	38	30	42	15	75	90%
B (n=20)	30	27	49,25	15	75	20%
C (n=16)	39	32,75	49,75	12	75	93,75%
E (n=20)	31	26,75	45	15	75	10,00%

A – Post-COVID syndrome

B – COVID, recovered

C – ME/CFS

E – Healthy

*clinically relevant MCA in more than 1 organ system

Mast cell Activation Tests

LAD2 cells (human mast cell line) stimulation with patients' plasma

Resting mast cell

Activated mast cell



https://www.frontiersin.org/files/Articles/52311/fimmu-04-00100-HTML/image_m/fimmu-04-00100-g001.jpg

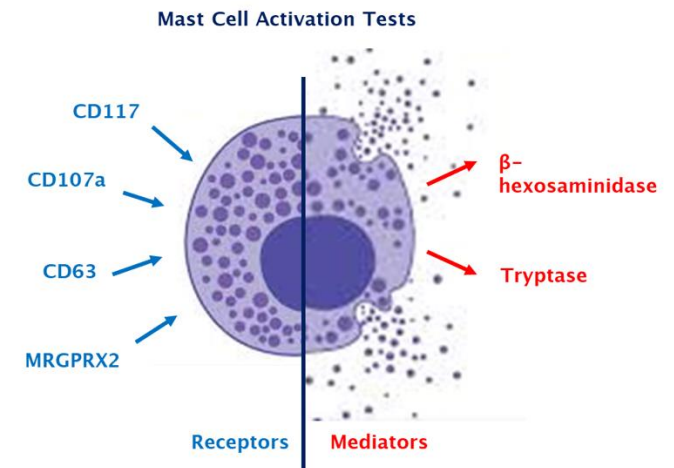
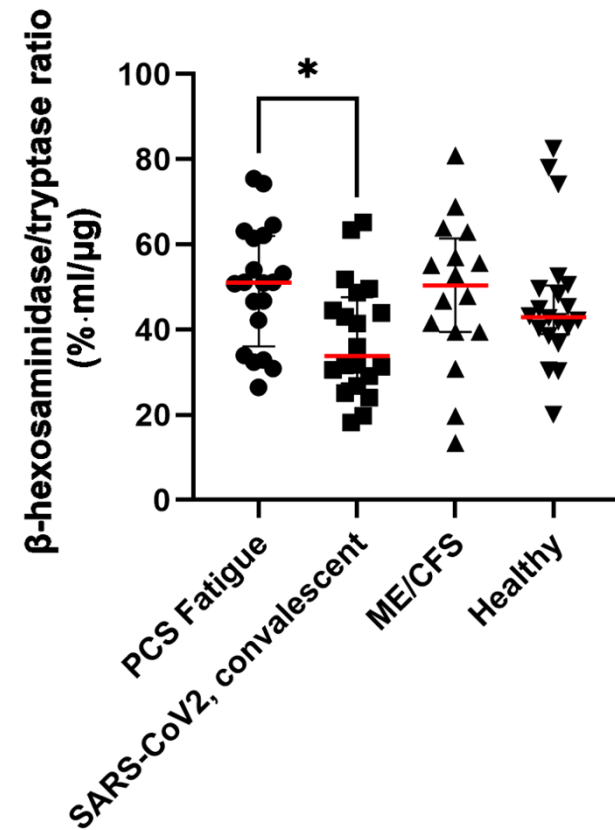


Figure: Johanna Rohrhofer

“Piecemeal” mast cell degranulation pattern in PAIS



FcεRI (IgE-mediated) ionophore/PMA stimulation, full anaphylactic degranulation	Tryptase (high), β-hex (high)	full granule fusion, strong release of everything, richer in proteases (tryptase/chymase)
MRGPRX2-mediated activation, weak FcεRI stimulation, partial/“piecemeal” degranulation, complement or neuropeptide activation	β-hex (high), tryptase (lower/variable)	vesicular transport from granules, selective mediator release, enriched in lysosomal enzymes (β-hex)
Complement receptors (C3aR, C5aR)	β-hex (low), tryptase (variable)	moderate, stimulus-dependent
Cytokine receptor-driven activation (IL-33), TLR activation, priming without degranulation	β-hex (low), tryptase (low), but high in cytokines	mainly cytokines, little degranulation

Rohrhofer J et al. Manuscript in preparation

Quantitative proteomics of stool samples followed by STRING-network analysis: Differentially regulated proteins linked to innate immune/MCAS-relevant axis in female ME/CFS patients compared to healthy controls

Key take home message

Mast cell activation can affect patients with post-acute infection syndrome

→ Diagnosis via medical history, laboratory testing and evaluation of treatment response

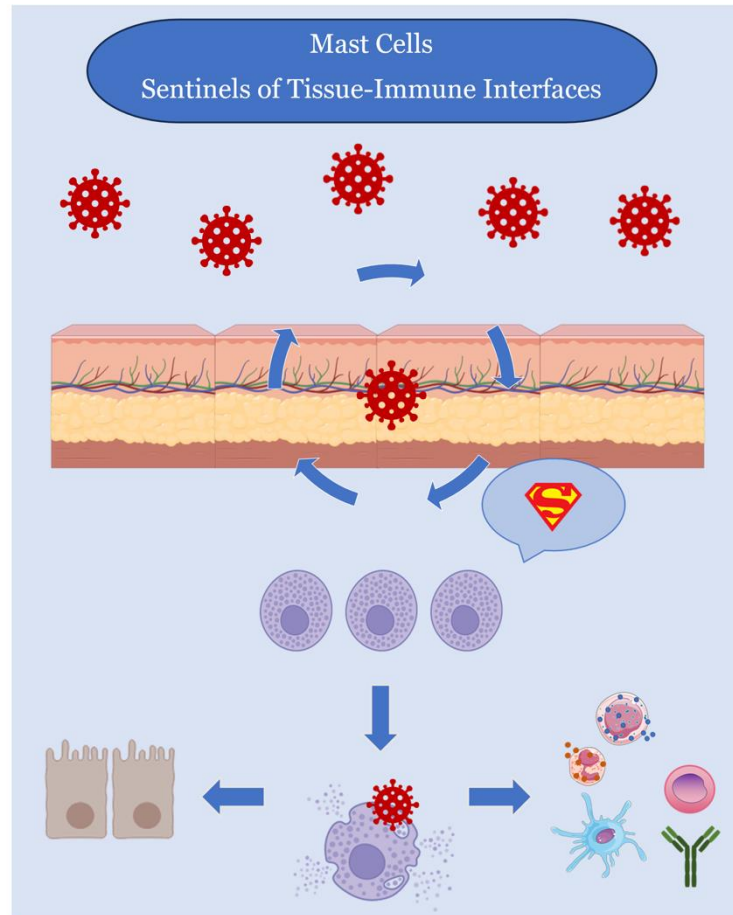
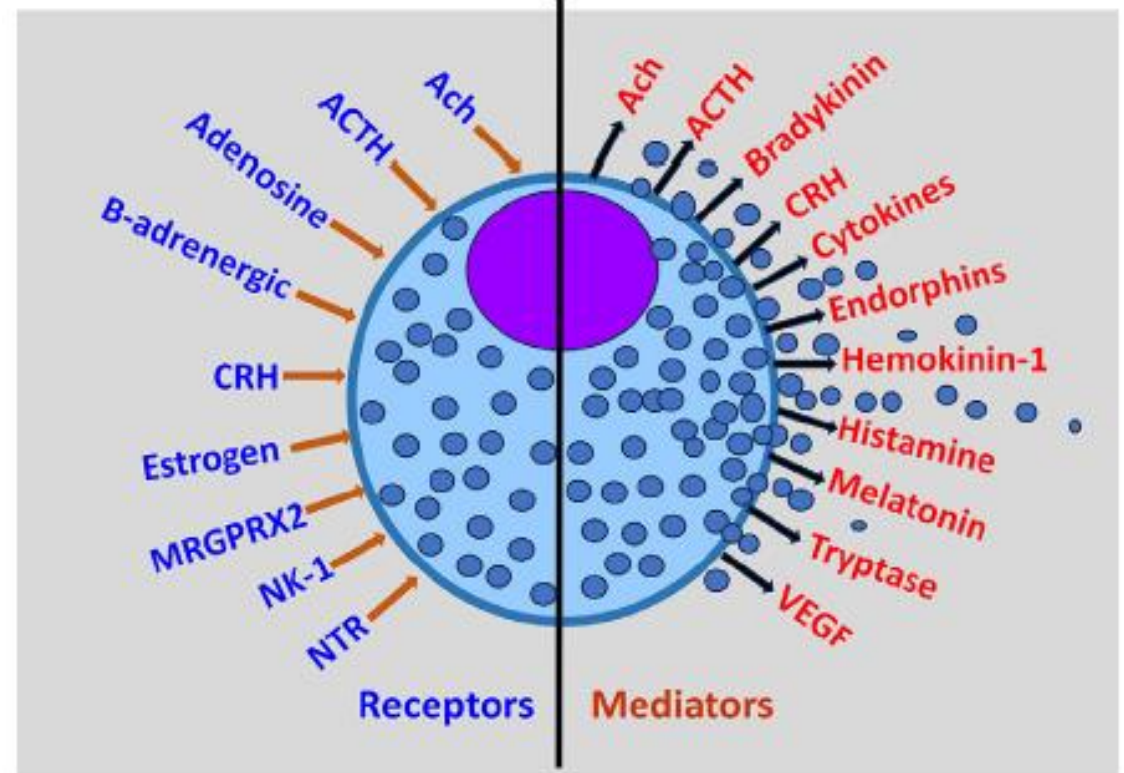


Figure: Johanna Rohrhofer

MCs may participate in autonomic dysfunction



Theoharides TC. et al Ann Allergy Asthma Immunol 2024

List of medications for ME/CFS and PAIS

NEU: Indikations (IND)– Medikamentenliste für ME/CFS und postakute Infektionssyndrome >

Mit der Indikation (IND): ME/CFS und postakute Infektionssyndrome wie z.B. das Post-COVID Syndrom werden die in der Liste genannten Medikamente ab sofort von der ÖGK, BVAEB und SVS übernommen.

Falls es anfangs noch Probleme mit der ÖGK geben sollte, bitte Mail an: sekre-md@oegk.at. Wenn Sie bei einer anderen Kasse krankenversichert sind (z.B. BVAEB, SVS), dann müssen Sie sich bitte diesbezüglich mit dem medizinischen Dienst dieser Kasse in Verbindung setzen!

Wichtig: Die Medikamente sind für diese Indikation nur off-label verfügbar, d.h. es handelt sich weiterhin um einen individuellen Heilversuch und braucht eine gründliche Aufklärung und Einverständnis der Patient:innen zur Therapie. Wie üblich muss auf Wechselwirkungen und Kontraindikationen geachtet werden. Bei ME/CFS hat es sich bewährt, immer mit einer sehr niedrigen Dosierung anzufangen und dann langsam hoch zu dosieren.

Nationales Referenzzentrum für postvirale Syndrome

> Für Ärzt:innen & Gesundheitsberufe

[Veranstaltungen & Fortbildungen](#)

[Kontakt](#)

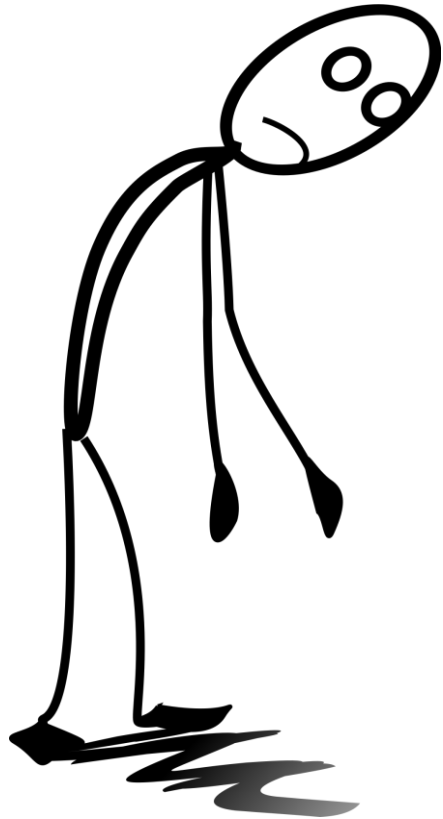
Mostly symptomatic therapy

Immune treatment included

Relevant patient stratification strategies in PAIS

- Stratification based on presence of post-exertional malaise → ME/CFS diagnosis
- Stratification based on sex and disease severity
- Stratification based on potential disease mechanisms eg. presence of immunodeficiencies
- Stratification based on presence co-morbidities

Summary of presented knowledge



<https://openclipart.org/detail/91513/al-tired>
by dominiquechappard

- PAIS are complex multisystem disorders
Relevance of the overlaps and differences with ME/CFS
 - Stratification is relevant for the care of PAIS patients
- Further research involving patient subgroups is urgently needed!
- Move from phenotypes to endotypes towards targeted treatment

New EU-funded research project in ME/CFS



Discovery of novel biomarkers and therapeutic targets that can be used for objective evidence-based diagnoses and treatments.

Identification of biological triggers and mechanisms underlying ME/CFS.

Improved diagnosis and distinguishing clinical subtypes.



Validation and subsequent integration of biomarker-based diagnosis in mainstream clinical practice.

Harmonization of existing and future datasets / patients' samples across the existing EU databases and biobanks to enable accelerated research, treatments and cures for ME/CFS.



Funded by
the European Union

New EU-funded research project in ME/CFS

Discover-ME



The first project to establish a fully integrated pipeline linking clinical phenotypes, validated biomarkers, mechanistic models, and testable therapeutic strategies.



Moving beyond symptom-based classifications to mechanistic, multi-omics-defined subtypes.



**Funded by
the European Union**

Thank you!

Cooperation partners

- Dr Michael Stingl
- Prof. Elisabeth Puchhammer,
Dr Marianne Graninger
- Prof. Sonja Zehetmayer
- **ÖG ME/CFS** | ÖSTERREICHISCHE
GESELLSCHAFT
FÜR ME/CFS
- Discover-ME project Team

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- WE&ME Foundation
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- LBG PPIE projects: CCCFS, Care for ME/CFS
- Horizon Europe: DISCOVER-ME



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