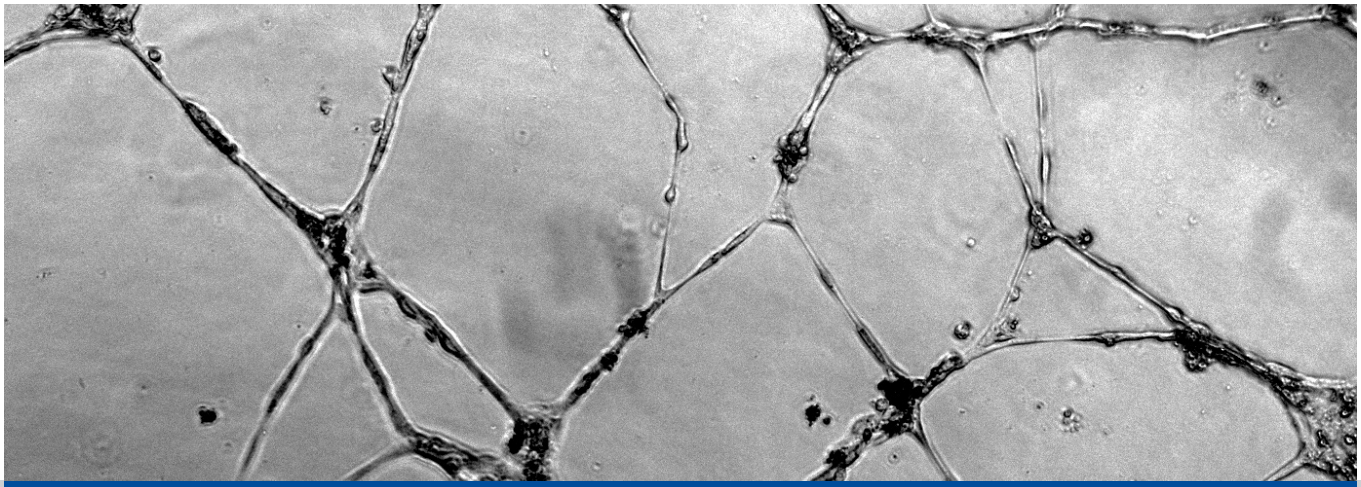


Novel Biomarkers of Endothelial Dysfunction and Angiogenesis Alterations in PCS and ME/CFS

Martina Seifert | May 11th 2023 |ME/CFS Conference 2023, Berlin

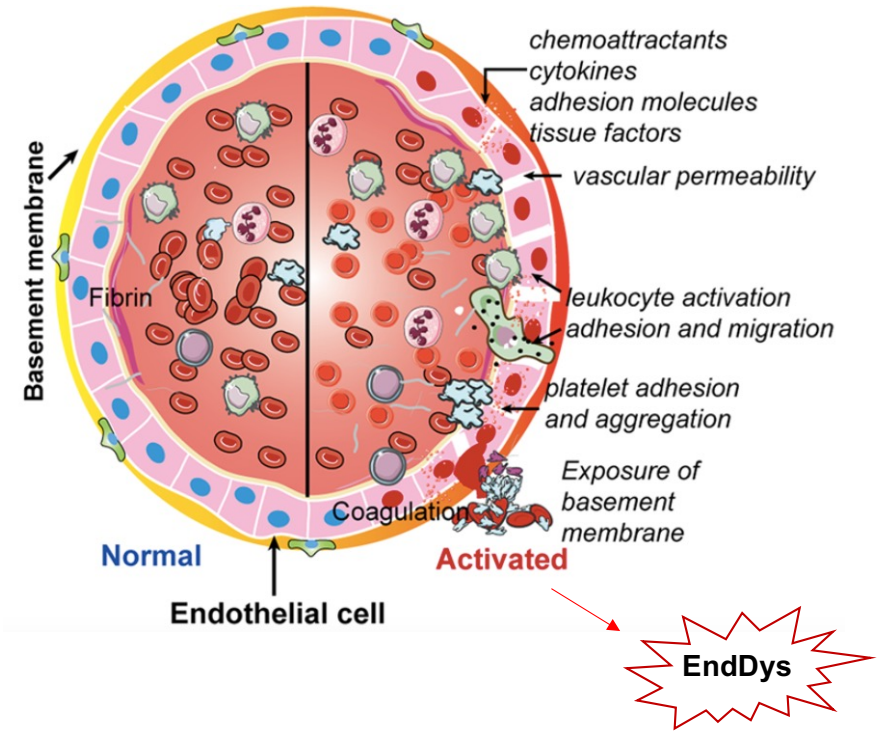
Campus Virchow Klinikum (CVK)

Institute of Medical Immunology

Group ´Vascular Immunology´

Introduction

Endothelial Dysfunction (EndDys) as an important pathogenetic mechansim in ME/CFS & PCS/CFS

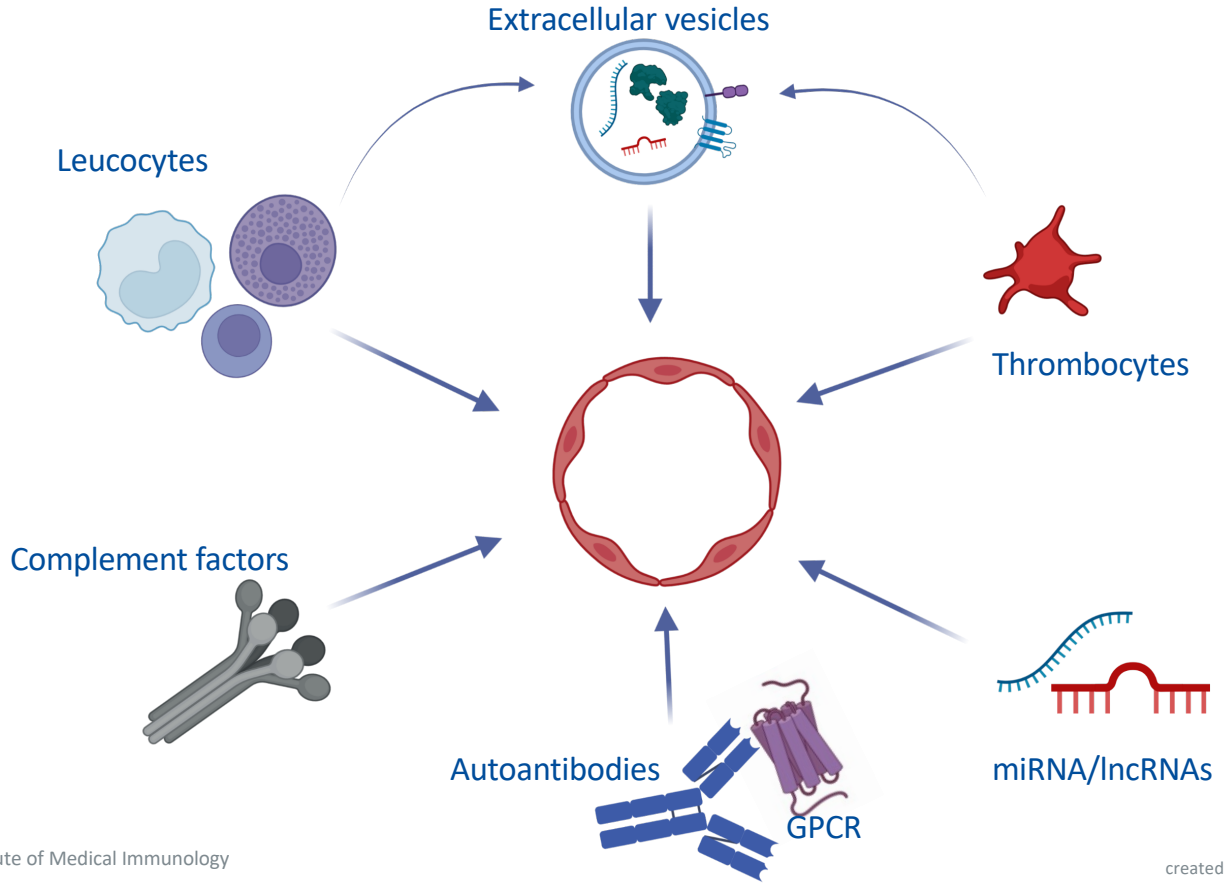


- ✓ Evidence for **vascular dysfunction in ME/CFS:** cerebral hypoperfusion, orthostatic intolerance, autonomic dysfunction
- ✓ EndDys **diagnosed in ME/CFS** patients *(Scherbakov et al. 2020)* and in **PCS/ME/CFS** patients (*Haffke et al. 2022*)
- ✓ EndDys and **altered EC biomarkers (ET-1**) in PCS/CFS patients *(Haffke et al. 2022)*
- ✓ Induction of autoimmune responses, e.g. **autoantibodies** to GPCRs *(Sotzny et al.2022)*
- ✓ **Decreased Nitric oxide (NO)** production in ECs exposed to plasma from **ME/CFS patients** (Bertinat et al. 2022)
- ✓ **Microclots** present in ME/CFS and PCS patients might block up capillaries and **disturb blood flow** *(Kell & Pretorius 2022)*

Figure adapted from: Jin *et al.*, 2020

Introduction

Serum components and blood cells as potential mediators of vascular inflammation and EndDys



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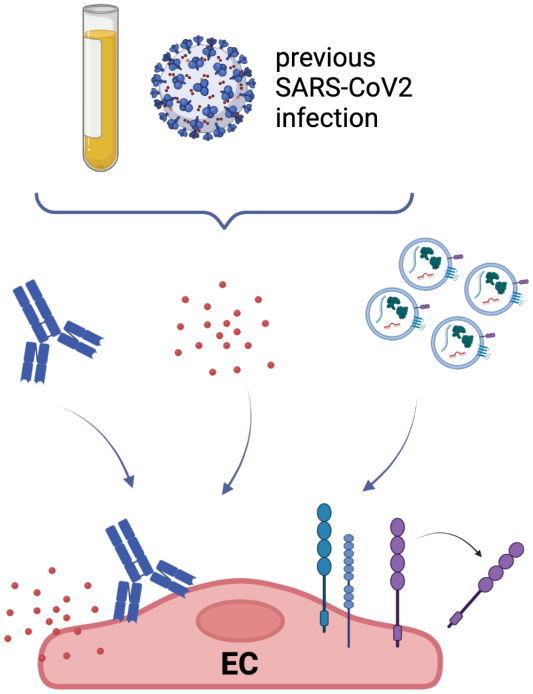
Question/Objective

Do serum factors from PCS and PCS/CFS patients show binding and functional effects on ECs in an *in vitro* culture model system?

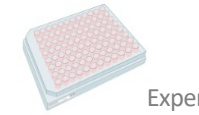


Patient and control group characteristics

	HC (n=14)	PCS (n=17)	PCS/CFS (n=13)
Age, mean (range)	45 (31-58)	42 (27-66)	43 (24-59)
Sex (f/m)	(12/2)	(15/1)	(12/2)
Months since COVID-19 infection, mean (range)	n/a	8.3 (4.3-11.6)	9.4 (8.2-11.1)
Bell Disability Scale, mean (range)	n/a	48.24 (10-80)	45.38 (20-80)
Chalder Fatigue Scale, mean (range)	n/a	24.76 (15-32)	26 (20-33)
PEM score, mean (range)	n/a	25.88 (17-46)	30.92 (16-44)

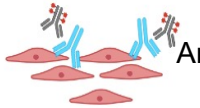
Results

- Anti-endothelial cell autoantibodies (AECA) of IgG type are more abundant in PCS/CFS patients
- Partial specificity of AECA-binding to human ECs



Experimental setup

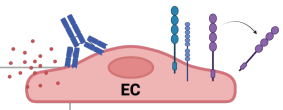
Serum AECAs?

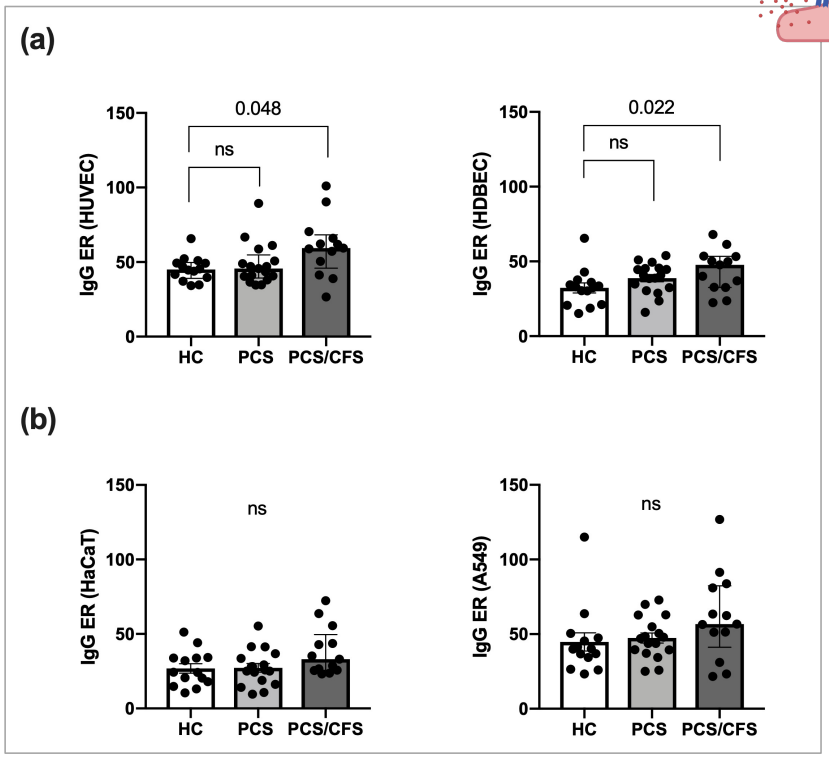


Anti-IgG-HRP

ELISA ratio (ER) = (Sample–Positive Control)/(Negative Control-Positive control) *Positive control = serum of autoimmune SSc patient*

Statistical testing: ANOVA with Tukey *posthoc*





Endothelial cells (HUVEC, HDBEC)

Epithelial cells (HaCaT, A549)

Flaskamp et al. 2022

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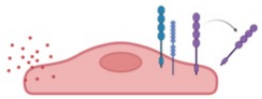
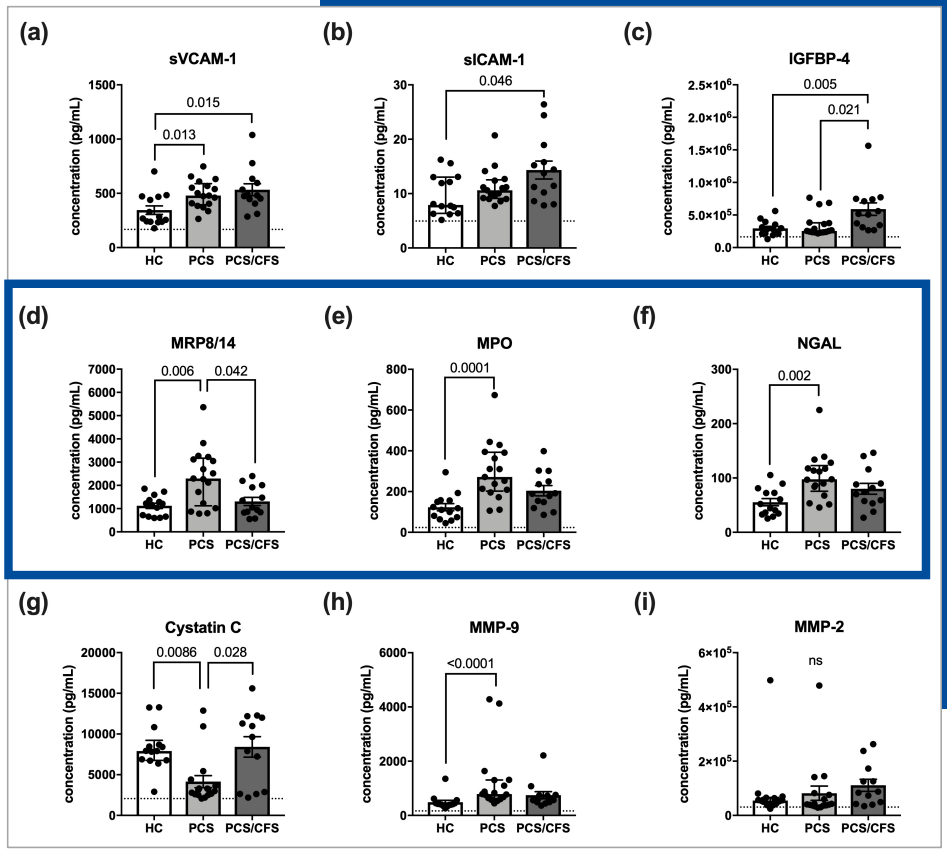
Results



- Patient serum induced changes in secretion profile of human ECs

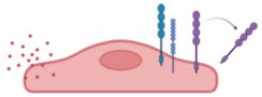
- **VCAM-1** (PCS and PCS/CFS) **shedding** and **MRP8/14 release** (PCS)
- **ICAM-1 shedding** and **IGFBP-4** release **specific for PCS/CFS;** IGFBP-4 putatively **interferes** with **NO synthesis** (vasodilation ↓?) and **angiogenesis**
- **MPO** enhanced in PCS group; implicated in **NO depletion** (vasodilation ↓?) and angiogenesis
- **NGAL and MMP-9 enhanced in PCS**; MMP9 complexes with NGAL and promotes its function including extracellular matrix remodelling
- **Cystatin C reduced** by PCS serum; known as prominent **protease inhibitor**

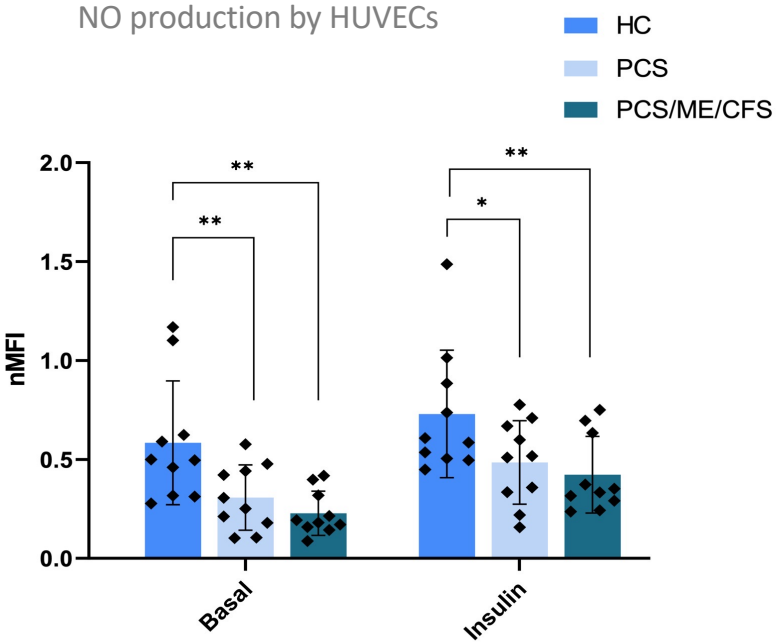


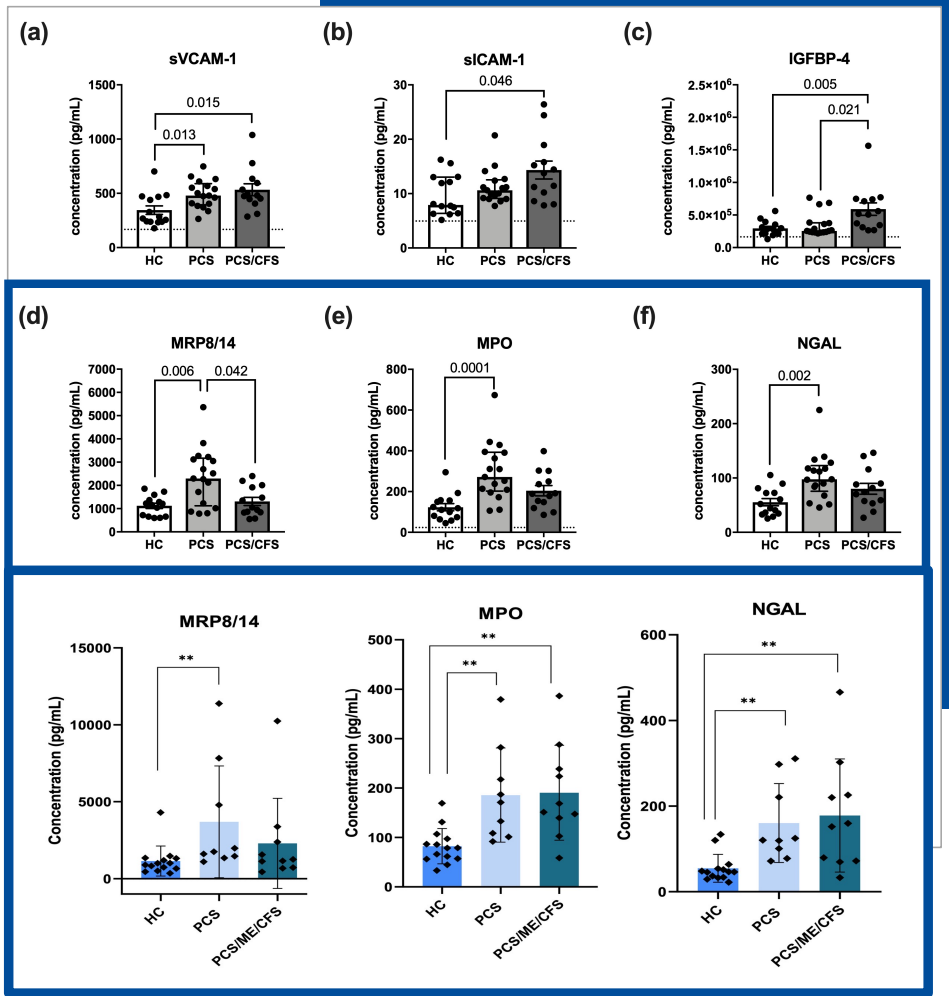
Flaskamp et al. 2022

VCAM, vascular cell adhesion molecule; ICAM, Intercellular adhesion molecule; IGFBP, insulin-like growth factor binding protein; MRP8/14, Myeloid related protein 8/14; NGAL, neutrophil gelatinase-associated lipocalin; MMP, matrix-metalloprotease; MPO, myeloperoxidase.

Results







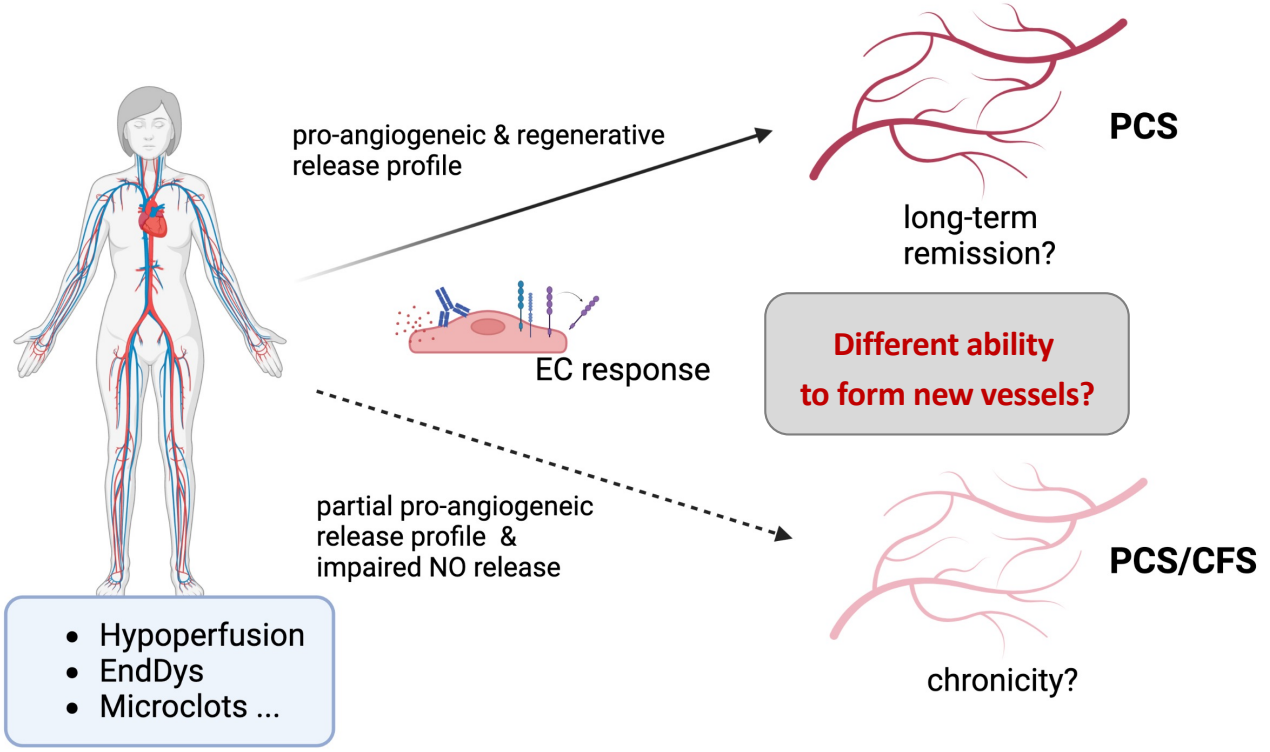
Poster No 23: Kanchan Dulal

CHARITÉ | Institute of Medical Immunology

Pro-angiogeneic EC signature in PCS

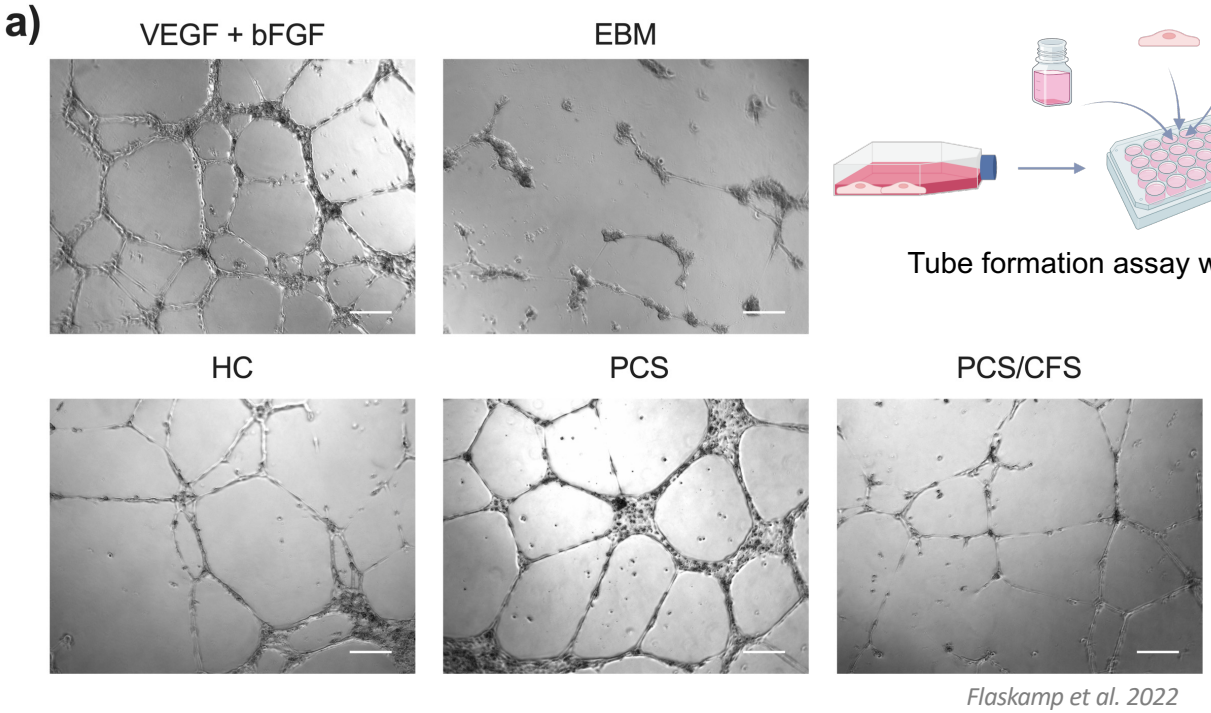
Differences between PCS & PCS/ME/CFS patients in:

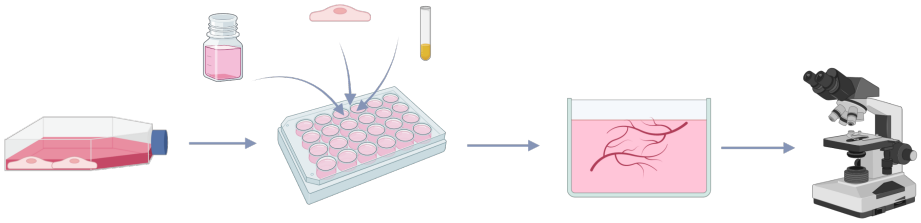
- Pro-angiogeneic/vascular remodeling capacities
- Nitric oxide bioavailability



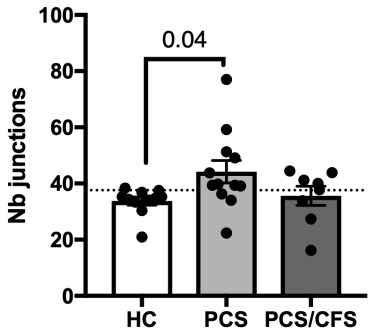
Results

- Significantly enhanced number of junctions in the PCS patients group





Tube formation assay with EC on Matrigel *in vitro*

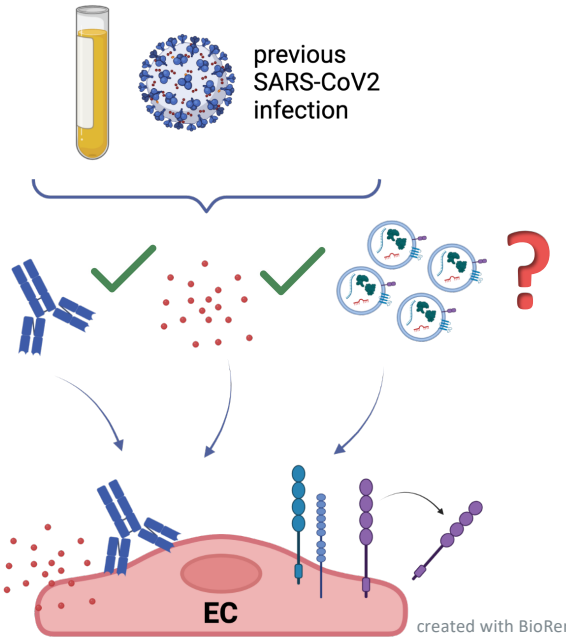


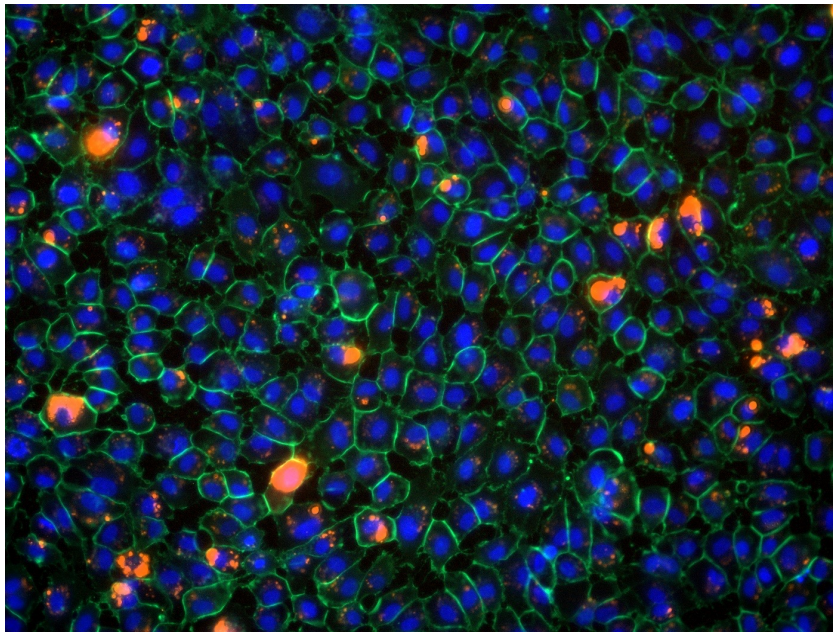
Flaskamp et al. 2022

Statistical testing: ANOVA with Tukey *posthoc*
Dotted lines denoted the 90th percentile of the HC group

Results

Serum extracellular vesicles as potential biomarkers for EndDys in ME/CFS and PCS and as mediators of effects on ECs?

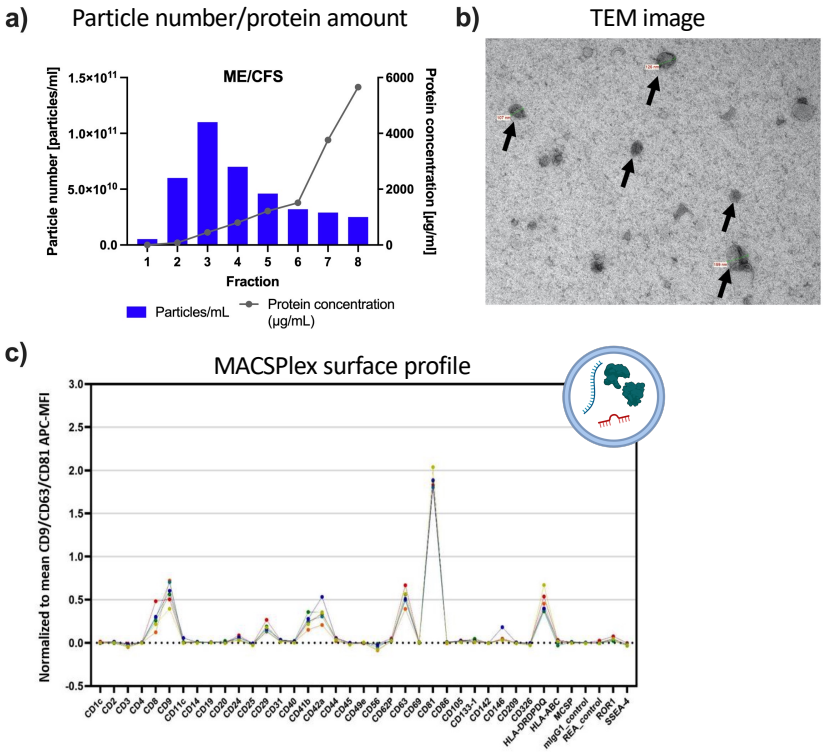


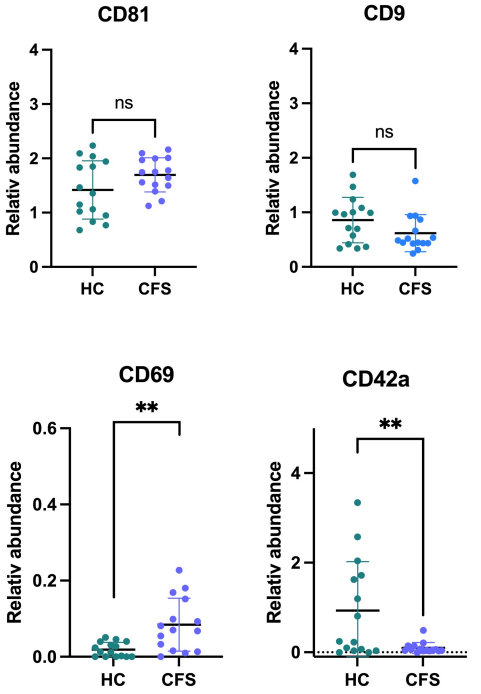


Uptake of labeled EVs (orange) by ECs (green/blue)
Into HUVECs

Result

EVs isolation by size exclusion chromatography from patients´plasma show characteristic EV features, but differ in their phenotype





Poster No 24: Diana Boristowski

Data by Steven Uddin & Diana Boristowski, unpublished

Results

- EV-proteom differences between post-infectiouse ME/CFS patients and Healthy Controls (HC)
- Candidate molecules as potential biomarkers

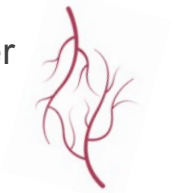
Data by Anne Birke, unpublished

Summary/Conclusions

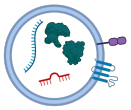
- **AECAs** show enhanced binding to ECs and thereby may attribute to EndDys development; Autoantibodies to GPCR involved?

- Serum from PCS patients with and without ME/CFS modulate differently the EC secretion profile indicating another pathomechanism for EndDys development.
- **Selected small molecules** released by EC might be used **as potential biomarkers** in PCS and PCS/CFS (sVCAM-1, MPO, MRP8/14, NGAL…)

- A **compensatory response** (**pro-angiogenic**) to a disturbed microcirculation, which appears to differ between PCS and PCS/CFS patients might be assumed.
- A missing/disrupted compensatory mechanism could lead to chronicity of the disease, as seen in PCS/CFS (and CFS).



- **Extracellular vesicles** might be promising biomarkers due to **differences in phenotype and proteome between ME/CFS and H**C and serve a the missing link for EC dysregulation.



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