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Proteomic profiling of ischemic stroke outcomes

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Outline

I. Biomarkers
II. Aims
III. Methods
IV. Research examples
V. Conclusions

I. What is a Biomarker?

"A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention."

Diagnostics

Detect or confirm presence of a disease

Stratification

Determine which treatment will work best

Prediction

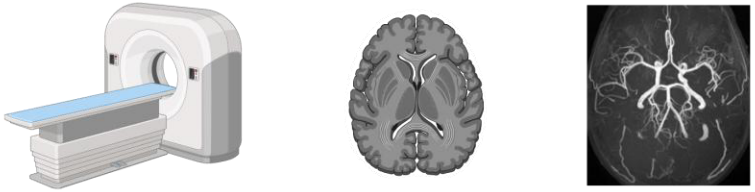
Indicate likelihood of disease recurrence

Prognosis

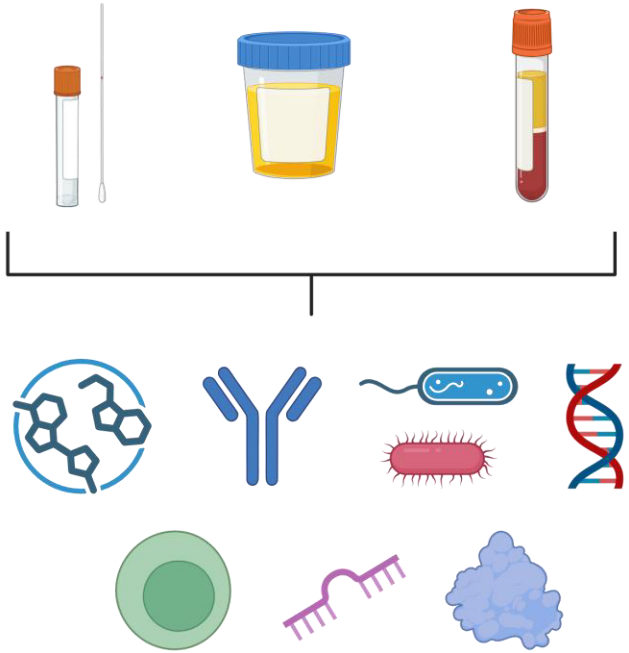
Indicate likelihood of an outcome

I. Types of Biomarkers

Imaging biomarkers

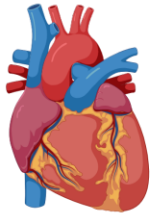


Biofluid biomarkers

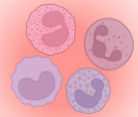


Physiological parameters

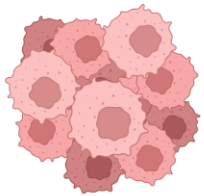
I. Commonly used blood-based biomarkers



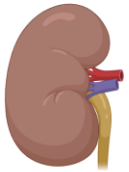
Heart
Troponin,
NT-proBNP



Inflammation
CRP, white
blood cell count



Cancers
PSA, BRCA,
ctDNA



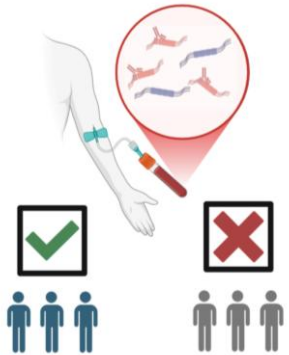
Kidney
Creatinine,
eGFR, cystatin C

Stroke is lagging behind!

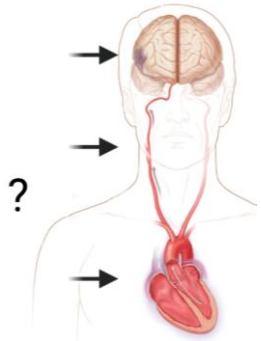
II. Aim

To identify blood-based biomarkers for diagnostics and outcome prediction after an ischemic stroke

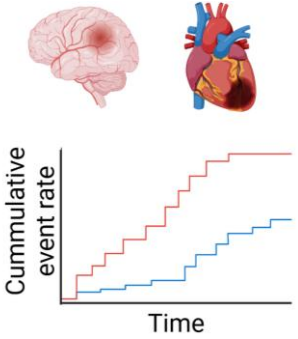
Diagnostics



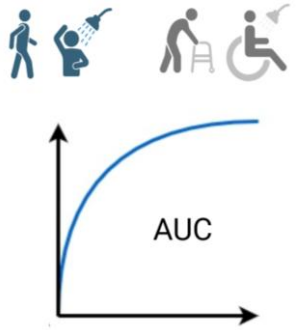
Stratification



Vascular recurrence



Prognostics



III. Methods: Biomarkers

Genomics

- DNA:
  - Whole genome arrays (GWAS)
  - Targeted DNA-seq

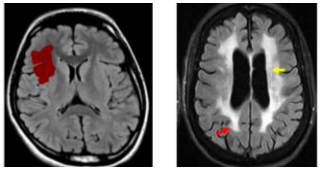
Proteomics

- Proteins:
  - Affinity-based (ELISA, SimoA, Olink Explore)
  - Mass spectrometry (Proteograph, Seer)

Transcriptomics

- RNA:
  - Bulk RNA-sequencing (leucocytes)
  - Small RNAseq (extracellular miRNA)

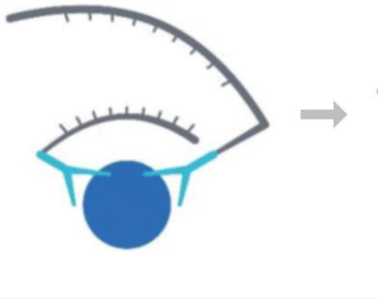
Radiomics



- MRI:
  - Lesion segmentation algorithms
  - Quantitative pipelines

III. Methods- Proximity Extension Assay (PEA)

1

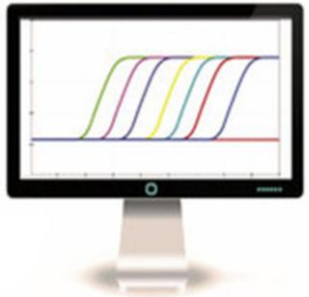


Antibody Pair Binding

2

Proximity Extension

3



Amplification (qPCR / NGS)

4

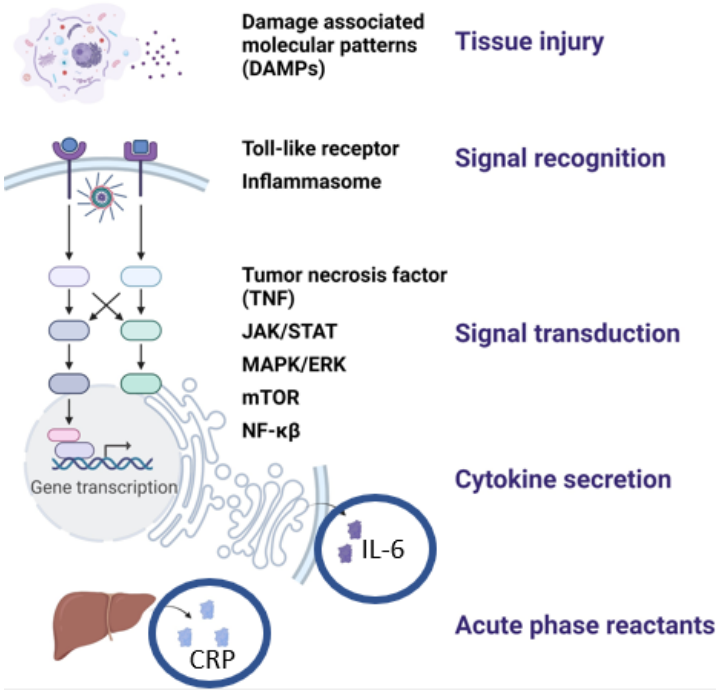
NPX Output

- Normalised Protein eXpression (NPX) on $\log_2$ scale
- Relative units; +1 NPX = doubling of protein level

Olink

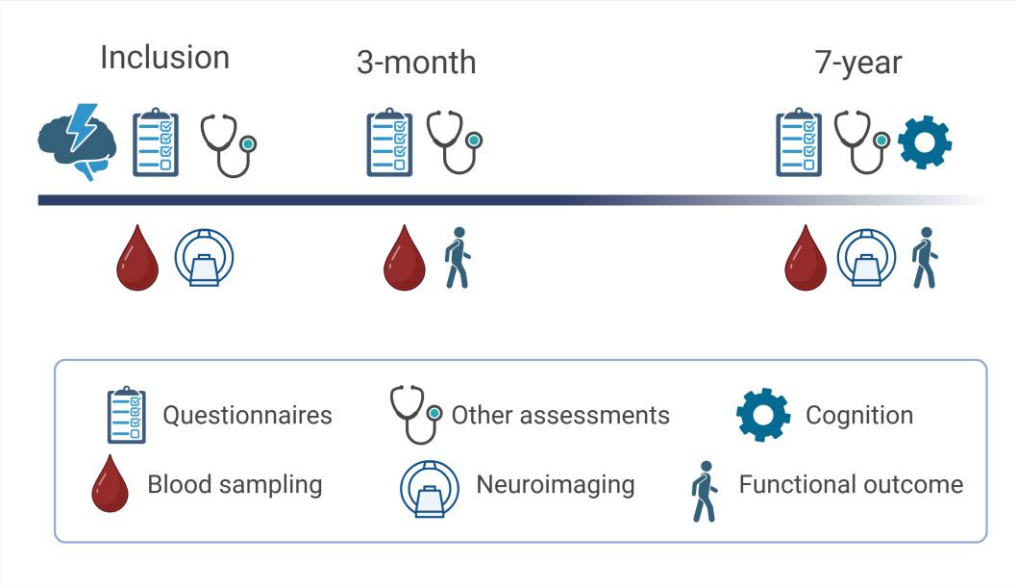
IV. Research Example - Inflammation

- Inflammation plays an important role in the acute response after stroke involved in both repair and damage
- Most studies focus on high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6)
- Complex pathway
- Other inflammation-related proteins may be more informative or specific to stroke
- Olink Target 96 Inflammation
  - chemokines, cytokines, interleukins, surface molecules, receptors



IV. Clinical cohorts

The Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS)



PI: Prof. Christina Jern
- 1998-2004
- Case-control, adults <70y
- N=600 longitudinal blood sampling

SAHLSIS phase 2:
- 2014-2025
- Adults >18 years
- N= 3,000 with acute blood sampling

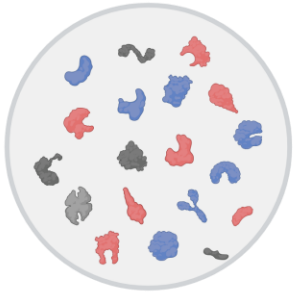
IV. Inflammation- Functional Outcome (mRS)

SAHLSIS < 70 years

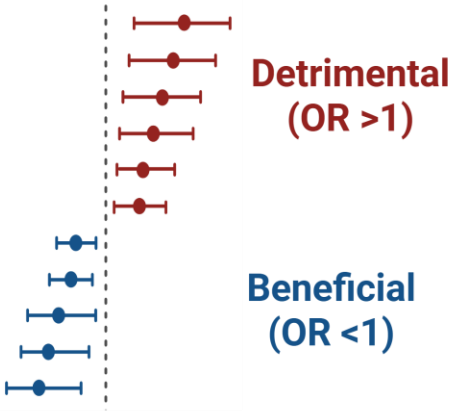
Favorable outcome (mRS 0-2)
n=415

Unfavorable outcome (mRS 3-6)
n=119

65 inflammation-related proteins



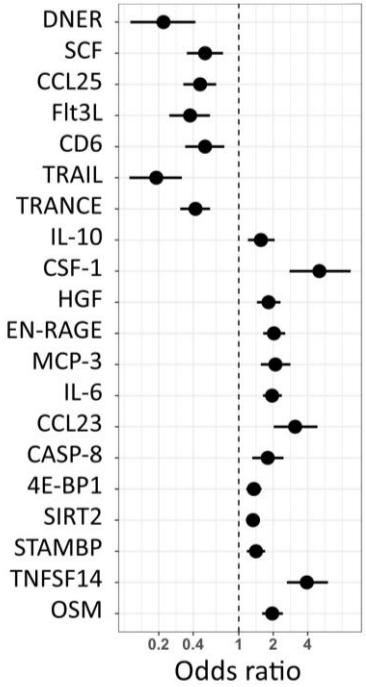
Call rate >80%

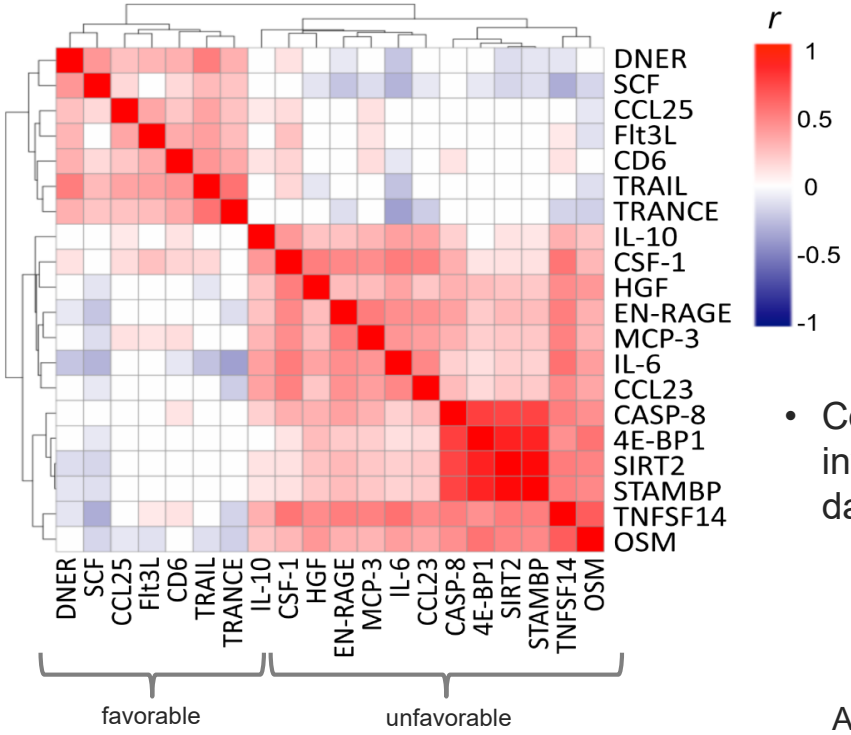


Odds ratio (OR) for unfavorable outcome

IV. Inflammation- Functional Outcome (mRS)

- n=20 proteins associated with functional outcome (FDR <0.05)





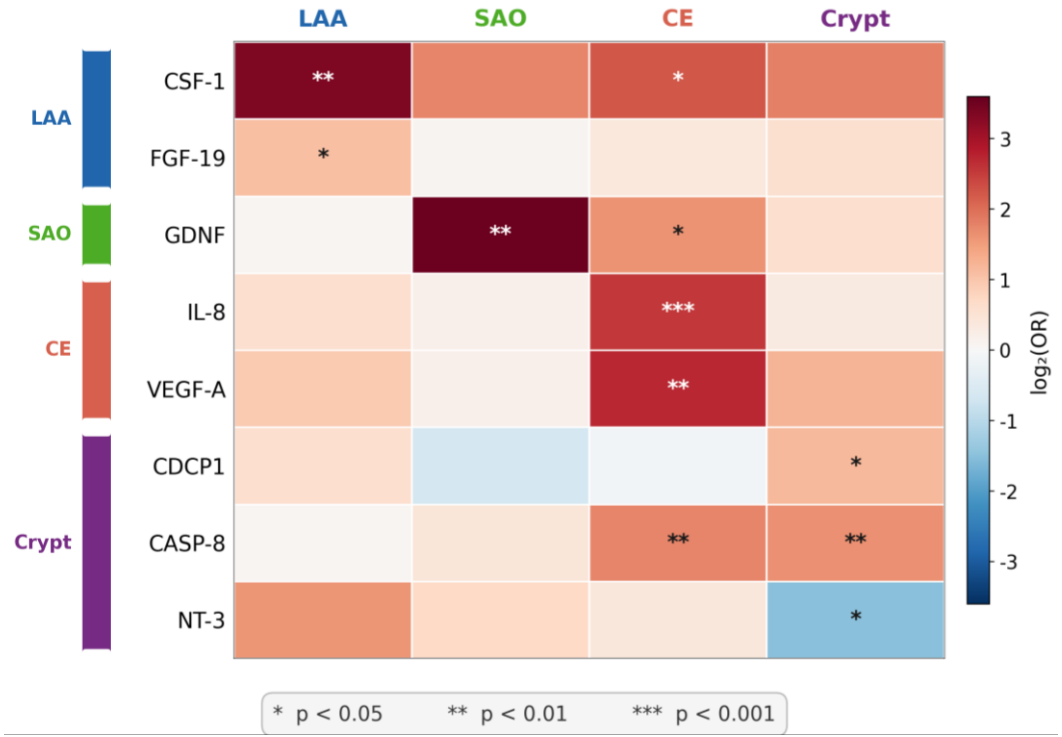
- Two main clusters

- Consistent with inflammation being involved in repair (protective) and damage (detrimental) after stroke

Angerfors et al, *J Neuroinflammation* 2023

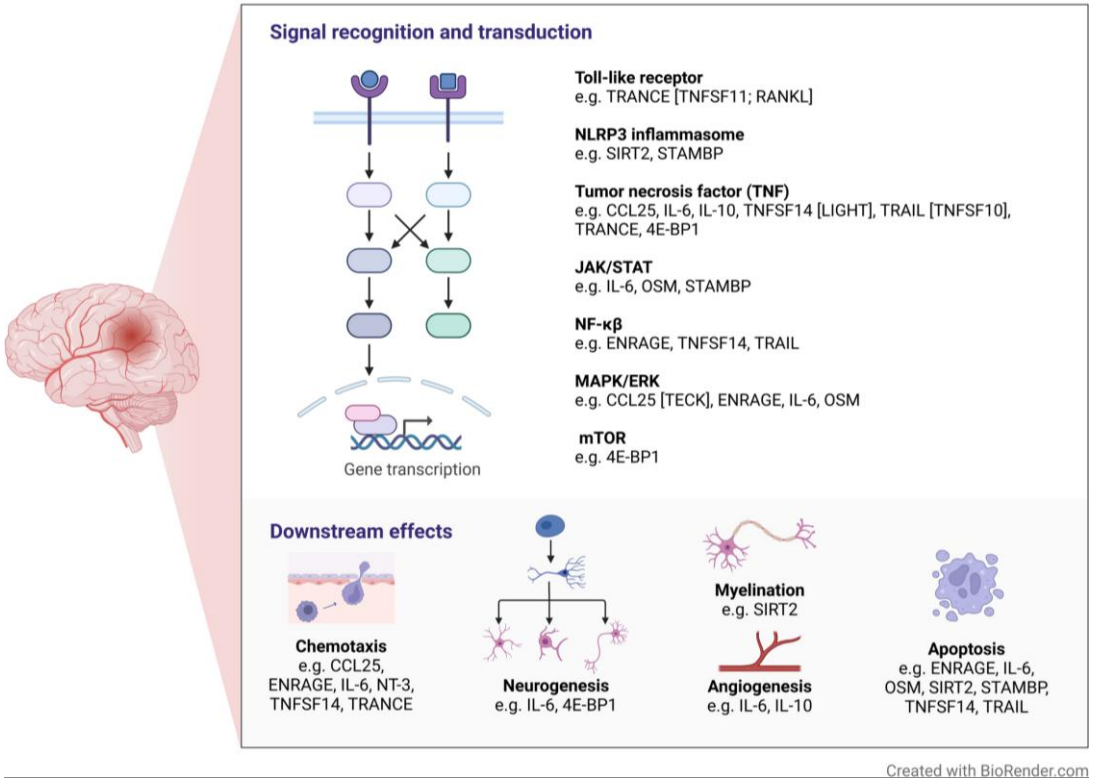
IV. Inflammation- Functional Outcome (mRS)

- Subtype-specific protein associations with functional outcome
- Replication in independent cohorts are needed
- Emphasis importance of larger studies with power for stratified analyses



Angerfors et al, *J Neuroinflammation* 2023

IV. Inflammation- Functional Outcome (mRS)

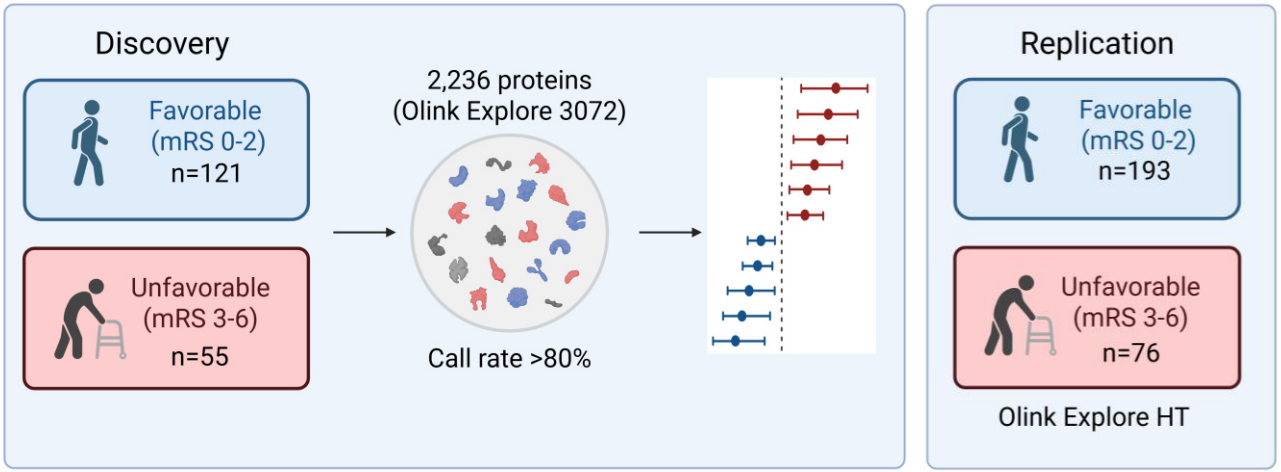


- Many upstream signals
- Approved drug target:
  - TRANCE (TNFSF11; RANKL)
- Several drug targets in Phase 3 trials
  - CD6
  - HGF
- Several in Phase 2 trials:
  - TNFSF14 (LIGHT)
  - CSF-1
  - IL-10
  - CASP-8

Angerfors et al, *J Neuroinflammation* 2023

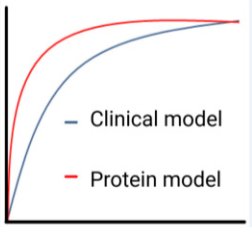
IV. Olink Explore - Functional Outcome (mRS)

SAHLSIS phase 2 (adult ischemic stroke)



Combined dataset

- 70% training + 30% test
- Machine Learning
- Evaluate discriminitive performance



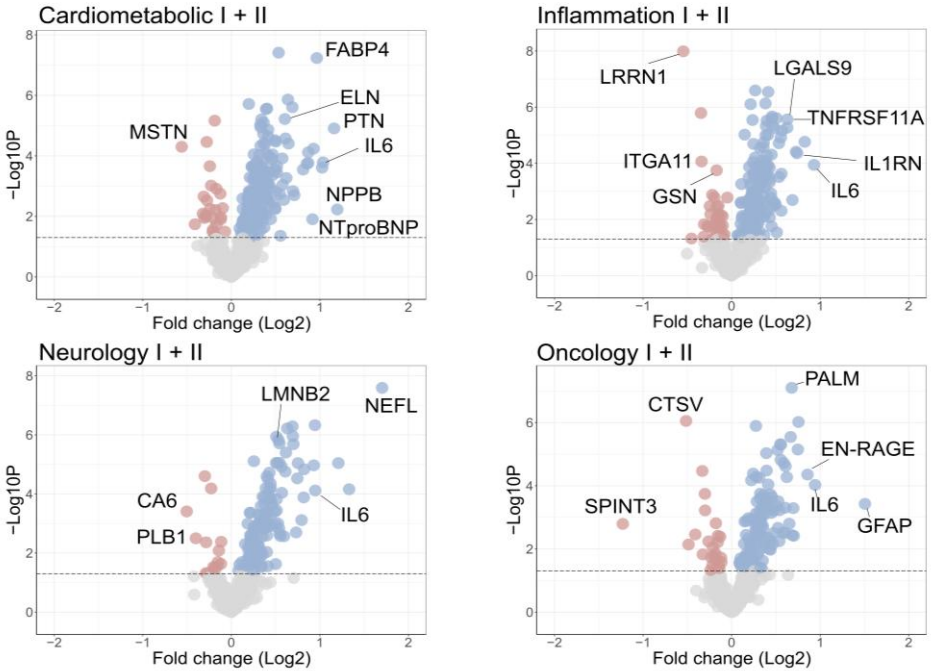
IV. Olink Explore - Functional Outcome (mRS)

Discovery (Olink Explore 3072)

- 204 proteins showed differential expression in favorable vs unfavorable outcome

Replication (Olink Explore HT)

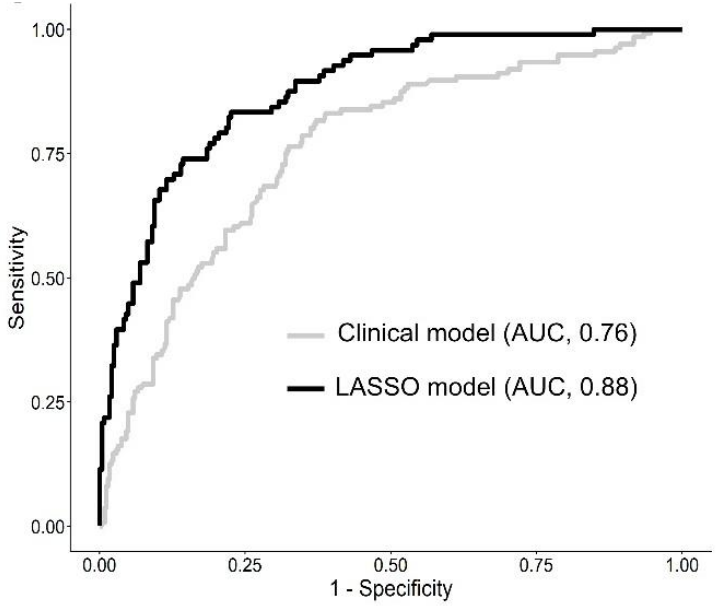
- Univariable: 100 proteins replicated
- Multivariable: 14 proteins replicated (P<0.0004)
  - 59 proteins P<0.05 P>0.0004
  - Candidates for mechanistic studies



IV. Olink Explore - Functional Outcome (mRS)

- Multiprotein modelling (LASSO)
  - 15 features selected (14 proteins + NIHSS)

- Strong discriminative performance for predicting unfavorable functional outcome
  - AUC = 0.88 in training; 0.89 in test



IV. Olink Explore- Functional Outcome (mRS)

Multi-pathway biology of stroke recovery

Neuronal injury & plasticity

- Strongest and most consistent signal
- Points to structural integrity and post-injury regeneration as key determinants

Vascular remodeling & extracellular matrix

- ELN (elastin) and PTN (pleiotropin) ranked above stroke severity in LASSO model
- Vascular integrity and regenerative signalling (angiogenesis) shape recovery

Immune activation

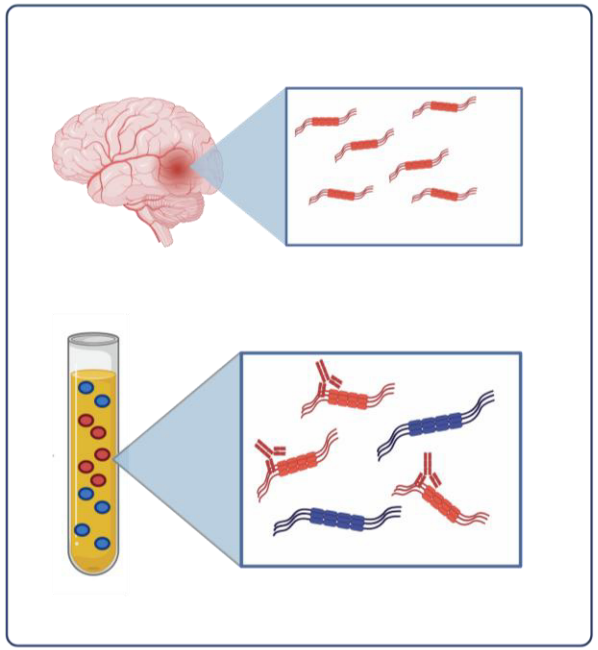
- Both detrimental (T-cell activation, NF-κB) and protective (gelsolin)

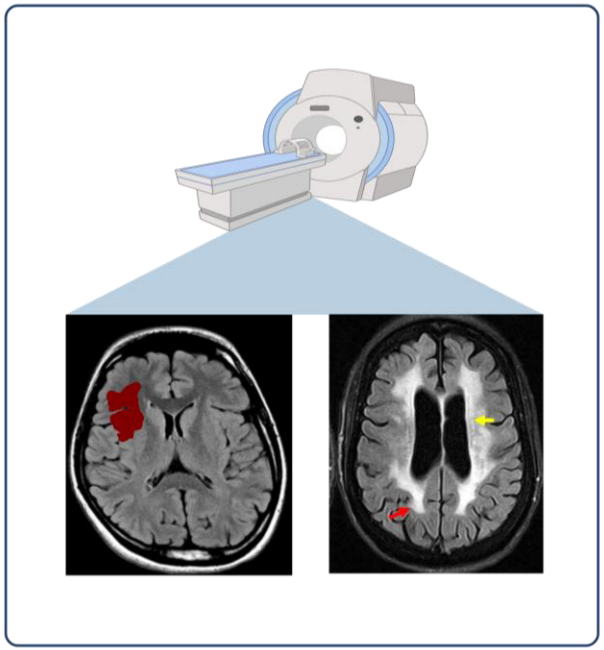
Lipid signaling

- PLB1 (phospholipase B1) = 2nd most important LASSO variable
- Generates lipid mediators that support vascular repair and inflammation resolution

IV. Integrating Neuroimaging & Blood Proteomics

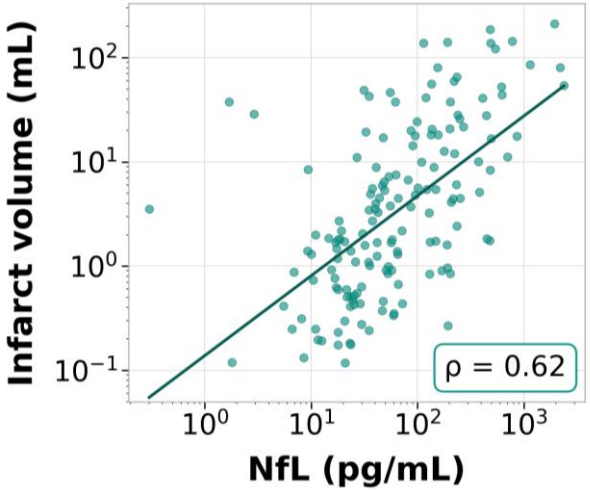
To identify plasma protein proxies of MRI phenotypes



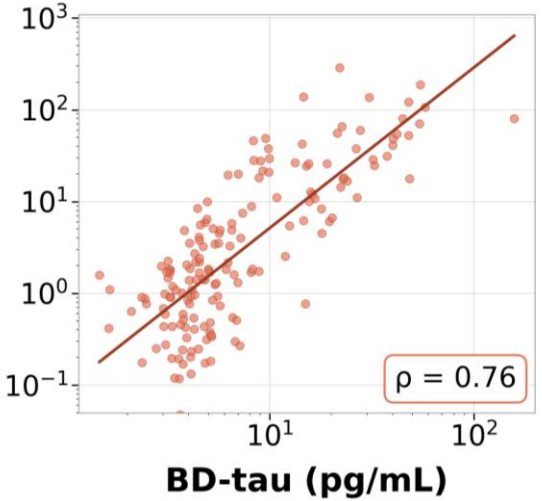


IV. Integrating Neuroimaging & Blood Proteomics

- Blood-based biomarker that proxy infarct volume
- *SAHLSIS*, manual infarct volume detemination
- Targeted proteins (SimoA, Quanterix)



Holmegaard et al, *Sci Rep* 2024

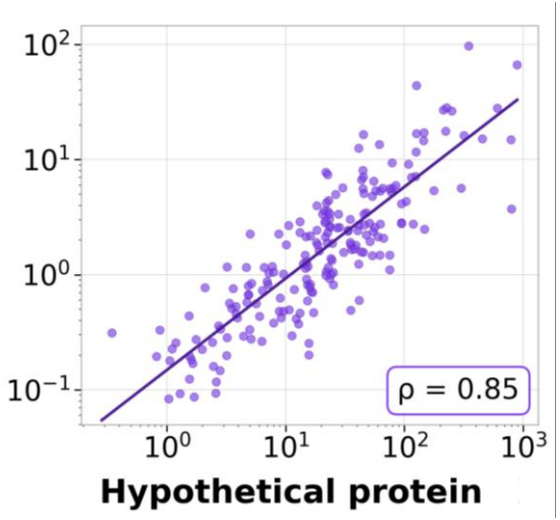


Gonzalez-Ortiz et al, *J Intern Med* 2025

- NfL expressed in central and peripheral nervous system
- Elevated in many neurological and neurodegenerative conditions
- Brain-derived tau, CNS isoform, stronger correlation

IV. Integrating Neuroimaging & Blood Proteomics

- *SAHLSIS phase 2*: AI-based neuroimaging pipelines
- Broad- scale proteomics : Olink Explore HT



- Can we identify an even better biomarker (or combination) to proxy infarct volume?
- Preliminary data looks promising!

Future goal to develop a diagnostic triage tool, ”a troponin of the brain”

IV. Integrating Neuroimaging & Blood Proteomics

DDLS PhD Student
Jesper Olsson, MSc Eng
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Markus Schirmer, PhD
Co-supervisor
Harvard Medical School

AI-Based Imaging Pipelines

e.g. infarct volume, white matter hyperintensities, brain health metrics, etc.

Broad-Scale Proteomics (+ other omics)

e.g. Olink Explore HT, mass-spectrometry (Seer Proteograph / Orbitrap Astral), miRNAseq

Integration & Modelling

Identify blood-based surrogates and mechanistic signatures of MRI-defined injury

V. Conclusions

- **Inflammation-related proteins (both protective and detrimental) are associated with functional outcome**
  - Stratified analyses revealed etiologic subtype-specific differences
- **Large-scale proteomics substantially improves outcome prediction**
  - Identified pathways of importance include neuronal injury, neuronal plasticity, inflammation, vascular remodelling and lipid signaling
- **BD-tau is more highly correlated to cerebral infarct volume than NfL**
- **Work in progress: To identify a protein (or combination) that is an even better proxy of infarct volume and proteins that mirror other neuroimaging phenotypes**

Taken together, plasma proteomics holds promise for identifying stroke biomarkers for diagnostics, stratification, and prognosis

Thank you

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Björn Granelli, MD, PhD student
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Katarina Jood, MD, PhD, Prof.
Lukas Holmegaard, MD, PhD

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Henrik Zetterberg, MD, PhD, Prof.
Fernando Gonzalez-Ortiz, MD, PhD

Markus D. Schirmer, PhD

… and all study participants and their families

Insamlingsstiftelsen for Neurological Research ISNF

Vetenskapsrådet

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STROKE CENTRUM VÄST

Hjärt-Lungfonden

King Gustaf V:s and Queen Victoria's Foundation

SAHLSIS- Baseline characteristics

	Controls	Ischemic stroke Acute-phase	Ischemic stroke 7y-follow up
n	600	600	223
Age, mean ± SD	56 ± 10	56 ± 10	61 ± 10‡
Male sex, n (%)	385 (64%)	385 (64%)	146 (65%)
NIHSS, median (IQR)	NA	3 (1-7)	2 (1-5)
BMI, mean ± SD	26 ± 4	26 ± 4	27 ± 4
Diabetes mellitus, n (%)	22 (5%)	114 (19%)‡	39 (17%)‡
Hypertension, n (%)	224 (37%)	354 (60%)‡	123 (55%)‡
Current smoking, n (%)	109 (18%)	233 (39%)‡	46 (20%)

Stanne TM et al (2022) *Stroke*

SAHLSIS- Functional Outcome (mRS)

	Total	Favorable	Unfavorable
n	534	415	119
Age, median [IQR], years	58 [52-64]	58 [52-64]	59 [53-65]
Male sex, n (%)	340 (64)	258 (62)	82 (69)
Time to blood draw, median [IQR]	4 [3-6]	4 [3-6]	4 [2-6]
Hypertension, n (%)	320 (60)	247 (59)	73 (63)
Diabetes mellitus, n (%)	100 (19)	73 (18)	27 (23)*
Hyperlipidemia, n (%)	379 (71)	295 (74)	84 (80)
Smoking, n (%)	208 (39)	164 (40)	44 (37)
NIHSS score, median [IQR]	3 [2-7]	2 [1-4]	12 [7-16]†

Angerfors et al, *J Neuroinflammation* 2023

Olink Explore - Functional Outcome (mRS)

	Discovery cohort			Replication cohort		
	Total	Favorable	Unfavorable	Total	Favorable	Unfavorable
n	176	121	55	269	193	76
Age, median [IQR], years	69 [57-80]	66 [53-74]	77 [68-84] †	66 [53-75]	64 [51-74]	73 [67-80] †
Male sex, n (%)	110 (63)	84 (69)	26 (47) *	175 (61)	130 (67)	34 (45) *
Hypertension, n (%)	68 (39)	39 (32)	29 (53) *	119 (41)	76 (39)	37 (49)
Diabetes mellitus, n (%)	18 (10)	8 (7)	10 (18) *	38 (13)	19 (10)	16 (21) *
Smoking, n (%)	21 (12)	17 (14)	4 (7)	28 (10)	14 (7)	10 (13)
Atrial fibrillation, n (%)	47 (27)	27 (22)	20 (36)	48 (17)	22 (11)	23 (30) †
Statin treatment, n (%)	26(15)	15 (12)	55 (20)*	70 (24)	40 (21)	26 (34)*
NIHSS score, median [IQR]	2 [1-5]	1 [0-3]	5 [2-8] †	2 [0-5]	1 [0-4]	5 [2-11] †
Recanalization therapy, n (%)	65 (37)	44 (36)	21 (38)	96 (33)	64 (33)	29 (38)

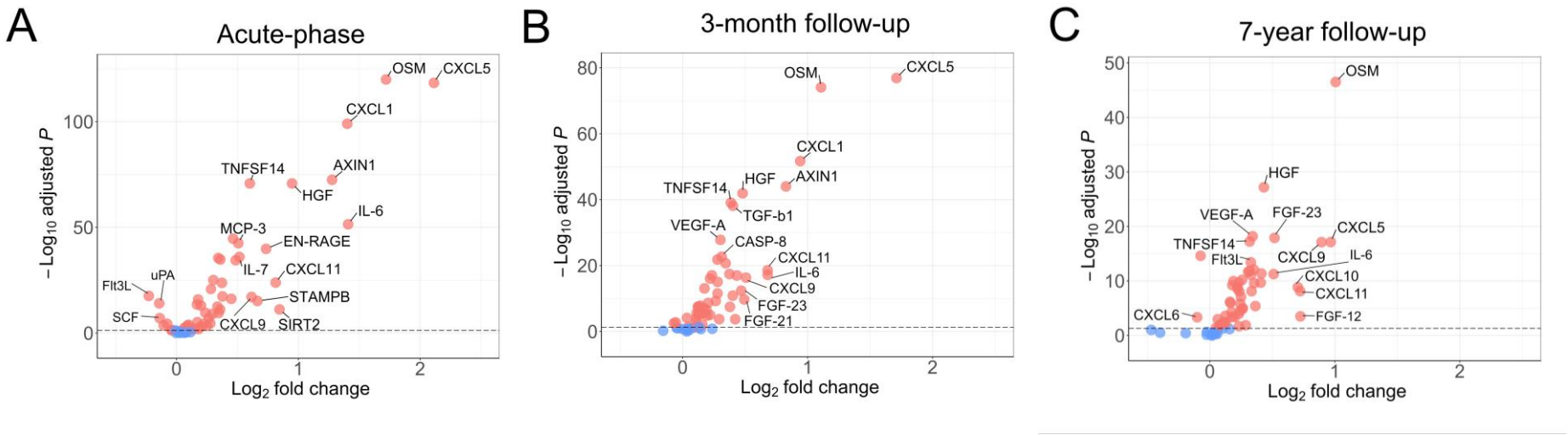
Olink Explore - Functional Outcome (mRS)

- Multiprotein modelling performed in the combined, bridging-normalized dataset (n=465)
  - Recursive feature elimination and LASSO logistic regression
  - 15 features selected (14 proteins + NIHSS)

Gene	Protein Name	384-plex panel	Direction (unfavorable)	Brain enriched
NEFL	Neurofilament light polypeptide	Neuro	↑	yes
PLB1	Phospholipase B1, membrane-associated	Neuro II	↓	-
LRRN1	Leucine-rich repeat neuronal protein 1	Inf	↓	yes
CRISP2	Cysteine-rich secretory protein 2	Onc	↓	-
ELN	Elastin	Cardio II	↑	-
PTN	Pleiotrophin	Cardio	↑	-
PALM	Paralemmin-1	Onc II	↑	yes
LEG1	Protein LEG1 homolog	Inf II	↓	-
CD302	CD302 antigen	Onc	↑	-
ITIH3	Inter-alpha-trypsin inhibitor heavy chain H3	Cardio	↑	-
LMNB2	Lamin-B2	Neuro II	↑	-
MRC1	Macrophage mannose receptor 1	Inf II	↑	-
RBFOX3	RNA binding protein fox-1 homolog 3	Neuro II	↑	yes
LGALS9	Galectin-9	Inf	↑	-

NIHSS

IV. Inflammation- Longitudinal



- N=51 proteins were differentially expressed in ischemic stroke vs controls in acute-phase
- N=34 proteins were upregulated in ischemic stroke at all 3 time points

- Subtype-differences, could reflect underlying pathophysiological processes

Stanne TM et al (2022) *Stroke*

IV. Olink Explore - Functional Outcome (mRS)

Discovery: N=176

Replication: N=269

Olink Explore 3072/384

Modular high-multiplex biomarker discovery with uncompromised data quality.

Proteins

2,900+

On 8 modular panels

Sample

1-6 µL

Plasma, serum & more

Olink Explore HT

Measure 5,400+ proteins with proven specificity to gain an understanding of disease at the protein level.

Proteins

5,400+

With proven specificity

Sample

2 µL

Plasma, serum & more

IV. Olink Explore - Functional Outcome (mRS)

- Discovery (Olink Explore 3072)
  - 2,236 proteins (call rate >80%)
- Many proteins highly correlated
- Effective multiplicity correction (M_{eff}) estimated at 125
- *P* <0.0004 (0.05/125)