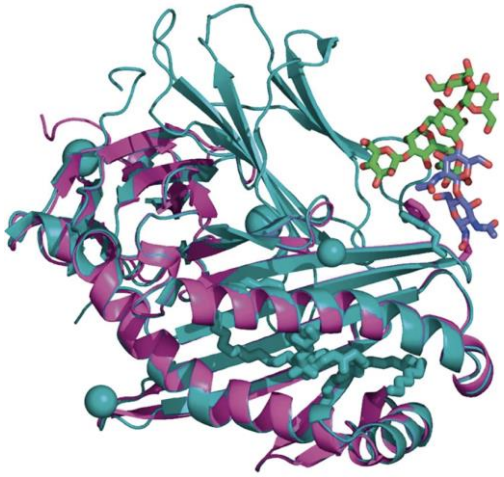
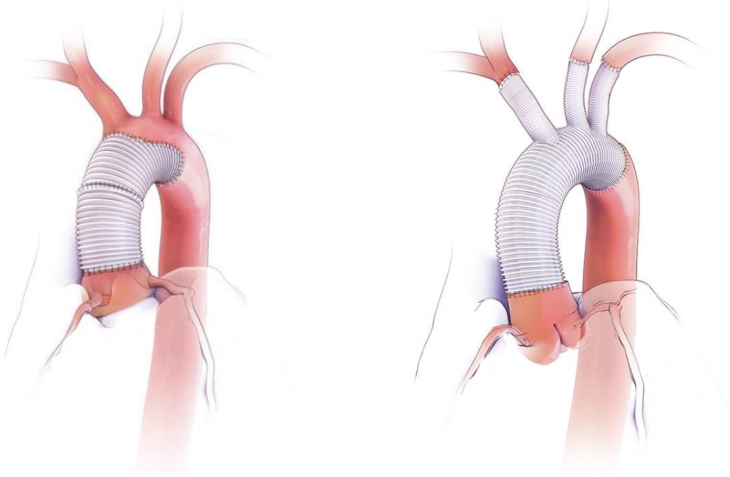
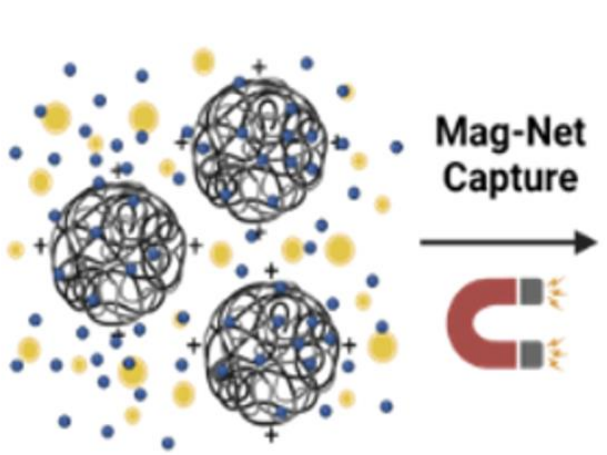




Protein signature discovery for ischemic stroke: A pilot study of aortic arch surgery

Christopher R. Burke, MD
Assistant Professor of Cardiac Surgery
Co-Director, Multidisciplinary Thoracic Aortic Program (MTAP)
University of Washington

Background

- Ischemic stroke is a major public health burden
- Delay in diagnosis can lead to irreversible neurological deficit
  - Rapid identification and initiation of therapy is critical
- Diagnosis is challenged by broad differential and lack of rapid, reliable lab test (“biomarker”) for ischemic stroke

Background

- Stroke biomarker studies in humans have been limited by lack of a “pre-stroke” control

- Aortic arch surgery with DHCA represents a unique population to study ischemic stroke
  - DHCA inherently leads to some degree of cerebral ischemia
  - 5-10% rate of clinical stroke[1,2]
  - 80-100% rate of DWI lesion on post-op MRI[3]

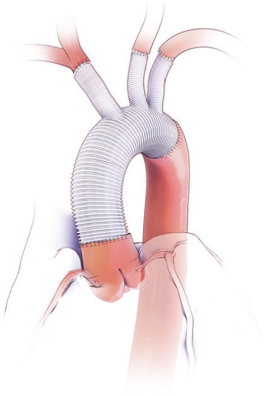
Hypothesis→ Can we use an aortic arch surgery model to perform ischemic stroke protein discovery?

1. Peterson MD, et al. A randomized trial comparing axillary versus innominate artery cannulation for aortic arch surgery. *J Thorac Cardiovasc Surg*. 2022;164:1426-1438 e1422. doi: 10.1016/j.jtcvs.2020.10.152.
2. Leshnower BG, et al. Deep Hypothermia With Retrograde Cerebral Perfusion Versus Moderate Hypothermia With Antegrade Cerebral Perfusion for Arch Surgery. *Ann Thorac Surg*. 2019;107:1104-1110. doi: 10.1016/j.athoracsur.2018.10.008.
3. Chen CH, et al. Acute Infarcts on Brain MRI Following Aortic Arch Repair With Circulatory Arrest: Insights From the ACE CardioLink-3 Randomized Trial. *Stroke*. 2022. doi: 10.1161/STROKEAHA.122.041612.

Methods

A total of 21 patients (DHCA n=17, CABG controls n=4) underwent study protocol.

Pre-op neuro exam

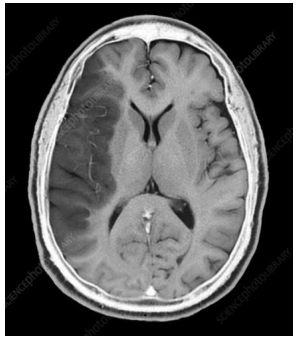


Surgery

Multiple blood samples obtained

- Pre-induction
- Pre-DHCA
- DHCA
- Post-DHCA
- POD 3

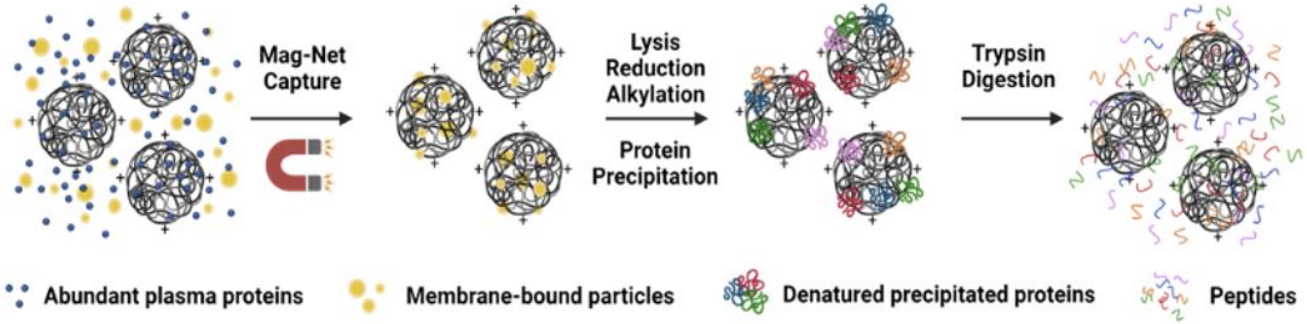
Post-op neuro exam

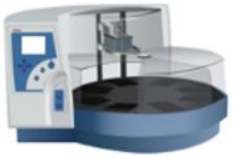


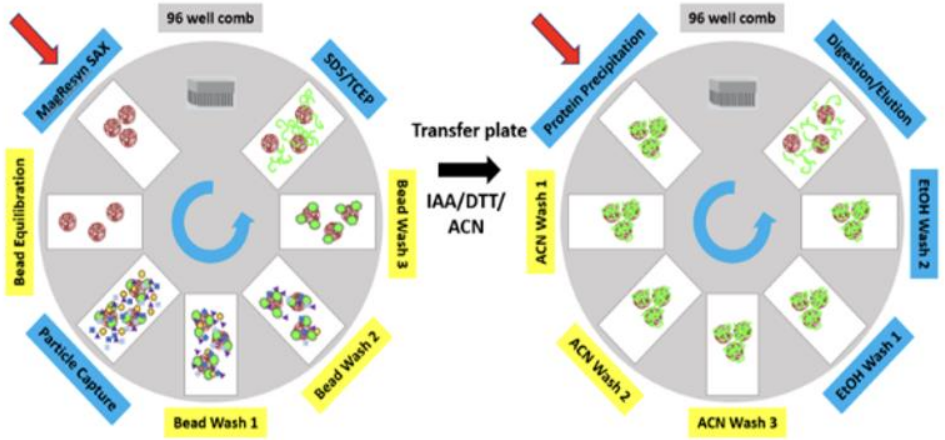
Post-op MRI

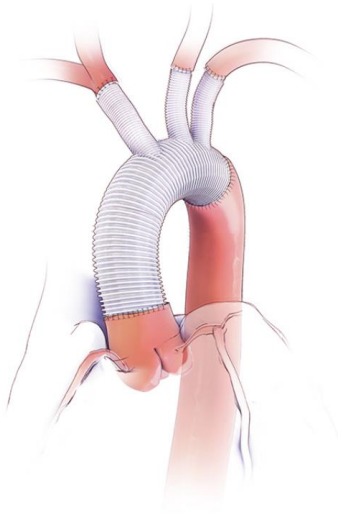
Protein Signature Discovery- Mag-Net

- Novel mass spectrometry (MS) technique utilizing extracellular vesicle (EV) enrichment to analyze membrane bound proteins
- Eliminates traditional MS limitations such as the “abundance problem” with plasma proteins such as albumin
- Plasma samples compared before and after DHCA



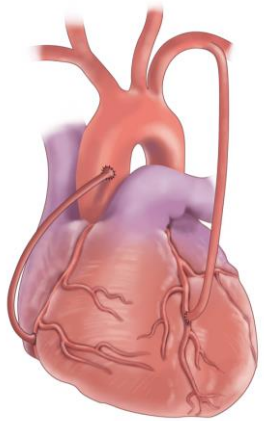






n=17 DHCA cohort

Distal Extent	
Hemiarch	**12**
Zone 1 arch	**1**
Zone 2 arch	**3**
Zone 3 arch	**1**
Proximal Extent	
Supracoronary anastomosis	**5**
Bentall	**4**
David V	**4**
Ross	**4**
CPB (min)	**195.5**
XC (min)	**147.5**
DHCA (min)	**22.5**



n=4 CABG controls

CPB (min)	90
XC (min)	71
DHCA (min)	0

Results

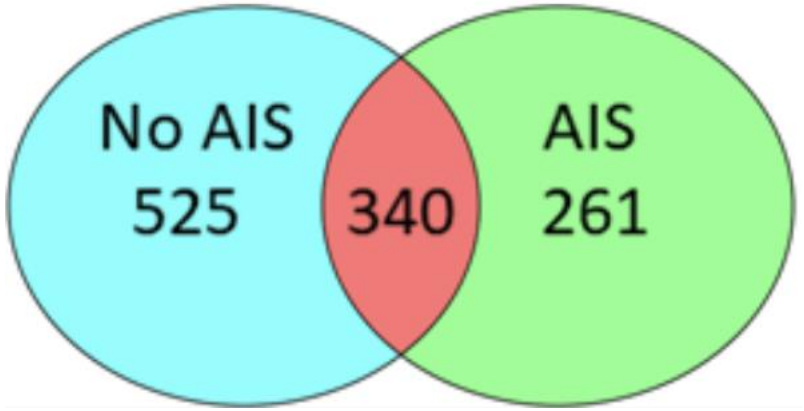
	DHCA (n=17)	CABG (n=4)	Total (n=21)	p-value
Age (median)	59	67	60	NS
Female (n,%)	3 (18)	0 (0)	3 (14)	NS
Race (n,%)				NS
White	14 (82)	3 (75)	17 (81)	
Black	1 (6)	0 (0)	1 (5)	
Asian	1 (6)	1 (25)	2 (9)	
Other	1 (6)	0 (0)	1 (5)	
BMI (median)	27	28	27	NS
Current smoker (n,%)	6 (35)	1 (25)	7 (33)	NS
Comorbidities (n,%)				
Atrial fibrillation	3 (18)	1 (25)	4 (19)	NS
Hypertension	10 (59)	4 (100)	14 (67)	NS
Diabetes	1 (6)	2 (50)	3 (14)	NS
History of CABG	1 (6)	0 (0)	1 (5)	NS
DWI lesion on MRI (n,%)	14 (82)	2 (50)	16 (76)	NS
Infarct volume (mm^3) (mean)	56	3	46	0.03*
Clinical stroke/TIA (n, %)	2 (12)	0 (0)	2 (10)	NS

DHCA = Deep hypothermic circulatory arrest, CABG = coronary artery bypass graft, NS = not significant, BMI = body mass index, DWI = diffusion weighted imaging, TIA = transient ischemic attack. P-value based on Fisher exact test for categorical variables and student's t-test for continuous variables. A significance threshold of $p<0.05$ was used.

Results

- A total of 5,376 proteins were identified, 1,125 of which showed a significant difference between paired pre- and post-operative concentrations.

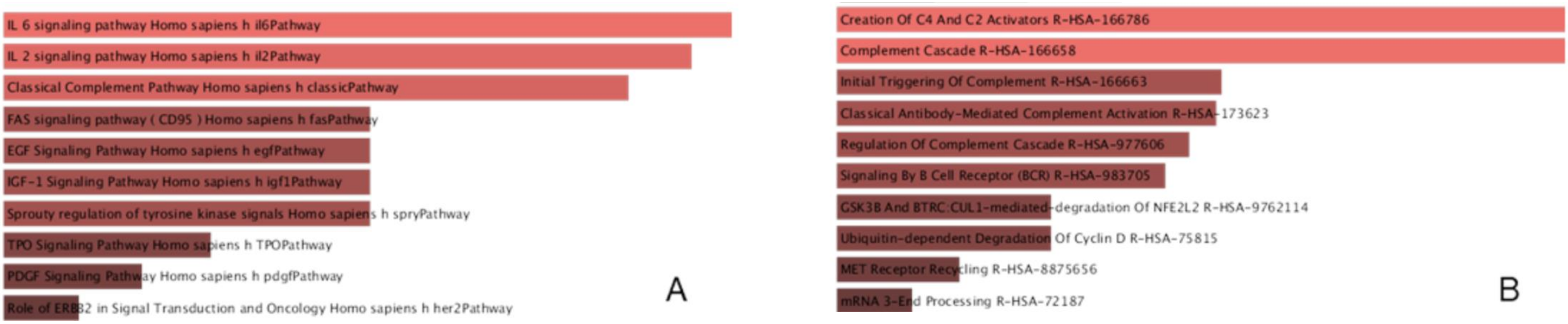
- 261 proteins had greater expression in the infarct group compared to the non-infarct group



The proteins unique to each group are those that have an false discovery rate (FDR) < 0.05 in that group and an FDR > 0.05 in the other. AIS = acute ischemic stroke.

Results

Analysis of enrichment pathways of these **261 unique proteins** revealed many known stroke pathways (interleukins, FAS, complement pathway, EGF, IGF-1, etc.), providing validation of this methodological approach.



Metabolic, signaling, and other pathway analysis for 261 proteins associated with AIS using Biocarta (A) and Reactome (B).

Conclusions

- Over 80% of patients undergoing aortic arch surgery with DHCA have DWI lesions on post-operative MRI.
- We have developed a research protocol for proteomic analysis of DHCA patients using a novel MS technique to assess EV proteins.
- Ischemic cerebral infarct elicits a unique proteomic expression as compared to non-infarct plasma.

Future Directions

- Expand current pilot study to significantly increase power to identify candidate biomarkers for ischemic stroke.
- Assay development to measure candidate biomarkers.
- This has tremendous potential to increase our understanding of stroke and safety of aortic arch surgery.