Integrated Analysis of Novel MRI and Blood Serum Biomarkers for the Estimation and Prediction of MS Disease Activity and Progression

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actrims 2021 FORUM VIRTUAL February 25-27

actrims AMERICAS COMMITTEE FOR TREATMENT AND RESEARCH IN MULTIPLE SCLEROSIS

Goal, Impact, and Significance

- High-frequency measurement of the state of a patient's MS would allow for more nimble clinical management of their disease.
- MRI scans are an important source of quantitative information in the diagnosis and management of MS.
- Blood draws are simpler, faster, and cheaper than MRIs.

Our goal is to extract some of the information available through MRI using a single blood draw and extended with a series of them.

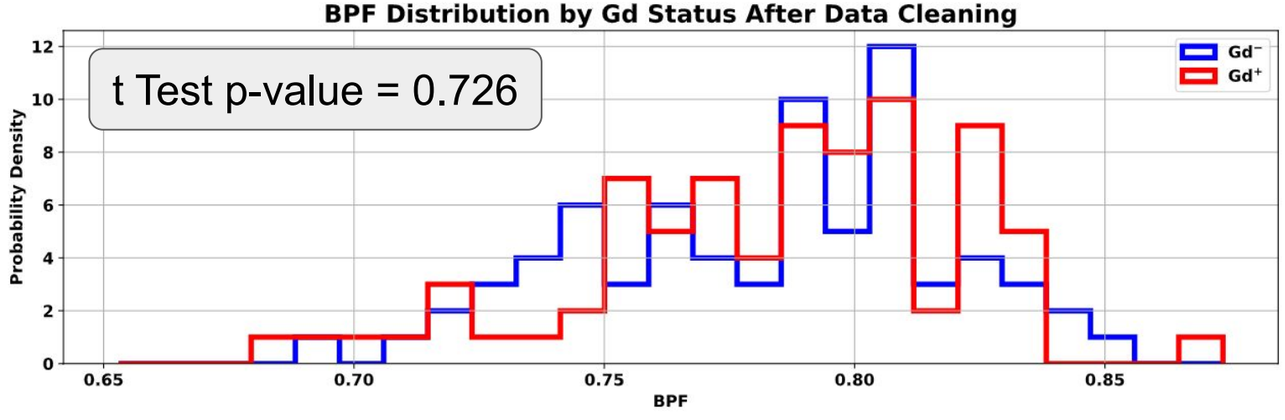
- Early detection of MS disease activity and progression facilitates responsive DMT adjustment and other clinical interventions for MS.
- More responsive management allows enhanced quality of life and longer healthspan.
- **This study will estimate Brain Parenchymal Fraction (BPF) using serum biomarkers because BPF is known to correlate with MS patients' disease progression.**

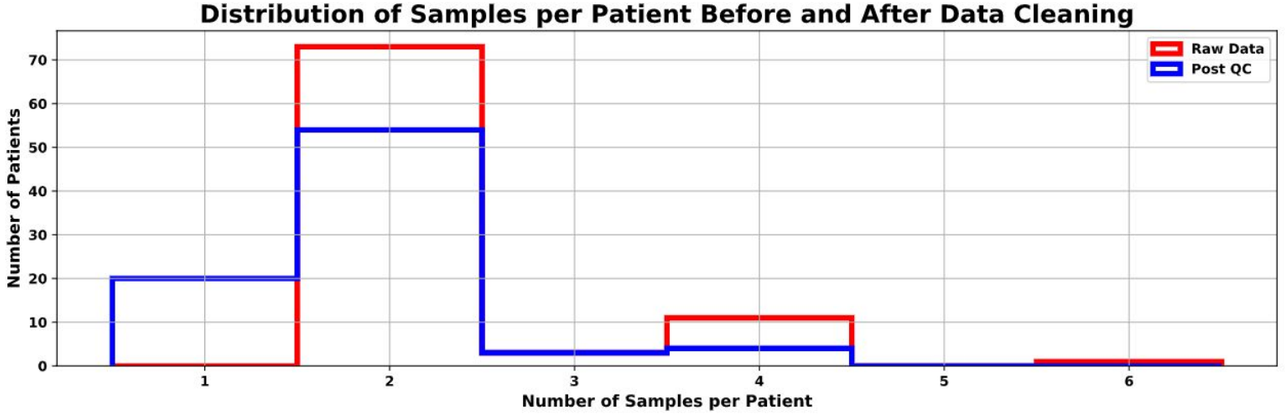
Disclosures: V.M. Gehman, M. Becich, A. Keshavan, K. Leyden, F. Qureshi, and F. Rubio da Costa are employees of Octave Bioscience. **J.Kuhle** received speaker fees, research support, travel support, and/or served on advisory boards by ECTRIMS, Progressive MS Alliance, Swiss MS Society, Swiss National Research Foundation (320030_189140/1), University of Basel, Bayer, Biogen, Celgene, Merck, Novartis, Roche, Sanofi. **Ö. Yaldizli** received grants from ECTRIMS/MAGNIMS, University of Basel, Pro Patient Stiftung, University Hospital Basel, Free Academy Basel, Swiss Multiple Sclerosis Society and advisory board fees from Sanofi Genzyme, Biogen, Almiralland Novartis. **D. Leppert** is CMO of GeNeuro; he has received personal compensation for consulting and speaking, and travel reimbursement from Quanterix, Roche, Novartis, Orion,GeNeuroand Sanofi. **C. Granziera**: The University Hospital Basel (USB), as the employer of Cristina Granziera has received the following fees which were used exclusively for research support: (i) advisory board and consultancy fees from Actelion, Novartis, Genzyme-Sanofi and F. Hoffmann-La Roche; (ii) speaker fees from Biogen, Genzyme-Sanofi and F. Hoffmann-La Roche; (iii) research support by F. Hoffmann-La Roche Ltd. Before my employment at USB, I have also received speaker honoraria and travel funding by Novartis.

Methods: Cohort

- 205 samples from 88 patients (67 female, 21 male) at University Hospital Basel.
- 153 samples from 81 patients (63 female, 18 male) after data cleaning/quality control on images and assay results.
- Samples banked between July 2012 to August 2019 with paired MRI scans.
- Patients have between 2-6 longitudinal samples (all patients have at least one sample with Gd enh. lesions and one without).
- Cohort selected to be balanced across age, sex, disease duration, EDSS for a study whose primary endpoint was Gd lesion count.

	Raw Data	Post-QC
Age [y]	40.8 ± 11.6	40.1 ± 11.1
Dis. Dur. [y]	11.9 ± 10.5	11.1 ± 9.7
% Female	77%	78%
EDSS	2.4 ± 1.6	2.29 ± 1.6
Blood draw within 30 days of MRI	75.6%	75.8%
Sample Count	205	153
Patient Count	88	81
Gd^+ Samples	106	78
Time From 1st to Last Patient Samples [y]	1.25 ± 0.93	1.16 ± 0.89





Methods: Serum Biomarkers

- We have developed a panel of 21 proteins that provides insight into the state of MS disease activity and progression.
- Also spans a number of physiological pathways affected by MS: neurodegeneration, inflammation, myelin integrity, immune modulation, and cerebrovascular function.
- Custom assay panel built on the Olink™ platform (proximity extension assay method) allowing rapid, accurate measurement of absolute protein concentrations in blood serum.
- Serum protein concentrations have demonstrated accurate, reliable disease activity detection by Gd lesions in MRI (AUROC > 0.9, Kuhle et al. P0055 MS Virtual 2020).
- Analytical validation study complete (P010 ACTRIMS 2021).
- Clinical validation study in progress (P014 ACTRIMS 2021).
- Disease progression and individual pathway scores are under development.

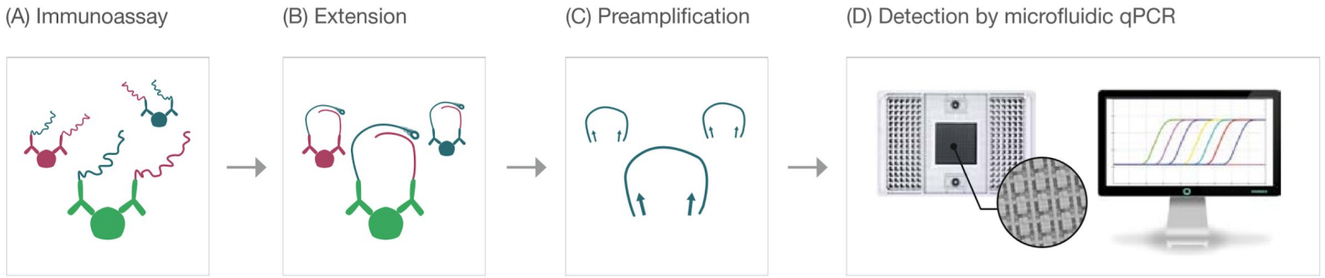
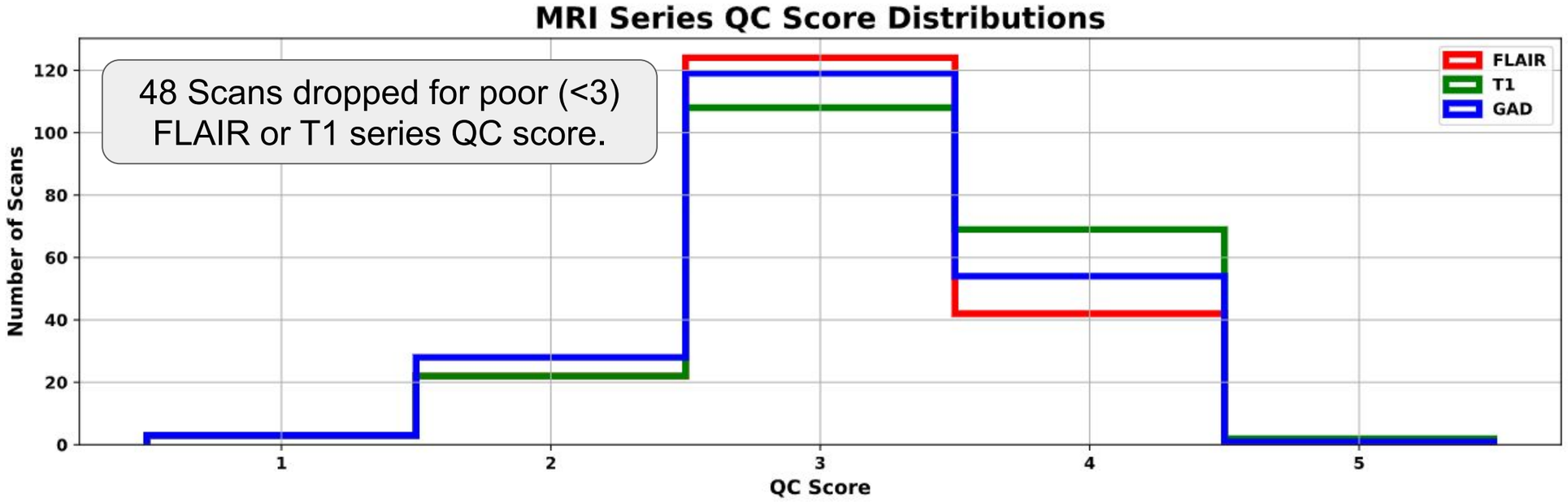


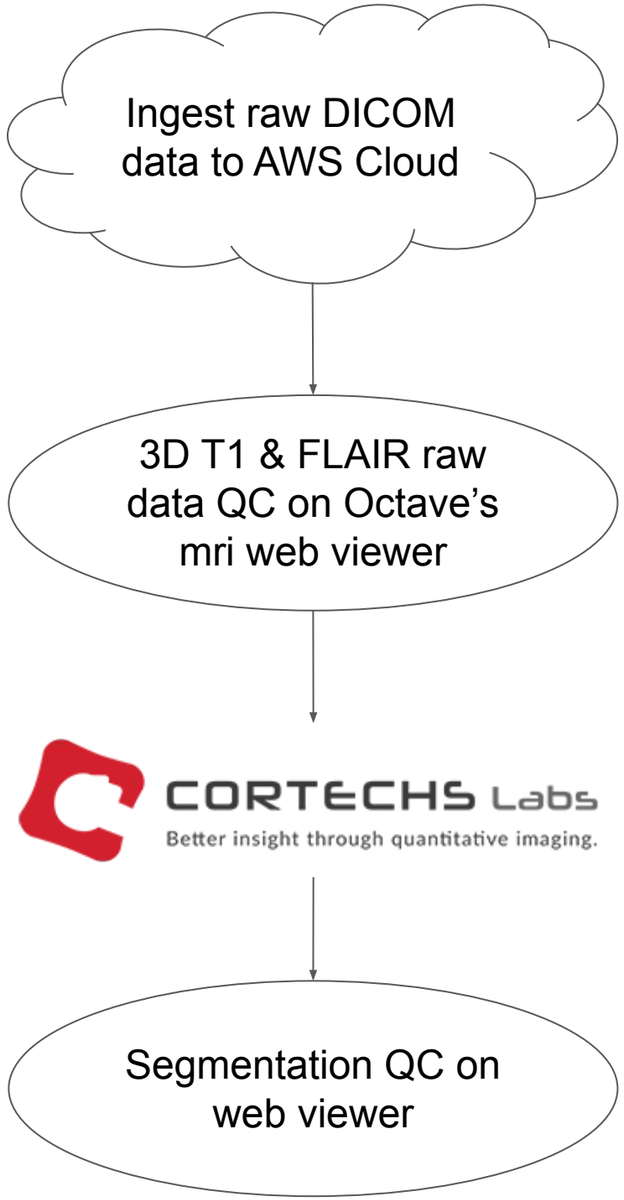
Fig 1. Overview of the PEA technology. (A) 92 Antibody pairs, labelled with DNA oligonucleotides, bind target antigen in solution. (B) Oligonucleotides that are brought into proximity hybridize, and are extended by a DNA polymerase. (C) This newly created piece of DNA barcode is amplified by PCR. (D) The amount of each DNA barcode is quantified by microfluidic qPCR.

Biomarkers Measured in the Custom Assay Panel	
ANALYTE	**PROTEIN NAME AND ALIAS**
APLP1	Amyloid Beta Precursor Like Protein 1
CCL20	MIP-3 alpha
CD6	Cluster of Differentiation 6
CDCP1	CUB domain-containing protein 1
CNTN2	Contactin 2
COL4A1	Collagen alpha-1(IV) chain
CXCL13	C-X-C Motif Chemokine Ligand 13, BLC
CXCL9	MIG, Monokine Induced by Gamma Interferon
FLRT2	Leucine-rich repeat transmembrane protein
GFAP	Glial Fibrillary Acidic Protein
GH	Growth Hormone, Somatotropin
IL-12B	Interleukin 12B
MOG	Myelin-Oligodendrocyte Glycoprotein
NEFL	NFL, Neurofilament Light
OPG	Osteoprotegerin, TNFRSF11B
OPN	Osteopontin
PRTG	Protogenin
SERPINA9	Serpin Family A Member 9
TNFRSF10A	TRAILR1, DR5 - Death Receptor 5
TNFSF13B	BAFF, B-cell activating factor
VCAN	Versican, Versican Proteoglycan

Methods: Imaging Biomarker Measurements

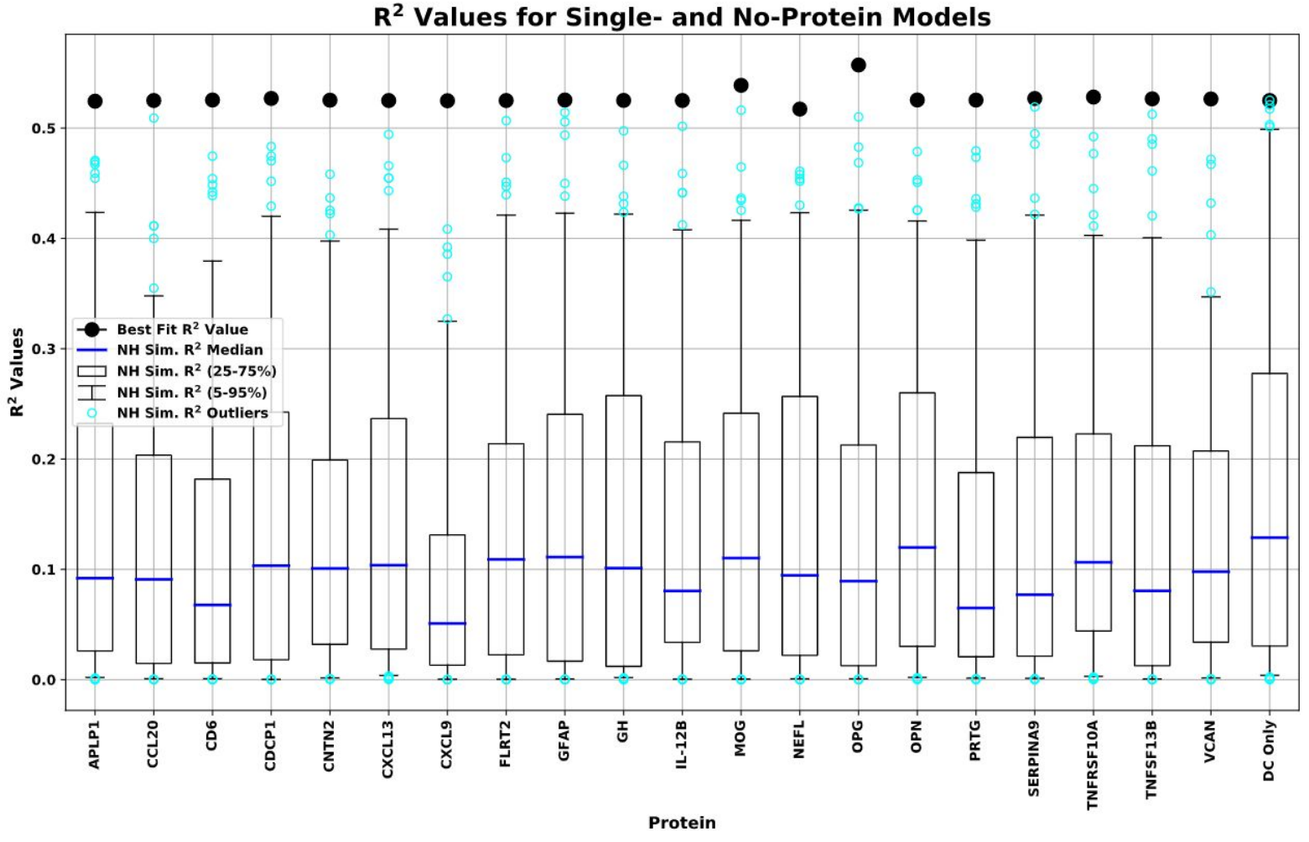
- 3D T1 and FLAIR images were uploaded in Octave's cloud environment.
- QC of raw data by two experienced raters.
- Data rated for quality on 1-5 scale (1=poor, 3=average, 5=excellent).
- Image segmentation of T1 and FLAIR through Cortechs' FDA-approved LesionQuant software.
- Second QC of segmentation by raters.





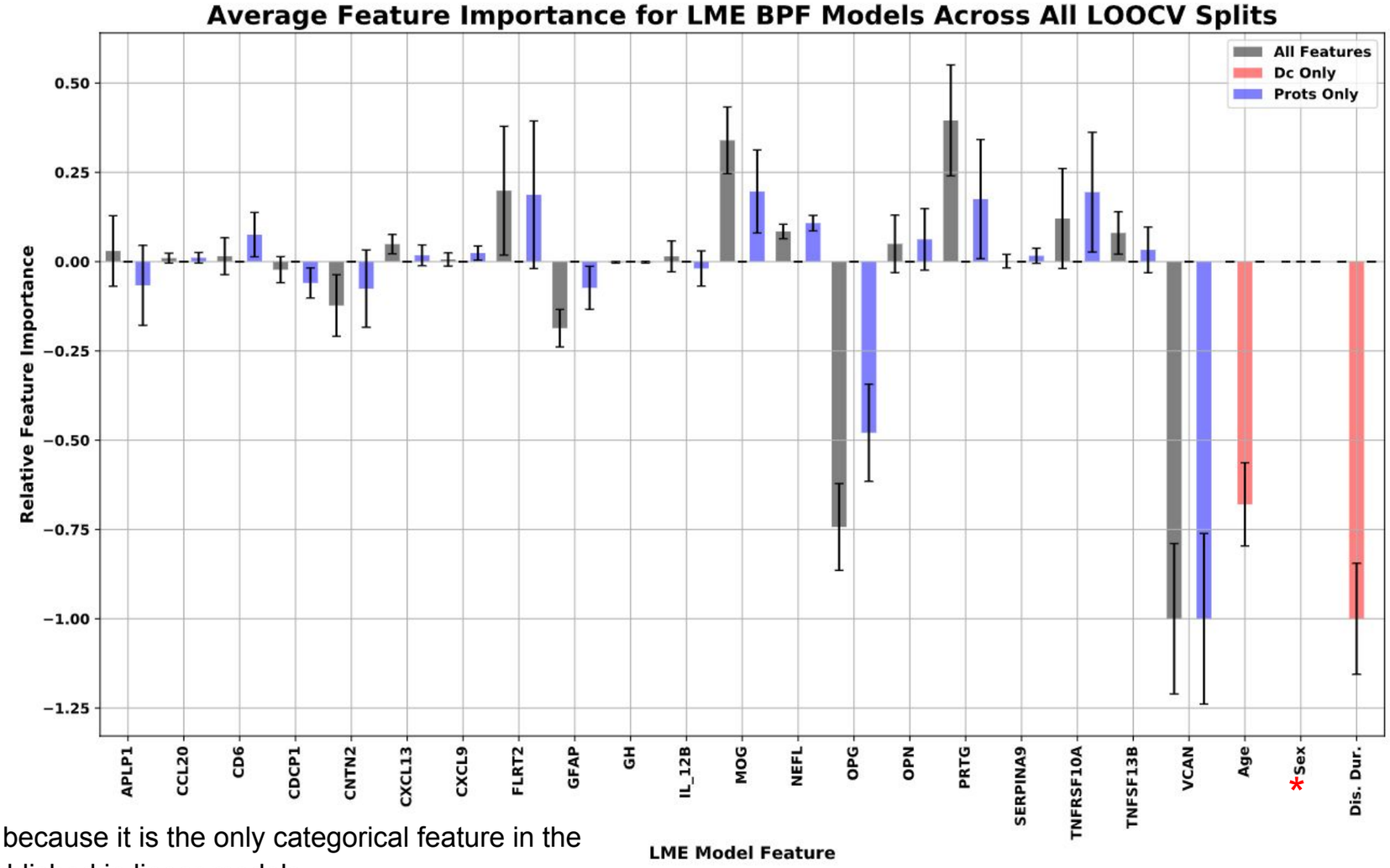
Single-Protein Analysis

- Cohort is a mix of longitudinal and cross sectional samples.
- Used a linear mixed effects (LME) model to account for <u>both</u> the variation within and across patients.
- Extracted the R^2 for predictions of BPF for models using one protein plus demographic/ clinical data, and one using only DC data).
- Also built 100 “Null Hypothesis Simulation” (NH Sim.) models, where BPF values had been randomized.
- All models significantly outperform the null hypothesis, but no model with a protein significantly outperforms The demographic only (“DC Only”) model.
- For this data, BPF is more strongly determined by the demographic information than by any single protein.



Multi-Protein Analysis: Regression

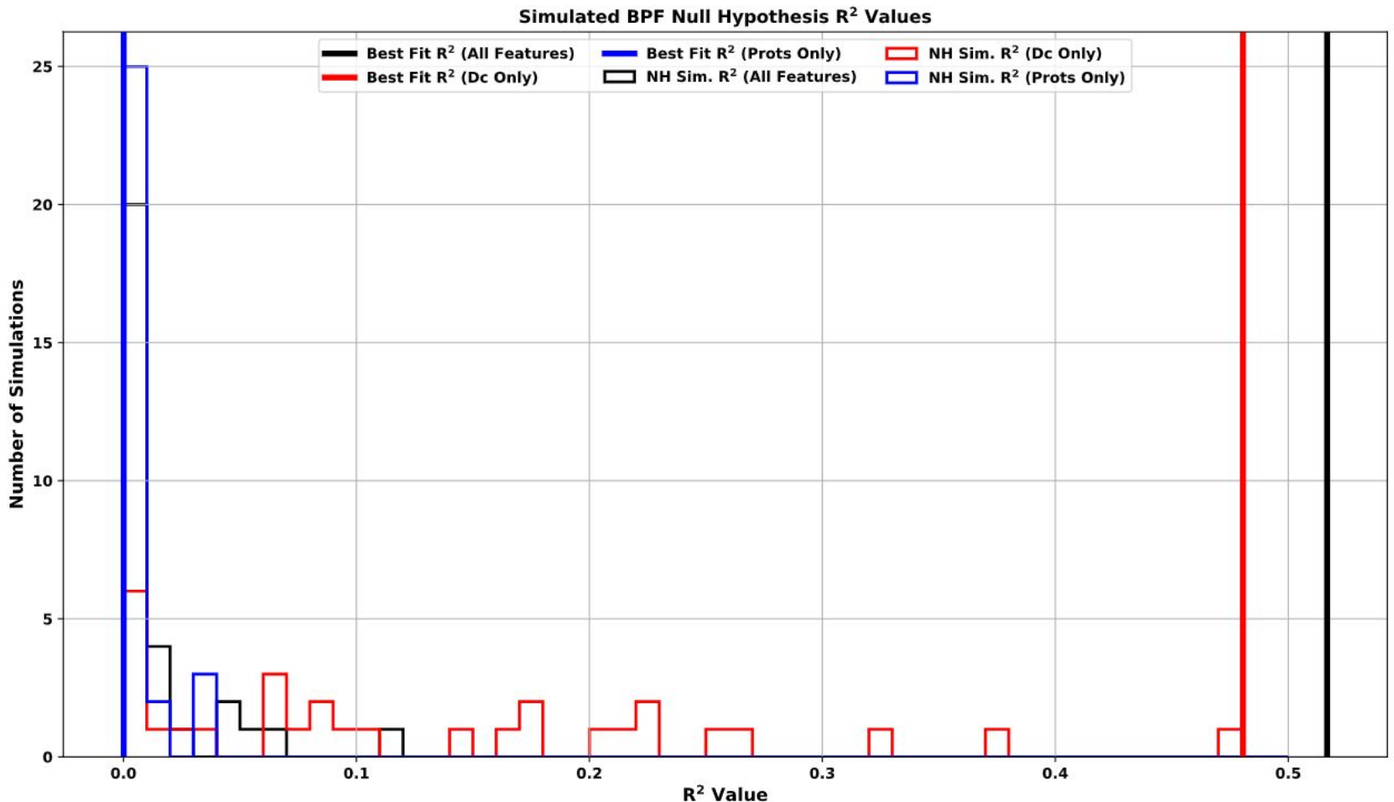
- Constructed an ensemble of LME models that predict BPF using ALL proteins, age, disease duration, and sex using Leave One Out Cross Validation (LOOCV) to look out for overfitting.
- Constructed same ensemble of models using only demographic and only protein features.
- Extracted the importance of each feature (variance weighted coefficient) from each model across LOOCV splits.
- Used these three models to predict BPF...

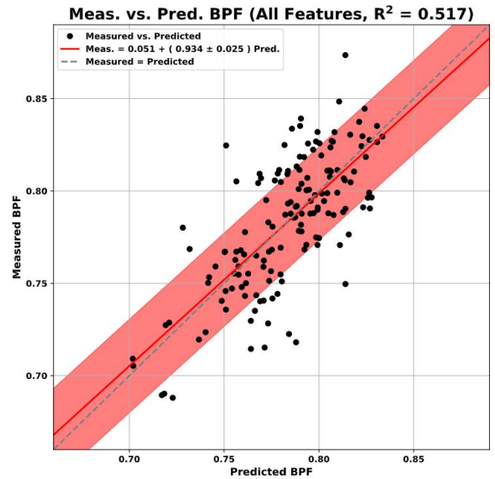


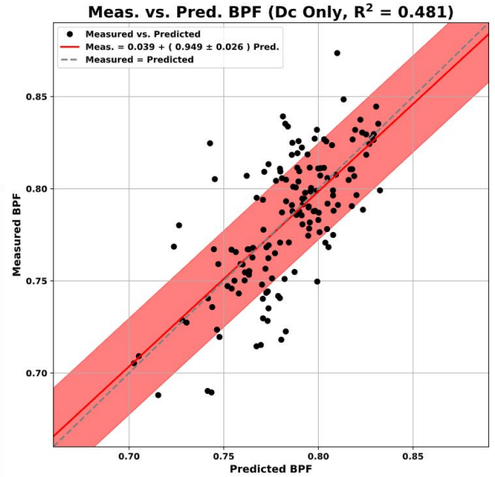
* Sex feature importance has been removed from this figure because it is the only categorical feature in the model. Overrepresentation of categorical features is well established in linear models.

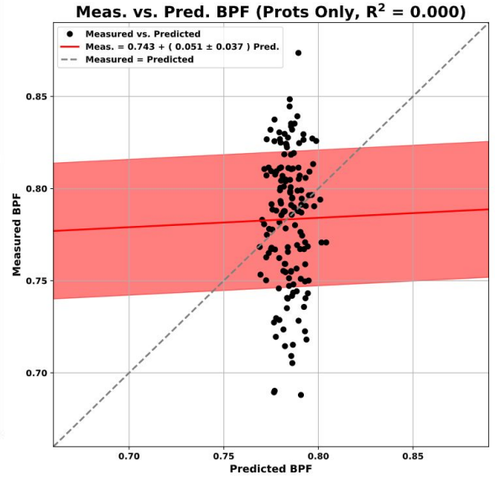
Multi-Protein Analysis: Regression

- The demographic features still strongly determine modeling power.
- Slight R^2 improvement from adding protein concentrations.
- Protein-only model has almost no predictive power.
- Null hypothesis simulations are well separated from both models with DC features as covariates, but not for the protein only-model.
- BPF is still very strongly determined by demographic features compared to serum protein concentrations.







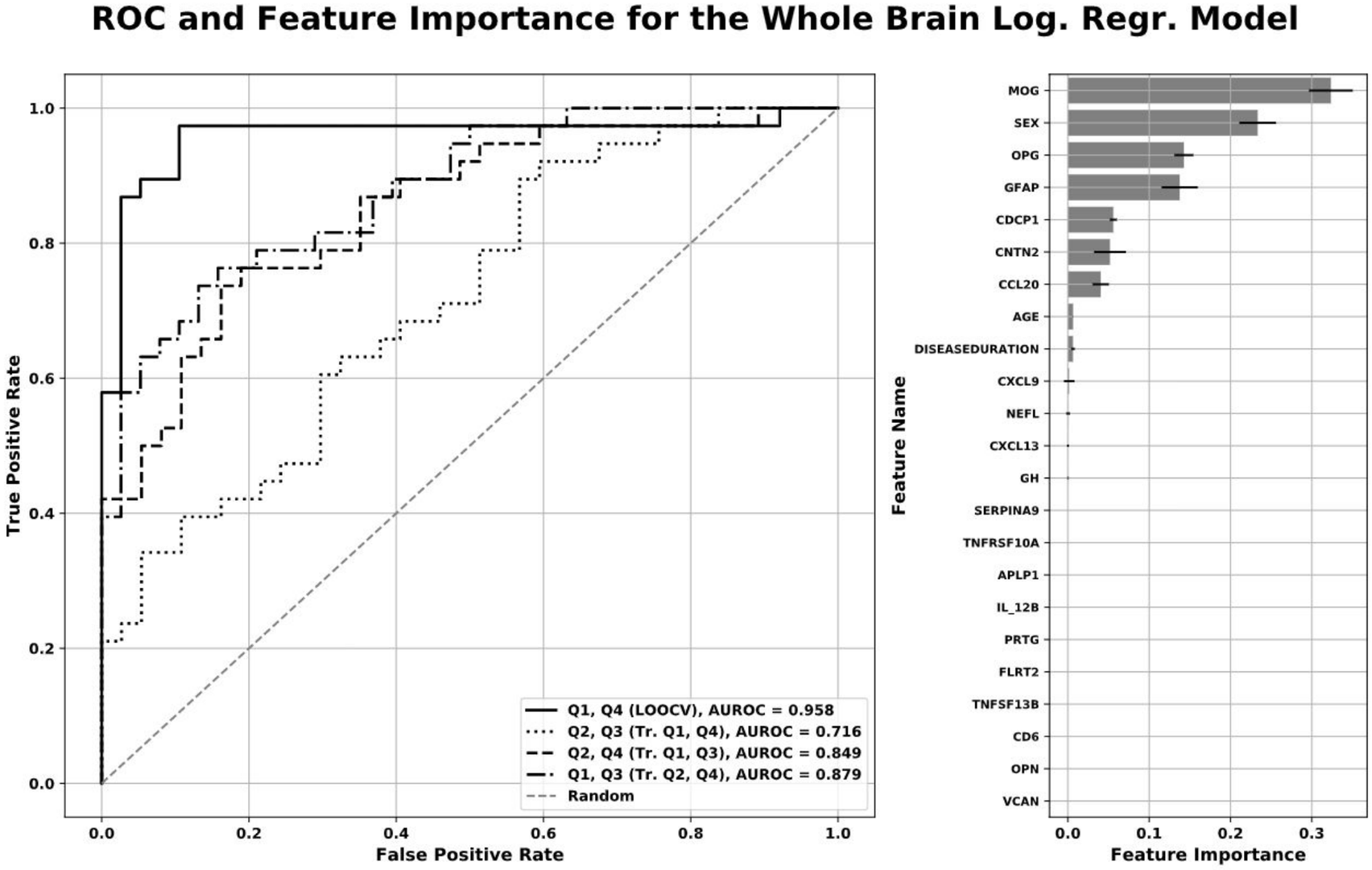


Multivariate Analysis: Classification

Can also frame this as a classification problem by separating samples into BPF quartiles, then using proteins (and DC features) to predict scan quartile.

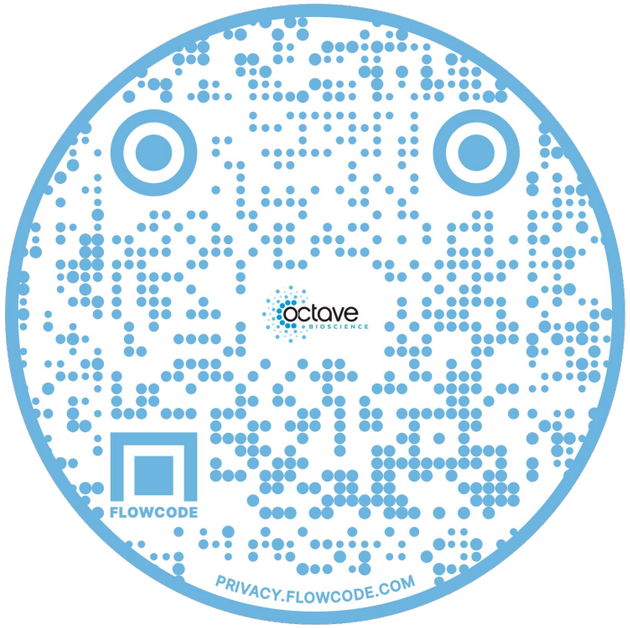
Looked at four ways to split the data:

- Separate Q1 from Q4 and LOOCV to check overfitting.
- Train on Q1 vs. Q4, separate Q2 from Q3.
- Train on Q1 vs. Q3, separate Q2 from Q4.
- Train on Q2 vs. Q4, separate Q1 from Q3.



Discussion and Conclusions

- Predicting imaging biomarkers using serum protein concentration remains a subject of active research.
  - BPF is strongly determined by demographic features on the *single-year* time scales in this data set.
  - There is a slight improvement in predictive power from adding serum protein concentrations to demographic features.
- While this dataset was constructed for the investigation of acute disease activity, we see hints of a signal for this first MS disease progression endpoint.
- Disease progression metrics are a major research focus, and will require an independent data set curated for them, principally requiring more samples per patient collected *over a longer time period* and more endpoints that correlate with progression.



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