Serum N-glycan Biomarkers Predict Patient Response to Biologics for Crohn's Disease

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Introduction

Crohn's Disease and Treatment

Several therapeutic antibodies are approved to treat Crohn's Disease (CD), for example, Vedolizumab (anti-$\alpha_4\beta_7$), Ustekinumab (targets IL-12 and IL-23), Adalimumab (humanized anti-TNF) and Infliximab (chimeric anti-TNF). These therapies area slow-acting, often requiring 6 months before its effects are detectable. A reliable predictor of a patient's response would not only educate the type of treatment a patient should receive, but would also prevent a, consequently, less effective secondary treatment.

Crohn's Disease and Glycans

Glycosylation is a post-translational modification of proteins that impacts their biological activity. Blood serum contains many types of glycoproteins related to immune and inflammatory activities. Changes in glycosylation have been observed in patients suffering from CD, for example, glycan biomarkers can distinguish CD patients from healthy controls. Total serum N-glycan (TSNG) analysis has the potential to predict patient response to biologics. In addition, comparing patients' glycan profiles after treatment may provide insight into the cellular pathways involved in remission, assessed by HBI score.

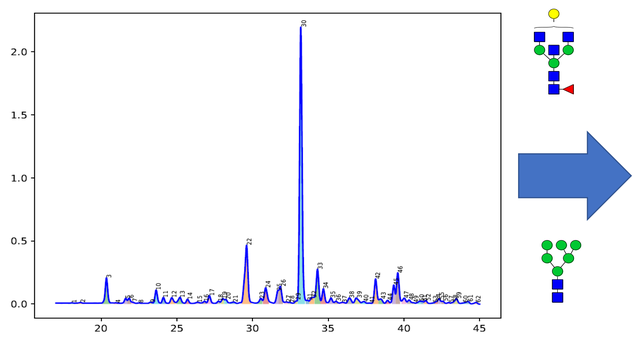
Workflow

Sample Processing

Enzymatic release (PNGase F) of serum glycans

Fluorescently label glycans (Procainamide)

Hydrophilic interaction liquid chromatography with fluorescence detection



Data Processing

HappyTools quantifies **direct traits** (individual glycan types)

Batch effect tested with PCA and corrected with

Biologics	Responder	Non-Responder
Infliximab	25	8
Adalimumab	18	11
Ustekinumab	23	23
Vedolizumab	34	24

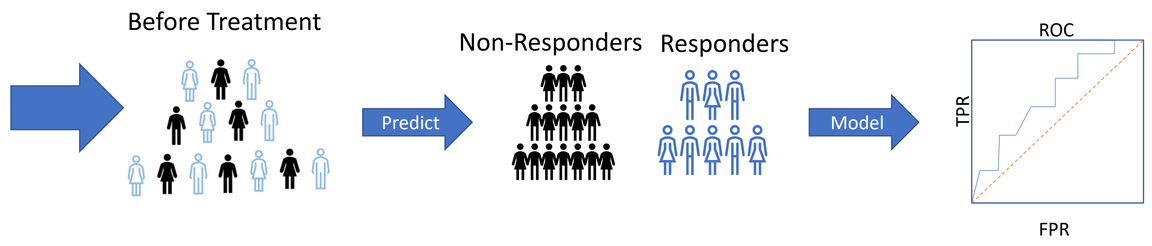
Statistical Analysis

Traits before and after treatment:

- Univariate analysis
- Logistic regression for response correcting for age and sex with 5-fold cross-validation

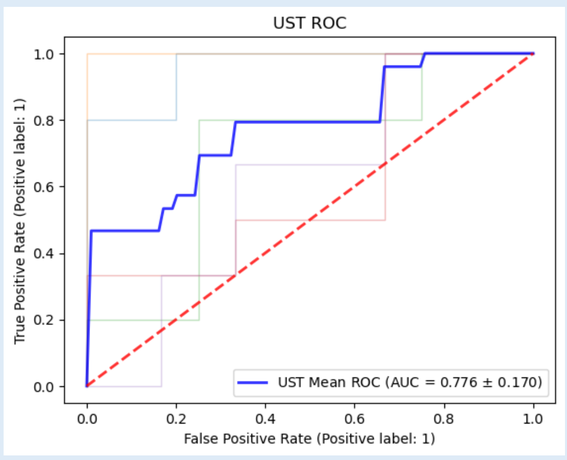
Response across biologics

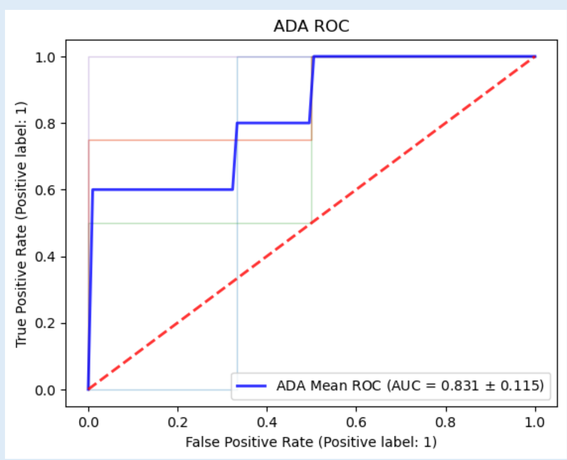
- Isolating responders before treatment and comparing against biologics with Kruskal test

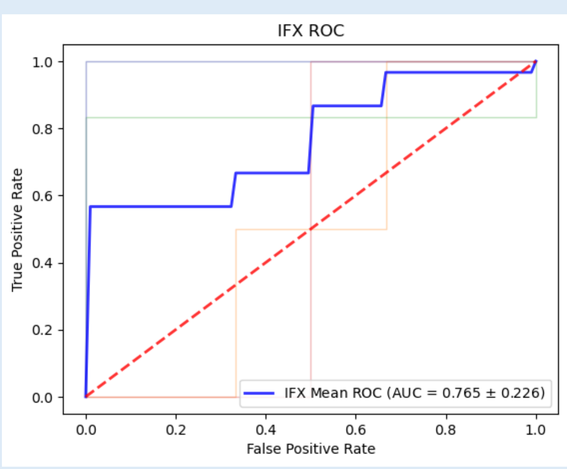


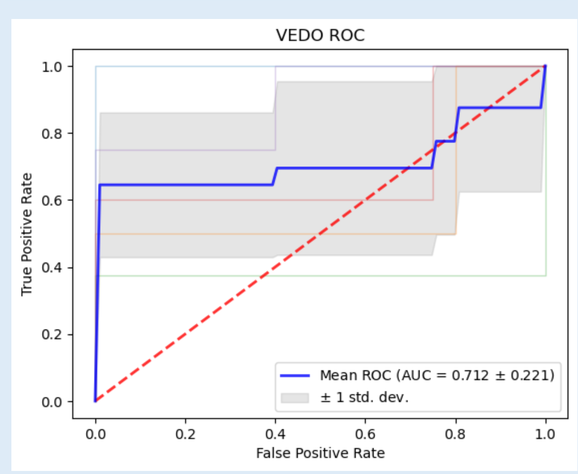
Results

1. Predicting Response Prior to Treatment









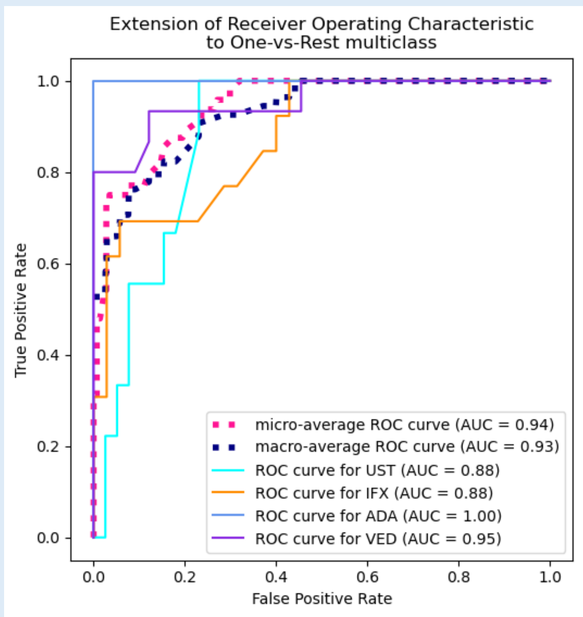
The classification models predicting response using N-glycans for all four sub-cohorts under different biologics, **Ustekinumab** (Gaussian NB, FA1,A1G1S1,Man6,Man7,A2BG2S1,A2G2S1, 0.78 AUC), **Adalimumab** (Logistic Regression,FA1, 0.82 AUC), **Infliximab** (Gaussian Process, FA2G2,0.75 AUC), **Vedolizumab** (LDA, A3G3S2,A2G2S2, 0.71 AUC), performed moderately well at predicting therapy response prior to treatment.

2. Markers of Response After Treatment

After treatment, traits which significantly differed across response, indicate the varied changes in glycan composition experienced by responders compared to non-responders. Considering these changes in each biologic:

- **Ustekinumab**: Logistic Regression, FA3G3S2,0.70 AUC
- **Adalimumab**: Logistic Regression, Man7, A4G4S2, A2G1, A1G1S1, FA3G3S2, FA2B, A2G2,Man5, A2G1S1, Man5BA1G1S1,Man6,0.86 AUC
- **Infliximab**: Random Forest Classifier, Peaks FA2G2S1, F2A3G3S3, A4G4S3, FA4G4S4, F2A4G4S4,0.85 AUC
- **Vedolizumab**: Gaussian NB, A3G3S3, FA2G2, A3G3S2, FA2G2S2, A2BG2S2, FA4G4S4, A2G1S1, FA3G3S2, A2G2S2, FA3G3S3, A3G3S2, 0.84 AUC

3. Comparing Response Across Biologics



Prior to treatment, patients who responded were strongly distinguished by the therapy type (0.96 AUC) through a combination of 13 significant glycan peaks with a Random Forest Classifier.

Conclusion & Future Work

- N-glycans proved to be good predictors of response prior to treatment
- N-glycan composition alters after response differently across biologics
- Biologics are strongly distinguished across responders (0.96 AUC) prior to treatment
- We intend to replicate analysis with additional treatments and refine the instrumentation used to acquire data

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