Serum *N*-glycans analysis by LC-MS allows the prediction of patients' response to Vedolizumab treatment for Crohn's Disease

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Introduction

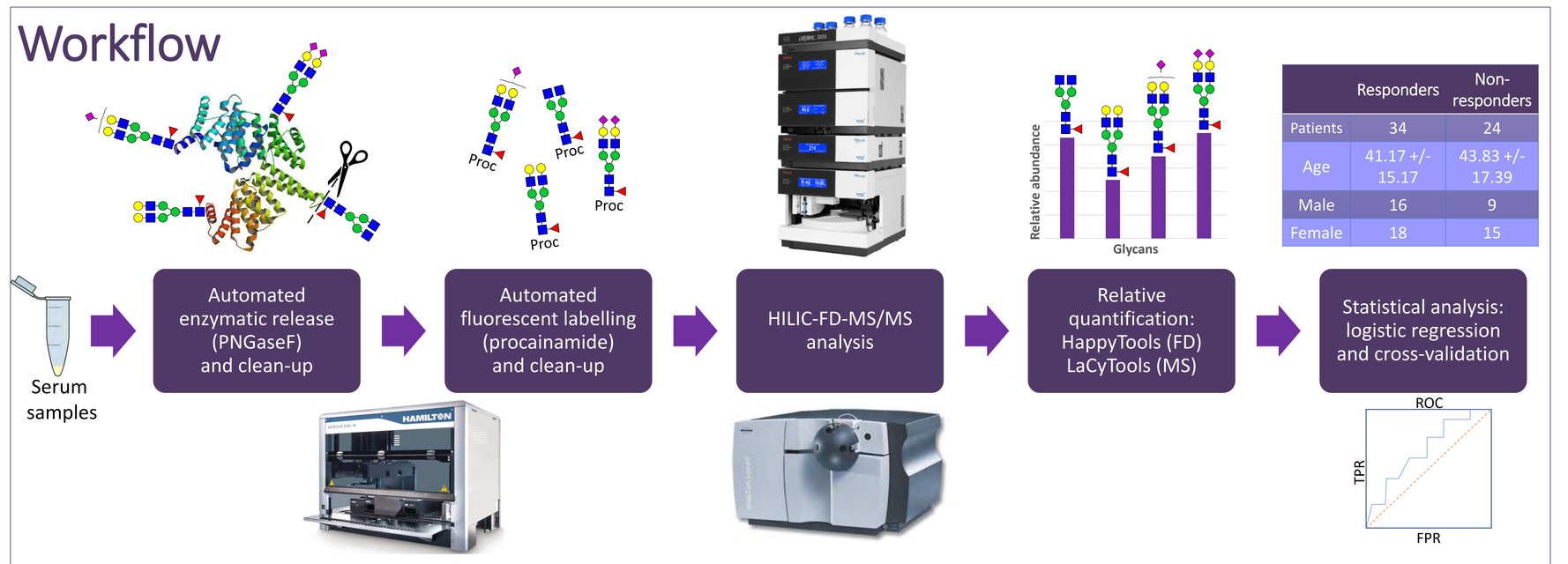
Crohn's disease (CD): common type of inflammatory bowel disease (IBD) – prompt need for patient access to correct treatment.

Vedolizumab (VDZ): monoclonal antibody directed against α4β7 integrin used to treat CD – efficacious in phase 3 clinical trials[1] and in real-world studies[2] - need for reliable biomarkers to predict treatment response.

Association of glycosylation of serum protein and CD – never been studied as potential predictors of remission to biological therapy[3].

Comparison of serum N-glycan profiles between responders and non-responders to Vedolizumab (VDZ) to detect potential biomarkers for treatment response.

Workflow



	Responders	Non-responders
Patients	34	24
Age	41.17 +/- 15.17	43.83 +/- 17.39
Male	16	9
Female	18	15

Figure 1: N-glycans analysis workflow. Serum proteins are digested with PNGaseF, their released glycans are then fluorescently labelled with procainamide. Labelled glycans are subsequently analyzed by HILIC-FD-MS. After quantification of peaks (FD) or of individual glycans (MS), direct and derived traits were analyzed statistically using logistic regression and cross-validation with correction for age and sex.

Results

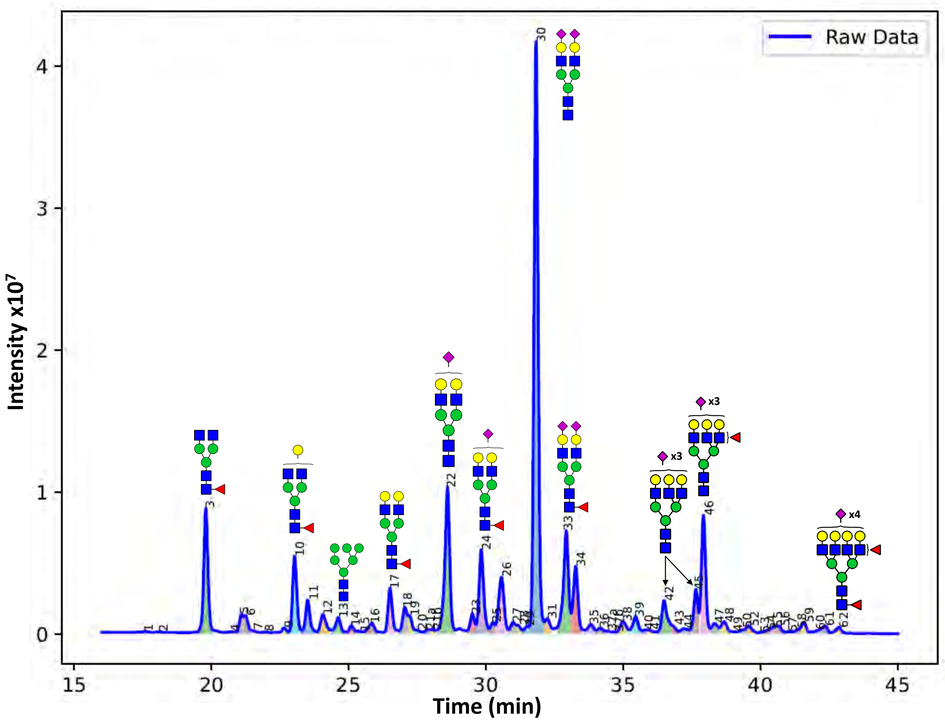


Figure 2: HILIC-FD analysis of a serum sample from a CD patient. Released glycans were separated based on their structures. After treating the fluorescent trace with our in-house software, HappyTools, 62 peaks were detected and quantified. Major glycans of some of the main peaks are presented here, neutral species are eluting first while highly sialylated species elute last. Conditions: BEH amide column; mobile phases, A: 50mM ammonium formate, B: 100% acetonitrile.

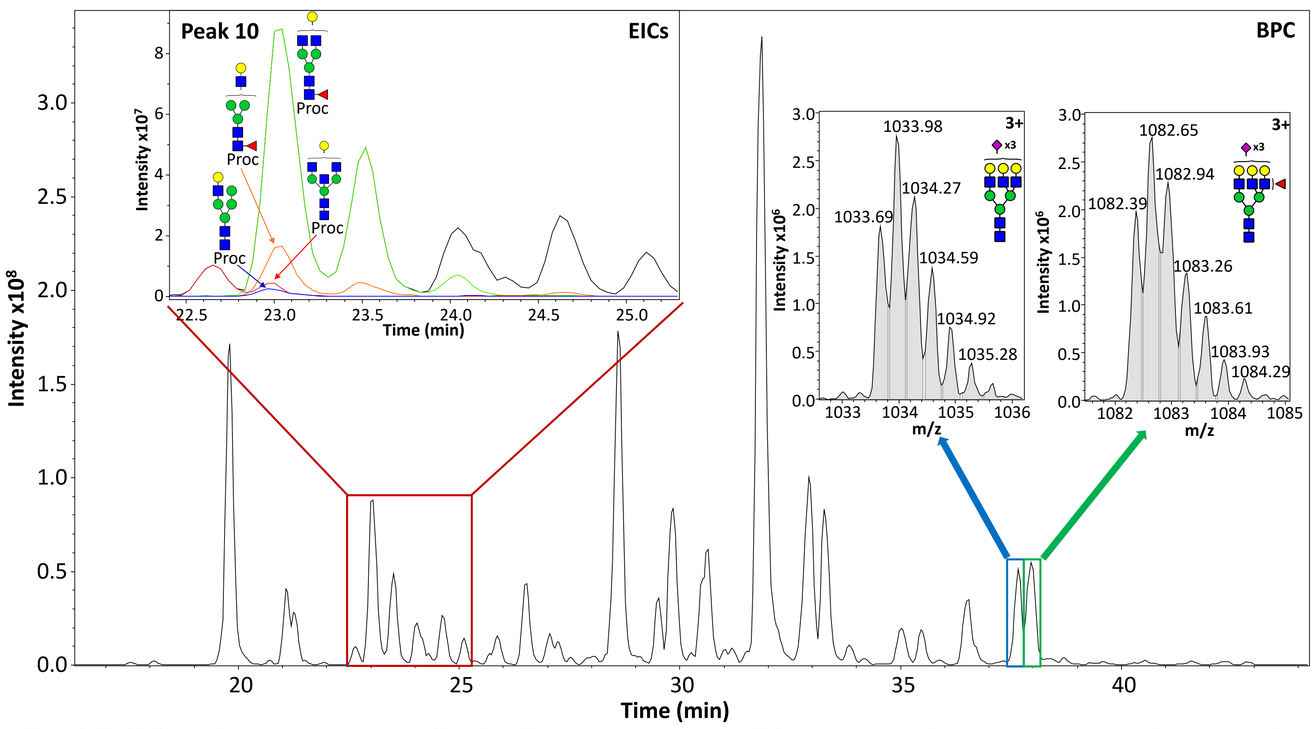


Figure 3: HILIC-MS analysis of a serum sample from a CD patient. The base peak chromatogram (BPC) shows the separation of the released glycans while the extracted ions chromatograms (EICs) from peak 10 exhibit the co-elution of several glycans species. Sum spectra at charge state +3 show similar intensities for H6N5S3 (left panel) and H6N5F1S3 (right panel). MS conditions: m/z range 600-1500; source: 4.5kV, 25psi, 10L/min, 300˚C.

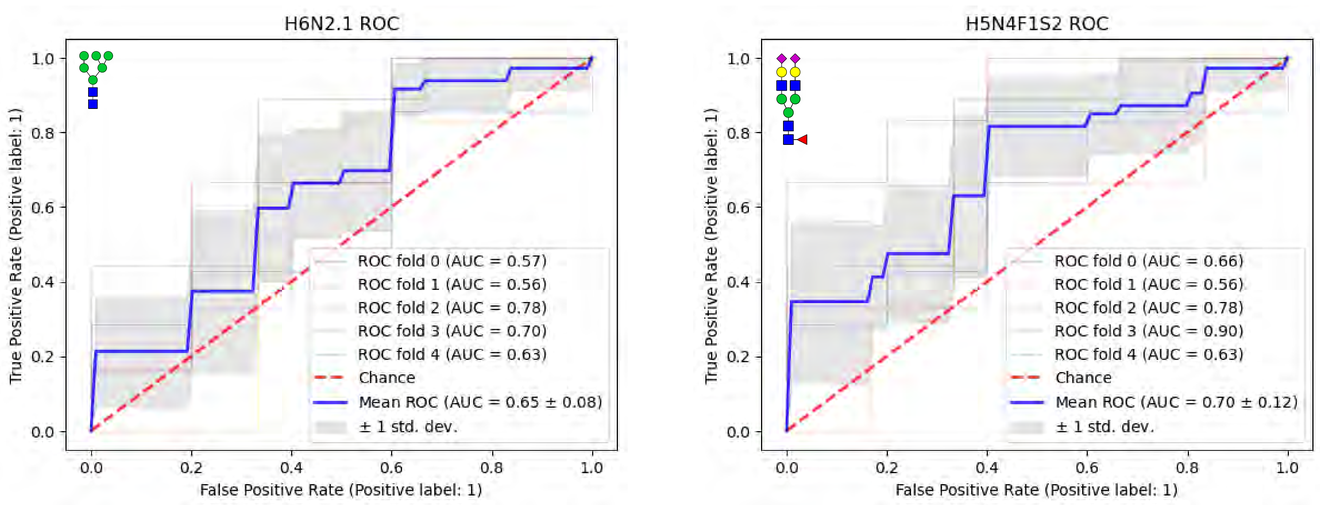


Figure 4: Direct traits before treatment are moderate predictors of response. Significant traits (p-value < 0.05) before treatment, H6N2 (left panel) and H5N4F1S2 (right panel) were subjected to a 5-fold cross-validation logistic regression correcting for age and sex. Receiver operating characteristic (ROC) curves were produced with their corresponding area under the curve (AUC).

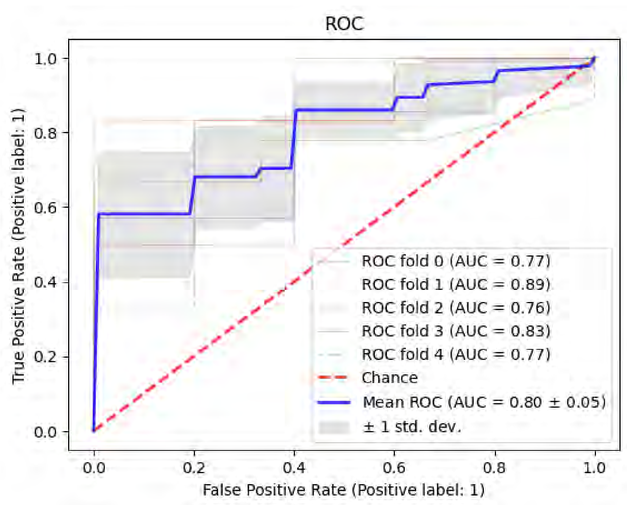


Figure 5: Direct traits after treatment are good predictors of response. Significant traits (p-value <0.05) after treatment were combined and subjected to a 5-fold cross-validation random forest correcting for age and sex. ROC curves with their corresponding AUC were produced.

Conclusions

- MAN6 (0.65 AUC) and FA2G2S2 (0.7 AUC) are higher in responders prior to treatment and moderate predictors of response.
- F-calprotectin, a clinical marker is a weaker predictor than glycan direct traits (0.43 AUC).
- Galactosylation levels found to increase in responders after treatment.
- 17 direct traits and 4 derived traits are markers of response after treatment and altogether good predictors of response (0.80 AUC).
- N-glycans are indicators of future response to VDZ and could potentially help clinicians determine the most appropriate treatment for individuals.
- Future work will be performed on an Orbitrap Exploris 120 mass spectrometer to obtain more accurate and sensitive MS data and to increase the number of quantified glycans.

Contact for more information

Thank you for viewing my poster. If you'd like a copy or want to know more about our work, then please send me an email to **thomas.senard@ludger.com** or connect with me on LinkedIn

References

[1]Bruce E. Sands, *et al.,* Effects of Vedolizumab Induction Therapy for Patients With Crohn's Disease in Whom Tumor Necrosis Factor Antagonist Treatment Failed. Gastroenterology, 2014 Sep; 147(3):618-627.e3. doi: 10.1053/j.gastro.2014.05.008.

[2]Bressler B, *et al.,* Vedolizumab and Anti-Tumour Necrosis Factor α Real-World Outcomes in Biologic-Naïve Inflammatory Bowel Disease Patients: Results from the EVOLVE Study. J Crohns Colitis. 2021 Oct 7;15(10):1694-1706. doi: 10.1093/ecco-jcc/jjab058.

[3]Šimurina M, *et al.,* Glycosylation of Immunoglobulin G Associates With Clinical Features of Inflammatory Bowel Diseases. Gastroenterology. 2018 Apr;154(5):1320-1333.e10. doi: 10.1053/j.gastro.2018.01.002.

Acknowledgements

The LaCyTools software can be found here: "https://github.com/Tarskin/LaCyTools" and was developed by Bas Jansen.