

Serum N-glycomic biomarkers predict treatment escalation in Inflammatory Bowel Disease



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Introduction

The clinical management of Inflammatory Bowel Disease (IBD) would benefit from biomarkers that would allow treatment choices to be optimised for each individual patient.

In this study, we have investigated and validated composite serum N-glycan biomarkers in predicting response to primary treatment following diagnosis in patients with IBD, Crohn's disease (CD) and Ulcerative colitis (UC).

What are glycans?

Glycans are oligosaccharides that are found in free form or bound to a wide variety of biological molecules through an enzymatic process called glycosylation. Most secreted proteins are glycosylated, largely as a result of post-translational modification, and these glycans play a vital role in regulation of key biological processes including protein folding, immune cell migration, cell adhesion and recognition of pathogens – all of which are implicated in IBD.

Recent glycomics studies have shown that aberrant glycosylation is associated with cancers, neurodegenerative and inflammatory diseases such as IBD. The total serum N-glycome is influenced by genetic, environmental and pathological factors, making it an attractive source for discovery of clinical biomarkers.

Current Glycan Biomarker Study

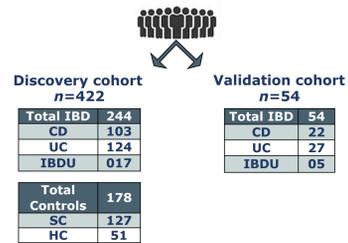


Figure 1: Cohort details for current glycan biomarker study

Current glycan biomarker study:

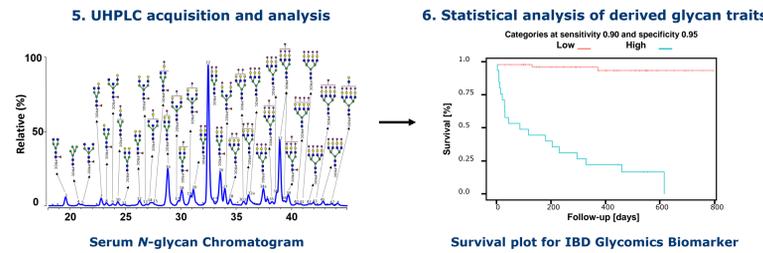
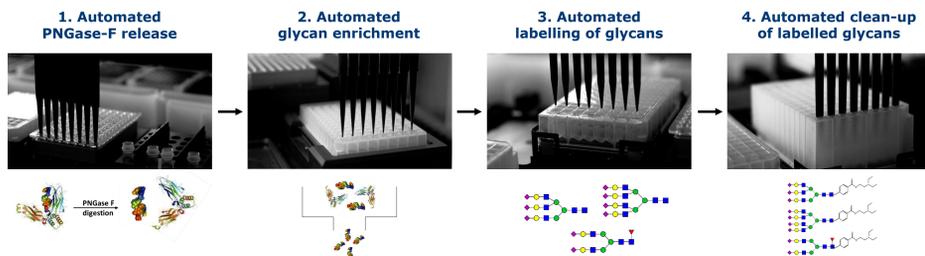
We analyzed total serum N-glycans (TSNG) by using an ultra-high performance liquid chromatography (UHPLC) analytical platform. The study was performed in a discovery cohort that was recruited at the Western General Hospital, in Edinburgh (UK) which consisted of 103 CD patients, 124 UC patients, 17 IBD unclassified (IBDU), 127 symptomatic controls (SC), and 51 healthy controls (HC). Our validation cohort was recruited independently at Örebro University, Sweden which consisted of 22 CD patients, 27 UC patients and 5 IBDU patients as depicted in Figure 1.

Methods

Total N-glycans from 227 patients and 195 controls (Edinburgh, UK) were assessed in a 10µl serum sample collected at the index clinic visit and analyzed by automated high-throughput fluorescent labeling of glycans using UHPLC. Scheme 1 below illustrates the automated N-glycan analysis and data analysis workflow used for biomarker discovery:

- 1) N-Glycans from serum were released using peptide-N-glycosidase F enzyme;
- 2) The released glycans were enriched using a protein binding membrane plate;
- 3) Fluorescently labeled with a procainamide tag;
- 4) Post-labeling clean-up was performed to remove excess procainamide dye and other impurities;
- 5) Samples were analyzed using UHPLC followed by logistic regression analysis using 24 glycomics traits, based on structurally related glycoforms known as derived traits.
- 6) Statistical analysis: Survival analysis was performed using Cox proportional hazard (Cox PH) models with age, sex, and age-sex interaction terms, with leave-one-out (LOO) validation. Cox PH models for all single derived glycomics traits in predicting treatment escalation in IBD, and in UC and CD separately were tested. Extended models comprising combinations of up to 7 derived traits were also tested. Model performance was ranked using area under the curve.

The best performing biomarkers for predicting treatment escalation of IBD patients was tested in the independently recruited validation cohort of 49 patients recruited in Örebro (Sweden) using age, sex and age-sex interaction corrected Cox PH models with LOO validation.



Scheme 1: Automated serum N-glycan analysis and data analysis used for biomarker discovery using the discovery and validation cohorts.

Results

The cox proportional hazard models were created to characterize biomarkers to predict the need for treatment escalation – defined as a requirement for anti-TNF, other biologics, or surgery.

a) Discovery cohort:

Models including all combinations of up to 7 derived glycan traits were tested for the prediction of treatment escalation in all IBD patients, and in CD patients and UC patients separately in the discovery cohort. See Figure 2 for survival curves and hazard ratios (HR) for CD, UC and IBD respectively.

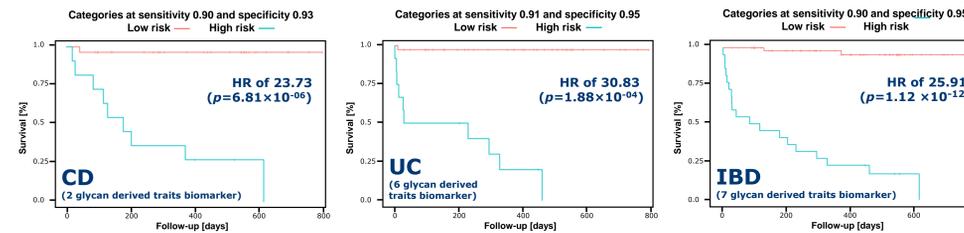


Figure 2: Kaplan-Meier survival plots for prediction of disease escalation using glycomics biomarkers for IBD patients from discovery cohort

b) Validation cohort:

The best performing glycomics biomarker (7 glycan derived traits) for predicting treatment escalation in IBD patients was reapplied to an independent cohort using an age, sex and age-sex interaction corrected Cox PH model with LOO validation (See Figure 3 for the survival curve and hazard ratio for IBD patients from the replication cohort). Sub-analyses for CD and UC biomarkers separately were hindered by sample numbers in the replication cohort.

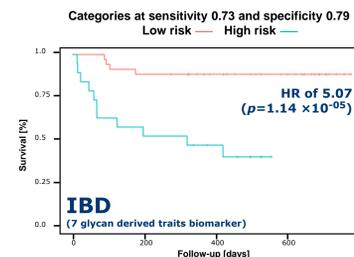


Figure 3: Kaplan-Meier survival plot for prediction of disease escalation using glycomics biomarker for IBD patients from validation cohort

c) Associations of glycans with subtypes of IBD:

Additionally, we characterized associations of glycosylation changes found between controls and patients with IBD sub-types.

Examples shown here include:

- galactosylation of diantennary glycans (A2F0S0G): lower in both CD and UC vs. controls
- bisection of diantennary glycans (A2S0B): generally higher in both CD and UC vs. controls
- fucosylation of triantennary glycans (A3F): higher in both CD and UC vs. controls
- galactosylation arm linkage feature (FA2[r6]B0G1): more abundant in UC in comparison with CD patients.

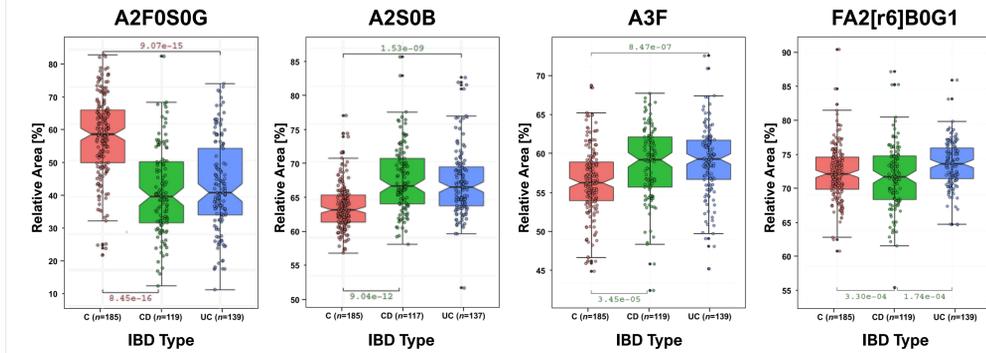


Figure 4: Association of derived glycan traits in controls, CD and UC patients.

d) Association of glycans with clinical markers:

Furthermore, we tested clinical blood markers such as albumin and high-sensitivity C-reactive protein (hsCRP) to assess their capability of prediction of treatment escalation. The survival curves for the best performing models are illustrated in Figure 5 for CD, UC and IBD patients. Based on this data we conclude that the glycan based biomarkers outperformed clinical markers in this study.

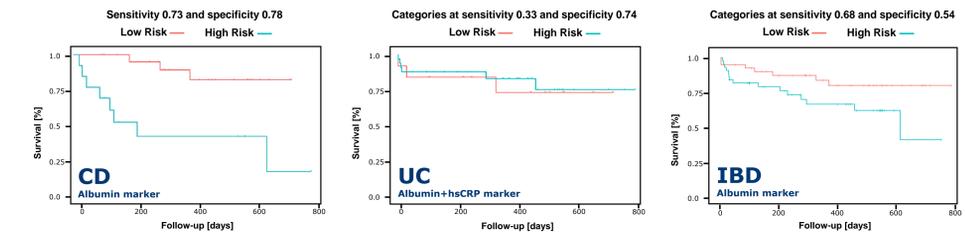


Figure 5: Kaplan-Meier survival plots for prediction of disease escalation using clinical blood biomarkers applied to CD, UC and IBD patients

Conclusions

- Using glycomics biomarkers we show that the future need for surgical intervention or escalation of medical treatment can be predicted in IBD patients at diagnosis based on a small serum sample (10 µL) taken at diagnosis.
- The glycomics biomarker has been validated in an independent replication cohort and therefore could potentially be selected for an individualized top-down approach to therapy in IBD.
- To the best of our knowledge, we are the first group to identify glycomics biomarkers for prediction of treatment escalation in IBD and these biomarkers could prove valuable in personalizing treatment of IBD.
- Future work: We intend to work on prediction of specific drug responses using glycomics and look forward to collaborating with you!

Acknowledgements

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Disclosures

- Ludger Ltd is a commercial bioscience company that offers products and services for glycoprofiling technology. Some of Ludger's products are used in this study.
- IP statement: A patent application for aspects of the work presented here has been submitted. (GB 1901708.6)

Contacts

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