Neu5Ac and Neu5,9Ac$_2$ in Human Plasma: Potential Biomarkers of Cardiovascular Disease

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Introduction

- *N*-Acetyl neuraminic acid (Neu5Ac) has previously been identified as a potential biomarker for the prediction of cardiovascular disease (CVD). This research aimed expanded scope to also investigate 9-*O*-acetyl-*N*-acetyl neuraminic acid (Neu5,9Ac$_2$)
- Sialic acids were quantified by labelling with 1,2-diamino-4,5-methylenedioxybenzene (DMB) followed by analysis using reverse-phase ultra-high pressure liquid chromatography (RP UHPLC)
- N-glycans were also investigated as many are highly sialylated structures, these were labelled with procainamide and analysed using hydrophilic interaction liquid chromatography (HILIC)
- Receiver operator curves (ROC) were prepared to determine the predictive power of sialic acids and N-glycans with regards to CVD. These were compared to a well-established biomarker: c-reactive protein (CRP)

Workflow

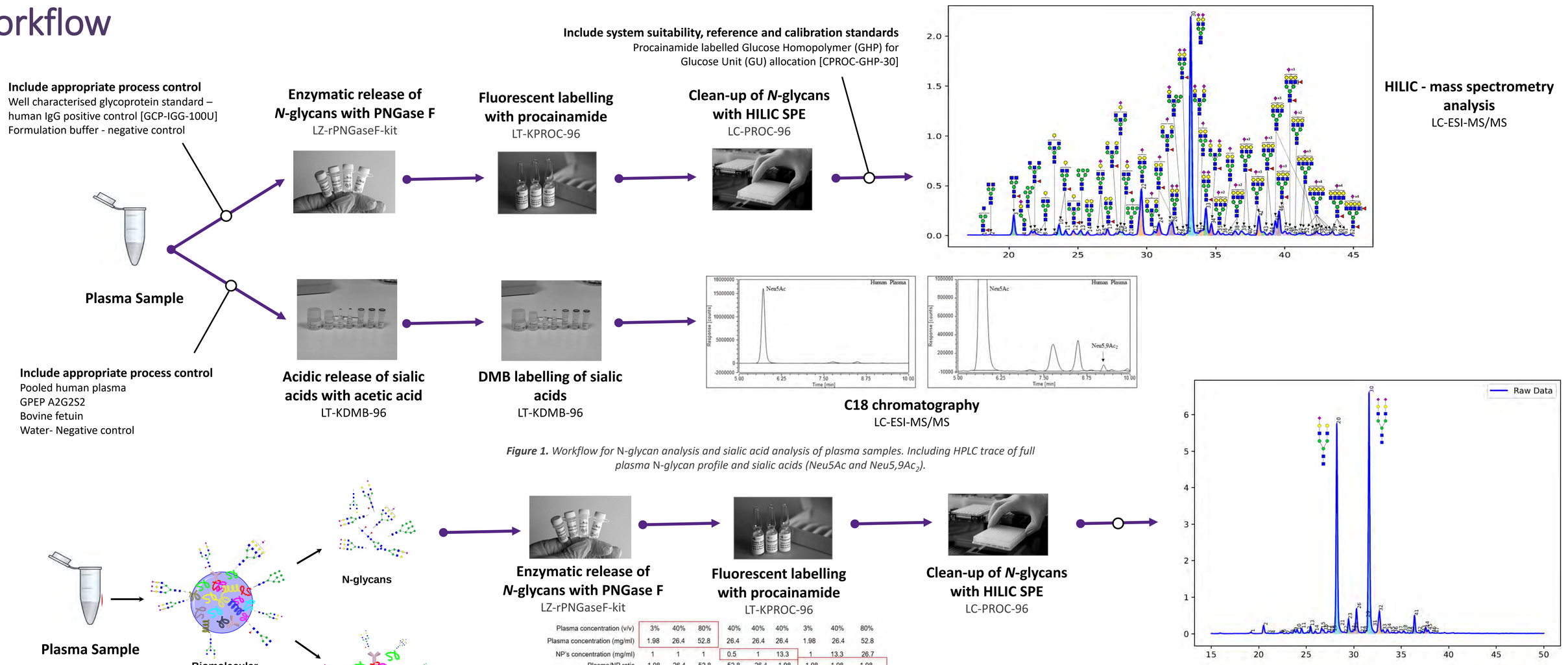


Figure 1. *Workflow for* N*-glycan analysis and sialic acid analysis of plasma samples. Including HPLC trace of full plasma* N*-glycan profile and sialic acids (Neu5Ac and Neu5,9Ac$_2$).*

Figure 2. *Workflow for NP prefractionation of plasma samples. The NP surface becomes coated in glycoproteins from the plasma with a particular affinity for fibrinogen at plasma/NP ratios of 1.98. This is then followed by* N*-glycan analysis. Note the enrichment of A2G2S1 in the* N*-glycan profile, a particular feature of fibrinogen. The enrichment of fibrinogen is also shown in the SDS-page. The enrichment is notable in that higher levels of fibrinogen in the sample will result in higher levels of enrichment of the protein, allowing for investigation as a biomarker.*

Results

	Healthy Controls	CVD Patients
n	30	30
Age (Years)	65 ± 13	59 ± 22
Male:Female Ratio	14:16	14:16
Mean Plasma Neu5Ac (mg/100 mL)	45.19 ± 8.46	63.55 ± 17.49
Mean Plasma Neu5,9Ac$_2$ (mg/100 mL)	0.32 ± 0.06	0.49 ± 0.19
Mean Plasma CRP (mg/L)	1.85 ± 2.37	6.21 ± 15.25

Table 1. *Plasma sample cohort details*

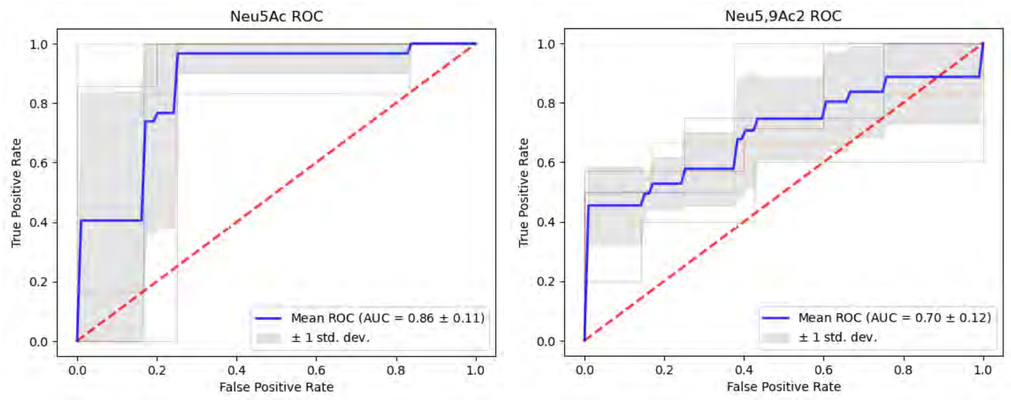


Figure 4. *ROC analysis of sialic acids in plasma. A combination marker of Neu5Ac and Neu5,9Ac$_2$ shows very good predictive power for CVD with a value of 0.92.*

	Sensitivity	Specificity	F-Score	AUC
Neu5Ac	0.82 ± 0.16	0.81 ± 0.17	0.80 ± 0.12	0.86 ± 0.12
Neu5,9Ac2	0.44 ± 0.22	0.82 ± 0.17	0.51 ± 0.20	0.71 ± 0.12
Neu5Ac + Neu5,9Ac2	0.87 ± 0.13	0.90 ± 0.12	0.88 ± 0.13	0.93 ± 0.10
C-reactive protein				0.50 ± 0.14

Table 2. *Summary of ROC analysis of Neu5Ac, Neu5,9Ac$_2$ and combined Neu5Ac/Neu5,9Ac$_2$ as well as CRP.*

Figure 3. *Box plots showing the differences in concentration of plasma Neu5Ac and Neu5,9Ac$_2$ between CVD patients and healthy controls. Both compounds are significantly elevated in the CVD cohort as compared to healthy controls.*

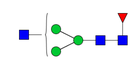
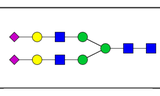
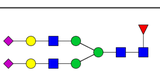
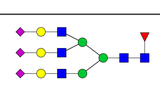
N-Glycan	P-value	AUC	Structure
FA1	0.0046	0.79	
A2G2S2	0.0012	0.8	
Man3A1BG1S1	0.0049	0.81	
FA2G2S2	0.016	0.68	
FA3G3S3	0.0049	0.71	

Table 3. *Summary of statistical significance and ROC analysis of* N*-glycans between the two groups of samples. Sialylated glycans showing elevation in CVD patients supports the elevation of sialic acid concentrations. Furthermore, the N-glycans show good levels of predictive power for CVD versus healthy controls as determined by ROC analysis.*

Conclusions

- Sialic acids and sialylated N-glycan structures are significantly elevated in patients with CVD versus healthy controls
- Both sialic acids and N-glycans can be used to distinguish between CVD patients and healthy controls with high levels of predictive power
- The predictive power of these markers is much higher than that of a well-established inflammatory marker (CRP)
- Fibrinogen can be preferentially enriched on NP surfaces giving insight into other inflammatory biomarkers

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References

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Acknowledgements

We would like to thank the MRC (MR/P015786/1) and Ludger Ltd. for funding this research. We thank the Irish Research Council for supporting the study (Enterprise Partnership Scheme Project EPSPG/2019/511).