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NMR-Based Quantification and Glycoprofiling of Acute-Phase Glycoproteins from Native Blood Serum

Keywords: nuclear magnetic resonance (NMR), acute-phase proteins, glycoproteins, N-glycans, GlycA/GlycB, spectral deconvolution, cancer

INVENTION NOVELTY

Many acute-phase proteins are abundant blood glycoproteins whose concentrations and glycosylation patterns change during inflammation, infection and cancer, partly in a disease-specific manner. Clinical immunoassays, nephelometry or turbidimetry typically measure one protein per test and largely disregard glycosylation. Mass spectrometry-based glycomics resolves glycan structures but requires glycan release or digestion, chromatographic separation and considerable effort. In standard 1H-NMR analysis of serum, glycoproteins appear mainly as the composite signals GlycA and GlycB. Building on a revised assignment of GlycA to sialic acid (Neu5Ac) and GlycB to N-acetylglucosamine (GlcNAc) residues of N-glycans, the invention decodes these signals into contributions of individual acute-phase glycoproteins and specific glycan features. Serum or plasma is analyzed with minimal sample preparation, without glycan release or separation, and non-destructively.

VALUE PROPOSITION

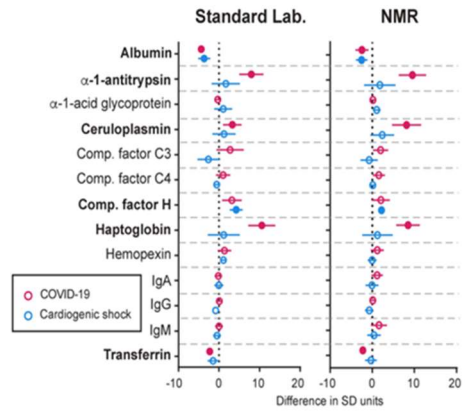
One automated series of NMR experiments on a single sample provides three levels of information: estimated concentrations of several acute-phase glycoproteins; a detailed N-glycan profile, including α2,3- and α2,6-sialylation, galactosylation, fucosylation and Lewis A/X antigens; and, combined with metabolite and lipoprotein parameters from the same sample, multiparametric signatures for patient stratification. Changes in glycosylation can thus be distinguished, at least in part, from changes in protein concentration – information provided neither by GlycA/GlycB nor by single-protein assays. In an exploratory hepatocellular carcinoma (HCC) study, glycan features discriminated patients from controls, whereas GlycA/GlycB alone did not.

TECHNOLOGY DESCRIPTION

Glycoprotein signals are separated from metabolite and lipoprotein signals by diffusion-, relaxation- and/or J-coupling-based spectral editing and by frequency-selective excitation of glycan resonances (selective TOCSY). Reference frequency matrices of isolated glycoproteins are used in a line-shape fitting algorithm to deconvolute the edited spectrum into the contributions of major acute-phase glycoproteins; selective TOCSY additionally resolves individual glycan features. The workflow runs on commercially available 600 MHz NMR spectrometers with automated sample handling.

COMMERCIAL OPPORTUNITY

The technology adds a glycoprotein and glycan layer to established NMR platforms for lipoprotein and metabolite profiling, currently used mainly in clinical research, cohort studies and biobanking. It can be implemented as a software and method module on these platforms or offered as a central laboratory service. Near-term applications include biomarker discovery and patient stratification in clinical and pharmaceutical studies; in the longer term, disease-specific signatures could be developed into diagnostic tests, subject to clinical validation. The invention is available for licensing and co-development, e.g. through a joint validation study.



Standard deviation (SD) of glycoprotein concentrations in blood serum of COVID-19 and cardiogenic shock patients using either standard laboratory methods or the NMR-based approach (from Mallagaray et al., 2023).

DEVELOPMENT STATUS

Analytical proof of concept has been established. In serum from patients with COVID-19 and cardiogenic shock, NMR-derived contributions of individual acute-phase glycoproteins correlated with clinical laboratory values (see Fig.); glycan profiles were confirmed by UHPLC-MS. Detailed glycoprofiling was applied in an exploratory HCC case–control study, and NMR-based proteometabolomic signatures were investigated in MASLD and further cohorts, e.g. inflammatory bowel disease and Parkinson's disease.

PATENT SITUATION

The German patent DE102023110285.0 has been granted. The international application WO2024/217637 has entered the national/ regional phases in AU, EP, and the US.

FURTHER READING

Mallagaray et al. (2023) Angew. Chem. Int. Ed. 62: e202306154; Rudolph et al. (2025) Anal. Chem. 97: 9367; Jegodzinski et al. (2025) JHEP Reports 7: 101450.

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