Clinical Chemistry 61:5
681–683 (2015)

Editorials

Serum Nuclear Magnetic Resonance Spectroscopy: One More Step toward Clinical Utility

Mika Ala-Korpela[1,2*]

Quantitative high-throughput nuclear magnetic resonance (NMR)[3] approaches, often referred to as metabolomics or metabolic phenotyping when a multitude of molecular measures are analyzed simultaneously, are increasingly applied in epidemiology, genetics, and medicine *(1)*. The work of Otvos et al. in this issue of *Clinical Chemistry (2)* adds to the accumulating evidence that automated quantitative analytics provided by modern NMR platforms is a powerful tool to reveal potential new biomarkers for disease risk assessment and clinical applications. The authors applied advanced spectral analysis and presented quantification of a composite NMR resonance reflecting glycoprotein acetylation, termed GlycA. The major contributions to the GlycA signal arise from 5 inflammatory markers, including α_1-acid glycoprotein, haptoglobin, α_1-antitrypsin, α_1-antichymotrypsin, and transferrin. These 5 glycoproteins, the circulating concentrations of which change due to inflammatory stimuli, have mobile glycan chains that in NMR produce the GlycA signal in proportion to their glycan *N*-acetylglucosamine concentrations.

Markers of inflammation are clinically important and widely used diagnostic and prognostic tools. C-reactive protein (CRP) is in routine use worldwide owing to its associations with a broad pattern of inflammation and its favorable clinical chemistry characteristics. Otvos et al. noted that GlycA concentrations were correlated with high-sensitivity CRP ($r = 0.56$; n = 5537; $P < 0.0001$) *(2)* at a similar level as noted previously ($r = 0.61$; n = 27 491 women; $P < 0.0001$) *(3)*. Akinkuolie et al. have previously shown that increased GlycA relates to the risk of future cardiovascular disease events with a small additive association of GlycA and high-sensitivity CRP, suggesting that these biomarkers may play both overlapping and partly distinct roles *(3)*. Unlike specific molecular markers of inflammation, GlycA is a composite marker that reflects the integrated concentrations of several of the most abundant glycoproteins in the circulation. In relation to this, Otvos and coworkers speculated that GlycA concentrations may provide a more stable measure of low-grade systemic inflammation and respond more uniformly to diverse inflammatory stimuli than individual inflammatory reactants *(2)*. Intriguingly, Fischer et al. have shown that the association of GlycA with all-cause mortality was only slightly attenuated when CRP was included in the prediction model *(4)*.

The detection of glycosylated proteins by NMR spectroscopy is not novel. The resonance was assigned by Bell et al. in 1987 *(5)* and has been shown to relate to the inflammatory status of patients *(6)*. More recently, in the NMR-based metabolomics-type of applications, increased circulating glycoproteins have been linked to rheumatoid arthritis and its severity *(7)* as well as to cardiovascular and cancer mortality *(4)*. Differences in glycoprotein concentrations have also been seen during pregnancy *(8)*, in relation to liver fibrosis *(9)*, after exercise *(10)*, and owing to long-term physical activity *(11)*. Increased adiposity causally affects increased circulating glycoprotein concentrations *(12)*. The above findings suggest potential utility of the NMR-based GlycA as a marker of systemic inflammation that provides complementary information in various clinically important biomarker-based risk assessment models.

NMR-based approaches in biomarker quantification have 2 essential advantages. First, the experimental NMR spectroscopy backbone used by Otvos et al. to quantify GlycA *(2)* is exactly the same as they have already used in multiple clinical studies to assay lipoprotein subclasses and lipids *(13)*. The original NMR data being stored therefore enable retrospective analysis of GlycA in all the archived studies. This, and existing extensive quantitative serum NMR metabolomics data collections *(1)*, will most likely expedite further studies to clarify and define the role of GlycA as a systemic inflammation marker. Second, proton NMR spectroscopy inherently provides multimetabolic phenotyping of all the abundant molecules with mobile protons *(14)*. With appropriate data analyses, this can lead to quantitative information on multiple lipoprotein subclasses, their lipid concentrations and compositions, apolipoprotein A-I and B, multiple cholesterol and triglyceride measures, albumin, and various fatty acids, as well as numerous low molecular weight metabolites, includ-

[1] Computational Medicine, Institute of Health Sciences, University of Oulu and Oulu University Hospital, Oulu, Finland; [2] Computational Medicine, School of Social and Community Medicine and Medical Research Council Integrative Epidemiology Unit, University of Bristol, Bristol, UK.

* Address correspondence to this author at: University of Oulu, Faculty of Medicine, Computational Medicine, Institute of Health Sciences, Aapistie 5 A, P.O. Box 5000, 90014 University of Oulu, Oulu, Finland. E-mail mika.ala-korpela@computationalmedicine.fi.

Received February 13, 2015; accepted February 19, 2015.
Previously published online at DOI: 10.1373/clinchem.2015.238279

[3] Nonstandard abbreviations: NMR, nuclear magnetic resonance; CRP, C-reactive protein.

ing amino acids, glycolysis-related measures, and ketone bodies *(1, 13, 14)*. Current NMR technology, coupled with automated sample handling, high-sensitivity probes, and stable electronics, is providing unprecedented opportunities in large-scale biofluid analytics. Consequently, a key challenge associated with the high-throughput experimentation is how to identify and quantify the molecular information from the spectral data with sufficient speed and automation to match the current experimental sophistication. Otvos et al. have adopted advanced deconvolution analyses to quantify the molecular resonances for both lipoprotein subclasses and GlycA *(2, 13, 14)*. There is also no reason that the analytical setting used by this group would not allow quantification of additional molecular components and development of the platform toward more comprehensive metabolic phenotyping.

Quantitative NMR metabolomics has already been applied in large-scale epidemiological study settings, and the comprehensive molecular data have revealed new biomarkers for early atherosclerosis, type 2 diabetes, diabetic nephropathy, cardiovascular disease, and all-cause mortality *(1)*. NMR provides a very cost-effective methodology for detailed molecular studies in large-scale epidemiology. It is to be stressed here, however, that absolute quantification of the NMR-based biomarkers is the essence for the clinical applications of NMR spectroscopy. The still widely applied "spectral-based metabolomics," in which only the overlapping and complex spectral data are used as the basis for multivariate statistical analyses, does not provide a reliable basis for clinical applications. The new method to quantify GlycA in physiological units, in this case via automated spectral deconvolution applying in-house software, illustrates how quantitative NMR-based metabolic measures can be treated as any other quantitative biomarker *(2)*. There is no requirement for any special knowledge in spectroscopy, but the epidemiological or clinical analyses of the metabolic measures of interest can be initiated with standard medical statistics. This approach also greatly facilitates the interpretation and replication of the findings—if a metabolite has not previously been associated with the risk factor or disease outcome, its association magnitudes can be studied in terms of, e.g., regression coefficients and odds ratios, which are adjustable for potential confounders. The effect estimates may then be compared to those of standard risk factors and replicated in independent studies. Quantitative NMR metabolomics data are also straightforward to use according to the recent recommendations of diagnostic and prognostic modeling *(15)*.

Otvos et al. took an additional step toward diverse clinical applications of quantitative NMR spectroscopy by incorporating GlycA as a part of their methodology *(2)*, which has previously explicitly focused on lipoprotein subclass and lipid analytics *(1, 13, 14)*. Additional developments toward automated high-throughput applications of quantitative NMR metabolomics are likely to take place in the near future. NMR is capable of providing quantitative data on >200 molecular measures, including multiple biomarkers already used in health care and clinical medicine such as cholesterol carried in various lipoproteins, triglycerides, creatinine, albumin, fatty acids, and glucose *(1)*. If these types of methodologies become widely available, they will most likely be used, at least initially, in large epidemiological studies and biobanks to maximize the scientific information from the valuable samples in a cost-effective manner. Whether NMR metabolomics will continue its journey to become a routine instrument depends on a rather long list of factors. The practical use of the extensive lipoprotein and metabolite panel is conditional on automated experimentation as well as data analyses, high sample throughput, accurate and precise measures that are comparable with existing methods and cost-effectiveness. It is also obvious that epidemiological and clinical studies must show clear evidence that the standard biomarkers quantified by NMR are robust and that the potential new biomarkers, such as GlycA, provide sufficient additional biological and predictive value to be incorporated into new clinical protocols.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: M. Ala-Korpela, Brainshake Ltd.
Stock Ownership: M. Ala-Korpela, Brainshake Ltd.
Honoraria: None declared.
Research Funding: None declared.
Expert Testimony: None declared.
Patents: None declared.

References

1. Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circ Cardiovasc Genet. 2015;8:192–206.
2. Otvos JD, Shalaurova I, Wolak-Dinsmore J, Connelly MA, Mackey RH, Stein JH, Tracy RP. GlycA: a composite nuclear magnetic resonance biomarker of systemic inflammation. Clin Chem 2015;61:714–23.
3. Akinkuolie AO, Buring JE, Ridker PM, Mora S. A novel protein glycan biomarker and future cardiovascular disease events. J Am Heart Assoc. 2014;3:e001221.
4. Fischer K, Kettunen J, Würtz P, Haller T, Havulinna AS, Kangas AJ, et al. Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons. PLoS Med. 2014;11:e1001606.

5. Bell JD, Sadler PJ, Macleod AF, Turner PR, La Ville A. ^{1}H NMR studies of human blood plasma: assignment of resonances for lipoproteins. FEBS Lett 1987;219: 239–43.
6. Torri GM, Torri J, Gulian JM, Vion-Dury J, Viout P, Cozzone PJ. Magnetic resonance spectroscopy of serum and acute-phase proteins revisited: a multiparametric statistical analysis of metabolite variations in inflammatory, infectious and miscellaneous diseases. Clin Chim Acta 1999;279:77–96.
7. Lauridsen MB, Bliddal H, Christensen R, Danneskiold-Samsøe B, Bennett R, Keun H, et al. ^{1}H NMR spectroscopy-based interventional metabolic phenotyping: a cohort study of rheumatoid arthritis patients. J Proteome Res 2010;9:4545–53.
8. Pinto J, Barros AS, Domingues MRM, Goodfellow BJ, Galhano E, Pita C, et al. Following healthy pregnancy by NMR metabolomics of plasma and correlation to urine. J Proteome Res 2015;14:1263–74.
9. Sands CJ, Guha IN, Kyriakides M, Wright M, Beckonert O, Holmes E, et al. Metabolic phenotyping for enhanced mechanistic stratification of chronic hepatitis C-induced liver fibrosis. Am J Gastroenterol 2015;110:159–69.
10. Pechlivanis A, Kostidis S, Saraslanidis P, Petridou A, Tsalis G, Veselkov K, et al. ^{1}H NMR study on the short- and long-term impact of two training programs of sprint running on the metabolic fingerprint of human serum. J Proteome Res 2013;12: 470–80.
11. Kujala UM, Mäkinen V-P, Heinonen I, Soininen P, Kangas AJ, Leskinen TH, et al. Long-term leisure-time physical activity and serum metabolome. Circulation 2013; 127:340–8.
12. Würtz P, Wang Q, Kangas AJ, Richmond RC, Skarp J, Tiainen M, et al. Metabolic signatures of adiposity in young adults: Mendelian randomization analysis and effects of weight change. PLoS Med 2014;11:e1001765.
13. Mallol R, Rodriguez MA, Brezmes J, Masana L, Correig X. Human serum/plasma lipoprotein analysis by NMR: application to the study of diabetic dyslipidemia. Prog Nucl Magn Reson Spectrosc 2013;70:1–24.
14. Ala-Korpela M. ^{1}H NMR spectroscopy of human blood plasma. Prog Nucl Magn Reson Spectrosc 1995;27:475–554.
15. Collins GS, Reitsma JB, Altman DG, Moons KGM. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD Statement. Circulation 2015;131:211–9.