Clinica Chimica Acta 459 (2016) 177–186

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Review

Inflammatory glycoproteins in cardiometabolic disorders, autoimmune diseases and cancer

Margery A. Connelly [a], Eke G. Gruppen [b,c], James D. Otvos [a], Robin P.F. Dullaart [b,*]

[a] LipoScience, Laboratory Corporation of America® Holdings, Raleigh, NC, USA
[b] Department of Endocrinology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[c] Department of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 7 April 2016
Received in revised form 8 June 2016
Accepted 11 June 2016
Available online 13 June 2016

Keywords:
Biomarker
Inflammation
Glycan
Glycomics
Glycoproteins
Glycosylation

ABSTRACT

The physiological function initially attributed to the oligosaccharide moieties or glycans on inflammatory glycoproteins was to improve protein stability. However, it is now clear that glycans play a prominent role in glycoprotein structure and function and in some cases contribute to disease states. In fact, glycan processing contributes to pathogenicity not only in autoimmune disorders but also in atherosclerotic cardiovascular disease, diabetes and malignancy. While most clinical laboratory tests measure circulating levels of inflammatory proteins, newly developed diagnostic and prognostic tests are harvesting the information that can be gleaned by measuring the amount or structure of the attached glycans, which may be unique to individuals as well as various diseases. As such, these newer glycan-based tests may provide future means for more personalized approaches to patient stratification and improved patient care.

Here we will discuss recent progress in high-throughput laboratory methods for glycomics (i.e. the study of glycan structures) and glycoprotein quantification by methods such as mass spectrometry and nuclear magnetic resonance spectroscopy. We will also review the clinical utility of glycoprotein and glycan measurements in the prediction of common low-grade inflammatory disorders including cardiovascular disease, diabetes and cancer, as well as for monitoring autoimmune disease activity.

Contents

Abbreviations: 2-AB, 2-aminobanzamide; AFP, α-fetoprotein; AGP, α1-acid glycoprotein; BMI, body mass index; CDG, congenital disorders of glycosylation; CEA, carcinoembryonic antigen; CRP, C-reactive protein; CVD, cardiovascular disease; DAS28, Disease Activity Score based on 28 joints; ELISA, enzyme-linked immunosorbent assay; ESR, erythrocyte sedimentation rate; GlcNAc, *N*-acetylglucosamine; GWAS, genome-wide association studies; HCC, hepatocarcinoma; hsCRP, high-sensitivity C-reactive protein; HILIC, hydrophilic interaction liquid chromatography; HOMA-IR, homeostatic model assessment of insulin resistance; HPLC, high performance liquid chromatography; IgG, immunoglobulin G; JUPITER, Justification for the Use of Statins in Prevention: an Interventional Trial Evaluating Rosuvastatin; LDL-C, low density lipoprotein cholesterol; MBDA, multi-biomarker disease activity; MS, mass spectrometry; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NMR, nuclear magnetic resonance spectroscopy; PREVEND, Prevention of Renal and Vascular End-stage Disease study; PSA, prostate specific antigen; RA, rheumatoid arthritis; SAA, serum amyloid A; SLe^a, sialyl Lewis antigen; SLE, systemic lupus erythematosus; T2DM, type 2 diabetes mellitus; UPLC, ultraperformance liquid chromatography; WHS, Women's Health Study.

* Corresponding author at: Department of Endocrinology, University Medical Center Groningen, University of Groningen, P.O. Box 30.001, Groningen 9700 RB, The Netherlands.
E-mail address: r.p.f.dullaart@umcg.nl (R.P.F. Dullaart).

http://dx.doi.org/10.1016/j.cca.2016.06.012

1. Introduction

Given the imperfections in the armamentarium of conventional biomarkers for diagnosis, prognosis, or risk prediction and disease prevention at the individual patient level, there is an ongoing effort using novel high-precision laboratory techniques to discover new biomarkers that will increase the sensitivity and specificity above current clinical tests [1–4]. Glycoproteins play key roles in inflammatory and pathological processes [5–9]. Thus, it is not surprising that investigation of the clinical utility of assays that measure inflammatory glycoproteins has received much attention [10–13]. Besides the clinical information that can be gleaned by quantifying circulating levels of glycoproteins, it is now clear that measurements based on the glycan structures of circulating proteins represent another avenue for improving diagnosis, prognosis and risk prediction of common inflammatory disorders [4,7,13–19]. Here we will briefly review the biochemistry and metabolism of glycoproteins, provide insight into the glycoprotein assays that are currently available for clinical use and describe newer high-throughput technologies that are being employed for identifying new glycan-based biomarkers that will add to the current armamentarium and are expected to improve patient care.

2. Glycoprotein biochemistry and rationale for measuring glycoproteins and glycans

Protein glycosylation is the enzyme-mediated post-translational process responsible for the attachment of glycan chains either to the nitrogen of an asparagine residue (N-linkage) or the oxygen of a serine or threonine residue (O-linkage) [8,20]. While most O-linked glycoproteins remain intracellular or are secreted and become part of the extracellular matrix, most of the abundant proteins in the circulation are N-linked glycoproteins. N-linked glycosylation is initiated in the endoplasmic reticulum and the oligosaccharide chains are further modified via a set of glycosyltransferases in the Golgi apparatus to form the basic

Table 1
Human inflammatory glycoproteins modified during an acute phase response.

Category	Positive acute phase proteins	Molecular weight (kDa)	Glycosylation sites (#)	UniProt number[a]	Adult concentrations in serum[b]
Binding or transport proteins	α1-Acid glycoprotein (AGP/orosomucoid)	41–43	5	P02763	0.5–1.2 mg/mL
	Haptoglobin	100	4	P00738	0.3–3.0 mg/mL
	Ceruloplasmin	151	6	P00450	0.2–0.6 mg/mL
	Mac-2 (or galectin-3) binding protein	85–97	7	Q08380	1.4–16.1 μg/mL
Antiproteases	α1-Antitrypsin	52	3	P01009	0.9–2.0 mg/mL
	α2-Macroglobulin	179	8	P01023	1.3–3.0 mg/mL
	α1-Antichymotrypsin	68	6	P01001	1.5–3.5 mg/mL
	Kallistatin	58	4	P29622	10 μg/mL
Complement system	C2	83	8	P06681	0.02–0.4 mg/mL
	C3	185	3	P01024	0.9–1.8 mg/mL
	C5	190	4	P01031	0.02–0.4 mg/mL
	C1 esterase inhibitor	105	7 N-, 8 O-linked	P05155	0.21–0.39 mg/mL
Coagulation system	Fibrinogen α, β, γ	340	5 N-, 2 O-linked	P02671, -75, -79	1.5–4.0 mg/mL
	Plasminogen	92	1 N-, 2 O-linked	P00747	plasma 120–200 μg/mL
	Vitronectin	140	3	P04004	plasma 110–140 μg/mL
	α2-Antiplasmin	70	4	P08697	70 μg/mL in plasma, 47.6 μg/mL in serum
	Prothrombin	72	3	P00734	Detection range 0.031–32 μg/mL
	Plasminogen activator inhibitor-1 (PAI-1)	43	3	P05121	Plasma 5–40 ng/mL
	Tissue plasminogen activator (tPA)	72	3 N-, 1 O-linked	P00750	1–18 ng/mL
Miscellaneous	Fibronectin	220–440	7 N-, 3 O-linked	P02751	0.3 mg/mL
	Lipoprotein phospholipase A2 (Lp-PLA2)	45	2	P13093	0.5–100 ng/mL
	C-reactive protein (CRP), pentamer	115–120	1[c]	P02741	hsCRP <1.0 μg/mL; ≥3.0 μg/mL risk for CVD
	Serum amyloid A (SAA)	13.5	0	P0DJ18	0.41–300 ng/mL; SAA is not glycosylated

Category	Negative acute phase proteins	Molecular weight (kDa)	Glycosylation sites (#)	UNIPROT number[a]	Adult concentrations in serum
Miscellaneous	Transferrin	76–81	3 N-, 1 O-linked	P02787	2.1–3.6 mg/mL
	Transthyretin	55	1	P02766	0.2–0.4 mg/mL
	α2-HS-glycoprotein (fetuin)	58	2 N-, 4 O-linked	P02765	0.21–0.45 mg/mL
	α-Fetoprotein (AFP)	70	1	P02771	<15 ng/mL
	Thyroxine binding protein	54	5	P05543	0.011–0.021 mg/mL
	Coagulation Factor XII	80	2 N-, 7 O-linked	P00748	Plasma 0.1–100 ng/mL

[a] Confirmation of contribution to the acute phase response and the number of sites that are glycosylated was obtained using the UniProtKB/Swiss-Prot database. http://www.uniprot.org/. The UniProt Consortium. UniProt: a hub for protein information Nucleic Acids Res. 43: D204–D212 (2015). For a more comprehensive review of plasma protein glycosylation see reference [4].

[b] Reference for adult (age 20–60 years) concentrations: C.A. Burtis, E.R. Ashwood, and D.E. Bruns. eds., Tietz Textbook of Clinical Chemistry and Molecular Diagnostics (Fourth edition) Philadelphia, WB Saunders, 2006, Chapter 56 pg. 2251–2302. If no standardized assay is available, a normal detection range was reported from a commercially available ELISA assay.

[c] Das T. et al., Biochem J. 2003 Jul. 15; 373(2): 345–55.