Metabolism Open 9 (2021) 100074

Contents lists available at ScienceDirect

Metabolism Open

journal homepage: www.journals.elsevier.com/metabolism-open

Mapping of type 2 diabetes proteins to COVID-19 biomarkers: A proteomic analysis

Keywords:
biomarkers
type 2 diabetes
COVID-19
SARS-CoV-2

To the Editor:

To determine predictive biomarkers for COVID-19 disease and infection severity, large scale multi-omic analyses have been undertaken in patients with respiratory disease, with and without COVID-19 disease [1]. Biomarkers involved in vessel damage, platelet degranulation, the coagulation cascade and the acute phase response were identified in COVID-19 disease and shown to differ further with increasing COVID-19 disease severity [1]. However, differences in protein expression may differ between patients with type 2 diabetes (T2D) and controls [2] and T2D patients may have altered markers of coagulation together with altered platelet function resulting in a prothrombotic propensity [3]. Biomarkers, or a combination of biomarkers, specific for COVID-19 disease in T2D would necessarily be independent of differentially expressed proteins in T2D versus controls. Therefore, this proteomic analysis was undertaken in subjects with and without T2D to compare these with the COVID-19 disease-related proteomic biomarkers that have been identified by using shotgun proteomics followed by parallel reaction monitoring [1], and to determine if any of the protein changes were dependent on glycemia.

Type 2 diabetes (T2D) (n = 23) and control subjects (n = 23) were enrolled in a case-controlled study, approved by Yorkshire and Humber Research Ethics Committee. A hyperinsulinemic clamp was performed as reported [4]; all subjects underwent a 10-h fast prior to the clamp. T2D: baseline glucose 7.6 ± 0.4mmol/l (136.8 ± 7.2mg/dl), reduced to 4.5 ± 0.07mmol/l (81 ± 1.2mg/dl) for 1-h. Controls: 4.9 ± 0.1mmol/l (88.2 ± 1.8mg/dl). Proteins that had been reported as biomarkers in COVID-19 disease for vessel damage (16 proteins), platelet degranulation (11 proteins), coagulation cascade (24 proteins) and acute phase response (9 proteins), shown in Table 1, were determined by Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement [4]. Statistics were performed using Graphpad Prism 8.0.

Table 1
Proteins identified as being altered in COVID-19 disease categorized according to biological processes: vessel damage (16 proteins), platelet degranulation (11 proteins), coagulation cascade (24 proteins) and acute phase response (9 proteins) in T2D and control subjects.

Target Full Name	Target	UniProt	Entrez Gene Symbol	T-test Baseline Control vs T2D
Vessel Damage				
Angiotensinogen	Angiotensinogen	P01019	AGT	0.0480
Angiopoietin-1	Angiopoietin-1	Q15389	ANGPT1	0.0190
Angiogenin	Angiogenin	P03950	ANG	0.0680
EGF-containing fibulin-like extracellular matrix protein 1	FBLN3	Q12805	EFEMP1	0.2190
Gelsolin	Gelsolin	P06396	GSN	0.0420
Hemopexin	Hemopexin	P02790	HPX	0.3050
Inter-alpha-trypsin inhibitor heavy chain H4	ITI heavy chain H4	Q14624	ITIH4	0.5620
Lumican	Lumican	P51884	LUM	0.4600
Nidogen-1	Nidogen	P14543	NID1	0.1250
Neuropilin-1	NRP1	O14786	NRP1	0.8850
Periostin	Periostin	Q15063	POSTN	0.1030
Ras-related C3 botulinum toxin substrate 1	RAC1	P63000	RAC1	0.1550
Kallistatin	Kallistatin	P29622	SERPINA4	0.0790
Pigment epithelium-derived factor	PEDF	P36955	SERPINF1	0.5110
Transforming growth factor-beta-induced protein ig-h3	BGH3	Q15582	TGFBI	0.4880
Tenascin	Tenascin	P24821	TNC	0.3090
Vitronectin	Vitronectin	P04004	VTN	0.2940
Platelet degranulation				
Alpha-2-macroglobulin	a2-Macroglobulin	P01023	A2M	0.9240
Clusterin	Clusterin	P10909	CLU	0.1590
Fibronectin	Fibronectin	P02751	FN1	0.9950

(*continued on next page*)

https://doi.org/10.1016/j.metop.2020.100074

Table 1 (*continued*)

Target Full Name	Target	UniProt	Entrez Gene Symbol	T-test Baseline Control vs T2D
Platelet glycoprotein Ib alpha chain	GP1BA	P07359	GP1BA	0.4170
Histidine-rich glycoprotein	HRG	P04196	HRG	0.4440
Integrin alpha-IIb: beta-3 complex	gpIIbIIIa	P08514 P05106	ITGA2B	0.5640
Neutrophil-activating peptide 2	NAP-2	P02775	PPBP	0.0140
Plasma serine protease inhibitor	PCI	P05154	SERPINA5	0.6080
Corticosteroid-binding globulin	CBG	P08185	SERPINA6	0.4170
Thyroxine-binding globulin	Thyroxine-Binding Globulin	P05543	SERPINA7	0.2970
Transgelin-2	Transgelin-2	P37802	TAGLN2	0.6080
von Willebrand factor	vWF	P04275	VWF	0.9860
Coagulation Cascade				
Carboxypeptidase B2	TAFI	Q96IY4	CPB2	0.7930
Prothrombin	Prothrombin	P00734	F2	0.7080
Coagulation Factor V	Coagulation Factor V	P12259	F5	0.1820
Coagulation factor VII	Coagulation Factor VII	P08709	F7	0.8670
Coagulation factor IX	Coagulation Factor IX	P00740	F9	0.0490
Coagulation factor Xa	Coagulation Factor Xa	P00742	F10	0.4140
Coagulation Factor XI	Coagulation Factor XI	P03951	F11	0.8400
Fibrinogen	Fibrinogen	P02671, P02675 P02679	FGA	0.3330
D-dimer	D-dimer	P02671, P02675 P02679	FGA	0.2790
Fibrinogen gamma chain	Fibrinogen g-chain dimer	P02679	FGG	0.3640
Hepatocyte growth factor activator	HGFA	Q04756	HGFAC	0.9840
Plasma kallikrein	Prekallikrein	P03952	KLKB1	0.3700
Kininogen-1	"Kininogen, HMW"	P01042	KNG1	0.0500
Plasminogen	Plasminogen	P00747	PLG	0.3980
Vitamin K-dependent protein S	Protein S	P07225	PROS1	0.0200
Vitamin K-dependent protein C	Protein C	P04070	PROC	0.0500
Alpha-1-antitrypsin	a1-Antitrypsin	P01009	SERPINA1	0.2700
Protein Z-dependent protease inhibitor	protein Z inhibitor	Q9UK55	SERPINA10	0.8930
Antithrombin-III	Antithrombin III	P01008	SERPINC1	0.4490
Heparin cofactor 2	Heparin cofactor II	P05546	SERPIND1	0.0070
Plasminogen activator inhibitor 1	PAI-1	P05121	SERPINE1	0.0060
Alpha-2-antiplasmin	a2-Antiplasmin	P08697	SERPINF2	0.2770
Acute Phase Response				
Serum albumin	Albumin	P02768	ALB	0.0310
Macrophage mannose receptor 1	Macrophage mannose receptor	P22897	MRC1	0.2720
Hepatocyte growth factor-like protein	MSP	P26927	MST1	0.5680
Protein S100-A9	calgranulin B	P06702	S100A9	0.9930
Serum amyloid A-1 protein	SAA	P0DJI8	SAA1	0.6680
Alpha-1-antichymotrypsin	a1-Antichymotrypsin	P01011	GIG25	0.3330
Superoxide dismutase [Cu−Zn]	SOD	P00441	SOD1	0.8690
Serotransferrin	Transferrin	P02787	TF	0.4430
Transketolase	Transketolase	P29401	TKT	0.9740

T2D had higher BMI ($p = 0.0012$) with duration of diabetes 4.5 ± 2.9 years.

For the 60 protein biomarkers reported [1], 11 were found to differ in T2D: for vessel damage, 3 of 16 proteins differed (Angiotensinogen, Angiopoietin-1 and Gelsolin ($p < 0.05$)); for platelet degranulation, 1 of 11 proteins differed (Neutrophil-activating peptide 2 [Pro-platelet basic protein] ($p < 0.014$); for the coagulation cascade, 6 of 24 proteins differed (Coagulation factor IX, Kininogen-1, Vitamin K-dependent protein S, Vitamin K-dependent protein C ($p < 0.05$); Heparin cofactor 2 and Plasminogen activator inhibitor 1 ($p < 0.01$); and for the acute phase response, 1 of 9 proteins differed (Serum albumin ($p < 0.03$)) (Table 1). None of the 11 proteins that differed between T2D and controls altered in response to glucose normalization in the T2D cohort. The functions of the proteins that differed between subjects with and without type 2 diabetes (T2D) are shown in Table 2.

Eleven of the 60 potential biomarkers reported for COVID-19 differed between subjects with T2D and controls, indicating that these potential biomarkers of COVID-19 disease and its severity need to be validated before they can be said to be specifically related to COVID-19 disease. It perhaps is not surprising that significant protein biomarkers described for COVID-19 patients and its disease severity were also found in T2D, affecting biological processes resulting in vessel damage, platelet degranulation, coagulation cascade dysregulation and the acute phase response, perhaps indicating why patients with T2D may be at higher risk for severe COVID-19 disease [5]. The proteins that differed appeared to be independent of changes in glycemia.

Limitations of the study include the small number of subjects and that a different method of proteomic analysis was undertaken compared to others and these may not be directly comparable [1].

In conclusion, of the 60 protein biomarkers that may be of interest in COVID-19 disease and its severity, 11 were found to differ between T2D and controls, and these were unaffected by glycemic changes. These results indicate that stringent validation of proposed biomarkers must be undertaken.

Table 2
The functions of the proteins that differed between subjects with and without type 2 diabetes (T2D).

Protein	Function
Angiotensinogen	Precursor protein of all angiotensin peptides and therefore central to the renin-angiotensin system (RAS) that is primarily involved in the regulation of blood pressure and sodium-water balance. Cleavage of angiotensinogen by renin is the rate limiting step to release Angiotensin I. [6].
Angiopoietin-1	A member of the angiopoietin family of growth factors; required for proper development and maturation of newly forming vessels. Promotes vessel survival, inhibits vascular leakage and suppresses inflammation [7].
Gelsolin	Expressed in the cytoplasm of most cells, functions in cytoskeleton remodelling. Plasma gelsolin acts as part of the actin scavenger system, removing circulating actin filaments released from dead cells; serves as a biomarker of inflammation [8].
Neutrophil-activating peptide 2 (NAP-2)	A cytokine that promotes neutrophil degranulation and chemotaxis. NAP-2 precursors are found in platelets and in the circulation [9]. NAP-2 induces inflammatory responses, may play a role in atherogenesis [10].
Coagulation factor IX (Christmas factor)	A vitamin K-dependent plasma protein involved in the intrinsic blood coagulation pathway; converts factor X to its active form in the presence of Ca^{2+} ions, phospholipids, and factor VIIIa. Factor IX deficiency (haemophilia B) is X-linked and causes a bleeding tendency [11].
Kininogen-1 (HMWK-kallikrein factor)	Part of the blood coagulation system and the kinin-kallikrein system. Kininogen-1 is the precursor protein for high molecular weight kininogen (HMWK), low molecular weight kininogen (LMWK), and bradykinin. HMWK is essential for blood coagulation and in the kallikrein-kinin system. Bradykinin, released from HMWK, influences smooth muscle contraction and is a mediator of inflammation causing increased vascular permeability, stimulation of nociceptors and release of other inflammatory mediators such as prostaglandins; it is cardioprotective and shows antibacterial and antifungal activity [12].
Vitamin K-dependent protein S	An essential anticoagulant protein. Cofactor for activating protein C (APC) to inactivate coagulation factors Va and VIIIa [13]. Mutations in the PROS1 gene cause thrombophilia, with impaired regulation of blood coagulation and a tendency for recurrent venous thrombosis [14]
Vitamin K-dependent protein C	An essential anticoagulant protein, it regulates blood coagulation by inactivating factors Va and VIIIa. Mutations cause thrombophilia, with impaired regulation of blood coagulation and a tendency for recurrent venous thrombosis [15].
Heparin cofactor II	An anti-coagulation factor that inhibits Factor IIa; a cofactor for heparin and dermatan sulfate. Deficiency causes increased thrombin generation and a hypercoagulable state [16].
Plasminogen activator inhibitor 1	A serine protein inhibitor secreted in response to inflammatory reactions. Platelets contain large amounts, and release it during vascular injury; assists in fibrin clot stability. PAI-1 is the main inhibitor of tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA), therefore it is important in regulation of fibrinolysis. Elevated levels of PAI-1 cause deficient plasminogen activation and are associated with a thrombotic tendency [17].
Albumin	The most abundant serum protein; transports hormones, fatty acids, and other compounds, buffers pH, maintains oncotic pressure. Low albumin is caused by liver disease, nephrotic syndrome, burns, protein-losing enteropathy, malabsorption, malnutrition, late pregnancy and malignancy. High albumin is usually caused by dehydration. [18].

Ethics approval and consent to participate

Yorkshire and Humber Research Ethics Committee approved this study that was conducted according to the Declaration of Helsinki. All study participants signed an informed consent form prior to participation.

Consent for publication

All authors gave their consent for publication.

Availability of data and materials

All the data for this study will be made available upon reasonable request to the corresponding author.

Funding

No funding was received to perform this study.

Author contributions

ASMM and AEB analyzed the data and wrote the manuscript. AAQ contributed to study design, performed experiments, collected, analyzed, and interpreted data and edited the manuscript. TS supervised clinical studies and edited the manuscript. SLA contributed to study design, data interpretation and the writing of the manuscript. All authors reviewed and approved the final version of the manuscript. Alexandra E Butler is the guarantor of this work.

Declaration of competing interest

No authors have any conflict of interest or competing interests to declare.

References

[1] Overmyer KA, Shishkova E, Miller IJ, Balnis J, Bernstein MN, Peters-Clarke TM, Meyer JG, Quan Q, Muehlbauer LK, Trujillo EA, He Y, Chopra A, Chieng HC, Tiwari A, Judson MA, Paulson B, Brademan DR, Zhu Y, Serrano LR, Linke V, Drake LA, Adam AP, Schwartz BS, Singer HA, Swanson S, Mosher DF, Stewart R, Coon JJ, Jaitovich A. Large-scale multi-omic analysis of COVID-19 severity. Cell Syst. 2020 Oct 8;S2405-4712(20):30371−9. https://doi.org/10.1016/j.cels.2020.10.003. Epub ahead of print. PMID: 33096026; PMCID: PMC7543711.

[2] Moin ASM, Al-Qaissi A, Sathyapalan T, Atkin SL, Butler AE. Do biomarkers of COVID-19 severity simply reflect a stress response in type 2 diabetes: biomarker response to hypoglycemia. Metab, Clin Exp 2020:154417.

[3] Kahal H, Aburima A, Spurgeon B, Wraith KS, Rigby AS, Sathyapalan T, et al. Platelet function following induced hypoglycaemia in type 2 diabetes. Diabetes Metabol 2018;44:431−6.

[4] Kahal H, Halama A, Aburima A, Bhagwat AM, Butler AE, Grauman J, et al. Effect of induced hypoglycemia on inflammation and oxidative stress in type 2 diabetes and control subjects. Sci Rep 2020;10(1):4750.

[5] Singh AK, Gupta R, Ghosh A, Misra A. Diabetes in COVID-19: prevalence, pathophysiology, prognosis and practical considerations. Diabetes Metab Syndr 2020;14(4):303−10.

[6] Lu H, Cassis LA, Kooi CW, Daugherty A. Structure and functions of angiotensinogen. Hypertens Res 2016;39(7):492−500.

[7] Brindle NP, Saharinen P, Alitalo K. Signaling and functions of angiopoietin-1 in vascular protection. Circ Res 2006;98(8):1014−23.

[8] Piktel E, Levental I, Durnas B, Janmey PA, Bucki R. Plasma Gelsolin: indicator of inflammation and its potential as a diagnostic tool and therapeutic target. Int J Mol Sci 2018;19(9).

[9] Cohen AB, Stevens MD, Miller EJ, Atkinson MA, Mullenbach G. Generation of the neutrophil-activating peptide-2 by cathepsin G and cathepsin G-treated

human platelets. Am J Physiol 1992;263(2 Pt 1):L249–56.
[10] Smith C, Damas JK, Otterdal K, Oie E, Sandberg WJ, Yndestad A, et al. Increased levels of neutrophil-activating peptide-2 in acute coronary syndromes: possible role of platelet-mediated vascular inflammation. J Am Coll Cardiol 2006;48(8):1591–9.
[11] Taylor SA, Duffin J, Cameron C, Teitel J, Garvey B, Lillicrap DP. Characterization of the original Christmas disease mutation (cysteine 206––serine): from clinical recognition to molecular pathogenesis. Thromb Haemostasis 1992;67(1):63–5.
[12] Kerbiriou DM, Griffin JH. Human high molecular weight kininogen. Studies of structure-function relationships and of proteolysis of the molecule occurring during contact activation of plasma. J Biol Chem 1979;254(23):12020–7.
[13] Heeb MJ. Role of the PROS1 gene in thrombosis: lessons and controversies. Expet Rev Hematol 2008;1(1):9–12.
[14] Gomez E, Poort SR, Bertina RM, Reitsma PH. Identification of eight point mutations in protein S deficiency type I–analysis of 15 pedigrees. Thromb Haemostasis 1995;73(5):750–5.
[15] Kovacs KB, Pataki I, Bardos H, Fekete A, Pfliegler G, Haramura G, et al. Molecular characterization of p.Asp77Gly and the novel p.Ala163Val and p.Ala163-Glu mutations causing protein C deficiency. Thromb Res 2015;135(4):718–26.
[16] Salem HH, Thompson EA. The role of heparin cofactor II in the modulation of hemostasis. Dev Biol Stand 1987;67:67–72.
[17] Kruithof EK, Gudinchet A, Bachmann F. Plasminogen activator inhibitor 1 and plasminogen activator inhibitor 2 in various disease states. Thromb Haemostasis 1988;59(1):7–12.
[18] Busher JT. Serum albumin and globulin. In: rd, Walker HK, Hall WD, Hurst JW, editors. Clinical methods: the history, physical, and laboratory examinations. Boston1990.

Abu Saleh Md Moin
Diabetes Research Center (DRC), Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), PO Box 34110, Doha, Qatar
E-mail address: amoin@hbku.edu.qa.

Ahmed Al-Qaissi
Academic Endocrinology, Diabetes and Metabolism, Hull York Medical School, Hull, UK

Leeds Medical School, Leeds, UK
E-mail address: ahmedsattar1978@yahoo.com.

Thozhukat Sathyapalan
Academic Endocrinology, Diabetes and Metabolism, Hull York Medical School, Hull, UK
E-mail address: Thozhukat.Sathyapalan@hyms.ac.uk.

Stephen L. Atkin
Royal College of Surgeons in Ireland Bahrain, Adliya, Bahrain
E-mail address: satkin@rcsi.com.

Alexandra E. Butler*
Diabetes Research Center (DRC), Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), PO Box 34110, Doha, Qatar

* Corresponding author.
E-mail address: abutler@hbku.edu.qa (A.E. Butler).

7 December 2020
Available online 13 December 2020