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

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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 ± 0.4 mmol/l (136.8 ± 7.2 mg/dl), reduced to 4.5 ± 0.07 mmol/l (81 ± 1.2 mg/dl) for 1-h. Controls: 4.9 ± 0.1 mmol/l (88.2 ± 1.8 mg/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 | ITIH4 | Q14624 | ITIH4             | 0.5620                        |
| Lumican           | Lumican | P51884 | LUM               | 0.4600                        |
| Nidogen-1         | Nidogen | P14543 | NID1              | 0.1250                        |
| Neuregulin-1      | NRPI   | Q14786 | NRPI              | 0.8850                        |
| Peristin          | Peristin | 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 | TGFBI | Q15582 | TGFBI            | 0.4880                        |
| Tenasin           | Tenasin | P24821 | TNC               | 0.3090                        |
| Vitronectin       | Vitronectin | P04004 | VTN               | 0.2940                        |
| **Platelet degranulation** |        |         |                   |                               |
| Alpha-2-macroglobulin | a2-Macroglobulin | P01023 | A2M               | 0.9240                        |
| Clusterin         | Clusterin | P10099 | CLU               | 0.1590                        |
| Fibronectin       | Fibronectin | P02751 | FN1               | 0.9950                        |

(continued on next page)
T2D had higher BMI ($p = 0.0012$) with duration of diabetes $4.5 \pm 2.9$ 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 ($p < 0.014$)); 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 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-1b: beta-3 complex | gpIIbIIIa | P08514 | ITGA2B | 0.5640 |
| Neutrophil-activating peptide 2 | NAP-2 | P02775 | PPBP | 0.0140 |
| Plasma serine protease inhibitor | PCI | P05154 | SERPINA5 | 0.6800 |
| Corticosteroid-binding globulin | CBG | P08185 | SERPINA6 | 0.4170 |
| Thromboglobulin | Thromboglobulin | P05543 | SERPINA7 | 0.2970 |
| Transgelin-2 | Transgelin-2 | P37802 | TAGLN2 | 0.6800 |
| von Willebrand factor | vWF | P04275 | vWF | 0.9860 |
| Coagulation Cascade | | | |
| Carboxypeptidase B2 | TAFI | Q6604 | 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 | P26761, P2675, P2679 | FGA | 0.3330 |
| D-dimer | D-dimer | P26761, P2675, P2679 | FGA | 0.2790 |
| Fibrinogen gamma chain | Fibrinogen g-chain dimer | P26769 | FGG | 0.3640 |
| Hepatocyte growth factor activator | HGF | Q04756 | HGFA | 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 | P01008 | SERPINA10 | 0.8930 |
| Antithrombin-III | Antithrombin III | P01008 | SERPIN1C | 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 | MRCl | 0.2720 |
| Hepatocyte growth factor-like protein | MSP | P26027 | MST1 | 0.5680 |
| Protein S100-A9 | calgranulin B | P06702 | S100A9 | 0.9930 |
| Serum amyloid A-1 protein | SAA | P0DJ18 | SAA1 | 0.6680 |
| Alpha-1-antichymotrypsin | a1-Antichymotrypsin | P01011 | GI25 | 0.3330 |
| Superoxide dismutase [Cu-Zn] | SOD | P00441 | SOD1 | 0.8690 |
| Serum transferrin | Transferrin | P02787 | TF | 0.4430 |
| Transketolase | Transketolase | P29401 | TKT | 0.9740 |
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. |
| Angiopoietin-I                                    | A member of the angiopoietin family of growth factors; required for proper development and maturation of newly forming vessels.                                                                                                                                 |
| Gelsolin                                          | Promotes vessel survival, inhibits vascular leakage and suppresses inflammation.                                                                                                                                                                                  |
| 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.                                                                                                                                     |
| 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 |
| 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 nucleopores and release of other inflammatory mediators such as prostaglandins; it is cardioprotective and shows antibacterial and antifungal activity. |
| Vitamin K-dependent protein S                     | An essential antiocoagulant protein. Cofactor for activating protein C (APC) to inactivate coagulation factors Va and Vila. Mutations in the PROS1 gene cause thrombophilia, with impaired regulation of blood coagulation and a tendency for recurrent venous thrombosis. |
| Vitamin K-dependent protein C                     | An essential antiocoagulant protein, it regulates blood coagulation by inactivating factors Va and Vila. Mutations cause thrombophilia, with impaired regulation of blood coagulation and a tendency for recurrent venous thrombosis. |
| 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. |
| 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. |
| 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. |

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.

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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.

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