LETTER TO THE EDITOR

COVID-19 biomarkers for severity mapped to polycystic ovary syndrome

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To the Editor,

Large scale multi-omics analysis has identified significant differences in the biomarkers between COVID-19 disease and control subjects [1]. These protein panels target biological processes involved in vessel damage, platelet degranulation, the coagulation cascade and the acute phase response [1], with greater protein changes dependent on the COVID-19 severity. However, it is observed that in metabolic conditions such as polycystic ovary syndrome expressed proteins differ compared to control women [2] and PCOS patients have increased platelet aggregation and decreased plasma fibrinolytic activity, resulting in a prothrombotic propensity [3, 4], with elevated coagulation markers [5]. Therefore, any biomarkers reflecting COVID-19 disease and its severity would necessarily have to be independent of differentially-expressed proteins relating to other conditions; therefore, this proteomic analysis was undertaken in women with and without PCOS to compare with the proteomic biomarkers recently described in COVID-19 using shotgun proteomics followed by parallel reaction monitoring [1].

146 PCOS and 97 control women who presented sequentially to the Department of Endocrinology, Hull and East Yorkshire Hospitals NHS Trust were recruited to the local PCOS biobank (ISRCTN70196169) [2]. PCOS diagnosis was based on all three Rotterdam consensus diagnostic criteria. Proteins that were identified as being altered 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 [6]. Statistics were performed using Graphpad Prism 8.0.

As reported previously [2], cohorts were age-matched, but PCOS women had increased insulin resistance, androgens and CRP (p < 0.001); systolic and diastolic blood pressure, and waist circumference were higher (p < 0.05).

For the 60 protein biomarkers described previously, 21 were found to differ in PCOS: for vessel damage 7 of 16 proteins differed, for platelet degranulation 2 of 11 proteins differed, for the coagulation cascade 9 of 24 proteins differed and for the acute phase response 3 of 9 proteins differed (Table 1).

These data show that altered protein expression relative to controls may occur in other conditions such as PCOS, and that COVID-19 biomarker changes found between respiratory patients with and without COVID-19 require validation before they can be confirmed to be related to COVID-19 disease and its severity. It is of concern that a number of significant protein biomarkers described for COVID-19 patients and its severity were also found in PCOS, spanning the biological processes involved in vessel damage, platelet degranulation, the coagulation cascade and the acute phase response, perhaps indicating why these patients may be at risk of more severe COVID-19 disease [7].

Limitations of the study include utilizing a different method of proteomic analysis compared to others that may not be directly comparable [1].
| Target full name                                      | Target full name                                      | UniProt   | Entrez gene ID | Entrez gene symbol | T-test PCOS vs control |
|-------------------------------------------------------|-------------------------------------------------------|-----------|----------------|--------------------|------------------------|
| Vessel damage                                         | Vessel damage                                         |           |                |                    |                        |
| Angiotensinogen                                        | Angiotensinogen                                        | P01019    | 183            | AGT                | 0.0240                 |
| Angiopoietin-1                                         | Angiopoietin-1                                         | Q15389    | 284            | ANGPT1             | 0.0760                 |
| Angiogenin                                             | Angiogenin                                             | P03950    | 283            | ANG                | 0.2540                 |
| EGF-containing fibulin-like extra-cellular matrix protein 1 | EGF-containing fibulin-like extra-cellular matrix protein 1 | Q12805    | 2202           | EFEMP1             | 0.4220                 |
| Gelsolin                                               | Gelsolin                                               | P06396    | 2934           | GSN                | 0.0002                 |
| Hemopexin                                              | Hemopexin                                              | P02790    | 3263           | HPX                | 0.4390                 |
| Inter-alpha-trypsin inhibitor heavy chain H4           | Inter-alpha-trypsin inhibitor heavy chain H4           | Q14624    | 3700           | ITIH4              | 0.5360                 |
| Lumican                                                | Lumican                                                | P51884    | 4060           | LUM                | 0.3790                 |
| Nidogen-1                                               | Nidogen-1                                              | P14543    | 4811           | NID1               | 0.9600                 |
| Neuropilin-1                                           | Neuropilin-1                                           | Q14786    | 8829           | NRP1               | 0.0090                 |
| Periostin                                              | Periostin                                              | Q15063    | 10631          | POSTN              | 0.0490                 |
| Ras-related C3 botulinum toxin substrate 1            | Ras-related C3 botulinum toxin substrate 1            | Q15582    | 7045           | TGFBI              | 0.9890                 |
| Tenascin                                               | Tenascin                                               | P24821    | 3371           | TNC                | 0.0020                 |
| Vitronectin                                             | Vitronectin                                             | P04004    | 7448           | VTN                | 0.3930                 |
| Platelet degranulation                                 | Platelet degranulation                                 |           |                |                    |                        |
| Alpha-2-macroglobulin                                  | Alpha-2-macroglobulin                                  | P01023    | 2              | A2M                | 0.2660                 |
| Clusterin                                              | Clusterin                                              | P10909    | 1119           | CLU                | 0.6900                 |
| Fibronectin                                             | Fibronectin                                             | P02751    | 2335           | FN1                | 0.0098                 |
| Platelet glycoprotein lb alpha chain                   | Platelet glycoprotein lb alpha chain                   | GP1BA     | 2811           | GP1BA              | 0.1560                 |
| Histidine-rich glycoprotein                            | Histidine-rich glycoprotein                            | P04196    | 3273           | HRG                | 0.5890                 |
| Integrin alpha-1b-beta-3 complex                      | Integrin alpha-1b-beta-3 complex                      | P08514    | 3674/3690      | ITGA2B/ITGB3       | 0.8370                 |
| Neutrophil-activating peptide 2                       | Neutrophil-activating peptide 2                       | P02775    | 5473           | PPBP               | 0.2740                 |
| Plasma serine protease inhibitor                       | Plasma serine protease inhibitor                       | P05154    | 5104           | SERPINA5           | 0.0380                 |
| Corticosteroid-binding globulin                       | Corticosteroid-binding globulin                       | P08185    | 866            | SERPINA6           | 0.1790                 |
| Thyroxine-binding globulin                             | Thyroxine-binding globulin                             | P05543    | 6906           | SERPINA7           | 0.5730                 |
| Transgelin-2                                           | Transgelin-2                                           | P37802    | 8407           | TAGLN2             | 0.8690                 |
| von Willebrand factor                                  | von Willebrand factor                                  | vWF       | 7450           | vWF                | 0.0770                 |
| Coagulation cascade                                    | Coagulation cascade                                    |           |                |                    |                        |
| Carboxypeptidase B2                                    | Carboxypeptidase B2                                    | TAFI      | Q964Y4         | CPB2               | 0.0170                 |
| Prothrombin                                             | Prothrombin                                             | P00734    | 2147           | F2                 | 0.0920                 |
| Coagulation Factor V                                   | Coagulation Factor V                                   | P12259    | 2153           | F5                 | 0.3020                 |
| Coagulation factor VII                                 | Coagulation factor VII                                 | P08709    | 2155           | F7                 | 0.2520                 |
| Coagulation factor IX                                  | Coagulation factor IX                                  | P00740    | 2158           | F9                 | <0.00001               |
| Coagulation factor Xa                                  | Coagulation factor Xa                                  | P00742    | 2159           | F10                | 0.1620                 |
| Coagulation Factor XI                                  | Coagulation Factor XI                                  | P03951    | 2160           | F11                | 0.5190                 |
| Fibrinogen                                             | Fibrinogen                                             | P02671 P02675 P02679 | 2243 2244 2266 | FGA FGB FGG         | 0.0126                 |
| D-dimer                                                | D-dimer                                                | P02671 P02675 P02679 | 2243 2244 2266 | FGA FGB FGG         | 0.00002                |
| Fibrinogen gamma chain                                 | Fibrinogen gamma chain                                 | P02679    | 2266           | FGG                | 0.00002                |
In conclusion, 21 of 60 protein biomarkers reported in respiratory patients with COVID-19 were found to differ between women with and without PCOS, showing the necessity for validation of such biomarkers, and suggesting that more severe COVID-19 disease may occur in PCOS.

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Authors’ contributions
ASMM and AEB analyzed the data and wrote the manuscript. TS supervised clinical studies and edited the manuscript. SLA contributed to study design, data interpretation and the writing of the manuscript. AEB is the guarantor of this work. All authors read and approved the final manuscript.

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

Ethics approval and consent to participate
The Newcastle & North Tyneside 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.

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

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Table 1 (continued)

| Target full name | Target | UniProt  | Entrez gene ID | Entrez gene symbol | T-test PCOS vs control |
|------------------|--------|---------|----------------|--------------------|------------------------|
| Hepatocyte growth factor activator | HGFA | Q04756  | 3083 | HGFAC | 0.0830 |
| Plasma kallikrein | Prekallikrein | P03952  | 3818 | KLKB1 | 0.0015 |
| Kininogen-1 | Kininogen, HMW | P01042  | 3827 | KNG1 | 0.7970 |
| Plasminogen | Plasminogen | P00747  | 5340 | PLG | 0.6740 |
| Vitamin K-dependent protein S | Protein S | P07225  | 5627 | PROS1 | 0.00003 |
| Vitamin K-dependent protein C | Protein C | P04070  | 5624 | PROC | 0.5110 |
| Alpha-1-antitrypsin | a1-Antitrypsin | P01009  | 5265 | SERPINA1 | 0.1500 |
| Protein Z-dependent protease inhibitor | protein Z inhibitor | Q9UK55  | 51156 | SERPINA10 | 0.1720 |
| Antithrombin-III | Antithrombin III | P01008  | 462 | SERPINC1 | 0.0003 |
| Heparin cofactor 2 | Heparin cofactor II | P05546  | 3053 | SERPIND1 | 0.00003 |
| Plasminogen activator inhibitor 1 | PAI-1 | P05121  | 5054 | SERPINE1 | 0.1680 |
| Alpha-2-antiplasmin | a2-Antiplasmin | P08697  | 5345 | SERPINF2 | 0.7340 |
| Acute phase response | | | | | |
| Serum albumin | Albumin | P02768  | 213 | ALB | 0.0660 |
| Macrophage mannose receptor 1 | | | | | |
| Hepatocyte growth factor-like protein | | | | | |
| Protein S100-A9 | calgranulin B | P06702  | 6280 | S100A9 | 0.0156 |
| Serum amyloid A-1 protein | SAA | P0DJ8  | 6288 | SAA1 | 0.5095 |
| Alpha-1-antichymotrypsin | a1-Antichymotrypsin | P01011  | 12 | SERPINA3 | 0.2672 |
| Superoxide dismutase [Cu–Zn] | SOD | P00441  | 6647 | SOD1 | 0.9448 |
| Serotransferrin | Transferrin | P02787  | 7018 | TF | 0.0185 |
| Transketolase | Transketolase | P29401  | 7086 | TKT | 0.8309 |
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