Association of endopeptidases, involved in SARS-CoV-2 infection, with microbial aggravation in sputum of severe asthma

To the Editor,

COVID-19 can be a serious multisystem disease caused by the SARS-CoV-2 coronavirus, and the current pandemic has affected more than 80 million people and caused nearly two million deaths worldwide. The SARS-CoV-2 virus attaches to angiotensin-converting enzyme 2 (ACE2) receptors on the host cell membrane, with the help of dipeptidyl peptidase 4 (DPP4), both exopeptidases. Cleavage of the virus spike protein (S-protein) by endopeptidases, such as transmembrane protease, serine 2 (TMPRSS2) and furin, occurs following which the virus enters the host cell leading to virus replication. Other enzymes, such as the sialyltransferases, ST6GAL1 and ST3GAL4, play a role for the synthesis of influenza A virus entry receptors; however, their role in SARS-CoV-2 infection has not been elucidated.

Asthma is a chronic inflammatory airway disease affecting 350 million people worldwide. It has not been linked to serious outcomes when presenting with COVID-19 infection, although a higher risk of death has been reported in severe asthma populations. The heterogeneous inflammatory nature of asthma raises the possibility that the type of asthmatic inflammation might determine the outcome of SARS-CoV-2 infection in asthma. Type 2 (T2) inflammatory markers and metagenomics -diversity measures. The median ES computed between endopeptidase ES and sputum inflammatory α markers and metagenomics -diversity measures. The median ES computed between endopeptidase ES and sputum inflammatory α markers and metagenomics -diversity measures. The median ES computed between endopeptidase ES and sputum inflammatory α markers and metagenomics -diversity measures. The median ES computed between endopeptidase ES and sputum inflammatory α markers and metagenomics -diversity measures. 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is equal to zero. Subsequently, subjects were subdivided into two groups according to their ESs, that is, endopeptidase-high (ES >0, n = 60) and endopeptidase-low expression group (ES <0, n = 60). These were compared according to sputum inflammatory markers, metagenomics α-diversity measures, and gene expression of the exopeptidases, ACE2, DPP4, and sialyltransferases (ST3GAL4 and ST6GAL1). The two groups were also compared with respect to current intake of antibiotics, oral corticosteroid (OCS), OCS normalized dosage (in mg), and history of hypertension and diabetes diagnoses. The differential bacterial abundance between endopeptidase groups was computed using edgeR after relative log expression normalization, while proteomics differential abundance was computed using limma. Pathway enrichment analysis of differentially abundant proteins in the endopeptidase-high group was performed using the Reactome database in g: Profiler (https://biit.cs.ut.ee/gprofiler/gost).

Severe nonsmoking (n = 61) and smoking (n = 23) asthmatics showed the highest median expression ES of endopeptidase as compared to mild-moderate asthmatics (n = 20) and healthy controls (n = 16) (Figure 1A), consistent with previous findings. The endopeptidases ESs were significantly correlated with sputum neutrophil absolute counts (r = 0.55, p = 7.7 × 10−11) and percentages (r = 0.58, p = 2.4 × 10−12), which suggests that the endopeptidases were neutrophil-derived. The endopeptidases ESs were inversely associated with bacterial α-diversity measures (r, for observed species = −0.44, Shannon = −0.38, Chao1 = −0.46, Simpson = −0.35, all ps < 1 × 10−5).

**FIGURE 1** (A) Protease (endopeptidases) genes enrichment scores (ES) in induced sputum were compared between the 4 U-BIOPRED adult subcohorts. (B) Sputum neutrophils (in absolute counts) were compared between endopeptidase-high and endopeptidase-low groups. (C) Sputum eosinophils (in absolute counts) were compared between endopeptidase-high and endopeptidase-low groups. (D) Different metagenomics α-diversity measures (observed, Shannon, Chao1, and Simpson) were compared between endopeptidase-high and protease-low groups. (E) ACE2 and DPP4 expression in induced sputum was compared between endopeptidase-high and protease-low groups. (F) ST3GAL4 and ST6GAL1 gene expression in induced sputum were compared between endopeptidase-high and endopeptidase-low groups. Analysis was performed using two-tailed Mann-Whitney U and Kruskal-Wallis H tests as appropriate.
The endopeptidase-high group (mean age = 50.9 ± 13.2, 53.3% females) had higher sputum neutrophils (Figure 1B), with no differences in sputum eosinophils (Figure 1C), and exhibited reduced bacterial α-diversity measures as compared with the endopeptidase-low group (mean age = 48.2 ± 14.6 years, 53.3% females) (Figure 1D). In addition, the endopeptidase-high group had a higher abundance of pathogenic bacteria, such as Moraxella catarrhalis and Haemophilus influenzae, displaying a pattern of pathogenic bacterial aggravation compared with endopeptidase-low group (Figure 2A), while the latter had a higher abundance of commensal bacteria, such as Rothia and Prevotella species. The endopeptidase-high group showed higher sputum expression of the exopeptidases, ACE2 and DPP4 (Figure 1E), and...
inadequate phagocytic capacity of macrophages, which might lead to a sequence of the disturbed immune system in severe asthma such as late that the presence of airway bacterial imbalances might be a consequence of innate immunity, neutrophil degranulation, cytokines signaling, Toll-like receptor, and platelet activation (Figure 2C). In serum, there was a higher levels of IL-6, IL-18, and C-reactive protein in the endopeptidase-high group. These findings suggest that appropriate stratification of asthma patients is necessary to adequately estimate risk and/or morbidity of SARS-CoV-2 infection. The neutrophilia observed in the endopeptidase-high group might be directly associated with pathogenic bacteria aggravation in this group. This may suggest that these pathogenic bacteria presence or “blooming” is aggravating the immune system and changing the overall microbial population. In addition, we speculate that the presence of airway bacterial imbalances might be a consequence of the disturbed immune system in severe asthma such as inadequate phagocytic capacity of macrophages, which might lead to higher risk of infections. In this cohort, clusters of severe asthma patients that exhibited bacterial aggravation were relatively stable after 12-18 months, which suggest impairment of immune system over relatively long periods of time. Second, this bacterial aggravation might be associated with comorbid conditions, such as hypertension and diabetes, which are known risk factors for more severe COVID-19. In our study, the endopeptidase-high group showed higher gene expression of exopeptidases ACE2 (associated with hypertension) and DPP4, and the sialyltransferase ST3GAL4 (associated with diabetes) compared with the endopeptidase-low group, which might indicate the pathophysiologic involvement of both diseases in the endopeptidase-high group. However, there were no significant associations between endopeptidases high/low groups and reported history of diabetes and hypertension diagnosis in the included subjects (data not shown). Therefore, future studies are needed to explore whether both diseases may influence the airway microbiome composition in asthmatics.

The present findings suggest that personalized therapies, such as those targeting neutrophils (eg, anti-IL-17), endopeptidase inhibitors (eg, nephrilysin inhibitors), and/or antimicrobial compounds, might be tailored to asthma patients with high risk of SARS-CoV-2 infection.

In conclusion, these findings in sputum highlight that it is important to assess overall microbial profile in relation to SARS-CoV-2-associated proteases in order to adequately assess risk of infection in patients with severe neutrophilic asthma.

ACKNOWLEDGEMENTS
We would like to thank all the patients who gave written and signed consent to take part in the U-BIOPRED study. The study is registered on ClinicalTrials.gov (identifier: NCT01976767). IA is supported by the EPSRC (EP/T003189/1), the UK MRC (MR/T010371/1) and by the Wellcome Trust (208340/Z/17/Z).

FUNDING INFORMATION
Innovative Medicines Initiative, Grant/Award Number: 115010; European Federation of Pharmaceutical Industries and Associations; Seventh Framework Programme, Grant/Award Number: FP7/2007–2013

CONFLICT OF INTEREST
SED reports personal fees from AZ, Cayman Chemicals, GSK, Merck, Novartis, Regeneron, Sanofi, Teva, outside the submitted work. RD reports receiving fees for lectures at symposia organized by Novartis, AstraZeneca and TEVA, consultation for TEVA and Novartis as member of advisory boards, and participation in a scientific discussion about asthma organized by GlaxoSmithKline. RD is a co-founder and current consultant, and has shares in Synairgen, a University of Southampton spin out company. PJS reports grants from Innovative Medicines Initiative (IMI) covered by the European Union and the European Federation of Pharmaceutical industries and Associations (EFPIA), during the conduct of the study. AHM has received research grants outside the submitted work from GSK, Boehringer Ingelheim, and Vertex, and she is the PI of a P4O2 (Precision Medicine for more Oxygen) public-private partnership sponsored by Health Holland involving many private partners that contribute in cash and/or in kind (Boehringer Ingelheim, Breathomix, Fluidia, Ortec LogicCare, Philips, Quantib-U, Smartfish, SODAQ, Thirona, TopMD, and Novartis), and she has served in advisory boards for AstraZeneca, GSK, and Boehringer Ingelheim with money paid to her institution. KFC has received honoraria for participating in Advisory Board meetings of GSK, AZ, Roche, Novartis, Merck, BI, and Shionogi regarding treatments for asthma, chronic obstructive pulmonary disease, and chronic cough and has also been remunerated for speaking engagements. All other co-authors have nothing to disclose.

FUNDING INFORMATION
U-BIOPRED has received funding from the Innovative Medicines Initiative (IMI) Joint Undertaking under grant agreement no. 115010, resources of which are composed of financial contributions from the European Union’s Seventh Framework Programme (FP7/2007–2013), and European Federation of Pharmaceutical Industries and Associations (EFPIA) companies’ in-kind contributions (www.imi.europa.eu).

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Additional supporting information may be found online in the Supporting Information section.