Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

BJA

British Journal of Anaesthesia, xxx (xxx): xxx (xxxx)

CORRESPONDENCE

Sex differences in immunological responses to COVID-19: a cross-sectional analysis of a single-centre cohort

Nishkantha Arulkumaran[1], Timothy A. C. Snow[1], Adarsh Kulkarni[1], David Brealey[2,3], Hannah M. Rickman[4], Chloe Rees-Spear[5,6,7], Moira J. Spyer[6], Judith Heaney[8], Edmund Garr[7], Bryan Williams[9], Peter Cherepanov[10], George Kassiotis[8,11], Michael P. Lunn[2,4], Catherine Houlihan[4], Laura E. McCoy[2,5], Eleni Nastouli[1] and Mervyn Singer[1,*]

[1]Bloomsbury Institute of Intensive Care Medicine, University College London, London, UK, [2]Department of Clinical Virology, University College London, London, UK, [3]Clinical Research Department, London School of Hygiene and Tropical Medicine, London, UK, [4]Department of Clinical Virology, University College London Hospital and Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK, [5]Department of Infection, Immunity and Inflammation, UCL Great Ormond Street Institute of Child Health, London, UK, [6]Advanced Pathogen Diagnostics Unit, Department of Clinical Virology, University College London Hospitals NHS Trust, London, UK, [7]Biology of Infection Laboratory, The Francis Crick Institute, London, UK, [8]Neuroimmunology and CSF Laboratory, University College London Hospitals, National Hospital of Neurology and Neurosurgery, London, UK, [9]NIHR University College London Hospitals (UCL) Biomedical Research Centre, UK, [10]Retroviral Immunology Laboratory, The Francis Crick Institute, London, UK and [11]Centre for Neuromuscular Disease, National Hospital for Neurology and Neurosurgery, London, UK

*Corresponding author. E-mail: nisharulkumaran@doctors.org.uk

Keywords: ARDS; COVID-19; cytokines; inflammation; sex

Editor—COVID-19 is associated with greater severity of illness and mortality in men compared with women. Although many lifestyle factors and co-morbidities may be more prevalent among men, most COVID-19 deaths are independently associated with advancing age, male sex, and comorbidity burden.[1,2] Differences in immune responses to COVID-19 may underpin sex-specific outcome differences. We hypothesised that this might contribute to the pathophysiology of COVID-19, and examined sex differences in physiology, viral loads, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific antibody titres, and plasma cytokines on hospital admission in patients with COVID-19 who had not received immunomodulatory therapies.

Ethical approval was received from the London—Westminster Research Ethics Committee, the Health Research Authority and Health and Care Research Wales on July 2, 2020 (REC reference 20/HRA/2505, IRAS ID 284088). Blood samples taken from patients ≥18 yr old within 5 days of admission to University College London Hospitals with polymerase chain reaction-proven COVID-19 from March 1 to June 30, 2020 were used for cytokine and antibody quantification (Supplementary data). Outcomes were determined using the WHO COVID-19 ordinal severity scale, with a score of 1 defined by no limitation of activities, increasing to 6 for those requiring noninvasive ventilation and additional organ support, and 10 for death.[3]

We included 86 patients, including 30 women and 56 men, with available serum samples (Supplementary Table S1). There were no differences in age, days from symptom onset to hospital admission, SpO_2/FiO_2 ratio, temperature, lymphocyte count, neutrophil count, or viral load between male and female patients. Compared with females, male patients had higher levels of C-reactive protein (CRP) ($P=0.03$), creatinine ($P<0.001$), and haemoglobin ($P<0.001$) and a lower platelet

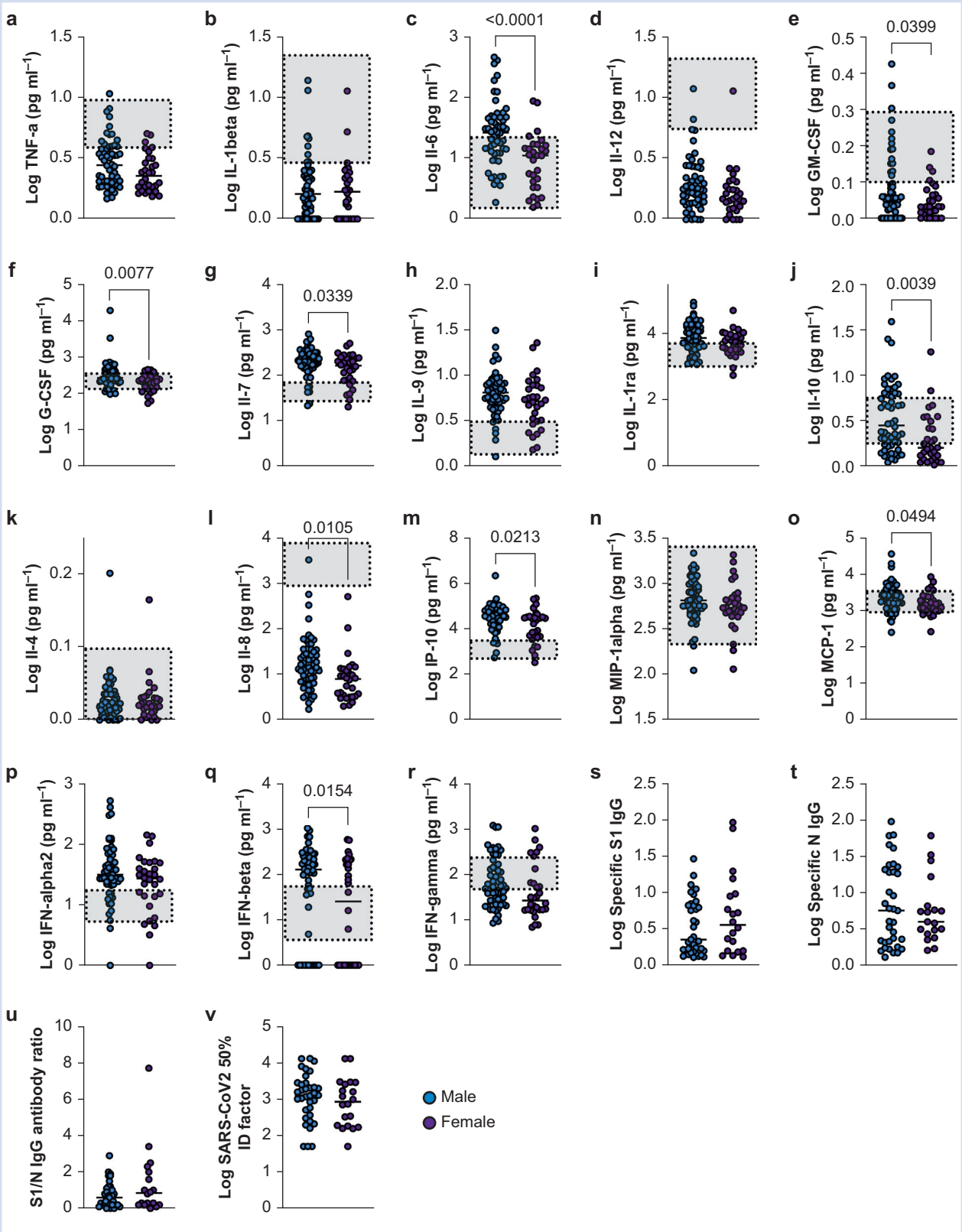

Fig 1. A number of cytokines were significantly lower among female patients (n=30) compared with male patients (n=56), including IFN-beta (−0.5636 [0.2279]; P=0.0154), G-CSF (−0.2029 [0.07437]; P=0.008), GM-CSF (−0.03877 [0.01857]; P=0.040), IL-6 (−0.4667 [0.1134]; P<0.001), IL-8 (−0.3376 [0.1289]; P=0.011), IL-7 (−0.1602 [0.07425]; P=0.034), IP-10 (−0.3448 [0.1469]; P=0.021), MCP-1 (−0.1622 [0.08133]; P=0.049), and IL-10 (−0.2284 [0.07701]; P=0.004). Data are presented as mean differences; differences between groups were analysed using the Mann–Whitney test. The grey box represents the range seen in healthy volunteers. G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IP-10, interferon-gamma inducible protein 10; MCP-1, monocyte chemoattractant protein-1.

count (P=0.01) (Supplementary Fig. S1). Similar proportions of male and female patients had mild disease, diabetes mellitus, or hypertension, were smokers, or required organ support. A similar proportion of males and females died in hospital (27% *vs* 17%; P=0.27). A total of 55 (65%) patients seroconverted on admission to hospital, with no significant difference in proportion of men and women. However, levels of granulocyte colony-stimulating factor (G-CSF; P=0.01), granulocyte–macrophage colony-stimulating factor (GM-CSF; P=0.04), interleukin (IL)-6 (P<0.001), IL-8 (P=0.01), IL-7 (P=0.03), interferon-gamma inducible protein 10 (IP-10; P0.02), monocyte chemoattractant protein-1 (MCP-1; P=0.049), and IL-10 (P=0.004) were higher in males than females (Fig. 1).

On stratification of patients with mild disease (WHO <6) or those who progressed to severe disease or death (WHO ≥6), differences between sexes persisted for levels of interferon (IFN)-beta, IL-9, IL-6, IL-10, and IP-10 among patients with mild disease. Females with severe disease had cytokine levels comparable with those of male patients (Supplementary Fig. S2). Level of correlation between different cytokine levels, biochemical results, and physiological variables was higher among women compared with men (Supplementary Fig. S2). Correlation between IP-10, IL-6, and IL-10 levels was significant in women. In contrast, correlation between these cytokines was minimal or non-existent among men.

Higher cytokine levels among males compared with females, despite similar age, viral load, degree of hypoxaemia at presentation, and requirement for organ support, may represent an exaggerated host immune response in males. Among female patients with severe disease, levels of cytokines were similar to those of male patients. This is congruent with recent clinical trials investigating either anti-IL-6 monoclonal antibodies or steroids, in which the benefits were seen predominantly in males and in patients with greater illness severity who required advanced respiratory support.[4,5]

Greater expression of virus entry factors (angiotensin-converting enzyme 2 [ACE2]) and accessory proteases (transmembrane serine protease 2 [TMPRSS2] and cathepsin L [CTSL]) in airway secretory cells and alveolar type 2 cells may explain the greater cytokine levels in male patients.[6] However, we found similar viral titres (C_t values) between males and females, suggesting that increased viral burden does not completely explain the differences in host response between males and females. In addition to differences in cytokine levels between sexes, poor T cell response is associated with worse disease outcome in male patients, but not in female patients.[7] Therapies aimed at modulating sex hormones show promise and warrant further attention.[8] A higher correlation between most cytokines was seen in female but not in male patients, in particular IL-6, IL-10, and IP-10, cytokines associated with increased mortality risk in patients with COVID-19.[9] The clinical implications of this are unclear, but may represent a dysregulated host response to COVID-19 among male patients.

The lack of statistical significance in mortality difference between sexes may be explained by the relatively small sample size in this study. Our data are observational and are hypothesis generating, and limited by the small sample size. We have focused on serological markers on hospital admission but not the trajectory of cytokines over time. Although we cannot exclude the possibility that measured cytokine levels represent different time points in the illness between different patients, the times from symptom onset to hospital admission were similar between males and females. In addition, we lack data on more diverse elements of the immune system including immune cell responses. However, the advantage of studying serological markers is the potential to apply them as therapeutic and prognostic biomarkers.

Despite these limitations, we provide detailed analysis of a panel of cytokines and anti-SARS-CoV-2 antibodies in a cohort of patients with COVID-19 who are naïve to immunomodulators. Our findings provide an important basis to further investigate a sex-based approach to the stratification and treatment of patients with COVID-19.

Acknowledgements

We thank Chris Wilson and Atul Goyale at UCLH Biochemistry for their assistance with patients' sera identification.

Declarations of interest

MeS reports grants and advisory board fees from NewB, grants from the Defense Science and Technology Laboratory, Critical Pressure, Apollo Therapeutics, advisory board and speaker fees (paid to his institution) from Amormed, Biotest, GE, Baxter, Roche, and Bayer, and an honorarium for chairing a data monitoring and safety committee from Shionogi.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2021.05.013.

References

1. Ioannou GN, Locke E, Green P, et al. Risk factors for hospitalization, mechanical ventilation, or death among 10131 US veterans with SARS-CoV-2 infection. *JAMA Netw Open* 2020; **3**, e2022310
2. Peckham H, de Gruijter NM, Raine C, et al. Male sex identified by global COVID-19 meta-analysis as a risk factor for death and ITU admission. *Nat Commun* 2020; **11**: 6317
3. WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection. A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis* 2020; **20**: e192–7
4. Recovery Collaborative Group, Horby P, Lim WS, et al. Dexamethasone in hospitalized patients with Covid-19. *N Engl J Med* 2021; **384**: 693–704
5. Recovery Collaborative Group. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet* 2021; **397**(10285): 1637–45
6. Muus C, Luecken MD, Eraslan G, et al. Single-cell meta-analysis of SARS-CoV-2 entry genes across tissues and demographics. *Nat Med* 2021; **27**: 546–59

7. Takahashi T, Ellingson MK, Wong P, et al. Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature* 2020; **588**: 315–20
8. Ghandehari S, Matusov Y, Pepkowitz S, et al. Progesterone in addition to standard of care vs standard of care alone in the treatment of men hospitalized with moderate to severe COVID-19: a randomized, controlled pilot trial. *Chest* 2021. https://doi.org/10.1016/j.chest.2021.02.024. Advance Access published on February 20
9. Laing AG, Lorenc A, Del Molino Del Barrio I, et al. A dynamic COVID-19 immune signature includes associations with poor prognosis. *Nat Med* 2020; **26**: 1623–35

doi: 10.1016/j.bja.2021.05.013