Received: 13 October 2021 | Revised: 21 November 2021 | Accepted: 29 November 2021

DOI: 10.1111/dom.14608

RESEARCH LETTER

WILEY

Preserved C-peptide in survivors of COVID-19: Post hoc analysis

Sophie A. Clarke MBBS[1,2] | Maria Phylactou MBBS[1,2] | Bijal Patel MBBS[1] | Edouard G. Mills MBChB[1] | Beatrice Muzi MSc[1] | Chioma Izzi-Engbeaya MBBS[1,2] | Bernard Khoo MBChB Cantab[3] | Karim Meeran MBBS[1,2] | Alexander N. Comninos MBBS[1,2] | Ali Abbara MBBS[1,2] | Tricia Tan MBChB[1,4] | Nick Oliver MBBS[1,2] | Waljit S. Dhillo MBBS[1,2]

[1]Division of Diabetes, Endocrinology and Metabolism, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, UK

[2]Department of Endocrinology, Imperial College Healthcare NHS Trust, London, UK

[3]Department of Endocrinology, Division of Medicine, Faculty of Medical Sciences, Royal Free Campus, University College London, London, UK

[4]Department of Clinical Biochemistry, Imperial College Healthcare NHS Trust, London, UK

Correspondence
Waljit S. Dhillo, MBBS, Section of Endocrinology and Investigative Medicine, Division of Diabetes, Endocrinology and Metabolism, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, W12 0NN, UK.
Email: w.dhillo@imperial.ac.uk

Funding information
National Institute for Health Research, Grant/Award Number: CS-2018-18-ST2-002

1 | INTRODUCTION

Hyperglycaemia[1] and new diagnoses of diabetes (including autoantibody-negative type 1 diabetes[2]) have been reported in patients with coronavirus disease 2019 (COVID-19).[1] Moreover, 77% of patients with COVID-19 and ketoacidosis (a condition typically associated with type 1 diabetes or pancreatic destruction) had type 2 diabetes.[3] The receptor necessary for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cellular entry, ACE2, and its permissive protein, TMPRSS2, are present in the pancreatic microvasculature[4] and, at postmortem, SARS-CoV-2 viral RNA has been detected in pancreatic beta cells.[5] In islets infected with SARS-CoV-2, in vitro insulin secretion, particularly glucose-stimulated insulin secretion, is attenuated.[6] Given this, it has been speculated that insulin secretory capacity may be curtailed by direct cytopathic action of SARS-CoV-2 on beta cells. However, it is unknown whether such changes are of clinical relevance in survivors of COVID-19.

Random non-fasting C-peptide (rCP) levels correlate with stimulated C-peptide levels derived during mixed meal tests,[7] and have high sensitivity and specificity compared with the gold standard threshold of 600 pmol/L considered to indicate insulin deficiency.[8] It remains to be determined whether there is evidence for deficient insulin secretion, consistent with beta cell destruction, in survivors of COVID-19. Therefore, we assessed rCP in patients at least 3 months after COVID-19 infection to assess longer term beta cell secretory capacity.

2 | METHODS

Ethical approval was provided by the London Bridge Research Ethics Committee (REC ref. 20/HRA/4110). This study was registered with the international standard randomised controlled trial number (ISRCTN) registry (ISRCTN15615697) and performed in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to inclusion.

The study protocol has previously been described.[9] Participants were survivors of COVID-19 aged 18 years or older diagnosed with

COVID-19 who attended Imperial College London NHS Healthcare Trust during March-November 2020, or who had responded to adverts placed on social media asking for patients who had tested positive for COVID-19 and then attended at least 3 months following diagnosis with COVID-19. Diagnosis of COVID-19 was confirmed using either real-time RT-PCR testing of a nasopharyngeal swab, confirmatory imaging (chest radiograph or CT scan), or a positive serum SARS-CoV-2 IgG antibody test after symptom onset. Exclusion criteria included those prescribed steroids following recovery from COVID-19, and those taking other medications known to affect cortisol-binding globulin (including the combined oral contraceptive pill, and hormone replacement therapy). Similarly, patients with underlying health conditions or states known to influence cortisol-binding globulin (including pregnancy, end-stage renal failure, or underlying malignancy) were also excluded.

Severity of COVID-19 was determined according to World Health Organization (WHO) classification.[10] Serum glucose and insulin were measured using an Abbott Alinity ci-series analyser. The lower limits of detection were as follows: insulin 2.9 pmol/L, glucose 0.03 mmol/L. The inter-assay coefficients of variation were: insulin 2.1%, glucose 1%. The intra-assay coefficients of variation were: insulin 2.2%, glucose 1.2%. Serum C-peptide was measured using an Abbott Architect analyser. The lower limit of detection was 3.3 pmol/L, the inter-assay coefficient of variation was 4.0%, and the intra-assay coefficient of variation was 2.8%. Serum fructosamine was measured using the Roche Cobas kit on the Abbott Architect platform (colorimetric method); the intra-assay coefficient of variation was less than 0.5% at 299 and 256 μmol/L. The reference range was 205-500 μmol/L.

Serum antibodies to SARS-CoV-2 N protein (IgG) were measured using Abbott Architect assay. For those with an indeterminate result, additional testing for antibodies to SARS-CoV-2 spike protein (RBD) (IgG) was performed using Imperial Hybrid DABA.[11]

Study visits commenced 8:00 AM-9:30 AM and were non-fasted. Serum samples were taken for measurement of insulin, glucose, and C-peptide levels. Fifty-four of 55 patients (98%) had complete insulin, C-peptide, and glucose measurements (one participant had missing data for C-peptide levels and was excluded from further analysis).

Statistical analysis was performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA). Distribution of data was assessed by D'Agostino and Pearson normality tests. Data were presented as mean ± standard deviation (SD) if parametrically distributed, or median with interquartile range (IQR) if not. Student's *t*-test, or one-way analysis of variation (ANOVA), was used to compare two or more groups respectively, of parametrically distributed data. Mann–Whitney *U* test, or Kruskal–Wallis test with post hoc Dunn's test, was used to compare two or more groups respectively, of non-parametrically distributed data. Multivariable linear regression was used to assess the ability of selected variables to predict C-peptide, including age, sex, ethnicity, pre-existing diabetes, body mass index (BMI) and severity of COVID-19 disease, according to the WHO severity index.

3 | RESULTS

3.1 | Baseline characteristics

In total, 55 participants attended for assessment at a median of 233 days post–COVID-19 infection. Of these, 15 (27.8%) had pre-existing type 2 diabetes; none had type 1 diabetes. No participants received a new diagnosis of type 2 diabetes following COVID-19 infection (Table 1). All participants with pre-existing diabetes were on metformin but none were treated with insulin.

3.2 | C-peptide is preserved at follow-up at ≥3 months following presentation with COVID-19

At the study visit appointment, which took place at least 3 months after initial presentation with COVID-19, median (IQR) C-peptide was 1319 (849, 1905) pmol/L and no patients had undetectable C-peptide levels (<3.3 pmol/L). Of the study cohort, 14.8% ($n = 8$) had C-peptide levels of less than 600 pmol/L with normoglycaemic contemporaneous glucose concentrations of 4.7-5.7 mmol/L, none of whom had known diabetes (Figure 1A).

3.3 | C-peptide levels and pre-existing diabetes

C-peptide values at follow-up at least 3 months after initial presentation with COVID-19 compared with those without (median [IQR] C-peptide [pmol/L]: pre-existing diabetes 1550 [1285, 2638], no diabetes 1239 [675, 1622], $P = .008$) (Figure 1B). There was no relationship between C-peptide and pre–COVID-19 HbA1c levels for those for whom data were available ($n = 12$) ($P = .81$), or with convalescent fructosamine levels ($P = .85$) (Figure S1B).

3.4 | C-peptide and COVID-19 disease severity and management

C-peptide levels were higher in those who had severe or critical disease compared with those with mild disease (median [IQR] C-peptide mild: 762 [510, 1289] pmol/L, severe/critical: 1538 [1143, 2906] pmol/L, $P = .004$) (Figure 1C). These data remained similar when those with diabetes were excluded (median [IQR] C-peptide mild: 675 [503, 1152] pmol/L, severe/critical: 1510 [1007, 2906] pmol/L). There was no correlation between C-peptide and either peak CRP or procalcitonin levels during acute admission with COVID-19 infection for those for whom data were available ($n = 25$). Similarly, C-peptide levels were not related to admission glucose level for those for whom data were available ($n = 26$).

The majority ($n = 33$, 61.1%) did not receive steroid treatment for acute COVID-19. However, there was no relationship between

TABLE 1 Baseline characteristics of patients attending ≥3 months following presentation with COVID-19

Participant characteristics	Total cohort ($n = 55$)
Age (y)	54.5 (12.8)
Sex:	
Male	35 (63.6%)
Female	20 (36.4%)
Ethnicity:	
Asian	16 (29.1%)
Black	4 (7.3%)
Mixed	2 (3.6%)
Other—not stated	10 (18.2%)
White	23 (41.8%)
Co-morbidities:	
Hypertension	18 (32.7%)
Cardiovascular disease	6 (10.9%)
Diabetes	
Type 1	0 (0.0%)
Type 2	15 (27.3%)
Unspecified	1 (1.8%)
Obesity (BMI > 30 kg/m^2)	
Yes	17 (30.9%)
No	33 (60%)
Unknown	5 (9.1%)
Smoking status:	
Current smoker	2 (3.6%)
Ex-smoker	2 (3.6%)
Never smoked	50 (90.9%)
Unknown	1 (1.8%)
Disease outcome:	
Hospitalized	41 (74.5%)
NIV	2 (3.6%)
ITU admission	4 (7.3%)
Non-hospitalized	14 (25.5%)
Disease severity:	
Mild	12 (21.8%)
Moderate	21 (38.1%)
Severe	17 (30.9%)
Critical	5 (9.1%)
Dexamethasone treatment	20 (36.4%)
Cumulative dose dexamethasone treatment (mg)	0.0 (0.0, 36.0)
Additional treatments:	
Remdesivir	13 (23.6%)
Tocilizumab	1 (1.8%)
Conv. plasma	0 (0.0%)
Other	(5.5%)
Diabetes treatment:	
Metformin	16 (100%)
Sulphonylurea	1 (6.3%)
DPP4 inhibitor	3 (18.8%)
GLP-1 agonist	1 (6.3%)
Duration of admission (d)	5 (0, 8)
Time since presentation (d)	233 (99.8, 283)

Note: Data are means (SD) for continuous variables parametrically distributed and medians (lower quartile, upper quartile) for continuous non-parametrically distributed variables. For categorical data, numbers of patients (percentages) are presented. Disease severity was determined by the World Health Organization severity index. For further details, please see the supporting information (supplementary methods).

Abbreviations: BMI, body mass index; Conv. plasma, convalescent plasma; DPP4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; ITU, intensive therapy unit; NA, not applicable; NIV, non-invasive ventilation; other, additional treatments include anakinra (an interleukin-1 receptor antagonist), Kaletra (lopinavir/ritonavir), and namilumab.

C-peptide levels and cumulative dexamethasone dose ($P = .32$) (Figure 1D). When ethnicity, sex, age, disease severity, BMI, and pre-existing diabetes were assessed using multivariable linear regression, only a history of diabetes predicted C-peptide at follow-up ($P = .001$). The proportion of participants with diabetes was not different between those with mild, moderate, or severe/critical disease ($P = .20$).

4 | DISCUSSION

Hyperglycaemia in patients with COVID-19 is associated with increased mortality rates,[12] and results from both impaired insulin secretion and increased peripheral insulin resistance.[13] However, the extent to which these causes of dysglycaemia persist beyond acute infection is unclear.

Both critical[14] and viral illnesses increase peripheral insulin resistance, particularly in people with pre-existing metabolic disorders.[6] Furthermore, the viruses themselves may contribute to beta cell destruction and dysfunction,[15] with cytomegalovirus,[16] Epstein–Barr virus,[17] rotavirus,[18] rubella virus, and enterioviruses (including coxsackievirus B) all being associated with pancreatic beta cell apoptosis and subsequent type 1 diabetes.[15] Similarly, SARS-CoV-2 induces pancreatic islet cell apoptosis,[19] and thus has the potential to result in beta cell destruction and resultant insulinopaenia. These data assess the clinical relevance of such findings.

In this analysis of 54 survivors of COVID-19, all participants (including those with pre-existing type 2 diabetes) had detectable C-peptide levels.

Although C-peptide levels were not associated with peak procalcitonin or CRP levels, C-peptide values were greater in participants with severe or critical disease compared with those with mild disease. Similar to data from national cohorts,[20] BMI was also greater in those who had had critical disease compared with those with mild disease (Figure S1), which may explain this observation.

Although not all the participants received steroid treatment, C-peptide levels at follow-up were not different in those who had received dexamethasone. While the use of steroids probably contributes to insulin resistance during acute COVID-19, it does not appear to have a deleterious legacy effect on insulin secretion at follow-up.

Finally, while critical and viral illness-induced insulin resistance[14] probably explains a large proportion of the dysglycaemia observed, given that C-peptide levels were preserved at follow-up, these data suggest that any insulinopaenia during acute COVID-19 is not apparent at 3 months or more after presentation. These data are consistent with findings from other studies where it has been shown that, while a minority of patients with acute COVID-19 display characteristics of beta cell failure, in contrast to other causes of acute respiratory distress syndrome, the predominant cause of hyperglycaemia observed in patients with acute COVID-19 results from insulin resistance.[21] Furthermore, patients with COVID-19 have been observed to have reduced adiponectin levels, consistent with adipose tissue dysfunction, providing a potential mechanism for the insulin resistance observed.[21] Taken together, given the deleterious impact of hyperglycaemia on outcomes in patients with COVID-19,[22] agents that improve insulin resistance, such as metformin or thiazolidinediones, could have clinical utility, and hold potential as a focus for future research.

This post hoc analysis of data prospectively collected from survivors of COVID-19 is limited in that participants were not fasted. However non-fasted C-peptide is reflective of stimulated C-peptide levels in other clinical settings, and can provide a measure of pancreatic islet cell insulin secretory capacity. While the study cohort is small, and subject to survivor bias, and therefore must be interpreted with caution, it offers initial insights into COVID-19–related hyperglycaemia, and some reassurance that while dysglycaemia may persevere post initial infection, islet cell insulin secretory capacity is not permanently impacted. Further research into the underlying pathophysiology of hyperglycaemia in patients with COVID-19 is underway;

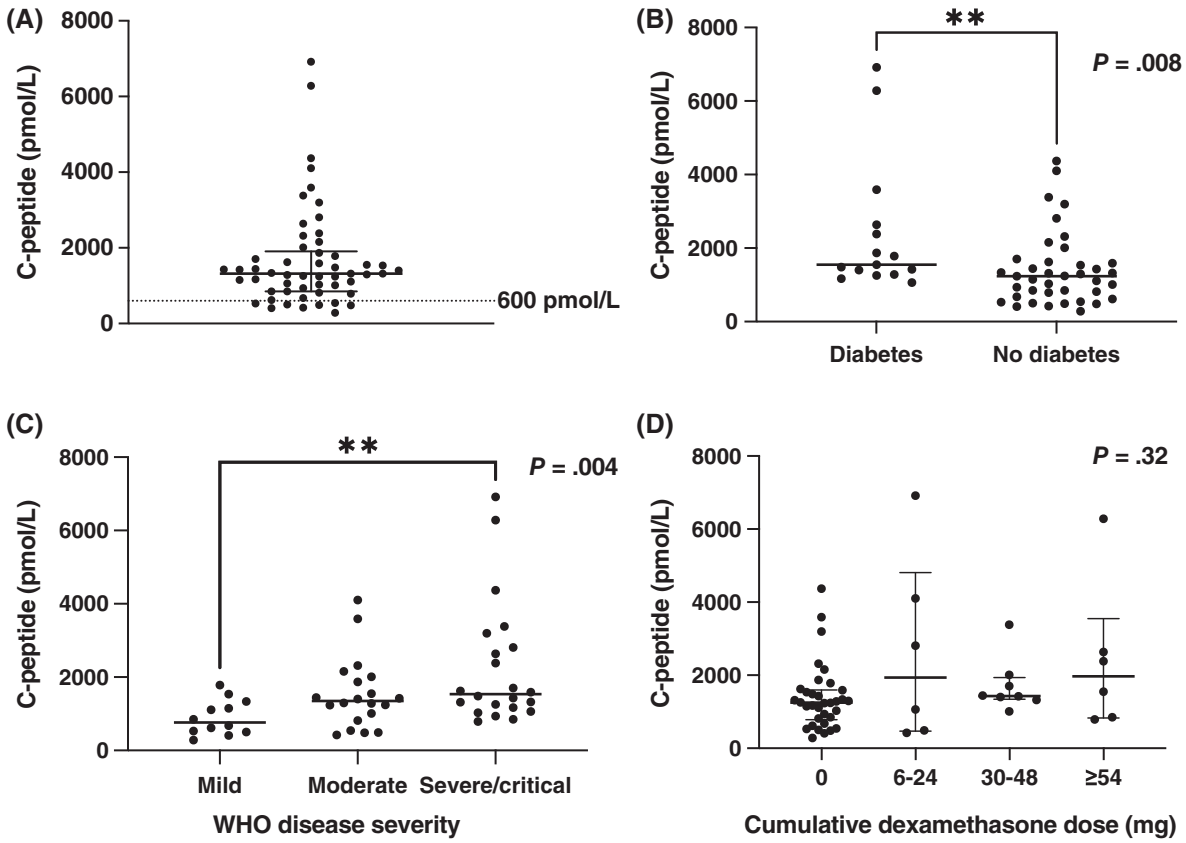

FIGURE 1 A, Individual random C-peptide levels (pmol/L) are presented. A value of 600 pmol/L (correlates with insulinopaenia) is shown. B, Random C-peptide levels (pmol/L) in patients with pre-existing diabetes and no pre-existing diabetes are presented. Median (IQR) C peptide (pmol/L): pre-existing diabetes 1550 (1285, 2638), no diabetes 1239 (675, 1622), $P = .008$. C, Random C-peptide levels (pmol/L) are presented according to World Health Organization (WHO) disease severity (mild: $N = 12$, moderate: $N = 20$, severe/critical: $N = 22$). Median (IQR) C-peptide mild: 762 (510, 1289) pmol/L, severe/critical: 1538 (1143, 2906) pmol/L, $P = .004$. D, Random C-peptide levels (pmol/L) are presented according to cumulative dose of dexamethasone received (0 mg: $N = 34$, 6-24 mg: $N = 6$, 30-48 mg: $N = 8$, ≥54 mg: $N = 6$), $P = .32$. Median (IQR) C-peptide mild: 762 (510, 1289) pmol/L, severe/critical: 1538 (1143, 2906) pmol/L, $P = .004$. ** denotes $P \leq 0.01$

however, we provide important data to aid clinicians managing survivors of COVID-19.

ACKNOWLEDGEMENTS

This work was supported by the NIHR Imperial Clinical Research Facility and NIHR Imperial Biomedical Research Centre at Imperial College Healthcare NHS Trust. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health and Social Care. The Section of Endocrinology and Investigative Medicine is funded by grants from the UK Medical Research Council (MRC), Biotechnology and Biological Sciences Research Council, and NIHR. S.A.C. is funded by an NIHR Clinical Lectureship. A.A. is funded by an NIHR Clinician Scientist Award (CS-2018-18-ST2-002); and W.S.D. is funded by an NIHR Senior Investigator Award. N.O. is funded by the Wynn Chair of Human Metabolism.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

S.A.C., T.T., N.O., and W.S.D. conceived and designed the study. S.A.C., M.P., B.P., E.G.M., B.M., and C.I.-E. undertook study visits and performed data collection. S.A.C., B.K., K.M., A.N.C., A.A., T.T., N.O., and W.S.D. undertook analysis of the data. S.A.C. prepared the manuscript and all the authors reviewed and adjusted the manuscript as necessary. W.S.D. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/dom.14608.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are not publicly available due to patient confidentiality but are available from the corresponding author on reasonable request.

ORCID

Chioma Izzi-Engbeaya https://orcid.org/0000-0001-7599-0166
Tricia Tan https://orcid.org/0000-0001-5873-3432
Waljit S. Dhillo https://orcid.org/0000-0001-5950-4316

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Clarke SA, Phylactou M, Patel B, et al. Preserved C-peptide in survivors of COVID-19: Post hoc analysis. *Diabetes Obes Metab*. 2022;24(3):570-574. doi:10.1111/dom.14608