THE LANCET
Diabetes & Endocrinology

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Tan T, Khoo B, Mills E G, et al. Association between high serum total cortisol concentrations and mortality from COVID-19. *Lancet Diabetes Endocrinol* 2020; published online June 18. https://doi.org/10.1016/S2213-8587(20)30216-3.

Supplementary: Higher serum total cortisol levels are associated with increased mortality from COVID-19

Methods

This was a cohort study of patients admitted to Imperial College Healthcare NHS Trust (ICHNT) hospitals (St Mary's Hospital, Charing Cross Hospital, Hammersmith Hospital) between Mar 9, 2020 to Apr 22, 2020 who were suspected to have COVID-19 infection. As part of the workup, a standardised panel of blood tests were performed including full blood count, creatinine, CRP, D-dimer and serum total cortisol. Clinical and demographic data was extracted from patient records. Pre-selected demographics and co-morbidities of interest (age, sex, history of diabetes, hypertension, chronic kidney disease (CKD), cardiovascular disease (CVD), endocrine disease, current diagnosis of cancer at time of admission, obstructive pulmonary disease including asthma and chronic obstructive pulmonary disease, current pregnancy) were recorded, and contemporaneous pre-selected laboratory values of interest (cortisol, creatinine, D-dimer, CRP, neutrophil to leukocyte ratio or N:L ratio) were recorded.

All analytes were measured at North West London Pathology, a UK Accreditation Service accredited laboratory, using Abbott Alinity series analysers except for D-dimer which was measured using Stago STA R Max3 analysers. Cortisol was measured by an Abbott Alinity ci-series analyser utilising a chemiluminescent microparticle immunoassay. The precision of the cortisol assay was ≤10% total coefficient of variation (CV) for serum samples with cortisol levels ≥83 to ≤966 nmol/L, and the lower limit of detection was 22 nmol/L. The cross-reactivity of cortisone in this assay is minimal (2·7% at 1000 µg/dl).

COVID-19 infection was defined as a positive diagnosis based on a real time RT-PCR confirmation of infection from a nasopharyngeal swab, or if patients exhibited typical radiological and clinical characteristics of COVID-19 infection despite a negative result from swabbing. The first cortisol measurement from each admission episode was utilised for analysis. Cases were excluded from analysis if the measurement was not taken within 48 hours of admission or of diagnosis of COVID-19, if patients were documented as having received exogenous glucocorticoid treatment at the time of measurement, having a confirmed diagnosis of hypoadrenalism, or if the cortisol measurements were done as part of a dynamic function test (e.g. Synacthen test). Mortality outcomes were recorded as of a database lock date of May 8, 2020.

Data processing and statistical analysis were conducted using R 3.6.3 (R Foundation for Statistical Computing) and the packages "tidyverse 1.3.0", "survival 3.1–12", "survminer 0.4.6", "ggpubr 0.3.0". Where noted, a log2 transformation was used for laboratory analytes exhibiting non-Gaussian distributions with right skew. For the Cox proportional hazards modelling, diagnostic plots for residuals and the Schoenfeld test were used to check for the influence of outliers and that the assumption of proportional hazards was met, and these were found to be satisfactory (data not shown). Values that were missing were assumed 'missing at random'. In the multivariable Cox proportional hazards model, cases with missing values were excluded as noted in the results.

Results

Demographics and outcomes

Supplementary Table 1 summarises the demographics, clinical characteristics and laboratory values of the cohort analysed in this study. Supplementary Table 2 summarises the outcomes during the study period (to May 8, 2020) for patients diagnosed with COVID-19 in each category.

Supplementary Table 1: Characteristics of patients diagnosed with COVID-19 and without COVID-19. COPD = chronic obstructive pulmonary disease. Categorical data shown as number (percentage). Normally distributed continuous variables displayed as mean (SD), otherwise as median [IQR]. * p<0·05, ** p<0·01, ***p<0·001, **** p<0·0001 (Student t-test or Wilcoxon test). †††† p<0·0001 (Chi-square test)

COVID-19 status		**No**	**Yes**
Number (%)		132 (25%)	403 (75%)
SARS-CoV2 swab	**Positive**	N/A	356 (88%)
	Negative: clinical diagnosis		47 (12%)
Age (yr)		64·5 (19·1)	66·3 (15·7)
Age (stratified)	**<45**	26 (19·7%)	33 (8·2%)
	45–59	21 (15·9%)	109 (27·1%)
	60–74	36 (27·3%)	115 (28·5%)
	≥75	49 (37·1%)	146 (36·2%)
Weight (kg)		74·0 (21·0)	79·0 (21·6)
Sex	**Male**	70 (53·0%)	240 (59·6%)
	Female	62 (47·0%)	163 (40·4%)
Diabetes		30 (22·8%)	160 (39·7%)
Hypertension		54 (40·9%)	191 (47·4%)
Current diagnosis of cancer		17 (12·9%)	38 (9·4%)
COPD/asthma		26 (19·7%)	63 (15·6%)
Chronic kidney disease		19 (14·4%)	50 (12·4%)
Cardiovascular disease		37 (28·0%)	94 (23·3%)
Pregnancy		0	2 (0·5%)
Endocrine disease		12 (9·1%)	37 (9·2%)
Length of stay (days)		5 [3–9]	7 [4–12]
Deaths (to May 8, 2020)		9 (6·8%)	112 (27·8%)††††
Cortisol (nmol/L)		519 [378–684]	619 [456–833]***
CRP (mg/L)		42·0 [9·5–123·5]	117·8 [58·2–180·4]****
D-dimer (ng/ml)		1365 [627–3163]	1403 [773–2851]
Neutrophil:Leukocyte (N:L) ratio		5·8 [3·3–11·3]	6·4 [3·9–10·7]
Creatinine (µmol/L)		87·5 [67–128·3]	84 [68·5–116·5]

Supplementary Table 2: Outcomes (as of May 8, 2020) in patients diagnosed with COVID-19 organised by category. COPD = chronic obstructive pulmonary disease. CKD = chronic kidney disease. CVD = cardiovascular disease.

Category		**Discharged**	**In patient**	**Deaths**
All patients diagnosed with COVID-19		271 (67%)	20 (5%)	112 (28%)
SARS-CoV-2 swab status	**Positive**	231 (65%)	20 (6%)	105 (29%)
	Negative	40 (85%)	0	7 (15%)
Age (stratified)	**<45**	31 (94%)	1 (3%)	1 (3%)
	45–59	83 (76%)	12 (11%)	14 (13%)
	60–74	79 (69%)	4 (3%)	32 (28%)
	≥75	78 (53%)	3 (2%)	65 (45%)
Sex	**Male**	157 (65%)	13 (5%)	70 (29%)
	Female	114 (70%)	7 (4%)	42 (26%)
Co-morbidities	**Diabetes**	91 (57%)	11 (7%)	58 (36%)
	Hypertension	110 (58%)	10 (5%)	71 (37%)
	Current diagnosis of cancer	20 (53%)	1 (3%)	17 (45%)
	COPD/asthma	45 (71%)	3 (5%)	15 (24%)
	CKD	19 (38%)	1 (2%)	30 (60%)
	CVD	56 (60%)	3 (3%)	35 (37%)
	Pregnancy	2 (100%)	0	0
	Endocrine disease	22 (59%)	3 (8%)	12 (32%)

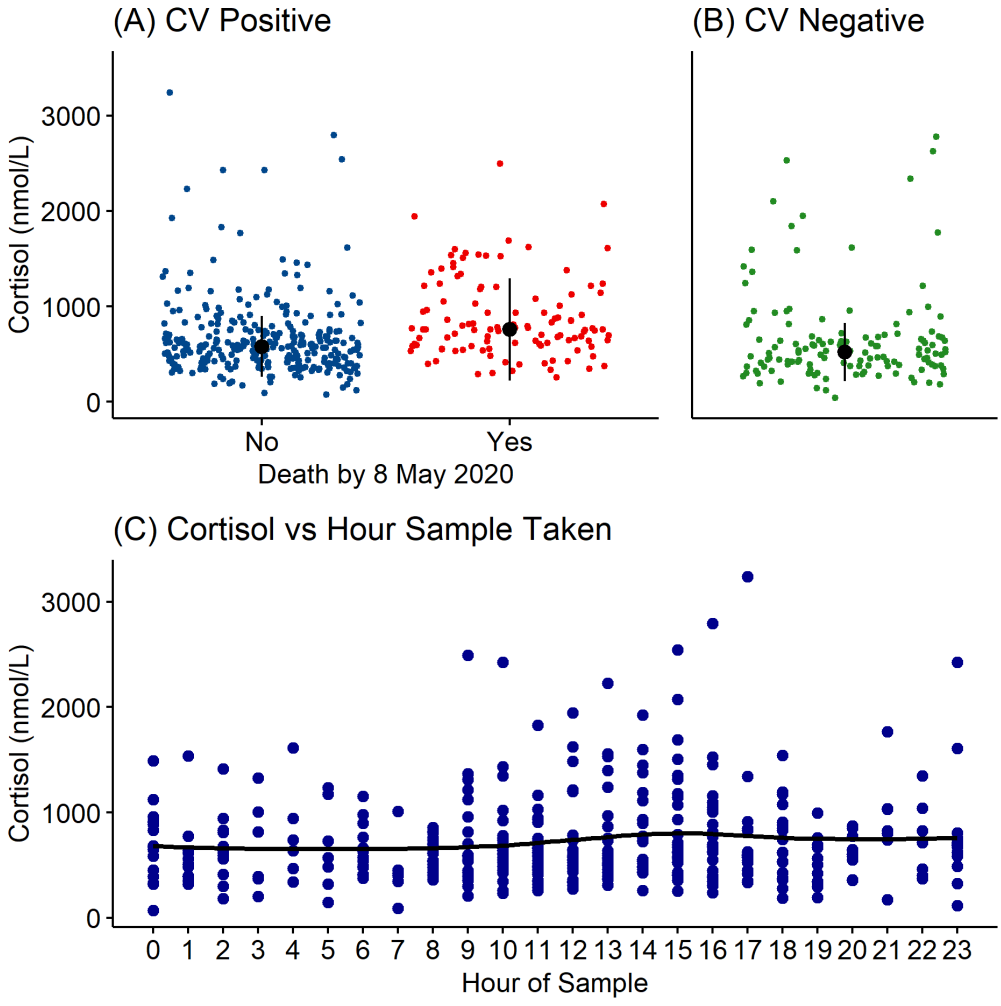
Acute patient mortality from COVID-19 is associated with higher baseline cortisol levels, as well as other markers of inflammation and renal dysfunction

To examine the simple relationship of the selected laboratory parameters to acute mortality from COVID-19 (without adjustment for covariates), we looked at the distribution of laboratory values comparing those patients who survived vs those who died in the 403 patients diagnosed with COVID-19. In those that died, we observed:

- significantly higher levels of cortisol (median [IQR] for survivors 578 [429–747] vs non-survivors 760 [592–1128] nmol/L, Wilcoxon rank sum test p<0·0001, Supplementary Figure 1A and B);
- significantly higher levels of creatinine (survivors 77 [66–102] vs non-survivors 118 [85–294] µmol/L, p<0·0001);
- significantly higher levels of CRP (survivors 102·95 [53·45–171·68] vs non-survivors 142·95 [75·3–210·9] mg/L, p=0·0003);
- significantly higher levels of D-dimer (survivors 1259 [709–2614] vs non-survivors 1752 [1068–3485] ng/ml, p=0·0017); and
- significantly higher N:L ratio (survivors 5·95 [3·70–9·48] vs non-survivors 8·5 [4·80–13·35], p<0·0001).

There was no discernible diurnal relationship between the cortisol level and the time of day/night that the sample was taken (Supplementary Figure 1C).

Supplementary Figure 1: Cortisol levels in patients with and without COVID-19 infection, and in relation to time of sampling. Dot-plot of cortisol values in patients with (A) and without (B) COVID-19 infection (black dot indicates the median and the line indicates interquartile range). (C) Bottom panel shows cortisol values in patients with COVID-19 plotted in relation to the clock hour that the sample was taken (locally estimated scatterplot smoothing [LOESS] line in black shown).



Univariable analysis shows that age, presence of co-morbidities, higher cortisol, CRP, N:L ratio and creatinine are associated with acute mortality from COVID-19

Cox proportional hazards modelling was used to discern statistically significant associations between acute mortality from COVID-19 and individual demographic, clinical and laboratory parameters (utilising log2 transformations to take account of their skewed distributions). Supplementary Table 3 shows that age <75 yr was associated with reduced hazard ratios (HR) for mortality from COVID-19 in comparison to the reference mortality in patients ≥75. The presence of the co-morbidities diabetes, hypertension, current diagnosis of cancer, CKD, and CVD were associated with increased HR for mortality. Sex, body weight, the presence of obstructive pulmonary disease or previous endocrine disease (hypoadrenalism having been excluded) were not significantly associated with acute mortality in this cohort. The log2-transformed values of cortisol, CRP, N:L ratio and creatinine were associated with significantly increased HR for mortality but not D-dimer.

Supplementary Table 3: Univariable analysis of demographic, clinical, laboratory parameters with acute mortality in patients diagnosed with COVID-19.

Pregnancy was not assessed in the univariable analysis as no patients with pregnancy in the cohort died. Beta = natural log of hazard ratio. HR = hazard ratio. COPD = chronic obstructive pulmonary disease. CKD = chronic kidney disease. CVD = cardiovascular disease. + = incorporated into subsequent multivariable model as indicated.

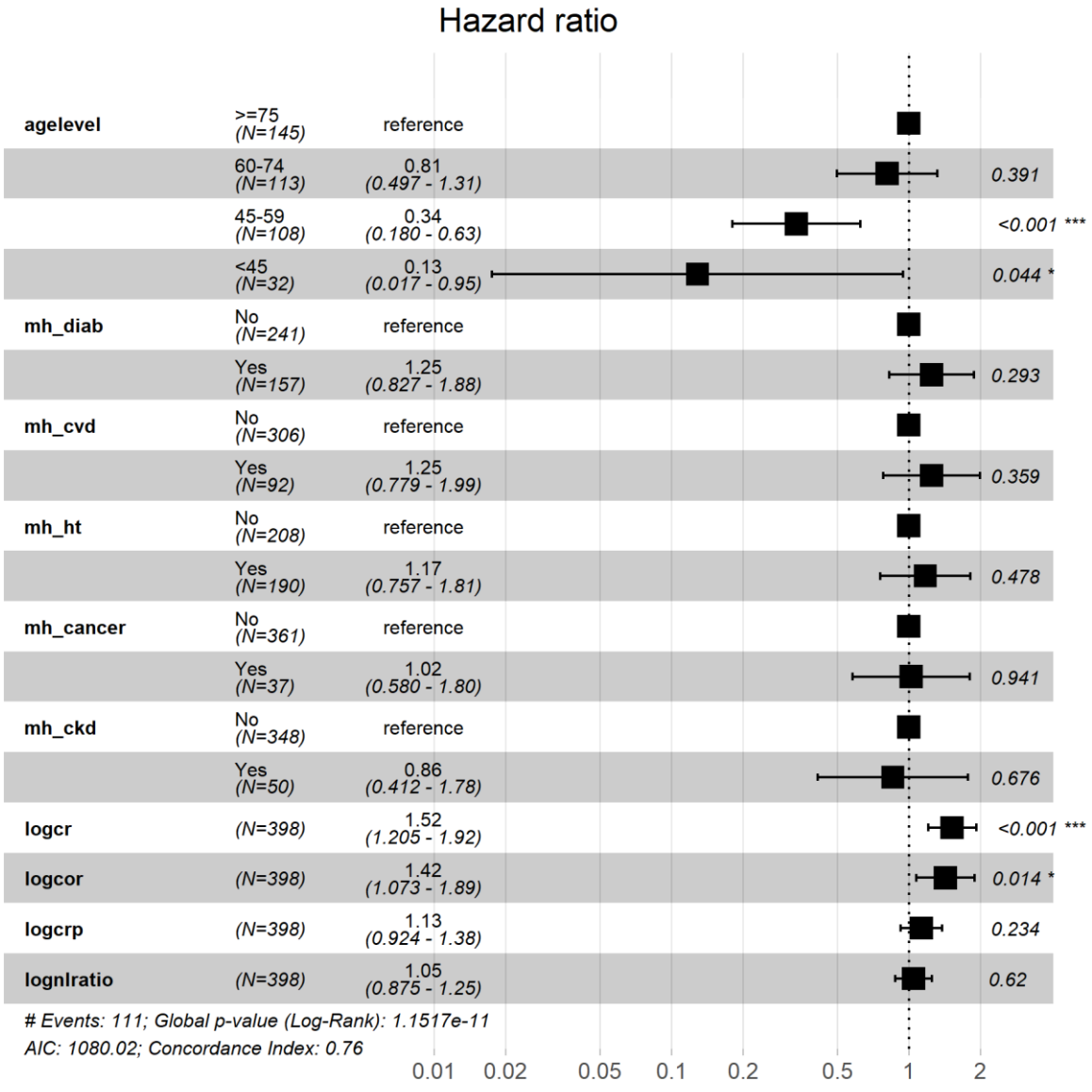
Predictors		beta	HR (95% CI for HR)	Wald test	P-value	Model
Age (stratified)	≥75	1·0 (reference)				+
	60–74	-0·54	0·58 (0·38–0·89)	1·23	0·0121	
	45–59	-1·29	0·28 (0·15–0·49)	1·99	<0·0001	
	<45	-2·56	0·08 (0·01–0·56)	2·54	0·011	
Sex (Female)		-0·24	0·79 (0·54–1·16)	1·44	0·229	
Weight		0·004	1·00 (0·99–1·02)	0·61	0·434	
Diabetes		0·43	1·54 (1·06–2·23)	5·14	0·023	+
Hypertension		0·59	1·81 (1·23–2·66)	9·11	0·003	+
Current cancer		0·57	1·76 (1·05–2·96)	4·62	0·032	+
COPD/asthma		-0·20	0·82 (0·47–1·42)	0·51	0·475	
CKD		0·99	2·69 (1·76–4·11)	20·80	<0·0001	+
CVD		0·53	1·71 (1·14–2·55)	6·83	0·009	+
Endo disease		0·03	1·03 (0·57–1·88)	0·01	0·917	
log2 cortisol		0·33	1·40 (1·11–1·75)	8·18	0·004	+
log2 D-dimer		0·04	1·04 (0·92–1·18)	0·37	0·542	
log2 CRP		0·17	1·18 (1·00–1·40)	3·99	0·046	+
log2 N:L ratio		0·20	1·23 (1·03–1·46)	5·27	0·022	+
log2 Creatinine		0·48	1·62 (1·42–1·84)	53·90	<0·0001	+

Multivariable analysis identifies age, creatinine and cortisol as significantly associated with mortality

A multivariable Cox proportional hazards model including only demographic and clinical characteristics identified as univariate predictors with p-values <0·2 was constructed (Supplementary Table 3). This model included 398 subjects and 111 events after removal of 5 subjects with missing data. After adjustment for these covariates, we identified age <60 as predictive of a reduced risk of acute mortality (HR for age <45 years 0·13 [0·017–0·95], p=0·044; age 45–59 years 0·34 [0·18–0·63], p<0·001). The presence of the co-morbidities of diabetes, CVD, hypertension, current diagnosis of cancer, or CKD were not associated with increased HR for acute mortality in this dataset, nor were the CRP and N:L ratios. For every doubling of creatinine, the HR increased by 52% (p<0·001). Per doubling of cortisol levels there is an increase of 42% in the adjusted hazard ratio for mortality after allowing for the above-mentioned covariates (p=0·014, Supplementary Figure 2).

Supplementary Figure 2: Forest plot of multivariable model

Estimated hazard ratios and 95% CI plotted. agelevel = age, stratified as shown; mh_diab = diabetes; mh_cvd = cardiovascular disease; mh_ht = hypertension, mh_cancer = current diagnosis of cancer, mh_ckd = chronic kidney disease, logcor = log2 cortisol, logcrp = log2 CRP, lognlratio = log2 N:L ratio, logcr = log2 creatinine.



Statements

Ethics: The study was approved by the Imperial College London and Imperial College Healthcare NHS Trust governance team who confirmed that as we are reporting on routinely collected non-identifiable clinical audit data, no approval from a research ethics committee was additionally required under the UK policy framework for Health and Social Care.

Author Contributions: TT, BK, KM, ANC, AA and WSD conceptualised the study. EGM, MP, BM, PCE, LT and BP collected and recorded the demographic, clinical and laboratory data. TT and BK analysed the data. TT, BK and EGM drafted the manuscript. BK, TT and WSD revised all subsequent versions of the manuscript. ATP provided advice on statistical analysis. All authors read and approved the final manuscript.

Acknowledgements: We are grateful to all the nursing, medical and allied healthcare professionals at Imperial College Healthcare NHS Trust for their dedication in caring for the patients included in this study. We would like to acknowledge the assistance of Paul Nacmanson (North West London Pathology).

Funding: This study presents independent work funded by the National Institute for Health Research (NIHR) and supported by the NIHR Imperial Clinical Research Facility and NIHR Imperial Biomedical Research Centre (BRC) 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 (BBSRC), and NIHR. TT is funded by the NIHR and the JP Moulton Charitable Foundation. BK is funded by the JP Moulton Charitable Foundation. EGM is funded by an MRC Clinical Research Training Fellowship (MR/T006242/1). AA is funded by an NIHR Clinician Scientist Award (CS-2018–18-ST2–002). WSD is funded by an NIHR Professorship (RP-2014–05–001).

Data sharing: An anonymized dataset and data analysis code is available upon application to the corresponding author.

Declaration of interest: We declare no competing interests.