PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Association between Biomarkers and COVID-19 Severity and Mortality: A Nationwide Danish Cohort Study
AUTHORS	Hodges, Gethin; Pallisgaard, Jannik; Schjerning Olsen, Anne-Marie; McGettigan, Patricia; Andersen, Mikkel; Krogager, Maria; Kragholm, Kristian; Køber, Lars; Gislason, Gunnar; Torp-Pedersen, Christian; Bang, Casper N.

VERSION 1 – REVIEW

REVIEWER	yan kang Department of Critical Care Medicine， West China Hospital, Sichuan University, Chengdu
REVIEW RETURNED	06-Jul-2020

GENERAL COMMENTS	1, The manuscript identifies the association between several biomarkers and poor outcomes in patients with COVID-19 in Denmark. 2, There are currently more than 20 studies of biomarkers for COVID-19 in the past 6 months. There is limited innovation in terms of the types of biomarkers and main results. 3, The author should add multi-factor regression analysis to identify the most possible risk factors for the outcomes. 4, More than 50% of the data were missed in D-dimer, ferritin, troponin and PCT, which makes the results less convinced in terms of the selection bias. 5, The mortality in this study is as high as 20.1%, which is relative higher comparing other studies. Therefore, it's better to analyze the reasons of death and describes the indication of ICU admission.

REVIEWER	Amer Harky UK
REVIEW RETURNED	28-Jul-2020

GENERAL COMMENTS	The methods and study design are clearly outlines by the authors, patient selection was appropriate. Your data summary is very to the point which I must commend you for this. I wonder if you can manage to do regression analysis and perhaps a multi-variate anaylsis correlating the biomarkers such as CRP, Leuck, Ferritin, Creatinine, D-Dimer, Trop...etc and death? a single variate analysis is often associated with lot so bias. We all know that old age, presence of cardiovascular comorbidities..etc are associated with increased mortality rate in patients with COVID-19, there is handful of evidence on this. There is a recent systematic review which discussed those

	biomarkers in more depth which I recommend you to read and cite it in your paper to strengthen your conclusion. Kermali M, Khalsa RK, Pillai K, et al. The role of biomarkers in diagnosis of COVID-19 - A systematic review. Life Sci. 2020;254:117788. doi:10.1016/j.lfs.2020.117788

REVIEWER	ZhibingLu Wuhan University, China
REVIEW RETURNED	09-Aug-2020

GENERAL COMMENTS	In this retrospective cohort study, 1310 inpatients with COVID-19 were analyzed to evaluate the association between common biomarkers, death and ICU admission. The authors should address the following points: 1.453 admitted patients were excluded from the analysis because of no available biochemistry data. What were the clinical characteristics of these patients? How did they compare to those included? 2.There are extensive data in the tables, which should be presented in a clearer way. Tables with three lines were recommended in the manuscript. 3.Most of the biological markers studied in this paper have been widely reported, the authors should give more prominence to the innovation of this article.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name

yan kang

Institution and Country

Department of Critical Care Medicine ， West China Hospital, Sichuan University, Chengdu

Please state any competing interests or state ‘None declared’:
None declared

Please leave your comments for the authors below
1, The manuscript identifies the association between several biomarkers and poor outcomes in patients with COVID-19 in Denmark.
R2, There are currently more than 20 studies of biomarkers for COVID-19 in the past 6 months. There is limited innovation in terms of the types of biomarkers and main results.

A2. Thank you for this comment. We recognise that the COVID-19 is a fast-moving topic and at the original time of submission there were already several studies involving biomarkers. However, we referred to the existing studies in the introduction and noted that despite many studies involving biomarkers, there were few large studies, especially with respect to well-defined endpoints. We maintain our position that our study is still of merit given it is a large, European cohort with well-defined endpoints.

R3, The author should add multi-factor regression analysis to identify the most possible risk factors for the outcomes.

A3. Thank you for this very relevant comment. We agree that a multi-factor regression analysis would be valuable. However, because of missing data for more specialist biomarkers such as D-dimer, procalcitonin and Troponin. Furthermore, the additional analysis required in building a multivariate risk model (including a validation cohort) was felt to be beyond the scope of this paper. We believe the study results in their current form are still of value and interest to the reader and may help identify clinical important biomarkers. In reference to your suggestion however, we have included a multivariable analysis of the most represented biomarker, CRP, to show that CRP remains strongly associated to the composite endpoint independent of several comorbidities. Please see the additional text and figure 3 and Table S3 below:

(Page 7, line 23)
"As part of a sensitivity analysis, we performed a Cox multivariate regression analysis to assess CRP in relation to the combined endpoint of all-cause mortality and ICU admission (adjusted for age, gender, diabetes, chronic obstructive pulmonary disease, hypertension and ischemic heart disease)."
(Page 9, line 17)
"In a multivariate model, elevated CRP was independently associated with death/ICU admission after adjusting for age, gender, diabetes, chronic obstructive pulmonary disease, hypertension and ischemic heart disease (Figure 4; Supplementals, Table S3*)."*

Figure 4: 30-day absolute risk for the composite outcome of death or ICU admission, adjusted for CRP level, age, gender, diabetes, chronic obstructive pulmonary disease, hypertension and ischemic heart disease. Legend: CRP, C-reactive protein; ICU, intensive care unit.

Variable	Hazard ratio (95% CI)	p
CRP 100-400 mmol/L	**10.32 (2.56 – 41.61)**	**0.001**
CRP elevated to 99 mmol/L	**4.89 (1.21-19.81)**	**0.026**
Age, years	**1.04 (1.03-1.05)**	**<0.001**
Male sex	**1.54 (1.23-1.93)**	**<0.001**
Hypertension	**1.01 (0.81-1.26)**	**0.924**
Ischemic heart disease	**0.91 (0.66-1.24)**	**0.533**
COPD	**1.26 (0.93-1.71)**	**0.142**
Diabetes	**1.25 (0.97-1.62)**	**0.087**
CI, confidence interval; COPD, chronic obstructive pulmonary disease; IQR, inter-quartile range.		

Table S3: Multivariable Cox regression analysis for the composite outcome of death or ICU admission, adjusted for CRP level, age, gender, diabetes, chronic obstructive pulmonary disease, hypertension and ischemic heart disease. Legend: CRP, C-reactive protein; ICU, intensive care unit.

R4, More than 50% of the data were missed in D-dimer, ferritin, troponin and PCT, which makes the results less convinced in terms of the selection bias.

A4. Thank you for this observation. We agree with this comment and acknowledge this in the limitations section, including reference to the selection bias as you mention:

"Furthermore, patients in the cohort may present to hospital at differing stages of their disease. A large proportion of patients with confirmed COVID-19 in Denmark had missing biochemistry data, which most likely represents patients who attended the emergency room with mild symptoms, which did not warrant admission or blood tests, and were not included in this study, thus leading to selection bias and limiting generalisability. Some biomarkers (particularly D-dimer, troponin and procalcitonin) are likely to be measured in those with the most severe disease (confounding by indication)."

R5, The mortality in this study is as high as 20.1%, which is relative higher comparing other studies. Therefore, it's better to analyze the reasons of death and describes the indication of ICU admission.

A5. Thank you again for this comment. We acknowledge the mortality percentage is higher in the present study compared to some other studies. We believe this is due to the inherent selection bias in only admitting those who experienced more severe COVID-19 symptoms which we have addressed in the existing text:

"This study included only patients admitted to the hospital with COVID-19 and with measured baseline biochemical data. Therefore, it is likely to represent symptomatic patients, who are more likely to be elderly or with more comorbidities and at the more severe end of the disease spectrum."

Reviewer: 2

Reviewer Name

Amer Harky

Institution and Country

Liverpool university, UK

Please state any competing interests or state 'None declared':
None

Please leave your comments for the authors below
The methods and study design are clearly outlines by the authors, patient selection was appropriate. Your data summary is very to the point which I must commend you for this.

R6. I wonder if you can manage to do regression analysis and perhaps a multi-variate anaylsis correlating the biomarkers such as CRP, Leuck, Ferritin, Creatinine, D-Dimer, Trop...etc and death? a single variate analysis is often associated with lot so bias. We all know that old age, presence of cardiovascular comorbidities..etc are associated with increased mortality rate in patients with COVID-19, there is handful of evidence on this.

A6. Thank you for this very relevant comment. We have used Cox regression analyses, adjusted for age and gender, for standardized absolute risk and average treatment effects curves to evaluate the association between individual biomarkers and the 30-day risk for each endpoint. We agree with your comment and the value of a multivariate analysis. Trying to address your comment, we have included a multivariable analysis for CRP (please also see response A3 above).

R7. There is a recent systematic review which discussed those biomarkers in more depth which I recommend you to read and cite it in your paper to strengthen your conclusion.

Kermali M, Khalsa RK, Pillai K, et al. The role of biomarkers in diagnosis of COVID-19 - A systematic review. Life Sci. 2020;254:117788. PubMed doi:10.1016/j.lfs.2020.117788

A7. Thank you for this useful comment. We have included your recommendation and included reference to this article in the text:

(Page 5, line 17)
"Furthermore, in a recently published systematic review, disease severity was associated with more prominent laboratory abnormalities including markers of inflammation and organ damage including elevated troponins, although much of the early research describes small case studies without clearly defined outcomes and the need for further research in more varied cohorts was highlighted"

Reviewer: 3

Reviewer Name

ZhibingLu

Institution and Country

Wuhan University, China

Please state any competing interests or state 'None declared':
None

Please leave your comments for the authors below
In this retrospective cohort study, 1310 inpatients with COVID-19 were analyzed to evaluate the association between common biomarkers, death and ICU admission. The authors should address the following points:

R8. 453 admitted patients were excluded from the analysis because of no available biochemistry data. What were the clinical characteristics of these patients? How did they compare to those included?

A8. Thank you for this comment. In the previous version of the paper we have tried to acknowledge this selection bias in the limitations section of the manuscript. However, we acknowledge that it could be interesting to elaborate on the differences between the patients with and without biocheistry. Therefore, we have included the following supplementary Table S2 below, which we believe will assist the reader in relation to this point.

R9. There are extensive data in the tables, which should be presented in a clearer way. Tables with three lines were recommended in the manuscript.

A9. Thank you for this point. We have now updated the table characteristics with the subheadings of "Prior comorbidities; Prior medication; Baseline laboratory values" to aid the reader (see updated Table 1 below).

Table S2: Characteristics of all patients with coronavirus disease 2019 (admitted and not-admitted) stratified by those with or without recorded biochemistry data.

	No biochemistry data	Biochemistry data	p
Characteristics	**2683 (62.0%)**	**1647 (38.0%)**	
Age, years (median [IQR])	**47.50 [36.10, 58.50]**	**72.20 [58.15, 80.95]**	**<0.001**
Male sex, n (%)	**1181 (44.0)**	**898 (54.5)**	**<0.001**
Prior comorbidities:			
Ischemic stroke, n (%)	**41 (1.5)**	**115 (7.0)**	**<0.001**
Diabetes, n (%)	**97 (3.6)**	**261 (15.8)**	**<0.001**
Ischemic heart disease, n (%)	**91 (3.4)**	**189 (11.5)**	**<0.001**
COPD, n (%)	**57 (2.1)**	**164 (10.0)**	**<0.001**
Atrial fibrillation, n (%)	**95 (3.5)**	**253 (15.4)**	**<0.001**
Chronic kidney disease, n (%)	**89 (3.3)**	**168 (10.2)**	**<0.001**

Hypertension, n (%)	**274 (10.2)**	**575 (34.9)**	**<0.001**
Cancer, n (%)	**123 (4.6)**	**243 (14.8)**	**<0.001**
Heart failure, n (%)	**42 (1.6)**	**118 (7.2)**	**<0.001**
COPD, chronic obstructive pulmonary disease; IQR, inter-quartile range.			

Table 1: Characteristics of patients with coronavirus disease 2019 for total cohort, and stratified by death/ICU admission (within 30-days of diagnosis).

	Total	**No Death/ICU admission**	**Death/ICU admission**	**p**
Characteristics	1310	958 (73.1)	352 (26.9)	
Age, years (median [IQR])	73.60 [60.50, 81.90]	71.15 [56.52, 79.80]	77.50 [70.18, 84.53]	<0.001
Male sex, n (%)	715 (54.6)	489 (51.0)	226 (64.2)	<0.001
Prior comorbidities:				
Ischemic stroke, n (%)	96 (7.3)	63 (6.6)	33 (9.4)	0.094
Diabetes, n (%)	221 (16.9)	145 (15.1)	76 (21.6)	0.008
Ischemic heart disease, n (%)	165 (12.6)	116 (12.1)	49 (13.9)	0.398
COPD, n (%)	135 (10.3)	86 (9.0)	49 (13.9)	0.010
Atrial fibrillation, n (%)	212 (16.2)	132 (13.8)	80 (22.7)	<0.001
Chronic kidney disease, n (%)	131 (10.0)	88 (9.2)	43 (12.2)	0.119
Hypertension, n (%)	474 (36.2)	323 (33.7)	151 (42.9)	0.002
Cancer, n (%)	194 (14.8)	140 (14.6)	54 (15.3)	0.727
Heart failure, n (%)	95 (7.3)	60 (6.3)	35 (9.9)	0.030
Prior medication				
Glimepiride, n (%)	245 (18.7)	164 (17.1)	81 (23.0)	0.017
Aspirin, n (%)	174 (13.3)	111 (11.6)	63 (17.9)	0.004
NSAID, n (%)	140 (10.7)	103 (10.8)	37 (10.5)	1.000
Beta blocker, n (%)	174 (13.3)	111 (11.6)	63 (17.9)	0.004
ACEi, n (%)	203 (15.5)	144 (15.0)	59 (16.8)	0.439
ARB, n (%)	463 (35.3)	323 (33.7)	140 (39.8)	0.044
Loop diuretic, n (%)	217 (16.6)	138 (14.4)	79 (22.4)	0.001
Thiazide diuretic, n (%)	116 (8.9)	87 (9.1)	29 (8.2)	0.742
CCB, n (%)	241 (18.4)	157 (16.4)	84 (23.9)	0.003
Spironolactones, n (%)	69 (5.3)	41 (4.3)	28 (8.0)	0.012
Baseline laboratory values				
CRP, n (%)	1256 (95.9)	906 (94.6)	350 (99.4)	<0.001
Leucocytes, n (%)	1300 (99.2)	952 (99.4)	348 (98.9)	0.472
eGFR, n (%)	915 (69.8)	619 (64.6)	296 (84.1)	<0.001
Urea, n (%)	1067 (81.5)	781 (81.5)	286 (81.2)	0.936
ALAT, n (%)	1167 (89.1)	850 (88.7)	317 (90.1)	0.549
Ferritin, n (%)	252 (19.2)	205 (21.4)	47 (13.4)	0.001
D-dimer, n (%)	449 (34.3)	332 (34.7)	117 (33.2)	0.646
Troponin, n (%)	258 (19.7)	197 (20.6)	61 (17.3)	0.210
Procalcitonin, n (%)	249 (19.0)	164 (17.1)	85 (24.1)	0.005
CRP, mmol/L (median [IQR])	88.00 [43.00, 160.00]	74.50 [35.25, 130.00]	131.00 [68.25, 218.00]	<0.001
Leucocytes, 10E9/L (median [IQR])	7.40 [5.50, 10.20]	7.10 [5.30, 9.60]	8.40 [6.12, 11.90]	<0.001
eGFR, mL/min/1.73m2	73.23 [56.39,	74.80 [60.97,	66.45 [48.43,	<0.001

(median [IQR])	83.55]	84.17]	81.33]	
Urea, mmol/L (median [IQR])	6.50 [4.60, 9.60]	5.90 [4.20, 8.30]	9.05 [6.60, 12.47]	<0.001
ALAT, U/L (median [IQR])	31.00 [21.00, 52.00]	31.00 [21.00, 51.00]	32.00 [22.00, 53.00]	0.169
Ferritin, µg/L (median [IQR])	266.00 [143.00, 446.75]	264.00 [130.00, 439.00]	311.00 [189.50, 464.00]	0.110
D-dimer, mg/L (median [IQR])	0.94 [0.55, 1.80]	0.86 [0.51, 1.63]	1.40 [0.77, 2.40]	<0.001
Troponin ratio (median [IQR])	1.00 [0.57, 1.79]	0.92 [0.39, 1.34]	2.14 [1.29, 3.43]	<0.001
Procalcitonin, µg/L (median [IQR])	0.20 [0.11, 0.49]	0.15 [0.08, 0.28]	0.40 [0.20, 0.92]	<0.001

ACEi, angiotensin-converting enzyme inhibitor; ALAT, alanintransaminase; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; IQR, inter-quartile range; NSAID, nonsteroidal anti-inflammatory drug.

R10. Most of the biological markers studied in this paper have been widely reported, the authors should give more prominence to the innovation of this article.

A10. Thank you for this comment. In Denmark, health care is free, and therefore the private health care section is very limited. We believe a key strength of our study is that the data are from a nationwide cohort, including all regions and all social classes. Furthermore, the present study is a European cohort with clearly defined endpoints, hence we believe the results are of value and interest to the reader and add value to the current literature base. Please also see comment A2 above.

VERSION 2 – REVIEW

REVIEWER	Yan Kang West China Hospital, Sichuan University
REVIEW RETURNED	09-Oct-2020

GENERAL COMMENTS	1, The manuscript identifies the association between several biomarkers and poor outcomes in patients with COVID-19 in Denmark. 2, There are currently more than 20 studies of biomarkers for COVID-19 in the past 6 months. There is limited innovation in terms of the types of biomarkers and main results. 3, More than 50% of the data were missed in D-dimer, ferritin, troponin and PCT, which makes the results less convinced in terms of the selection bias. 4, The mortality in this study is as high as 20.1%, which is relative higher comparing other studies. Therefore, it's better to analyze the reasons of death and describes the indication of ICU admission.

REVIEWER	Amer Harky UK
REVIEW RETURNED	24-Sep-2020

GENERAL COMMENTS	The authors responded to my comments appropriately and made necessary changes.

REVIEWER	Zhibing Lu Wuhan University
REVIEW RETURNED	30-Sep-2020

GENERAL COMMENTS	The authors have addressed my concerns and I have no more comments.