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.

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Potential impact of serpin peptidase inhibitor clade (A) member 4 SERPINA4 (rs2093266) and SERPINA5 (rs1955656) genetic variants on COVID-19 induced acute kidney injury

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ABSTRACT

Background: SARS-CoV-2 has a number of targets, including the kidneys. Acute Kidney Injury (AKI) might develop in up to a quarter of SARS-CoV-2 patients. In the clinical environment, AKI is linked to a high rate of death and leads to the progression of AKI to chronic renal disease.
Aim: We aimed to investigate rs2093266 and rs1955656 polymorphisms in *SERPINA4* and *SERPINA5* genes, respectively, as risk factors for COVID-19 induced AKI.
Subjects and methods: A case-control study included 227 participants who were divided into three groups: 81 healthy volunteers who served as controls, 76 COVID-19 patients without AKI and 70 COVID -19 patients with AKI. The TaqMan assay was used for genotyping the *SERPINA4* (rs2093266) and *SERPINA5* (rs1955656) polymorphisms by real-time PCR technique.
Results: Lymphocytes and eGFR showed a significantly decreasing trend across the three studied groups, while CRP, d-Dimer, ferritin, creatinine, KIM-1and NGAL showed a significantly increasing trend across the three studied groups ($P < 0.001$). Rs2093266 (AG and AA) genotypes were significant risk factors among non-AKI and AKI groups in comparison to controls. Rs1955656 (AG and AA) were significant risk factors among the AKI group, while AA was the only significant risk factor among the non-AKI group. Recessive, dominant, co-dominant, and over-dominant models for genotype combinations were demonstrated. The GG v AA, GG + AG v AA, and GG v AG + AA models of the rs2093266 were all significant predictors of AKI, whilst only the GG v AA model of the rs1955656 SNP was a significant predictor. The logistic regression model was statistically significant, $\chi 2 = 56.48$, $p < 0.001$. AKI was associated with progressed age (OR = 0.95, 95% CI: 0.91–0.98, $p = 0.006$), suffering from chronic diseases (OR = 3.25, 95% CI: 1.31–8.01, $p = 0.010$), increased BMI (OR = 0.89, 95% CI: 0.81–0.98, $p = 0.018$), immunosuppressive (OR = 4.61, 95% CI: 1.24–17.16, $p = 0.022$) and rs2093266 (AG + AA) (OR = 3.0, 95% CI: 1.11–8.10, $p = 0.030$).
Conclusion: Single nucleotide polymorphisms (rs2093266) at *SERPINA4* gene and (rs1955656) at *SERPINA5* gene were strongly linked to the development of AKI in COVID-19 patients.

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1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-caused global pandemic of coronavirus disease 2019 (COVID-19) is rapidly moving from moderate respiratory tract infection to acute respiratory distress syndrome (ARDS) and multiple organ failure, with a high fatality rate (Zhou et al., 2020). Kidney affection became prevalent in COVID-19, and acute kidney injury (AKI) influences about 20–40% of COVID-19 patients admitted to intensive care in Europe and the USA and is emerging as a potential indicator of disease severity and decreased patient's survival (Ronco et al., 2020).

The cause of kidney affection in COVID-19 is complex. Different reasons have been proposed for the progression of AKI, with concomitant cardiovascular disease and predisposing factors such as sepsis, dehydration, and nephrotoxic drug as significant elements (Ronco et al., 2019). In critically ill patients, sepsis is the most common condition predisposing them to AKI. It affects up to 50% of patients with sepsis and up to 60% of individuals with septic shock (Poukkanen et al., 2013). Through T cell apoptosis driven by type I interferon, the cytokine storm syndrome (CRS) inhibits the immune system and causes lymphopenia (reduction of CD8 and CD4 T cells) in persons with COVID-19. IFN-stimulated T cells also produce less ATP and undergo apoptosis (Channappanavar et al., 2016). In COVID-19, the beginning of cytokine storms is linked to a rise in apoptosis in the kidney and lung. Apoptosis decreases the efficiency of virus recognition via receptors by increasing the availability of nucleic acids (Devaux et al., 2020).

Apoptosis-related genes, such as serpin peptidase inhibitor clade A (alpha-1 antiproteinase, antitrypsin) member 4 (*SERPINA4*) and serpin peptidase inhibitor clade A member 5 (*SERPINA5*), and salt-inducible kinase family 3 (*SIK3*), are good candidates for AKI because apoptosis appears to be a key mechanism in AKI (Vilander et al., 2017). The serpins (serine protease inhibitors) are a family of endogenous proteins of molecular weight about 40–60 kDa. They are classified into 16 clades (A–P) (Kelly-robinson et al., 2021). Clade A serpins are plasma, antitrypsin-like proteins and encoded by genes found on chromosome 14 (Mkaouar et al., 2019).

The SNP rs2093266 is found in the *SERPINA4* gene that encodes kallistatin, a serine proteinase inhibitor with many regulatory functions in biological processes (Vilander et al., 2017). Kallistatin has two functional domains: an active site and a heparin-binding site, both of which influence a variety of signaling and metabolic pathways. It contains anti-inflammatory, antioxidant, vasodilator, and angiogenesis inhibitory properties and its apoptosis-related actions (Chao et al., 2016). SERPINA5 is a 57 kDa glycoprotein and is identified as a protein C inhibitor (PCI). PCI is a heparin-dependent serpin that exhibits both pro-and anti-coagulant actions and is involved in various anti-inflammatory and anti-coagulant mechanisms (Kelly-robinson et al., 2021).

There is a lot of diversity in disease behavior among COVID-19 infected individuals; a multifactorial analysis might help uncover the probable risk factors for AKI induction in COVID-19 patients. In order to estimate the COVID-19 patients' genetic predisposition to develop AKI and as per the previous analyses of Vilander et al., 2017 and Frank et al., 2012 who reported that both SNPs rs2093266 of SERPINA4 and rs1955656 of SERPINA5 were related to the risk of AKI in severely ill patients with septic shock. Also, both SERPINA4 and SERPINA5 have anti-inflammatory and apoptosis-related activities (Chao et al., 2016; Kelly-robinson et al., 2021). Accordingly, we aimed to evaluate the link between genetic polymorphisms in the apoptosis-related genes *SERPINA4* (rs2093266) and *SERPINA5* (rs1955656) and AKI risk COVID-19 patients.

2. Patients and methods

The departments of Chest Disease, Medical Biochemistry & Molecular Biology, Clinical Pathology, Public Health, Microbiology, and Internal Medicine at Menoufia Faculty of Medicine in Egypt collaborated on a case-control study from October 2020 to January 2022. Our hypothesis was tested on 227 volunteers enrolled in a case-control study from October 2020 to January 2022. They were distributed to three groups: 81 healthy controls, 76 COVID-19 patients without AKI and 70 COVID-19 patients with AKI.

Patients having a positive Reverse Transcription polymerase chain reaction (RT-PCR) for SARS-COV-2 utilizing a nasopharyngeal swab specimen were included in the COVID-19 patients' both groups. COVID-19 patients who developed AKI during hospitalization were included in group3. On the other hand patients with end-stage kidney disease, kidney transplantation, pregnancy and patients with AKI on admission due to any other reason were excluded from the study. Negative RT-PCR patients and patients with a proved other concurrent acute illness were also excluded from the study. At the same time, control subjects were confirmed to be negative by RT-PCR for COVID-19.

Our study comprised adult patients with mild, moderate, severe, and critical COVID-19 infection, as defined by World Health Organization (WHO) criteria for COVID-19 clinical care (World Health Organization, 2021).

The KDIGO (Kidney Disease Improving Global Outcomes) criteria will be used to define AKI based on both urinary output and serum creatinine (KDIGO, 2012). For patients without a baseline creatinine, we used the hospital admission creatinine as a baseline, and eGFR was calculated using the MDRD ml/min per 1.73 m^2 equation. Inflammatory indicators such as serum ferritin, C-reactive protein (CRP), D-dimer, and markers of AKI such as kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) are all evaluated in the lab, as well as a complete blood count (CBC) with differential. The chest imaging of these individuals was evaluated using X-rays and computed tomography.

3. Approval on ethical grounds

Before blood samples were taken, all individuals completed a permission form authorized by "the Local Ethics & Human Rights committee in Research at Faculty of Medicine, Menoufia University."

4. Collection and processing of blood samples

Six millilitres of fresh venous blood were drawn using sterile venipuncture. Two millilitres of blood were transferred to an EDTA tube and separated into two aliquots, one for CBC and the other for DNA extraction for further SNP analysis. 1.8 ml was transferred to a sodium citrate tube for the D-dimer assay. The remaining 2.2 ml was transported to a plain tube and centrifuged for 20 min at 4000 rpm; the serum was then frozen at −20 °C until serum ferritin, C-reactive protein (CRP), and KIM-1 & NGAL were assayed.

5. Methods

The nephelometric approach was utilized to determine CRP using Mispa-i2 (Agape Diagnostics, Switzerland). Using a chemoilumincence immunoassay, the Architect plus i1000SR immunoassay analyzer was utilized to assess serum ferritin (Abbott, Illinois, USA).

The D- dimer was measured using the stago (STA Compact Max Analyzer, Fully Automated Coagulation System) (Diagnostic Stago, France). WBCs and lymphocytes were measured using the Sysmex XN-1000 Automated Hematology Analyzer (Sysmex Corporation, Kobe, Japan). Quantikine, Canada, USA, supplied human enzyme-linked immunosorbent assay (ELISA) kits for evaluating blood levels of KIM-1 and NGAL. A quantitative sandwich enzyme immunoassay was utilized by the researchers.

6. Rs2093266 G/A polymorphism in *SERPINA4* gene and rs1955656 G/A SNP in *SERPINA5* gene genotyping

Thermo Fisher Scientific's GeneJET Whole Blood Genomic DNA Purification extraction Kit was used to extract DNA from peripheral blood. A real-time PCR technique with a TaqMan probe from Applied Biosystems in the United States was used for genotyping the G/A polymorphism at the *SERPINA4* and *SERPINA5* genes.

Based on the Allele Frequency of the reference SNP (rs) report (https://www.ncbi.nlm.nih.gov/snp/rs2093266?horizontal_tab=true#frequency_tab), the minor allele frequency or MAF (A alleles) of SERPINA4 rs2093266 are 0.1687 and 0.2141in the general population globally and in African respectively. Moreover, the MAF (A alleles) of SERPINA5 rs1955656 are 0.1663 and 0.2050 globally and in African respectively (https://www.ncbi.nlm.nih.gov/snp/rs1955656?horizontal_tab=true#frequency_tab).

The primers, probes, and Master Mix were also provided by Thermo Scientific (40×). The probe sequences were created as follows: GAGGCAATAGGTTTTGAGGGGCATG[G/A]GGACGGGGTTCAGCCTCCAGG GTCC was the [VIC/FAM] for rs1955656, whereas TAACAAT CTTGTCTCACTTTTCATT[G/A]AGAAAACAGAATCAGTTAAACAGGA was the [VIC/FAM] for rs2093266. 1.25 μl of primer/probe combination, 10 μl of Master Mix, 3.75 μl of nuclease-free water, and 5 μl of purified DNA were used in each reaction. The cycling conditions were as follows: 10 min at 94 °C for the first denaturation, then 50 cycles (30 s at 95 °C for the second denaturation, 60 s at 50 °C for primer annealing, and 1.5 min at 72 °C for primer extension), followed by 1 min at 72 °C for the final extension step.

The ABI 7500 real-time PCR software, version 2.0.1, was used to analyse the results. (Figure 1 a & b).

7. Statistical analysis

SPSS version 28.0 [SPSS Inc., Chicago, IL, USA] was used to conduct the analyses. The significance of the connection between two categories was determined using Pearson's Chisquare [χ2] test. To determine the normality of the distribution, the Shapiro-Wilk test was employed as one of the normality tests, and one-way ANOVA was utilized for parametric data. The Jonckheere-Terpstra test was used to determine if there was an increasing or declining trend across the ordered groups using linear trend analysis. By measuring the effect size following the Jonckheere-Terpstra [J-T] test, the Mann-Kendall [M-K] test is used to identify the presence of linear or non-linear trends [steadily increasing/decreasing or unchanged] in a series of data. Hardy Weinberg equilibrium [HWE] was examined in patients and controls for the two SNPs rs2093266 and rs1955656.

95% confidence intervals [95% CI] and the odds ratio [OR] were generated in dominant, recessive1 and 2, co-dominant, and over-dominant genetic models for further study of the connection between SNPs rs2093266 and rs1955656 and disease progression to AKI. To find the independent determinants of illness severity, a binary logistic regression analysis was used. Holm Bonferroni Sequential Correction: An EXCEL Calculator was used to assess multiple comparisons" © Justin Gaetano, 2013. After this modification, the *P*-values are statistically significant.

8. Sample size

Bujang et al., 2018 and Peduzzi et al., 1996 supported the notion of a variable per 10 patients (Event per variable (EPV)) to be suitable for regression analysis. In our study the greatest number of predictors that might be included was five; hence the sample size was computed and it

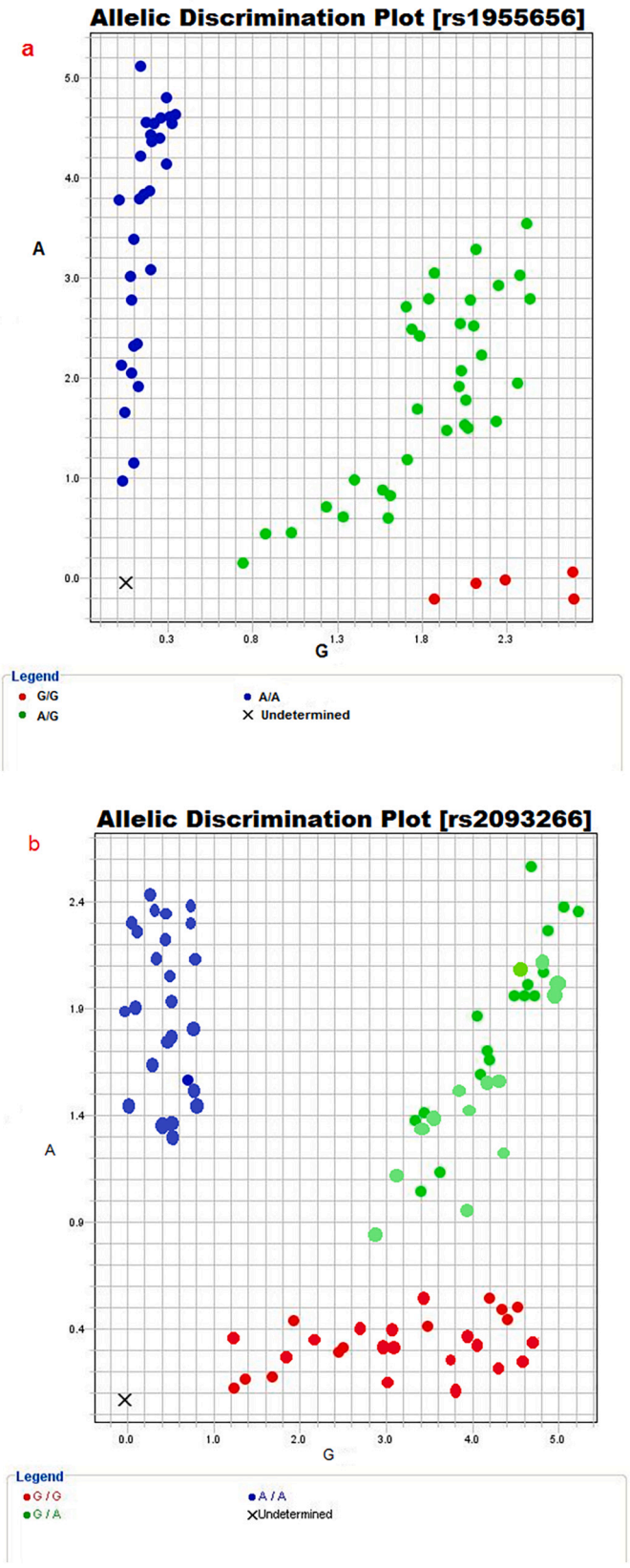

Fig. 1. a- Allelic discrimination plot (rs1955656) showing different genotypes. b- Allelic discrimination plot (rs2093266) showing different genotypes.

was 150 participants but according to Austin and Steyerberg, 2017 who recommended EPV of 20 instead of 10, the sample size for regression analysis was determined at power = 0.80 and CI 95% and it was 200 participants. In every case, in our study we included a total sample size of 227 which was sufficient for the primary outcome and regression analysis.

9. Results

Age and BMI were significantly higher among AKI than other groups ($P < 0.001$). Chronic diseases were more prevalent in the AKI group (74.3%) than COVID non-AKI group (27.6%) ($P < 0.001$). Fever and Dyspnea were more common in the AKI group, while muscle ache, abdominal pain, and anorexia were more prevalent in the non-AKI group ($P < 0.05$). Death was reported among 55.7% of the AKI group versus 5.3% among the Non-AKI group ($P < 0.001$) (Table 1).

Lymphocytes and eGFR showed a significantly decreasing trend across the three studied groups, while CRP, d-Dimer, ferritin, creatinine, KIM-1and NGAL showed a significantly increasing trend across the three studied groups ($P < 0.001$) (Table 2).

Hardy-Weinberg Equilibrium calculation should non-significance for rs1955656, and rs205 ($P > 0.05$) among all studied groups except rs1955656 showed weak significance among AKI group ($P = 0.038$) (Table 3).

Rs2093266 (AG and AA) genotypes were significant risk factors among non-AKI and AKI groups compared to controls. Rs1955656 (AG and AA) were significant risk factors among the AKI group, while AA was the only significant risk factor among the non-AKI group ($P < 0.001$) (Table 4).

Up on comparing different gene models between the AKI and the non-AKI groups, recessive, dominant, co-dominant, and over-dominant models for genotype combinations were demonstrated. The GG v AA, GG + AG v AA, and GG v AG + AA models of the rs2093266 were all significant predictors of AKI, while only the GG v AA model of the rs1955656 SNP was a significant predictor (Table 5).

In our sample, the potential predictors of AKI in COVID-19 patients in comparison to No AKI group were analyzed. The potential predictors for AKI development were Age, co-morbidities, BMI, rs2093266 (AG + AA) and being on immunosuppressive. With age adjustment, it was evident that co-morbidities were the major risk factors of AKI development (OR = 6.63[3.01–14.52, $P < 0.001$], followed by rs2093266 (AG + AA) (OR = 3.40, CI95%:1.28–9.0, $p = 0.014$], and being on immunosuppressive (OR = 4.61, CI95%:1.24–17.16, $p = 0.018$] (Table 6).

10. Discussion

Despite the specific cause of kidney affection in COVID-19 being indistinct, it is commonly associated with sepsis (Zaim et al., 2020). Also, AKI in COVID-19 patients may occur due to systemic endothelial injury, and extensive production of cytokines and inflammatory mediators in CRS may cause renal tubular cell injury. Medications or hyperventilation-stimulated rhabdomyolysis can similarly participate in COVID-19-induced AKI (Ertuğlu et al., 2020).

Serpins are a family of circulatory proteins that control proteases enrolled in coagulation, inflammation, and immune response (Gatto et al., 2013). SERPINA4 or kallistatin is a serine proteinase inhibitor and kallikrein inhibitor with anti-inflammatory and apoptosis-related activities (Chao et al., 2016). SERPINA5 or PCI is tangled in several anti-inflammatory and coagulant pathways and inhibits plasma kallikrein (Kelly-robinson et al., 2021). It is expressed in body fluids and various organs as the kidney (Yang and Geiger, 2017).

This study evaluated the link between genetic polymorphisms in the apoptosis-related genes *SERPINA4* (rs2093266) and *SERPINA5* (rs1955656) and AKI risk COVID-19 patients. Our results demonstrated

Table 1
Distribution of the studied groups regarding their demographic data, past history and signs and symptoms of COVID-19:

		Controls (No. = 81)		COVID-19				*P* value
				Without AKI (No. = 76)		With AKI (No. = 70)		
		no	%	no	%	no	%	
Demographic data	Age (y) mean ± SD	52.60 ± 7.20		45.94 ± 16.58		59.41 ± 7.92		<0.001*
	BMI(kg/m2) mean ± SD	21.82 ± 2.27		33.17 ± 5.12		35.40 ± 4.21		<0.001*
	Sex							
	Male	47	58.0	44	57.9	48	68.6	0.317
	Female		42.0	32	42.1		31.4	
		34				22		
Past history	Smoking	29	35.8	31	40.8	27	38.6	0.813
	Co-morbidities			21	27.6	52	74.3	<0.001*
	• Diabetes Mellitus			17	22.4	38	54.3	<0.001*
	• Hypertension			15	19.7	21	30.0	0.151
	• Chest disease			4	5.3	14	20.0	0.007*
	• Heart disease			5	6.6	8	11.4	0.304
	• Liver disease			3	3.9	6	8.6	0.312
	Immunosuppressive			5	6.6	20	28.6	<0.001*
Signs & symptoms of COVID1–9	Fever			59	77.6	70	100.0	<0.001*
	Cough			70	92.1	57	81.4	0.055
	Sore throat			42	55.3	45	64.3	0.267
	Muscle ache			65	85.5	49	70.0	0.023*
	Dyspnea			24	31.6	52	74.3	<0.001*
	Headache			50	65.8	38	54.3	0.156
	Abdominal pain			17	22.4	16	22.9	0.944
	Anorexia			45	59.2	29	41.4	0.032*
	Diarrhea			37	48.7	21	30.0	0.021*
	Severity							<0.001*
	▪ Mild			24	31.6	0	0.0	
	▪ Moderate			28	36.8	14	20.0	
	▪ Severe			16	21.1	14	20.0	
	▪ Critical ill			8	10.5	42	60.0	
	Mortality			4	5.3	39	55.7	<0.001*

* Significant.

Table 2
Distribution of the studied groups regarding their demographic data, past history and signs and symptoms of COVID-19:

	Controls (No. = 81)	COVID-19		P value	Effect size(95%CI)
		Without AKI (No. = 76)	WithAKI (No. = 70)		
	mean ± SD	mean ± SD	mean ± SD		
WBC*10^3	7.4 ± 2.3	5.0 ± 2.3	9.71 ± 2.46	0.001*	0.18[0.08–0.27]
Lymphocytes%	31.2 ± 6.1	21.2 ± 8.9	16.5 ± 6.9	<0.001*	−0.50[(−0.57)-(0.43)]
CRP	6.1 ± 1.9	57.1 ± 46.6	59.2 ± 17.4	<0.001*	0.63[0.56–0.68]
d-Dimer(Median (IQR)	0.20(0.10–0.30)	0.50(0.30–1.30)	0.75(0.71–0.81)	<0.001*	0.52[0.45–0.58]
Ferritin (Median (IQR)	10(72.5–146)	78 (45–331)	526 (412–788)	<0.001*	0.44[0.37–0.50]
Creatinine	0.9 ± 0.1	0.8 ± 0.2	4.3 ± 0.5	<0.001*	0.56[0.48–0.63]
eGFR	104.3 ± 12.3	104.5 ± 12.5	52.3 ± 7.7	<0.001*	−0.55[(−0.62)-(−0.46)]
KIM-1(Median (IQR)	55(31–82)	62 (44–82.)	390(296.5–484.5)	<0.001*	0.53[0.45–0.60]
NGAL(Median (IQR)	1.3(0.7–2)	1.5 (1–2)	7.5 (3.7–8.5)	<0.001*	0.56[0.48–0.63]

* Significant.

Table 3
Hardy-Weinberg Equilibrium calculation for SNP rs2093266and rs1955656:

	Controls (No. = 81)		P value	COVID-19					P value
				Without AKI (No. = 76)		P value	With AKI (No. = 70)		
	Observed	Expected		Observed	Expected		Observed	Expected	
rs2093266	57	56.3	0.548	25	21.1	0.069	11	7.2	0.038
				30	37.9		23	30.5	
GG®	21	22.5		21	17.1		36	32.2	
AG	3	2.3							
AA									
rs1955656	43	44.4	0.403	20	16.1	0.073	12	10.0	0.317
							29	32.9	
				30	37.8		29	27.0	
GG®	34	31.1		26	22.1				
AG	4	5.4							
AA									

Table 4
Distribution of the studied groups regarding SNPrs2093266 and rs1955656:

	Controls (No. = 81)		COVID-19						Test /P value	OR (95%CI)
			Without AKI (No. = 76)		Test /P value	OR (95%CI)	With AKI (No. = 70)			
	no	%	no	%			no	%		
rs2093266	57	70.4	25	32.9	-10.41/0.001*	1.0	11	15.7	-16.46/<0.001	1.0
GG®	21	25.9	30	39.5						5.68[2.36–13.62]
AG	3	3.7	21	27.6			23	32.9	58.32/<0.001*	62.18[16.23–238.21]
AA					24.57/<0.001*	3.26[1.57–6.75] 15.96[4.36–58.44]	36	51.4		
G	135	83.3	80	52.6	-24.24/<0.001*	1.0	45	32.1	81.73/<0.001*	10.56[6.12–18.20]
A	27	16.7	72	47.4		4.50[2.67–7.58]	95	67.9		
rs1955656	43	53.1	20	26.3	-3.04/0.081	1.0	12	17.1	-	1.0
GG®	34	42.0	30	39.5	24.52/<0.001*	1.90[0.92-3.91]	29	41.4	7.59/<0.001*	3.06[1.36-6.87]
AG	4	4.9	26	34.2		13.98[4.30–45.43]	29	41.4	36.17/<0.001*	25.98[7.63–88.50]
AA										
G	120	74.1	70	46.1	-	1.0	53	37.9	40.26/<0.001*	4.69[2.87–7.66]
A	42	25.9	82	53.9	25.77/<0.001*	3.35[2.08–5.38]	87	62.1		

* Significant.

an increasing trend in KIM-1 and NGAL across our groups. Rs2093266 the (AG and AA) genotypes were significant risk factors in non-AKI and AKI patients, and rs1955656 (AG and AA) were significant risk factors in AKI patients. In contrast, AA was the only significant risk factor among non-AKI COVID-19 patients. Additionally, only rs2093266 (AG + AA) was found as independent predictors of AKI progression.

The SARS-CoV-2 virus causes the production of various cytokines and inflammatory interleukins, resulting in an inflammatory reaction in lung tissues and subsequently ARDS and multiple organ failure (Xu et al., 2020b).

Coagulation and inflammatory reactions are marked in COVID-19 patients and considered as indicators of endothelial damage. Both thromboembolic and inflammatory reactions might have essential participation in COVID-19 progression and extra-pulmonary complications (Bernard et al., 2020). Kallikreins are initiated by inflammatory responses and enhance IL-1production via nuclear factor kappa B (NF-kB), which augments the inflammatory reaction in viral infection. So, proteases such as SERPINA4 or Kallistatin (kallikrein inhibitor) are important in viral infections like influenza and SARS-CoV (Leu et al., 2015). Reduced circulatory SERPINA4 has been reported in patients

Table 5
Distribution of the studied COVID groups regarding SNP rs2093266 and rs1955656 in different genetic model:

	OR(95%CI)	P value
rs2093266		
GG vs. AA (Recessive-1)	3.90[1.60–9.49]	0.002*
GG + AG vs. AA(Recessive-2)	2.77[1.39–5.51]	0.003*
GG vs. AG + AA(Dominant)	2.63[1.18–5.86]	0.016*
GG vs. AG(co-dominant)	1.74[0.71–4.26]	0.221
AG vs. GG + AA	1.33[0.68–2.63]	0.406
rs1955656		
GG vs. AA (Recessive-1)	2.50[1.05–5.97]	0.036*
GG + AG vs. AA(Recessive-2)	1.36[0.70–2.66]	0.368
GG vs. AG + AA(Dominant)	1.73[0.77–3.86]	0.180
GG vs. AG(co-dominant)	1.61[0.67–3.88]	0.286
AG vs. GG + AA	1.08[0.56–2.10]	0.809

* Significant, OR: Odds ratio, CI95%: Confidence interval at 95%.

Table 6
Univariate and multivariate analysis of potential predictors of associated with AKI development.

Variables	P value	Unadjusted OR [95% CI]	P value	Adjusted OR [95% CI]
Age	<0.001*	0.92 [0.90–0.95]	–	–
Co-morbidities	<0.001*	7.56 [3.62–15.77]	<0.001*	6.63 [3.01–14.52]
BMI	0.006*	0.92 [0.84–0.97]	0.033*	0.91 [0.83–0.99]
rs2093266 (AG + AA)	0.018*	2.62 [1.17–5.86]	0.014*	3.40[1.28–9.0]
Immunosuppressive	0.034*	3.23 [1.09–9.62]	0.018*	4.61 [1.24–17.16]

* Significant. Adjusted for Age.

with severe community-acquired pneumonia that progressed to ARDS (Lin et al., 2013). SERPINA4 has been related to better outcomes and survival in the sepsis-related acute lung. SERPINA4 gene transfer or SERPINA4 administrations reduced apoptosis, repressed reactive oxygen species (ROS), and inhibited ROS- NF-κB activation and inflammation (Lin et al., 2015).

Also, SERPINA5 or PCI induced expression in mice lungs displayed a marked reduction in proinflammatory cytokines like necrosis factor-alpha (TNF-α) and reduced coagulation activity in lung tissues (Nishii et al., 2006). All may explain our finding of an association of SERPINA5 rs1955656 and SERPINA4 rs2093266 variants with the risk of COVID-19.

Our current analysis revealed an association of rs1955656 and rs2093266 with the risk of AKI patients among COVID-19 patients. Additionally, rs2093266 was found as an independent predictor of AKI progression.

Vilander et al., in their analysis, reported a similar finding, as both SNPs rs2093266 of SERPINA4 and rs1955656 of SERPINA5 were found to be related to the risk of AKI in severely ill patients with septic shock (Vilander et al., 2017). On the same line, **Frank and colleagues,** in their study, showed rs2093266 and rs1955656 as significant risk factors of AKI in ICUs admitted patients with septic shock (Frank et al., 2012).

Previous analyses supposed that AKI in COVID-19 is analogous to sepsis-induced AKI triggered by a complex interaction between inflammation, cell death, and microvascular dysfunction as microemboli or microthrombi as part of the coagulopathy state (Kellum et al., 2020). Progression of AKI in COVID-19 may also be related to SARS-CoV-2-related immune responses like CRS (Ronco and Reis, 2020). Additionally, AKI may develop due to endothelial damage, enhanced vascular permeability, and renal inflammatory reactions (Ertuğlu et al., 2020). COVID-19-associated endotheliitis enhances organ damage, and thrombosis and systemic endotheliitis may clarify multi-organ injury in severe COVID-19 cases (Nägele et al., 2020). Moreover, pulmonary endotheliitis and microvascular dysfunction may explain the occurrence of severe hypoxia despite relatively conserved lung mechanics in COVID-19 (Gattinoni et al., 2020).

SERPINA4 has a vasodilator effect and can repress vascular inflammation by inducing eNOS and NO production. Moreover, SERPINA4 exhaustion enhances glomerular endothelial cell loss (Chao et al., 2018). SERPINA4 prevents vascular and organ injury by suppressing inflammation, apoptosis, and oxidative stress. Furthermore, it had a positive association with the anticoagulation markers and a negative correlation with the inflammatory markers (Lin et al., 2013). SERPINA4 administration enhances renal performance in rats (Shen et al., 2008). It has been correlated with renal dysfunction in diabetic patients (Jenkins et al., 2010) and shown to have a protective role in diabetic nephropathy (Yiu et al., 2016). It inhibits TNF-α enhanced apoptosis in endothelial cells (Shen et al., 2010).

Additionally, SERPINA5 or PCI in blood was diminished in patients with hyper-coagulopathy, sate-like pulmonary embolism. PCI suppresses circulatory kallikrein, which induces vascular inflammation and enhances vascular permeability (Suzuki, 2008). SERPINA5 or PCI is expressed in renal tubular cells (Radtke et al., 1994) and has been correlated with metastasis in renal cell carcinoma (Wakita et al., 2004).

The age is a potential predictor to development of AKI, but when adjusted it was evident that co-morbidities were the major risk factors of AKI development (OR = 6.63[3.01–14.52], followed by rs2093266 (AG + AA) (OR = 6.63, CI95%:3.01–14.52, p = 0.014], and being on immunosuppressive (OR = 4.61, CI95%:1.24–17.16, p = 0.018].

This is in agreement with Naser et al., 2021 who concluded that age is a major potential predictor of AKI-induced COVID-19 compared to non-AKI-induced COVID-19 patients. As concluded by Kane-Gill et al., 2015 and Coca et al., 2011 indicates that age is a major potential predictor of AKI compared to patients without AKI. Acute kidney injury is strongly associated with advanced age, and the recent rise in AKI incidence is linked to the growing size of the elderly population (Coca et al., 2011; Ishani et al., 2009; Feest et al., 1993). Elderly patients are at risk of AKI due to decreased renal reserve and altered kidney function inhibiting kidney function recovery following acute injury (Himmelfarb, 2009).

Our results are concurrent with previous analyses, verifying a relationship of COVID-19 severity, progression, and unfavorable consequence with the presence of comorbidities as diabetes mellitus (Zhang et al., 2020), higher body mass index, and chronic pulmonary disease (Xu et al., 2020a).

We realize the limitations of a relatively small sample size, as well as the difficulties of making additional inferences about the relationships between these SNPs and other study indices. Nonetheless, our findings are significant in biomedical research since they demonstrate the ability of the SERPINA4 gene polymorphism rs2093266 to predict COVID-19-related AKI.

11. Conclusion

Based on the results of the current analysis, we can conclude that the genetic variants rs2093266 of *SERPINA4* gene and rs1955656 of *SERPINA5* might participate in the pathogenesis of COVID-19 and were strongly linked to the development of AKI in COVID-19 patients, specifically rs2093266 (AG + AA) was found as independent predictors of AKI progression.

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CRediT author statement

SME*:* Supervision- Methodology, **ZAK:** review, formal analysis, **HAE**: data curation. **ISE:** methodology, **RGM:** Writing Original draft. **TAO:** Review and Editing, **HEK**: Data curation, **EMG:** conceptualization, **MMG:** validation, **AAS:** methodology, editing.

Declaration of Competing Interest

There is no conflict of interest among authors.

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References

Austin, P.C., Steyerberg, E.W., 2017. Events per variable (EPV) and the relative performance of different strategies for estimating the out-of-sample validity of logistic regression models. Stat. Methods Med. Res. 26, 796–808. https://doi.org/10.1177/0962280214558972.

Bernard, I., Limonta, D., Mahal, L.K., Hobman, T.C., 2020. Endothelium infection and dysregulation by SARS-CoV-2: evidence and caveats in COVID-19. Viruses 13. https://doi.org/10.3390/V13010029.

Bujang, M.A., Saat, N., TMI, Tg Abu Bakar Sidik, Lim, C.J., 2018. Sample size guidelines for logistic regression from observational studies with large population: emphasis on the accuracy between statistics and parameters based on real life clinical data. Malays J Med Sci 25, 122–130. https://doi.org/10.21315/MJMS2018.25.4.12.

Channappanavar, R., Fehr, A.R., Vijay, R., Mack, M., Zhao, J., Meyerholz, D.K., Perlman, S., 2016. Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. Cell Host Microbe 19, 181–193. https://doi.org/10.1016/J.CHOM.2016.01.007.

Chao, J., Bledsoe, G., Chao, L., 2016. Protective role of Kallistatin in vascular and organ injury. Hypertens. (Dallas, Tex. 1979) 68, 533. https://doi.org/10.1161/HYPERTENSIONAHA.116.07861.

Chao, J., Guo, Y., Chao, L., 2018. Protective role of endogenous Kallistatin in vascular injury and senescence by inhibiting oxidative stress and inflammation. Oxidative Med. Cell. Longev. 2018 https://doi.org/10.1155/2018/4138560.

Coca, S.G., Cho, K.C., Hsu, C.Y., 2011. Acute kidney injury in the elderly: predisposition to chronic kidney Disease and vice versa. Nephron. Clin. Pract. 119, c19 https://doi.org/10.1159/000328023.

Devaux, C.A., Rolain, J.M., Colson, P., Raoult, D., 2020. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? Int. J. Antimicrob. Agents 55. https://doi.org/10.1016/J.IJANTIMICAG.2020.105938.

Ertuğlu, L.A., Kanbay, A., Afşar, B., Elsürer Afşar, R., Kanbay, M., 2020. COVID-19 and acute kidney injury. Tuberk. Toraks 68, 407–418. https://doi.org/10.5578/TT.70010.

Feest, T.G., Round, A., Hamad, S., 1993. Incidence of severe acute renal failure in adults: results of a community based study. BMJ 306, 481–483. https://doi.org/10.1136/BMJ.306.6876.481.

Frank, A.J., Sheu, C.C., Zhao, Y., Chen, F., Su, L., Gong, M.N., Bajwa, E., Taylor Thompson, B., Christiani, D.C., 2012. BCL2 genetic variants are associated with acute kidney injury in septic shock*. Crit. Care Med. 40, 2116–2123. https://doi.org/10.1097/CCM.0B013E3182514BCA.

Gattinoni, L., Coppola, S., Cressoni, M., Busana, M., Rossi, S., Chiumello, D., 2020. COVID-19 does not Lead to a "typical" acute respiratory distress syndrome. Am. J. Respir. Crit. Care Med. 201, 1299–1300. https://doi.org/10.1164/RCCM.202003-0817LE.

Gatto, M., Iaccarino, L., Ghirardello, A., Bassi, N., Pontisso, P., Punzi, L., Shoenfeld, Y., Doria, A., 2013. Serpins, immunity and autoimmunity: old molecules, new functions. Clin. Rev. Allergy Immunol. 45, 267–280. https://doi.org/10.1007/S12016-013-8353-3.

Himmelfarb, J., 2009. Acute kidney injury in the elderly: problems and prospects. Semin. Nephrol. 29, 658–664. https://doi.org/10.1016/J.SEMNEPHROL.2009.07.008.

Ishani, A., Xue, J.L., Himmelfarb, J., Eggers, P.W., Kimmel, P.L., Molitoris, B.A., Collins, A.J., 2009. Acute kidney injury increases risk of ESRD among elderly. J. Am. Soc. Nephrol. 20, 223–228. https://doi.org/10.1681/ASN.2007080837.

Jenkins, A.J., Januszewski, A.S., Karschimkus, C.S., Zhang, B., O'Neal, D.N., Nelson, C.L., Chung, J.S., Harper, C.A., Lyons, T.J., Ma, J.X., 2010. Increased serum kallistatin levels in type 1 diabetes patients with vascular complications. J. Angiogenes. Res. 2 https://doi.org/10.1186/2040-2384-2-19.

Kane-Gill, S.L., Sileanu, F.E., Murugan, R., Trietley, G.S., Handler, S.M., Kellum, J.A., 2015. Risk factors for acute kidney injury in older adults with critical illness: a retrospective cohort study. Am. J. Kidney Dis. 65, 860–869. https://doi.org/10.1053/J.AJKD.2014.10.018.

Kellum, J.A., Olivier van Till, J.W., Mulligan, G., 2020. Targeting acute kidney injury in COVID-19. Nephrol. Dial. Transplant. 35, 1652–1662. https://doi.org/10.1093/NDT/GFAA231.

Kelly-robinson, G.A., Reihill, J.A., Lundy, F.T., McGarvey, L.P., Lockhart, J.C., Litherland, G.J., Thornbury, K.D., Martin, S.L., 2021. The serpin superfamily and their role in the regulation and dysfunction of serine protease activity in COPD and other chronic lung diseases. Int. J. Mol. Sci. 22 https://doi.org/10.3390/IJMS22126351.

Kidney Disease: Improving Global Outcomes (KDIGO), 2012. Acute Kidney Injury (AKI). Available at: https://kdigo.org/guidelines/acute-kidneyinjury/. Accessed April 10, 2020.

Leu, C.H., Yang, M.L., Chung, N.H., Huang, Y.J., Su, Y.C., Chen, Y.C., Lin, C.C., Shieh, G. S., Chang, M.Y., Wang, S.W., Chang, Y., Chao, J., Chao, L., Wu, C.L., Shiau, A.L., 2015. Kallistatin ameliorates influenza virus pathogenesis by inhibition of kallikrein-related peptidase 1-mediated cleavage of viral hemagglutinin. Antimicrob. Agents Chemother. 59, 5619–5630. https://doi.org/10.1128/AAC.00065-15.

Lin, W.C., Lu, S.L., Lin, C.F., Chen, C.W., Chao, L., Chao, J., Lin, Y.S., 2013. Plasma kallistatin levels in patients with severe community-acquired pneumonia. Crit. Care 17. https://doi.org/10.1186/CC12507.

Lin, W.C., Chen, C.W., Huang, Y.W., Chao, L., Chao, J., Lin, Y.S., Lin, C.F., 2015. Kallistatin protects against sepsis-related acute lung injury via inhibiting inflammation and apoptosis. Sci. Report. 51 (5), 1–16. https://doi.org/10.1038/srep12463.

Mkaouar, H., Akermi, N., Kriaa, A., Abraham, A.L., Jablaoui, A., Soussou, S., Mokdad-Gargouri, R., Maguin, E., Rhimi, M., 2019. Serine protease inhibitors and human wellbeing interplay: new insights for old friends. PeerJ 7. https://doi.org/10.7717/PEERJ.7224.

Nägele, M.P., Haubner, B., Tanner, F.C., Ruschitzka, F., Flammer, A.J., 2020. Endothelial dysfunction in COVID-19: current findings and therapeutic implications. Atherosclerosis 314, 58. https://doi.org/10.1016/J.ATHEROSCLEROSIS.2020.10.014.

Naser, M.N., Al-Ghatam, R., Darwish, A.H., Alqahtani, M.M., Alahmadi, H.A., Mohamed, K.A., Hasan, N.K., Perez, N.S., 2021. Risk factors, predictions, and progression of acute kidney injury in hospitalized COVID-19 patients: an observational retrospective cohort study. PLoS One 16, e0257253. https://doi.org/10.1371/JOURNAL.PONE.0257253.

Nishii, Y., Gabazza, E.C., Fujimoto, H., Nakahara, H., Takagi, T., Bruno, N., D'Alessandro-Gabazza, C.N., Maruyama, J., Maruyama, K., Hayashi, T., Adachi, Y., Suzuki, K., Taguchi, O., 2006. Protective role of protein C inhibitor in monocrotaline-induced pulmonary hypertension. J. Thromb. Haemost. 4, 2331–2339. https://doi.org/10.1111/J.1538-7836.2006.02174.X.

Peduzzi, P., Concato, J., Kemper, E., et al., 1996. A simulation study of the number of events per variable in logistic regression analysis. J. Clin. Epidemiol. 49, 1373–1379. https://doi.org/10.1016/S0895-4356(96)00236-3.

Poukkanen, M., Vaara, S.T., Pettilä, V., Kaukonen, K.M., Korhonen, A.M., Hovilehto, S., Inkinen, O., Laru-Sompa, R., Kaminski, T., Reinikainen, M., Lund, V., Karlsson, S., 2013. Acute kidney injury in patients with severe sepsis in Finnish intensive care units. Acta Anaesthesiol. Scand. 57, 863–872. https://doi.org/10.1111/AAS.12133.

Radtke, K.P., Fernández, J.A., Greengard, J.S., Tang, W.W., Wilson, C.B., Loskutoff, D.J., Scharrer, I., Griffin, J.H., 1994. Protein C inhibitor is expressed in tubular cells of human kidney. J. Clin. Invest. 94, 2117–2124. https://doi.org/10.1172/JCI117566.

Ronco, C., Reis, T., 2020. Kidney involvement in COVID-19 and rationale for extracorporeal therapies. Nat. Rev. Nephrol. 16, 308–310. https://doi.org/10.1038/S41581-020-0284-7.

Ronco, C., Bellomo, R., Kellum, J.A., 2019. Acute kidney injury. Lancet (London, England) 394, 1949–1964. https://doi.org/10.1016/S0140-6736(19)32563-2.

Ronco, C., Reis, T., Husain-Syed, F., 2020. Management of acute kidney injury in patients with COVID-19. Lancet Respir. Med. 8, 738–742. https://doi.org/10.1016/S2213-2600(20)30229-0.

Shen, B., Hagiwara, M., Yao, Y.Y., Chao, L., Chao, J., 2008. Salutary effect of kallistatin in salt-induced renal injury, inflammation, and fibrosis via antioxidative stress. Hypertens. (Dallas, Tex. 1979) 51, 1358–1365. https://doi.org/10.1161/HYPERTENSIONAHA.107.108514.

Shen, B., Gao, L., Te Hsu, Y., Bledsoe, G., Hagiwara, M., Chao, L., Chao, J., 2010. Kallistatin attenuates endothelial apoptosis through inhibition of oxidative stress and activation of Akt-eNOS signaling. Am. J. Physiol. Heart Circ. Physiol. 299 https://doi.org/10.1152/AJPHEART.00591.2010.

Suzuki, K., 2008. The multi-functional serpin, protein C inhibitor: beyond thrombosis and hemostasis. J. Thromb. Haemost. 6, 2017–2026. https://doi.org/10.1111/J.1538-7836.2008.03181.X.

Vilander, L.M., Kaunisto, M.A., Vaara, S.T., Pettilä, V., Laru-Sompa, R., Pulkkinen, et al., 2017. Genetic variants in SERPINA4 and SERPINA5, but not BCL2 and SIK3 are associated with acute kidney injury in critically ill patients with septic shock. Crit. Care 21. https://doi.org/10.1186/S13054-017-1631-3.

Wakita, T., Hayashi, T., Nishioka, J., Tamaru, H., Akita, N., Asanuma, K., Kamada, H., Gabazza, E.C., Ido, M., Kawamura, J., Suzuki, K., 2004. Regulation of carcinoma cell invasion by protein C inhibitor whose expression is decreased in renal cell carcinoma. Int. J. Cancer 108, 516–523. https://doi.org/10.1002/IJC.11594.

World Health Organization, 2021. Clinical Management of COVID-19: patients: living guidance. World Health Organization, pp. 13–15 v0.16.

Xu, L., Mao, Y., Chen, G., 2020a. Risk factors for 2019 novel coronavirus disease (COVID-19) patients progressing to critical illness: a systematic review and meta-analysis. Aging (Albany NY) 12, 12410. https://doi.org/10.18632/AGING.103383.

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., Liu, S., Zhao, P., Liu, H., Zhu, L., Tai, Y., Bai, C., Gao, T., Song, J., Xia, P., Dong, J., Zhao, J., Wang, F.S., 2020b. Pathological findings of COVID-19 associated with acute respiratory distress

syndrome. Lancet Respir. Med. 8, 420–422. https://doi.org/10.1016/S2213-2600(20)30076-X.

Yang, H., Geiger, M., 2017. Cell penetrating SERPINA5 (ProteinC inhibitor, PCI): more questions than answers. Semin. Cell Dev. Biol. 62, 187–193. https://doi.org/10.1016/J.SEMCDB.2016.10.007.

Yiu, W.H., Wong, D.W.L., Wu, H.J., Li, R.X., Yam, I., Chan, L.Y.Y., Leung, J.C.K., Lan, H. Y., Lai, K.N., Tang, S.C.W., 2016. Kallistatin protects against diabetic nephropathy in db/db mice by suppressing AGE-RAGE-induced oxidative stress. Kidney Int. 89, 386–398. https://doi.org/10.1038/KI.2015.331.

Zaim, S., Chong, J.H., Sankaranarayanan, V., Harky, A., 2020. COVID-19 and multiorgan response. Curr. Probl. Cardiol. 45, 100618 https://doi.org/10.1016/J.CPCARDIOL.2020.100618.

Zhang, B., Zhou, X., Qiu, Y., Song, Y., Feng, F., Feng, J., Song, Q., Jia, Q., Wang, J., 2020. Clinical characteristics of 82 cases of death from COVID-19. PLoS One 15. https://doi.org/10.1371/JOURNAL.PONE.0235458.

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., Guan, L., Wei, Y., Li, H., Wu, X., Xu, J., Tu, S., Zhang, Y., Chen, H., Cao, B., 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet (London, England) 395, 1054–1062. https://doi.org/10.1016/S0140-6736(20)30566-3.