

Association between Calprotectin and Cystatin C levels with Kidney Injury Molecule-1 and their Effects on Developments of COVID-19 Complications

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Abstract

Objectives: This work aimed to study the association between KIM-1, Calprotectin and Cystatin C and other biomarkers and their roles for early detection of the severity of Covid-19 associated with kidney injury complications that lead to death in severe Covid-19 patients.

Methods: This study included 91 samples patients with Covid-19 divided into two group, 46 patients of them with severe Covid-19 and 45 patients of them with moderate infected Covid-19 include (56 male, 35 female) with a matched age ranged between (22–88) years patients. The study was conducted from Al-hayat unit, Al-Hussein Teaching Hospital, Al-Hussein Medical City during the period from (Oct., 2020 to July., 2021). The biomarkers determined include: ferritin, C-reactive protein (CRP), total lactate dehydrogenase (LDH) activity, creatinine, urea, cystatin C (Cys C), calprotectin (CLP), and kidney injury molecule-1 (KIM-1) beside complete blood count test (CBC) which were determined by using various biochemical techniques including ELISA.

Results: The results of data which include 46 patients with severe Covid-19 group and another 45 patients with moderate Covid-19 infection, there is significant data obtained for ferritin, LDH and RDW-CV when compare between severe and moderate group of Covid-19 patients ($P < 0.05$) while other parameter showed a non-significant increased result. There is positive correlation between KIM-1 and Cys C in severe and moderate group ($P < 0.01$) and with creatinine ($P < 0.01$) and urea ($P < 0.05$) in severe Covid-19 group, and significant correlation between Cys C and creatinine in severe group ($P < 0.05$).

Conclusion: There is a significant correlation between KIM-1 and CysC and serum creatinine with Cys. C and KIM-1. Also, the obtained results illustrated that CLP, Cys C and KIM-1 were non-significant increase in Covid-19 patients groups, but ferritin and LDH had increment significantly when compare between severe and moderate group and can be served as parameter predictor and increased mortality risk for Covid-19 patients.

Keyword: Leukocyte L1 antigen complex, cystatin C, COVID-19, pathogenesis

Introduction

Coronavirus is one of the major viruses which primarily affecting the respiratory system in human.¹ However, coronaviruses have been also diagnosed in animals and can cause a range of severe diseases such as gastroenteritis and pneumonia.² The hallmark of severe Covid-19 is the hyper-inflammatory host response due to the so-called “cytokine storm”, defined as an uncontrolled systemic inflammatory response due to the release of large amounts of pro-inflammatory cytokines, resulting from the SARS-CoV-2 induced activation of both natural and cellular immunity.³ Moreover, circulating mediators could interact with kidney-resident cells resulting in endothelial dysfunction, microcirculatory derangement, and tubular injury.⁴

Calprotectin (CLP) is a heterodimeric complex formed by two binding proteins of the calcium ion, which belong to the S-100 protein family, S100A8 and S100A9, having both anti-inflammatory and anti-bacterial properties. The first applied name to CLP was major leukocyte protein L1 or 27E10.⁵ It is expressed as an anti-bacterial agent, mainly but not exclusively by neutrophils, when activated.⁶ Previous studies have reported significantly elevated levels of calprotectin in patients with severe Covid-19 and the possible ability of calprotectin to discriminate between mild and severe form of the disease.⁶

There are three distinct types of cystatins that share sequence and structure homology but differ in size, site of action, and disulfide bond topology.⁷ Renal tubular epithelial cells do not secrete sCys C into the lumen, therefore its serum concentration is mainly determined by glomerular filtration rate which is an important indicator of glomerular filtration.⁸ Furthermore, cystatin C was suggested as an independent predictive factor of high mortality among the elderly population.⁹ Several studies reported kidneys alterations, as reflected by increased serum creatinine, in Covid-19 patients. SARS-CoV-2 could directly infect kidney tubular cells, which express the ACE-2 receptor on their cellular surface.⁵

Kidney Injury Molecule-1 (KIM-1) was identified by our group as the most up-regulated protein in the kidney proximal tubule after a wide variety of injurious influences including ischemia, nephrotoxicants, sepsis and immune related injury and its cleaved ectodomain is often used as a serum and urine marker for kidney injury.¹⁰ KIM-1 also functions as a scavenger receptor, mediating the uptake of modified low-density lipoprotein and necrotic-cell debris.¹¹ The ability of the virus to bind the ubiquitous angiotensin-converting enzyme (ACE)-2 receptors allows SARS-CoV-2 to target organs other than the lungs, such as the heart, central nervous system, gastrointestinal tract, etc.¹²

Materials and Methods

This cross-sectional study included 91 samples (56 male, 35 female) infected with Covid-19 divided into two groups according to their clinical and biochemical investigations, 46 patients of them were infected with severe Covid-19 and the other 45 patients with moderate Covid-19 with match age ranged between (22–88) years. The patients were admitted Al-hayat unit, Al-Hussein Teaching Hospital, Al-Hussein Medical City, Kerbala Health Directorate/Kerbala - Iraq during Oct., 2020 to July, 2021). The study proposal was approved by local medical ethics and all participants, information consent before the onset of study. The study excluded patients with any chronic or immune diseases like diabetes mellitus, infection and inflammation, and receiving long term oral corticosteroid, anti-IL-6 or anti-TNF therapy and patients had history of vasculitis connective tissue disease. The patients were registered and handed over a file for recording their data such as name, age, gender, weight, height, smoking states, current chronic diseases (diabetes mellitus, hypertension). Serum calprotectin, cystatin C, kidney injury molecule-1, C-reactive protein, ferritin, creatinine, urea concentration was investigated by ELISA (for Cys. C and CLP), chemical and spectrophotometric procedure Maglumi technique and (S. Cr, blood urea and CRP) and by chemiluminescence technique (CLIA).

Results

The clinical demographic characteristics and laboratory parameters of both patients' groups of Covid-19 were summarized in Table 1.

Table 1 illustrates the mean \pm SD of participants age which were within the age group of (22–88) years and they are higher in severe Covid-19 as compared with moderate. Gender distribution among the studied groups was: 62% male, 38% female for patients' group. The diabetes mellitus, hypertension and oxygen saturation were higher in severe Covid-19 than that found in moderate. The patient's group was divided into (moderate and severe) based on severity of disease based on WHO guideline. The data was collected through self-reported technique (student questionnaire).

This study showed significant positive correlation between KIM-1 and Cys C in moderate group infected with Covid-19 ($r = 0.44$, $P = 0.002$) and also in severe group ($r = 0.54$, $P = 0.000$). Also, positive correlation between KIM-1 and creatinine ($r = 0.46$, $P = 0.001$) and blood urea ($r = 0.32$, $P = 0.03$) in severe Covid-19 group were obtained. These findings are shown in Figures 1–5.

Figures 6 and 7 showed no correlation between KIM-1 and CLP in moderate group of Covid-19 patients ($r = 0.27$, $P = 0.06$) and severe group of Covid-19 patients ($r = 0.02$, $P = 0.8$).

Table 2 showed the biochemical changes in the concentrations of different biomarkers in both severe and moderate groups. There were significant increments in levels of ferritin and LDH activity (0.02) respectively. There was a non-significant level in CLP, Cys. C, KIM-1 and other biomarkers as compared between moderate Covid-19 and severe Covid-19.

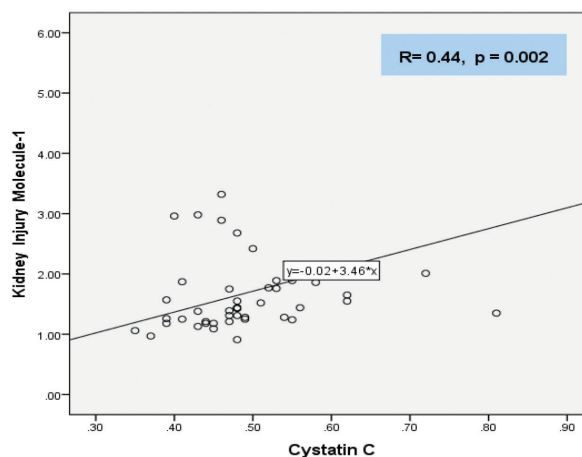


Fig. 1 Correlation between Kidney Injury Molecule-1 and Calprotectin in moderate group of Covid-19 patients.

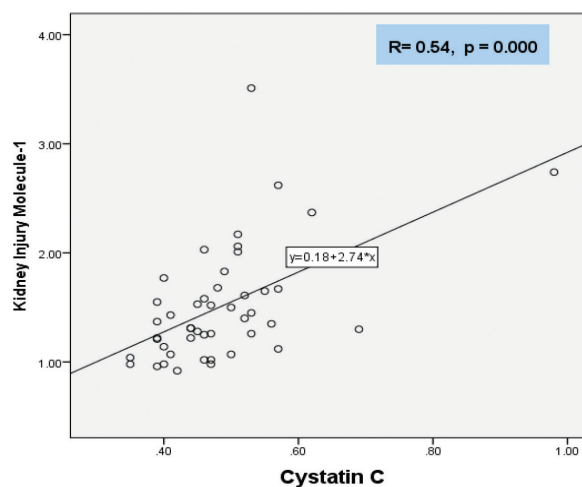


Fig. 2 Correlation between Kidney Injury Molecule-1 and Calprotectin in severe group of Covid-19 patients.

Table 1. Demographic characteristics of Covid-19 patients in severe and moderate groups

Characteristics	Patient group		
	Moderate	Severe	
Demographics	Age	56.82 \pm 12.574	63.04 \pm 11.143
	Gender (Male/Female)	(28/17)	(29/17)
Medical history	Smoking (Yes/No)	(6/39)	(8/38)
	DM (Yes/No)	(17/28)	(22/24)
	HT (Yes/No)	(19/26)	(26/20)
	Sat. O ₂	91.87 \pm 3.181	70.87 \pm 10.340

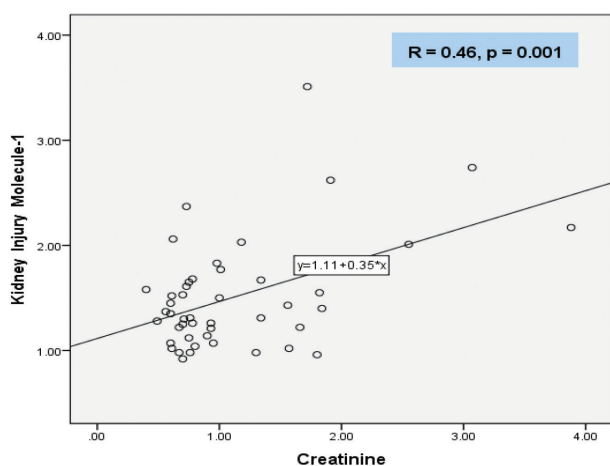


Fig. 3 Correlation between Kidney Injury Molecule-1 and Creatinine in severe group of Covid-19 patients.

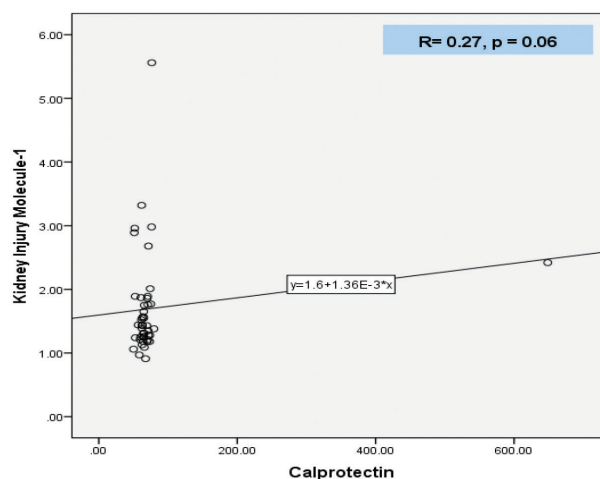


Fig. 6 Correlation between Kidney Injury Molecule-1 and Calprotectin in moderate group of Covid-19 patients.

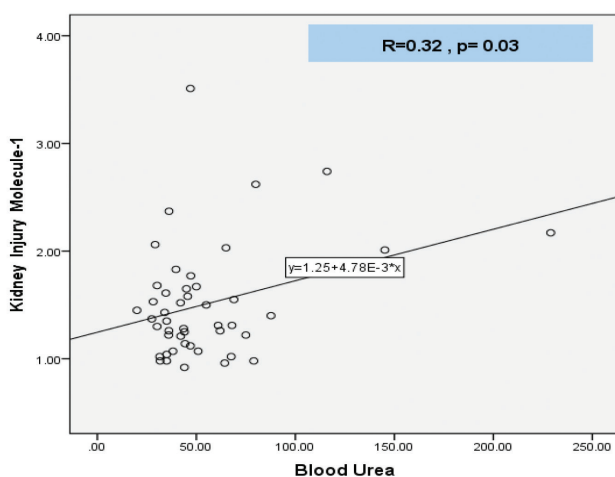


Fig. 4 Correlation between Kidney Injury Molecule-1 and Blood Urea in severe group of Covid-19 patients.

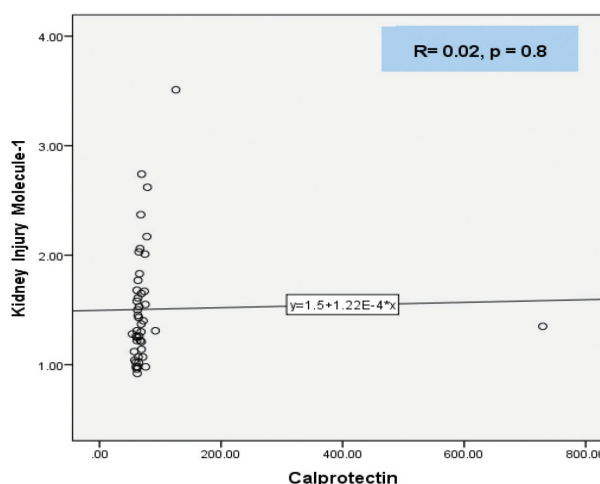


Fig. 7 Correlation between Kidney Injury Molecule-1 and Calprotectin in severe group of Covid-19 patients.

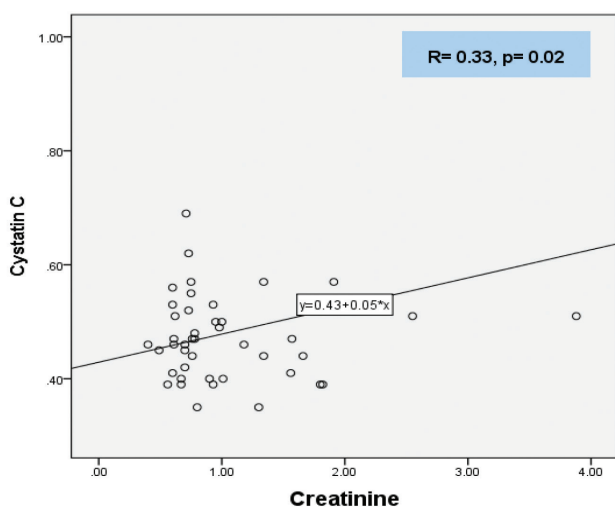


Fig. 5 Correlation between Cystatin C and Creatinine in severe group of Covid-19 patients.

Discussion

Various biomarker and genetic polymorphism studies on sera of Covid-19 have been performed in Iraqi pandemic.^{13,14} Most data on Covid-19 are from China, and although most confirmed cases have been classified into mild or moderate, 14% are severe and 5% critical.¹⁵ Another report showed that the most frequent comorbidities in patients with Covid-19 who developed the acute respiratory distress syndrome were hypertension (27%), diabetes (19%), and cardiovascular disease (6%).¹⁶ In this study found male more than female that is infected with Covid-19 agreed with Zhao et al. found cell type-specific expression of the ACE2 receptor in type II alveolar epithelial cells is higher in males than in females.¹⁷ In Italy, the reported death rate in men (16.6%) is significantly higher than that in women 9.1%.¹⁸ With regard to coronaviruses, in particular, smoking is associated with increased susceptibility and mortality in MERS-CoV infection, potentially due to upregulation of dipeptidyl peptidase-IV, the host receptor for MERS-CoV, in smokers.¹⁹ In addition, a meta-analysis performed failed to find a relationship between active smoking

Table 2. Change in biochemical parameters between severe and moderate Covid-19 groups

Variables with normal range	Moderate Covid-19 Cases Mean ± SD	Severe Covid-19 Cases Mean ± SD	P-value
CRP, (0–6 mg/l)	85.58 ± 122.14	63.65 ± 59.89	0.27
Ferritin, (20–350 ng/ml)	598.63 ± 502.53	894.99 ± 712.82	0.02
LDH, (240–480 U/l)	409.31 ± 217.30		0.02
S. Creatinine, (0.7–1.2 mg/dl)	2.67 ± 10.28	1.11 ± 0.69	0.3
Blood urea, (12–45 mg/dl)	57.70 ± 35.45	54.45 ± 35.32	0.6
Calprotectin, (31.64–126.97 ng/ml)	78.24 ± 130.38	89.27 ± 49.90	0.5
Cystatin C, (0.24–0.76 mg/dl)	0.49 ± 0.104	0.48 ± 0.103	0.5
Kidney Injury Molecule-1, (0.76–3.26 ng/ml)	1.50 ± 0.52	1.702 ± 0.104	0.1

and severe Covid-19 on Chinese patients, and another meta-analysis indicates that active smoking is not a predisposing factor for hospitalization.²⁰ The ratio of oxygen saturation as measured by pulse oximetry divided by the fraction of inspired oxygen is a simple measure, which has been previously used in the setting of acute respiratory distress syndrome instead of more complex variables,²¹ and thus can be evaluated in each patient with Covid-19 pneumonia to help identify patients at higher risk of severe disease.

During the pandemic period, it has been shown that 56.9% of hospitalized COVID-19 positive patients and 37.2% of COVID-19 negative patients developed AKI.²² In this meta-analysis, although the rate of patients with increased serum creatinine levels was 9.6%, proteinuria was found in 57.2% of all patients.²³ For now, it is well known that KIM-1 expression is elevated in various etiologies of AKI, chronic kidney disease, the kidney transplant population, and renal cell carcinoma.²⁴ Most of the cases present with complications and is diagnosed by blood levels of conventional marker, which is serum creatinine. Estimation of serum cystatin C can help early diagnosis of CKD, compared with traditional renal function indexes, such as serum creatinine and urea nitrogen, Cys-C can reflect glomerular filtration rate more accurately.²⁵ An increase in urea level is seen when there is damage to the kidney. Increase in blood urea level in the presence of high blood sugar level in diabetic patient indicates damage to the kidney. Studies conducted by Anjaneyulu et al had found that increase urea and serum creatinine in diabetic rats indicates progressive renal damage.²⁶

Calprotectin could play a pivotal regulatory role in controlling the inflammatory cascades and leukocyte trafficking to yield more coordinated immune reactions.²⁷ Serum calprotectin was significantly increased in patients with AKI compared to patients without AKI. Serum calprotectin was also significantly increased in patients with sepsis compared to patients without sepsis.²⁸ In another study, they concluded that KIM-1 is a blood biomarker that specifically reflects acute and chronic kidney injury.²⁹

In our study ferritin is increased in severe than moderate group Table 2 and it is significant when compare between it and that is agreement with Qin et al. reported that Covid-19 patients with high levels of ferritin have greater proportions of severe and deceased cases ($P = 0.0016$).³⁰ Theoretically, elevated serum LDH is an important laboratory indicator for evaluating Covid-19.³¹ In this study showed significant increment in LDH levies in severe and moderate group that is agreed with Zhou et al. showed that the levels of serum ferritin,

D-dimer, lactate dehydrogenase, and interleukin-6 (IL-6) are increased during the worsening of the disease, providing an indication of the risk of mortality.³² Tan et al. and other studies concluded that CRP was associated with disease progression and predicted early severe Covid-19.¹⁵ In present study there is a non-significant increment when measure the kidney function biomarkers serum (Cr, urea, Cys C and KIM-1) and the mechanism of kidney injury following Covid-19 infection remains unclear, although the co-expression of angiotensin-converting enzyme 2 (ACE2) receptors and transmembrane serine proteases (TMPRSSs) is key for the entry of SARS-CoV-2 into host cells.³³ Calprotectin is a protein that is especially secreted by neutrophils secondary to inflammation. It has recently been reported that calprotectin can be used as a biomarker of inflammation for assessing the activity of some inflammatory disorders.³⁴ In addition, according to that study, a significant elevation of serum calprotectin was associated with high mortality in Covid-19 patients.³⁵ Studies are ongoing, but there is not enough evidence at this time to support this finding and no scientific consensus whether calprotectin can serve as a prediction of how serious the virus will be in some patients. Researchers will continue studying calprotectin in COVID-19 patients.³⁶

Conclusion

There is a positive correlation between KIM-1 with Cys C in severe and moderate Covid-19 groups, but only in severe group with serum Creatinine and blood urea. Also a significant positive correlation between Cys C and serum creatinine in severe group of Covid-19 patients. There is a non-significant elevation in CLP, Cys C and KIM-1 and they cannot serve as indicator biomarkers, but. Hyperferritinemia can serve as predictor of severe and fatal Covid-19 and extent of cytokine storm and its risk factor of poor prognosis in patients with Covid-19. Also elevated levels of LDH activity served as parameter for evaluating Covid-19 and increased during acute lung damage.

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Conflicts of Interest

None. ■

References

- Chen, Yu; Liu, Qianyun; Guo, Deyin. Emerging coronaviruses: genome structure, replication, and pathogenesis. *Journal of Medical Virology*, 2020, 92.4: 418–423.
- Gralinski, Lisa E.; Menachery, Vineet D. Return of the Coronavirus: 2019-nCoV. *Viruses*, 2020, 12.2: 135.
- Ciaccio, Marcello; Agnello, Luisa. Biochemical biomarkers alterations in Coronavirus disease 2019 (Covid-19). *Diagnosis*, 2020, 7.4: 365–372.
- Joaninidis, Michael, et al. Lung–kidney interactions in critically ill patients: consensus report of the Acute Disease Quality Initiative (ADQI) 21 Workgroup. *Intensive Care Medicine*, 2020, 46.4: 654–672.
- Ometto, Francesca, et al. Calprotectin in rheumatic diseases. *Experimental Biology and Medicine*, 2017, 242.8: 859–873.
- Chen, Liting, et al. Elevated serum levels of S100A8/A9 and HMGB1 at hospital admission are correlated with inferior clinical outcomes in Covid-19 patients. *Cellular and Molecular Immunology*, 2020, 17.9: 992–994.
- Barrett, A. J. The cystatins: a diverse superfamily of cysteine peptidase inhibitors. *Biomedica biochimica acta*, 1986, 45.11-12: 1363–1374.
- Liu, J. Evaluation of serum cystatin C for diagnosis of acute rejection after renal transplantation. In: *Transplantation Proceedings*. Elsevier, 2012. p. 1250–1253.
- Ingelfinger, Julie R.; Marsden, Philip A. Estimated GFR and risk of death—is Cystatin C useful?. *The New England journal of medicine*, 2013, 369.10: 974.
- Brooks, Craig R., et al. KIM-1-/TIM-1-mediated phagocytosis links ATG 5-/ULK 1-dependent clearance of apoptotic cells to antigen presentation. *The EMBO Journal*, 2015, 34.19: 2441–2464.
- Ichimura, Takaharu, et al. Kidney injury molecule-1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells. *The Journal of Clinical Investigation*, 2008, 118.5: 1657–1668.
- Carfora, Vincenzo, et al. Anticoagulant treatment in Covid-19: a narrative review. *Journal of Thrombosis and Thrombolysis*, 2021, 51.3: 642–648.
- Noman W. A., Ali H. A., Mahdi A. B. Zabibah R. S., and Ali R. A. The Association between Serum Levels of Ferritin and D-Dimer with Liver Function Tests in Patients with Covid-19, 2021, *Clin Schizophr Relat Psychoses*, 15(4):2–6.
- Mohsin HS, Ali HA, Al-Tu'ma FJ. Assessment of von Willebrand Factor/ADAMTS13 Ratio and Vitamin K Levels as Predictor Markers for Severity of COVID-19 Patients. *J. Contemp. Med. Sci.*, 2021, 7. 5: 303–307.
- Wu, Zunyou; McGoogan, Jennifer M. Characteristics of and important lessons from the coronavirus disease 2019 (Covid-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA*, 2020, 323.13: 1239–1242.
- Wu, Chaomin, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Internal Medicine*, 2020, 180.7: 934–943.
- Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCoV. *bioRxiv*. 2020 Jan 1.
- Gagliardi, Maria Cristina, et al. ACE-2 expression and sex disparity in Covid-19. *Cell Death Discovery*, 2020, 6.1: 1–2.
- Seys, Leen JM, et al. DPP4, the Middle East respiratory syndrome coronavirus receptor, is upregulated in lungs of smokers and chronic obstructive pulmonary disease patients. *Clinical Infectious Diseases*, 2018, 66.1: 45–53.
- Lippi, Giuseppe; Henry, Brandon Michael. Active smoking is not associated with severity of coronavirus disease 2019 (Covid-19). *European Journal of Internal Medicine*, 2020, 75: 107–108.
- Schmid, Marcello FS; Gernand, Jill; Kakaral, Radhika. The use of the pulse oximetry saturation to fraction of inspired oxygen ratio in an automated acute respiratory distress syndrome screening tool. *Journal of Critical Care*, 2015, 30.3: 486–490.
- Fisher, Molly, et al. AKI in hospitalized patients with and without Covid-19: a comparison study. *Journal of the American Society of Nephrology*, 2020, 31.9: 2145–2157.
- Yang, Xianghong, et al. Prevalence and impact of acute renal impairment on Covid-19: a systematic review and meta-analysis. *Critical Care*, 2020, 24.1: 1–8.
- Fontanill, John; Han, Won K. Kidney injury molecule-1 as an early detection tool for acute kidney injury and other kidney diseases. *Expert Opinion on Medical Diagnostics*, 2011, 5.2: 161–173.
- Iyare, Rachel Nkem; Volskiy, Vladimir; Vandenbosch, Guy AE. Study of the correlation between outdoor and indoor electromagnetic exposure near cellular base stations in Leuven, Belgium. *Environmental Research*, 2019, 168: 428–438.
- Anjaneyulu, Muragundla; Chopra, Kanwaljit. Quercetin, an anti-oxidant bioflavonoid, attenuates diabetic nephropathy in rats. *Clinical and Experimental Pharmacology and Physiology*, 2004, 31.4: 244–248.
- Kopec-Medrek, Magdalena; Widuchowska, Małgorzata; Kucharz, Eugeniusz J. Calprotectin in rheumatic diseases: a review. *Reumatologia*, 2016, 54.6: 306.
- Nikolakopoulou, Zacharoula, et al. Plasma S100A8/A9 heterodimer is an early prognostic marker of acute kidney injury associated with cardiac surgery. *Biomarkers in Medicine*, 2019, 13.3: 205–218.
- Sabbisetti, Venkata S., et al. Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. *Journal of the American Society of Nephrology*, 2014, 25.10: 2177–2186.
- Qin, Lu, et al. Gendered effects on inflammation reaction and outcome of Covid-19 patients in Wuhan. *Journal of Medical Virology*, 2020, 92.11: 2684–2692.
- Liu, Yingxia, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Science China Life Sciences*, 2020, 63.3: 364–374.
- Tan, Chaochao, et al. C-reactive protein correlates with computed tomographic findings and predicts severe Covid-19 early. *Journal of Medical Virology*, 2020, 92.7: 856–862.
- Zhang, Haibo, et al. Angiotensin-converting enzyme 2 (ACE-2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Medicine*, 2020, 46.4: 586–590.
- Wirtz, Theresa H., et al. Association of serum calprotectin concentrations with mortality in critically ill and septic patients. *Diagnostics*, 2020, 10.11: 990.
- Chen, Liting, et al. Elevated serum levels of S100A8/A9 and HMGB1 at hospital admission are correlated with inferior clinical outcomes in Covid-19 patients. *Cellular and Molecular Immunology*, 2020, 17.9: 992–994.
- Fisher, Molly, et al. AKI in hospitalized patients with and without Covid-19: a comparison study. *Journal of the American Society of Nephrology*, 2020, 31.9: 2145–2157.

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