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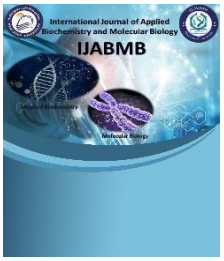

Role of Forkhead box protein O1 (FOXO-1) and Clinical Laboratory Investigations Assist to Investigate the Severity of COVID-19

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Running Title: FOXO-1 gene biomarker for COVID-19 patients.

Abstract

Background: The transmission of diseases across nations and continents is a significant disadvantage of globalization. In December 2019, COVID-19 emerged in Wuhan, China, as a new viral illness caused by a novel beta coronavirus. The forkhead box O1 (FoxO-1) transcription factors are reported to play a significant role in maintaining normal cell physiology by regulating survival, apoptosis, and oxidative stress.

Objective: we aimed to detect severity of COVID-19 by clinical laboratory investigation and epigenetic biomarkers FOXO-1.

Patients and methods: This study is a prospective case-control investigation involving 50 participants divided into two groups. Group I consists of 30 patients diagnosed with COVID-19. While Group II includes 20 healthy controls. The expression levels of FOXO-1 were evaluated using real-time quantitative polymerase chain reaction (RT-qPCR).

Results: In comparison between COVID-19 patients and healthy individuals, we use many clinical laboratory investigations such as Systolic, Diastolic blood pressure, RBCs, Hemoglobin, Hematocrit (%), MCHC (g/dL), PLTs, WBCs, INR, ALBUMIN, ALT, AST, LDH, PT, Creatinine, Sodium (Na), Random Blood Sugar (mg/dL) and FOXO-1. There are statistically significant differences in these parameters. The analysis of the ROC curve demonstrated along with AUC curve notable diagnostic performance at a cut-off value of 0.34-fold, yielding an AUC of 0.874, with a sensitivity of 88.0% and a specificity of 95.0%.

Conclusion: Then conclude from these results the expression levels of FOXO-1 and clinical laboratory investigation include hematological, serological and molecular tests serves as biomarkers in determining the severity of COVID-19.

Keywords: Coronavirus-2019 (COVID-19), Forkhead box protein O1 (FOXO-1).

Introduction

Coronaviruses are classified within the subfamily Coronavirinae, the family Coronaviridae, and the order Nidovirales. These viruses are enveloped and possess a single-stranded positive-sense RNA genome. The term "corona," which translates to "crown," refers to the distinctive club-shaped spikes that protrude from the virus's surface, along with its notably large RNA genome and unique replication mechanism. Coronaviruses are categorized into four genotypes: alpha, beta, gamma, and delta. Among these, alpha and beta are known to be pathogenic to humans, with a total of twenty-six distinct species exhibiting various forms of antigenic cross-reactivity (1). Coronaviruses have historically been recognized as significant viral pathogens in veterinary medicine, impacting a wide range of mammalian and avian species and leading to respiratory and gastrointestinal illnesses. Nevertheless, these viruses have been known to infect humans for a period of 500 to 800 years, with evidence suggesting that their origins can be traced back to bats (2).

People of all ages can contract SARS-CoV-2. Nevertheless, the likelihood of experiencing severe COVID-19 increases for individuals aged 65 and older, residents of nursing homes or long-term care facilities, those who are unvaccinated against COVID-19 or have inadequate responses to the vaccines, as well as individuals with specific chronic health conditions (3).

Data regarding comorbid health conditions in patients diagnosed with COVID-19 reveal that individuals with cardiovascular disease, chronic kidney disease, chronic obstructive pulmonary disease, diabetes with complications, neurocognitive disorders, and obesity face a heightened risk of experiencing severe COVID-19. This risk is notably greater among patients who have multiple comorbidities.

Additionally, other health issues that may contribute to an increased risk of severe COVID-19 include cancer, cystic fibrosis, conditions that compromise the immune system, liver disease (particularly in those with cirrhosis), pregnancy, and sickle cell disease. Furthermore, transplant recipients and individuals on immunosuppressive therapies are also considered to be at a significant risk for severe COVID-19 (4).

While COVID-19 vaccination does not completely eradicate the possibility of SARS-CoV-2 infection, it notably decreases the likelihood of morbidity and mortality associated with COVID-19, especially among those who are at a higher risk of developing severe illness (5).

The Forkhead Box O (FoxO) subfamily of transcription factors has been shown to have essential roles in maintaining pulmonary homeostasis, in addition to their participation in a range of cellular biochemical processes (6). FoxO factors play a crucial role in the adaptive immune system, particularly in the maturation and differentiation of B and T lymphocytes. Consequently, the interaction between FoxOs and SARS-CoV-2 may represent a significant opportunity for intervention, potentially addressing the harmful inflammatory response that follows SARS-CoV-2 infection. This review highlights the critical function of FoxO proteins in the mechanistic regulation of the host's inflammatory and immune responses to SARS-CoV-2 (7).

This review thus underscores the importance of expression levels FoxO-1 and other clinical laboratory analyses as effective biomarker to detect severity of SARS-CoV-2 and mechanistically regulating the host inflammatory and immunological response to SARS-CoV-2.

Patients and methods

This study was carried out at the Department of Medical Biochemistry and Molecular Biology at the Faculty of Medicine at Cairo University in

Cairo, Egypt. It is a prospective case-control study involving 50 participants divided into two groups: Group I includes 30 patients diagnosed with COVID-19, and Group II consists of 20 healthy control subjects. All participants gave informed consent before being included in the research. The study protocol was approved by the Ethical Committee of the Faculty of Medicine at Cairo University (N-448-2023). The diagnosis of COVID-19 in patients was confirmed using the RT-qPCR Detection Kit (Taq Path™ COVID-19 CE-IVD RT-PCR; Thermo Fisher), in conjunction with comprehensive history-taking, thorough clinical assessments, and routine laboratory tests. A 10 mL sample of peripheral blood was drawn into EDTA anticoagulated tubes (BD Vacutainer) from both COVID-19 patients at the time of diagnosis and healthy volunteers. This sample was kept at 4 ℃ until it was processed, which occurred within two hours of collection. The plasma samples underwent a two-step centrifugation process (2500 ×g for 10 minutes followed by 16,000 ×g for an additional 10 minutes, both at 4 °C) to extract the plasma. After separation, the cell-free plasma samples were homogenized, aliquoted, and stored at −80 °C for future analysis. Total RNA extraction was performed from serum samples using GeneJET RNA Purification Kit (Thermo Fisher Scientific, Inc.) following the instruction. Checking of RNA quality was done using NanoDrop® 1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA). Then, synthesis of complementary DNA (cDNA) was done using the High- Capacity cDNA Reverse Transcription Kits (Thermo Fisher Scientific) according to the instructions. Subsequently, real time-qPCR was performed for amplification of the genes of FOXO-1, using SYBR Premix Ex TaqTM II (Perfect Real Time, TaKaRa, Japan).

The PCR reaction was initiated with a step at 95°C for 5 minutes, followed by 40 cycles comprising 15 seconds at 95°C and 60 seconds at 60°C. The results were quantitatively assessed using the 2-ΔΔCt method, with GAPDH utilized as internal controls

for the normalization of the other genes.

FOXO-1: forward primer 5′-CTA CGA GTG GAT GGT CAA GAG C-3′ and reverse primer 5′-CCA GTT CCT TCA TTC TGC ACA CG-3′. Primer of housekeeping gene GAPDH forward sequence 5′-GTC TCC TCT GAC TTC AAC AGC G-3′ and reverse sequence 5′-ACC ACC CTG TTG CTG TAG CCA A-3′.

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS v23). For quantitative parametric data, an Independent Student's t-test was used to compare measurements between two independent groups. In contrast, One-way ANOVA was applied to evaluate differences among more than two independent groups, with the Bonferroni Post-Hoc test utilized to determine significance at a p-value of less than 0.05. For quantitative non-parametric data, the Kruskal-Wallis test and the Mann-Whitney test were applied to compare multiple independent groups. To evaluate the correlation among qualitative data, a Bivariate Pearson correlation test was conducted to analyze the relationships between different groups, employing a two-tailed method to assess significance. Additionally, sensitivity and specificity analyses were carried out to evaluate a new test through the ROC curve (Receiver Operating Characteristic). A significance threshold was set at a p-value of less than 0.05 (8).

Results

Demographic, Clinical, and laboratory characteristics of COVID-19 Patients: According to demographic data, there is no statistically significant difference between COVID-19 patients and the controls in age and sex. Additionally, statistically significant difference between COVID-19 patients and the controls in Systolic, Diastolic blood pressure, RBCs, Hemoglobin, Hematocrit (%), MCHC (g/dL), PLTs, WBCs, INR, ALBUMIN, ALT, AST, LDH, PT, Creatinine, Sodium (Na), Random Blood Sugar (mg/dL) and FOXO-1.

Table 1. Demographic, clinical and laboratory characteristics of studied participants

Parameters	COVID-19 (N=30)	Control (N=20)	P-value
Age (years)	61.46±10.72	59.70±9.02	0.30
Gender Female Male	13 (43.3%) 17 (56.7%)	12 (60%) 8 (40%)	0.25
Systolic blood pressure	132.07±27.42	103.50±10.89	**0.001***
Diastolic blood pressure	79.87±12.56	70.0±7.25	**0.032***
RBCs	4.32±0.85	4.90±0.05	**0.004***
Hemoglobin	11.75±2.60	12.28±1.30	**0.001***
Hematocrit (%)	36.03±7.36	38.90±0.85	**0.001***
MCHC (g/dL)	32.40±1.29	34.29±0.57	**0.001***
PLTs	212.60±78.46	290.75±60.18	**0.0001***
WBCs	8.97±6.53	5.68±1.51	**0.033***
INR	1.41±0.40	1.01±0.03	**0.0001***
ALBUMIN	2.90±0.53	4.50±0.21	**0.003***
ALT	72.71±16.64	13.35±1.12	**0.0001***
AST	74.70±16.54	16.40±1.42	**0.0001***
LDH	455.03±214.08	161.50±26.21	**0.002***
PT	20.34±7.29	11.50±0.51	**0.0001***
Creatinine	1.98±0.44	0.65±0.28	**0.004***
Sodium (Na)	139.99±1.97	136.40±1.72	**0.001***
Random Blood Sugar (mg/dL)	279.63±20.54	130.83±15.42	**0.004***
CRP	44.40±4.52	1.45±0.25	**0.0001***
RDW (%)	14.61±0.40	13.82±0.14	**0.003***
Partial pressure of Oxygen (pO2)	54.69±2.27	89.50±1.39	**0.001***
FOXO-1	3.37±1.22	1.10±0.01	**0.001***

RBCs Red blood cells, MCHC Mean corpuscular hemoglobin, PLTs platelets, WBCs White blood cells, INR International normalized ratio, ALT Alanine transferase, AST Aspartate transferase, LDH lactate dehydrogenase, CRP C-reactive protein, RDW Red cell distribution width.

Table 2 presented the results for Foxo-1, indicating statistically significant differences related to the duration of admission, Heart Rate >100, drug treatment, hypertension, consolidation, patterns of bronchial dilation, and the distribution observed in chest CT scans.

Table 3 demonstrated that FOXO displayed significant positive correlations with the duration of patient admission, MCH (Mean corpuscular hemoglobin), Red cell distribution width (RDW), and respiratory rate.

FOXO displayed non- significant correlations with Age, RBCs, Hemoglobin, Hematocrit (%),MCV (Mean Corpuscular Volume), MCHC (Mean corpuscular hemoglobin concentration), PLTs, WBCs, INR (International normalized ratio), Neutrophil, Lymphocyte, Lymphocyte, Monocyte, ALBUMIN, ALT (Alanine transferase), AST

FOXO displayed non- significant correlations with heart Rate <60 bpm and Between 60 and 100 bpm, RSNA (Radiological Society of North America), Diabetes mellitus, GGO (Ground-glass opacity), mixed GGR and consolidation, pl (Pleural) effusion, nodal enlargement and No. of lobe affected.

(Aspartate transferase), LDH (Lactate dehydrogenase), PT, Creatinine, Sodium (Na), Potassium (K), Random Blood Sugar, CRP (C-reactive protein), Heart Rate, Partial pressure of carbon dioxide (PCO2), Partial pressure of Oxygen (pO2), Bicarbonate (HCO3), Oxygen Saturation level, Temperature, TSS (Total severity score), GGO (Ground-glass opacity), CO-RADS (Coronavirus disease 2019 Reporting Data System) and CXR score (score Chest x-ray).

Table 2. Relationship between FOXO-1 expression levels versus different characteristics in COVID-19 patients

Parameters		**FOXO-1 mean±SE**	**p-value FOXO-1**
Duration of admission	≤10 days (N=23) >10 days (N=7)	1.94±7.46 8.05±4.44	**0.001***
Heart Rate	>100 (N=7)	2.155±2.02	**0.441c**
Drug treatment	Negative (N=22)	4.52±1.61	**0.005***
	Positive (N=8)	0.185±0.099	
Comorbidity			
Hypertension	Negative (N=6)	2.08±0.89	**0.021***
	Positive (N=24)	5.29±2.74	
CT Findings			
Consolidation	Negative (N=27)	3.717±1.35	**0.017***
	Positive (N=3)	0.24±0.18	
Pattern bronchial dilatation	Negative (N=28)	3.59±1.30	**0.019***
	Positive (N=2)	0.27±0.24	
Chest CT distribution	Central (N=4)	7.91±3.02	**0.006x**
	Peripheral (N=10)	2.90±1.42	**0.927y**
	Mixed (N=16)	2.52±1.96	**0.903z**

FOXO-1 Forkhead box Protein O1, CT Computed tomography.
Significant p in bold. significant between- a (<60 bpm, 60-100 bpm), b between (<60 bpm, >100), c between (60-100 bpm, >100).
significant between chest CT distribution- x between (central, Peripheral) y between (Central, mixed) z between (Peripheral and mixed) significant between the number of affected lobes-aa between (0, 3) bb between (0,5) cc between (3,5).

Table 3. Correlations of FOXO expression levels with descriptive/laboratory and clinical data of COVID-19 patients

Parameters	FOXO r (p-value)
Duration of Hospital Admission	0.386(**0.035***)
MCH (pg)	-0.481(**0.007***)
RDW (%)	0.657(**0.0001***)
Respiratory Rate	0.526(**0.003***)

MCH Mean Corpuscular Hemoglobin, RDW Red cell distribution width.

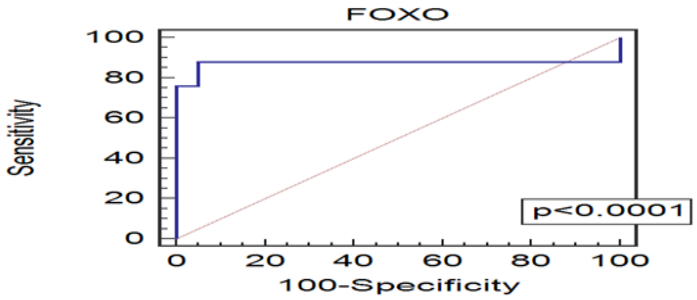

Figure 1. ROC Curve for FOXO for COVID-19 patient group

Figure (1) presents the analysis of receiver operating characteristic (ROC) curves, along with the calculated area under the curve (AUC), to evaluate the diagnostic efficacy of FOXO-1 in patients with COVID-19. The ROC curve analysis for FOXO indicated a notable diagnostic performance at a cut-off value of 0.34-fold, yielding an AUC of 0.874, with a sensitivity of 88.0% and a specificity of 95.0%.

Discussion

Researchers have developed a range of methods for diagnosing COVID-19. Typically, these techniques utilize biomarker genes, including FOXO-1, as a foundation for their approaches.

Forkhead box O1 (FOXO1) is a transcription factor involved in various physiological processes, including inflammation and immune systems. The FOXO1 could suppress viral load, a human airway epithelial cell line, infected with SARS-CoV-2. In addition, the expression of inflammatory-related cytokines was reduced, an anti-inflammatory cytokine, was increased by FOXO1. AS also reduced expressions of ACE2 and TMPRSS2, both host cell proteins required for SARS-CoV-2 infection [9].

According to our effects [10] Reported Given the top-notch defensive houses of the FoxO family, it's miles viable that SARS-CoV-2 will try to save you the host mobileular from receiving indicators from the FoxO signalling community so that it will sell viral development. By changing FoxO functions, extra studies in this subject matter might also additionally display a unique mechanism underlying the aetiology of COVID-19.

Sodium levels were found to be significantly higher than the control group, indicating a statistically significant difference. This finding aligns with the results reported by [11], which suggested a correlation between elevated serum sodium levels and patient symptoms. In the context of COVID-19, this relationship is particularly important, as SARS-CoV-2 infection can lead to gastrointestinal symptoms such as nausea, vomiting, and diarrhea, as well as increased insensible fluid losses due to fever and anorexia. These factors can contribute to hypernatremia resulting from dehydration or, in certain cases,

hypovolemic hyponatremia.

Hyperglycemia was observed to be significantly elevated in COVID-19 patients compared to the control group, indicating a statistically significant difference. This finding aligns with the report in [12], which states that blood sugar levels exceeding normal level at random times are classified as hyperglycemia. The condition arises when the body either lacks sufficient insulin or fails to regulate insulin levels effectively. The relationship between diabetes and hyperglycemia is well established; however, hyperglycemia can also occur in various other situations, such as stress induced by illness or infection. These findings correspond with [13], which identified multiple mechanisms contributing to hyperglycemia in COVID-19. These mechanisms can be categorized into two main types: the first being a direct effect of the virus on cells, and the second involving indirect pathways, such as the influence of pro-inflammatory cytokines, acute stressors, or prior glucocorticoid treatment.

The CRP level is significantly higher than that of the control group, a finding that is consistent with the observations made in [14]. This study noted that COVID-19 patients exhibited a notable increase in CRP levels. In cases of severe COVID-19, CRP levels were found to be elevated. Similarly, our research aligns with [15], which indicated that patients experiencing severe disease courses had significantly higher CRP levels compared to those with mild or non-severe cases. One study reported that patients with more severe symptoms had an average CRP concentration very high, while those with milder symptoms had low levels of CRP. Conversely, [16] noted that there were no differences in clinical outcomes, emphasizing that the acute-phase protein CRP serves as an early marker for infection or inflammation. This protein is

produced in the liver.

Red cell distribution width (RDW) was found to be significantly higher in critically ill and COVID-19 patients of late stage, a statistically significant difference. This is consistent with the idea that patients with severe COVID-19 illness have a higher RDW than patients with mild disease [17]. Patients with COVID-19 are known to suffer from significant inflammation, which can lead to multiorgan failure, according to another study [18]. The increased RDW in COVID-19 patients is most likely the result of this inflammatory response.

The partial pressure of oxygen (pO2) was found to be significantly lower in COVID-19 patients, indicating a statistically significant difference compared to the control group. This finding aligns with previous research [19], which noted that as the prevalence of COVID-19 increases; the positive predictive value (PPV) of pO2 and lymphocyte count also rises, while the negative predictive value (NPV) declines. A study conducted in a general hospital in the UK examined the effectiveness of pO2 and lymphocyte count as screening tools for COVID-19. This suggests that these measures can effectively serve as "rule-out" tests. However, it is important to note that only patients exhibiting respiratory symptoms should utilize pO2 and lymphocyte count for screening, as asymptomatic individuals or those presenting primarily with non-respiratory symptoms are unlikely to show low pO2 levels upon admission.

The relationship between the blood biomarker FOXO and various characteristics of COVID-19 groups was examined. The results indicated that patients hospitalized for more than 10 days exhibited higher levels of FOXO compared to those with shorter hospital stays, with significant p-values. These findings align with previous research [20], which suggested that FoxO proteins may be

modified to influence ACE2 accessibility during the entry of SARS-CoV-2 into cells. Typically, cellular stress leads to an upregulation of ACE2 expression, which can be triggered by factors such as exposure to the AMP mimic 5-amino-4-imidazole carboxamide riboside, IL-1 treatment, or hypoxia (AICAR). In instances of acute respiratory distress, IL-1 acts as an initial responder, providing protective effects and facilitating epithelial repair. Certain patients underwent therapeutic interventions; consequently, those who received medication exhibited lower FOXO levels compared to their counterparts. A significant difference was observed between the two groups, with hypertensive patients showing higher FOXO levels than non-hypertensive patients. These results align with our previous findings [20], suggesting that FoxO-1 may be instrumental in modulating ACE2 expression through SIRT1 and IL-1β. This further substantiates our prior recommendation to consider exogenous adjustments of FoxO to decrease ACE2 availability for SARS-CoV-2. Regarding the Chest X-Ray rating and the extent of FOXO in COVID-19 patients, the effects confirmed that slight patients, who have been accompanied with the aid of using moderate and extreme patients, had the very best degree of FOXO. Patients with consolidation have reduced FOXO levels, In step with the CT effects. Additionally, the ones who've tremendous Pattern bronchial dilatation additionally had decrease FOXO levels.

Conclusion

The diagnostic efficacy of the expression levels of Forkhead box protein O1 (FOXO-1), Sodium (Na), Random Blood Sugar (mg/dL), CRP, RDW (%), and Partial pressure of Oxygen (pO2) is evaluated for their potential use as biomarkers in determining the severity of COVID-19.

References

1. DE WILDE, Adriaan H., et al. Host factors in coronavirus replication. Roles of host gene and non-coding RNA expression in virus infection, 2017, 1-42.
2. BERRY, Michael; GAMIELDIEN, Junaid; FIELDING, Burtram C. Identification of new respiratory viruses in the new millennium. Viruses, 2015, 7.3: 996-1019.
3. CENTERS FOR DISEASE CONTROL AND PREVENTION, et al. Overview of testing for SARS-CoV-2, the virus that causes COVID-19. Cdc. Gov [Internet], 2022.
4. PATEL, Pragna. Information for persons who are immunocompromised regarding prevention and treatment of SARS-CoV-2 infection in the context of currently circulating Omicron sublineages—United States, January 2023. MMWR. Morbidity and Mortality Weekly Report, 2023, 72.
5. LOGUNOV, Denis Y., et al. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. The Lancet, 2021, 397.10275: 671-681.
6. AL-TAMARI, Hamza M., et al. FoxO3 an important player in fibrogenesis and therapeutic target for idiopathic pulmonary fibrosis. EMBO molecular medicine, 2018, 10.2: 276-293.
7. DELPOUX, Arnaud, et al. Continuous activity of Foxo1 is required to prevent anergy and maintain the memory state of CD8+ T cells. Journal of Experimental Medicine, 2018, 215.2: 575-594.
8. KOTHARI, Chakravanti Rajagopalachari. Research methodology: Methods and techniques. New Age International, 2004.
9. FURUKAWA, Ryutaro, et al. FOXO1 inhibitor has the antiviral and anti-inflammatory effects against SARS-CoV-2 infection in in vitro and in vivo models. The Journal of Immunology, 2024, 212.1_Supplement: 0143_4445-0143_4445.
10. OLAGNIER, David, et al. SARS-CoV2-mediated suppression of NRF2-signaling reveals potent antiviral and anti-inflammatory activity of 4-octyl-itaconate and dimethyl fumarate. Nature communications, 2020, 11.1: 4938.
11. GHEORGHE, Gina, et al. Is there a relationship between COVID-19 and hyponatremia?. Medicina, 2021, 57.1: 55.
12. ZHOU, Wan, et al. Clinical features of COVID-19 patients with diabetes and secondary hyperglycemia. Journal of diabetes research, 2020, 2020.1: 3918723.
13. MAMTANI, Manju, et al. Association of hyperglycaemia with hospital mortality in nondiabetic COVID-19 patients: A cohort study. Diabetes & metabolism, 2021, 47.3: 101254.
14. MO, Pingzheng, et al. Clinical characteristics of refractory coronavirus disease 2019 in Wuhan, China. Clinical infectious diseases, 2021, 73.11: e4208-e4213.

15. GAO, Yong, et al. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. Journal of medical virology, 2020, 92.7: 791-796.
16. NEHRING, S. M., et al. C reactive protein (CRP) Treasure Island. FL: StatPearls, 2020.
17. ZINELLU, Angelo; MANGONI, Arduino A. Red blood cell distribution width, disease severity, and mortality in hospitalized patients with SARS-CoV-2 infection: a systematic review and meta-analysis. Journal of Clinical Medicine, 2021, 10.2: 286.
18. LIPPI, Giuseppe; HENRY, Brandon M.; SANCHIS-GOMAR, Fabian. Red blood cell distribution is a significant predictor of severe illness in coronavirus disease 2019. Acta Haematologica, 2021, 144.4: 360-364.
19. FENG, Cong, et al. A Novel triage tool of artificial intelligence-assisted diagnosis aid system for suspected COVID-19 pneumonia in fever clinics. MedRxiv, 2020, 2020.03. 19.20039099.
20. MONTEIL, Vanessa, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. Cell, 2020, 181.4: 905-913. e7.