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*Scientific paper*

# The Predictive Value of Oxidative Stress Index in Patients with Confirmed SARS-COV-2 Infection

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## Abstract

Disbalance balance between oxidants and antioxidants is called oxidative stress and could be presented as oxidative stress index (OSI). OSI is determined by the reactive oxygen metabolites test (d-ROM test) to assess oxidants and the plasma antioxidant capacity test (PAT test) to measure antioxidants. The aim of the study was to evaluate the predictive value of OSI in the disease COVID-19. d-ROMs results were the highest in the SARS-CoV-2 POSITIVE group (365+/-112), lower in the SARS-CoV-2 NEGATIVE group (314+/-72.4), and the lowest in an INTENSIVE CARE UNIT group (ICU) (277+/-142) U.Carr. PAT test values were the lowest in the SARS-CoV-2 POSITIVE group (2762+/-387), higher in the ICU group (2772 +/-786), and the highest in the SARS-CoV-2 NEGATIVE group (2808+/-470), and are not statistically significantly different (P > 0.05), while OSI was: healthy with average value of 49 and the critical ill with average value of 109 (P = 0.016). Cut-offs for predicting ICUs admission was at OSI 62, with 80.0% sensitivity and 68.2% specificity.

**Keywords:** Oxidative stress; SARS-CoV-2; OSI Index

## 1. Introduction

Oxidative stress in cells and tissues is caused by an imbalance between the formation of reactive oxygen species (ROS) and the ability to detoxify ROS with the antioxidant system. The balance of ROS and antioxidants may be disturbed by the increased formation of ROS and/or decreased antioxidant activity. This imbalance leads to many spontaneous oxidations in the cell and, because ROS can be reducers of almost all cellular components, oxidation of biological macromolecules such as lipids, proteins, and nucleotides. Oxidation of macromolecules leads to their denaturation and, consequently, changes in their physiological functions. The chronic production of ROS causes toxic effects that lead to cell damage, long-term oxidative stress, accelerated aging, and many diseases including dementia, inflammation, cancer, diabetes, and cardiovascular disease. In contrast to these diseases, short-term acute oxidative stress does not yield typical clinical signs to divulge its presence. Symptoms of oxidative stress usually come to the fore only when chronic diseases develop.[1,2]

Although abnormal levels of ROS are detrimental, small amounts of ROS production occur normally and have physiological roles. In a homeostatic state, slightly more oxidants are present than antioxidants because small amounts of ROS are formed as by-products of oxygen metabolism. Normal levels of ROS can assist with cellular signaling by changing gene and protein expression, syn-

thesizing certain hormones, and defending against infections.[3]

Inflammation is the body's normal response to injuries, pathogens, irritants, and other toxins. The cells of the immune system that are involved in this process include neutrophils and monocytes during acute inflammation, and macrophages, especially in chronic inflammation. These phagocytes use very strong oxidants from ROS and reactive nitrogen species (RNS) groups when microbes invade.[1] The sudden production of large amounts of reactive species produced by phagocytes is called an oxidative eruption. This process is usually limited to the acute response to a pathogen, but if chronic inflammation occurs, it can cause chronic oxidative stress. In chronic inflammation, the increasing amounts of ROS and RNS lead to the oxidation of cellular components and, thus, damage and apoptosis.

The entry of a virus into the cell first triggers the activation of innate immune cells (macrophages, neutrophils) that arrive at the site of infection and trigger an inflammatory response. Macrophages secrete cytokines and produce several oxidants that they use to defend themselves against the virus. Production of oxidants by macrophages depends on NADPH oxidase, which leads to the formation of O2, and on myeloperoxidase, which catalyzes the formation of hypochlorous acid. ROS can activate epithelial cells and alveolar macrophages to generate chemotactic molecules that further attract neutrophils and, especially, monocytes and lymphocytes into the lungs, providing an ideal environment for the development of chronic inflammation. Inflammation is key in the progression of COVID-19 pathology. Presentations of SARS-CoV-2 infection have ranged from asymptomatic or mildly symptomatic to severe disease and death. Common symptoms include fever, headache, cough, and shortness of breath. Other symptoms, such as malaise and acute respiratory distress syndrome (ARDS), have also been described.[4]

Particular laboratory features have been associated with a more severe course of the disease and worse outcomes. A progressive decline in the lymphocyte count and rise in the D-dimer concentration was observed in those who succumbed to the disease, compared with survivors who exhibited more stable levels of the D-dimer.[5]

In severe cases of COVID-19, it is common to observe prolonged prothrombin time, elevated levels of lactate dehydrogenase, deficient cellular immune response, activation of coagulation, and damage to the heart, liver, and kidneys.[6,7]

The immune response plays a key role in controlling the SARS-CoV-2 infection, but excessive and uncontrolled activation of the immune response can contribute to a more severe course of the disease.[8]

Preclinical studies suggest that increased ROS production and decreased antioxidant responses play an important role in the pathogenesis of viral infection and also in disease progression and severity. The severe course of COVID-19 disease involves the connection of several pathophysiological processes such as cytokine storm, inflammation, cellular apoptosis, and redox imbalance, which contribute to the poor outcomes of COVID-19.[9]

Lymphocyte infiltration into the lungs may explain the lymphopenia and elevated neutrophil to lymphocyte ratio observed in critically ill COVID-19 patients. The elevated neutrophil to lymphocyte ratio is also used to predict the death of critically ill COVID-19 patients. The consequence of increased ROS secreted by neutrophils, macrophages, and other immune cells has so far had two outcomes: 1) ROS damages erythrocytes, which release heme into the bloodstream, which is broken down by heme oxygenase, which releases free iron; and 2) an oxidative eruption occurs, leading to the formation of a superoxide radical and hydrogen peroxide. Furthermore, oxidative stress and free iron convert fibrinogen into abnormal fibrin clots, leading to the formation of micro thromboses in the vascular system and pulmonary microcirculation.[7,10]

Increased ROS production also directly or indirectly triggers the NF-κB signaling pathway, and studies suggest that its activation is responsible for the more severe course of COVID-19 disease. NF-κB is one of the major mediators of cytokine and chemokine induction and is a central coordinator of the innate and adaptive immune responses.[7,11,12]

If over-activation of all these pathways occurs, likely depending on the amount of virus present, a cytokine storm can develop, leading to ARDS. The cytokine storm is triggered via these oxidative stress-signaling pathways by activated leukocytes, including B and T cells, macrophages, monocytes, neutrophils, dendritic cells, as well as epithelial and endothelial cells.[13–15]

Hydroperoxides are formed by the oxidation of various biological molecules such as amino acids, peptides, proteins, nucleotides, and, to the greatest extent, by the oxidation of lipids. Peroxides are only one of the groups of reactive oxygen species, but they are an early marker of lipid oxidation as they are formed in the initial stages of oxidative stress unlike other markers (malondialdehyde, isoprostane). Therefore, peroxides are early indicators of oxidative stress.[16–18]In this study, we wanted to investigate how the OSI index may be a good predictor of the severity of COVID-19 disease.

## 2. Materials and Methods

### 2. 1. Patients

Measurements of oxidants and antioxidants were performed on 171 (M/F = 42/129) samples taken at University Medical Centre Ljubljana (UMCL). Subjects were divided into 3 groups according to the course of the disease.

Group 1 (SARS-CoV-2 NEGATIVE): employees of UMCL who had a negative PCR test for SARS-CoV-2 infection (79).

Group 2 (SARS-CoV-2 POSITIVE): employees of UMCL who had a positive PCR test for SARS-CoV-2 infection without symptoms (51).

Group 3 (INTENSIVE CARE): A group of people who were hospitalized in the intensive care unit (ICU) of UMCL due to a severe course of COVID-19 (41).

## 2. 2. Methods

We used d-ROMs to measure oxidants and a PAT test to measure serum antioxidants. From the values of both tests, we then calculated the values of the oxidative stress index (OSI index) according to the FRAS5 analyzer algorithm, which summarizes the values of d-ROMs and PAT tests into one value to facilitate the evaluation of oxidative stress.

d-ROMs fast is a photometric test that gives us the status of oxidants in a biological sample by measuring hydroperoxides (ROOH).

The d-ROMs fast test is based on the Fenton reaction. Measurement with a FRAS5 photometer was performed at 505-546 nm. The color intensity was directly proportional to the ROS concentration in the sample.

The PAT test is a method that evaluates the antioxidant power of a biological sample. Measuring the antioxidant power of a sample is important as antioxidants are the first line of defense in the fight against oxidative damage.[16,18]

The PAT test is used to quantify water-soluble antioxidants in a biological sample by measuring its ability to reduce ferric ions ($Fe^{3+}$) to $Fe^{2+}$ ions. The measured antioxidants represent the main components of plasma in defense against oxidation: vitamin C, vitamin E, uric acid, and bilirubin.

The values of the OSI index are obtained by a certain arithmetic transformation and enable easier interpretation of oxidative stress for an individual sample. The OSI index does not have to replace the results of d-ROMs and PAT test, but complements them and presents the state of oxidative stress in the body.[19]

## 2. 3. Statistics

Statistical analyses were performed with IBM SPSS (version 22). We first established whether our data sets were normally distributed with the Shapiro-Wilk test for normality and established that the distribution of the oxidative stress index was nonparametric. The data were logarithmically transformed and a follow-up Shapiro-Wilk test determined that the logarithmically-transformed data were normally distributed. We used the one-factor ANOVA parametric test and the post hoc Bonferroni test and Dunn's Method test for further analysis of statistical significance. For descriptive statistics, we used mean and standard deviation (SD) to summarize our data.

# 3. Results and Discussion

**Table 1:** Basic statistics of d-ROMs, PAT, and OSI tests.

| | N | d-ROMs [U. Carr] | PAT [U. Cor] | OSI Median (IRQ) |
|---|---|---|---|---|
| **SARS-CoV-2 NEGATIVE** | 79 | | | |
| | Mean | 314 | 2808 | 46 |
| | SD | 72.4 | 470 | (28–61) |
| **SARS-CoV-2 POSITIVE** | 51 | | | |
| | Mean | 365 | 2762 | 56 |
| | SD | 112 | 387 | (31–84) |
| **INTENSIVE CARE** | 41 | | | |
| | Mean | 277 | 2772 | 109 |
| | SD | 142 | 786 | (60–134) |

* d-ROMs - ROS concentration assay
PAT- antioxidant concentration assay
OSI - index of oxidative stress
SARS-CoV-2 - SARS-associated coronavirus

## 3. 1. Comparison of Groups in the Coordinate System

We used a coordinate system to show where certain groups of patients are concentrated based on their OSI (Figure 1). The purpose of the OSI index is to integrate a single value based on d-ROMs and PAT test results despite different units of measure and different value ranges. With the OSI index, we can show exactly what the disease state of each group is, for example, the levels of d-ROMs = 500 U.Carr and PAT = 1800 U.Cor can show us the same OSI value (142) as the result d-ROMs = 95 U.Carr and PAT = 3900 U.Cor, although these are completely different conditions. Namely, the OSI value serves as a rough picture of oxidative stress; if the values are normal (below 40) we can ascertain that the patient's redox ratio is balanced, otherwise, when the values are higher (above 40) it is necessary to investigate the cause and look at the values of oxidants and antioxidants. We can most reliably interpret the patient's condition based on the results of all 3 parameters, clinical laboratory tests, and when the sample was taken during the patient's illness.

We entered d-ROMs test values on the y-axis and PAT test values on the x-axis. Based on these two tests, with the help of OB Manager Online copyright © H&D S.r.l. In: 2.0.16 calculated oxidative stress index values.

## 3. 2. The Interpretation of the Results in Specific Quadrants

The first quadrant includes individuals with normal or high values of d-ROMs test and normal or high values of PAT test:

– High values of d-ROMs and normal PAT values indicate

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | <40 | Normality | | | | | |
| | | | | | | | | | | | | | 41-65 | Borderline | | | | | |
| | | | | | | | | | | | | | 66-120 | Alert | | | | | |
| | | | | | | | | | | | | | >121 | Critical; evident unbalance | | | | | |
| d-ROMs | II. | | | | | | | | | | | | | | | | | | I. |
| 700 | 271 | 265 | 259 | 254 | 250 | 247 | 245 | 244 | 243 | 244 | 245 | 247 | 250 | 254 | 259 | 265 | 271 | 278 | |
| 675 | 258 | 251 | 246 | 241 | 237 | 233 | 231 | 230 | 229 | 230 | 231 | 233 | 237 | 241 | 246 | 251 | 258 | 265 | |
| 650 | 245 | 239 | 232 | 227 | 223 | 219 | 217 | 215 | 215 | 215 | 217 | 219 | 223 | 227 | 232 | 239 | 245 | 253 | |
| 625 | 233 | 226 | 219 | 214 | 209 | 205 | 203 | 201 | 200 | 201 | 203 | 205 | 209 | 214 | 219 | 226 | 233 | 241 | |
| 600 | 220 | 213 | 206 | 200 | 195 | 191 | 188 | 186 | 186 | 186 | 188 | 191 | 195 | 200 | 206 | 213 | 221 | 229 | |
| 575 | 208 | 200 | 193 | 187 | 181 | 177 | 174 | 172 | 171 | 172 | 174 | 177 | 181 | 187 | 193 | 200 | 209 | 218 | |
| 550 | 197 | 188 | 181 | 174 | 168 | 163 | 160 | 158 | 157 | 158 | 160 | 163 | 168 | 174 | 181 | 188 | 197 | 207 | |
| 525 | 186 | 177 | 168 | 161 | 155 | 150 | 146 | 144 | 143 | 144 | 146 | 150 | 155 | 161 | 168 | 177 | 186 | 196 | |
| 500 | 175 | 165 | 156 | 148 | 145 | 136 | 132 | 129 | 129 | 129 | 132 | 136 | 142 | 148 | 156 | 165 | 175 | 186 | |
| 475 | 165 | 154 | 145 | 136 | 129 | 123 | 118 | 115 | 114 | 115 | 118 | 123 | 129 | 136 | 145 | 154 | 165 | 176 | |
| 450 | 155 | 144 | 134 | 124 | 116 | 109 | 104 | 101 | 100 | 101 | 104 | 109 | 116 | 124 | 134 | 144 | 156 | 167 | |
| 425 | 146 | 135 | 123 | 113 | 104 | 97 | 91 | 87 | 86 | 87 | 91 | 97 | 104 | 113 | 123 | 135 | 147 | 159 | |
| 400 | 138 | 126 | 114 | 103 | 93 | 84 | 77 | 73 | 71 | 73 | 77 | 84 | 93 | 103 | 114 | 126 | 139 | 152 | |
| 375 | 132 | 118 | 106 | 94 | 82 | 72 | 64 | 59 | 57 | 59 | 64 | 72 | 82 | 94 | 106 | 118 | 132 | 146 | |
| 350 | 126 | 112 | 99 | 86 | 73 | 62 | 52 | 45 | 43 | 45 | 52 | 62 | 73 | 86 | 99 | 112 | 127 | 141 | |
| 325 | 122 | 108 | 93 | 79 | 66 | 53 | 41 | 32 | 29 | 32 | 41 | 53 | 66 | 79 | 93 | 108 | 122 | 137 | |
| 300 | 119 | 105 | 90 | 75 | 61 | 47 | 33 | 21 | 14 | 21 | 33 | 47 | 61 | 75 | 90 | 105 | 120 | 135 | |
| 275 | 119 | 104 | 89 | 74 | 59 | 44 | 30 | 15 | 0 | 15 | 30 | 44 | 59 | 74 | 89 | 104 | 119 | 134 | |
| 250 | 119 | 105 | 90 | 75 | 61 | 47 | 33 | 21 | 14 | 21 | 33 | 47 | 61 | 75 | 90 | 105 | 120 | 135 | |
| 225 | 122 | 108 | 93 | 79 | 66 | 53 | 41 | 32 | 29 | 32 | 41 | 53 | 66 | 79 | 93 | 108 | 122 | 137 | |
| 200 | 126 | 112 | 99 | 86 | 73 | 62 | 52 | 45 | 43 | 45 | 52 | 62 | 73 | 86 | 99 | 112 | 127 | 141 | |
| 175 | 132 | 118 | 106 | 94 | 82 | 72 | 64 | 59 | 57 | 59 | 64 | 72 | 82 | 94 | 106 | 118 | 132 | 146 | |
| 150 | 138 | 126 | 114 | 103 | 93 | 84 | 77 | 73 | 71 | 73 | 77 | 84 | 93 | 103 | 114 | 126 | 139 | 152 | |
| 125 | 146 | 135 | 123 | 113 | 104 | 97 | 91 | 87 | 86 | 87 | 91 | 97 | 104 | 113 | 123 | 135 | 147 | 159 | |
| 100 | 155 | 144 | 134 | 124 | 116 | 109 | 104 | 101 | 100 | 101 | 104 | 109 | 116 | 124 | 134 | 144 | 156 | 167 | |
| 75 | 165 | 155 | 145 | 137 | 129 | 123 | 119 | 116 | 115 | 116 | 119 | 123 | 129 | 137 | 145 | 155 | 165 | 176 | |
| 50 | 175 | 166 | 157 | 149 | 142 | 137 | 132 | 130 | 129 | 130 | 132 | 137 | 142 | 149 | 157 | 166 | 175 | 186 | |
| 25 | 186 | 177 | 169 | 161 | 155 | 150 | 146 | 144 | 143 | 144 | 146 | 150 | 155 | 161 | 169 | 177 | 186 | 196 | |
| PAT | 1000 | 1200 | 1400 | 1600 | 1800 | 2000 | 2200 | 2400 | 2600 | 2800 | 3000 | 3200 | 3400 | 3600 | 3800 | 4000 | 4200 | 4400 | |
| | III. | | | | | | | | | | | | | | | | | | IV. |

**Figure 1:** Coordinate system representing OSI and four different quadrants for interpretation. OSI values less than 40 represent normal levels. Values between 41-65 are borderline alert and normal. Values ranging from 66-120 signify a concerning imbalance between oxidants and antioxidants. Values above 121 signify a critical imbalance of oxidants and antioxidants.

* OSI - index of oxidative stress

an initial oxidative outbreak due to an innate immune response, but the patient still maintains a good antioxidant defense.

– High values of d-ROMs and high values of PAT are the result of an increase in oxidant species and an anomalous increase of the antioxidant reserve that might reflect a state of cellular destruction and release in circulation.

The second quadrant includes individuals with normal or high values for d-ROMs test and normal or low values for the PAT test:

– High values of d-ROMs and low values of PAT indicate the increase of the antioxidant species and the decrease of the antioxidant response, signs of possible inflammation onset, and hospitalization.
– The interpretation of high values of d-ROMs and normal PAT values remain the same as for the first quadrant.

The third quadrant includes individuals with normal or low values of d-ROMs and normal or low values of PAT:

– Low values of d-ROMs and normal PAT values indicate a long-term infection and exhaustion of the body. Thus, the body is unable to form normal ROS, the efficacy of the innate immune response declines, and antioxidant defenses decline due to pre-existing damage indicating a loss of redox signaling power.
– Low values of d-ROMs and low PAT values show the exhaustion of the ROS system and the antioxidant network.

The fourth quadrant is comprised of individuals with normal or low d-ROMs and normal or high PAT test values.

– Low values of d-ROMs and normal PAT values in the fourth quadrant indicate the same conditions as for the third quadrant.
– Low values of d-ROMs and high values of PAT indicate a long-term infection, which involves extensive inflammation and tissue damage.

For the statistical comparison of groups, we used the parametric test one-factor ANOVA and Bonferroni post hoc test. We first performed a test of homogeneity of variances and found that there was no statistically significant difference between all three groups; variances were homogeneous (P = 0.395). This indicated that we could go forward with the one-factor ANOVA and Bonferroni test. The ANOVA result showed that there was a statistically significant difference (P = 0.016) between the individual groups, as shown in Table 2.

**Table 2:** Calculated differences between groups.

| Group comparison for OSI | | P |
|---|---|---|
| SARS-CoV-2 POSITIVE | SARS-Cov-2 NEGATIVE | **0.272** |
| SARS-CoV-2 POSITIVE | INTENSIVE CARE | **0.471** |
| SARS-Cov-2 NEGATIVE | INTENSIVE CARE | **0.024** |

*OSI - index of oxidative stress
SARS-CoV-2 - SARS-associated coronavirus

We did not prove a statistically significant difference between the SARS-Cov-2 positive and negative groups (P = 0.272). The average oxidative stress index of the positive group was 17 units higher than the negative group. According to the reference table, a value of 17 is a concerning value, whereas the control group is in the range of the oxidative stress borderline state. Due to the less stressful course of the disease (from asymptomatic patients to patients with mild symptoms, which did not require hospitalization of patients), there was no critically impaired state of oxidants/antioxidants.

We demonstrated a statistically significant difference between the ICU COVID-19 patients and the SARS-Cov-2 negative group (P = 0.024). This difference was expected as the redox ratio of hospitalized persons in ICU was severely disrupted. Some patients had a very high amount of oxidants present, yet others had a very low amount of oxidants, both indicative of oxidative stress. Normal amounts of oxidants are necessary for the normal functioning of the patient.

We did not prove statistically significant differences between the SARS-Cov-2 positive group and the ICU group (P = 0.471). The SARS-CoV-2 positive group without symptoms had more oxidative stress than the SARS-CoV-2 negative group, but much less than the patients hospitalized in ICU.

### 3. 2. Interpretation of OSI Values for SARS-Cov-2 NEGATIVE Group:

The vast majority of patients had normal values of d-ROMs and PAT, and, consequently, the largest share of them (43.7%) had OSI values below 40, while only 2.3% had OSI above 121. These slight deviations were likely caused by other underlying conditions (such as obesity and differences in physical activity).

### 3. 3. Interpretation of OSI Values for SARS-CoV-2 POSITIVE Group:

Individuals from this group were concentrated in approximately the same part of the coordinate system, namely in quadrants I and II. Normal or high values of d-ROMs and normal or high values of PAT were measured. Most individuals (41.5%) of this group had an oxidative stress index below 40, i.e. they had normal levels without oxidative stress. Furthermore, 26.8% of them had values between 66-120 (alert state) and 22% of individuals had values between 41-65 (borderline). The last group of 66-120, which is already considered a warning state, included the fewest individuals (9.7%). The results, which were slightly above normal but not critical, were in agreement with the symptoms of the participants, which were mild although they tested positive for SARS-CoV-2. We hypothesize that the cause of high values of d-ROMs is an oxidative outbreak due to the innate immune response, while the antioxidant system also functions to fight high amounts of ROS.

### 3. 4. Interpretation of OSI Values for INTENSIVE CARE Group:

We observed the most diverse patient conditions in this group. The majority of individuals had OSI values below 40 (33.3%) and above 121 (26.7%). Based on the results of d-ROMs, PAT tests, and OSI values, this large variation in patient condition was expected in the ICU group. We observed very diverse values in intensive care patients and most (66.7%) completely disturbed the balance of oxidants/antioxidants. In quadrant II were individuals who had mostly elevated values from the d-ROMs test and normal or decreased values from the PAT test. Based on these two results, we concluded that these patients were in the initial stage of COVID-19 disease and had just been admitted to ICU.

Individuals in quadrants I and II were patients with very high values of d-ROMs and normal or elevated PAT values. The first quadrant includes patients who were in the initial stage of the disease. In those who had elevated levels of d-ROMs and PAT, there was an extensive immune response that triggered an oxidative outburst and consequently an increased response of antioxidants.

Individuals in quadrants III and IV +- were patients with very low d-ROMs scores and normal PAT scores, and patients with very low d-ROMs scores and high PAT scores. In both cases, these are samples taken during hospitalization in the ICU after the COVID-19 infection had been going on for some time. At this point during infection, the body is already exhausted and unable to form ROS, nor is there an effective innate immune response. The antioxidant system is also active, trying to remove the damage. In the group with low d-ROMs and high PAT scores, high PAT values indicated inadequate redox signaling and increasingly severe tissue damage.

Individuals in quadrant IV were critically ill patients with low d-ROMs and high PAT values. As in the above example, there was an increasing amount of tissue damage and slow organ failure.

The frequency of the OSI index is shown in Figure 2.

A receiver operating characteristics (ROC) curve was constructed and Youden Index was used to determine the optimal cut-off for predicting intensive care unit (ICU) admission. The ROC curve is presented in Figure 3.

## 4. Conclusions

The oxidative stress index serves as a predictor for the course of COVID-19 disease. Based on our data, it is reasonable to think that OSI is a good predictive index for ICU admission where a cut-off of 62 was identified.

The very low d-ROMs level observed in patients 1 and 2 can be explained by the pathological status of the subjects. In contrast, high PAT levels can be explained by hemolysis processes, through which a high amount of glutathione (GSH) is released from red blood cells. Simul-

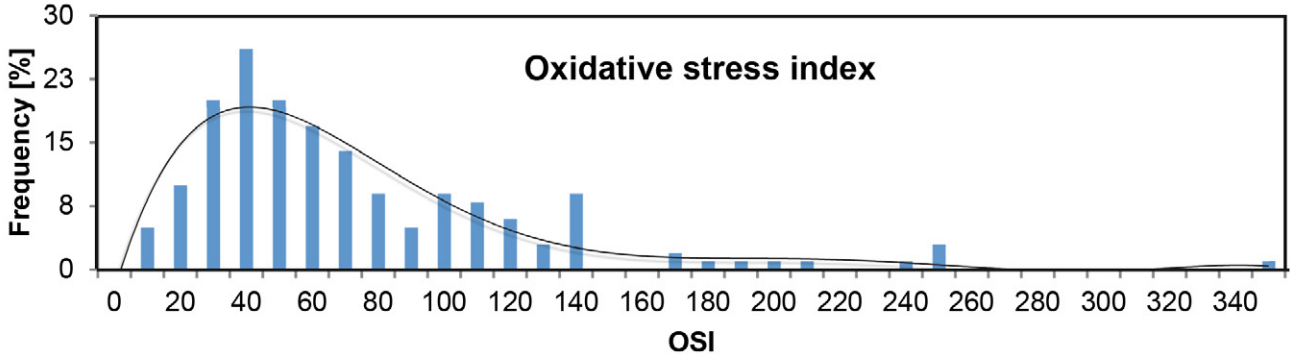


**Figure 2:** Calculated OSI of the whole study group.

* OSI - index of oxidative stress

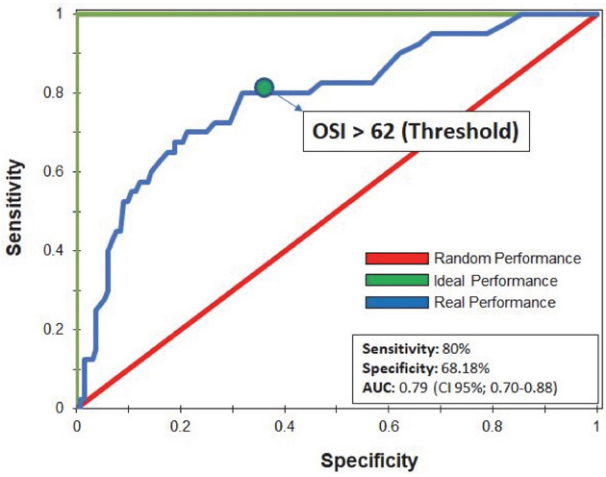


**Figure 3:** ROC curve for predicting ICU admission for COVID-19 positive patients.

* OSI - index of oxidative stress AUC - area under the ROC curve

taneously, the antioxidants are not used by the individual due to the lack of ROS species, which could contribute to why PAT levels are quite high in some cases. Moreover, this could be the reason why statistically significant differences in d-ROMs and PAT were not identified in the patients analyzed since the levels strongly depend upon the time course of the disease, and pathology can occur with low or high ratios of d-ROMs/PAT.

With the help of the coordinate system, we evaluated the disease status of the patients. We found that the PCR negative group was concentrated approximately in the middle of the coordinate system, signifying that most of the values of the measured parameters were within normal reference limits. The PCR positive group was primarily found in quadrants I and II, and the values of oxidative stress parameters indicated a shift from the normal reference limits. A wide variety of disease conditions were present in the ICU group, some of which were in the initial stage of the disease and had just been admitted to the ICU, and the results of d-ROMs, PAT, OSI were not as severe as in individuals with long-term hospitalization.

A comparison of the oxidative stress index in two ICU patients with biochemical and hematological parameters (supplemental data) showed that the values of the OSI index correlated very well with the patients' disease state and the inflammatory parameters. We compared CRP, lymphocyte and neutrophil count, IL-6, and oxidative stress index. The latter varied with a lag compared to the others, but this is consistent with studies by test manufacturers d-ROMs and PAT, where we found that oxidant levels rise when there is actual oxidative damage and thus reflect the current state of the body.

Doğan et al. published a study in which they calculated the OSI index from the parameters total oxidant status (TOS) and total antioxidant status (TAS). The calculated OSI levels were significantly different between severe moderate and mild groups of patients infected with SARS-CoV-2.[20]

The same method for calculating OSI was also used by Çakırca et al. Their results revealed that the increase in oxidative stress and decrease in antioxidant levels in COVID-19-infected patients were associated with worsening of disease.[21] The results of our study, in which we calculated OSI based on d-ROM and PAT determinations, are comparable to the results of both of these studies, which used TOS and TAS to calculate OSI.

In our study of oxidative stress, our results suggest that OSI could be a predictor of the course of SARS-CoV-2 infection and warrants further investigation. Our studies were conducted on a small number of samples so further research with more samples is necessary.

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## Author Contributions

Conceptualization JO; Writing – Original draft Preparation JO; Clinical data of the Patients ML, MJ; Laboratory Analysis SP, TF, EBA; Statistics TF; Writing – Review & Editing MJ.

### Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the National Ethics Committee; protocol number – 012-60/2021/5.

### Conflicts of interest

The authors declare no conflict of interest.

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## Povzetek


Neravnovesje med oksidanti in antioksidanti imenujemo oksidativni stres in ga lahko prikažemo kot indeks oksidativnega stresa (OSI). OSI določimo s testom reaktivnih presnovkov kisika (d-ROM) za oceno oksidantov in testom plazemske antioksidativne kapacitete (PAT) za merjenje antioksidantov. Namen študije je bil oceniti napovedno vrednost OSI pri bolezni COVID-19. Rezultati tasta d-ROM so bili najvišji v skupini SARS-CoV-2 pozitivni (365+/-112), nižji v skupini SARS-CoV-2 negativni (314+/-72,4) in najnižji v skupini kritično bolnih v enoti za intenzivno nego (ICU) (277+/-142) U.Carr. Vrednosti testa PAT so bile najnižje v skupini SARS-CoV-2 pozitivni (2762+/-387), višje v skupini kritično bolnih (2772 +/-786) in najvišje v skupini SARS-CoV-2 negativni (2808 +/-470). Skupine se med sabo statistično značilno ne razlikujejo (P>0,05). OSI se statistično značilno razlikuje med zdravimi s povprečno vrednostjo 49 in kritično bolnimi s povprečno vrednostjo 109 (P = 0,016). Določili smo mejno vrednost za napovedovanje sprejema pacienta v enoto intenzivne nege na osnovi analize OSI, in sicer 62, z 80,0 % občutljivostjo in 68,2 % specifičnostjo.