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 d-ROMs test for prooxidants, and the PAR test that measures antioxidants. The purpose of the study was to assess the predictive value of OSI in COVID-19 illness.

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 (AUC:0.79 (Cl95%; 0.70-0.88).

Keywords

Oxidative stress; SARS-CoV-2; OSI Index

Introduction

Oxidative stress is caused by a disturbed balance between the formation and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of the defence antioxidant system to remove these reactive products. The balance may be disturbed due to increased formation of reactive species and / or decreased antioxidant protection activity. This leads to many spontaneous oxidations in the cell and because they can be reducers of almost all cellular components, oxidation of biological macromolecules such as lipids, proteins, nucleotides begin, which leads to their denaturation and consequently changes their physiological functions. ROS cause toxic effects that lead to cell damage, long-term oxidative stress and even accelerated aging and many diseases such as dementia, inflammation, cancer, diabetes, cardiovascular disease, … In contrast to these diseases, oxidative stress in the early period it does not have a typical clinical picture to suggest its presence. Symptoms usually come to the fore only when chronic diseases develop. [1,2]

In the physiological state, slightly more prooxidants are present than antioxidants, as small amounts of ROS are formed as a by-product of oxygen metabolism. In normal amounts, they also participate in many physiological roles and adapt the body to environmental and body factors that increase the formation of ROS (cellular signalling through which they cause the expression of relevant genes and protein synthesis, synthesis of certain hormones and also act as a defence mechanism against infections). [3]

Inflammation is the body's normal response to injury, the intrusion of a pathogen, irritants and other toxins. The cells of the immune system are involved in this process: neutrophils and monocytes in acute inflammation and macrophages especially in chronic inflammation. These phagocytes use very strong oxidants from the ROS and RNS groups when microbes invade. [1]

Such a sudden appearance of large amounts of reactive substances produced by phagocytes is called an oxidative eruption. This process is to a limited extent necessary to fight pathogens, but if chronic inflammation occurs, it can cause chronic oxidative stress, as it can be self-sustaining. In chronic inflammation, due to increasing amounts of ROS and RNS, more and more cellular components begin to oxidize, leading to damage and apoptosis.

Presentations of SARS-CoV-2 infection have ranged from asymptomatic/mild symptoms to severe illness and death. Common symptoms have included fever, headache, cough, and shortness of breath. Other symptoms, such as malaise and respiratory distress syndrome (ARDS), have also been described.

Particular laboratory features have been associated with more severe course of the disease and worse outcomes. A progressive decline in the lymphocyte count and rise in the D-dimer were observed in nonsurvivors compared with more stable levels in survivors. [4]

In severe cases prolonged prothrombin time, elevated levels of lactate dehydrogenase, deficient cellular immune response, activation of coagulation and damage to the heart, liver and kidneys are usually found. [5,6]

The immune response plays a key role in controlling the infection, but in excessive and uncontrolled activation it can contribute to a more severe course of the disease. [7]

The T and B cellular responses occur approximately 1 week after the onset of symptoms. T CD8 + cells are important for the direct attack of infected cells, while T CD4 + cells are crucial for the binding of T CD8 + and B cells and for the production of cytokines. Autopsies have shown that T cells begin to accumulate at the site of infection in order to destroy the cells with the virus and therefore lymphopenia is present in the blood. [8]

The B cell response first appears against the SARS-CoV-2 virus N protein, and neutralizing antibodies against the S protein (RBD domain) begin to form within 2 weeks of the onset of symptoms. Studies have found that some patient populations do not develop long-lasting antibodies to SARS-CoV-2 and therefore it is not known whether re-infection with the virus may occur. [8]

Preclinical studies suggest that increased ROS production and decreased antioxidant response play a very important role in the pathogenesis of viral infection and also in disease progression and severity. The severe course of the disease involves the connection of several pathophysiological processes such as cytokine storm, inflammation, cellular apoptosis and redox imbalance, which affect the poor outcome of the disease. [9]

The entry of the virus into the cell first triggers the activation of natural immune cells (macrophages, neutrophils) that arrive at the site and trigger an inflammatory response. In doing their job, macrophages secrete cytokines and produce a number of oxidants that they use to defend themselves against the virus. Production depends on NADPH oxidase, which causes the formation of O^2, and on myeloperoxidase, which catalyses the formation of hypochlorous acid. ROS are able to 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. Lymphocyte infiltration into the lungs may explain lymphopenia and an elevated neutrophil to lymphocyte ratio observed in critically ill patients and is also used to predict hospital death. The consequence of increased ROS secreted by neutrophils, macrophages and other immune cells has so far had two consequences: 1) ROS damages erythrocytes from which heme is released into the bloodstream, which is broken down by heme oxygenase and free iron is released; and 2) an oxidative eruption occurs and a

superoxide radical and hydrogen peroxide are formed. Furthermore, oxidative stress and free iron convert fibrinogen into abnormal fibrin clots and consequently the formation of micro thrombosis in the vascular system and pulmonary microcirculation. [6,10]

Increased ROS production also directly or indirectly triggers the NF-κB signalling pathway, for which studies suggest that its activation is responsible for the more severe course of the disease. NF-κB is one of the major mediators of cytokine and chemokine induction. It is a central factor that coordinates the response of the natural immunity, the inflammatory response and also the maturation of lymphocytes, ie the acquired immune system. SARS-CoV-2 triggers the so-called signal 1, which leads to the activation of NF-κB and consequently also to the activation of NLRP3. [6,11,12]

If over-activation of all these pathways occurs (most likely depending on the exposed dose of the virus) this leads to a cytokine storm from which respiratory distress syndrome can develop. The cytokine storm is triggered via these signalling 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 compared to other markers (malondialdehyde, isoprostane). Therefore, they are an early indicator of oxidative stress. [16,17]

Materials and Methods

Patients

Measurements of prooxidants and antioxidants were performed on 171 (M/F = 42/129) samples taken in University Medical Centre Ljubljana (UMCL). Subjects were divided into 2 groups according to the course of the disease.

Group 1: SARS-CoV-2 POSITIVE: employees of UMCL who had a positive PCR test for SARS-CoV-2 infection without symptoms (51), and SARS-CoV-2 NEGATIVE: employees of UMCL with negative PCR test for SARS-CoV-2 infection (79).

Group 2: INTENSIVE CARE: A group of people who were hospitalized in the intensive care unit of UMCL due to a severe course (41).

The age distribution is not statistically different between male and female, while group 1 and group 2 have different distribution of age (group 1: 41 +/- 12 and group 2: 70 +/- 11). Average age was 47 years with median of 46. Population features are presented in Figure 1.

Methods

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

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

The principle of the test is based on the Fenton reaction. Hydroperoxides, which represent ROS, react with iron (II) ions in an acidic medium from a biological sample to form peroxyl ($ROO^{\cdot}$) and alkoxyl ($RO^{\cdot}$) radicals. As a hydroperoxide detector, chromogen N, N-diethyl-p-phenylenediamine is added, which is oxidized in the presence of radicals; from its structure of a neutral aromatic amine (it is colorless) it emits one electron to radicals (formed in a biological sample at low pH) and in the process turns into a pink colored cationic radical. Measurement with a FRAS5 photometer is performed at 505-546 nm. The color intensity is directly proportional to the ROS concentration in the sample.

The PAT test is a method that tells us what the antioxidant power of a biological sample is. Measuring the antioxidant power of a sample is important as antioxidants are the first line of defence 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 ferric ions (Fe^{2+}). The measured antioxidants represent the main components of plasma in defense against oxidation. These antioxidants are vitamin C, vitamin E, uric acid, bilirubin.

A solution of ferric ions (FeCl3) is mixed with a specific chromogenic substrate containing thiocyanate to obtain a red colored complex. This is followed by the addition of a sample and incubation at 37°C. During this time, there will be a reduction in ferric ions, which discolors the solution. After incubation, the FRAS5 photometer is measured at 505 nm. The change in color intensity is directly proportional to the ability of the biological sample to reduce ferric ions to ferric ions.

The purpose of the OSI index is to integrate a single value based on d-ROMs and PAT test results despite different units and different value ranges. 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]

Statistics

Statistical analyses were performed with IBM SPSS (version 22). We first performed normality tests with the Shapiro-Wilk test and found that the distribution of the oxidative stress index was nonparametric, after logarithmic transformation the distribution was normal. The test was performed again and since the result showed that the data were parametrically distributed, we used the one-factor ANOVA parametric test and the post hoc Bonferroni test and Dunn's Method test for further analysis. In descriptive statistics, we used mean and standard deviation (SD) to give results.

Results and Discussion

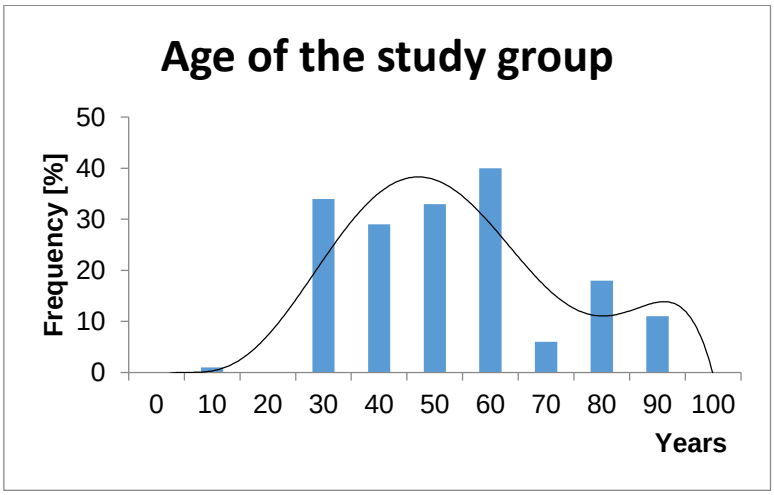

Figure 1: Age distribution of all patients (Group 1 and Group 2)

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

	N	d-ROMs [U. Carr]	PAT [U. Cor]	OSI Median (IRQ)
SARS-CoV-2 NEGATIVE	79			
	Mean	314	2808	46 (28-61)
	SD	72,4	470	
SARS-CoV-2 POSITIVE	51			

	Mean	365	2762	56 (31-84)
	SD	112	387	
INTENSIVE CARE	41			
	Mean	277	2772	109 (60-134)
	SD	142	786	

Comparison of groups in the coordinate system

We used a coordinate system to show where certain groups are concentrated (Figure 2). The purpose of this was to show exactly what the state of each group is, as for example the result d-ROMs = 500 U. Carr and PAT = 1800 U. Corr can show us the same OSI value (142) as the result d-ROMs = 95 U. Carr and PAT = 3900 U. Corr, 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 assume 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 prooxidants and antioxidants. We can most reliably interpret the patient's condition based on the results of all 3 parameters and data on sampling 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.

<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 2: Coordinate system representing OSI and four different quadrants for interpretation

The interpretation of the results in specific quadrant:

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 an initial state where an oxidative outbreak has occurred due to an innate immune response and at the same time the organism still maintains a good antioxidant defense.
- High values of d-ROMs and high values of PAT

The second quadrant concentrates individuals with normal or high values of d-ROMs test and normal or low values of PAT test:

- High values of d-ROMs and low values of PAT indicate the onset of infection and hospitalization because of an innate immune system response.
- High values of d-ROMs and normal PAT values indicate an initial state where an oxidative outbreak has occurred due to an innate immune response and at the same time the organism still maintains a good antioxidant defense.

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

- Low values of d-ROMs and normal PAT values indicate a long-term infection, the body is exhausted and unable to form ROS, the effectiveness of the innate immune response declines, and the antioxidant defense due to pre-existing damage (loss of redox signaling power).

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

- Low values of d-ROMs and normal PAT values indicate a long-term infection, the body is exhausted and unable to form ROS, the effectiveness of the innate immune response declines, and the antioxidant defense works due to pre-existing damage (redox signaling power is lost).
- Low values of d-ROMs and high values of PAT indicate a long-term infection, which involves extensive inflammation and damage to many tissues.

For the statistical comparison of groups, we used the parametric test one-factor ANOVA and Bonfferoni post hoc test. We first performed a test of homogeneity of variances and found that there was no statistically significant difference, variances were homogeneous ($P = 0.395$). This was a condition for us to continue with the one-factor ANOVA and Bonfferoni test. The ANOVA result showed that there was a statistically significant difference ($P = 0.016$) between the individual groups, shown in Table 2.

Table 2: Calculated differences between groups

Group comparison for OSI		P
SARS-CoV-2 POSITVE	SARS-Cov-2 NEGATIVE	**0,272**
SARS-CoV-2 POSITVE	INTENSIVE CARE	**0,471**
SARS-Cov-2 NEGATIVE	INTENSIVE CARE	**0,024**

We did not prove a statistically significant difference between the SARS-Cov-2 positive and negative group (P = 0.272). The average oxidative stress index of the positive group is 17 units higher. According to the reference table, this is a warning condition. While the control group is in the range of the oxidative stress limit state. Due to the easier 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 prooxidants / antioxidants.

We demonstrated a statistically significant difference between the intensive care and SARS-Cov-2 negative group (P = 0.024). This difference was expected as the redox ratio of hospitalized persons in intensive care was severely disrupted. Some had a very high amount of prooxidants present, yet others a very low amount of prooxidants, both indicative of oxidative stress. Normal amounts of prooxidants are necessary for the normal functioning of the organism, in these cases we cannot talk about it.

We did not prove statistically significant differences between the SARS-Cov-2 positive group and intensive care 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 intensive care.

Interpretation of OSI values for SARS-Cov-2 NEGATIVE group:

The vast majority have normal values of d-ROMs and PAT, and consequently the largest share of them (43.7%) has OSI values below 40, while only 2.3% has OSI above 121. These slight deviations are probably caused by some other present conditions (obesity, physical activity…).

Interpretation of OSI values for SARS-CoV-2 POSITIVE group:

Individuals from this group are concentrated in approximately the same part of the coordinate system, namely in I. and II. quadrant. Normal or high values of d-ROMs and normal or high

values of PAT were measured. Most individuals (41.5%) of this group have an oxidative stress index below 40, i.e. they have a normal state without oxidative stress. Furthermore, 26.8% of them have values between 66-120 (warning state) and 22% of individuals have values between 41-65 (limit state). The last group of 66-120, which is already considered a warning condition, includes the fewest persons (9.7%). The results are in good agreement with the symptoms of the participants, which were mild but the disease state was present, and the values of the tests, which are already slightly outside the reference values but still not critical, are also appropriate. 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 works well and fights high amounts of ROS.

Interpretation of OSI values for INTENSIVE CARE group:

We observed the most diverse conditions in this group. The largest share of individuals has OSI values below 40 (33.3%) and above 121 (26.7%). Based on the results of d-ROMs and PAT tests, and OSI values, this statistic is expected, as we observed very diverse values in intensive care patients and in most (66.7%) completely disturbed balance of prooxidants / antioxidants. In II. quadrant are individuals who have mostly elevated values of d-ROMs test and normal or decreased values of PAT test. Based on these two results, we can conclude that these patients were in the initial stage of the disease and had just been admitted to intensive care.

Individuals in I. and II. quadrant are patients with very high values of d-ROMs and normal or elevated PAT values. In the first case, these are patients who were in the initial stage of the disease. However, a sample of those who had elevated levels of d-ROMs and PAT was taken after a few days of hospitalization, as there was an extensive immune response that triggered an oxidative outburst and consequently an increased response of antioxidants.

Individuals in III. in IV. the quadrant, on the other hand, are 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 intensive care unit when the infection had been going on for some time. 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 second case, high PAT values already indicate inadequate redox signaling and increasingly severe tissue damage.

Individuals in a quadrant IV are critically ill patients with low d-ROMs and high PAT values.

As in the above example, there is an increasing number of tissue damage and slow organ failure.

The frequency of OSI index is shown in Figure 3.

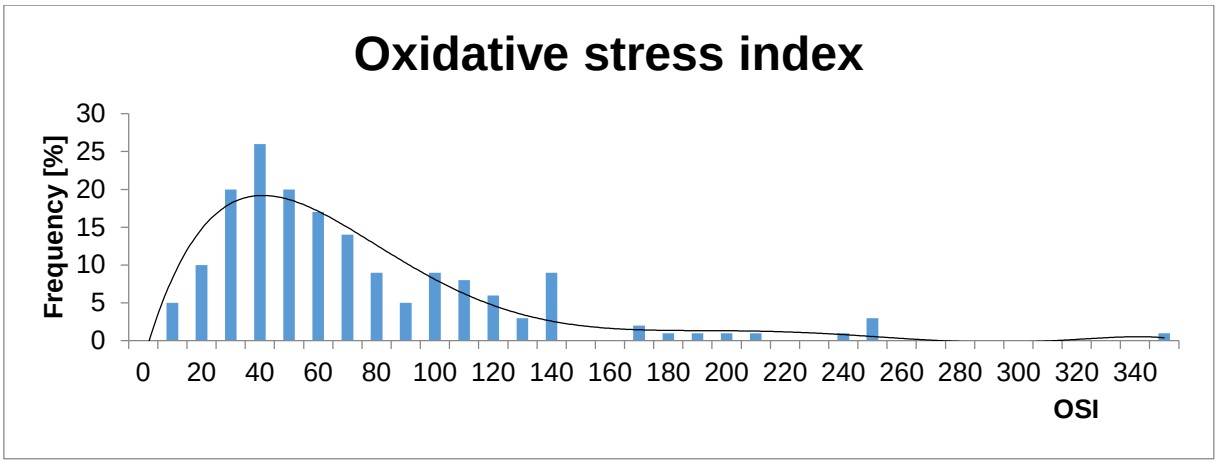

Figure 3: Calculated OSI of the whole study group

Receiver operating characteristics (ROC) curve was constructed and Youden Index was used to determine optimal cut-off for predicting intensive care unit (ICU) admission. ROC curve is presented in Figure 4.

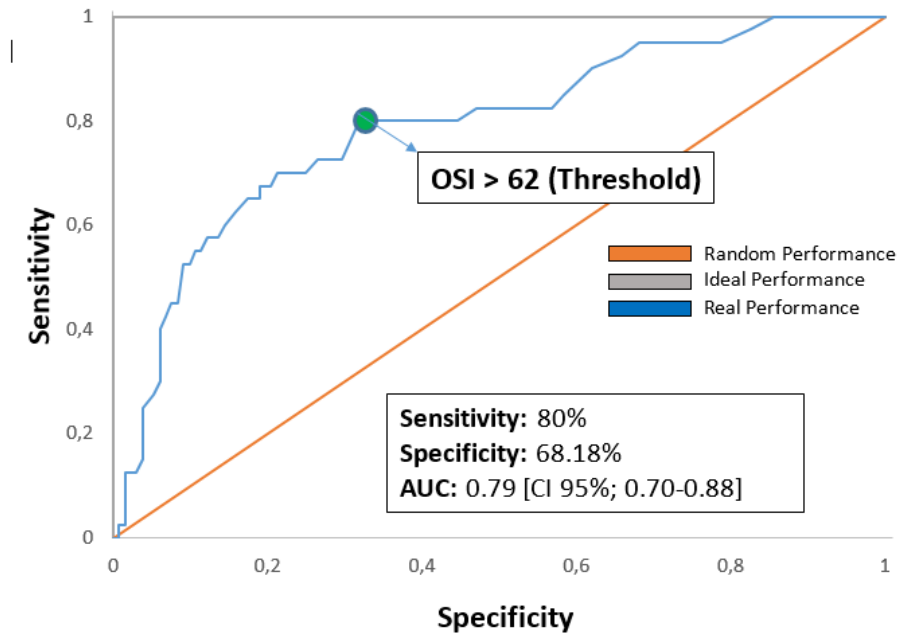

Figure 4: ROC curve for predicting ICU admission

From the group of patients in intensive care unit, two patients' oxidative stress analysis results were compared to biochemical and hematological laboratory values of the same date. Based on the results and literature, we focused on the following parameters: C-reactive protein (CRP), lymphocytes, neutrophils, interleukin-6 (IL-6), and compared them with our oxidative stress index results.

Patient 1: A 71-year-old woman with dilatative cardiomyopathy, arterial hypertension, chronic atrial fibrillation and depression presented to the emergency department due to dyspnea, unproductive cough, chest pain depending on body position, and temperature lasting for three days. Clinical examination showed a slightly elevated body temperature, regular heart pulse rate, eupneic breathing and oxygen saturation of 96% breathing room air. No typical covid pulmonary infiltrates or other pathological changes were seen on initial chest x-ray. A nasopharyngeal swab was positive for SARS-CoV-2 and she was admitted to the regular ward for additional diagnostics and observation. An ishemic myocardial event was ruled out. In the following days her condition deteriorated and due to respiratory failure on the fifth day of hospitalization she was admitted to the intensive care unit (ICU), where she was intubated and started on mechanical ventilation. A chest X-ray showed bilateral consolidations and a lung ultrasound showed diffuse B-lines. Laboratory results on admission to the ICU showed normal white blood cell (WBC) count (4200/mm^3) with low percentage of lymphocytes (17.8%), normal procalcitonin (PCT; 0,02 μg/L) and troponin (21 ng/l) levels. CRP (85 mg/L), IL-6 (43,6 ng/L), blood urea, creatinine, D-dimer, N-terminal brain natriuretic propeptide (NTproBNP), lactate dehydrogenase (LDH), and fibrinogen were elevated. Due to severe respiratory failure she was started on experimental antiviral therapy with hydroxychloroquine in accordance to interim local guidelines and antibiotic therapy with cefriaxone. Possible infections with hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) were excluded at the start of antiviral therapy. She received therapeutic doses of subcutaneous low-molecular weight heparin (LMWH) for chronic atrial fibrillation and stress ulcer bleeding prophylaxis has been prescribed. The patient remained intubated (70% fraction of inspired oxygen with positive end-expiratory pressure [PEEP] of 10 cmH_2O and ratio of arterial oxygen partial pressure to fraction of inspired oxygen bellow 100) and sedated. On the fifteenth day after admission to the ICU there was an additional deterioration of her condition. According to the increase of laboratorial inflammation markers (leukocyte, CRP and PCT) and hemodynamic instability antimicrobial treatment was modified and cefepime was started. After transient improvement

additional complications followed. Several nosocomial infections, deep vein thrombosis, and deterioration of cardiac function were confirmed. Treatment with glucocorticoids was started due to organizing pneumonia and tracheotomy was performed due to prolonged intubation. The patient's clinical and respiratory status then gradually improved. After 65 days of ICU treatment she was decannulated and transferred to the regular ward.

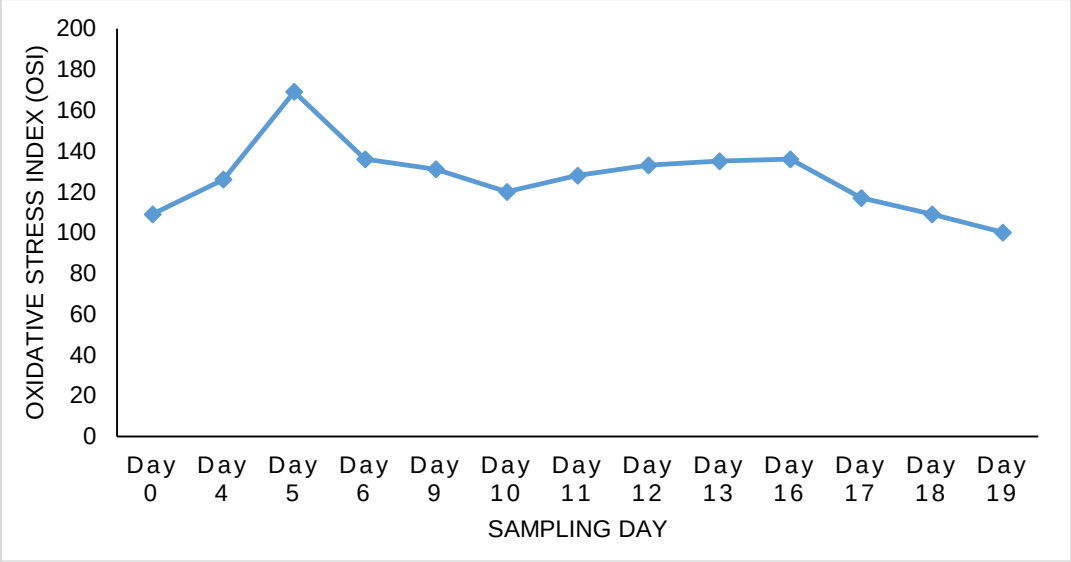

Figure 5: Oxidative stress index as a function of time. Day 0 is the first day in the critical care unit

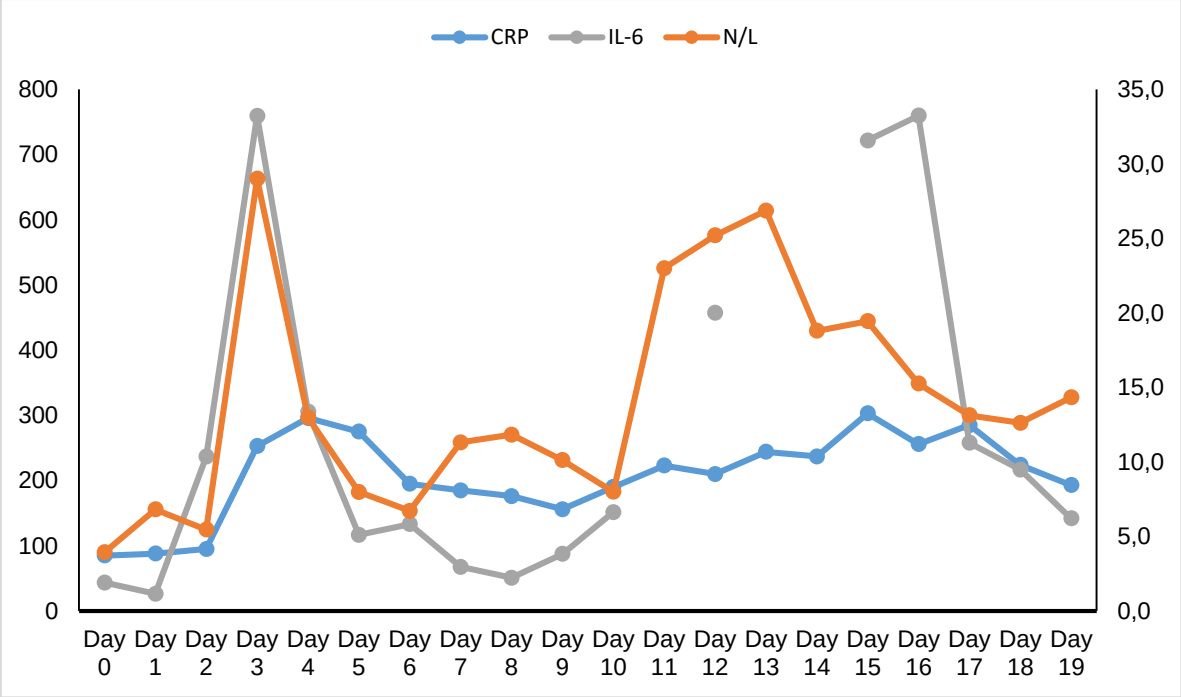

Figure 6: C-reactive protein (CRP), interleukin 6 (IL-6) and neutrophil to lymphocyte values as a function of time. Day 0 is the first day in the critical care unit.

CRP values, the proportion of neutrophils and lymphocytes and IL-6 values correlate very well. All parameters are completely outside the reference values in the first days of measurements and indicate extensive inflammation and an aggressive immune system that accelerates the synthesis of neutrophils, while the virus completely disables lymphocytes and inhibits their function. Further results show that the patient's body slowly began to fight back, as the results normalized at the end of the measurements.

We can try to identify a better correlation but we need to express neutrophil and lymphocyte counts in different units; as 10^9/l instead of % of change (Figure 7). Neutrophil to lymphocyte ratio worsening is anticipated in OSI worsening.

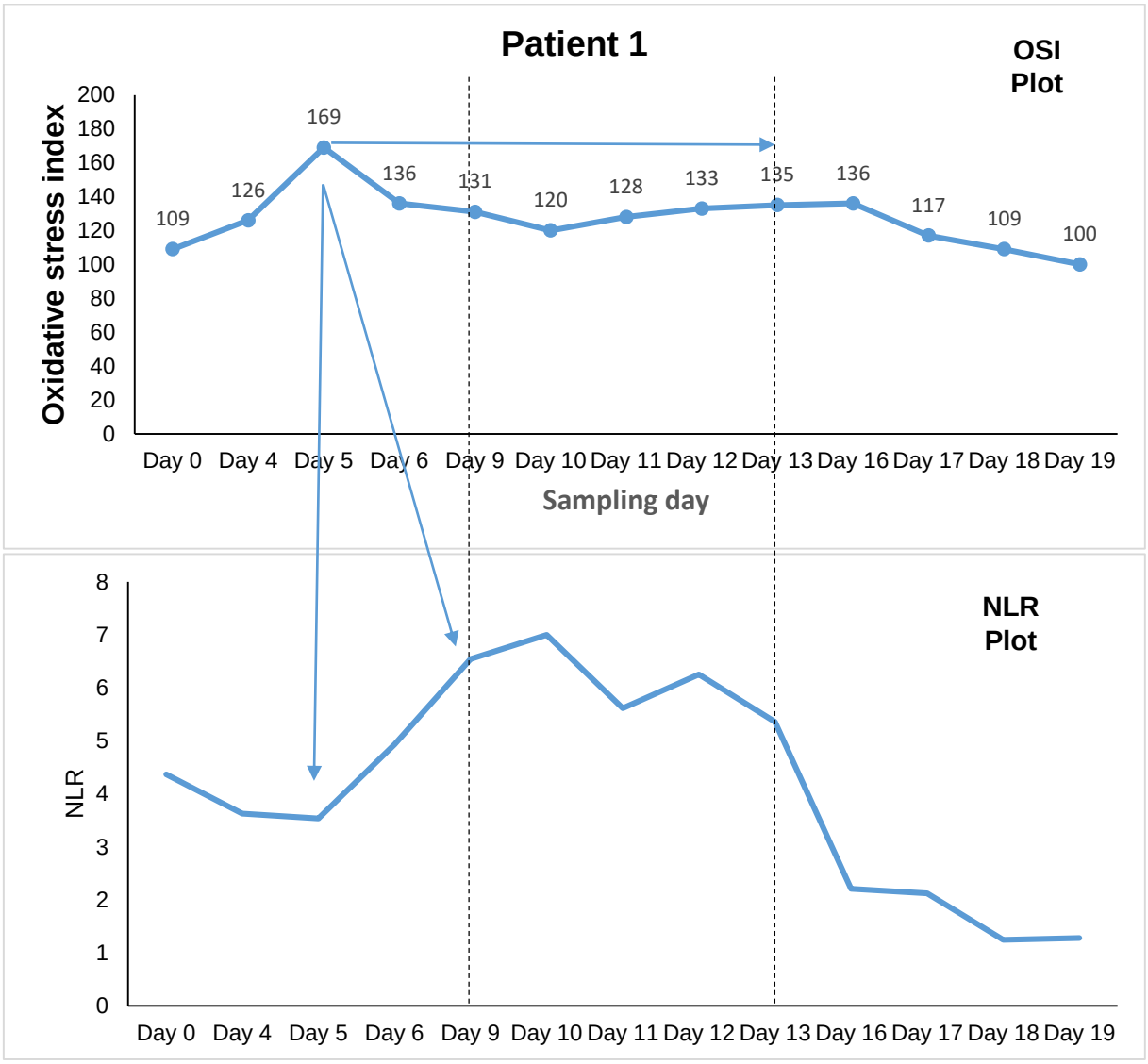

Figure 7: OSI index in relation to neutrophil lymphocyte ratio (NLR) for patient 1

Biochemical and hematological parameters helped us obtain a slightly broader picture of the patient's condition and correlation with our predictions based on the coordinate system were drawn. Our patient is classified in III. and IV. quadrant where low values of d-ROMs and normal values of PAT are located. These results indicate a long-term infection and exhaustion of the organism, but the antioxidant defense is still working. Also, the biochemical and hematological parameters in the initial days indicate a state of extensive inflammation and a poor prognosis for the patient, but the condition began to improve in the last days of the measurements. We can conclude that the patient still had enough antioxidant power to improve in the last few days.

Patient 2: A 75-year-old man with arterial hypertension, hypercholesterolemia, and after total right hip replacement, cholecystectomy and pacreatitis in the past, was admitted to the gastroenterology department due to fever, abdominal pain, nausea, and vomiting. Nasopharyngeal swab for SARS-CoV-2 virus was negative. A *Salmonella infantis*, and *Streptococcus mitis* were isolated from blood cultures and treatment with ceftriaxone was started. The clinical condition gradually improved and elevated laboratory inflammation markers normalized. However, after ten days of hospitalization he was transferred to the quarantine ward due to contact with the COVID positive patient. Eight days later, a repeat nasopharyngeal smear was positive for SARS-CoV-2, respiratory symptoms with cough and hypoxia occurred, and a week later he was admitted to the intensive care unit due to respiratory failure. On examination, he had a respiratory rate of 30 breaths per minute and oxygen saturation of 88% while receiving high flow oxygen (15 L/min) via a non-rebreather mask. Otherwise he was afebrile, normocardiac and normotensive. The patient was sedated, intubated, and mechanically ventilated. A chest X-ray showed extensive peripheral bilateral opacities and arterial oxygen partial pressure to fraction of inspired oxygen ratio was 105. Upon admission to the ICU the patient’s laboratory test results were 77mg/L for CRP, 5900/mm^3 for WBC with a normal percentage of neutrophils (58%) and of lymphocytes (25,4%). A high sensitive troponin level, blood urea, creatinine, fibrinogen, and PCT concentration were in normal range. However, IL-6, D-dimer, NTproBNP, LDH, and fibrinogen were elevated. An experimental antiviral therapy with lopinavir/ritonavir and hydroxychloroquine sulphate via nasogastric tube was initiated. However, antiviral treatment was discontinued after five days due to bradycardia and prolonged QTc interval. He received prophylactic doses of LMWH and stress ulcer bleeding prophylaxis as well. Three days after

ICU admission nosocomial pneumonia was suspected and antimicrobial treatment with piperacillin/tazobactam was started. A respiratory parameters of ventilation began to gradually improve, a lower FiO2 was required and lung compliance was adequate. However, laboratory indicators of inflammation increased and abdominal CT scan revealed thrombosis of the superior mesenteric artery, extensive splenic infarction and walled-off pancreatic necrosis was suspected. A renal function deteriorated as well and hemodialysis was required. The patient remained intubated and sedated. Subsequently, there was a massive bleeding from the upper gastrointestinal tract and gastroscopy confirmed extensive stress ulcers. His condition remained critical while being aggressively managed in the ICU and ultimately the patient's family decision was to pursue comfort measures and the patient passed away.

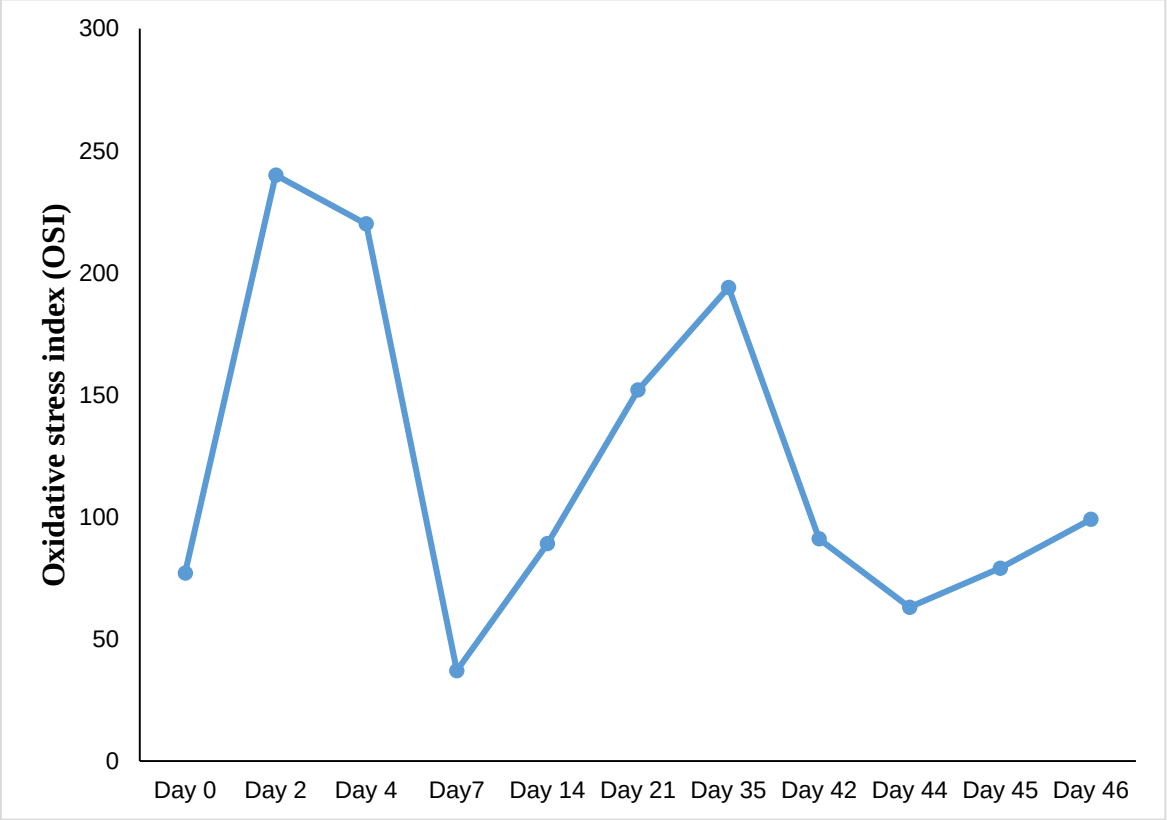

Figure 8: Oxidative stress index as a function of time. Day 0 is the first day in the critical care unit

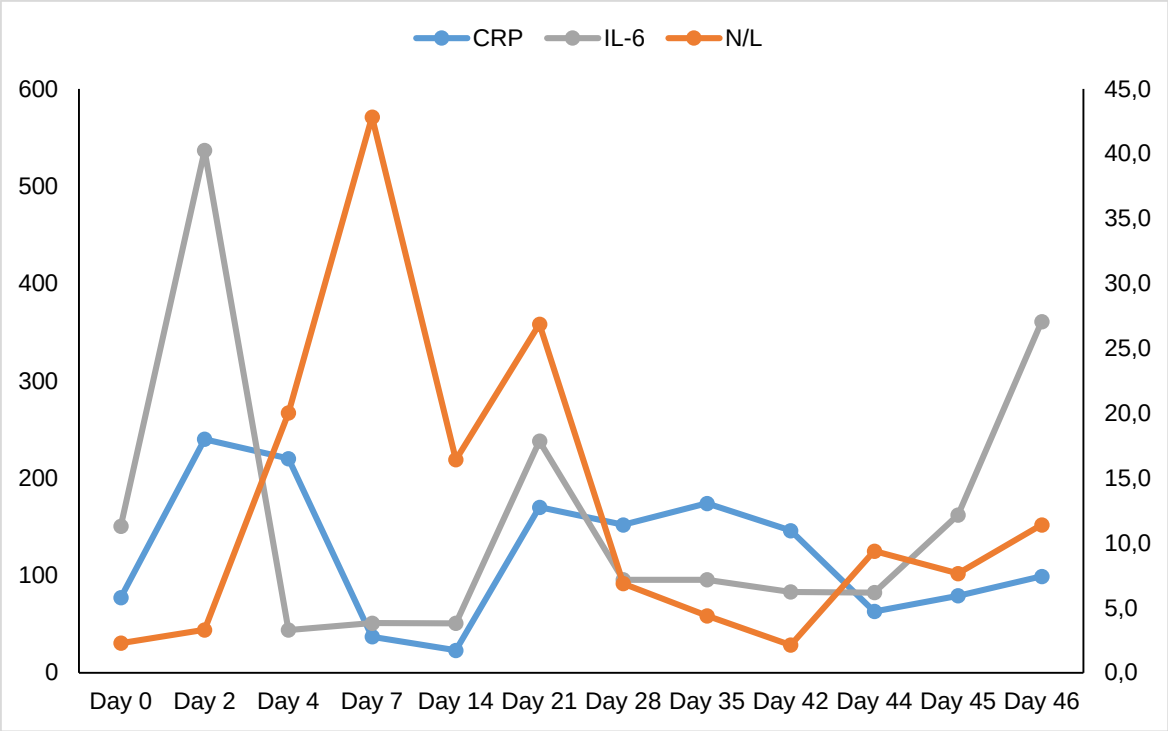

Figure 9: C-reactive protein (CRP), interleukin 6 (IL-6) and neutrophil to lymphocyte values as a function of time. Day 0 is the first day in the critical care unit

We see that the parameters correlate very well. High CRP and IL-6 values indicate extensive inflammation, and an elevated neutrophil to lymphocyte ratio suggests an aggressive immune response of neutrophils with characteristic lymphopenia observed in patients with COVID-19. The results in the initial days of the measurements indicate a very poor condition of the patient, which then improves around day 7, but deteriorates rapidly back in the following days of the measurements.

The state of oxidative stress shows us a very similar picture. The oxidative stress index slowly decreases and shows an improvement in the same way as the other parameters. Furthermore, it also starts to rise again with slight falls after day 35 to 42, and given the previously mentioned fact that the state of prooxidants and antioxidants later changes as CRP, we assume that in the following days the oxidative stress index began to rise steadily.

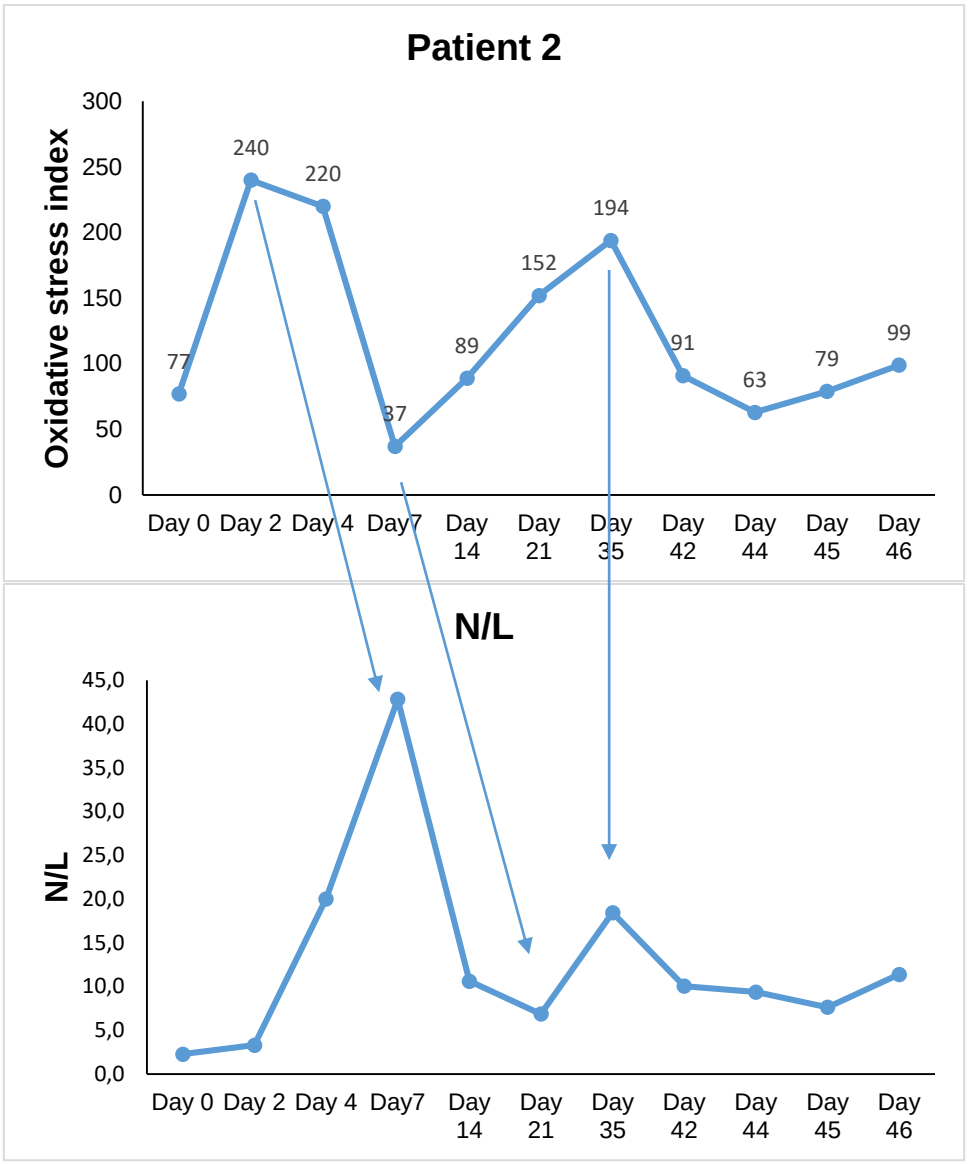

Figure 10: OSI index in relation to neutrophil lymphocyte ratio (NLR) for patient 2

We also made a comparison with the coordinate system in this patient. This is classified in IV. a quadrant where low levels of prooxidants and high levels of antioxidants are concentrated. This condition has the worst prognosis, as a long-term infection is already present, the organism is not able to form ROS, redox signaling is inadequate, and most likely multiple tissue damage is present. The biochemical and hematological parameters show the same and we can conclude that despite the intermediate improvement of the condition, the patient did not recover.

Conclusions

In the research we came to the following conclusions:

The oxidative stress index serves as a predictor for the course of the disease. On the basis of the data analysed it can be reasonable to think that OSI is a good predictive index for ICUs admission where a cut-off of 62 was identified.
The NLR is an index able to predict COVID-19 in-hospital mortality. A trend between OSI and NLR can exist, but we need Neutrophil and Lymphocyte counts expressed with a different unit and we need a way to better describe such correlation.
The very low d-ROMs level observed in patients 1 and 2 can be explained by the pathological status of the subjects, on the contrary high PAT levels can be explained by haemolysis processes, through which high amount of GSH are released from red blood cells, at the same time due to the lack of ROS species the antioxidants are not used by the organism and this can be another reason why the PAT is quite high in some cases. This can be the reason why statistically significant differences in d-ROMs and PAT were not identified in the patients analysed, since the evolution strongly depend upon the time evolution of the diseases, and pathological condition can occur at low and high d-ROMs/PAT level.
With the help of the coordinate system, we evaluated the condition of the patients and concluded the condition of each group. We found that PCR negative group is concentrated approximately in the middle of the coordinate system, which means that most of the values of the measured parameters are within normal reference limits. The PCR positive group is grouped into I. and II. quadrant, and the values of the oxidative stress parameters already indicate a shift from the normal reference limits. However, a wide variety of conditions were present in intensive care group, some of which were in the initial stage of the disease and had just been admitted to intensive care, and the results of d-ROMs, PAT, OSI were not as severe as in individuals with long-term hospitalization.Comparison of the oxidative stress index in two intensive care patients with biochemical and hematological parameters showed that the values correlated very well. 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 prooxidant levels rise when there is actual oxidative damage and thus reflect the current state of the body.

In our study of oxidative stress, we came to very interesting findings that can serve as a basis for further research. The limitation of our research is the small number of samples, however, we hope that the results have contributed to greater knowledge about the SARS-CoV-2 virus, which will also help in the further treatment of patients.

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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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