Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Original research article

Inflammatory and thrombotic parameters associated with the COVID-19 course in Poland (SARSTer study)

Piotr Czupryna [a,1], Anna Moniuszko-Malinowska [a,1,*], Magdalena Rogalska [b], Dorota Zarębska-Michaluk [c], Beata Lorenc [d], Marta Rorat [e,f], Dorota Kozielewicz [g], Magdalena Tudrujek-Zdunek [h], Katarzyna Sikorska [i], Aleksandra Berkan-Kawińska [j], Beata Bolewska [k], Włodzimierz Mazur [l], Justyna Kowalska [m], Barbara Oczko-Grzesik [n], Regina Podlasin [o], Anna Piekarska [j], Krzysztof Kłos [p], Aleksandra Szymczak [r], Piotr Leszczyński [s], Katarzyna Maciejewska [t], Sławomir Pancewicz [a], Jerzy Jaroszewicz [n], Robert Milewski [u], Monika Groth [a], Robert Flisiak [b]

[a] Department of Infectious Diseases and Neuroinfections, Medical University of Bialystok, Bialystok, Poland
[b] Department of Infectious Diseases and Hepatology, Medical University of Bialystok, Bialystok, Poland
[c] Department of Infectious Diseases, Jan Kochanowski University, Kielce, Poland
[d] Pomeranian Center of Infectious Diseases, Department of Infectious Diseases, Medical University of Gdańsk, Gdańsk, Poland
[e] Department of Forensic Medicine, Wrocław Medical University, Wrocław, Poland
[f] First Infectious Diseases Ward, Gromkowski Regional Specialist Hospital in Wrocław, Wrocław, Poland
[g] Department of Infectious Diseases and Hepatology, Faculty of Medicine, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Poland
[h] Department of Infectious Diseases and Hepatology, Medical University of Lublin, Lublin, Poland
[i] Department of Tropical Medicine and Epidemiology, Medical University of Gdańsk, Gdańsk, Poland
[j] Department of Infectious Diseases and Hepatology, Medical University of Łódź, Łódź, Poland
[k] Department of Infectious Diseases, University of Medical Sciences, Poznań, Poland
[l] Clinical Department of Infectios Diseases, Specialistic Hospital in Chorzów, Chorzów, Poland
[m] Department of Adult's Infectious Diseases, Medical University of Warsaw, Warsaw, Poland
[n] Department of Infectious Diseases in Bytom, Medical University of Silesia, Katowice, Poland
[o] Hospital of Infectious Diseases in Warsaw, Warsaw, Poland
[p] Department of Infectious Diseases and Allergology, Military Institute of Medicine, Warsaw, Poland
[r] Department of Infectious Diseases, Liver Diseases and Acquired Immune Deficiencies, Wrocław Medical University, Wrocław, Poland
[s] Department of Rheumatology, Rehabilitation and Internal Medicine, Poznan University of Medical Sciences, Poland
[t] Department of Infectious Diseases, Tropical Diseases and Acquired Immunodeficiency, Pomeranian Medical University in Szczecin, Szczecin, Poland
[u] Department of Statistics and Medical Informatics, Medical University of Bialystok, Bialystok, Poland

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ABSTRACT

Purpose: The aim of the study was to assess the coagulation and inflammatory markers connected with severe course of COVID-19 and no clinical improvement.

Material and methods: The study population included 2590 adult patients, diagnosed with COVID-19, selected from the SARSTer national database - an ongoing project led by the Polish Association of Epidemiologists and Infectiologists and supported by the Medical Research Agency. Clinical and laboratory parameters, such as C-reactive protein (CRP), white blood cells (WBCs), neutrophil and lymphocyte count, procalcitonin, ferritin, interleukin-6 (IL-6), D-dimer concentration and platelet (PLT) count were analyzed before and after treatment (remdesivir, tocilizumab, dexamethasone, anticoagulants).

Results: Significant differences between patients with mild and severe course of the disease were observed in all examined parameters before treatment ($p < 0.05$). After treatment only ferritin concentration did not differ significantly. In patients with pulmonary embolism, CRP concentration, neutrophil count, D-dimer and IL-6 concentration were significantly higher than in patients without embolism ($p < 0.05$). The significant differences between the groups with and without fatal outcome were observed within all analyzed parameters.

* Corresponding author. Department of Infectious Diseases and Neuroinfections, Medical University of Bialystok, Bialystok, Zurawia 14, 15-540, Bialystok, Poland.
E-mail address: anna.moniuszko-malinowska@umb.edu.pl (A. Moniuszko-Malinowska).
[1] These authors contributed equally to this work.

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Significant differences in all examined parameters before treatment were observed between patients with and without clinical improvement ($p < 0.05$).
Multivariate logistic regression showed that no clinical improvement was associated with: IL-6>100 pg/ml (OR-2.14), D-dimer concentration over 1000 ng/ml (OR-1.62) and PLT count below 150,000/μl (OR-1.57).
Conclusions: Severe course of the disease is associated with lower PLT and lymphocyte count, higher D-dimer, CRP, neutrophil count and IL-6 concentration. The best predictors of no clinical improvement in COVID-19 are: IL-6>100 pg/ml, D-dimer>1000 ng/ml and PLT<150,000/μl.

1. Introduction

According to current knowledge, inflammation, coagulation and thrombosis play an important role in the pathogenesis of 2019 coronavirus disease (COVID-19). At first, SARS coronavirus 2 (SARS-CoV-2) infection is usually accompanied by an aggressive pro-inflammatory response and insufficient control of an anti-inflammatory response. Systemic inflammation with elevated levels of C-reactive protein (CRP), fibrinogen and cytokines such as interleukin-6 (IL-6) is commonly observed [1]. The on-going inflammation may induce the endothelial cell dysfunction, resulting in excess thrombin production, which enhances fibrin formation and promotes platelet activation and aggregation, thus leading to increased coagulation state. Products of fibrinolysis, such as D-dimer and platelet (PLT) count are laboratory markers of coagulation disorders [2]. Secondly, the hypoxia found in severe COVID-19 course can stimulate thrombosis, not only by increasing blood viscosity, but also through a hypoxia-inducible transcription factor-dependent signaling pathway [3]. Additionally, interactions between various kinds of blood cells, macrophages, endothelial cells, platelets could play a crucial role in procoagulant effect of viral infection. The immune dysregulation characteristic for severe COVID-19 infection may be initiated by "pyroptosis", a form of apoptosis initially described in macrophages, with rapid viral replication leading to massive release of inflammatory mediators [4]. Furthermore, neutrophil extracellular traps (NETs) have also been observed in vessels in autopsy specimens of patients with COVID-19. These are associated with high circulating levels of cell-free DNA and histones, which in turn can activate pro-thrombotic pathways leading to increased thrombin production [5].

The immune response may participate in the formation of thrombi within blood vessels, particularly in microvessels in COVID-19. Immunothrombosis, accurately describes the intricate network between the coagulation system and the innate immune system [6,7]. In clinical practice it is usually associated with poor prognosis and complicated course of the disease. Elevated D-dimer concentration is always associated with unfavorable events.

The aim of the study was to assess the coagulation and inflammatory markers associated with severe course of COVID-19 and no clinical improvement.

2. Material and methods

This study is based on the SARSTer national database. This ongoing project, supported by the Polish Association of Epidemiologists and Infectiologists, is a national real-world experience, observational study assessing treatment in patients with COVID-19. Patients whose data are collected in the SARSTer database were hospitalized in 18 Polish centers, between 1 March 2020 and 31 January 2021. The decision about the treatment regimen was made entirely by the treating physician with respect to the state-of-the-art knowledge and recommendations of the Polish Association of Epidemiologists and Infectiologists [8,9].

Data of 2590 adult patients (1208 females and 1382 males, mean age 60 ± 16.9 years) diagnosed with COVID-19 from the SARSTer database were analyzed. Analysis included the whole database of patients enrolled in the study hospitalized between 1st March 2020 and 31st January 2021 in the infectious diseases wards located in 18 medical centers in Poland.

The most common concomitant chronic diseases reported within this group were: hypertension in n = 1194 (46.1%) patients, diabetes n = 456 (17.6%), ischemic coronary disease n = 266 (10.3%), neoplasms n = 167 (6.4%), chronic kidney disease n = 87 (3.4%), chronic obstructive pulmonary disease n = 86 (3.3%), hyperuricemia n = 47 (1.8%), rheumatoid arthritis n = 26 (1%), and AIDS/HIV infection n = 5 (1.9%).

Patients included in the study met the following criteria: cough, dyspnea, or fever (>38 °C); positive result of a polymerase chain reaction (PCR) test for SARS-CoV-2 from a nasopharyngeal swab; typical lesions visible in the chest computed tomography (CT; ground glass; thickened interlobular and interlobular lines in combination with a ground glass pattern - crazy paving; consolidation, widening of the vessels); need for continuous oxygen therapy; oxygen saturation ≤94% at any time during hospitalization.

The patients were treated with the following: n = 273 (9.23%) - convalescent plasma, n = 566 (19.1%) - remdesivir, n = 341 (11.5%) - tocilizumab, n = 730 (24.7%) - dexamethasone, n = 1628 (55%) - prophylactic dose of low-molecular-weight heparin, and n = 210 (7.1%) - therapeutic dose of low-molecular-weight heparin.

Analysis included juxtaposition of clinical and laboratory parameters. Laboratory parameters, such as D-dimer concentration, PLT count and CRP were analyzed on admission and on the 28th day.

Depending on the severity of the disease the patients were divided into 8 categories on an ordinal scale:

1 - not hospitalized, normal activity,
2 - not hospitalized, with impaired activity and/or requiring oxygen support,
3 - hospitalized, requiring neither oxygen support nor medical care (hospitalized because of epidemiological reasons),
4 - hospitalized, not requiring oxygen support but requiring medical care (related or not related to COVID-19),
5 - hospitalized, requiring oxygen support,
6 - hospitalized, requiring noninvasive high flow oxygen support (high-flow nasal cannula),
7 - hospitalized, requiring mechanical ventilation or extracorporeal membrane oxygenation (ECMO),
8 - death

All patients were assessed before and after treatment (remdesivir, tocilizumab, dexamethasone, anticoagulants).

On admission the median score on the ordinal scale in this group was 5 (range 3–7).

The analysis was focused on risk factors/predictors of the following:

- death,
- pulmonary embolism, based on the results of a computed tomography (CT) angiography of the chest,
- severe course of the disease (defined as score 5 or higher on the ordinal scale on admission),
- no clinical improvement defined as a drop by 2 points on the ordinal scale during the 28-day follow up period (only symptomatic, hospitalized patients who scored 4 or higher on admission, were included in the analysis).

2.1. Statistical analysis

Data were presented as mean and standard deviation (SD) or median

and interquartile ranges (IQR), as appropriate. Normal distribution of variables was examined using Shapiro-Wilk test. Depending on the data distribution, either ANOVA (normal distribution) or Mann Whitney U/ Kruskal-Wallis test (non-normal distribution depending on the number of groups compared), were applied. Statistically significant results were subjected to post hoc comparisons. Paired Wilcoxon singed-rank test was used to compare the frequency of variables before and after treatment.

Multivariate logistic regression model was built. Receiver operating characteristic (ROC) curves were calculated for measurable variables that differed significantly between the groups.

Correlations were measured with Spearman rank test.

A p-value < 0.05 was considered statistically significant.

2.2. Ethical issues

The SARSTer study was approved by the Ethics Committee of the Medical University of Bialystok, Poland (approval 29 October 2020, APK.002.303.2020). In each site participating in the study, the local Bioethics Committees approved the treatment and written informed consent was obtained from each patient if necessary. The study was conducted according to the guidelines of 1964 Declaration of Helsinki with its later amendments.

3. Results

3.1. General results

Demographic characteristics and laboratory findings on admission and after treatment are presented in Table 1. The significant differences were found in CRP concentration, white blood cells (WBC) count, procalcitonin (PCT) concentration, IL-6 concentration, PLT count, and ferritin concentration ($p < 0.05$).

CRP concentration correlated with IL-6 concentration ($R = 0.7$, $p < 0.05$), neutrophil count ($R = 0.45$, $p < 0.05$), D-dimer concentration ($R = 0.43$, $p < 0.05$), and ferritin concentration ($R = 0.54$, $p < 0.05$).

IL-6 concentration correlated with PLT count ($R = 0.17$, $p < 0.05$), neutrophil count ($R = 0.34$, $p < 0.05$), D-dimer concentration ($R = 0.36$, $p < 0.05$), and ferritin concentration ($R = 0.47$, $p < 0.05$).

PLT count correlated with neutrophil count ($R = 0.35$, $p < 0.05$), D-dimer concentration ($R = 0.07$, $p < 0.05$), and ferritin concentration ($R = -0.1$, $p < 0.05$).

Neutrophil count correlated with d-dimer concentration ($R = 0.25$, $p < 0.05$), and ferritin concentration ($R = 0.24$, $p < 0.05$).

D-dimer concentration correlated with ferritin concentration ($R = 0.3$, $p < 0.05$).

There was no statistically significant differences among analyzed parameters concerning treatment (remdesivir, tocilizumab, dexamethasone, anticoagulants), yet it should be emphasized that the treatment depended on initial parameters and clinical status of the patients according to the ordinal scale.

3.2. Results in adults before treatment depending on the score on the ordinal scale

In Table 2, we present laboratory parameters on admission before and after treatment depending on the score on the ordinal scale. Statistically significant differences were observed between patients with 3–4 points and patients with 5–7 points on the ordinal scale considering all examined parameters before treatment ($p < 0.05$). After treatment, only concentration of ferritin was not statistically significantly different.

3.3. Results of analyzed parameters in patients with pulmonary embolism

Pulmonary embolism was diagnosed in 42 patients (1.6%). Analysis of the parameters of patients with and without pulmonary embolism is presented in Table 3. Statistically significant difference was observed in CRP concentration, neutrophil count, D-dimer and IL-6 concentration ($p < 0.05$).

3.4. Analysis of selected parameters in patients with fatal outcome

Of the 2590 patients, 223 (8.61%) died. The juxtaposition of laboratory parameters of patients who died and survived is presented in Table 3. Statistically significant differences between the groups were observed in all analyzed parameters.

3.5. Analysis of selected parameters in patients with clinical improvement

In Table 3, we present results of laboratory parameters before treatment depending on the clinical improvement after 1 month. Statistically significant difference between patients with clinical improvement versus patients without clinical improvement in all examined parameters before treatment was observed ($p < 0.05$). After treatment, only concentration of ferritin was not statistically significantly different.

Multivariate logistic regression showed that D-dimer concentration over 1000 ng/ml increases the odds ratio (OR) of no clinical improvement 1.62 times compared to patients with D-dimer below 1000 ng/ml. PLT count below 150,000/µl decreases the OR for clinical improvement 1.57 times in comparison to patients with PLT count over 150,000/µl. IL-6 over 100 pg/ml decreases the OR for clinical improvement 2.14 times in comparison to IL-6 concentration below 100 pg/ml (Table 4).

ROC curves in Fig. 1 present differences between the groups according to the examined parameters. The proposed cut-off (criterion) is marked in the graph.

Table 1
Demographic characteristics and laboratory parameters at admission and after treatment (n = 2590).

	Before treatment					After treatment					p value
	Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max	
CRP (mg/l)	69.5	74.9	44.5	0.010	439	30.3	52.1	10.9	0.090	535	<0.05
WBC (1/µl)	6843.1	6509.9	5865.0	200.000	224000	8129.9	5647.2	6740.0	1730.000	73000	<0.05
Neutrophils (1/µl)	4693.97	3176.99	3900	0	40000	5238.0	4569.9	4000.0	500.000	62300	NS
PCT (ng/ml)	0.4	2.5	0.1	0.000	48	0.3	1.1	0.1	0.010	13	<0.05
IL-6 (pg/ml)	66.3	162.8	27.9	0.030	3636	172.8	648.8	9.2	0.040	8855	<0.05
PLT (1/µl)	219164.0	94497.2	201000.0	2000.000	905000	315384.3	131858.4	297000.0	6000.000	1035000	<0.05
D-dimer (ng/ml)	1789.4	5332.3	740.0	24.000	93239	1769.6	3442.4	828.0	28.000	40055	NS
Ferritin (µg/l)	930.4	1272.2	568.0	5.000	19257	1097.9	2726.2	672.0	29.500	33511	<0.05
Age (years)	60.0	16.9	62.0	18.000	98	60.0	16.9	62.0	18.000	98	–
BMI	28.1	5.2	27.7	12.440	56	28.1	5.2	27.7	12.440	56	–

Abbreviations: SD – standard deviation; NS – non-significant; CRP – C reactive protein; WBC – white blood cells; PCT – procalcitonin; IL-6 – interleukin 6; PLT – platelet count; BMI – body mass index.

Table 2
Results of laboratory parameters before and after treatment depending on the score on the ordinal scale at admission.

		Clinical scale score at admission - 3–4 (n = 1350)					Clinical scale score at admission - 5–7 (n = 1240)					p value
		Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max	
Before treatment	CRP (mg/l)	41.2	55.5	19.0	0.01	422	100.0	81.0	79.2	0.42	439	<0.05
	WBC (1/μl)	6201.0	4195.4	5470.0	200.00	111570	7536.4	8260.9	6340.0	660.00	224000	<0.05
	Neutrophils (1/μl)	3970.14	2583.84	3350	0	25840	5483.44	3554.32	4670	100	40000	<0.05
	PCT (ng/ml)	0.2	1.8	0.1	0.00	38	0.6	3.0	0.1	0.00	48	<0.05
	IL-6 (pg/ml)	39.2	105.9	13.9	0.03	1679	93.2	200.7	46.2	0.63	3636	<0.05
	PLT (1/μl)	221309.1	91773.7	206000.0	2000.00	691000	216840.1	97346.1	196000.0	10000.00	905000	<0.05
	D-dimer (ng/ml)	1273.8	3551.7	597.0	24.00	63564	2329.6	6668.2	879.5	74.00	93239	<0.05
	Ferritin (μg/l)	596.7	1091.6	404.0	5.00	19257	1223.3	1346.4	786.5	8.80	13468	<0.05
After treatment	CRP (mg/l)	19.7	38.6	5.5	0.09	371	38.2	58.9	16.0	0.25	535	<0.05
	WBC (1/μl)	6867.3	4538.0	6175.0	1920.00	73000	9095.8	6198.2	7500.0	1730.00	64570	<0.05
	Neutrophils (1/μl)	4001.2	3211.4	3410.0	500.00	48100	6189.9	5192.3	4880.0	550.00	62300	<0.05
	PCT (ng/ml)	0.2	0.7	0.1	0.01	5	0.4	1.3	0.1	0.01	13	<0.05
	IL-6 (pg/ml)	98.0	449.6	5.0	0.04	4164	226.8	756.9	24.4	0.20	8855	<0.05
	PLT (1/μl)	305069.1	126292.9	281000.0	12000.00	1025000	323319.2	135538.5	308000.0	6000.00	1035000	<0.05
	D-dimer (ng/ml)	1289.5	2326.1	660.0	34.00	26172	2090.6	3989.3	989.0	28.00	40055	<0.05
	Ferritin (μg/l)	938.2	2006.3	568.0	29.50	15278	1161.5	2968.8	691.0	59.16	33511	NS

Abbreviations: SD – standard deviation; NS – non significant; CRP – C reactive protein; WBC – white blood cells; PCT – procalcitonin; IL-6 – interleukin 6; PLT – platelet count.

Table 3
Parameters of patients with and without pulmonary embolism, patients who died and who survived, and depending on the clinical improvement in 1 month.

	With embolism (n = 42)					Without embolism (n = 2548)					p value
	Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max	
CRP (mg/l)	112.9	93.98	90.5	2.1	328.4	68.8	74.4	44.0	0.010	439	<0.05
WBC (1/μl)	7000.1	3571.65	6265.0	1006.0	15160.0	6840.5	6548.2	5850.0	200.000	224000	NS
Neutrophils (1/μl)	5578.75	3200.34	4935	600	14020	4679.52	3175.22	3900	0	40000	<0.05
PCT (ng/ml)	0.4	1.05	0.1	0.0	4.6	0.4	2.5	0.1	0.000	48	NS
IL-6 (pg/ml)	96.6	136.34	44.0	1.5	618.7	65.7	163.2	27.7	0.030	3636	<0.05
PLT (1/μl)	210869.0	95237.47	200500.0	27300.0	427000.0	219302.9	94497.7	202000.0	2000.000	905000	NS
D-dimer (ng/ml)	4533.5	7694.22	950.0	203.0	33433.0	1742.5	5272.9	737.0	24.000	93239	<0.05
Ferritin (μg/l)	1144.9	952.19	885.5	153.0	4135.0	924.7	1279.5	566.2	5.000	19257	NS
	Fatal outcome (n=223)					**Recovery (n=2367)**					
CRP (mg/l)	125.4	90.0	107.9	1.00	438.6	64.3	71.2	40.6	0.010	430	<0.05
WBC (1/μl)	10978.8	17989.5	7900.0	200.00	224000.0	6457.5	3812.9	5730.0	660.000	111570	<0.05
Neutrophils (1//μl)	7293.17	5243.83	5820	0	40000	4464.49	2814	3800	100	25840	<0.05
PCT (ng/ml)	2.0	6.2	0.3	0.01	48.4	0.3	1.6	0.1	0.000	38	<0.05
IL-6 (pg/ml)	193.4	418.8	88.0	1.50	3636.0	56.8	119.2	25.7	0.030	1679	<0.05
PLT (1/μl)	207713.8	107894.1	188000.0	22000.00	572000.0	220229.0	93107.9	202000.0	2000.000	905000	<0.05
D-dimer (ng/ml)	4770.1	10059.1	1500.0	162.00	69575.0	1521.1	4589.1	699.5	24.000	93239	<0.05
Ferritin (μg/l)	1377.7	1014.1	1043.0	8.80	4498.0	896.2	1283.9	541.0	5.000	19257	<0.05
	With clinical improvement in 1 month (n=1698)					**Without clinical improvement in 1 month (n=892)**					
CRP (mg/l)	67.1	70.6	44.3	0.01	430	97.3	90.2	70.1	0.36	438.6	<0.05
WBC (1/μl)	6506.3	3977.4	5790.0	660.00	111570	8517.4	12305.5	6380.0	200.00	224000.0	<0.05
Neutrophils (1/μl)	4550.4	2732.0	3900.0	100.00	25660	5761.8	4518.5	4450.0	0.00	40000.0	<0.05
PCT (ng/ml)	0.3	1.8	0.1	0.00	38	1.1	4.1	0.2	0.00	48.4	<0.05
IL-6 (pg/ml)	52.7	110.9	25.3	0.03	1679	130.5	294.2	52.5	0.64	3636.0	<0.05
PLT (1/μl)	221503.0	92759.7	202000.0	10000.00	905000	207769.4	101598.4	191000.0	7000.00	649000.0	<0.05
D-dimer (ng/ml)	1465.4	4642.9	694.5	24.00	93239	2992.2	7156.7	1114.0	128.20	69575.0	<0.05
Ferritin (μg/l)	904.0	1134.6	564.0	14.00	13468	1111.6	1102.7	721.0	8.80	8302.0	<0.05

Abbreviations: SD – standard deviation; NS – non significant; CRP – C reactive protein; WBC – white blood cells; PCT – procalcitonin; IL-6 – interleukin 6; PLT – platelet count.

Table 4
Multivariate logistic regression parameters presenting the odds ratios for no clinical improvement.

Parameter	OR	P	95% CI	
D-dimer >1000 ng/ml	1.62	0.0001	1.24	2.12
PLT <150,000/μl	1.57	0.003	1.17	2.10
IL-6 > 100 pg/ml	2.14	0.0001	1.54	2.98

4. Discussion

The inflammatory cytokine storm seems to be one of the most important pathomechanisms leading to death of COVID-19 patients. It is defined by the excessive and uncontrolled release of pro-inflammatory cytokines, including cytokines released by macrophages (IL-6, IL-10, and TNF-α), resulting in the damage to the lungs and other organs [10, 11]. Our study, conducted on the large group of patients, confirms the crucial role of inflammation and coagulopathy in the pathomechanism of

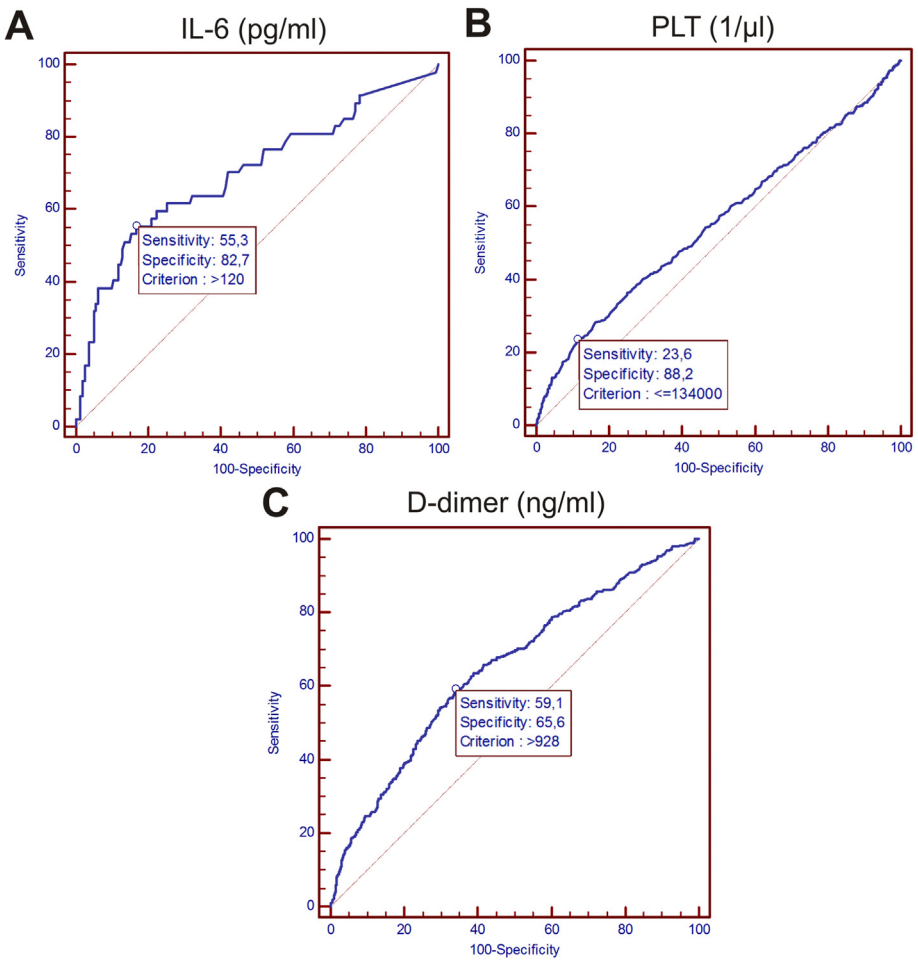

Fig. 1. ROC curves of IL-6, PLT, D-dimer, when considering clinical improvement (IL-6 AUC – 0.702; PLT AUC – 0.555; D-dimer AUC – 0.650).

COVID-19. Markers associated with these processes seem to be good predictors of the COVID-19 outcomes. Hariyanto et al. [12] performed a meta-analysis of 4848 patients from 23 studies and suggested that CRP and D-dimer can be used for predicting severe outcomes in COVID-19, which was also observed in our study. Our present study is the first study based on the Polish population concerning this topic. However, the results are comparable to the reports in other countries. During COVID-19 the increase of neutrophils is observed in the bloodstream and the lungs. Although the neutrophils can play a protective role, extensive and prolonged activation of these leukocytes can lead to harmful effects in the lungs and result in pneumonia and/or acute respiratory distress syndrome (ARDS) [13].

Neutrophilia has been described as an indicator of severe respiratory symptoms and poor outcome in patients with COVID-19. Wang et al. [14] also demonstrated that neutrophilia coexists with lung injury in severe COVID-19 patients. In our study, neutrophil count was significantly higher in patients with more severe presentation of COVID-19, patients with embolisms and those who did not improve after treatment. However, in multivariate logistic regression, neutrophil count did not have a significant influence on clinical recovery.

IL-6 is one of the most important cytokines released during the cytokine storm phase in COVID-19. It might be secreted from various types of cells, such as T cells, macrophages, endothelial cells, fibroblasts and monocytes [15]. Zhang et al. [16] reported that IL-6 concentration higher than 37.65 pg/ml was predictive of in-hospital death and according to Herold et al. [17] IL-6 concentration >80 pg/ml was highly predictive of respiratory failure. The results of our study show that IL-6 concentration >100 pg/ml is associated with more severe course of COVID-19, fatal outcome, and reduced chances for clinical improvement. In multivariate logistic regression, IL-6 over 100 pg/ml decreases the OR for clinical improvement 2.14 times in comparison to IL-6 concentration below 100 pg/ml.

CRP concentration can also be used in predicting the outcome of COVID-19. In many studies, CRP concentration strongly correlated with the severity of the COVID-19 course [17–20], which was also presented in our study. Significantly higher CRP concentration was observed in patients with more severe course of the disease, death and no clinical improvement. In multivariate logistic regression, CRP concentration did not have a significant influence on clinical recovery.

Ferritin is another marker of inflammation that seems to be strongly associated with COVID-19 outcome prognosis. According to Ahmed et al. [21], ferritin is a good marker of mortality in COVID-19. Extensive ferritin secretion in the course of COVID 19 might be caused by macrophages and cytokines such as IL-6 [22,23]. In our study, higher ferritin concentration was observed in patients with more severe disease course and fatal outcome, and it decreased the probability of clinical improvement. In multivariate logistic regression, ferritin concentration did not influence the clinical improvement significantly.

The presence of coagulopathy, as part of the systemic inflammatory response syndrome, is a common feature of severe COVID-19. Approximately 20%–50% of hospitalized patients with COVID-19 present hematologic abnormalities in coagulation tests. The most common hemostatic alterations in COVID-19 are thrombocytopenia and elevation of D-dimer [24]. Although these coagulation abnormalities mimic the pattern observed in disseminated intravascular coagulation (DIC), most of COVID-19 patients do not fulfill the criteria of typical forms of DIC. It

seems that coagulation abnormalities observed in COVID-19 may represent a completely distinct intravascular coagulation syndrome [25].

Ozen et al. [26] observed increased concentration of D-dimer in 63.3% of patients and reported that the severity of COVID-19 pneumonia was closely associated with D-dimer levels, which tended to increase as the clinical or radiological condition of patients with COVID-19 deteriorated.

Also, Yao et al. [27] reported that D-dimer is significantly increased in COVID-19 patients and may be a good prognostic factor for in-hospital mortality (D-dimer > 2.0 mg/L was associated with increased OR of mortality - 10.17). Zhang et al. [28] observed that at the same cut off value of D-dimer concentration (>2.0 mg/L), the increase in mortality was even higher (hazard ratio 51.5).

In our present study, we observed that D-dimer was higher in patients with more severe course of the disease, unfavorable outcome of COVID-19, and it decreased the likelihood of clinical improvement. In multivariate logistic regression, D-dimer of over 1000 ng/ml significantly increased the probability of no clinical improvement (OR – 1.62).

The other important marker of coagulopathy is PLT count. In our study, PLT count was significantly lower in patients with no clinical improvement and fatal outcome. In multivariate logistic regression, PLT <150,000/μl significantly increased the OR for no clinical improvement (OR – 1.57). This result is in accordance with a meta-analysis performed by Jiang et al. [29] who reported that in patients with severe COVID-19 PLT count is significantly lower than in patients with mild course of the disease.

4.1. Strengths and limitations of the study

The most important advantage of our study was a relatively large number of symptomatic patients included in the study, which allowed us do draw helpful and practical conclusions, concerning the usefulness of inflammatory and coagulation parameters in predicting the course of COVID-19. The multicenter nature of our study allows for screening of a larger population than in a regional study, yet, on the contrary, it is also one of the limitations of our report due to laboratory test differences depending on each laboratory (although standardized form was used and, if necessary, the data were unified and re-calculated).

In every case of suspected pulmonary embolism, CT angiography was performed. However, it seems probable that some of the pulmonary embolism cases were not diagnosed or were diagnosed after the follow up. Although these patients were included in overall statistics, they were not considered as pulmonary embolism group. The SARSTer study is continued, so future analyses will verify the conclusions from the current study.

5. Conclusions

Severe course of the disease is associated with lower PLT and lymphocyte count, higher D-dimer, CRP, neutrophil count and IL-6 concentrations. In COVID-19, the best predictors of no clinical improvement are: IL-6 over 100 pg/ml, D-dimer >1000 ng/ml and PLT <150,000/μl.

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The author contribution

Study Design: Robert Flisiak, Piotr Czupryna, Anna Moniuszko-Malinowska.

Data Collection: Piotr Czupryna, Anna Moniuszko-Malinowska, Magdalena Rogalska, Dorota Zarębska-Michaluk, Beata Lorenc, Marta Rorat, Dorota Kozielewicz, Magdalena Tudrujek-Zdunek, Katarzyna Sikorska, Aleksandra Berkan-Kawińska, Beata Bolewska, Włodzimierz Mazur, Justyna Kowalska, Barbara Oczko-Grzesik, Regina Podlasin, Anna Piekarska, Krzysztof Kłos, Aleksandra Szymczak, Piotr Leszczyński, Katarzyna Maciejewska, Sławomir Pancewicz, Jerzy Jaroszewicz, Robert Flisiak.

Statistical Analysis: Piotr Czupryna.

Data Interpretation: Robert Flisiak, Piotr Czupryna, Anna Moniuszko-Malinowska Manuscript Preparation: Robert Flisiak, Piotr Czupryna, Anna Moniuszko-Malinowska.

Literature Search: Robert Flisiak, Piotr Czupryna, Anna Moniuszko-Malinowska.

Funds Collection: Robert Flisiak.

Declaration of competing interest

The authors declare no conflict of interests.

References

[1] Wong JP, Viswanathan S, Wang M, Sun LQ, Clark GC, D'Elia RV. Current and future developments in the treatment of virus-induced hypercytokinemia. Future Med Chem 2017;9:169–78.
[2] Levy M, van der Poll T. Coagulation and sepsis. Thromb Res 2017;149:38–44.
[3] Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. J Thromb Haemostasis 2020. https://doi.org/10.1111/jth.14817.
[4] Sakr Y, Giovini M, Leone M, Pizzilli G, Kortgen A, Bauer M, Tonetti T, Duclos G, Zieleskiewicz L, Buschbeck S, Ranieri VM, Antonucci E. Pulmonary embolism in patients with coronavirus disease-2019 (COVID-19) pneumonia: a narrative review. Ann Intensive Care 2020 Sep 16;10:124. https://doi.org/10.1186/s13613-020-00741-0.
[5] Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, Daßler-Plenker J, Guerci P, Huynh C, Knight JS, Loda M, Looney MR, McAllister F, Rayes R, Renaud S, Rousseau S, Salvatore S, Schwartz RE, Spicer JD, Yost CC, Weber A, Zuo Y, Egeblad M. Targeting potential drivers of COVID-19: neutrophil extracellular traps. J Exp Med 2020 Jun 1;217(6):e20200652. https://doi.org/10.1084/jem.20200652.
[6] Price LC, McCabe C, Garfield B, Wort SJ. Thrombosis and COVID-19 pneumonia: the clot thickens. Eur Respir J 2020 Jul 30;56(1):2001608. https://doi.org/10.1183/13993003.01608-2020.
[7] Fang XZ, Wang YX, Xu JQ, He YJ, Peng ZK, Shang Y. Immunothrombosis in acute respiratory dysfunction of COVID-19. Front Immunol 2021;12:651545. https://doi.org/10.3389/fimmu.2021.651545.
[8] Flisiak R, Horban A, Jaroszewicz J, Kozielewicz D, Pawłowska M, Parczewski M, Piekarska A, Simon K, Tomasiewicz K, Zarębska-Michaluk D. Management of SARS-CoV-2 infection: recommendations of the polish association of Epidemiologists and Infectiologists as of March 31, 2020. Pol Arch Intern Med 2020 Apr 30;130(4):352–7. https://doi.org/10.20452/pamw.15270. Epub 2020 Mar 31. Update in: Pol Arch Intern Med. 2020 Jun 25;130(6):557-558.
[9] Flisiak R, Parczewski M, Horban A, Jaroszewicz J, Kozielewicz D, Pawłowska M, Piekarska A, Simon K, Tomasiewicz K, Zarębska-Michaluk D. Management of SARS-CoV-2 infection: recommendations of the polish association of Epidemiologists and Infectiologists. Annex no. 2 as of october 13, 2020. Pol Arch Intern Med 2020 Oct 29;130(10):915–8. https://doi.org/10.20452/pamw.15658. Epub 2020 Oct 29.
[10] Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, Wang T, Zhang X, Chen H, Yu H, Zhang X, Zhang M, Wu S, Song J, Chen T, Han M, Li S, Luo X, Zhao J, Ning Q. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest 2020 May 1;130(5):2620–9. https://doi.org/10.1172/JCI137244.
[11] Gómez-Pastora J, Weigand M, Kim J, Wu X, Strayer J, Palmer AF, Zborowski M, Yazer M, Chalmers JJ. Hyperferritinemia in critically ill COVID-19 patients - is ferritin the product of inflammation or a pathogenic mediator? Clin Chim Acta 2020;509:249–51. https://doi.org/10.1016/j.cca.2020.06.033.
[12] Hariyanto TI, Japar KV, Kwenandar F, Damay V, Siregar JI, Lugito NPH, Tjiang MM, Kurniawan A. Inflammatory and hematologic markers as predictors of severe outcomes in COVID-19 infection: a systematic review and meta-analysis. Am J Emerge Med 2021;41:110–9. https://doi.org/10.1016/j.ajem.2020.12.076.
[13] Cavalcante-Silva LHA, Carvalho DCM, Lima ÉA, Galvão JGFM, da Silva JSF, Sales-Neto JM, Rodrigues-Mascarenhas S. Neutrophils and COVID-19: the road so far. Int Immunopharm 2021;90:107233. https://doi.org/10.1016/j.intimp.2020.107233.
[14] Wang J, Li Q, Yin Y, Zhang Y, Cao Y, Lin X, Huang L, Hoffmann D, Lu M, Qiu Y. Excessive neutrophils and neutrophil extracellular traps in COVID-19. Front Immunol 2020;11:2063. https://doi.org/10.3389/fimmu.2020.02063.
[15] Vatansever HS, Becer E. Relationship between IL-6 and COVID-19: to be considered during treatment. Future Virol 2020. https://doi.org/10.2217/fvl-2020-0168. 10.2217/fvl-2020-0168.
[16] Zhang J, Hao Y, Ou W, Ming F, Liang G, Qian Y, Cai Q, Dong S, Hu S, Wang W, Wei S. Serum interleukin-6 is an indicator for severity in 901 patients with SARS-

CoV-2 infection: a cohort study. J Transl Med 2020 Oct 29;18(1):406. https://doi.org/10.1186/s12967-020-02571-x.
[17] Herold T, Jurinovic V, Arnreich C, Lipworth BJ, Hellmuth JC, von Bergwelt-Baildon M, Klein M, Weinberger T. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. J Allergy Clin Immunol 2020;146(1):128–36. https://doi.org/10.1016/j.jaci.2020.05.008. e4.
[18] Yang M, Chen X, Xu Y. A retrospective study of the C-reactive protein to lymphocyte ratio and disease severity in 108 patients with early COVID-19 pneumonia from january to March 2020 in wuhan, China. Med Sci Mon Int Med J Exp Clin Res 2020;11(26):e926393. https://doi.org/10.12659/MSM.926393.
[19] Ahnach M, Zbiri S, Nejjari S, Ousti F, Elkettani C. C-reactive protein as an early predictor of COVID-19 severity. J Med Biochem 2020;39(4):500–7. https://doi.org/10.5937/jomb0-27554.
[20] Luo X, Zhou W, Yan X, Guo T, Wang B, Xia H, Ye L, Xiong J, Jiang Z, Liu Y, Zhang B, Yang W. Prognostic value of C-reactive protein in patients with coronavirus 2019. Clin Infect Dis 2020;71(16):2174–9. https://doi.org/10.1093/cid/ciaa641. 19.
[21] Ahmed S, Ansar Ahmed Z, Siddiqui I, Haroon Rashid N, Mansoor M, Jafri L. Evaluation of serum ferritin for prediction of severity and mortality in COVID-19- A cross sectional study. Annal Med Surg 2021;63:102163. https://doi.org/10.1016/j.amsu.2021.02.009.
[22] Rosário C, Zandman-Goddard G, Meyron-Holtz EG, D'Cruz DP, Shoenfeld Y. The Hyperferritinemic Syndrome: macrophage activation syndrome, Still's disease, septic shock and catastrophic antiphospholipid syndrome. BMC Med 2013;11:185.
[23] Gómez-Pastora J, Weigand M, Kim J, Wu X, Strayer J, Palmer AF, Zborowski M, Yazer M, Chalmers JJ. Hyperferritinemia in critically ill COVID-19 patients - is ferritin the product of inflammation or a pathogenic mediator? Clin Chim Acta 2020;509:249–51. https://doi.org/10.1016/j.cca.2020.06.033.
[24] Gómez-Mesa JE, Galindo-Coral S, Montes MC, Muñoz Martin AJ. Thrombosis and coagulopathy in COVID-19. Curr Probl Cardiol 2021;46(3):100742. https://doi.org/10.1016/j.cpcardiol.2020.100742.
[25] Levi M, Iba T. COVID-19 coagulopathy: is it disseminated intravascular coagulation? Internal Emerge Med 2021;16(2):309–12. https://doi.org/10.1007/s11739-020-02601-y.
[26] Ozen M, Yilmaz A, Cakmak V, Beyoglu R, Oskay A, Seyit M, Senol H. D-Dimer as a potential biomarker for disease severity in COVID-19. Am J Emerg Med 2021;40:55–9. https://doi.org/10.1016/j.ajem.2020.12.023.
[27] Yao Y, Cao J, Wang Q, Shi Q, Liu K, Luo Z, Chen X, Chen S, Yu K, Huang Z, Hu B. D-dimer as a biomarker for disease severity and mortality in COVID-19 patients: a case control study. J Intensive Care 2020;10(8):49. https://doi.org/10.1186/s40560-020-00466-z.
[28] Zhang L, Yan X, Fan Q, Liu H, Liu X, Liu Z, Zhang Z. D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. J Thromb Haemostasis 2020;18(6):1324–9. https://doi.org/10.1111/jth.14859.
[29] Jiang SQ, Huang QF, Xie WM, Lv C, Quan XQ. The association between severe COVID-19 and low platelet count: evidence from 31 observational studies involving 7613 participants. Br J Haematol 2020;190(1):e29–33. https://doi.org/10.1111/bjh.16817.