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A biological profile for diagnosis and outcome of COVID-19 patients

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Abstract

Objectives: As severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) pandemic is increasing its victims on a global scale with recurring outbreaks, it remains of outmost importance to rapidly identify people requiring an intensive care unit (ICU) hospitalization. The aim of this study was to identify Coronavirus Disease 2019 (COVID-19) biomarkers, to investigate their correlation with disease severity and to evaluate their usefulness for follow-up.

Methods: Fifty patients diagnosed with SARS-Cov-2 were included in March 2020. Clinical and biological data were collected at admission, during hospitalization and one month after discharge. Patients were divided into two severity groups: non-ICU (28) and ICU and/or death (22) to stratify the risk.

Results: Blood parameters in COVID-19 patients at admission showed increased C-reactive protein (CRP) (100%), ferritin (92%), lactate dehydrogenase (LDH) (80%), white blood cell (WBC) count (26%) with lymphopenia (52%) and eosinopenia (98%). There were significant differences in levels of CRP, ferritin, D-dimers, fibrinogen, lymphocyte count, neutrophil count and neutrophil-to-lymphocyte ratio (NLR) among the two severity groups. Mapping of biomarker's kinetics distinguished early and late parameters. CRP, ferritin, LDH, lymphopenia and eosinopenia were present upon admission with a peak at the first week. Late biomarkers such as anemia, neutrophilia and elevated liver biomarkers appeared after one week with a peak at three weeks of hospitalization.

Conclusions: We confirmed that high-values of CRP, NLR, D-dimers, ferritin as well as lymphopenia and eosinopenia were consistently found and are good markers for risk stratification. Kinetics of these biomarkers correlate well with COVID-19 severity. Close monitoring of early and late biomarkers is crucial in the management of critical patients to avoid preventable deaths.

Keywords: biomarkers; COVID-19; risk stratification.

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Introduction

The new severe acute respiratory syndrome coronavirus (SARS-CoV-2), discovered in December 2019 in China, is related to the virus strain that caused the pandemic of 2003 in southern China, SARS-CoV. The long incubation period and high contagiousness of the virus together with current international air travel are some of the major aspects that have facilitated the rapid spread of SARS-CoV-2. This has had an unprecedented magnitude of impact on health systems and global economics in our connected and globalized world [1].

At the time of preparing this article, all European countries had cases of contamination with a total of

206,823 deaths for 3,573,453 cases individuals detected [2]. Despite the public health measures taken by governments to control SARS-CoV-2 transmission in Europe, the virus continues to spread.

A big challenge is the lack of awareness of positive cases [3]. One of the reasons for this issue is the clinical spectrum, ranging from no symptoms to mild flu-like symptoms to severe acute respiratory illness or death [4]. The usual clinical signs of Coronavirus Disease 2019 (COVID-19) are fever, cough, shortness of breath, expectorations and anosmia. Gastrointestinal symptoms including diarrhea and nausea, have also been reported [4].

The pathophysiology of the virus is reminiscent of previous SARS-CoV epidemics. It is known that SARS-CoV-2 binds preferentially to the angiotensin metallopeptidase 2 converting enzyme (ACE2) via its surface protein S. ACE2 is present in alveolar epithelial cells but also in enterocytes of the small intestine, in the brush border of the proximal tubular cells of the kidney, in arterial smooth muscle cells of certain organs and in endothelial cells of veins and arteries [5, 6]. Since ACE2 exerts a protective role against vasoconstriction, pro-inflammatory and pro-fibrotic phenomena, the binding of the virus to its ACE2 target appears to result in decreased ACE2 activity. This decrease in activity has been reported to be a factor in the aggravation of COVID-19-induced inflammatory organ damage, particularly pulmonary [7].

The objectives of this study were to: 1) identify key biomarkers associated to the coronavirus SARS-CoV-2 that can be easily tested in the clinical laboratories; 2) explore the correlation between these biomarkers and disease severity and 3) select biomarkers useful for follow-up and risk stratification.

Materials and methods

Data collection and study population

In March 2020, we retrospectively recruited hospitalized patients (50) at the Cliniques Universitaires Saint-Luc (CUSL) diagnosed with "viral pneumonia" based on their clinical symptoms, in most cases accompanied with the typical changes in chest imaging.

The Ethics Board of CUSL approved the present study '2020/30AVR/255'.

In this study, all patients were confirmed as having SARS-CoV-2. After a positive diagnosis by RT-PCR (real-time reverse transcriptase polymerase chain reaction), patients were divided into two severity groups according to whether they were admitted to intensive care and/or died (28) or were treated in conventional care units (22). Only one of the deceased patients did not enter intensive care unit because he was not admissible at that time of the outbreak.

The body mass index (BMI) was mainly estimated based on patients' reports. Clinical data such as medical history, exposure history, co-morbidities, signs and symptoms, radiological characteristics (CT and X-ray results) and laboratory results were obtained from our own hospital's electronic medical record systems. Laboratory results were collected at admission, weekly for the first three weeks and one month after discharge. The date of onset of illness and date of admission were also recorded, as well as the duration of hospitalization. As the disease progressed, the clinical course of each patient's hospital stay was analyzed.

Finally, we performed a literature review, using two databases (PubMed and Cochrane Library) to assess laboratory features and outcomes of COVID-19.

Laboratory testing

Detection of the virus in nasal and pharyngeal swabs was carried out in our laboratory by the department of Molecular Microbiology using RT-PCR test during the first two weeks of illness. The extraction was done using a SPS solid phase extraction (Abbott, IL, USA).

Blood samples were collected in microtubes containing EDTA (ethylenediaminetetraacetic acid) for the complete blood count, lithium heparin for biochemistry tests and citrated samples for hemostasis testing. Biochemistry and complete blood count results were obtained by using the automated chemistry, Cobas® 8100 (Roche, Basel, Switzerland), and hematology, XN9000 (Sysmex, Kobe, Japan), chain. Hemostasis parameters were performed on ACL TOP 750 (Werfen, Barcelona, Spain). All analyses were carried out at the clinical laboratory of CUSL. The following parameters were assessed: absolute count and percentage of leukocytes, neutrophils, lymphocytes and eosinophils, the neutrophils to lymphocyte ratio (NLR), the concentration of C-reactive protein (CRP), lactate dehydrogenase (LDH), ferritin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin (BIL), serum creatinine (sCr), N-terminal pro Brain natriuretic peptide (NT-proBNP), high-sensitive cardiac troponin T (hs-TnT), fibrinogen, D-dimers, prothrombin time (PT) and activated partial thromboplastin time (aPTT). For each patient, lithium-heparin samples were processed for measurement of each parameter using the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) approved methods [8, 9].

In total, the results on admission as well as the weekly biology results were collected for each patient. In order to detect the presence of reactive lymphocytes, blood smears were reviewed with the Cellavision (Sysmex, Kobe, Japan) at patient entry. All slides were examined by the same operator in order to minimize the inter-operator bias.

Statistical analysis

All data were collected in Excel by Microsoft Office (Windows, Washington, USA) and MedCalc (MedCalc, New York, USA). For each parameter the median was calculated. Each median was compared with the reference values used in our laboratory. In-between group comparison was performed by Mann-Whitney U test for independent samples. Values of $p<0.05$ were considered statistically significant. All the graphs were made with Prism version 6 software (GraphPad, California, USA).

Results

Clinical characteristics of patients infected with SARS-CoV-2

During March 2020, 50 patients that had been admitted to the hospital in COVID units or the ICU were enrolled. The most frequent symptoms at admission were fever (68%), shortness of breath (58%), expectoration (12%), fatigue (8%), dry cough (58%) and myalgia (24%). The median age of our analyzed population was 59.5 years old (ranging from 18 to 98) and contained 58% of male patients (Table 1). The median weight of these patients was 80 kg with a minimum of 40 kg and a maximum of 140 kg with 50% of hospitalized patients suffering from overweight (BMI >25) and 20% from obesity (BMI >30). There was no difference in BMI between patients in the normal care unit and those in intensive care. In 88% of cases patients presented with comorbidities, the most significant being hypertension (44%), diabetes (14%) and obesity (20%) (Table 1). Patients with chronic diseases were more often in the ICU group than in the non-ICU group. The overall mortality rate was 6%.

Biochemical markers

At admission, the most prevalent results were increased levels of C-reactive protein (CRP) (100%; 17.6-fold), ferritin (92%; 3.2-fold) and lactate dehydrogenase (LDH) (80%; 1.1-fold) while albuminemia was decreased (50%, 0.9-fold) (Table 2). Blood creatinine, ALT, AST, ALP and total bilirubin were within the reference range (Table 3, Figure 1). NT-proBNP and hs-TnT values were higher in the ICU group than in the non-ICU group (Table 3).

The follow-up of these parameters on the long term revealed that the levels of CRP differed significantly ($p<0.05$) between ICU patients and non-ICU patients for at least two weeks after admission (Figure 1). We have even noticed a continued elevation in CRP values for the ICU patients during the first two weeks. Another remarkable difference between the two groups was the level of LDH in the ICU population, which is significantly higher ($p<0.05$) in in the first two weeks of hospitalization. Although AST, GGT and ALP increased progressively after the first week of hospitalization, reaching the highest level between the second and the third week, there was no significant difference between the two groups (Figures 1 and 2). Most of recent studies reported that the alteration of specific liver biomarkers, such as AST, ALT, bilirubin and albumin is a common laboratory finding in COVID-19 patients [10].

No other significant changes in biochemical markers were highlighted during the follow-up.

Blood cell count

The initial white blood cell count (WBC) showed leukocytosis in only 26% cases due to absolute neutrophilia while rare cases of transient leukopenia were also observed. Altogether, the median WBC value remained always within the reference range. Patients admitted in the ICU showed a significantly higher WBC ($p<0.05$) than non-ICU patients. One month after discharge, both groups converged to normal WBC.

The neutrophil count at admission was not elevated but we observed a boost of neutrophils in the ICU group during their stay.

At admission, 52% of all patients showed an absolute lymphopenia. The absolute lymphocyte count remained

Table 1: Characteristics of 50 patients with Coronavirus disease 2019.

Characteristics		All (n50)	Non-ICU (n28)	ICU and deaths (n22)
Age, years	Median	59.5	61	59.5
	≤18	1	0	0
	19–65	26	7	13
	>65	23	1	10
Sex, n (%)	Male	29 (58%)	14 (50%)	15 (68%)
	Female	21 (42%)	14 (50%)	7 (32%)
Coexisting conditions, n (%)	Any	6 (12%)	1 (4%)	5 (23%)
	Hypertension	22 (44%)	9 (32%)	13 (59%)
	Diabetes	7 (14%)	5 (19%)	2 (9%)
	Obesity (BMI>30)	10 (20%)	4 (14%)	6 (27%)
	Chronic cardiac disease	2 (4%)	1 (4%)	1 (5%)
	Chronic pulmonary disease	4 (8%)	1 (4%)	3 (17%)
	Immunodepression	8 (18%)	3 (11%)	5 (23%)

Table 2: Biological changes in coronavirus disease 2019 patients at the admission time.

Parameters	Limit value	Percentage of patients, %
↑ C-reactive protein, mg/L	>5	100
↑ Ferritin, µg/L	Women > 150 Men > 400	92
↑ Lactate dehydrogenase, U/L	>250	80
↓ Albuminemia, g/L	<35	50
↑ Aspartate aminotransferase, U/L	>36	52
↑ Alanine aminotransferase, U/L	>35	32
↑ Gamma-glutamyl-transferase, U/L	>40	46
↑ Alkaline phosphatase, U/L	>105	8
↑ Creatinine kinase, U/L	>200	34
↓ Hemoglobin, g/dL	Women < 12 Men < 13	34
↑ Neutrophil count, 109/L	>7.0	26
Relative neutrophilia, %	>70	56
↓ Lymphocyte count, 109/L	<0.8	52
Relative lymphopenia, %	<20	62
↓ Eosinophil count, 109/L	<0.03	98
Relative eosinopenia, %	<0.5	98
↓ Platelet count, 109/L	<150	34
↑ Fibrinogen, g/L	>400	83
↑ INR[a]	>1.20	26
↑ D-dimers, µg/L	>500	100

[a]INR, International normalized ratio.

slightly below normal values for the ICU which was not the case with the non-ICU group. On the other hand, the relative lymphopenia, was significantly ($p<0.05$) below normal values and stayed low throughout the hospital stay in ICU patients only. Reactive lymphocytes and natural killer (NK) cells were observed in peripheral blood smears in 40.9 and 60.4% of cases, respectively. Furthermore, in 28% of the cases, lymphocytes with a spliced nucleus and basophilic cytoplasm were observed. The NLR for non-ICU patients was only slightly elevated at admission but was two times higher in ICU patients. This ratio continued to gradually rise during the first two weeks of ICU hospitalization before it progressively normalized. The monocyte count remained within the normal range at admission and follow-up. Eosinopenia was the most frequent hematological finding (98% of all patients) on admission and correlated with the onset of lymphopenia. The initial low eosinophil count and its recovery after week 2 were observed in both ICU and non-ICU patients.

Anemia is a late hematologic marker only visible as early as the second and third week in ICU patients and non-ICU patients respectively (Table 3, Figure 2).

Thrombocytopenia (platelet count $<150 \times 10^9$/L) has only been documented in 26% of patients on admission and did not appear to be more prevalent in the more critical patients (ICU).

Hemostasis

Fibrinogen and D-dimers (when available) were respectively elevated in 83.72% (1.3 times) and 100% (2.7 times) of the COVID-19 patients at admission. Due to a lack of follow-up data for fibrinogen, a long-term statistical comparison could not be made. The level of D-dimers followed a comparable trend, with a median of 819 µg/L in the non-ICU and 894 µg/L in the ICU group at admission. In the ICU patients, a rapid rise in D-dimer level was seen after admission right until the second week of hospitalization, where it was 25 times higher than in the non-ICU population. The aPTT and PT/INR were both slightly elevated, yet without a difference between the subpopulations.

Discussion

The current COVID-19 pandemic is challenging the healthcare capacities and system resilience but also attracting attention on the importance of identifying patients with an increased risk of severe disease. Our study showed biological features related to severity of COVID-19.

Early biomarkers of COVID-19

We first observed marked changes in the biochemistry profile. The activation of the innate and adaptive immune response, results from the release of chemokines and other cytokines inducing the inflammatory response. This includes the increase of acute-phase proteins [11]: CRP, LDH, ferritin and fibrinogen were significantly elevated at admission.

Specific liver enzymes, such as ALT, AST, GGT and ALP showed no significant changes at admission for both groups, while LDH was significantly increased in most patients. Most commonly a hallmark of liver disease, LDH is also a marker of lung injury. Comparable result were published by Chen et al. [12].

We have seen significant changes in the blood cell count. Most patients suffering from SARS-CoV-2 show a deregulation of their immune system. The production of cytotoxic T-lymphocytes (CTL) represents a major response of our immune system during a viral infection [13]. However, like its predecessors, SARS-CoV-2 infection is

Table 3: Comparison of main biomarkers in coronavirus disease 2019 patients.

Parameters	All			Non-ICU patients					ICU patients				Reference range
Chemistry	Median	n-fold URL	Admission	Week 1	Week 2	Week 3	Discharge	Admission	Week 1	Week 2	Week 3	Discharge	
C-reactive protein, mg/L	84.2	16.2	80.7	129	56.5	32.0	2.15	123	219	248	81.2	2.3	<5
Lactate dehydrogenase, U/L	371	1.3	353	376	311	299	228	452	494	397	396	222	–
Procalcitonin, ng/mL	1.34	2.68	0.12	–	–	–	–	1.84	–	–	–	–	–
High-sensitive troponin T, µg/L	18.6	1.9	16	18.5	27	15	–	19	28	32	–	–	–
N-terminal prohormone of brain natriuretic peptide, ng/L	384.6	1.3	126.9	–	–	–	–	504.2	–	–	–	–	–
Creatine kinase, U/L	108	0.6	132	120	50.3	26.3	110	153	171	126	62.3	59.3	20–200
Aspartate aminotransferase, U/L	37.7	1.1	34.7	40.1	37.7	20.4	21.0	45.5	41.9	50.9	37.7	24.0	9–36
Alanine aminotransferase, U/L	35.3	1.0	28.1	31.7	40.7	44.9	18.6	36.5	37.7	34.1	40.7	25.7	7–35
Alkaline phosphatase, U/L	77.8	0.7	59.3	62.9	98.8	102	62.9	55.7	60.5	89.2	121	64.1	35–105
Gamma-glutamyl-transferase, U/L	65.9	1.6	49.1	56.9	100	101	34.7	43.7	65.3	107	144	28.7	< 40
Blood creatinine, µmol/L	86.6	0.8	98.6	76.0	69.8	75.6	86.9	97.2	0.82	72.5	69.8	73.4	64–106
Hematology													
Hemoglobin, g/dL	12.0	1.1	12.95	12.1	12.1	10.4	12.55	13.5	11.95	9.55	9.1	12.3	12.0–16.0
Leukocytes, $\times 10^9$/L	6.07	0.6	5.50	5.36	5.57	5.08	6.25	6.54	8.3	8.8	8.76	6.31	4.0–10.0
Neutrophils, $\times 10^9$/L	4.53	0.6	3.76	3.95	4.18	3.01	3.16	5.0	6.60	7.03	7.35	3.31	1.6–7.0
Neutrophils, %	76.0	1.1	68.0	68.2	67.1	60.65	53.85	79.8	82.6	73.6	70.6	53.9	40–70
Lymphocytes, $\times 10^9$/L	0.89	0.2	1.13	1.01	1.17	0.96	2.12	0.9	0.67	0.84	1.01	2.19	0.8–5.0
Lymphocytes, %	16.1	0.3	21.3	23.25	20.0	20.3	33.85	12.75	10.3	10.9	9.35	30.8	20–50
Neutrophil to lymphocyte ratio	5.1	–	3.48	3.68	3.47	3.04	1.59	6.19	8.38	8.45	6.24	1.75	0.78–3.53
Eosinophils, $\times 10^9$/L	0	–	0	0.02	0.09	0.12	0.18	0	0.01	0.09	0.17	0.23	0.03–0.6
Eosinophils, %	0	–	0	0.2	1.6	2.5	2.9	0	0.1	1.0	2.5	2.6	0.5–6
Platelets, $\times 10^9$/L	197	0.4	167	237	326	281	220.5	203	236.5	275.5	349	290	150–450
Hemostasis													
Fibrinogen, g/L	584	1.5	522	485	584	480	–	601	696	685	675	366	150–400
D-dimers, nmol/L	1080	2.2	819	–	–	–	–	894	–	–	–	–	< 500

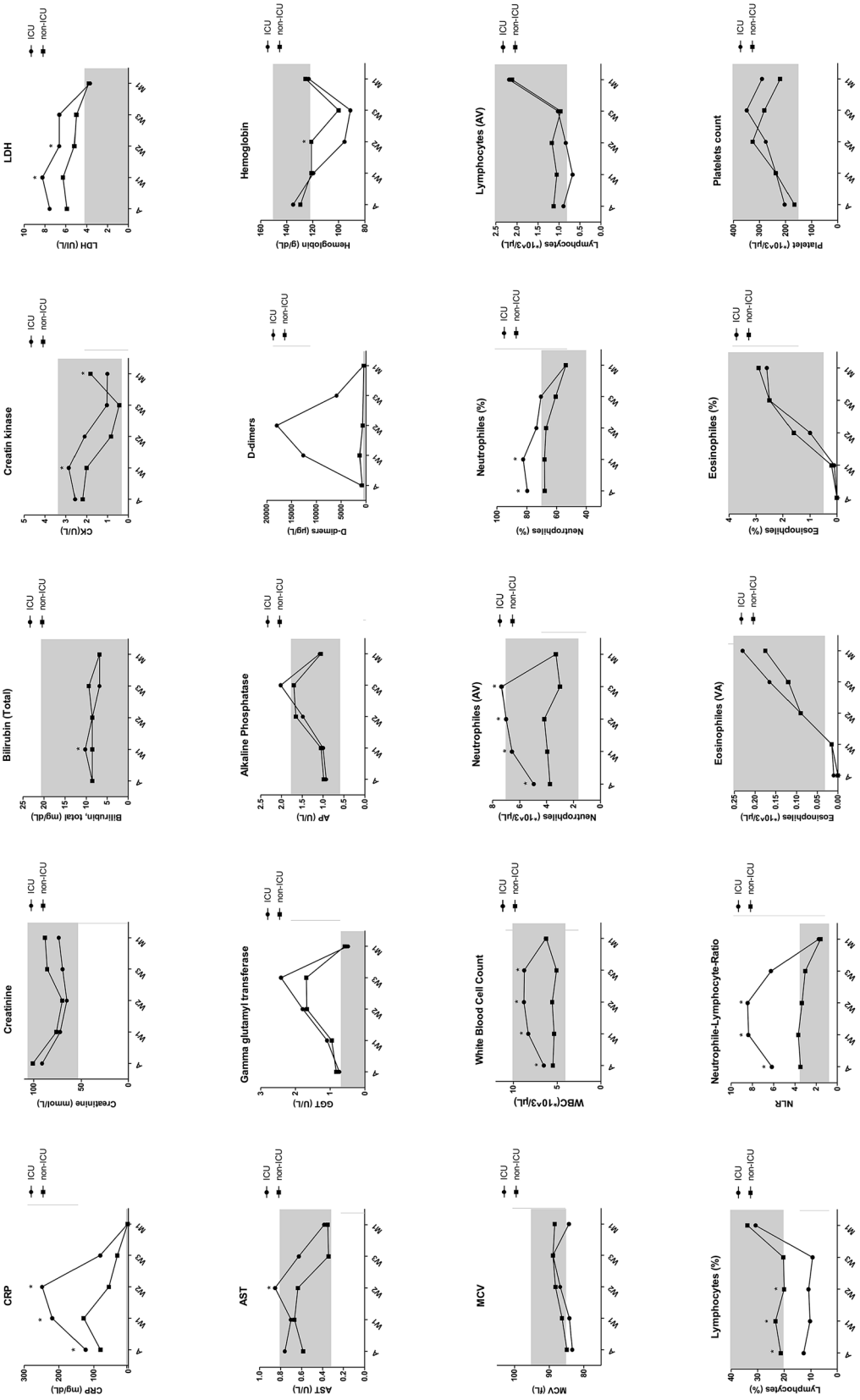

Figure 1: Biological changes in COVID-19.

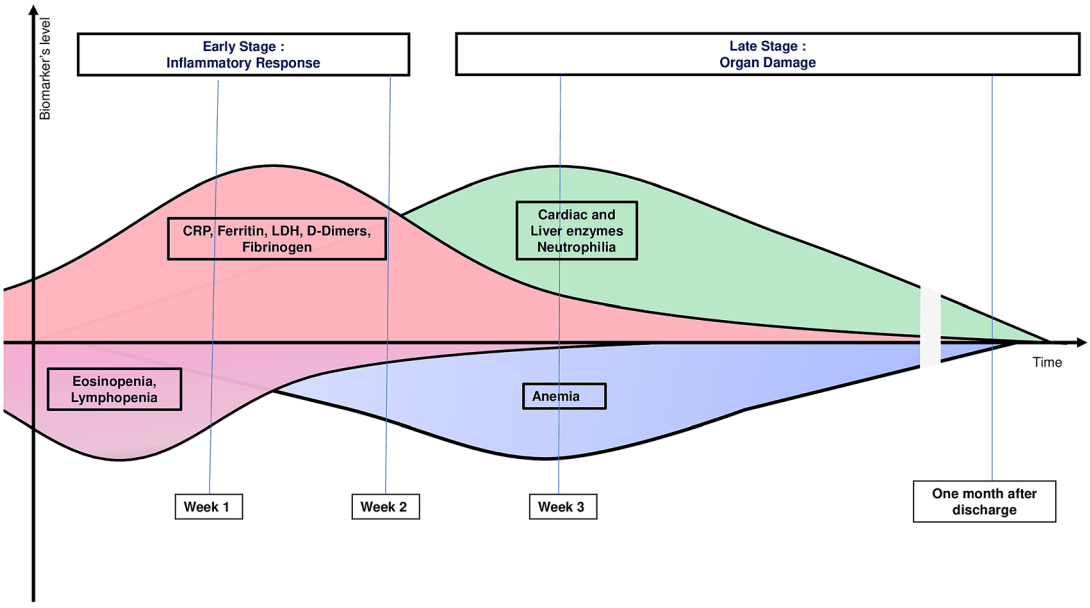

Figure 2: Kinetics of COVID-19 biomarkers.

associated with a rapid decrease in lymphocyte count more pronounced in the ICU group [14–17]. This lymphopenia is found in 52% of the cases at admission. The responsible mechanisms are still unknown, but the main hypotheses are either chemokine-mediated lymphocyte redistribution and sequestration in the lungs or bone marrow suppression via CD13 or CD66 [14–17].

Lymphopenia, appearing generally 8 days after the onset of symptoms, was frequent and more pronounced within the first week of hospitalization and continued right until the third week of hospitalization [18]. In order to determine its prognostic usefulness, we compared the lymphocyte values found in different patients' groups. According to our data, total lymphocyte count was significantly lower in severe cases during the first three weeks of hospitalization (Figure 1, Table 3). Both these findings suggest that lymphopenia is an early marker for COVID-19 diagnosis and a significant prognostic indicator.

The presence of reactive lymphocytes and NK cells in 40.9 and 61.4% patients respectively are evidence of the immune response. NK cells can directly destroy infected cells by producing perforins or, indirectly, by the production of IFN-γ (interferon-gamma) via JAK1/2 signaling cascade [19]. Moreover, we also noted the presence of cleaved nucleus lymphocytes in 28% the patients. These atypical lymphocytes do not appear to be related to the presence of reactive lymphocytes neither to the severity of the disease.

Eosinopenia is the most common hematological abnormality found in our study at admission. It appears transient and eosinophil count normalizes during the second week of hospitalization (Figures 1 and 2). It was previously described in Jin-jin Zhang's study and it is known to be associated with the acute inflammatory response [20, 21]. Eosinopenia plays a physiological role in the stress response and is dependent on the host, acute inflammatory and systemic response. It is therefore found in several clinical conditions including sepsis, viral infections, therapy with corticosteroids and catecholamines, physiological stress, psychiatric condition and in some allergies [22, 23]. It has been shown that the fall of circulating eosinophils occurs rapidly after the induction of an inflammatory process suggesting peripheral sequestration, mostly in the spleen and at the site of infection [20]. Eosinopenia is clearly an early marker of SARS-CoV2 infection. After the first week of hospitalization, the eosinophil count begins to recover.

Late biomarkers of COVID-19

After two to three weeks of hospitalization, AST, GGT and ALP began to rise and were often higher in the ICU group than in the non-ICU group. Indeed, specific liver enzymes appear to be a late biomarker of organ damage as multiples studies showed [10, 18, 24]. In this study AST rose

until the second week of hospitalization. Afterward, it began to decrease, while for GGT and ALP, a decrease was only observed after the third week of hospitalization. In contrast, other studies reported that patients who had abnormal liver test results had higher risk of poor outcome [25].

Another striking element was a tendency to neutrophilia pronounced in severe forms (ICU). The neutrophilia the first week of hospitalizations was only visible in the relative neutrophil count (%) and was later followed by an absolute increase at the second and third week of hospitalization. While the lymphopenia and eosinopenia are early markers, neutrophilia is a late onset maker of COVID-19. A recent study demonstrated that this increase in neutrophils in COVID-19 was not related to a secondary infection [26].

Moderate microcytic anemia due to the inflammatory state was found in 42% of admitted patients. It is no longer necessary to demonstrate the influence of inflammation and its cytokines on the development of anemia [27]. More severe anemia, without a transfusion need, was developed in both populations at the third week of infection.

Severity biomarkers

CRP was consistently increased during hospitalization, with higher values measured in the ICU patients. This observation confirms its usefulness as an early inflammatory biomarker. Indeed, Tan et al. and other studies concluded that CRP was associated with disease progression and predicted early severe COVID-19 [10, 11].

Ferritin was also higher in the severe group in comparison to other groups (Table 3, Figure 1). Evidence is increasing in the literature for ferritin as an early marker of severity for COVID-19 patients, which confirms our results.

Previous studies have found that NT-proBNP and hs-TnT are powerful and independent predictor of mortality in community-acquired pneumonia (CAP) [28–30]. Studies suggested that the prognostic effect of cardiac biomarkers in severe COVID-19 patients could be related to direct and indirect effects on the heart. SARS-CoV-2 binds with ACE2, resulting in high levels of angiotensin 2 and restricted synthesis of angiotensin 1-7 [28, 31]. The latter exerts anti-inflammatory effect to protect tissue while angiotensin 2 plays an opposite role and facilitate the secretion of hs-TnT and NT-proBNP [28, 31, 32]. Thus, cardiac biomarker levels are associated with the severity of infection [28].

We observed a severe lymphopenia in the ICU group. When an absolute lymphopenia was not present at the onset of disease, a relative one could be observed.

Neutrophils are known to be contributors to lung inflammation and are also identified as a key element in the outcome of viral infections. However, despite their beneficial effect on the initiation of the adaptive response, a pathogenic role for neutrophils in the development of ARDS (acute respiratory distress syndrome) has been proven [33, 34]. The strongest hypothesis is the state of "hyper-cytokinemia" or cytokine storm, demonstrated in COVID-19 patients [17].

The Neutrophil to Lymphocyte Ratio (NLR), is a simple parameter reflecting the inflammatory status of patients. It has already contributed to better stratify risk in inflammatory and infectious diseases [35]. The recent literature has established a cut-off for population in good health being 0.78 to 3.53 [35]. While NLR remains borderline throughout the hospitalization of non-ICU patients, severe patients show a high NLR on admission (Table 3). With a significant threshold observed at week 2, the NLR falls back and is no longer statistically significant by week 3 between the two groups. This observation highlights the fact that this parameter can be as useful as CRP to predict poor outcome at admission.

Eosinopenia correlates with the presence of absolute lymphopenia and appears to correlate with severity [23]. However, our study only included patients who were hospitalized and therefore were pre-selected leading to a falsely elevated rate of eosinopenic patients in comparison to non-hospitalized patients.

Lastly, changes in coagulation parameters were observed. Following Tang's publication suggesting a vascular component to SARS-COV-2 patients, we examined hemostasis parameters. Classical markers of DIC are platelet count, fibrinogen concentration, D-dimer concentration, prothrombin time and activated partial thromboplastin time [36].

It is known that infections can cause the activation and acceleration of the coagulation mechanisms in the human body. This leads to benefits because the coagulation system also has an immune function in cases of severe infection. This explains why in COVID-19 patients parameters such as fibrinogen and D-dimers are elevated [37]. This mechanism could explain normal levels or slightly increased levels even in the presence of peripheral consumption. The D-dimer levels are always increased showing peripheral coagulation activation and fibrinolysis. Both parameters showed a correlation with the severity of the disease and its prognosis, e. g. D-dimers can

be increased 25-fold in severe patients. Several studies also warn for the development of DIC, which on its own has a high mortality rate [37].

Limitations

The present study is limited by the number of hospitalized patients included. Another limitation is the lack of information for some biomarkers such as cardiac biomarkers which were not available for every patient at the admission and/or during the follow-up.

Conclusions

In this retrospective COVID-19 study of Belgian patients, followed from admission to three weeks hospitalization, we report that a panel of easily available biomarkers may help in diagnosis and risk stratification. This panel consists of early biomarkers such as CRP, ferritin, fibrinogen, lymphopenia and eosinopenia. These biomarkers were consistently found in all patients and some can also be used for the risk stratification (CRP, ferritin, lymphocyte count, eosinophilia count, NLR). Other biomarkers that can be used are NT-proBNP, hs-TnT, neutrophilia and D-dimers. NLR also correlates well with severity during the whole hospital stay. Late markers for COVID-19 are increased liver and cardiac enzymes and a decrease in hemoglobin.

These biomarkers may enable the clinician to classify the patients in the first weeks of illness.

Hypertension, diabetes, respiratory diseases, and cardiovascular disease were here identified as potentially important risk factors that should be included in future recommendations for the measurement of specific antibodies and vaccination programs. The contribution of blood tests remains underestimated despite high accessibility, fast results and low cost.

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