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Biomarkers associated with COVID-19 disease progression

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ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic is a scientific, medical, and social challenge. The complexity of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is centered on the unpredictable clinical course of the disease that can rapidly develop, causing severe and deadly complications. The identification of effective laboratory biomarkers able to classify patients based on their risk is imperative in being able to guarantee prompt treatment. The analysis of recently published studies highlights the role of systemic vasculitis and cytokine mediated coagulation disorders as the principal actors of multi organ failure in patients with severe COVID-19 complications. The following biomarkers have been identified: hematological (lymphocyte count, neutrophil count, neutrophil-lymphocyte ratio (NLR)), inflammatory (C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin (PCT)), immunological (interleukin (IL)-6 and biochemical (D-dimer, troponin, creatine kinase (CK), aspartate aminotransferase (AST)), especially those related to coagulation cascades in disseminated intravascular coagulation (DIC) and acute respiratory distress syndrome (ARDS). New laboratory biomarkers could be identified through the accurate analysis of multicentric case series; in particular, homocysteine and angiotensin II could play a significant role.

Abbreviations: ACE: angiotensin-converting enzyme; ALT: alanine aminotransferase; Ang: angiotensin; aPTT: activated partial thromboplastin time; ARDS: acute respiratory distress syndrome; AST: aspartate aminotransferase; AT2R: AT2 receptor; BK: bradykinin; CI: confidence interval; CK: creatine kinase; CKD: chronic kidney disease; COVID-19: coronavirus disease 2019; CRP: C-reactive protein; CT: computer tomography; CTL: cytotoxic T lymphocyte; DIC: disseminated intravascular coagulation; ESR: erythrocyte sedimentation rate; FDP: fibrin degradation product; G-CSF: granulocyte-colony stimulating factor; Hcy: homocysteine; HPLC: high-performance liquid chromatography; HR: hazard risk; ICU: intensive care unit; IL: interleukin; INF: interferon; IP: interferon- γ inducible protein; LDH: lactate dehydrogenase; MasR: Mas receptor; MCP: monocyte chemoattractant protein; MIP: macrophage inflammatory protein; MOF: multiple organ failure; NCP: novel coronavirus pneumonia; NK: natural killer; NLR: neutrophil-lymphocyte ratio; NO: nitric oxide; OR: odds ratio; ORF: open reading frame; PCT: procalcitonin; PLR: platelet-to-lymphocyte ratio; PT: prothrombin time; RAS: renin-angiotensin system; ROCK: RhoA/Rho kinase; S: spike; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; TNF: tumor necrosis factor; WBC: white blood cell

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1. Introduction

The scientific community is in urgent need for reliable biomarkers related to coronavirus disease 2019 (COVID-19) disease progression, in order to stratify high risk patients. The rapid disease spread necessitates the immediate categorization of patients into risk groups following diagnosis, to ensure optimal resource allocation. Novel biomarkers are needed to identify patients who will suffer rapid disease progression to severe complications and death. The identification of novel

biomarkers is strictly related to the understanding of viral pathogenetic mechanisms, as well as cellular and organ damage. Effective biomarkers would be helpful for screening, clinical management, and prevention of serious complications.

Preliminary studies describe vasculitic processes underlying organ damage in seriously ill patients, induced by the activation of inflammatory cascades, complement activation and pro-inflammatory cytokines (i.e. interleukin (IL)-6) [1,2]. Vasculitic damage causes

edema and acute respiratory distress syndrome (ARDS) in the lung, and plays a significant role in cardiovascular damage (ischemia, deep venous thrombosis, pulmonary thromboembolism) and cerebral injuries (embolism); its severity is unfortunately not easily predictable through currently used laboratory biomarkers such as D-dimer or prothrombin time/activated partial thromboplastin time (PT/aPTT) [3,4]. Epidemiological observations have associated a critical role of cardiovascular damage in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) patients, with ischemic heart disease and hypertension among the most frequent preexisting comorbidities associated with SARS-CoV-2 mortality [5,6]. Current clinical practice suggests determining IL-6, D-dimer, lactate dehydrogenase (LDH), and transaminases in addition to routine laboratory tests, in order to identify patients at risk of fatal complications and those who will potentially benefit from anti-IL6 immunotherapies with tocilizumab [7]. However, as costly cytokine analysis is not routinely performed in most laboratories, surrogate markers of infection (ferritin, C-reactive protein (CRP)) correlated to IL-6 will be of increasing interest for prognostic value. Beyond D-dimer, prothrombin time (PT) and fibrin degradation product (FDP) [1], there are no specific predictive parameters of severe ischemic and thrombo-embolic disease. For this reason, it is not easy to cluster patients in risk categories for an appropriate early anticoagulant or fibrinolytic therapy.

According to the most recently published Diagnosis and Treatment Program of 2019 New Coronavirus Pneumonia (trial version seven) [8], COVID-19 patients are divided into mild, moderate, severe, and critical classifications. Some hematological parameters, including white blood cell (WBC), lymphopenia, CRP, and some biochemical parameters, such as LDH, creatine kinase (CK), and troponin were reported to be associated with COVID-19 severity [9,10].

Concerning new predictive parameters of specific cardiovascular risk, very recent data report that homocysteine (Hcy) (together with age, monocyte-lymphocyte ratio (MLR), and period from disease onset to hospital admission) may be a specific cardiovascular risk predictive parameter for severe pneumonia observed at chest computed tomography (CT) during the first week of COVID-19 infection; however, these observations did not report any additional organ involvement [11]. The aim of this review is to report the current state of knowledge regarding known biomarkers for COVID-19 infection, focusing on those potentially predictive of organ damage in patients with severe complications and death.

2. The mechanisms of action of COVID-19

The knowledge of molecular mechanisms related to virus damage on human cells is necessary to define efficacious pharmacologic strategies and to identify novel biomarkers predictive of severe cardiovascular damage or fatality.

The main mechanism for SARS-CoV-2 infection is the binding of the virus to membrane-bound form of angiotensin-converting enzyme 2 (ACE2) and the internalization of complex by the host cell.

ACE2, a glycoprotein and metalloprotease, exists in both membrane-bound and soluble forms [12]. The membrane-bound form contains a transmembrane domain which anchors its extracellular domain to the plasma membrane, whereas in its soluble form, it is cleaved and secreted, as the N-terminal ectodomain is barely measurable in circulation.

The significance of circulating ACE2 is unclear, although levels may be increased in chronic diseases such as diabetes, chronic kidney disease (CKD), and hypertension [13,14]. ACE2 has kinins, apelin, neurotensin, dynorphin, ghrelin, amyloid, and angiotensin as substrates. The main function of ACE2 is to physiologically counterbalance ACE and regulate angiotensin II (Ang II) by converting Ang I into Ang-(1-9), and by converting Ang II into Ang-(1-7), which is tissue-protective [15].

Recently, it has been demonstrated that the receptor-binding domain in the novel coronavirus spike (S) protein binds strongly to ACE2 receptors [16]. SARS-CoV-2 uses ACE2 and the serine protease TMPRSS2 for S protein priming. ACE2 and TMPRSS2 are not only expressed in lung, but also in the small intestinal epithelia, in the upper esophagus, liver, colon [17], in organs involved in blood pressure regulation (blood vessels, heart, kidneys) as well as in the ovaries and testes [15]. This wide distribution of the COVID-19 receptor could provoke systemic failure due to direct organ injury [18].

An additional SARS-CoV-2 mechanism of action was suggested by Wenzhong and Hualan who demonstrated that the open reading frame (ORF8) and surface glycoprotein may both bind to the porphyrin. At the same time, orf1ab, ORF10, and ORF3a proteins are presumed to coordinate an attack on the heme of the 1-beta chain of hemoglobin to dissociate the iron, forming the porphyrin and reducing the capacity of hemoglobin to carry oxygen and carbon dioxide [19] (not peer-reviewed). This mechanism of the virus inhibits the normal metabolic pathway of heme and provokes disease symptomatology.

Greater consensus exists on the pathogenetic mechanisms triggered by COVID-19 after entering the human body: inflammatory cascades, cytokine storms, and the activation of coagulation cascades. These are common in systemic vasculitis (pulmonary, renal, and cerebral), and lead to severe and even fatal complications, such as sepsis, disseminated intravascular coagulation (DIC), and acute cardiovascular events.

DIC has been identified in the majority of SARS-CoV-2 infected deceased patients. Patients with viral infection may develop sepsis associated with organ dysfunction. DIC, most commonly caused by sepsis, develops when monocytes and endothelial cells are activated, and following injury cytokines are released, with the expression of tissue factor and the secretion of von Willebrand factor. Free thrombin, uncontrolled by natural anticoagulants, can activate platelets and stimulate fibrinolysis. At late stages of novel coronavirus pneumonia (NCP), fibrin-related markers (D-dimer and FDP) were reported to be moderately or markedly elevated in all SARS-CoV-2 deaths, suggesting a common coagulation activation and secondary hyperfibrinolysis in these patients [1].

3. Markers of COVID-19 infection and severe progression

A pattern of hematologic, biochemical, inflammatory, and immune biomarker abnormalities has been identified in patients with severe disease compared to mild systemic disease, and warrant inclusion in risk stratification models (Table 1). Additionally, authors report the observation of significantly increased Hcy in patients with severe COVID-19 disease.

3.1. Hematologic biomarkers

Hematologic biomarkers used to stratify COVID-19 patients include WBC count, lymphocyte count,

neutrophil count, neutrophil–lymphocyte ratio (NLR), platelet count, eosinophil count, and hemoglobin.

Yang et al. [20] reported lymphopenia in 80% of critically ill adult COVID-19 patients, whereas Chen et al. [4] reported a rate of only 25% of patients with mild COVID-19 infection. These observations suggest that lymphopenia may correlate with infection severity. Qin et al. analyzed markers related to dysregulation of immune response in a cohort of 450 COVID-19 positive patients, reporting that severe cases tended to have lower lymphocyte-, higher leukocyte-counts and higher NLR, as well as lower percentages of monocytes, eosinophils, and basophils compared to mild cases [21]. Similarly, Henry et al. also concluded in a meta-analysis on 21 studies including 3377 COVID-19 positive patients that patients with severe and fatal disease had significantly increased WBC, and decreased lymphocyte and platelet counts compared to non-severe disease and survivors [22].

In COVID-19 patients, both helper T cells and suppressor T cells were below normal levels, with the lowest helper T cells levels associated with severe cases. Further, in severe cases, the percentage of naïve helper T cells were reportedly increased, and memory helper T cells were reportedly decreased. Patients with COVID-19 also have lower level of regulatory T cells, which are more obviously damaged in severe cases [21,23]. Cytotoxic lymphocytes, such as cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, are necessary for the control of viral infection, and the functional exhaustion of cytotoxic lymphocytes is correlated with disease progression [24]. On confirmed COVID-19 cases, laboratory testing showed that mean lymphocyte counts were below normal [3,25–27]. Changes in lymphocyte populations in patients severely affected by COVID-19 indicate a low T cells count, an increase in naïve helper T cells and a decrease in memory helper T cells [23]. The total number of NK, T cells, and B cells was decreased markedly in patients with SARS-CoV-2 infection

Table 1. Biomarker abnormalities in COVID-19 patients with severe systemic disease and potential new biomarkers.

Hematologic biomarkers		Biochemical biomarkers		Coagulation biomarkers	Inflammatory biomarkers	Potential new biomarkers	
↑	↓	↑	↓	↑	↑	↑	↓
WBC count	Lymphocyte count	ALT	Albumin	PT	ESR	Hcy	Ang-(1-7)
Neutrophil count	Platelet count	AST		D-dimer	CRP	Ang II	Ang-(1-9)
	Eosinophil count	Total bilirubin			Serum ferritin	NLR	Alamandine
	T cell count	Blood urea nitrogen			PCT	MLR	
	B cell count	CK			IL-2		
	NK cell count	LDH			IL-6		
		Myoglobin			IL-8		
		CK-MB			IL-10		
		Cardiac troponin I					
		Creatinine					

WBC: white blood cell; NK: natural killer; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CK: creatine kinase; LDH: lactate dehydrogenase; PT: prothrombin time; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; PCT: procalcitonin; IL: interleukin; Hcy: homocysteine; Ang: angiotensin; NLR: neutrophil–lymphocyte ratio; MLR: monocyte–lymphocyte ratio.

[21,28,29]. Lower CD8⁺ T cells count tended to be an independent predictor for COVID-19 severity and treatment efficacy [29]. Xu et al. reported that a decrease of specific T lymphocyte subsets is related to in-hospital death and severe illness. Lower counts of T lymphocyte subsets; lymphocyte (<500/ μ L), CD3 + T-cell (<200/ μ L), CD4⁺ T-cell (<100/ μ L), CD8⁺ T-cell (<100/ μ L), and B-cell (<50/ μ L) were associated with higher risks of in-hospital death of COVID-19. The warning values to predict in-hospital death of lymphocyte, CD3⁺ T-cell, CD4⁺ T-cell, CD8⁺ T-cell, and B-cell were 559/ μ L, 235/ μ L, 104/ μ L, 85/ μ L and 82/ μ L, respectively [30].

In a study of 32 COVID-19 patients, decreased eosinophil count was registered in 66% [31]. Eosinophil counts have been positively correlated to lymphocyte count ($r = 0.305$, $p < .001$) [32]. In another study of 140 COVID-19 patients, eosinopenia was reported in 52.9% ($< 0.02 \times 10^9/L$), and the eosinophil count was positively associated with lymphocyte count in mild ($r = 0.449$, $p < .001$) and severe ($r = 0.486$, $p < .001$) cases of COVID-19 [33]. Du et al. reported very low eosinophil counts in 81.2% patients at admission, which may indicate poor prognosis [9]. Liu et al. also reported low eosinophil values on initial hospitalization, which reportedly returned to normal before discharge, concluding that increasing eosinophils may be an indicator of clinical COVID-19 improvement [34]. However, results from a systematic literature review concluded that “eosinopenia may not be associated with unfavorable progression of COVID-19” [35]. Therefore, the diagnostic value of eosinopenia in COVID-19 requires further investigation with larger patient cohorts to establish the sensitivity and specificity of the eosinophil count.

The NLR, calculated simply by the ratio of neutrophils count/lymphocytes count, is an inflammatory marker that can predict the probability of death in patients with various cardiovascular diseases [36,37]. Moreover, NLR has been identified in a meta-analysis as a prognostic biomarker for patients with sepsis [38]. For COVID-19 patients, NLR has been shown to be an independent risk factor for severe disease [39–41]. Fifty (75.8%) patients with disease progression during hospitalization had a NLR ≥ 2.973 [42], which may indicate COVID-19 infection severity [43]. Binary logistic analysis identified elevated NLR (hazard risk (HR): 2.46, 95% confidence interval (CI): 1.98–4.57) as an independent factor for poor COVID-19 clinical outcome [44], which was confirmed by a meta-analysis which reported that NLR values were significantly increased in severe COVID-19 patients [45]. NLR elevation may be due to dysregulated expression of inflammatory cytokines, aberrant increase of pathological low-density neutrophil and the

upregulation of genes involved in lymphocyte cell death pathway, caused by the mechanism of SARS-CoV-2 infection [46].

Lymphopenia, excessive activation of the inflammatory cascade, and cardiac involvement are all crucial features of COVID-19 disease and have high prognostic value. However, the understanding of the underlying mechanisms is still limited [47]. Based on the observations derived from clinical practice, it has also been postulated that coronaviruses may directly infect bone marrow precursors, resulting in abnormal hematopoiesis, or trigger an auto-immune response against blood cells [48,49].

As platelet count is a simple, cheap, and easily available biomarker and has been independently associated with disease severity and mortality risk in intensive care unit (ICU) [50–52], it has been rapidly adopted as a potential biomarker for COVID-19 patients. The number of platelets was reported to be significantly reduced in COVID-19 patients [11,53] and was lower in non-survivor patients compared to survivors [54]. Low platelet count has been associated with increased risk of severe disease and mortality for COVID-19 patients, and can serve as an indicator of clinical disease worsening during hospitalization [55]. Another research group found that patients with severe pneumonia induced by SARS-CoV2 had higher platelet count than those induced by non-SARS-CoV2 [56]. The patients with significantly elevated platelets and higher platelet-to-lymphocyte ratio (PLR) during treatment had longer average hospitalization days [57]. Damaged lung tissue and pulmonary endothelial cells may activate platelets in the lungs, resulting in the aggregation and formation of microthrombi, thereby increasing platelet consumption [58].

In severe disease, WBCs show lymphocytopenia, affecting both CD4⁺ and CD8⁺ cells, as well as a decrease in monocytes and eosinophils, and a clear increase in neutrophils and NLR. These simple parameters can be used for early diagnosis and identification of critically ill patients [59,60].

3.2. Biochemical biomarkers

The main laboratory changes in severe or fatal COVID-19 patients were recently explored in a meta-analysis, including three large studies comparing survivors to non-survivors. A significant increase in total bilirubin and CK, together with serum ferritin, WBC count, and IL-6 was registered in non-survivors compared to survivors [20,26,61]. Further, given the strong association between thrombo-embolism and COVID 19 and to a

lesser extent, myocardial injury, D-dimer, and cardiac markers are crucial in COVID-19 patient monitoring.

Markers of muscular and in particular cardiac injury were elevated in patients with both severe and fatal COVID-19. At presentation, non-survivors had significantly higher cardiac troponin levels (weighted mean difference (WMD): 32.7 ng/L), which is probably due to both viral myocarditis and cardiac injury from disease progression to multiple organ failure (MOF). In MOF, significant elevation in liver enzymes (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) is associated with critical changes in renal function parameters (blood urea nitrogen, creatinine) and coagulation markers [60].

Chen et al. observed in a cohort of 799 patients (113 non-survivors and 161 recovered) markedly higher concentrations of ALT, AST, creatinine, CK, LDH, cardiac troponin I, N-terminal pro-brain natriuretic peptide, and D-dimer in non-survivors compared to recovered patients [62]. Du et al., in a prospective study of 179 patients with COVID-19 pneumonia (including 21 non-survivors), identified cardiac troponin $I \geq 0.05$ ng/mL as among the four risk factors predictive of mortality (age ≥ 65 years, preexisting concurrent cardiovascular or cerebrovascular diseases, $CD3 + CD8 + T$ cells (≤ 75 cell/ μ L) [9].

Liver function has also been identified as an important predictor for COVID-19 patient mortality. A recent study suggested that SARS-CoV-2 may directly bind to ACE2-positive cholangiocytes, and therefore, liver abnormalities in COVID-19 patients may be due to cholangiocyte dysfunction and other causes, such as drug-induced and systemic inflammatory response-induced liver injuries [63]. Regarding the specific and dynamic pattern of liver injury parameters, Lei et al., in a wide retrospective multicenter study involving a COVID-19 cohort-derived data set of 5771 patients, reported that AST is strongly associated with mortality risk compared to other parameters, reflecting liver injury [64]. This evidence is in contrast with the evidence of ALT elevation in other hepatitis-induced liver injury.

3.3. Inflammatory biomarkers

The increase in inflammation markers is the critical point underlying the systemic vasculitic processes and the defects in the coagulation processes that cause most parenchymal lesions in vital organs. The main inflammatory and immune biomarkers correlated with COVID-19 disease are summarized in Table 1.

The CRP marker was found to be significantly increased in the initial phases of the infection for severe

COVID-19 patients, also prior to indications of critical findings with CT. Importantly, CRP has been associated with disease development and is an early predictor for severe COVID-19 [65]. The authors also reported by correlation analysis that CRP ($R = 0.62$, $p < .01$), erythrocyte sedimentation rate (ESR) ($R = 0.55$, $p < .01$) and granulocyte/lymphocyte ratio ($R = 0.49$, $p < .01$) were positively associated with CT severity scores.

The immunological biomarkers of IL-6 and serum ferritin are reported to be significantly increased in non-survivors vs. survivors (WMD: 4.6 pg/mL and 760.2 ng/mL, respectively) and as compared to severe vs. non-severe disease (WMD: 1.7 pg/mL and 408.3 ng/mL, respectively) [60]. The significant increase of inflammatory cytokines, such as IL-6, is connected to a so-called "Cytokine Storm," behind acute lung injury and ARDS and can lead to further tissue damage and MOF [66]. This hyperbolic systemic inflammation relates to lymphopenia and is associated with severe disease [67]. Important inflammatory markers include IL-6, IL-2, IL-7, tumor necrosis factor (TNF)- α , interferon- γ inducible protein (IP)-10, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP) 1- α , granulocyte-colony stimulating factor (G-CSF), CRP, procalcitonin (PCT), and ferritin [3,26,47,61,67,68].

Some of the above-mentioned parameters not only appear to be related to disease severity, but also to mortality. In a retrospective clinical series, non-survivors had higher levels of IL-6, ferritin, and CRP [26,61] compared to survivors. Current clinical practice suggests that the determination of IL-6, D-dimer, LDH, and transaminases in addition to routine laboratory tests, is useful for the stratification of high risk patients and the identification of those who might potentially benefit from anti-IL-6 immunotherapies with tocilizumab [7].

3.3.1. Procalcitonin

PCT, a glycoprotein, is the pro-peptide of calcitonin devoid of hormonal activity. Under normal circumstances, it is produced in the C-cells of the thyroid gland. In healthy humans, PCT levels are undetectable (< 0.1 ng/mL). During severe infection (bacterial, parasitic, and fungal) with systemic manifestations, PCT levels may rise to over 100 ng/mL, produced mostly by extra-thyroid tissue [69]. Although its biological action is largely unknown, the sequence homologies between PCT and other human cytokines, such as TNF- α family, IL-6, etc., support the hypothesis that PCT is a mediator of inflammation [70].

The synthesis of PCT can be increased as a result of endotoxins and/or cytokines (e.g. IL-6, TNF- α , and IL-1b). The extra-thyroid synthesis of PCT has been found to occur in the liver, pancreas, kidney, lung, intestine, and within leukocytes. However, the synthesis of PCT has been shown to be suppressed within these tissues in the absence of bacterial infection. In contrast, cytokines, such as interferon (INF)- γ , which are released following viral infection, lead to down-regulation of PCT, thus highlighting another advantage of PCT assays [71]. PCT levels are either unmodified or only moderately increased in systemic inflammatory response to viral or to noninfectious stimuli (non-viral infections). Therefore, PCT values were more discriminative than WBC count and CRP in distinguishing a bacterial infection from another inflammatory process [72]. As for COVID-19 patients, more severe cases showed a more marked increase of PCT compared with non-severe cases [33,73–76]. A slight increase (much less than 0.5 ng/mL) in PCT levels is an important indicator to distinguish between SARS-CoV-2-positive and SARS-CoV-2-negative patients [77] and increased PCT values have been associated with a nearly fivefold higher risk of severe SARS-CoV-2 infection (odds ratio (OR): 4.76; 95% CI: 2.74–8.29). PCT value remains within reference ranges in patients with non-complicated SARS-CoV-2 infection; any substantial increase reflects bacterial coinfection and the development of a severe form of disease and a more complicated clinical picture [78]. PCT elevation has also been found in pediatric cases with lower respiratory tract infection, reflecting bacterial coinfection [79].

Although initial PCT value may be helpful in the determination of illness severity, it may not always be a reliable prognostic indicator. As PCT values may be influenced by preexisting comorbid conditions, such as CKD and congestive heart failure, baseline values may be high. However, PCT can provide invaluable information if considered within the clinical context [80].

3.4. Coagulation biomarkers

Abnormal coagulation parameters are associated with poor prognosis. Specifically, markedly elevated D-dimer and FDP are common in COVID-19 non-survivor patients [1].

D-dimer appears to be frequently increased in patients with COVID-19 (36–43%) [81] and may be related to severe complications and death. However, currently the interpretation of D-dimer during disease monitoring is unclear, as it may not be directly related

to disease severity. Similarities may exist with troponins (8), whose range does not always correspond to acute cardiac ischemia; not all increases in cardiac troponin require invasive assessments in the absence of clinical symptoms [82,83]. However, in some large-scale studies, PT has been shown to be correlated to disease severity. In a retrospective study involving 296 COVID-19 patients (with 17 non-survivors), the non-survivor group had higher D-dimer and thrombin time and lower aPTT than the survivor group [84]. In a retrospective, multicenter cohort study including 191 COVID-19 patients who had either been discharged or had died, factors associated with non-survival were PT, high-sensitive cardiac troponin I, CK, and D-dimer [61]. Wang et al. showed that 58% of patients with COVID-19 had prolonged PT [68]. Tang et al. investigated 207 non-survivor COVID-19 patients and revealed that non-survivors had remarkably higher D-dimer and FDP levels and longer PT at admission compared with survivors [1].

The activation of coagulation processes reaches its peak in the DIC, which appeared to occur before most of the COVID-19 positive patients' death. In fact, such patients may evolve to sepsis, which is one of the most common causes of DIC. DIC is the result of activation of monocytes and endothelial cells to release cytokines following injury, with expression of tissue factor and secretion of von Willebrand factor. Circulation of free thrombin, uncontrolled by natural anticoagulants, can activate platelets and stimulate fibrinolysis. At the late stages of NCP, levels of fibrin-related markers (D-dimer and FDP) are moderately or markedly elevated in all deaths, suggestive of a common coagulation activation and secondary hyperfibrinolysis condition.

This evidence could explain the rapid disease progression to death and the limited efficacy of mechanical ventilation in the treatment of COVID-19 patients. ARDS mechanical ventilation protocols are not always of significant benefit and may even cause additional lung damage. Conversely, the rapid and positive response of some patients to full anti coagulation therapy can be explained.

Terpos et al. showed that blood hypercoagulability is common among hospitalized COVID-19 patients. They reported that coagulation abnormalities in PT, aPTT, FDP, and D-dimer, along with severe thrombocytopenia, are associated with life-threatening DIC, which necessitates continuous vigilance and prompt intervention [85]. In large scale studies, D-dimer and PT have been found to be associated with severe disease and death [61,86].

3.5. Potential Novel Markers in COVID-19 positive patients

3.5.1. Homocysteine

Hcy has been under a lot of speculation since its discovery in 1932. The heating of the amino acid methionine with sulfuric acid led to this amino acid of interest [87].

High plasma levels of Hcy significantly increase the incidence of vascular damage in both small and large vessels [88,89]; concentrations above the 90th percentile are associated with increased risk of degenerative and atherosclerotic processes [90] in the coronary, cerebral and peripheral circulatory systems. Although Hcy is an effective cardiovascular risk biomarker, and the cardiovascular complications are critical in hospitalized COVID-19 patients, this parameter has not been adopted and studied in this clinical setting and in neither of the published prospective studies focused on laboratory markers useful for clinical evaluation of COVID-19.

The definition of hyperhomocysteinemia differs between studies [91]. Hyperhomocysteinemia is defined as a medical condition characterized by an abnormally high level ($>15 \mu\text{mol/L}$) of Hcy in the blood [92]. Total concentration of Hcy in plasma of healthy humans (fasting) is low, between 5.0 and $15.0 \mu\text{mol/L}$ when assessed with the use of high-performance liquid chromatography (HPLC), or 5.0 – $12.0 \mu\text{mol/L}$ when immunoassay methods are used [93]. When the level is between 16 and $30 \mu\text{mol/L}$, it is classified as moderate, 31 – $100 \mu\text{mol/L}$ is considered as intermediate and a value $>100 \mu\text{mol/L}$ is classified as severe hyperhomocysteinemia [87,94]. Recent observations related hyperhomocysteinemia to cardiovascular disease, diabetes, CKD, and fatty liver disease [91,92,94].

Very recent data demonstrated a predictive value of Hcy (together with age, MLR, and period from disease onset to hospital admission) for severe pneumonia on chest CT at first week from COVID-19 patients, but did not report on additional organ involvement [11]. In the same study, the authors reported that MLR was significantly higher in imaging progression patients compared to that in imaging progression-free ones ($p < .001$).

3.5.2. Angiotensin II, Ang-(1-7), Ang-(1-9), and alamandine

ACE2 functions as a regulator of the renin–angiotensin system (RAS), modulating endogenous levels of Ang I and Ang II. Ang II levels were found to be significantly increased in the kidneys, hearts, and plasma of ACE2 null mice [95]. The level of Ang II was also significantly increased in the avian influenza A infected patients, indicating that Ang II is a biomarker for lethality in flu infections [96,97]. A strong correlation has been found

between increases in IL-6 and vascular macrophage accumulation and the degree of endothelial dysfunction produced by Ang II [98].

In an animal model, ACE2 and Ang-(1-7) infusion were shown to be protective via downregulation of RhoA/Rho kinase (ROCK) pathway. This pathway is deeply involved in changes of vascular tone and structure leading to hypertension and cardiovascular-renal remodeling, and it has a relevant role in the induction of lung fibrosis [99].

ACE2 converts Ang II to Ang-(1-7) and Ang I to Ang-(1-9). Ang-(1-7) and Ang-(1-9) produce biological effects through the Mas receptor (MasR) and AT2 receptor (AT2R), respectively. Ang-(1-7) induces regional and systemic vasodilation, diuresis, and natriuresis. Ang-(1-9) increases nitric oxide (NO) bioavailability by stimulating bradykinin (BK) release [100]. Activation of these pathways mediates anti-inflammatory and anti-fibrotic effects leading to cardiovascular, renal-protective actions, and acute lung injury protection [101,102].

Alamandine is generated by the catalytic action of ACE2 on Ang A or through a decarboxylation reaction on Ang-(1-7) in the N-terminal aspartate amino acid residue. Alamandine produces the same effects as Ang-(1-7), such as vasodilation and antifibrosis [103]. It modulates peripheral and central blood pressure regulation and cardiovascular remodeling [104].

Mechanistic evidence from related coronaviruses suggests that SARS-CoV-2 infection may downregulate ACE2, leading to toxic over-accumulation of Ang II that induces ARDS and fulminant myocarditis [105]. The Ang II level in the plasma samples from SARS-CoV-2 infected patients was markedly elevated and linearly associated to viral load and lung injury [106].

Up to date, there are no data regarding Ang-(1-7), Ang-(1-9), and alamandine plasma levels in COVID-19 patients. Following the loss of ACE2 function, due to the role of ACE2 as the viral binding site by SARS-CoV-2, we expect elevated level of Ang II and lower levels of Ang-(1-7), Ang-(1-9), and alamandine in severely infected patients than in mild ones.

4. Limitation of the study

A large proportion of the primary research is based on Asian patients; therefore, further verification is needed in populations in other areas. Some biomarkers, such as Hcy, have been evaluated in a few scientific reports and further analysis is needed in a large cohort of COVID-19 patients. There appears to be geographic variability in the percentage of patients with lymphopenia as shown in a retrospective study [107]. Further investigations

should be done to assess this geographic variability. This is a review of the current scientific literature with no statistical outcome measures. Therefore, these results may not be generalizable to all populations. Finally, with COVID-19 being a very novel disease, many clinical studies are still ongoing or undergoing publication.

5. Conclusions

Since the emergency pandemic situation began, it is of high scientific significance to analyze the discriminative ability of hematologic, biochemical, inflammatory, and immunologic biomarkers in patients with and without the severe or fatal forms of COVID-19. It is necessary to determine risk categories following COVID-19 diagnosis, to ensure an optimal resource allocation and to improve clinical management and prevention of serious complications.

To sum up, we can conclude from the analysis of published studies that hematological (lymphocyte count, neutrophil count, and NLR), inflammatory (CRP, ESR, IL-6), and especially biochemical (D-dimer, Troponins, CK) parameters correlate with severe prognosis or exitus in COVID-19 patients and can therefore be used as predictive biomarkers. Coagulation and liver parameters might play a crucial role in identifying severe cases of COVID-19.

Understanding the weight of the pathophysiological processes of systemic cardiovascular damage (vasculitis, DIC, myocardial infarction) and metabolic processes associated to the critical course of the infection, also thanks to autopsy cohorts [68,108–110], sets new light on biochemical biomarkers related to coagulation disorders. These are in fact not only predictive of disease severity, but are also helpful for the therapeutic management, based on drugs preventing the activation of coagulation processes. A laboratory score, taking into account hematological, inflammatory, biochemical and immunological parameters, would help to stratify COVID-19 positive patients into risk categories, which would be of outmost importance in the clinical setting and therapeutic management.

In addition to above discussed laboratory parameters, which are currently used in clinical practice, novel biomarkers potentially useful for screening, clinical management, and prevention of serious complications are under investigation. These include Hcy, Ang II, Ang-(1-7), Ang-(1-9), and alamandine, which need to be evaluated in larger case series in order to clearly determine their predictive clinical value as indicators of severe prognosis in COVID-19 patients.

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Author contributions

GP, TO conceived of the idea for the review, searched the scientific literature and drafted the manuscript. MM searched the literature and drafted the manuscript. TO, CR and AT revised the manuscript. All authors read and approved the final manuscript.

Disclosure statement

The authors report no conflict of interest.

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