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Biomarkers for Prognosis and Treatment Response in COVID-19 Patients

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During a severe infection such as coronavirus disease 2019 (COVID-19), the level of almost all analytes can change, presenting a correlation with disease severity and survival; however, a biomarker cannot be translated into clinical practice for treatment guidance until it is proven to have a significant impact. Several studies have documented the association between COVID-19 severity and circulating levels of C-reactive protein (CRP) and interleukin-6, and the accuracy of the CRP level in predicting treatment responses has been evaluated. Moreover, promising findings on prothrombin and D-dimer have been reported. However, the clinical usefulness of these biomarkers in COVID-19 is far from proven. The burst of data generation during this pandemic has led to the publication of numerous studies with several notable drawbacks, weakening the strength of their findings. We provide an overview of the key findings of studies on biomarkers for the prognosis and treatment response in COVID-19 patients. We also highlight the main drawbacks of these studies that have limited the clinical use of these biomarkers.

Key Words: Biomarkers, Coronavirus, COVID-19, Predictive value, Severity

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INTRODUCTION

Although coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is well known worldwide, it is impossible to predict how the disease will manifest in an individual. The manifestations of symptomatic COVID-19 vary widely from mild fever (>37.5°C) and cough to acute respiratory distress syndrome (ARDS) and death, and the disease follows an unpredictable course. This variability has led to an urgent search for biomarkers of disease severity to appropriately manage patients and prevent fatal complications.

Severe COVID-19 and other critical diseases have a common inflammatory pathophysiology involving a cytokine storm, which refers to massive inflammatory activation in response to infection. In addition, organ damage and multi-organ failure (MOF) due to vasculitis have been commonly reported in COVID-19 patients [1]. Accordingly, most biomarkers investigated in COVID-19 patients, such as C-reactive protein (CRP), interleukin (IL)-6, procalcitonin (PCT), white blood cell (WBC) count, neutrophil count (NC), lymphocyte count (LC), neutrophil:lymphocyte ratio (NLR), D-dimer, prothrombin time (PT), and activated partial thromboplastin time (aPTT), belong to the immune-inflammatory and coagulation pathways. Other non-specific biomarkers of cellular damage and inflammation include lactate dehydrogenase (LDH) and transaminases [2, 3]. Moreover, severe COVID-19 often involves cardiac, liver, and kidney failure; hence, organ-specific biomarkers have also been evaluated in these patients (Fig. 1). Finally, new molecules, including sepsis bio-

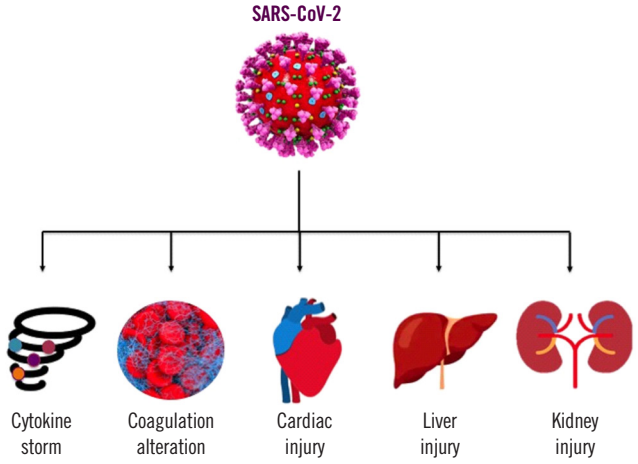

Fig. 1. Alterations induced by SARS-CoV-2 infection.
Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

markers and microRNAs (miRNAs), have been assessed as potential COVID-19 biomarkers [4, 5].

Among these candidates, only a few biomarkers reliably predict a worse outcome in COVID-19 patients, and even fewer molecules display the ability to predict treatment responses. This review aims to define the biological markers that are clinically useful in predicting a severe disease course in COVID-19 patients and to identify molecules that can be used to predict treatment responses. The main limitations hindering the usefulness of biomarkers in these patients are also described.

TRADITIONAL BIOMARKERS

CRP level

A surprisingly high number of papers have focused on circulating CRP levels in COVID-19 patients, with multiple lines of evidence showing the prognostic value of this biomarker [6–24]. Studies addressing the clinical usefulness of CRP have mostly reported a positive association between disease severity and baseline values. For instance, CRP has been shown to be superior to NC, LC, and the erythrocyte sedimentation rate (ESR) and to correlate with computed tomography (CT) scan severity scores [13, 19, 22]. In a retrospective single-center study on 145 COVID-19 patients, CRP was defined as an early detector of disease severity and a suitable biomarker for guiding therapy [13]. Despite the retrospective single-center design of this study, variables with missing values were not included in the analysis, which strengthens the findings. Yang, *et al*. [19] analyzed CRP levels in 108 COVID-19 patients to assess its effectiveness as a biomarker of disease severity. The CRP level and CRP-to-LC ratio had high prognostic value in the early disease stage. Based on these findings, the authors inferred that CRP has an "outstanding ability" to predict a severe course of COVID-19 in the early stage. Ali [21] showed that the CRP level could predict disease worsening among non-severe cases, reporting a 5% risk of developing a severe course for every unit increase in the CRP level. Ali [21] highlighted a study by Luo, *et al*. [10], who identified independent predictors of death based on a logistic regression model and then compared the predictors by ROC curve analysis. CRP emerged as the best predictor, over NC, D-dimer, and platelet count. Additionally, CRP levels in patients who died from COVID-19 were 10-fold higher than those in survivors [10]. It is worth mentioning that Ali [21] only included studies addressing the positive association between the CRP level and disease severity in his review.

Other studies documented no significant differences in the CRP level among mild, severe, and critical patients [14, 24]. However, the sample sizes in these studies were relatively small (29 patients in Chen, *et al*. [24] and 25 patients in Luo, *et al*. [14]). In contrast, studies reporting remarkable changes in the CRP level across various degrees of severity had larger sample sizes [8, 18, 19] (Table 1).

The CRP level has also been reported to be a reliable biomarker for treatment responses in COVID-19 patients [25–28]. In a study on 15 COVID-19 patients with respiratory failure who were undergoing treatment with the IL-6 receptor antagonist, sarilumab, sharp differences in median CRP levels were observed between responders and non-responders, and non-responders never displayed a decrease below the highest values in the responder group [25]. Ponti, *et al*. [26] and Zhang, *et al*. [27] pointed out that the CRP level could be used to identify patients who benefit from treatment with tocilizumab, another IL-6 receptor blocker similar to sarilumab. Xu, *et al*. [28] reported that the CRP level returned to normal after treatment, which is slightly different from predicting the treatment response.

PCT level

The PCT level reportedly is increased in patients with severe disease compared with non-severe COVID-19 patients, reflecting bacterial super-infection. PCT levels do not rise above the normal range in patients with non-complicated COVID-19, thereby representing a candidate marker for serious disease progression [29–32]. However, the prognostic value of PCT in COVID-19 patients is disputed, since it is within the normal range in most patients at initial presentation [33].

Table 1. Main studies and findings on the prognostic role of CRP level in COVID-19 severity

Reference	Study design	Cut-off	Sample size	Main findings
Zeng, *et al.* [52]	Meta-analysis	NS	2,984 patients for assessing severity 393 for assessing mortality	CRP levels increased in severe and fatal COVID-19 patients.
Qin, *et al.* [7]	Retrospective	NS	452	CRP levels were significantly higher in patients with severe COVID-19 than in patients with non-severe disease [57.9 (20.9–103.2) mg/L vs. 33.2 (8.2–59.7) mg/L].
Liu, *et al.* [8]	Retrospective	8 mg/L	140	CRP levels could effectively assess disease severity and predict outcome in COVID-19 patients.
Wang, *et al.* [20]	Cross-sectional	64.79 mg/L	143	CRP levels above the cut-off value were associated with a high risk of progression of COVID-19 to a critical stage.
Luo, *et al.* [14]	Retrospective	41.4 mg/L	298	Increased CRP levels on hospital admission correlated with disease severity, representing a good predictor of adverse outcome.
Gao, *et al.* [12]	Retrospective	NS	43	CRP levels showed poor accuracy for predicting severe disease (AUC = 0.60, 95% CI = 0.44–0.75)
Ahnach, *et al.* [13]	Retrospective	10 mg/L	145	CRP levels measured on admission showed good accuracy for predicting severity (AUC = 0.87). The CRP level was an independent predictor of disease severity in multivariate analysis.
Luo, *et al.* [17]	Retrospective	NS	25	CRP levels were not associated with severe COVID-19 pathology. CRP levels were not associated with disease severity.
Villard, *et al.* [18]	Retrospective	NS	44	CRP levels were significantly higher in patients with a severe clinical course [152 (34–389) mg/L] than in those with a mild or moderate course [83 (3–298) mg/L; $P = 0.03$]. In multivariate analyses, CRP levels remained positively associated with disease severity.
Yang, *et al.* [19]	Retrospective	26.3 mg/L	108	The CRP level showed good prognostic accuracy in assessing the severity of COVID-19 (AUC = 0.79, 95% CI = 0.70–0.86, $P < 0.001$)
Xie, *et al.* [6]	Retrospective	27.8 mg/L	140	Increased CRP levels (median = 76.5 mg/L) were associated with low oxygen saturation (≤ 90%)

Abbreviations: AUC, area under the curve; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; CI, confidence interval; NS, not specified.

Immunological markers

The WBC count, encompassing the NC, LC, NLR, and lymphocyte subsets, has been assessed in COVID-19 patients, along with the cytokine profile. Several studies have reported that neutrophilia, lymphopenia, T-helper ($CD4^+$) and T-cytotoxic ($CD8^+$) lymphocyte depletion, and NLR increase are strongly associated with disease severity [7, 11, 34, 35]. Other studies have reported that LC and NC have lower prognostic accuracy than CRP in distinguishing severe and non-severe COVID-19 cases [13, 18, 19]. The reliability of WBC count, NC, and LC is somewhat disputed since immunological markers can be affected by many factors, including glucocorticoid therapy and other viral or bacterial infections targeting the lymphoid tissues [36, 37]. Hence, variability in these indices cannot be equivocally attributed to the degree of COVID-19 severity.

Among the cytokines, IL-6 has attracted particular attention with respect to COVID-19. Several studies have shown an association between IL-6 levels and disease severity in COVID-19 patients [38, 39]. Higher baseline IL-6 levels in severe COVID-19 patients were strongly correlated with the need for mechanical ventilation, lung damage on CT scans, and other inflammatory markers, including CRP, ferritin, and D-dimer [39]. A recent meta-analysis revealed that IL-6 levels were nearly three-fold higher in severe COVID-19 patients than in non-severe patients. However, multiple outcomes were considered in the studies evaluated in this meta-analysis (ARDS, intensive care unit [ICU] admission, and death), making it difficult to determine specific IL-6 levels that lead to a given outcome [11, 40]. Regarding the reliability of IL-6 as a treatment response marker, Montesarchio, *et al*. [25] showed that IL-6 levels do not significantly vary between sarilumab responders and non-responders. Thus, the usefulness of IL-6 as a marker of the treatment response is not proven. Liu, *et al*. [39] reported that IL-6 levels decreased after treatment with antibiotics, antivirals, and glucocorticoids, but did not specify whether baseline levels could predict treatment response.

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Coagulation pathway biomarkers

D-dimer and PT levels have been assessed in COVID-19 patients to establish their ability to predict a worse outcome, defined as ARDS development, ICU admission, and death [32, 33, 41–44]. Wu, *et al*. [35] demonstrated PT and D-dimer levels to be significantly associated with ARDS development in a cohort of 201 patients. Coagulation indices were significantly higher in patients who developed ARDS and died than in patients who survived. Similarly, Perlman, *et al*. [41] and Han, *et al*. [42], showed that D-dimer and fibrin/fibrinogen degradation products were significantly higher in mild disease than in severe disease. However, Han, *et al*. [42] did not confirm the association of PT with disease severity, reporting no differences in the levels of PT, aPTT, and PT-international normalized ratio (INR) among mild disease, severe disease, and control groups. Zhang, *et al*. [43] found that a D-dimer level ≥2.0 μg/mL on admission was the optimum cut-off to predict in-hospital mortality for COVID-19. Huang, *et al*. [33] found that D-dimer levels on admission were higher in ICU patients than in non-ICU patients and concluded that D-dimer could be used to triage patients into critical care. Although a few studies indicated that D-dimer has lower prognostic accuracy than CRP, analyses of coagulation indices in the prognosis of COVID-19 patients suggested that PT and D-dimer are useful indicators of a severe disease course [14–20].

Platelet count

The platelet count is considered a reliable biomarker for disease severity and is decreased in patients with severe disease compared with those with mild disease [11, 45]. Platelet count has also been proposed as an independent risk factor for mortality in COVID-19 patients. However, compared with CRP, platelet count reportedly has worse prognostic value [14]. Notably, an increased platelet count during SARS-CoV-2 infection has also been reported, albeit in a limited proportion of patients [38].

OTHER NON-SPECIFIC BIOMARKERS

LDH and serum amyloid A (SAA) are also relevant candidate biomarkers for COVID-19. Several studies have shown that ICU patients had significantly higher LDH levels than non-ICU patients and that LDH levels correlated with tissue damage and CT scan scores, reflecting disease severity [46–48]. Further, LDH levels were higher in patients needing mechanical ventilation as well as additional corticosteroid and antiviral treatment [49]. Among these studies, only one study is prone to selection bias as a single-center study with a small sample size, which weakens the results [46]; the other studies had a multicenter design and included more than 1,000 patients [47].

SAA was able to distinguish severe from mild cases of COVID-19 in a 132-patient cohort based on an area under the ROC curve (AUC) of 0.74 [50]. Although 0.74 is not an excellent AUC score, Li, *et al*. [51] independently confirmed this result, demonstrating a good accuracy of SAA in predicting disease progression. A recent meta-analysis suggested that SAA and ferritin levels were higher in the severe COVID-19 group than in the non-severe group [52]. However, the authors did not conclude that SAA is associated with COVID-19 severity given the low number of studies evaluated (N=3) and the fact that sensitivity analysis changed the conclusion (see further discussion on sensitivity analysis in

Table 2. Non-specific prognostic biomarkers of COVID-19

Pathway	Biomarkers
Hematological	Elevated WBC count Elevated neutrophil count Decreased lymphocyte count Elevated neutrophils-to-lymphocyte ratio Elevated monocyte-to-lymphocyte ratio Elevated platelet volume Elevated monocyte distribution width Elevated red cell distribution width
Inflammation	Elevated serum amyloid A Elevated ESR Elevated ferritin Decreased sphingosine-1-phosphate Elevated IL-2 Elevated IL-8 Elevated IL-10
Coagulation	Elevated fibrin/fibrinogen degradation products
Necrosis	Elevated lactate dehydrogenase
Cardiac injury	Elevated cTn Elevated NT-pro-BNP Elevated D-dimer Elevated homocysteine
Liver injury	Elevated ALT Elevated AST Elevated gamma-GT Elevated total bilirubin
Kidney injury	Elevated creatinine Elevated blood urea nitrogen Proteinuria
Muscular injury	Elevated CK Elevated myoglobin
Organ failure	Elevated MR-pro-ADM

Abbreviations: CK, creatine kinase; COVID-19, coronavirus disease 2019; cTn, cardiac troponin; ESR, erythrocyte sedimentation rate; IL, interleukin; MR-pro-ADM, mid-regional pro-adrenomedullin; GT, glutamate transferase; NT-pro-BNP, N-terminal pro-B-type natriuretic peptide; WBC, white blood cell.

the limitations section below).

Many other non-specific biomarkers have been evaluated for the prediction of severity in COVID-19 patients, but there is insufficient evidence to prove their clinical usefulness (Table 2).

ORGAN-SPECIFIC BIOMARKERS

Cardiac markers

Epidemiological evidence suggests that cardiovascular comorbidities, including hypertension and ischemic heart disease, are frequently associated with COVID-19 mortality [11]. Cardiac troponin I (cTnI) has been proposed as a marker of symptom severity and mortality in COVID-19 patients [53–55]. The cytokine storm can increase the occurrence of viral myocarditis and cardiac injury and can exacerbate coronary artery disease [56, 57]. Cardiovascular disease frequently occurs in COVID-19 patients requiring ICU admission, and cTnI is a good predictor of mortality in many other respiratory diseases and sepsis [58–60]. Thus, cTnI can be used as a predictor of severity in COVID-19 patients. According to Zhou, *et al*. [53], cTnI was superior to D-dimer and LC in predicting severity. Patients with a high cTnI level at presentation needed invasive or non-invasive ventilation and developed ARDS more frequently than those with a normal cTnI level. Despite the evidence, the soundness of measuring cTnI level in these patients is somewhat disputed, since the American College of Cardiology has recommended measuring this biomarker only in cases of clinical suspicion of myocardial infarction [61]. The main concern related to the eventual misuse of cTnI as a COVID-19 biomarker is the inappropriate use of cardiology consultation. However, most researchers consider cTnI measurement as a reliable tool for predicting mortality in COVID 19 patients with ischemic and non-ischemic heart injury, allowing clinicians to timely stratify and appropriately treat these patients [1, 62].

Liver markers

The levels of liver enzymes, including transaminases and gamma-glutamyl transferase (GGT), are commonly elevated in COVID-19 patients [38, 47, 63]. The elevation in GGT level is not accompanied by a rise in the alkaline phosphatase level; thus, liver involvement in COVID-19 seems similar to that of drug-induced injury [64]. However, there is no robust evidence of a correlation to disease severity, and the relevance of testing for liver indices in these patients is not confirmed [1].

Kidney markers

In a prospective cohort study on 701 COVID-19 patients, Cheng, *et al*. [65] found that baseline serum creatinine and blood urea nitrogen levels were independent risk factors for in-hospital death after adjusting for confounders (age, sex, disease severity, comorbidity, and WBC count). In addition, creatinine levels were higher in patients requiring ICU admission and mechanical ventilation. COVID-19 patients with kidney disease have a higher mortality risk, but further confirmation is needed to define kidney indices as reliable markers of severity in these patients.

NEW BIOMARKERS

Mid-regional pro-adrenomedullin (MR-pro-ADM)

Adrenomedullin (ADM) and its surrogate, MR-pro-ADM, are organ damage biomarkers, whose predictive values have been mostly investigated in infected patients for identifying those at risk of developing sepsis [66]. MR-pro-ADM is also considered a good prognostic biomarker for predicting mortality in ICU patients, independent of the cause of ICU admission [67, 68]. Spoto, *et al*. [68] assessed MR-pro-ADM levels in 69 COVID-19 patients, demonstrating that an MR-pro-ADM level ≥2 nmol/L at presentation was significantly associated with higher mortality risk. The authors also reported that CRP was a better predictor for ARDS than MR-pro-ADM. Since data on this biomarker in COVID-19 are sparse, no conclusion can be drawn about its potential role in predicting prognosis in these patients.

Monocyte distribution width (MDW)

MDW is a novel biomarker of sepsis, whose prognostic value has been recently highlighted [69–71]. Ognibene, *et al*. [72] reported that MDW is a good analyte for predicting positivity in a molecular diagnostic testing for SARS-CoV-2. The median MDW level was higher in patients requiring ICU admission than in patients who did not. However, it should be noted that the prognostic value was assessed using a small sample size (23 ICU vs. 8 non-ICU patients). Data on this biomarker are too sparse to conclude on its prognostic value for COVID-19 patients.

MiRNAs

MiRNAs are non-coding RNAs that bind to the target mRNA sequence, regulating gene expression at the post-transcriptional level. Many cellular processes, including differentiation, proliferation, and survival, are regulated by miRNAs [73]. During infections, host cell miRNAs can interact with viruses and may play a role in the antiviral immune response [74, 75]. Thus, the role of

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miRNAs as potential biomarkers in COVID-19 has been studied, revealing 34 positive-sense and 45 negative-sense miRNAs that strongly bind to key SARS-CoV-2 genes [73]. The authors hypothesized that miRNAs may be useful to monitor the disease at different stages and predict the disease course. However, supportive evidence remains to be provided.

NEW APPROACHES AND TECHNOLOGIES FOR BIOMARKER MEASUREMENT

Salivary biomarker measurement

Saliva sample collection is rapid, easy, and non-invasive. The usefulness of saliva has been suggested for diagnosis during the COVID-19 pandemic, and the possibility of measuring salivary inflammatory biomarkers has attracted some attention [76]. Based on evidence that the salivary CRP level reflects the serum CRP level, Spanish researchers have recently proposed using saliva to measure acute-phase reactants such as CRP, ILs, and ferritin for assessing disease severity in COVID-19 patients [77, 78]. However, no sufficient data are available regarding the usefulness of salivary inflammatory biomarkers in COVID-19 patients.

Digital immunoassays

The digital immunoassay is a next-generation protein detection method; however, the high cost and large size of the instrumentation limits its application in clinical practice [79, 80]. Microfluidic platforms for laboratory-on-a-chip digital assays, including a mobile phone-based microfluidic immunoassay as a point-of-care device, have been developed. Recently, digital assay technology has been proposed to measure cytokines in COVID-19 patients. An automated platform named pre-equilibrium digital ELISA (PEdELISA microarray) has been used for rapid multiplex monitoring of IL-6, tumor necrosis factor-α, IL-1β, and IL-10 in COVID-19 patients [79]. Along with cytokines, the circulating levels of surrogate biomarkers of inflammation were also measured. When patients had low CRP levels, CRP was associated with IL-6. However, such association was not detected in patients with high CRP levels. One of the advantages of this method is that the results can be obtained within four hours, which encourages the use of such devices for rapid measurement of cytokine levels.

Non-conventional methods for biomarker measurement

Besides conventional methods such as ELISA for detecting ILs and other biomarkers, non-conventional methods for measuring inflammatory markers and detecting SARS-CoV-2, including chip-, paper-, thread-, and film-based biosensors, have been described [81]. Electro-chemical, optical, and microfluidic biosensors have been considered promising tools for CRP, PCT, IL-6, and ferritin level measurements [82]. Advantages of these methods include high sensitivity and reliability, and a relatively low cost. However, further efforts are required to establish biosensors that can be used in clinical settings.

CONSIDERATIONS OF BIOMARKERS IN COVID-19 PATIENTS AND LIMITATIONS OF RELATED STUDIES

Although the usefulness of biomarkers for the prediction of disease severity and treatment response in COVID-19 patients is a fascinating prospect, at present, their applicability in clinical practice remains conceptual. Scientific data production and publication have blown up following the COVID-19 outbreak, opening the perspective for writing hundreds of papers. Consequently, most of these studies exhibit many flaws that diminish the strength of their findings. The main limitations can be summarized as follows.

First, most studies had a retrospective design, which provides a lower level of evidence than prospective and interventional studies. Prospective studies are rare and, unavoidably, have short-term follow-up. Therefore, there are insufficient data to prove the usefulness of a certain biomarker for therapy guidance and appropriate patient management. Second, the assay methods, cut-offs, time points of measurement, and end points chosen in the studies reviewed herein varied greatly. Third, differences in cut-offs and outcomes limit the possibility of drawing definitive conclusions about the usefulness of a certain biomarker in predicting prognosis. In particular, it is not clear which cut-off determines which outcome. Fourth, the retrospective design of and heterogeneity among studies strongly limit the strength of a meta-analysis, since sensitivity analysis often alters the results obtained in a first evaluation. Sensitivity analysis is needed to avoid bias for arbitrary selection or omission, which requires repeating the analysis after excluding studies reporting unknown or unclear data. When results change after sensitivity analysis, the main conclusions from a meta-analysis should be interpreted with caution. Fifth, selection bias can affect the reproducibility and robustness of results when the study subjects are recruited at a single center, limiting their extrapolation to other geographic areas and ethnicities. Sixth, although many studies adjusted their analysis for various factors, it should be noted that unmeasured confounders cannot be excluded. Finally, meta-analysis often report a quality assessment according to the New Castle-

Ottawa scale (NOS) of the studies reviewed, showing that most studies have a low-quality score [3, 6], while few have a high-quality score [9–11].

CONCLUSIONS

Theoretically, some biomarkers can predict a worse outcome during any disease or condition, supporting clinical management. Practically, the clinical usefulness of a given biomarker is not proven until it helps clinicians manage patients and make treatment decisions. The biomarker pipeline involves many steps that are often prone to defeat; thus, the evaluation and validation of a certain molecule require rigorous studies with faultless methods and homogeneous features. In this perspective, studies on the usefulness of biomarkers in COVID-19 have failed to prove an effect on treatment decision-making and are affected by several restraints, including discrepancies in the methods used and weaknesses in the study design. Based on these considerations, promising findings have been reported on the potential usefulness of CRP, PT, and D-dimer levels as biomarkers of COVID-19 severity. However, the clinical usefulness of these biomarkers remains to be established. Further, the data on the efficacy of these biomarkers in predicting the treatment response are sparse, necessitating confirmatory studies.

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Bivona G conceived the review and wrote the manuscript; Agnello L revised the manuscript and produced the tables and figures; Ciaccio M supervised the work.

CONFLICT OF INTEREST

None declared.

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