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Marta Cancella de Abreu[a,b], Jacques Ropers[c], Nathalie Oueidat[d], Laurence Pieroni[e], Corinne Frère[f], Michaela Fontenay[g,h], Krystel Torelino[c], Anthony Chauvin[i], Guillaume Hekimian[j], Anne-Geneviève Marcelin[k], Beatrice Parfait[l], Florence Tubach[c] and Pierre Hausfater[a,b] for the COVIDeF study group

Background **During a pandemic like COVID-19, hospital resources are constrained and accurate severity triage of the patients is required.**

Objective **The objective of this study is to estimate the predictive performances of candidate biomarkers for short-term worsening (STW) of COVID-19.**

Design **Prospective, multicenter (20 hospitals in Paris) cohort study of consecutive COVID-19 patients with systematic biobanking at admission, during the first waves of COVID-19 in France in 2020 (COVIDeF cohort).**

Setting and participants **Consecutive COVID-19 patients were screened for inclusion. They were excluded in presence of severity criteria defined by either an ICU admission, mechanical ventilation (including noninvasive ventilation), acute respiratory distress, or in-hospital death before sampling. Routine blood tests measured during usual care and centralized systematic measurement of creatine kinase, C-reactive protein (CRP), procalcitonin, soluble urokinase plasminogen activator receptor (suPAR), high-sensitive troponin T (TnT-hs), N terminal pro-B natriuretic peptide (NT-proBNP), calprotectin, platelet factor 4, mid-regional pro-adrenomedullin (MR-proADM), and proendothelin were performed.**

Outcome measures and analyses **The primary outcome was STW, defined by a severity criteria within 7 days. A backward stepwise logistic regression model and a 'best subset' approach were used to identify independent association, and the area under the receiving operator characteristics (AUROC) was computed.**

Results **Five hundred and eleven patients were analyzed, of whom 60 (11.7%) experienced STW. Median time to occurrence of a severity criteria was 3 days. At admission, lower values of eosinophils, lymphocytes, platelets, alanine aminotransferase, and higher values of neutrophils, creatinine, urea, CRP, TnT-hs, suPAR, NT-proBNP, calprotectin, procalcitonin, MR-proADM, and proendothelin were predictive of worsening. Stepwise logistic regression identified three biomarkers significantly associated with worsening: CRP [adjusted odds ratio (aOR): 1.10, 95% confidence interval (95% CI): 1.06–1.15 for a 10-unit increase, AUROC: 0.73 (0.66–0.79)], procalcitonin [aOR: 0.42, 95% CI: 0.22–0.81, AUROC: 0.69 (0.64–0.88)], and MR-proADM [aOR: 2.85, 95% CI: 1.74–4.69, AUROC: 0.75 (0.69–0.81)]. These biomarkers outperformed clinical variables except diabetes and cancer comorbidities.**

Conclusion **In this multicenter prospective study that assessed a large panel of biomarkers for COVID-19 patients, CRP, procalcitonin, and MR-proADM were independently associated with the risk of STW.**

Trial registration **ClinicalTrials.gov NCT04352348.**

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Keywords: calprotectin, COVID-19, C-reactive protein, mid-regional pro-adrenomedullin, N terminal pro-B natriuretic peptide, procalcitonin, proendothelin, prognosis, soluble urokinase plasminogen activator receptor, troponin

[a]Emergency Department, Hôpital Pitié-Salpêtrière, AP-HP Sorbonne Université, [b]Groupe de Recherche Clinique (GRC)-14 BIOSFAST, Centre d'Immunologie et des Maladies Infectieuses (CIMI), UMR 1135, Sorbonne Université, [c]Département de Santé Publique, Unité de Recherche Clinique PSL-CFX, Hôpital Pitié-Salpêtrière, AP-HP Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, [d]Biochemistry Department, UF des Urgences Biologiques, Service de Biochimie métabolique, Hôpital Pitié-Salpêtrière, DMU BioGeM, AP-HP Sorbonne Université, [e]Unité de Biochimie, Département de Biochimie-Hormonologie-Suivi thérapeutique général, Hôpital Tenon, DMU BioGeM, AP-HP Sorbonne Université, [f]UMRS 1166, Hôpital Pitié-Salpêtrière, AP-HP Sorbonne Université, [g]Université Paris Cité, Institut Cochin, INSERM U1016, CNRS UMR 8104, [h]Hematology Laboratory, Assistance Publique-Hôpitaux de Paris Centre, Service d'hématologie biologique, Hôpital Cochin, [i]Emergency Department, Hôpital Lariboisoière, APHP-Université de Paris Cité, [j]Critical Care Department, Service de Médecine Intensive Réanimation, Hôpital La Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris (AP-HP), Institut de Cardiométabolisme et Nutrition (ICAN), Sorbonne Université, [k]Laboratoire de Virologie, Virology Laboratory Department, Hôpitaux Universitaires Pitié-Salpêtrière – Charles Foix, AP-HP Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique and [l]Centre de Ressources Biologiques – site Cochin, Fédération des CRB/PRB, DMU BioPhyGen, AP-HP, Centre-Université Paris Cité, Hopital Cochin, Paris, France

Correspondence to Pierre Hausfater, PhD, Emergency Department, APHP Sorbonne Université Hôpital Pitié-Salpêtrière, 83 boulevard de l'hôpital, 75651 Paris Cedex 13, France
Tel: +33 142177240; fax: +33 142177412; e-mail: pierre.hausfater@aphp.fr

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Introduction

The dynamics and burden of the COVID-19 pandemic on our health care system have highlighted the need for accurate triage of patients infected with severe acute respiratory syndrom-coronavirus 2 (SARS-CoV2). During the different waves of the pandemic, the available resources for hospitalization were constrained [1,2]. Therefore, the rapid identification of COVID-19 patients at low risk of worsening was crucial to optimize their medical care and to allocate the hospital resources efficiently.

Outside clinical prognostic parameters, blood biomarkers can be helpful in assessing disease severity. Therefore, several biomarkers such as C-reactive protein (CRP), procalcitonin, soluble urokinase plasminogen activator receptor (suPAR), have been reported to be individually associated with severe cases of COVID-19 [3–9]. However, the pathophysiology of COVID-19 is complex and involves not only a unique but simultaneous host-response mechanisms among notably inflammatory/anti-inflammatory balance, coagulation, and cardiovascular system [10].

The aim of this study was to identify among a large panel of blood biomarkers on samples collected at admission of COVID-19 patients, those that are independently associated with clinical short-term worsening (STW).

Methods

Study design, patients, and methods

The COVIDeF cohort study was constituted prospectively during the first year of COVID-19 pandemic in the Greater Paris area, France, from March 2020 to March 2021. Patients with suspected SARS-CoV2 infection admitted to the emergency departments (EDs), medical wards, or intensive care units of 20 hospitals of the Assistance Publique-Hôpitaux de Paris hospital trust were proposed to participate. After signing an informed consent, blood samples were collected at admission (day 0: D0) and, for hospitalized patients, at D3 and D7 after admission. In case of clinical worsening, an additional blood sample was collected on the day meeting worsening definition, Supplementary Data 1, Supplemental digital content 1, *http://links.lww.com/EJEM/A459*.

The final COVIDeF cohort comprises 1953 patients. A confirmed COVID-19 case was defined by either a positive SARS-CoV2 RT-PCR on a respiratory sample, a characteristic thoracic computed tomography scan (bilateral pure ground-glass opacities with multifocal lesions distributed mainly in the peripheral areas of the lower lobes), or a highly evocative clinical presentation together with a close contact with an index COVID-19 case, as during the first wave PCR testing materials for SARS-CoV2 was not available for all patients. The COVIDeF cohort is registered in ClinicalTrials.gov under the number NCT04352348. Ethical approval was obtained from the Comité de Protection des Personnes Île-de-France XI (ID RCB, 2020-A00754-35).

Patients

Criteria of inclusion

In this work, we screened the consecutive patients with confirmed COVID-19 included during the first two waves of COVID-19 pandemic in France. At the time of the selection of patients for this ancillary study, the COVIDeF cohort was made of 1086 patients.

Criteria of exclusion

We excluded the patients already meeting severity criteria before sampling [admission to an ICU, noninvasive or mechanical ventilation, acute respiratory distress syndrome (ARDS), or death at admission], as well as those with no or withdrawn consent or missing biobanked samples (Fig. 1).

Objectives and outcomes

The primary objective was to estimate the performances of candidate biomarkers to predict STW of COVID-19. Secondary objectives was to estimate the performances of candidate biomarkers to predict the absence of hospitalization requirement at the acute phase.

The primary outcome was STW defined as the occurrence of at least one of the following criteria: admission to ICU, noninvasive or mechanical ventilation, ARDS, or in-hospital death (considering index hospitalization if any). The secondary outcome was discharge from ED with no further admission to the hospital.

Selection of biomarkers candidates

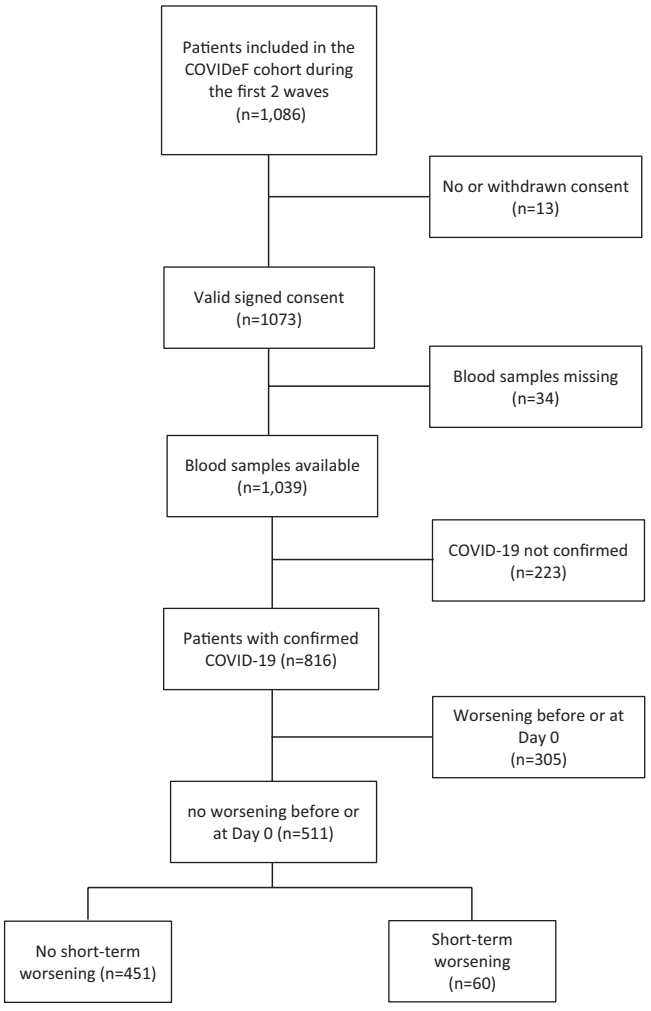
We first selected usual blood tests measured at admission as routine care: complete blood count with differential, creatinine, liver enzymes, ferritin, lactate dehydrogenase (LDH), and D-dimers. Secondly, we identified a panel of biomarkers of interest that either had been reported in the early phase of the pandemic as being associated with the prognosis of COVID-19, or might be a candidate of COVID-19 pathophysiological pathways surrogate: creatine kinase, CRP, procalcitonin, suPAR, high-sensitive troponin T (TnT-hs), N terminal pro-B natriuretic peptide (NT-proBNP), calprotectin, platelet factor 4, mid-regional pro-adrenomedullin (MR-proADM), and proendothelin [3–8,11–19]. All of these biomarkers were measured centrally by batch on the COVIDeF biobank material.

Sampling and dosage

Sampling

Blood samples were prepared, aliquoted, and stored locally for biobanking at –80 °C, and consisted of serum, EDTA plasma, citrate plasma, RNA, DNA, and peripheral blood mononuclear cells. Patients were followed for outcome up to 12 ± 2 months and clinical data were collected. EDTA plasma samples from the COVIDeF biobank were thawed and processed on the Cobas 8000 analyzer (Roche Diagnostics, Meylan, France) to measure suPAR, TnT-hs, NT-proBNP, creatine kinase, and CRP (all reagents

Fig. 1



Flowchart. Worsening was defined as at least one among: admission to an ICU, noninvasive or mechanical ventilation, acute respiratory distress syndrome (ARDS), or in-hospital death.

from Roche diagnostics). More precisely, TnT-hs and NT-proBNP tests require a sandwich electrochemiluminescent immunoassay and were performed on the Cobas e801 analyzer (Roche diagnostics). Both creatine kinase (using an UV test) and CRP (using an immunoturbidimetry assay) measurement were performed on Cobas c701 (Roche Diagnostics). suPAR test (Virogates, Birkerod, Danemark) used an immunoturbidimetry assay performed on the Cobas c502 (Roche Diagnostics) with a measuring range between 1.8 and 16 ng/ml.

Soluble calprotectin (diluted 1 : 100) was analyzed using a R-plex Human Calprotectin Antibody Set (Meso Scale Discovery, ref: F21YB-3) according to the manufacturer's instructions. Each sample was assayed once and results out-of-range were excluded.

Procalcitonin, CT-pro-ET-1, and MR-proADM plasma concentrations were measured by an automated Kryptor analyzer, using a time-resolved amplified cryptate emission (TRACE) technology assay (Thermo Fisher Scientific™; Brahms AG, Hennigsdorf, Germany), with commercially available immunoluminometric assays previously described. The limits of detection for procalcitonin, MR-proADM, and CT-pro-ET-1 were 0.02 ng/ml, 0.08 nmol/l, and 0.4 pmol/l, respectively. The imprecision of those assays were lower than 10%.

Citrated plasma samples were used to measure levels of platelet factor 4 (PLF4/CXCL4) by ELISA using a commercially available kit (ab189573; Abcam, Cambridge, UK) according to the manufacturer's instructions.

Statistical methods

The study population characteristics including biomarkers were reported as percentages for categorical variables and medians (Q1–Q3) for continuous variables. As COVIDeF was a prospective cohort, no sample size calculation was performed. Biomarkers that were not measured were not imputed. Conversely, unquantifiable values were imputed with numerical values as follows: concentrations below the limit of detection were imputed with half of that limit, whereas those above the upper limit of analytical determination were imputed with the value of that limit. Factors associated with STW (among clinical characteristics and biomarkers concentrations) were first assessed in univariate analysis, using the Wilcoxon rank test, Fisher's exact test, or Pearson's chi-squared test depending to the type of variable. The biomarkers with a *P*-value of less than 0.2 from Wilcoxon rank tests were candidates for multivariable analysis.

LDH, platelets, and D-dimer were excluded from the analysis due to numerous missing values. To identify biomarkers predictive of STW, only complete cases (with all biomarker values available at baseline) were selected. Two multivariable approaches were used: a backward stepwise logistic regression model and a 'best subset' approach, which consisted of identifying the subset of covariates that provided the lowest mean squared error in leave-one-out cross validation.

First, we adjusted the logistic models without clinical variables. Second, the following variables were taken into account: age, sex, obesity (BMI > 30), and past medical history (chronic respiratory disease, cardiovascular disease, hypertension, diabetes, cancer, chronic renal disease, chronic neurological disorder). We calculated the area under the receiving operator characteristics (ROC) curve with 95% confidence interval (CI), as well as sensitivity and specificity at the Youden's cut-point.

Funding

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Table 1 Population characteristics at inclusion, according to the primary outcome (short-term worsening)

	COVID-19 short-term worsening		
Variable	No ($n = 451$)	Yes ($n = 60$)	*P* value
Age (years)			<0.0001[a]
Med (Q1–Q3)	58 (44–74)	69 (59–83)	
N (NA)	451 (0)	60 (0)	
Sex			0.1597[c]
Men	219 (48.56%)	35 (58.33%)	
Women	232 (51.44%)	25 (41.67%)	
BMI			0.0885[a]
Med (Q1–Q3)	25.3 (22.2–28.98)	27.3 (22.15–30.75)	
N (NA)	401 (50)	55 (5)	
Department at inclusion			0.2099[b]
Emergency	184 (40.20%)	25 (41.67%)	
Medical wards	232 (51.44%)	34 (56.67%)	
Other	35 (7.76%)	1 (1.67%)	
Past medical history			
Asthma	32 (7.08%)	4 (6.67%)	1.0000[b]
COPD	24 (5.31%)	11 (18.33%)	0.0010[b]
Chronic heart failure	23 (5.09%)	2 (3.33%)	0.7554[b]
Hypertension	157 (34.73%)	31 (51.67%)	0.0106[c]
Coronary arterial disease	37 (21.02%)	8 (21.62%)	0.9353[c]
Stroke	26 (14.69%)	9 (24.32%)	0.1496[c]
Diabetes	85 (18.81%)	26 (43.33%)	<0.0001[c]
Obesity	82 (18.14%)	15 (25%)	0.2027[c]
Renal disease	52 (11.5%)	16 (26.67%)	0.0011[c]
Organ or BM graft	15 (3.32%)	4 (6.67%)	0.2618[b]
Auto-immune disease	32 (7.08%)	7 (11.67%)	0.1997[b]
Immunosuppressive Tt	36 (7.96%)	8 (13.33%)	0.1633[c]
Chronic neurological disease	46 (10.18%)	14 (23.33%)	0.0029[c]
Cancer	54 (11.95%)	22 (36.67%)	<0.0001[c]
Ongoing treatments before inclusion			
Antibiotics	99 (21.9%)	21 (35%)	0.0244[c]
ACE or ARA2	75 (16.59%)	15 (25%)	0.1079[c]
Antidiabetic	50 (11.06%)	14 (23.33%)	0.0069[c]
Corticosteroid	14 (3.1%)	4 (6.67%)	0.1485[b]
Anticoagulant	81 (17.92%)	13 (21.67%)	0.4813[c]
Antiaggregant	49 (10.84%)	10 (16.67%)	0.1842[c]
Vital parameters at inclusion			
Pulse rate (bpm)			0.8090[a]
Med (Q1–Q3)	84 (74–94)	81 (71–96)	
N (NA)	402 (50)	57 (3)	
Systolic blood pressure (mmHg)			0.4809[a]
Med (Q1–Q3)	129 (116–142)	126 (116.75–138.25)	
N (NA)	383 (69)	56 (4)	
SpO2 (%)			0.0035[a]
Med (Q1–Q3)	97 (95–99)	96 (93–97.75)	
N (NA)	406 (46)	54 (6)	
Temperature (°C)			0.0297[a]
Med (Q1–Q3)	37 (36.6–37.5)	37.25 (36.6–38.08)	
N (NA)	405 (47)	58 (2)	
Respiratory rate (/min)			0.0018[a]
Med (Q1–Q3)	20 (18–24)	23 (20–24.5)	
N (NA)	334 (118)	47 (13)	
Duration of symptoms before day 0 (days)			0.7377[a]
Med (Q1–Q3)	7 (4–12)	8 (4–10)	
N (NA)	430 (22)	58 (2)	
CURB65 upon admission			0.0079[a]
Med (IQR)	1 (0–1)	1 (0.5–2)	
N (NA)	280 (171)	47 (13)	

ACE, angiotensin-converting enzyme inhibitors; ARA2, angiotensin II receptor antagonists; BM, bone marrow; bpm, beat per minute; COPD, chronic obstructive pulmonary disease; IQR, interquartile range; Med, median; *N*, number; NA, not available; SpO2, peripheral pulse oximetry; Tt, treatment.

[a]Wilcoxon rank test.

[b]Fisher's exact test.

[c]Pearson's Chi-squared test.

Publique – Hôpitaux de Paris and from the Fondation de France.

Results

The cohort flowchart is shown in Fig. 1. From 31 March 2020 to 27 November 2020, 1086 consecutive patients were included in the COVIDeF cohort. After the exclusion of participants with no or invalid informed consent, no available blood samples stored in the biobank, the 223 participants for whom the diagnosis of COVID-19 was not confirmed and the 305 COVID-19 patients who already met worsening criteria at inclusion, 511 patients were included in the analyses (75.14% PCR-confirmed).

Sixty patients (11.7%) experienced STW and 451 (88.3%) not. The median time (Q1–Q3) to worsening was 3 days [2–4] after inclusion and D0 first blood sampling: 52 (86.7%) patients worsened between D0 and D3, 6 patients between D4 and D7, and 2 after D7. Among them, 36 patients (60%) were admitted to ICU, 22 (36.7%) patients underwent noninvasive ventilation, 16 (26.7%) patients underwent mechanical ventilation, 28 (46.7%) developed ARDS, and 23 (38.3%) died.

The main characteristics of the patients are detailed in Table 1. Patients who experienced STW were older, had more often chronic obstructive pulmonary disease, diabetes, renal disease, chronic neurological disorder or cancer, were more often already treated with antibiotics at inclusion in the study, had higher temperature and respiratory rate, and lower peripheral pulse oximetry at inclusion.

Biomarkers measurement at inclusion

The distribution of the values of the studied biomarkers, according to the primary outcome, is reported in Table 2 (for usual blood tests performed during the routine care) and in Table 3 (for biomarkers specifically measured for this study). At admission, patients who finally experienced STW had lower values of eosinophils, lymphocytes, platelets, alanine aminotransferase, and higher values of neutrophils, creatinine, and urea (Table 2). Similarly, many biomarkers of interest exhibited higher values in STW patients (Table 3): CRP, TnT-hs, suPAR, NT-proBNP, calprotectin, procalcitonin, MR-proADM, and proendothelin. The correlations between the different biomarkers are reported in Supplementary material 1, Supplemental digital content 2, *http://links.lww.com/EJEM/A460* and the optimal threshold maximizing the Youden index for each biomarker in Supplementary material 2, Supplemental digital content 2, *http://links.lww.com/EJEM/A460*.

Multivariable analysis

The analysis was performed on 461 patients for whom all biomarkers' values were available, of whom 56 (12.1%) experienced STW. Due to numerous missing values, LDH, platelets, and D-dimer were excluded from the

Table 2 Distribution of routine blood tests values according to the primary outcome (short-term worsening)

Variable	COVID-19 short-term worsening			*P* value[a]
	No (*n* = 451)	Yes (*n* = 60)	Total	
Hemoglobin (g/dl)				0.193
Med (Q1–Q3)	12.9 (11.6–14.1)	12.55 (10.85–14.07)	12.8 (11.5–14.1)	
Mean (std)	12.77 (1.96)	12.42 (1.98)	12.72 (1.97)	
N (NA)	341 (110)	56 (4)	397 (114)	
WBC (×10⁹/l)				0.230
Med (Q1–Q3)	6.01 (4.5–7.78)	6.24 (4.7–9.2)	6.06 (4.52–7.97)	
Mean (std)	7.4 (17.09)	7.32 (3.74)	7.39 (15.9)	
N (NA)	341 (110)	56 (4)	397 (114)	
Neutrophils (×10⁹/l)				0.027
Med (Q1–Q3)	4 (2.69–5.77)	4.44 (3.14–7.49)	4.05 (2.78–5.93)	
Mean (std)	4.58 (2.78)	5.71 (3.44)	4.74 (2.9)	
N (NA)	336 (115)	54 (6)	390 (121)	
Eosinophils (×10⁹/l)				<0.0001
Med (Q1–Q3)	0.04 (0–0.1)	0 (0–0.02)	0.03 (0–0.09)	
Mean (std)	0.08 (0.12)	0.02 (0.05)	0.07 (0.12)	
N (NA)	335 (116)	54 (6)	389 (122)	
Lymphocytes (×10⁹/l)				0.007
Med (Q1–Q3)	1.3 (0.89–1.72)	0.96 (0.75–1.32)	1.26 (0.86–1.7)	
Mean (std)	2.22 (16.54)	1.1 (0.49)	2.07 (15.36)	
N (NA)	336 (115)	54 (6)	390 (121)	
Monocytes (×10⁹/l)				0.261
Med (Q1–Q3)	0.51 (0.35–0.67)	0.44 (0.34–0.63)	0.51 (0.35–0.67)	
Mean (std)	0.55 (0.28)	0.55 (0.36)	0.55 (0.29)	
N (NA)	335 (116)	54 (6)	389 (122)	
Platelets (×10⁹/l)				*0.001*
Med (Q1–Q3)	239 (176.5–307)	187 (146.75–248.5)	230.5 (168–297)	
Mean (std)	248.72 (100.5)	210.96 (103.4)	243.41 (101.63)	
N (NA)	341 (110)	56 (4)	397 (114)	
Creatinine (µmol/l)				0.007
Med (Q1–Q3)	72 (61–87)	86 (65–114.5)	74 (62–92)	
Mean (std)	83.33 (55.16)	103.34 (65.21)	86.17 (57.04)	
N (NA)	338 (113)	56 (4)	394 (117)	
Urea (nmol/l)				0.012
Med (Q1–Q3)	5 (3.8–6.7)	6 (4.05–9.55)	5.1 (3.8–6.97)	
Mean (std)	6.08 (4.57)	7.63 (4.89)	6.3 (4.64)	
N (NA)	337 (114)	56 (4)	393 (118)	
AST (UI/l)				0.776
Med (Q1–Q3)	37 (26–54)	37 (26–50)	37 (26–53)	
Mean (std)	45.91 (32.46)	40.58 (19.55)	45.13 (30.94)	
N (NA)	284 (167)	49 (11)	333 (178)	
ALT (UI/l)				0.006
Med (Q1–Q3)	29 (19–48.25)	22 (15–32)	28 (19–46)	
Mean (std)	41.23 (41.71)	27.37 (17.1)	39.22 (39.39)	
N (NA)	287 (164)	49 (11)	336 (175)	
Bilirubin (µmol/l)				0.253
Med (Q1–Q3)	8 (6–11)	8 (5–10)	8 (6–11)	
Mean (std)	9.93 (6.94)	9.35 (8.22)	9.84 (7.13)	
N (NA)	283 (168)	49 (11)	332 (179)	
Ferritin (µg/l)				0.948
Med (Q1–Q3)	595 (312.5–1219)	564 (320.75–1429)	581 (313–1228)	
Mean (std)	964.66 (1072.68)	1060.31 (1284.32)	977.81 (1100.88)	
N (NA)	164 (289)	26 (34)	188 (323)	
LDH (UI/l)				0.565
Med (Q1–Q3)	309 (239–407)	336 (250–407)	316 (241–408)	
Mean (std)	345.79 (146.38)	368.77 (172.08)	349.02 (150.09)	
N (NA)	213 (238)	35 (25)	248 (263)	
D-dimers (ng/ml)				0.198
Med (Q1–Q3)	720 (461–1380)	950 (582.5–1802.5)	750 (481–1410)	
Mean (std)	1289.27 (1652.85)	1384.03 (1291.53)	1304.69 (1597.26)	
N (NA)	174 (277)	34 (26)	208 (303)	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; std: standard deviation; WBC, total white blood cell count.
[a]Wilcoxon rank test.

models. The results of the model comprising all biomarkers is shown in Table 4. The backward stepwise logistic regression showed that three biomarkers were independently associated with worsening: CRP [adjusted odds ratio (aOR): 1.10, 95% CI: 1.06–1.15 for a 10-unit increase], procalcitonin (aOR: 0.42, 95% CI: 0.22–0.81), and MR-proADM (aOR: 2.85, 95% CI: 1.74–4.69). The area under the ROC curve (AUC), sensitivity, and specificity at the Youden's cut-point were respectively 0.73 (95% CI: 0.66–0.79), 0.64 (0.49–0.76), and 0.70 (0.54–0.80) for CRP, 0.69 (0.64–0.88), 0.80 (0.66–0.88), and 0.57 (0.42–0.65) for procalcitonin, and 0.75 (0.69–0.81), 0.68 (0.51–0.78), and 0.72 (0.57–0.78) for MR-proADM. The characteristics of this model, estimated by leave-one-out

Table 3 Distribution of the values of the biomarkers specifically measured for this study, according to the primary outcome (short-term worsening)

Variable	COVID-19 worsening			*P* value[a]
	No (*n* = 451)	Yes (*n* = 60)	Total	
Creatine kinase (UI/l)				0.411
Med (Q1–Q3)	80.5 (48.25–131)	90 (44–192.5)	81 (48–136)	
Mean (std)	152.68 (548.31)	453.2 (1960.37)	187.79 (846.78)	
N (NA)	446 (5)	59 (1)	505 (6)	
CRP (mg/l)				<0.0001
Med (Q1–Q3)	16.42 (2.9–57.74)	68.25 (23.84–139.46)	20.88 (3.26–67.4)	
Mean (std)	41.83 (58)	92.87 (85.28)	47.8 (63.84)	
N (NA)	446 (5)	59 (1)	505 (6)	
suPAR (ng/l)				<0.0001
Med (Q1–Q3)	5.2 (3.3–8.67)	8.4 (6–13.1)	5.6 (3.5–9.4)	
Mean (std)	7.33 (6.6)	10.98 (7.96)	7.76 (6.87)	
N (NA)	442 (9)	59 (1)	501 (10)	
TnT-hs (µg/l)				<0.0001
Med (Q1–Q3)	6.96 (3.37–15.4)	20.1 (9.01–35.92)	7.5 (3.56–18.05)	
Mean (std)	13.51 (20.45)	31.98 (37.69)	15.64 (23.79)	
N (NA)	445 (6)	58 (2)	503 (8)	
NT-proBNP (pg/ml)				0.0007
Med (Q1–Q3)	93.6 (34.73–322.5)	189 (66.15–1189.5)	104 (37.1–381.5)	
Mean (std)	505.53 (1463.18)	1496.46 (3571.75)	619.57 (1854.7)	
N (NA)	446 (5)	58 (2)	504 (7)	
Calprotectin (pg/ml)				<0.0001
Med (Q1–Q3)	497 044.06 (205 537.57–1 078 667.09)	1 022 879.83 (568 197.39–1 562 454.36)	547 277.96 (232 890.5–1 161 499.64)	
Mean (std)	926 201.97 (1 347 698.57)	1 263 808.08 (1 120 952.83)	965 489.47 (1 326 756.76)	
N (NA)	448 (3)	59 (1)	507 (4)	
Platelet factor 4 (ng/l)				0.170
Med (Q1–Q3)	1515.11 (801.18–3025.07)	1202.61 (770.2–2028.4)	1420.67 (796.28–3012.6)	
Mean (std)	2014.17 (1567.56)	1734.32 (1523.5)	1980.14 (1563.38)	
N (NA)	419 (32)	58 (2)	477 (34)	
Procalcitonin (ng/ml)				<0.0001
Med (Q1–Q3)	0.09 (0.06–0.16)	0.17 (0.1–0.27)	0.09 (0.06–0.18)	
Mean (std)	0.94 (10.33)	0.4 (0.96)	0.88 (9.72)	
N (NA)	449 (2)	59 (1)	508 (3)	
MR-proADM (pg/ml)				<0.0001
Med (Q1–Q3)	0.68 (0.49–0.98)	1.09 (0.79–1.65)	0.71 (0.51–1.1)	
Mean (std)	0.84 (0.65)	1.41 (1.01)	0.91 (0.72)	
N (NA)	448 (3)	59 (1)	507 (4)	
Proendothelin (pg/ml)				<0.0001
Med (Q1–Q3)	62.81 (49.33–82.59)	89.23 (64.69–131.1)	63.85 (50.18–87.21)	
Mean (std)	71.14 (39.25)	103.8 (58.65)	74.96 (43.2)	
N (NA)	446 (5)	59 (1)	505 (6)	

CRP, C-reactive protein; MR-proADM, mid-regional pro-adrenomedullin; NA, not available; NT-proBNP, N terminal pro-B natriuretic peptide; std: standard deviation; suPAR, soluble urokinase plasminogen activator receptor; TnT-hs, high sensitive troponin T.
[a]Wilcoxon rank test.

Table 4 Biomarkers associated to short-term worsening: multivariable analysis (*n* = 461)

Biomarker	Odds ratio	95% CI	*P* value
CRP (mg/l)	1.11	1.05–1.16	0.0001
suPAR (ng/l)	1.02	0.98–1.06	0.46
Troponin (µg/l)	1.01	0.99–1.02	0.38
NT-proBNP (pg/ml)	1.00	0.99–1.00	0.56
Calprotectin (pg/ml)	1.00	1.00–1.00	0.42
Platelet factor 4 (ng/l)	0.99	0.99–1.00	0.29
Procalcitonin (ng/ml)	0.45	0.22–0.89	0.02
MR-proADM (pmol/l)	2.55	1.01–6.44	0.04
Proendothelin (pmol/l)	0.99	0.99–1.01	0.91

CI, confidence interval; CRP, C-reactive protein; MR-proADM, mid-regional pro-adrenomedullin; NT-proBNP, N terminal pro-B natriuretic peptide; suPAR, soluble urokinase plasminogen activator receptor.

cross validation, are: accuracy 0.88, sensitivity 0.14, specificity 0.99, positive predictive value 0.61, and negative predictive value 0.89. A sensitivity analysis on the subpopulation of patients under 65 years of age is represented on Supplementary material 3, Supplemental digital content 2, *http://links.lww.com/EJEM/A460*.

After including also the clinical variables in the model, CRP (aOR: 1.09, 95% CI: 1.04–1.14 for a 10-unit increase), procalcitonin (aOR: 0.44, 95% CI: 0.22–0.91), and MR-proADM (aOR: 2.39, 95% CI: 1.26–4.53) remained independently associated with worsening, along with diabetes (aOR: 2.31, 95% CI: 1.09–4.91) and cancer (aOR: 2.65, 95% CI: 1.27–5.55), whereas age, sex, obesity, chronic respiratory disease, cardiovascular disease, hypertension, chronic renal disease, and chronic neurological disorder were not (Supplementary material 4, Supplemental digital content 2, *http://links.lww.com/EJEM/A460*).

Biomarkers associated with early hospital discharge

A total of 174 patients were discharged from the ED with no further hospital admission. The values of creatine

kinase, CRP, suPAR, troponin, procalcitonin, NT-proBNP, calprotectin, platelet factor 4, MR-proADM, proendothelin, LDH, and D-dimers were significantly lower in these (Supplementary material 5, Supplemental digital content 2, *http://links.lww.com/EJEM/A460*). The multivariate analysis showed that CRP, troponin, NT-proBNP, calprotectin, platelet factor 4, and MR-proADM were independently associated with ED discharge with no further hospital admission (Supplementary material 6, Supplemental digital content 2, *http://links.lww.com/EJEM/A460*).

Discussion

In this study, by systematically measuring a large panel of potential biomarkers in a prospectively collected biobank of COVID-19 patients, we identified three biomarkers independently associated with STW: CRP, MR-proADM, and procalcitonin. Due to the complex pathophysiology, it is not surprising that biomarkers exploring different host-response pathways may be useful for prognostication in COVID-19 patients. CRP is a nonspecific biomarker of the acute phase of inflammation that is used worldwide [20]. Although its prognostic value in sepsis has been a matter of debate [21], since the beginning of the SARS-CoV2 pandemic, numerous publications have regularly confirmed its association with COVID-19 severity [3–6,11,14]. Of note, Stringer *et al.* proposed a threshold of 40 mg/l to predict mortality in hospitalized COVID-19 patients, similar to the one we identified with the Youden index (Supplementary material 2, Supplemental digital content 2, *http://links.lww.com/EJEM/A460*) to predict STW.

High adrenomedullin levels have been associated with increased vasodilation and severity of illness, particularly in systemic inflammation and sepsis, and have been reported to perform better than procalcitonin or CRP for sepsis prognosis in the ICU [22,23]. More recently, plasma MR-proADM (a more stable circulating precursor of adrenomedullin) was also reported to be a promising prognostic biomarker in an unselected ED population having blood test (area under the ROC curve: 0.83 for 30-day mortality) and moreover to perform better than clinical scores for those with suspected infection [24,25]. Finally, Del Castillo *et al.* recently reported that using MR-proADM at ED's triage of infectious disease significantly reduced the rate of hospitalization by 20% [26]. Interestingly, the 0.87 pmol/l MR-proADM threshold that was applied is very close to the cut-off of 0.93 pmol/l identified through the Youden index in our study (Supplementary material 2, Supplemental digital content 2, *http://links.lww.com/EJEM/A460*).

Procalcitonin is considered as an emergency room biomarker of host-response to bacterial infection as well as a sepsis diagnostic and prognostic biomarker [27–29]. Procalcitonin values usually remain low (<0.25 ng/ml) during uncomplicated viral infection like flu [30], but higher concentrations have been reported in case of bacterial superinfection. In the early phase of the pandemic, it was confirmed that uncomplicated COVID-19 had low procalcitonin values but that higher concentrations could be observed in relation with the cytokine storm that can complicate the course of disease in several patients [9,31]. We confirm here that procalcitonin values are usually low in COVID-19 [median: 0.09 (0.06–0.18), Table 2] and that higher concentrations are associated with the risk of worsening [median: 0.17 (0.1–0.27) in STW patients vs 0.09 (0.06–0.16) in the others].

Other biomarkers such as suPAR have been reported to be associated with COVID-19 severity and even more have been proposed to identify patients eligible for anti-inflammatory targeted biologics therapy [7,12,13,32]. In our study, although suPAR values were higher in STW patients on univariate analysis, it did not appear to be independently associated with worsening when all biomarkers tested were taken into account (multivariable analysis).

This was also the case for troponin, calprotectin, and NT-proBNP, for which concentrations have been reported to increase in severe COVID-19 cases [4,11,16,17,33]. Of note, several previous studies focused on a single specific biomarker [12,16,33,34] without applying a multimarker approach, which is more appropriate for the complex pathophysiology encountered during COVID-19. Indeed, we report that several biomarkers were correlated (Supplementary material 1, Supplemental digital content 2, *http://links.lww.com/EJEM/A460*).

Moreover, comparing our results to previous published cohort studies is difficult because of small sample size in some of them, heterogeneity in the definition of worsening, time of sampling (ED admission, ICU, wards), and particularly the mixed population of already severe COVID-19 and patients who will worsen.

However, Hodges *et al.* also reported that CRP and procalcitonin were associated with mortality or ICU admission in a retrospective study of 1310 hospitalized COVID-19 patients of mixed origin, along with leucocytes, urea, troponin, and D-dimer [4]. However, due to the retrospective design, there were numerous missing values for D-dimer, troponin, and procalcitonin. In present study, we choose to retain only the patients for whom we had all the biomarkers concentrations available. Cen *et al.* reported that procalcitonin, urea, α-hydroxybutyrate dehydrogenase, and D-dimer were associated with worsening, along with numerous clinical variables and comorbidities, in a retrospective cohort of 1007 hospitalized COVID-19 patients [15]. In our study, CRP, procalcitonin, and MR-proADM outperformed the usual clinical variables and routine blood tests associated with COVID-19 STW, in that only cancer and diabetes were independently associated with worsening, whereas age, sex, cardiovascular disease,

hypertension, obesity, chronic renal disease, chronic neurological disease, or chronic respiratory disease – the main risk factors reported in the literature – were not (Tables 2 and 3, Supplementary material 4, Supplemental digital content 2, *http://links.lww.com/EJEM/A460*).

Limitation

This study presented some limitation. First the exclusion of D-dimer, LDH, and platelets due to a too high rate of missing values. Second, the relatively small sample and low rate of patients meeting the worsening criteria with subsequent large confidence intervals. Therefore it cannot be excluded that a larger sample size would have yielded other significant associations between worsening and other parameters among those that did not reach significance in the multivariable model.

Third, we decided to exclude the patients already meeting severity criteria before blood sampling, although measurement of the biomarkers tested would had been interesting.

Fourth, although there was an independent association between CRP, MR-proADM, procalcitonin, and COVID-19 worsening the performances of the three biomarkers were moderate, with an AUC below 0.80. Additional studies are warranted to determine their usefulness to triage patients at risk of worsening.

Conclusion

In this multicenter prospective study that assessed a large panel of biomarkers in COVID-19 patients, CRP, procalcitonin, and MR-proADM were independently associated with the risk of STW.

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Conflicts of interest

M.C.A. received travel and congress fees reimbursement from bioMerieux. P.H. received consultant fees from Beckman Coulter, lecture honoraria from Beckman Coulter, Thermo Fisher, Siemens, Abbott, bioMerieux, and support for attending meeting from bioMerieux. For the remaining authors, there are no conflicts of interest

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