Journal of Infection and Public Health 17 (2024) 102589

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: www.elsevier.com/locate/jiph

Original Article

Circulating inflammatory cytokines predict severity disease in hospitalized COVID-19 patients: A prospective multicenter study of the European DRAGON consortium

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ARTICLE INFO

Article history:
Received 5 March 2024
Received in revised form 28 October 2024
Accepted 4 November 2024

Keywords:
COVID-19
Biomarker
Disease severity
Cytokines

ABSTRACT

Background: COVID-19 has put a huge strain on the healthcare systems worldwide, requiring unprecedented intensive care resources. There is still an unmet clinical need for easily available biomarkers capable of predicting the risk for severe disease. The main goal of this prospective multicenter study was to identify biomarkers that could predict ICU admission and in-hospital mortality.
Methods: We prospectively recruited COVID-19 PCR positive patients in two hospitals, in Belgium and Italy. Blood samples were collected at hospital admission and 20 potential biomarkers were measured with the Luminex technology. Logistic regression models were performed to identify the biomarkers that, alone or together, were associated with patient disease severity.
Results: Our study demonstrates that elevated levels of circulating inflammatory cytokines were associated with disease severity in COVID-19 hospitalized patients. CXCL10, IL-4, IL-6 and MCP-1 values were predictive of ICU admission. Elevated levels of IL-6 and MCP-1 were also associated with in hospital death in COVID-19 hospitalized patients.
Conclusion: Altogether, elevated and correlated inflammatory cytokines in the blood of COVID-19 patients at hospital admission are predictive of disease severity and suggest a dysregulated inflammation induced by SARS-CoV-2 infection.

Introduction

In December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) was detected in Wuhan (China) and was

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https://doi.org/10.1016/j.jiph.2024.102589

identified as the etiological agent of the coronavirus infectious disease 2019 (COVID-19). Since, COVID-19 became a pandemic disease and a global public health issue, responsible for the death of more than 7 million people worldwide till January 2024. Next to asymptomatic SARS-CoV2 infections, the World Health Organization (WHO) guidelines divided COVID-19 patients into mild, moderate or severe based of clinical presentation [1]. Most infected patients experienced mild to moderate disease but 15 % of the infected patients developed severe symptoms and required oxygen support [1,2]. In the critical cases (up to 5 %), patients exhibited a severe disease requiring high flow oxygen therapy and in the most severe form, a respiratory failure typically recognized as an acute respiratory distress syndrome (ARDS) [1,2]. Out of the respiratory insufficiency, cytokine storm could lead to multiple complications mimicking septic shock with an increased risk of thromboembolism, and in the most extreme forms in a multi-organ failure.

As a result, COVID-19 has put a huge strain on the healthcare systems worldwide, requiring unprecedented intensive care resources. In this context, the identification of early biological prognostic markers is a major research focus. Nowadays, there is still an unmet clinical need for easily available biomarkers capable of predicting the risk for severe disease, including intensive care unit (ICU) admission and in-hospital mortality. Specific biomarkers can help guide patient management and clinical workflow, allowing early referral to the most appropriate unit and administration of specific targeted therapies.

On one hand, imaging-based biomarkers have been extensively explored in multiple studies to help clinicians to identify patients with more advanced disease [3,4]. On the other hand, the study of circulating biomarkers is non-invasive and they reflect inflammation and organ damage [5]. Accumulating evidence indicates that severe COVID-19 is associated with immune dysregulation [6]. Hyperinflammation has been identified as one of hallmark of the COVID-19 immune response that predispose to severe disease [7]. The cytokine storm, a state of uncontrolled release of inflammatory cytokines, is also thought to be involved in severe COVID-19 [8]. Therefore, inflammatory-associated adhesion proteins and cytokines and may provide useful prognostic information. The goal of the study was to identify early biomarkers that could predict ICU admission and in-hospital mortality in COVID-19 patients. We analyzed cytokines and chemokines involved in inflammation, leukocyte recruitment and markers involved in lung fibrosis and scarring to assess the whole disease pathway, from epithelial injury to recovery [9–18]. The secondary objective was to map the heterogeneity of inflammatory proteins in COVID-19 infection.

Material and methods

We present here the results of a prospective multicenter study, part of the DRAGON consortium (DRAGON for The RapiD and SecuRe AI enhAnced DiaGnosis, Precision Medicine and Patient EmpOwerment Centered Decision Support System for Coronavirus PaNdemics, https://europeanlung.org/dragon/). The DRAGON consortium was initiated in October 2020 with the main objective of providing new perspectives of patient stratification with innovative tools and approaches [19,20]. As a result, a prototype of a multifactorial decision support system has been developed that can integrate patient input with clinical, imaging, and molecular phenotyping data (the article is under review).

Patient cohort and study design

We prospectively recruited COVID-19 patients hospitalized in two hospitals, namely Careggi University Hospital (Florence, Italy) and University Hospital Center of Liège (Liège, Belgium). Patients were recruited in two centers, in Belgium and Italy. Inclusion criteria were 1) patient aged 18 years and older; 2) patient able to consent to inclusion; 3) PCR-proven COVID-19 infection within 48 h; 4) hospitalized for COVID-19 infection. The first enrolled patient was hospitalized on the 14th of March 2020 and the last patient left the hospital on the 23rd of February 2022. In total, blood samples of 301 patients were collected, 50 patients were recruited in Italy and 251 patients in Belgium. Day 0 was considered as the day of the first positive SARS-CoV2 nucleid acid amplification test (NAAT).

ICU admission criteria were either compromised respiratory exchanges (PaO2/FiO2 ratio less than 100, corresponding to severe ARDS) or compromised respiratory dynamics (high respiratory rate at rest, paradoxical abdominal breathing, or asynchrony with non-invasive ventilation), associated with intubation. Similarly, patients with hemodynamic instability (hypovolemic/hemorrhagic shock, septic shock, severe renal or hepatic failure) and patients with altered consciousness were admitted to ICU.

Biomarker measurement

Blood samples for analysis of biological parameters were collected within the first 72 h of COVID-19 positive testing. The time between the positive COVID-19 NAAT and the biomarker blood tests is depicted in Table S1. Serum was extracted and stored at −80 °C before cytokine quantification. The choice of 20 inflammatory cytokines and adhesion proteins as potential biomarker was based on literature review [9–18]. The level of the 20 selected biomarkers were measured using the Human Inflammation 20-Plex ProcartaPlex Panel (Invitrogen by Thermo Scientific, Belgium) with the Luminex xMAP (multi-analyte profiling) technology on the MAGPIX instrument (Biotechne). All samples were analyzed at a single site, the clinical chemistry laboratory of the CHU Liège hospital. Biomarkers were analyzed by groups of 40, called “runs”. The limit of detection (LOD) values were determined during the validation process.

Statistical analysis

A power analysis was performed to determine the appropriate sample size for a biomarker study in COVID-19 patients. In order to take into account low concentrations of biomarkers, in statistics and calculations, the measurements below LOD were replaced by a random value coming from a triangular distribution between zero and the threshold. A logarithmic transformation of the variables has been made for statistical analyses, for all biomarkers and most of biological parameters.

For descriptive statistics, qualitative variables were described with a frequency table (number and percentage) while continuous quantitative variables were described with median and quartiles (Q1; Q3) and extreme values (min; max). The group homogeneity for qualitative variables was tested using either the Chi-square test or Fisher’s exact test. ANOVA test (or nonparametric Kruskal-Wallis test if necessary) was used to compare continuous variables. Tukey test was used for pairwise mean comparison of continuous variables. Chi-square test or Fisher’s exact test were used for pairwise mean comparison of qualitative variables. The association between biomarkers was quantified with the Pearson correlation coefficient based on log-transformed serum levels. A principal component analysis (PCA) was performed to summarize the variability of all measurements.

For biomarker analyses, simple and multiple logistic regression models were performed to identify the biomarkers associated alone or together to disease severity. The significant effect of the variable was summarized with the odds ratio (OR), 95 % confidence interval (95 % CI) and the p-value. Haldane correction and Firth logistic regression were used when OR was not calculable for simple logistic regressions.

To validate the statistic models, the samples were randomly divided in two groups, the training dataset (n = 201) and the validation dataset (n = 100). The model validation was based on the area under the ROC curve (AUC) and on the Hosmer and Lemeshow test, which allows to evaluate the adjustment of the logistic models on the two datasets.

Results with a p-value of less than 0.05 were considered statistically significant. However, to take the multiple testing into account, p-values were compared to the Bonferroni-corrected significance thresholds, which are mentioned in the figure legends.

Calculations were performed with the 9.4 SAS/STAT® analytic software and the graphs were made with the 4.3.1 R software.

Ethical considerations

All procedures were performed in compliance with relevant laws and institutional guidelines and have been approved by the appropriate institutional committees. The protocol was approved by the ethics committee of the University Hospital of Liege (reference number 2021/89) and the ethics committee of the UNFI (#18085/OSS). Informed consent was obtained for every participant.

Results

Blood inflammatory proteins were analyzed to identify biomarkers predictive of ICU admission and in-hospital death in patients with COVID-19.

Patient characteristics

Blood samples of hospitalized patients were collected in the DRAGON study. Patient characteristics are listed in Table 1. The median age of the study population was 67 years old, 195 were male (64.8 %) and 106 were female (35.2 %). High blood pressure (64.8 %) and diabetes (45.8 %) were the most common comorbidities. 123 patients (40.9 %) were admitted to ICU and amongst them, 88 (71.5 %) underwent mechanical ventilation. The median length of hospital stay was 13 days. Unfortunately, 73 patients (24.2 %) died in hospital. The most common symptoms observed at day 0, which corresponds to the day of the first positive SARS-CoV2 PCR test, were cough (80.4 %), dyspnea (72.8 %) and fever (72.6 %). Biological parameters are described in supplemental Table S2.

Table 1
Patient demographic and clinical characteristics.

	n	n (%)	Med (Q1; Q3)	Min; Max
Demographics				
Age (years)	301		67 (57; 75)	26; 104
BMI (kg/m²)	280		27.9 (24.9; 31.3)	12.8; 58.8
Gender, male	301	195 (64.8)		
Smoking status	264			
Nonsmoker		208 (78.8)		
Ex-smoker		30 (11.4)		
Current		26 (9.8)		
Cancer	301	44 (14.6)		
Chronic renal disease	235	35 (14.9)		
Chronic pulmonary disease	262	60 (22.8)		
Diabetes	264	121 (45.8)		
Type 2 diabetes (vs type 1)	50	47 (94.0)		
High blood pressure	287	186 (64.8)		
Center, UNIFI (vs CHU)	301	50 (16.6)		
Delay from March 2020	301			
1st wave*		148 (49.2)		
Later		153 (50.8)		
Hospitalization				
Hospitalization (days)	301		13 (8; 28)	0; 530
ICU (yes vs no - days)	301	123 (40.9)	14 (7; 27)	7; 164
Ventilation in ICU (yes vs no - days)	123	88 (71.5)	16.5 (9; 26)	1; 138
Death in hospital (yes vs no)	301	73 (24.2)		
Symptoms at day 0				
Cough	92	74 (80.4)		
Dry cough	57	34 (59.6)		
Wet cough	57	14 (24.6)		
Dyspnea	92	67 (72.8)		
Fever	102	74 (72.6)		
Nausea	62	19 (60.6)		
Myalgia	66	30 (45.5)		
Diarrhea	69	26 (37.7)		
Headaches	67	24 (35.8)		
Rhinorrhea	62	19 (30.6)		
Pharynx pain	56	10 (17.9)		
Chest pain	72	12 (16.7)		
Vomiting	63	6 (9.5)		

Qualitative variables are described with a frequency table (number and percentage). Continuous quantitative variables are described with median and quartiles (Q1; Q3). n = 301. BMI: body mass index, ICU: intensive care unit. *The first wave is defined by hospitalization before 15 May 2020. BMI: body mass index, ICU: intensive care unit.

Inflammatory biomarker levels in COVID-19 hospitalized patients

To identify biomarkers predictive of COVID-19 severity, the serum levels of 20 selected inflammatory biomarkers were measured with the Luminex technology, in hospitalized COVID-19 patients (see the time between the positive COVID-19 NAAT and the biomarker blood test in Table S1). Protein blood values are provided in Table S3. We wondered if inflammatory biomarkers were positively correlated. We observed that most markers were closely associated (see Fig. S1). TNFα was significantly correlated with most markers and revealed the highest correlations with IL-1β, IL-17A and IFNγ (r = 0.63, 0.62 and 0.50, respectively). Additionally, CXCL10 was significantly correlated with IL-4 (r = 0.34, $p < 0.0001$), IL-6 (r = 0.19, p = 0.0007) and MCP-1 (r = 0.44, $p < 0.0001$). A highly significant correlation was also observed between IL-4 and IL-6 (r = 0.30, $p < 0.0001$) and between IL-4 and MCP-1 (r = 0.27, $p < 0.0001$). IL-6 and MCP-1 were significantly correlated, too (r = 0.37, $p < 0.0001$). It is worth noting that GM-CSF and ICAM-1 showed poor association with the other inflammatory markers, GM-CSF being detected in the blood of 2.7 % patients. Principal component analysis (PCA) was used to summarize all measurements in two dimensions (Fig. S2). PC1 was defined by a high value of all biomarkers except GM-CSF and explained 25.4 % variability of all biomarkers. PC2 distinguished 2 groups of biomarkers with opposed effect and explained 9.9 % variability of all biomarkers (Fig. S2). In summary, all inflammatory markers quantified in the serum of hospitalized COVID-19 patients were found to be correlated. High values of all biomarkers were summarized with PC1.

Inflammatory biomarkers predictive of ICU admission

First, we analyzed the predictive effect of circulating inflammatory biomarkers on disease severity by looking at ICU admission. To validate the statistic model, the samples were randomly divided in two groups, the training dataset (n = 201) and the validation dataset (n = 100). After confirming the homogeneity between the two datasets (Table S4), analyses were performed on the training dataset.

Multiple cofactors other than the inflammatory cytokines could influence disease severity and ICU hospitalization. Hence, we started by studying the association between the cofactors and the probability of ICU admission. Demographics, comorbidities and biological parameters at day 0 were considered as cofactors. Simple logistic regression revealed that CRP ($p < 0.0001$), LDH ($p < 0.0001$), TGO ASAT ($p < 0.0001$) and TGP ALAT (p = 0.0004) blood levels at day 0 were statistically associated with ICU admission (Table S5). Trends were observed for BMI, chronic renal disease and diabetes status

Table 2
Individual predictive effect of the biomarkers on ICU admission.

Biomarker [a]	OR	CI95%	p-value [b]
CD62E	2.19	1.20 ; 3.99	0.011
CD62P	0.95	0.64 ; 1.41	0.80
CXCL10	1.75	1.27 ; 2.42	**0.0007**
GM-CSF	0.93	0.48 ; 1.78	0.82
ICAM-1	1.45	0.98 ; 2.15	0.061
IFNα	1.21	0.78 ; 1.88	0.39
IFNγ	1.80	0.99 ; 3.25	0.053
IL-1α	1.49	1.04 ; 2.13	0.031
IL-1β	1.30	0.83 ; 2.03	0.25
IL-4	3.93	2.05 ; 7.55	**<0.0001**
IL-6	2.07	1.41 ; 3.04	**0.0002**
IL-8	1.10	0.87 ; 1.38	0.42
IL-10	1.02	0.66 ; 1.59	0.91
IL-12p70	1.21	0.68 ; 2.14	0.52
IL-13	2.19	0.99 ; 4.85	0.052
IL-17A	2.20	1.22 ; 3.96	0.0087
MCP-1	1.83	1.30 ; 2.59	**0.0006**
MIP-1α	0.89	0.66 ; 1.19	0.42
MIP-1β	1.03	0.65 ; 1.61	0.91
TNFα	1.76	0.95 ; 3.25	0.071

Individual predictive effect with demographical cofactor adjustment by logistic regression. Log-transformed biomarker serum values. All cofactor information was provided for 163 patients amongst the 201 patients in the training dataset. n = 163. OR: odds ratio, CI: confidence interval. a: pg/ml, log-transformed values. b: adjusted 5 % threshold with Bonferroni correction is 0.05/20 = 0.0025. If p < 0.0025, it is considered as statistically significant.

(Table S5). Then, logistic regression models adjusting for covariates separated for each biomarker were used to determine the individual effect of biomarkers on ICU admission (Table 2). Taking demographic covariates into account, a significant association between ICU admission and the circulating level of four inflammatory cytokines was observed, namely CXCL10, IL-4, IL-6 and MCP-1 (p = 0.0007, p < 0.0001, p = 0.0002, p = 0.0006, respectively). The circulating levels of these four inflammatory markers in ICU vs non-ICU patients are depicted in Fig. 1A-D. In addition, the ROC curves were calculated, demonstrating the good discriminative ability of the 4 biomarkers (Fig. 1E). The AUC of CXCL10, IL-4, IL-6 and MCP-1 roc curves were respectively 73.8 %, 75.7 %, 77.9 % and 72.3 %.

All these models analyze the individual effect of each biomarker on ICU admission. A joint analysis was also conducted to consider the combined effect of the biomarkers. To avoid collinearity due to the correlation between most of the biomarkers and cofactors, a selected joint model with PC1 was applied instead of all biomarkers. After adjustment with the COVID-19 wave, BMI, CRP and TGO ASAT, we can conclude that elevated biomarker levels (PC1) were associated with ICU admission, although not statistically significant (p = 0.067, OR= 1.20) (data not shown).

Finally, the Hosmer and Lemeshow test was performed on the validation dataset and allowed to validate the above-mentioned results (Table S6). Altogether, our study shows that elevated levels of circulating inflammatory cytokines were associated with disease severity in COVID-19 hospitalized patients. CXCL10, IL-4, IL-6 and MCP-1 values were predictive of ICU admission.

Inflammatory biomarkers predictive of in-hospital death

Second, we analyzed the predictive effect of the same circulating inflammatory biomarkers on mortality during the hospital stay using the same procedure. We used the same training and validation datasets, as well as the same statistical method. As for ICU admission, multiple cofactors are associated with COVID-19 mortality, including comorbidities, demographical and biological parameters. Simple logistic regression identified chronic renal disease as a cofactor significantly associated with mortality in COVID-19 hospitalized patients (p < 0.0001). Age was also a risk factor associated with death from COVID-19, although, due to the multiple testing correction, not statistically significant in our training cohort (p = 0.0030, Table S7). Using a logistic regression model adjusted with demographic covariates, we found that IL-6 and MCP-1 cytokines were significantly associated with in-hospital mortality (p < 0.0001, p = 0.0003, respectively, see Table 3). The IL-6 and MCP-1 circulating levels at day 0 in patients who died vs survived during their hospital stay is depicted in Fig. 2A-B. We also generated the ROC curve (Fig. 2C), in which the AUC of IL-6 is shown, demonstrating a very good predictive effect of COVID-19 in-hospital death (IL-6 AUC=80.9 % and MCP-1 AUC=76.2 %).

To study the joint effect of the biomarkers on the probability of dying in hospital after COVID-19 hospitalization, and to avoid the collinearity bias due to the correlation between the biomarkers, selected joint models with PC1 were applied. They did not reveal any significant data (data not shown).

It suggests that PC1 alone is not predictive of in-hospital death, which means that mortality is already explained by other demographic cofactors rather than by elevated inflammatory cytokines alone.

Finally, the Hosmer and Lemeshow test was performed on the validation dataset and allowed to validate the above-mentioned results (Table S8). Altogether, our study shows that elevated levels of the circulating inflammatory cytokines IL-6 and MCP-1 were associated with in-hospital death in COVID-19 hospitalized patients.

Discussion

This prospective multicenter study investigated circulating inflammatory cytokines in hospitalized COVID-19 patients at hospital admission. The objective was to identify biomarkers that were predictive of ICU admission and in-hospital mortality. The secondary objective was to map the heterogeneity of inflammatory proteins in COVID-19 infection. Our study demonstrates that elevated levels of circulating inflammatory cytokines were associated with disease severity in COVID-19 hospitalized patients. Precisely, CXCL10, IL-4, IL-6 and MCP-1 values were predictive of ICU admission. Furthermore, elevated levels of IL-6 and MCP-1 were also associated with in-hospital death in COVID-19 hospitalized patients.

The predictive value of IL-6 blood levels on COVID-19 severity is in line with previous studies. Indeed, increased levels of IL-6 have been associated to COVID-19 severity in several articles [21–24]. IL-6 blood levels were found to be predictive of ICU admission [25–28] and in-hospital mortality [25] when measured at hospital admission, at the RNA and protein level.

In addition to IL-6, we observed that elevated levels of MCP-1 were associated with COVID-19 severity [29]. MCP-1, also known as CCL2 (chemokine ligand 2), is a key chemokine that regulates the migration of monocytes and macrophages [30]. Years ago, increased MCP-1 blood levels were observed in SARS-CoV-1 patients [31] and in MERS-CoV patients admitted to ICU [32]. During the COVID-19 pandemic, higher levels of MCP-1 were found in critically ill patients compared to severe COVID-19 patients, indicating a predictive value of mortality [29,33,34]. As well, increased MCP-1 blood levels were observed in patients with respiratory failure and in those who were admitted to ICU [27,33]. Notably, the MCP-1 increase was not as high as in other acute inflammatory cytokine-associated diseases such as sepsis [35]. Our study strengthens the predictive value of IL-6 and MCP-1 levels on COVID-19 severity with our large multicentric cohort.

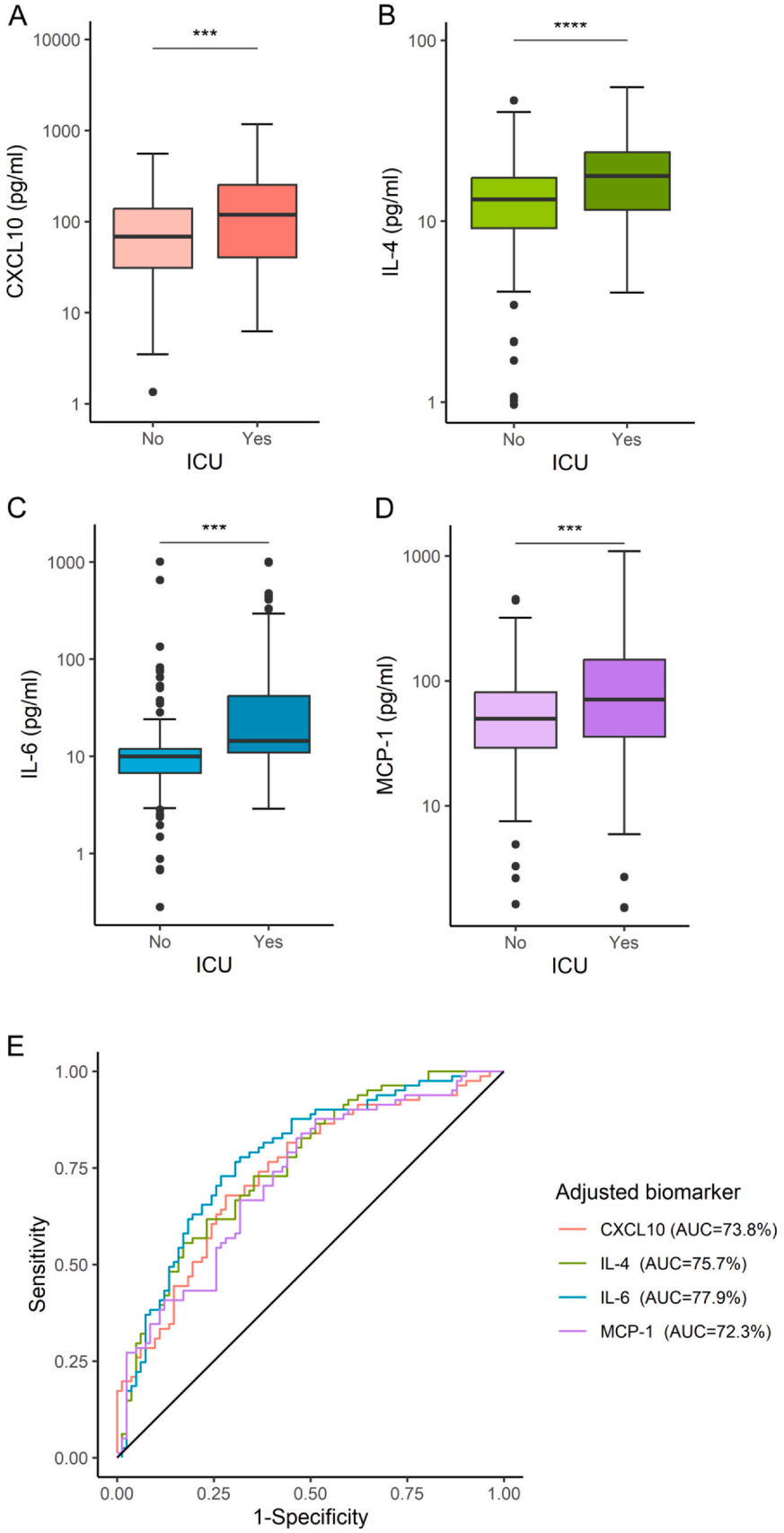

Fig. 1. Predictive effect of CXCL10, IL-4, IL-6 and MCP-1 circulating levels on ICU admission. CXCL10 serum level in ICU vs non-ICU hospitalized COVID-19 patients (A). IL-4 serum level in ICU vs non-ICU hospitalized COVID-19 patients (B). IL-6 serum level in ICU vs non-ICU hospitalized COVID-19 patients (C). MCP-1 serum level in ICU vs non-ICU hospitalized COVID-19 patients (D). ROC curve analysis of the predictive effect of CXCL10, IL-4, IL-6 and MCP-1 circulating levels on ICU admission (E). *p < 0.05, **p < 0.01, ***p < 0.001, **** p < 0.0001. (A-D) n = 301. AUC: area under the curve, ICU: Intensive care unit, IL: interleukin, MCP: Monocyte chemoattractant protein.

Table 3
Individual predictive effect of the biomarkers on in-hospital death.

Biomarker [a]	OR	CI95%	p-value [b]
CD62E	1.29	0.69 ; 2.41	0.43
CD62P	0.84	0.29 ; 2.43	0.75
CXCL10	1.48	1.05 ; 2.07	0.024
GM-CSF	1.15	0.54 ; 2.46	0.72
IFNα	2.04	1.20 ; 3.47	0.0082
IFNγ	0.89	0.47 ; 1.66	0.71
ICAM-1	1.21	0.76 ; 1.90	0.42
IL-1α	1.20	0.81 ; 1.78	0.36
IL-1β	0.61	0.37 ; 1.01	0.055
IL-4	1.44	0.77 ; 2.70	0.25
IL-6	2.00	1.42 ; 2.82	**<0.0001**
IL-8	1.17	0.89 ; 1.54	0.25
IL-10	2.39	1.33 ; 4.30	0.0036
IL-12p70	0.70	0.38 ; 1.28	0.24
IL-13	0.89	0.38 ; 2.06	0.78
IL-17A	1.37	0.70 ; 2.70	0.36
MCP-1	2.11	1.40 ; 3.18	**0.0003**
MIP-1α	1.06	0.74 ; 1.51	0.74
MIP-1β	0.94	0.53 ; 1.67	0.84
TNFα	1.08	0.52 ; 2.23	0.83

Individual predictive effect with demographical cofactor adjustment by logistic regression. Log-transformed biomarker serum values. All cofactor information was provided for 163 patients amongst the 201 patients in the training dataset. n = 163. OR: odds ratio, CI: confidence interval. a: pg/ml, log-transformed values. b: adjusted 5 % threshold with Bonferroni correction is 0.05/20 = 0.0025. If $p < 0.0025$, it is considered as statistically significant.

The literature is less unanimous regarding circulating IL-4 in COVID-19 patients. We observed increased IL-4 serum levels in ICU patients compared to patients who were not admitted to ICU. Our results support another study showing that IL-4 blood levels were correlated to SARS-CoV2 viral load and increased in severe COVID-19 cases compared to mild disease [23]. Higher IL-4 levels were also observed in patients with respiratory failure and patients admitted to ICU [33]. However, some researchers did not observe any significant difference in IL-4 blood levels between ICU or non-ICU patients or between COVID-19 survivors and non survivors [23,24]. A meta-analysis revealed that IL-4 serum levels were increased in COVID-19 patients compared to healthy patients but did not find any difference between severity groups [25]. The discrepancies might be explained by different cohort sizes, different timing of samples and criteria used to define COVID-19 severity.

Additionally, we observed that CXCL10 serum levels were predictive of ICU admission in hospitalized patients. CXCL10, or IFNγ-induced protein (IP-10), is a pleiotropic cytokine inducing the chemotaxis of CXCR3 cells, apoptosis and angiogenesis [36]. CXCL10 increased levels were observed in severe COVID 19 patients compared to mild disease [12,26]. As well, CXCL10 elevated levels were associated to COVID-19 mortality and ICU admission [27–29]. In prospective studies, cytokines levels were measured at hospital admission of COVID-19 patients and the researchers found that CXCL10 was predictive ICU admission and in-hospital death [37,38]. In line with our observations, CXCL10 is part of an inflammatory signature in COVID-19 hospitalized patients with other cytokines and chemokines. It has been suggested that CXCL10 might be a key regulator of the cytokine storm in COVID-19 patients [39,40]. Importantly, Coperchini and colleagues proposed that IL-6, CXCL10 and infiltrating macrophages would have a crucial role in the COVID-19 cytokine storm [39]. Laing et al. also observed increased IL-6 and CXCL10 as part of an immune signature anticipating clinical progression [40]. Our results showing that IL-6 and CXCL10 elevated levels are predictive of COVID-19 severity corroborate these studies.

In terms of statistical analysis, this article presents results based on imputation with triangular distribution. Analyses were also performed using a classic 0.5 *LOD imputation for undetected concentrations and the results remained consistent, which confirms our conclusions. Moreover, we evaluated the type and proportion of missing values for the cofactors and we performed additional analyses to ensure that the missing values have no impact on our conclusions.

Our study has certain limitations. The first one is the nature of the dataset. This study only included hospitalized patients. Consequently, the predictive biomarkers and the observed dysregulated inflammation presented here only apply to hospitalized patients. Second, the data were not compared or validated with healthy patient biomarker values. No clinical data were available for our healthy cohort; hence, we decided not to include them in the study. Third, the Italian cohort and the Belgian cohort were not homogeneous. The patients included in the Italian cohort were generally in a better condition, with less diabetes, chronic renal insufficiency, chronic pulmonary disease than in the Belgian cohort. Importantly, no patient in the Italian cohort was admitted to ICU and the hospitalization length of stay was shorter in the Italian cohort. To avoid any bias due to the heterogeneity between the exploratory and the validation cohorts, the three cohorts were mixed then, the samples were randomly divided in two groups, the training and the validation datasets. Homogeneity between the two datasets was tested and confirmed. The statistical analyses were performed on the training and validation datasets rather than on the exploratory and validation cohorts. The fourth limitation concerns the timing of the blood tests. On one hand, biomarker measurements were made with a serum sample taken at hospital admission, not at symptom onset, which would be better for a predictive biomarker. On another hand, cytokine levels were measured only once, at hospital admission. Therefore, our results do not consider the disease dynamic. It would have been interesting to perform cytokine measurement at other timepoints to involve the biomarker kinetics in the predictive analysis. Moreover, cytokine kinetics would have been important for the evaluation of the inflammation dysregulation, which has been linked to COVID-19 [22,24]. The fifth limitation of the study is the lack of information on the COVID-19 strain. This information was not available in our dataset. Given the evolution of the strain dominance during the recruitment period of the patients, this could have influenced the results of the study. Alternatively, we considered the wave in which the patients were hospitalized (first wave vs later, Table 1) as a patient characteristic and included it in our analyses. To verify that the wave did not influence the results of our study, we tested the predictive effect of the cofactors on ICU admission and in-hospital death (including the wave). The tests showed no significant effect of the wave on either ICU admission or in-hospital death (see Tables S5 and S7).

On the other hand, this study has strengths that increase its reliability compared to the previously published results mentioned above. Mainly, we studied a large cohort of more than 300 patients, recruited in two different countries. We present a combination of 4 cytokines that are predictive of ICU admission, two of which are also predictive of in-hospital death, strengthening the coherence of their predictive value. Consequently, a simple test quantifying only 4 cytokines would predict disease severity and improve the allocation of critical care resources.

To conclude, our multicentric prospective study identified CXCL10, IL-4, IL-6 and MCP-1 as biomarkers predictive of ICU admission in COVID-19 hospitalized patients. IL-6 and MCP-1 could also predict in-hospital mortality. We observed elevated and correlated inflammatory cytokines in the blood of COVID-19 patients at

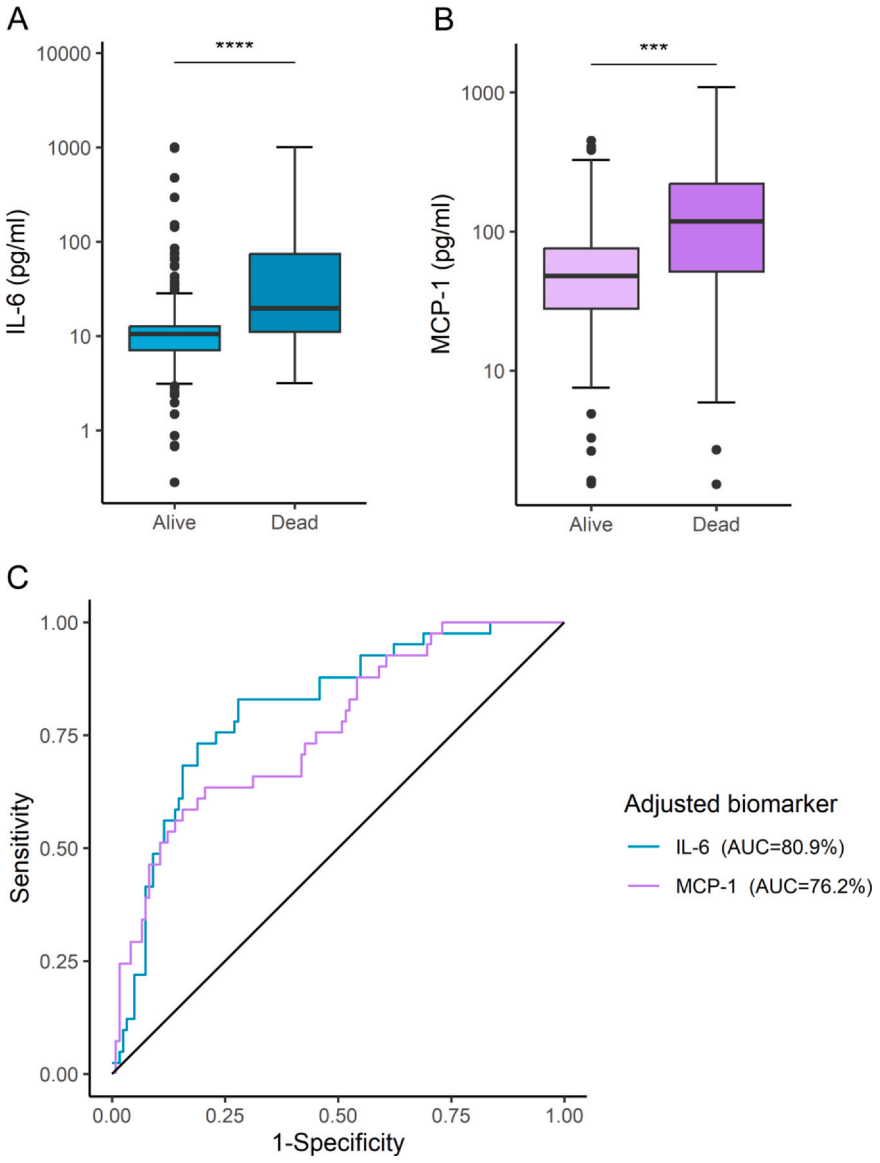

Fig. 2. Predictive effect of IL-6 and MCP-1 circulating levels on in-hospital death. IL-6 serum level in survivor vs dead hospitalized COVID-19 patients (A). MCP-1 serum level in survivor vs dead hospitalized COVID-19 patients (B). n = 301 for A-B. ROC curve analysis of the predictive effect of IL-6 and MCP-1 circulating levels on in-hospital death (E). * p < 0.05, * *p < 0.01, * **p < 0.001, * *** p < 0.0001. (A-B) n = 301. AUC: area under the curve.

hospital admission, suggesting a heterogeneous dysregulated inflammation induced by SARS-CoV-2 infection.

Ethical approval statement

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All procedures were performed in compliance with relevant laws and institutional guidelines and have been approved by the appropriate institutional committees. Informed consent was obtained for every participant.

Funding sources

The DRAGON project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 101005122. The JU receives support from the European Union's Horizon 2020 research and innovation program and EFPIA. This publication reflects the author's view. Neither IMI nor the European Union, EFPIA or the DRAGON consortium, are responsible for any use that may be made of the information contained therein. This work was also supported by a Leon Fredericq Foundation grant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. JS, RPR, PS and EP are employees of TopMD with JS, PS and FS also being directors and shareholders. JG reports personal fees for advisory board, work and lectures from Boehringer Ingelheim, Janssen, SMB, GSK, Roche, AstraZeneca, Aquilon, Volition, Oncoradiomics, and Chiesi, non-financial support for meeting attendance from AstraZeneca, Chiesi, MSD, Roche, Boehringer Ingelheim and Janssen. He is in the permanent SAB of Radiomics (Oncoradiomics SA) for the SALMON trial without any specific consultancy fee for this work. He is co-inventor of one issued patent on radiomics licensed to Radiomics (Oncoradiomics SA). He confirms that none of the above entities or funding was involved in the preparation of this work.

Acknowledgements

The authors would like to thank the study participants.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2024.102589.

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