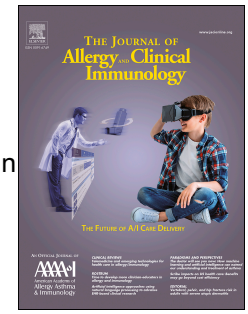

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Elevated levels of interleukin-6 and CRP predict the need for mechanical ventilation in COVID-19

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Author contributions

T.H. and T.W. conceived and designed the study. T.H., C.A., M. K. and T.W. were responsible for clinical care and collected patient data. J.C.H. was responsible for the ethical approval of the study. Statistical analysis was conducted by V.J.. M.v.B.-B. supervised all aspects of the study. B.L. corrected and helped write the manuscript and added important aspects to the analysis. T.H. and T.W. wrote the first draft. All authors contributed to data interpretation, critical revision of the manuscript and approved the final version of the manuscript.

Competing interests

B. L. reports grants and personal fees from Sanofi, AstraZeneca, and Teva; reports personal fees from Cipla, Glenmark, and Lupin; reports grants, personal fees, and other from Chiesi, outside the submitted work; and reports that his son is an employee of AstraZeneca.

M.v.B-B. is the local principal investigator of the currently conducted COVACTA-Trial (A Study to Evaluate the Safety and Efficacy of Tocilizumab in Patients With Severe COVID-19 Pneumonia; NCT04320615, Sponsor: Hoffmann-La Roche). He has previously received honoraria and research funding from Hoffman-La Roche unrelated to this project.

M. K received speakers' fees from BioMerieux and served on the advisory board of BioMerieux.

The other authors declare no conflict of interest.

Abstract

Background: COVID-19 can manifest as a viral induced hyperinflammation with multi-organ involvement. Such patients often experience rapid deterioration and need for mechanical ventilation. Currently, no prospectively validated biomarker of impending respiratory failure is available.

Objective: We aimed to identify and prospectively validate biomarkers that allow the identification of patients in need of impending mechanical ventilation.

Methods: Patients with COVID-19 hospitalized from February 29th to April 09th, 2020 were analyzed for baseline clinical and laboratory findings at admission and during the disease. Data from 89 evaluable patients were available for the purpose of analysis comprising an initial evaluation cohort (n=40) followed by a temporally separated validation cohort (n=49).

Results: We identified markers of inflammation, LDH and creatinine as most predictive variables of respiratory failure in the evaluation cohort. Maximal interleukin-6 (IL-6) levels before intubation showed the strongest association with the need of mechanical ventilation followed by maximal CRP. Respective AUC values for IL-6 and CRP in the evaluation cohort were 0.97 and 0.86 and similar in the validation cohort 0.90 and 0.83. The calculated optimal cutoff values in the course of disease from the evaluation cohort (IL-6> 80 pg/ml and CRP> 97 mg/l) both correctly classified 80% of patients in the validation cohort regarding their risk of respiratory failure.

Conclusion: Maximal levels of IL-6 followed by CRP were highly predictive of the need for mechanical ventilation. This suggests the possibility of using IL-6 or CRP levels to guide escalation of treatment in patients with COVID-19 related hyperinflammatory syndrome.

Clinical Implications: IL-6 followed by CRP strongly predicted patients at risk of respiratory deterioration and might be pivotal for risk-adapted escalation of treatment.

Capsule summary: We studied laboratory parameters as predictors of impending respiratory failure in COVID-19. Maximum levels of interleukin-6 over the course of disease, followed by CRP, were the best predictors of respiratory failure in two separate cohorts.

Key words: Interleukin-6, IL-6, CRP, COVID-19, respiratory failure, mechanical ventilation, prediction, hyperinflammation

Abbreviations: COVID-19: Coronavirus Disease 2019; SARS-CoV-2: Severe Acute Respiratory Syndrome coronavirus 2; SARS: severe acute respiratory syndrome; H7N9: avian-origin influenza; H1N1: influenza A; BAL: Bronchoalveolar lavage; ROC: Receiver operating characteristic; AUC: Area under the curve; CI: Confidence interval; BMI: Body mass index; CT: Computed Tomography; CRP: C-Reactive Protein; WBC: White blood cell count; LDH: Lactate Dehydrogenase; PCT: Procalcitonin; IL6: Interleukin-6; qSOFA score: quick sequential organ failure assessment score - predicts mortality in sepsis; CURB-65 score: predicts mortality in community-acquired pneumonia; MuLBSTA score: predicts mortality in patients with viral pneumonia; q-values represent the Benjamini-Hochberg adjusted p-values

Introduction

The pandemic Coronavirus-disease 19 (COVID-19) is characterized by a highly variable course. While most patients experience only mild symptoms, a relevant proportion develops severe disease progression up to respiratory failure. Interestingly, many patients do not show signs of respiratory distress, despite severe hypoxemia in blood gas analysis [1]. About 5% of patients require intensive care including mechanical ventilation [2, 3].

Recently published large retrospective analyses provide a detailed characterization of COVID-19 and identify variables associated with disease severity and high mortality [4, 5]. One of the largest studies so far shows that age, quick sequential organ failure assessment score (qSOFA score) and D-Dimer correlate with in-hospital death in a multivariate analysis [2]. Another group showed a correlation of obesity and increased inflammatory markers in the blood with respiratory failure [6].

In many aspects, severe COVID-19 may be regarded as a viral induced hyperinflammatory condition with multi-organ involvement due to a cytokine cascade [7]. Of these various cytokines, the presence of raised circulating levels of interleukin-6 (IL-6) appears to be key and is closely connected to disease severity not only in COVID-19 [8] but also in avian-origin H7N9 influenza infections [9] and the common seasonal H1N1 influenza A [10].

While these studies identify the correlation of parameters with disease severity, prospective factors predicting impending deterioration of patients are not yet established. The broad spectrum of the disease courses and silent hypoxia make identification of patients at risk difficult. We aimed to identify variables that allow the prediction of COVID-19 patients with a high risk of respiratory failure.

Methods

Patients and study design

All patients with PCR proven COVID-19 hospitalized at our institution from February 29th to April 09th, 2020 (n=115) were screened and analyzed for baseline clinical and laboratory findings. In total, 26 patients were excluded from the study and the depicted cohort consisted of 89 patients (Table 1). Patients with palliative treatment (n=3) or hospitalization due to other medical reasons and nosocomial Sars-CoV2-infection on the ward (n=13) were excluded from this study. Additionally, patients already mechanically ventilated at admission (n=8) and those receiving anti-IL-6 antibody treatment (n=2) were excluded (Figure 1).

Of the 89 evaluable patients, 40 were part of an initial evaluation cohort hospitalized from February 29th to March 27th, 2020 (Supplementary Table E1). This cohort was used to identify predictive markers of respiratory failure.

Following an interim analysis of the initial evaluation cohort[11], we performed a power analysis to estimate the number of patients needed to validate our findings. Assuming the need of mechanical ventilation to be 20% in the validation cohort and the risks for mechanical ventilation to be 70% and 20% in the high-risk and the low-risk group, respectively, the total sample size for a two-sided test was determined to be 40. We defined an additional safety margin of 10%. This subsequent validation cohort consisted of patients hospitalized from March 27th to April 09th, 2020 (n=49) (Supplementary Table E2). Follow up for all patients was complete through April 12th, 2020. A comparison of both cohorts is shown in Supplementary Table E3.

Use of compassionate medication was low in the study cohort before mechanical ventilation (5 patients received lopinavir/ritonavir, 8 patients received hydroxychloroquine).

Decision on endotracheal intubation was made following internationally accepted recommendations (PaO2/FiO2 <150mmHg or <200mmHg in case of anticipated difficult airway) [12].

Patients are part of the COVID-19 Registry of the Ludwig-Maximilian-University Hospital Munich (CORKUM). Patient data were anonymized for analysis and the study was approved by the local ethics committee (Ethics committee of the LMU Munich, No: 20-245).

IL-6 and CRP measures

The fully automated Elecsys® system on a cobas e801 platform (Roche Diagnostics, Switzerland) was used to measure single levels of IL-6, as described previously [13, 14]. The Elecsys® IL-6 immunoassay has been standardized against the NIBSC 1st IS 89/548 Standard. CRP values were measured on a cobas c702 platform using the Tina-quant® C-Reactive Protein assay (Roche Diagnostics, Switzerland).

Statistical analysis

All variables with less than 50% of missing data in the initial cohort were tested for the association with respiratory failure. Categorical variables were tested with the χ^2 test, and numerical variables with the Mann-Whitney U test. When appropriate, a paired test was performed. All tests were two-sided. The p-values were adjusted for multiple testing with the Benjamini-Hochberg-method to avoid inflating the alpha error. An adjusted p-value (q-value) of ≤0.05 was considered significant. We constructed receiver operating characteristic (ROC) curves and calculated the area under the curve (AUC) to compare the predictive ability of continuous variables. The AUC can be interpreted as the probability that the predictor's value for a randomly chosen patient requiring intubation will be higher than its value for a randomly chosen patient not requiring intubation. The optimal cut off was defined as the one maximizing the Youden's Index [15]. Statistical analyses were performed using the R software package (version 3.6.2). Figures were drawn using Graphpad Prism® (Version 6.0).

Results

Initial identification of IL-6 and CRP as strongest predictors of respiratory failure

To initially evaluate predictors of respiratory failure, 40 patients with confirmed COVID-19 were recruited from February 29th to March 27th, 2020 and served as an evaluation cohort (Figure 1). Thirteen (32.5%) patients deteriorated during hospitalization and required mechanical ventilation. The time from hospital admission to intubation varied from less than two hours to 9 days (median 2 days). Patients requiring mechanical ventilation did not differ in age, comorbidities, radiological findings, respiratory rate or qSofa score (Supplementary Table E1).

Heart rate, markers of inflammation, LDH and creatinine at admission were significantly associated with respiratory failure (Supplementary Table E1). Elevated IL-6 showed the strongest association with the need for mechanical ventilation (Figure 2A, $p=1.2x10^{-5}$).

In addition to values at first assessment, follow-up data were available for laboratory variables. These follow-up data were used to test if there are critical laboratory values that are associated with respiratory failure once they have been reached during disease course. For each patient, we assessed the maximum level of each parameter during disease (for patients requiring ventilation, only values before intubation were used). The maximal values were correlated with respiratory failure (Table 2). Maximal IL-6 levels predicted respiratory failure with highest accuracy (Figure 2, AUC=0.97, CI [0.93, 1.0]), followed by CRP (Figure 3 AUC=0.86, CI [0.74, 0.98]) and creatinine (AUC=0.85, CI [0.74, 0.97]). The optimal cutoff for maximal IL-6 was 80 pg/ml. After reaching an IL-6 value of 80 pg/ml, the median time to mechanical ventilation was 1.5 days (range 0–4 days). The optimal cutoff for maximal CRP was 97 mg/l, with the median time to mechanical ventilation of 0 days after reaching the cutoff (range 0–4 days).

Prospective validation of calculated cutoffs for IL-6 and CRP

A cohort of 40 patients was estimated to have an adequate power to validate our findings (see Methods). The validation cohort prospectively recruited 49 patients from March 27th to April 09th, 2020, of which 19 (39%) required mechanical ventilation. As in the initial cohort, creatinine, LDH, and several markers of inflammation were significantly elevated in patients requiring intubation (Table 2 and Supplementary Table E2). Again, IL-6 at assessment was strongly associated with respiratory failure (Figure 2B), and maximal IL-6 was the best predictor of future respiratory failure among all parameters (Figure 2D, AUC 0.90, CI [0.81, 0.98], Table 2). CRP values at initial assessment were significantly associated with respiratory failure (Figure 2F and Figure 3 AUC=0.86, CI [0.75, 0.96]). Follow-up values of CRP during the disease course did not improve the prediction of respiratory failure in the validation cohort (Table 2, AUC=0.83, CI [0.72, 0.95]).

To validate our findings from the initial cohort, we analyzed the number of patients correctly classified regarding their need of mechanical respiratory support by the determined cutoffs of IL-6 and CRP at presentation and in the course of disease (Table 3). At presentation, IL-6 >35 pg/ml as well as CRP >32.5 mg/l showed high sensitivity to detect patients at risk for respiratory failure (84% and 95%) with moderate specificity (63% for both parameters). Measuring IL-6 and CRP values in the course of disease (cutoffs 80 pg/ml and 97 mg/l) increased the specificity for both parameters (83% and 77%) accompanied with a decrease in sensitivity (74% vs. 84%). In detail, nineteen (39%) patients exceeded the calculated maximal IL-6 cutoff (>80 pg/ml) in the validation cohort, compared to 23 (47%) patients exceeding the CRP cutoff (>97mg/l). Of these patients, 74% and 70% were correctly classified by IL-6 and CRP, respectively, as being at risk for respiratory failure (positive predictive value). Of the 30 patients with values below the IL-6 cutoff, 83% did not require mechanical ventilation, while this was the case for 88% of the 26 patients remaining below the CRP cutoff of 97 mg/l (negative predictive value). In total, the calculated cutoffs for maximal IL-6 and CRP both correctly classified 80% of patients regarding their risk of respiratory failure (Table 3), while values at assessment show poorer predictor properties owing to the moderate specificity (correct classification of 71% for IL-6 and 76% for CRP)

Taken together, while both values have a strong sensitivity at assessment, specificity is gained when examining values in the course of disease. The risk ratios for the cutoffs of IL-6 and CRP were 4.4 and 6.0 in the validation cohort, with corresponding p-values of 0.00022 and 0.00011. The optimal cut point in the validation cohort was slightly lower for IL-6 (60 pg/ml) and identical for CRP (97 mg/l).

Predictive values of the combined cohort

To further evaluate positive and negative predictive values (PPV/NPV) of IL-6 and CRP we combined the two cohorts (Table 1). We calculated predictive values across the range of all possible cutoffs. The PPV of CRP was consistently lower compared to IL-6 in the overall study cohort (Figure 4). In other words, increased CRP misclassified more patients as being at risk for respiratory failure than IL-6. However, the predictive values strongly depend on the selected cutoff (Figure 4). For cutoffs <50 pg/ml for IL-6 and <40 mg/l for CRP (dotted line), the risk of intubation for patients with sub-threshold levels is roughly zero, while patients with levels above these values show a dramatic increase in the risk of respiratory failure. The risk for respiratory failure in patients with IL-6 levels exceeding 210 pg/ml was 100% (dashed line). The NPV of IL-6 and CRP parameters was comparable. In the combined cohort, the optimal threshold value (maximal Youden index[15]) is highest at 65 pg/ml for IL-6 and for CRP at 97 mg/l (corresponding risk ratio of 18.1 and 6.9).

Furthermore, we analyzed the time lag from reaching the cutoff values to intubation in the combined cohort. Patients reached the cutoff of IL-6 (>65 ng/ml) and CRP (>97 mg/l) at a median of 23.2 and 15.7 hours before intubation, resulting in a significant time difference between the two values of 7.5 hours in favor for IL-6 (Figure 5; p=0.014).

Discussion

Our study in hospitalized patients with COVID-19 has provided three key findings: First, circulating levels of IL-6 as well as CRP were highly predictive of the need for invasive ventilation, with corresponding AUC values of 0.97 and 0.90 for IL-6 and 0.86 and 0.83 for CRP in the first and the second cohorts, respectively. Secondly, we defined cutoffs for IL-6 (at presentation >35 pg/ml; maximal value >80 pg/ml) and CRP (at presentation >32.5 mg/l; maximal value >97 mg/l) in the evaluation cohort. Cutoff values at assessment correctly classified 71% (for IL-6) and 76% (for CRP) of patients in the validation cohort with a further increase when measuring maximal values in the course of disease (80% for both parameters). Thirdly, elevated IL-6 levels in the course of disease predicted respiratory failure significantly earlier than CRP (23.2 vs. 15.7 hours). Therefore, IL-6 and CRP are useful markers that predict impending respiratory failure with high accuracy and can help physicians correctly allocate patients who might benefit from early treatment escalation, for example using anti-cytokine strategies. We believe that having these data reproduced across the two separate cohorts enhances the strength of our conclusions. It is important to note that the commercial diagnostic IL-6 assay used in our study allows the measurement of Il-6 in a comparable time scale as CRP. Since it uses the broadly available Cobas platform it can be implemented in most laboratories.

Our study also has several limitations. It is still unclear whether elevated inflammatory markers merely represent an epiphenomenon or a causal pathogenic element of severe COVID-19 [16]. It is likely that elevated IL-6 reflects the cytokine mediated hyperinflammatory state as evidenced by the similarly predictive values for CRP. Further, even though IL-6 and CRP levels are significantly elevated in patients requiring ventilation, they are relatively low compared to levels observed in patients with septic shock [17]. However, earlier studies in severe acute respiratory syndrome (SARS) or H7N9 influenza patients show that inflammatory cytokines are highly expressed in lung tissues. Autopsy reports from SARS patients showed a high amount of inflammatory cytokines in cells expressing angiotensin-

converting enzyme 2 [18], the functional receptor for SARS-CoV and in even higher affinity for SARS-CoV2 [19]. Bronchoalveolar lavage (BAL) in H7N9 influenza patients showed 10^3 times higher concentrations of different cytokines including IL-6 compared to plasma levels, hinting towards a massively increased local concentration of inflammatory cytokines in the diseased lung [9]. Recent preprints provide detailed single cell RNA-sequencing data from immune cells in peripheral blood as well as BAL from COVID-19 patients. The authors report that peripheral monocytes did not substantially express proinflammatory cytokines [20], while there was high expression in monocyte derived macrophages in BAL [21]. Taken together, these data possibly suggest that circulating levels of IL-6 might be a putative surrogate for the burden of lung tissue damage and provide a "window" into the lung [9].

IL-6 and CRP have been associated with severity of COVID-19 (in most cases defined by the Chinese National Health Commission) and mortality before [22-24]. However, to our knowledge our study is the first to demonstrate a prospective prediction of the end point "mechanical ventilation", which is of high clinical relevance not only for patient treatment but also for resource planning. Very recent publications provide additional data that strengthen the role of IL-6 and CRP in COVID-19 as predictive markers [22, 23]. Unfortunately, these studies did not include a prospective validation cohort and sometimes did not mention analysis platforms [22]. A further difference between our and other studies is the dramatic discrepancy in mortality of severely diseased patients. We are not able to analyze mortality as an end point because only two patients had died until April 12th. This number has only increased by one until May 6th (overall mortality 3.4%). While still some patients are in critical condition and the mortality rate in our cohort is likely to increase in the next weeks it will be significantly below those reported. We can only speculate about the reasons for this huge difference but argue that overwhelmed hospitals and patient selection might have contributed to the increased mortality observed in other studies. As we did not perform sequential CT-scans after 24-48 hours in our patients due to radiation hygiene, we are not able to precisely calculate severity of COVID-19 according the Chinese National Health Commission classification to compare our patient cohort to the cohorts of the mentioned studies. However, our validation cohort at

least exists of 63% of severe patients due to the available parameters (2% with mild and 35% with moderate symptoms), which exceeds the recently published cohorts [22, 23].

Since the start of the pandemic, hundreds of research articles on COVID-19 have been published [25]. To our knowledge, we report the first predictive marker for respiratory failure that was prospectively validated in an independent cohort. Although our sample sizes were small, the large difference in risk for respiratory failure between the high-risk and the low-risk group made it possible to successfully validate our findings. Interestingly, a study of 134 patients with avian-origin H7N9 influenza in 2013 also showed a strong correlation of IL-6 and disease severity. In analogy to our findings, this study reports that IL-6 plasma levels >80 pg/ml were found in all patients with lethal outcome compared to only 8.3% in surviving patients [9]. The combined cohort (n=89) produced an only slightly lower cutoff for IL-6 (65 pg/ml) while the cutoff for CRP levels remained the same at 97 mg/l when calculated from the combined cohort. However, even the combined sample size is probably too small to determine an optimal cutoff value. Furthermore, the acceptable proportion of falsely identified low-risk patients, and therefore the set threshold, is largely dictated by the availability of health care resources. Future prospective studies with larger sample sizes are needed to formally address this issue. We want to stress that IL-6 and CRP should be used as a predictor not an indication for invasive respiratory support, as mechanical ventilation per se has several unintended adverse consequences and may support inflammation of distal airways in COVID-19 patients.

Immunologically, CRP and IL-6 are closely intertwined. IL-6 is known to induce gene expression and release of CRP from the liver [26, 27] and also from immune cells [28]. A functional connection has been shown in different trials using IL-6 inhibition, in which CRP-levels rapidly normalized after blocking IL-6 [29]. In analogy, we found that IL-6 levels predicted respiratory failure significantly earlier than CRP-levels, which is essential for a predictive marker. While inhibition of inflammatory pathways represents a promising approach to treat hyperinflammatory COVID-19 patients, inhibition of IL-6 could be detrimental in the immune

response to virus-induced pneumonias [30, 31]. Thus, our study does not facilitate any recommendations for or against IL-6 inhibition. Ongoing randomized controlled clinical trials of IL-6-antibodies in the treatment of COVID-19 will shed light on this question (e.g. NCT04320615 and NCT04331795). More importantly, in times of missing established therapeutic options, best supportive care is essential [32].

In summary, we were able to validate our finding that IL-6 and CRP levels serve as strong predictors of patients in need of ventilator support. In the current situation with overwhelmed intensive care units and overcrowded emergency rooms, correct identification of patients in need of intensive care is crucial. Assessing these parameters to identify patients at risk of respiratory failure at an early stage might be helpful for triage planning and timely allocation of critically ill patients as well as a guide to escalation of treatment strategies in COVID-19 patients.

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Tables

Table 1: Combined Cohort

Variable	Evaluable	Median (range) / n (%)	Mechanical ventilation		p-value	q-value
			No (n = 57)	Yes (n = 32)		
Baseline Characteristics *						
Age (years)	89	61 (18 - 84)	58 (18 - 84)	65 (45 - 81)	**0.031**	0.067
Respiratory rate (/min)	74	18 (11 - 40)	17 (13 - 39)	25 (11 – 40)	**0.0024**	**0.0073**
Heart rate (/min)	66	86 (54 - 130)	85 (54 - 130)	89 (64 - 112)	0.32	0.47
BMI	71	26.9 (18.1 – 45.7)	26.0 (18.1 – 36.2)	27.6 (18.3 – 45.7)	0.074	0.15
Male gender	89	62 (70)	33 (58)	29 (91)	**0.0029**	**0.0073**
Any comorbidities	87	70 (80)	43 (77)	27 (87)	0.38	0.53
Hypertension	86	45 (52)	25 (45)	20 (65)	0.14	0.25
Diabetes mellitus	86	13 (15)	7 (13)	6 (19)	0.61	0.68
Coronary heart disease	85	7 (8)	4 (7)	3 (10)	>0.99	>0.99
Chronic obstructive lung disease	86	9 (10)	7 (13)	2 (6)	0.54	0.67
Computed Tomography[#]						
Consolidation	78	46 (59)	30 (59)	16 (59)	>0.99	>0.99
Ground glass opacity	78	72 (92)	47 (92)	25 (93)	>0.99	>0.99
Bilateral infiltration	78	70 (90)	44 (86)	26 (96)	0.32	0.47
Scores[§]						
qSOFA score [33]	71	30 (42)	13 (28)	17 (68)	**0.0028**	**0.0073**
CURB-65 score[34] ≥ 1	47	22 (47)	11 (41)	11 (55)	0.50	0.67
MuLBSTA score[35]	68	11 (0 - 15)	9 (0 - 15)	11 (5 - 15)	0.090	0.17
Laboratory parameters[#]	**Evaluable**	**Median (range)**	**Mechanical ventilation**		**p-value**	**q-value**
			No (n = 57)	**Yes (n = 32)**		
Lymphocyte count (G/l)	67	0.92 (0.20 – 2.84)	0.85 (0.31 – 2.36)	0.94 (0.20 – 2.84)	0.60	0.68
CRP (mg/l)	89	36 (0 - 369)	20 (0 - 315)	93 (16 - 369)	**$1.9 \cdot 10^{-7}$**	**$2.6 \cdot 10^{-6}$**
Bilirubin (mg/dl)	84	0.5 (0.2 – 1.9)	0.5 (0.2 – 1.2)	0.6 (0.2 – 1.9)	0.19	0.32
WBC (G/l)	89	5.86 (0.15 – 308)	5 (1.92 – 12.4)	7.26 (0.15 - 308)	**0.0024**	**0.0073**
LDH (U/l)	88	311 (153 - 1121)	278 (153 - 619)	462 (240 - 1121)	**$1.5 \cdot 10^{-6}$**	**0.000010**
PCT (ng/ml)	87	0 (0 - 5)	0 (0 – 0.6)	0.2 (0 - 5)	**$8.7 \cdot 10^{-7}$**	**$8.1 \cdot 10^{-6}$**
IL-6 (pg/ml)	86	34 (0 - 430)	23.2 (0 - 209)	95.4 (14.2 - 430)	**$2.3 \cdot 10^{-9}$**	**$6.5 \cdot 10^{-8}$**
Thrombocyte count (G/l)	89	194 (0.12 - 450)	194 (0.27 - 383)	202 (0.12 - 450)	0.55	0.67
Troponin T (ng/ml)	78	0 (0 – 0.178)	0 (0 – 0.143)	0 (0 – 0.178)	**0.00010**	**0.00047**
Creatinine (mg/dl)	89	0.9 (0.4 – 7)	0.9 (0.4 – 5.6)	1.1 (0.8 – 7)	**$5.2 \cdot 10^{-6}$**	**0.000029**
D-Dimer	76	0.7 (0 – 35.2)	0.6 (0 – 35)	0.9 (0 – 35.2)	**0.0079**	**0.018**
Ferritin (ng/ml)	79	703 (30 - 3577)	545 (30 - 2578)	1392 (237 - 3577)	**0.00023**	**0.00092**

* respiratory rate and heart rate and BMI (Body mass index) were measured at admission; existing comorbidities were evaluated by patient history at admission; # CT-scans and laboratory parameters at admission; § scores were calculated at admission. CRP = C-Reactive Protein; WBC= White blood cell count; LDH = Lactate Dehydrogenase; PCT = Procalcitonin; IL6 = Interleukin-6; qSOFA score = predicts mortality in sepsis, CURB-65 score = predicts mortality in community-acquired pneumonia, MuLBSTA score = predicts mortality in patients with viral pneumonia; q-values represent the Benjamini-Hochberg adjusted p-values

Table 2: p-values, AUC's and optimal cutoffs in evaluation, validation and combined cohort

Variable	Evaluation set						Validation set						Combined cohort					
	At presentation			Maximal			At presentation			Maximal			At presentation			Maximal		
	p-value	AUC [CI]	Cutoff	p-value	AUC [CI]	Cutoff	p-value	AUC [CI]	Cutoff	p-value	AUC [CI]	Cutoff	p-value	AUC [CI]	Cutoff	p-value	AUC [CI]	Cutoff
IL-6 pg/ml	0.000012	0.94 [0.86, 1.00]	35	$5.4 \cdot 10^{-8}$	0.97 [0.93, 1.00]	80	0.000076	0.84 [0.73, 0.95]	48.9	$4.9 \cdot 10^{-7}$	0.90 [0.81, 0.98]	60	$2.3 \cdot 10^{-9}$	0.89 [0.81, 0.96]	48.9	$2.6 \cdot 10^{-11}$	0.93 [0.88, 0.98]	65
CRP mg/l	0.0031	0.79 [0.65, 0.93]	32.5	0.00027	0.86 [0.74, 0.98]	97	0.000032	0.86 [0.75, 0.96]	32.5	0.000097	0.83 [0.72, 0.95]	97	$1.9 \cdot 10^{-7}$	0.83 [0.75, 0.92]	32.5	$7.0 \cdot 10^{-8}$	0.85 [0.76, 0.93]	97
PCT ng/ml	0.0043	0.74 [0.58, 0.90]	0.05	0.0084	0.74 [0.57, 0.91]	0.25	0.000073	0.81 [0.69, 0.93]	0.05	0.00015	0.80 [0.67, 0.93]	0.25	$8.7 \cdot 10^{-7}$	0.78 [0.68, 0.88]	0.05	$4.2 \cdot 10^{-6}$	0.78 [0.67, 0.88]	0.25
LDH U/l	0.00062	0.83 [0.70, 0.97]	320	0.071	0.68 [0.50, 0.86]	590	0.00032	0.81 [0.67, 0.95]	410	0.0076	0.73 [0.60, 0.89]	440	$1.4 \cdot 10^{-6}$	0.81 [0.72, 0.91]	410	0.0015	0.70 [0.59, 0.82]	380.5
WBC G/l	0.0028	0.80 [0.66, 0.93]	4920	0.010	0.75 [0.58, 0.93]	9860	0.13	0.63 [0.45, 0.81]	6190	0.30	0.59 [0.41, 0.77]	10510	0.0024	0.69 [0.57, 0.81]	6190	0.015	0.66 [0.53, 0.78]	9860
Creatinine mg/dl	0.00051	0.84 [0.72, 0.96]	0.95	0.00028	0.85 [0.74, 0.97]	1.05	0.0023	0.76 [0.63, 0.89]	0.95	0.026	0.69 [0.54, 0.84]	1.05	$5.2 \cdot 10^{-6}$	0.79 [0.70, 0.88]	0.95	0.000070	0.75 [0.65, 0.86]	1.05
Troponin ng/ml	0.0053	0.72 [0.56, 0.88]	0.005	0.0079	0.72 [0.55, 0.90]	0.005	0.0078	0.72 [0.57, 0.87]	0.005	0.020	0.69 [0.54, 0.85]	0.005	0.00010	0.73 [0.62, 0.83]	0.005	0.00027	0.72 [0.61, 0.83]	0.005
Ferritin ng/ml	0.064	0.72 [0.52, 0.91]	766	0.12	0.68 [0.47, 0.89]	530	0.0026	0.76 [0.62, 0.90]	1285	0.010	0.72 [0.58, 0.87]	1510	0.00023	0.75 [0.64, 0.86]	1285	0.0024	0.71 [0.59, 0.83]	1610

CRP = C-Reactive Protein; WBC= White blood cell count; LDH = Lactate Dehydrogenase; PCT = Procalcitonin; IL6 = Interleukin-6; AUC = area under the curve; CI = confidence interval

Table 3: Contingency table for high-risk and low-risk groups as defined by IL-6 and CRP in the validation cohort

Variable	Value	Mechanical ventilation		p-value
		No	Yes	
IL-6 at presentation	**≤35**	19	3	0.0030
	>35	11	16	
Maximal IL-6	**≤80**	25	5	0.00022
	>80	5	14	
CRP at presentation	**≤32.5**	19	1	0.00019
	>32.5	11	18	
Maximal CRP	**≤97**	23	3	0.00011
	>97	7	16	

Figure legends

Figure 1: Consort Diagram:

Consort Diagram. DNR/DNI: do-not-resuscitate and do-not-intubate order.

Figure 2: IL-6 at presentation, maximal IL-6 levels before mechanical ventilation and ROC-analysis of different parameters in the evaluation and validation cohort

Box plots showing IL-6 levels at first assessment (A, B) and maximal IL-6 levels before mechanical ventilation (C, D) in the evaluation cohort and in the validation cohort; dashed lines represents the cutoff calculated from the evaluation cohort (IL-6 at initial assessment >35 pg/ml, maximal IL-6 >80 pg/ml). Mean ± SD is shown. Receiver operating characteristic (ROC) curve of maximal follow-up levels before mechanical ventilation in the evaluation (E) and validation cohorts (F).

Figure 3: CRP levels at presentation and maximal CRP levels before mechanical ventilation

Box plot showing CRP levels at first assessment (A, B) and maximal IL-6 levels before mechanical ventilation (C, D) in the evaluation cohort and in the validation cohort; dashed lines represents the cutoff calculated from the training cohort (CRP at assessment >32.5 mg/l, maximal CRP>97 mg/l). Mean ± SD is shown.

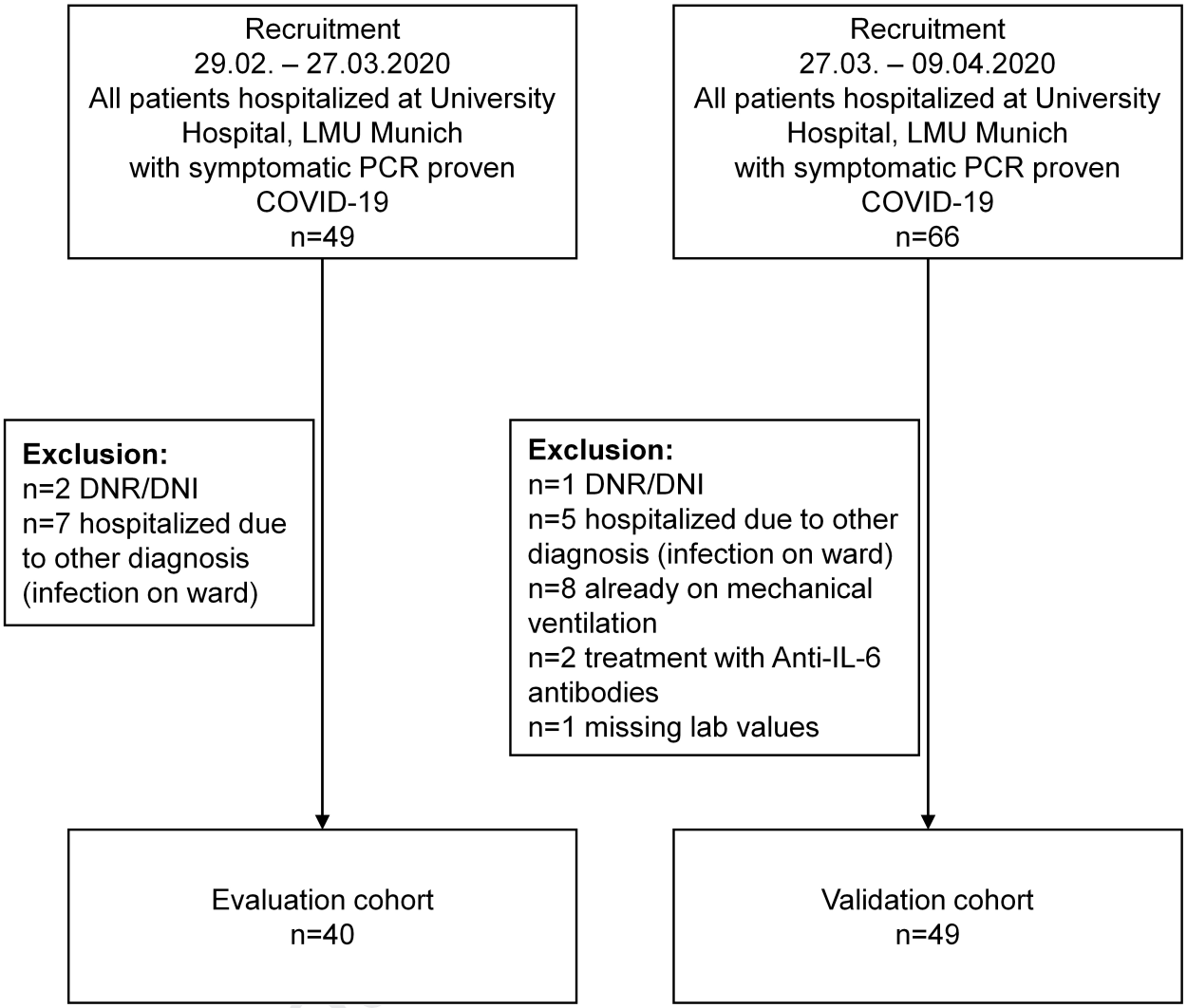
Figure 4: Cutoffs and predictive values of maximal IL-6 and CRP values in the combined cohort

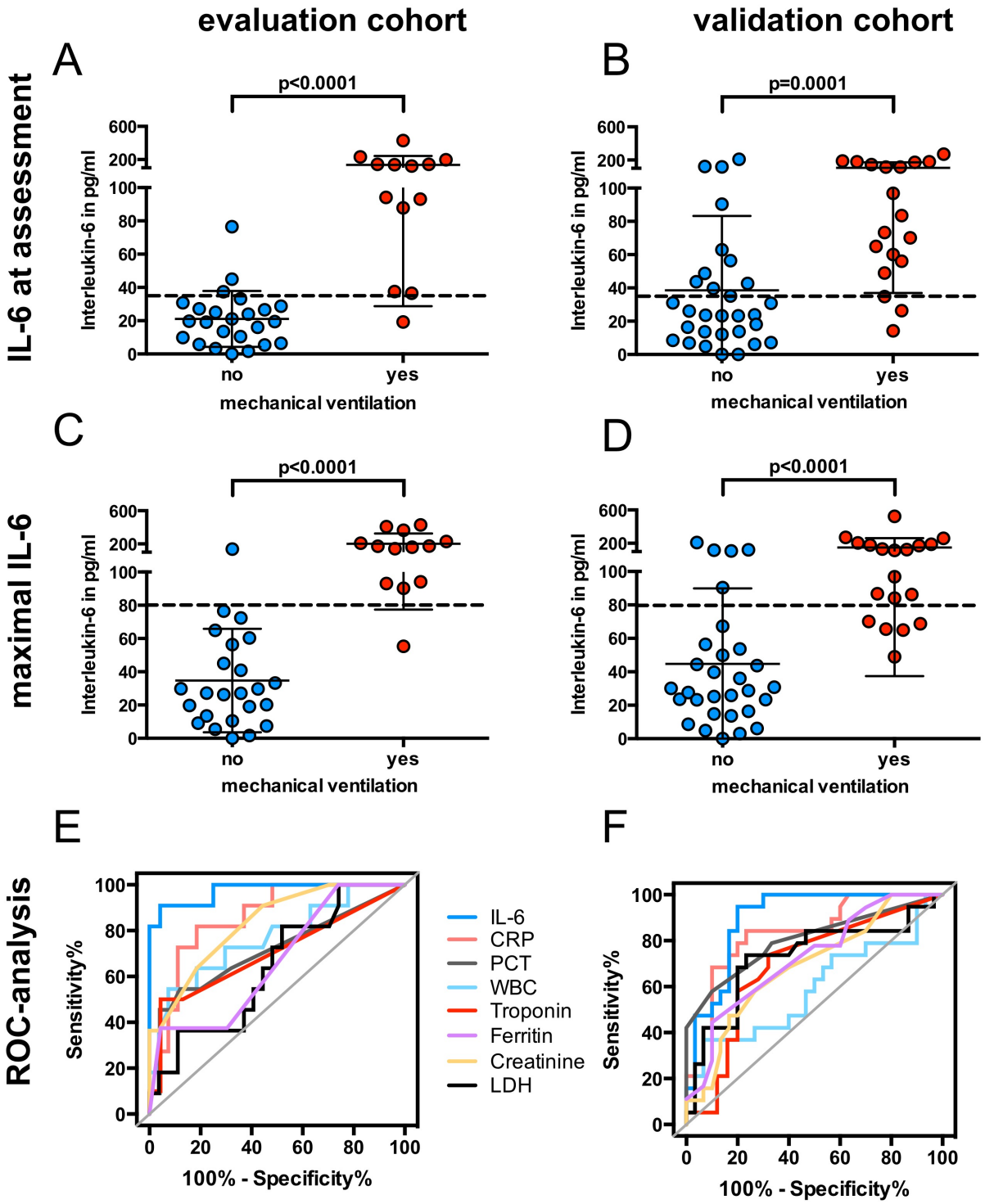
Box plots depicting the maximal values of IL-6 and CRP in the overall cohort (A, B); dashed line represents the validated cutoff; dotted line represents the calculated improved cutoff from all patients (applicable only for IL-6). Positive predictive value (PPV) and negative

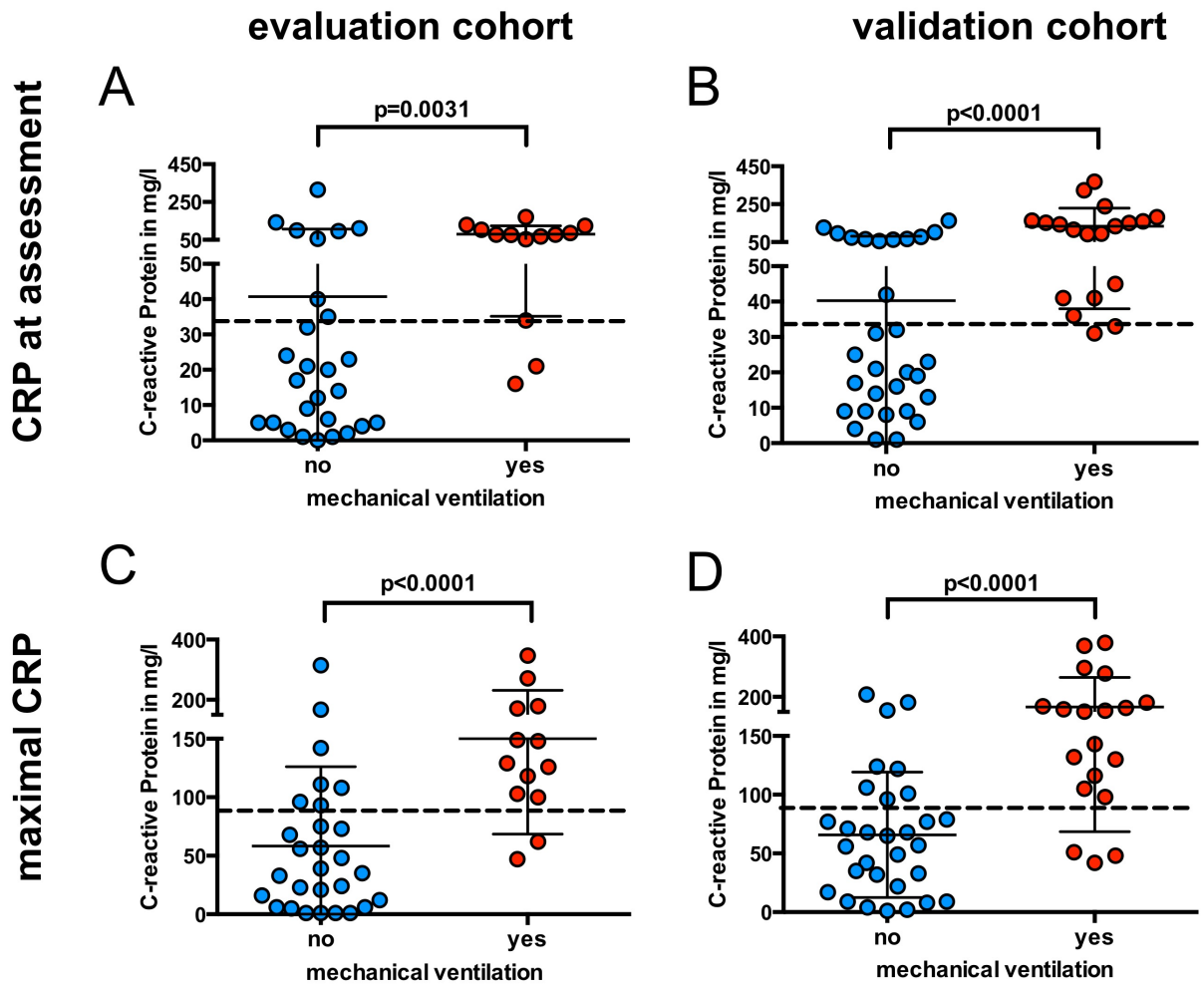
predictive value (NPV) as a function of different cutoffs is shown for IL-6 (C) and CRP (D) values (dotted line represents cutoff for perfect NPV; dashed line represents cutoff for perfect PPV)

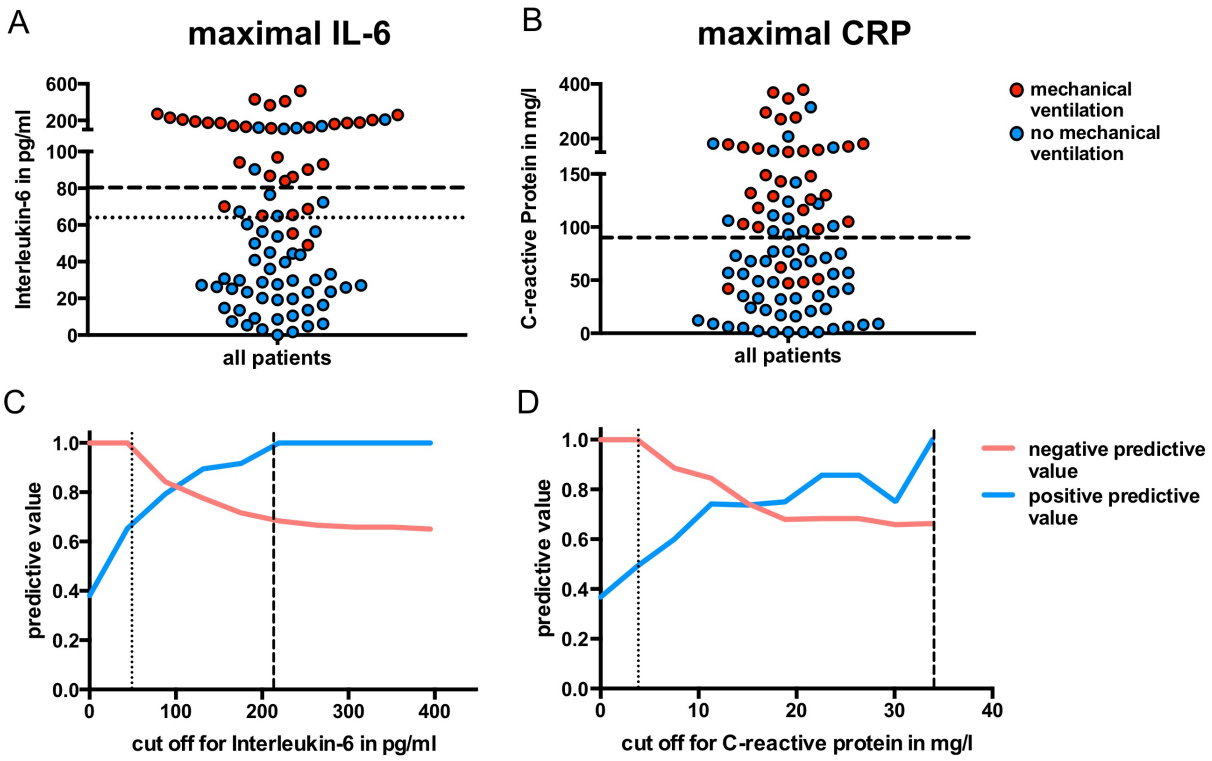
Figure 5: Time from exceeding the maximal cutoff value of IL-6 or CRP to intubation in the combined cohort

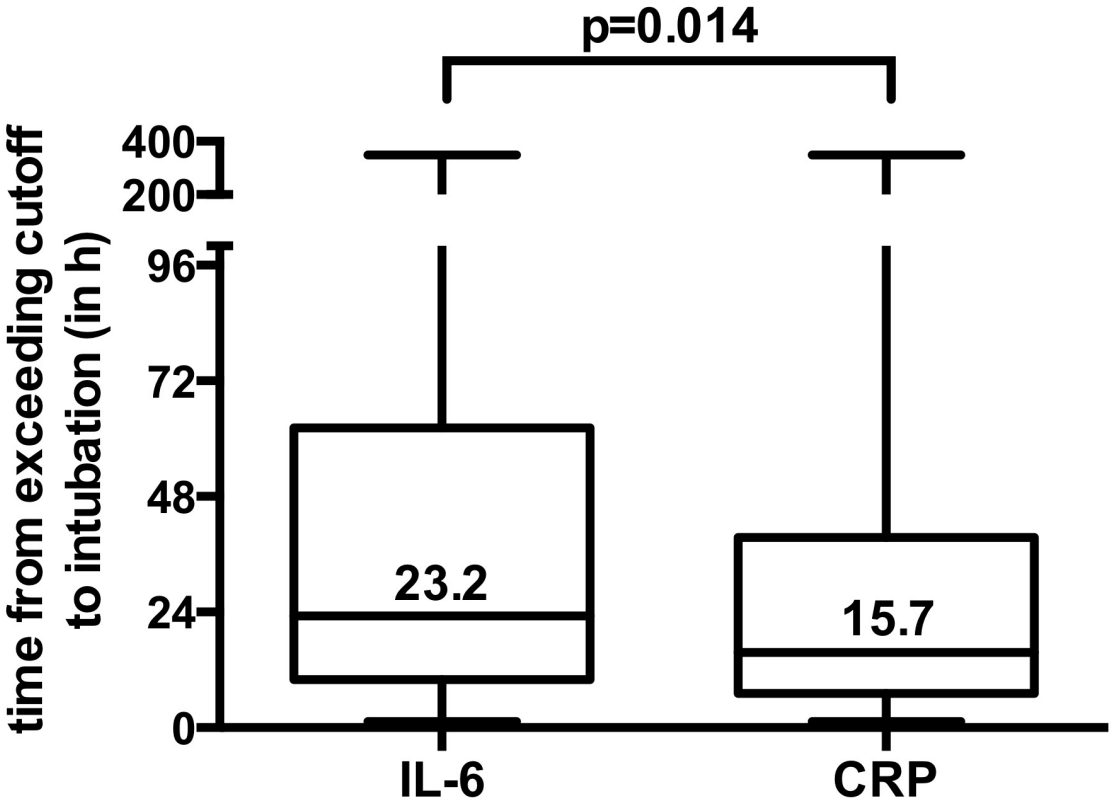
Box plot depicting the time from exceeding the IL-6 (>65 ng/ml) and CRP (>97 mg/l) cutoff to intubation in hours in the combined cohort. Median ± min/max is shown.


Recruitment
29.02. – 27.03.2020
All patients hospitalized at University Hospital, LMU Munich with symptomatic PCR proven COVID-19
n=49
Recruitment
27.03. – 09.04.2020
All patients hospitalized at University Hospital, LMU Munich with symptomatic PCR proven COVID-19
n=66
Exclusion:
n=2 DNR/DNI
n=7 hospitalized due to other diagnosis (infection on ward)
Exclusion:
n=1 DNR/DNI
n=5 hospitalized due to other diagnosis (infection on ward)
n=8 already on mechanical ventilation
n=2 treatment with Anti-IL-6 antibodies
n=1 missing lab values
Evaluation cohort
n=40
Validation cohort
n=49


evaluation cohort
validation cohort
IL-6 at assessment
maximal IL-6
ROC-analysis
A
B
C
D
E
F
p<0.0001
p=0.0001
Interleukin-6 in pg/ml
no
yes
mechanical ventilation
Sensitivity%
100% - Specificity%
IL-6
CRP
PCT
WBC
Troponin
Ferritin
Creatinine
LDH


evaluation cohort
validation cohort
CRP at assessment
maximal CRP
A
B
C
D
p=0.0031
p<0.0001
p<0.0001
p<0.0001
C-reactive Protein in mg/l
mechanical ventilation
no
yes
0
10
20
30
40
50
100
150
200
250
400
450




p=0.014
time from exceeding cutoff to intubation (in h)
400
200
96
72
48
24
0
23.2
15.7
IL-6
CRP

Supplementary

Elevated levels of interleukin-6 and CRP predicts the need for mechanical ventilation in COVID-19

Tobias Herold and, Vindi Jurinovic et. al.

Table E1: Evaluation Cohort

Variable	Evaluable	Median (range) / n (%)	Mechanical ventilation		p-value	q-value
			No (n = 27)	Yes (n = 13)		
Baseline Characteristics *						
Age (years)	40	57 (19 - 81)	54 (19 - 80)	64 (45 - 81)	0.15	0.29
Respiratory rate (/min)	34	18 (14 - 40)	18 (14 - 32)	23 (15 - 40)	0.066	0.14
Heart rate (/min)	32	81 (54 - 112)	77 (54 - 111)	94 (80 - 112)	**0.0069**	**0.022**
BMI	30	25.9 (19.0 – 45.7)	23.7 (19.0 – 34.7)	30.5 (24.8 – 45.7)	**0.0030**	**0.014**
Male gender	40	29 (72)	16 (59)	13 (100)	**0.020**	0.051
Any comorbidities	39	32 (82)	20 (77)	12 (92)	0.46	0.81
Hypertension	38	19 (50)	10 (40)	9 (69)	0.17	0.32
Diabetes mellitus	38	3 (8)	1 (4)	2 (15)	0.55	0.82
Coronary heart disease	36	3 (8)	3 (12)	0 (0)	0.52	0.82
Chronic obstructive lung disease	37	3 (8)	2 (8)	1 (8)	>0.99	>0.99
Computed Tomography[#]						
Consolidation	36	21 (58)	14 (61)	7 (54)	0.95	>0.99
Ground glass opacity	36	31 (86)	20 (87)	11 (85)	>0.99	>0.99
Bilateral infiltration	36	33 (92)	21 (91)	12 (92)	>0.99	>0.99
Scores[§]						
qSOFA score [1]	32	12 (37)	7 (32)	5 (50)	0.55	0.82
CURB-65 score[2] ≥ 1	24	7 (29)	5 (31)	2 (25)	>0.99	>0.99
MuLBSTA score[3]	29	9 (4 - 15)	9 (4 - 13)	7 (5 - 15)	0.89	>0.99
Laboratory parameters[#]	**Evaluable**	**Median (range)**	**Mechanical ventilation**		**p-value**	**q-value**
			No (n = 27)	**Yes (n = 13)**		
Lymphocyte count G/l	31	0.99 (0.45 – 2.50)	0.99 (0.45 – 1.80)	0.95 (0.57 – 2.50)	**0.92**	>0.99
CRP (mg/l)	40	28 (0 – 315)	17 (0 – 315)	77 (16 – 171)	**0.0031**	**0.014**
Bilirubin (mg/dl)	37	0.5 (0.2 – 1.9)	0.5 (0.2 – 1.2)	0.5 (0.4 – 1.9)	0.78	>0.99
WBC (G/l)	40	5.04 (2.12 - 308)	4.67 (2.12 – 10.8)	7.38 (4.67 - 308)	**0.0028**	**0.014**
LDH (U/l)	39	285 (153 - 1078)	258 (153 - 619)	381 (252 - 1078)	**0.00062**	**0.0058**
PCT (ng/ml)	38	0 (0 - 5)	0 (0 – 0.6)	0.1 (0 - 5)	**0.0043**	**0.017**
IL-6 (pg/ml)	37	27.1 (0 - 430)	19.6 (0 – 76.5)	121 (19.2 - 430)	**0.000012**	**0.00034**
Thrombocyte count (G/l)	40	161 (0.12 - 440)	162 (0.27 - 334)	160 (0.12 - 440)	0.74	>0.99
Troponin T (ng/ml)	34	0 (0 – 0.032)	0 (0 – 0.022)	0 (0 – 0.032)	**0.0053**	**0.019**
Creatinine (mg/dl)	40	0.9 (0.4 – 2.1)	0.9 (0.4 – 1.3)	1.0 (0.9 – 2.1)	**0.00051**	**0.0058**
D-Dimer	31	0.7 (0 – 2.9)	0.6 (0 – 2.2)	1.1 (0.6 – 2.9)	**0.019**	0.051
Ferritin (ng/ml)	31	626 (46 - 2153)	553 (46 - 1748)	810 (431 - 2153)	0.064	0.14

* respiratory rate and heart rate and BMI (Body mass index) were measured at admission; existing comorbidities were evaluated by patient history at admission; # CT-scans and laboratory parameters at admission; § scores were calculated at admission. CRP = C-Reactive Protein; WBC= White blood cell count; LDH = Lactate Dehydrogenase; PCT = Procalcitonin; IL6 = Interleukin-6; qSOFA score = predicts mortality in sepsis, CURB-65 score = predicts mortality in community-acquired pneumonia, MuLBSTA score = predicts mortality in patients with viral pneumonia; q-values represent the Benjamini-Hochberg adjusted p-values

Table E2: Validation cohort

Variable	Evaluable	Median (range) / n (%)	Mechanical ventilation		p-value	q-value
			No (n = 30)	Yes (n = 19)		
Baseline Characteristics *						
Age (years)	49	64 (18 - 84)	61 (18 - 84)	65 (46 - 81)	0.18	0.31
Respiratory rate (/min)	34	18 (11 - 40)	17 (13 - 39)	26 (11 – 40)	**0.027**	0.083
Heart rate (/min)	34	90 (64 - 130)	94 (74 - 130)	86 (64 - 107)	**0.033**	0.091
BMI	41	27.5 (18.1 – 36.2)	27.6 (18.1 – 36.2)	27.0 (18.4 – 34.7)	**0.58**	0.71
Male gender	49	33 (67)	17 (57)	16 (84)	**0.091**	0.21
Any comorbidities	48	38 (79)	23 (77)	15 (83)	0.85	0.96
Hypertension	48	26 (54)	15 (50)	11 (61)	0.65	0.76
Diabetes mellitus	48	10 (21)	6 (20)	4 (22)	>0.99	>0.99
Coronary heart disease	49	4 (8)	1 (3)	3 (16)	0.31	0.46
Chronic obstructive lung disease	49	6 (12)	5 (17)	1 (5)	0.46	0.61
Computed Tomography[#]						
Consolidation	42	25 (59)	16 (57)	9 (64)	>0.99	0.98
Ground glass opacity	42	41 (98)	27 (96)	14 (100)	>0.99	>0.99
Bilateral infiltration	42	37 (88)	23 (82)	14 (100)	0.24	0.37
Scores[§]						
qSOFA score [1]	39	18 (46)	6 (25)	12 (80)	**0.0025**	**0.010**
CURB-65 score[2] ≥ 1	23	15 (65)	6 (55)	9 (75)	0.55	0.71
MuLBSTA score[3]	39	11 (0 - 15)	10 (0 - 15)	13 (9 - 15)	**0.038**	0.096
Laboratory parameters[#]	**Evaluable**	**Median (range)**	**Mechanical ventilation**		**p-value**	**q-value**
			No (n = 30)	**Yes (n = 19)**		
Lymphocyte count G/l	36	0.80 (0.20 – 2.84)	0.73 (0.31 – 2.36)	0.94 (0.20 – 2.84)	0.43	0.60
CRP (mg/l)	49	42 (1 – 369)	22 (1 – 163)	134 (31 – 369)	**0.000032**	**0.00068**
Bilirubin (mg/dl)	47	0.5 (0.2 – 1.2)	0.4 (0.2 – 1.2)	0.6 (0.2 – 1.1)	0.16	0.30
WBC (G/l)	49	6.0 (0.15 – 25.8)	5.79 (1.92 – 12.4)	7.22 (0.15 – 25.8)	0.13	0.26
LDH (U/l)	49	336 (181 - 1121)	278 (181 - 502)	474 (240 - 1121)	**0.00032**	**0.0022**
PCT (ng/ml)	49	0 (0 – 2.3)	0 (0 – 0.3)	0.2 (0 – 2.3)	**0.000073**	**0.00068**
IL6 (pg/ml)	49	42.7 (0 - 272)	23.7 (0 - 209)	83.5 (14.2 - 272)	**0.000072**	**0.00068**
Thrombocyte count (G/l)	49	216 (93 - 450)	212 (112 - 383)	220 (93 - 450)	0.23	0.37
Troponin T (ng/ml)	44	0 (0 – 0.178)	0 (0 – 0.143)	0.022 (0 – 0.178)	**0.0078**	**0.027**
Creatinine (mg/dl)	49	0.9 (0.5 – 7.0)	0.9 (0.5 – 5.6)	1.1 (0.8 – 7.0)	**0.0023**	**0.010**
D-Dimer	45	0.8 (0 – 35.2)	0.6 (0 - 35)	0.9 (0 – 35.2)	**0.11**	0.24
Ferritin (ng/ml)	48	789 (30 - 3577)	508 (30 - 2578)	1692 (237 - 3577)	**0.0026**	**0.010**

* respiratory rate and heart rate and BMI (Body mass index) were measured at admission; existing comorbidities were evaluated by patient history at admission; # CT-scans and laboratory parameters at admission; § scores were calculated at admission. CRP = C-Reactive Protein; WBC= White blood cell count; LDH = Lactate Dehydrogenase; PCT = Procalcitonin; IL6 = Interleukin-6; qSOFA score = predicts mortality in sepsis, CURB-65 score = predicts mortality in community-acquired pneumonia, MuLBSTA score = predicts mortality in patients with viral pneumonia; q-values represent the Benjamini-Hochberg adjusted p-values

Supplementary Table E3: Comparison Evaluation and Validation cohort

Variable	Cohort		p-value
	Evaluation (n = 40)	Validation (n = 49)	
Baseline Characteristics *			
Age (years)	57 (19 - 81)	64 (18 - 84)	0.15
Respiratory rate (/min)	18 (14 - 40)	18 (11 - 40)	0.76
Heart rate (/min)	81 (54 - 112)	90 (64 - 130)	**0.017**
BMI	25.9 (19.0 – 45.7)	27.5 (18.1 – 36.2)	**0.18**
Male gender	29 (72)	33 (67)	0.77
Any comorbidities	32 (82)	38 (79)	0.95
Hypertension	19 (50)	26 (54)	0.87
Diabetes mellitus	3 (8)	10 (21)	0.17
Coronary heart disease	3 (8)	4 (8)	>0.99
Chronic obstructive lung disease	3 (8)	6 (12)	0.79
Computed Tomography[#]			
Consolidation	21 (58)	25 (60)	>0.99
Ground glass opacity	31 (86)	41 (98)	0.14
Bilateral infiltration	33 (92)	37 (88)	0.89
Scores[§]			
qSOFA score [1]	12 (37)	18 (46)	0.62
CURB-65 score[2] ≥ 1	7 (29)	15 (65)	**0.029**
MuLBSTA score[3]	9 (4 - 15)	11 (0 - 15)	0.13
Laboratory parameters[#]	**Cohort**		**p-value**
	Evaluation (n = 40)	**Validation (n = 49)**	
Lymphocyte count G/l	0.99 (0.45 – 2.5)	0.8 (0.2 – 2.84)	0.27
CRP (mg/l)	28 (0 – 315)	42 (1 – 369)	0.10
Bilirubin (mg/dl)	0.5 (0.2 – 1.9)	0.5 (0.2 – 1.2)	0.71
WBC (G/l)	5.04 (2.12 - 308)	6 (0.15 – 25.8)	0.47
LDH (U/l)	285 (153 - 1078)	336 (181 - 1121)	0.18
PCT (ng/ml)	0 (0 – 5)	0 (0 – 2.3)	0.32
IL-6 (pg/ml)	27.1 (0 - 430)	42.7 (0 - 272)	0.34
Thrombocyte count (G/l)	161 (0.12 - 440)	216 (93 - 450)	**0.0084**
Troponin T (ng/ml)	0 (0 – 0.032)	0 (0 – 0.178)	**0.016**
Creatinine (mg/dl)	0.9 (0.4 – 2.1)	0.9 (0.5 – 7.0)	0.82
D-Dimer	0.7 (0 – 2.9)	0.8 (0 – 35.2)	0.57
Ferritin (ng/ml)	626 (46 - 2153)	789 (30 - 3577)	0.20

* respiratory rate and heart rate and BMI (Body mass index) were measured at admission; existing comorbidities were evaluated by patient history at admission; [#] CT-scans and laboratory parameters at admission; [§] scores were calculated at admission. CRP = C-Reactive Protein; WBC= White blood cell count; LDH = Lactate Dehydrogenase; PCT =

Procalcitonin; IL6 = Interleukin-6; qSOFA score = predicts mortality in sepsis, CURB-65 score = predicts mortality in community-acquired pneumonia, MuLBSTA score = predicts mortality in patients with viral pneumonia; q-values represent the Benjamini-Hochberg adjusted p-values

Supplementary References

1. Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A*, et al.* Assessment of Clinical Criteria for Sepsis: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* **315**, 762-774 (2016).
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3. Guo L, Wei D, Wu Y, Zhou M, Zhang X, Li Q*, et al.* Clinical Features Predicting Mortality Risk in Patients With Viral Pneumonia: The MuLBSTA Score. *Front Microbiol* **10**, 2752 (2019).