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► **To cite this version:**

Victor Gravrand, François Mellot, Felix Ackermann, Marie-Christine Ballester, Benjamin Zuber, et al.. Stratification of COVID-19 Severity Using SeptiCyte RAPID, a Novel Host Immune Response Test. *Viruses*, 2023, 15 (2), pp.419. 10.3390/v15020419 . hal-04488758

**HAL Id: hal-04488758**

**<https://hal.science/hal-04488758v1>**

Submitted on 4 Mar 2024

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1 Type of the Paper: Article

# 2 Stratification of COVID-19 severity using SeptiCyte RAPID, a 3 novel host immune response test.

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**Abstract:** SeptiCyte<sup>®</sup> RAPID is a gene expression assay measuring the relative expression levels of host response genes PLA2G7 and PLAC8, indicative of a dysregulated immune response during sepsis. As severe forms of COVID-19 may be considered viral sepsis, we evaluated SeptiCyte RAPID in a series of 94 patients admitted to Foch Hospital (Suresnes, France) with proven SARS-CoV-2 infection. EDTA blood was collected in the emergency department (ED) in 67 cases, in the intensive care unit (ICU) in 23 cases and in conventional units in 4 cases. SeptiScore (0-15 scale) increased with COVID-19 severity. Patients in ICU had the highest SeptiScores, producing values comparable to 8 patients with culture-confirmed bacterial sepsis. Receiver operating characteristic (ROC) curve analysis had an area under the curve (AUC) of 0.81 for discriminating patients requiring ICU admission from patients who were immediately discharged or from patients requiring hospitalization in conventional units. SeptiScores increased with the extent of the lung injury. For 68 patients, a chest computed tomography (CT) scan was performed within 24 hours of COVID-19 diagnosis. SeptiScore > 7 suggested lung injury ≥ 50 % (AUC = 0.86). SeptiCyte RAPID was compared to other biomarkers for discriminating Critical + Severe COVID-19 in ICU, versus Moderate + Mild COVID-19 not in ICU. The mean AUC for SeptiCyte RAPID was superior to that of any individual biomarker or combination thereof. In contrast to C-reactive protein (CRP), correlation of SeptiScore with lung injury was not impacted by treatment with anti-inflammatory agents. SeptiCyte RAPID can be a useful tool to identify patients with severe forms of COVID-19 in ED, as well as during follow-up.

**Citation:** To be added by editorial staff during production.

Academic Editor: Firstname  
Lastname

**Keywords:** COVID-19; gene expression; severity; viral sepsis

Received: date

Revised: date

Accepted: date

Published: date



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## 36 1. Introduction

37 Sepsis is a complex immuno-pathological disorder characterized by a  
38 pro-inflammatory response that may be followed by an anti-inflammatory, immuno-  
39 suppressive state, or possibly a cycling between these states over several weeks [1]. Sepsis-3 now defines sepsis as “a life-threatening organ dysfunction caused by a dysregulated host immune response to infection” [2]. It is now well known that SARS-CoV-2 infection can progress to a severe disease characterized by a dysregulated immune response and a maladaptive cytokine release [3], leading to multiple complications including acute respiratory distress syndrome (ARDS), myocarditis, acute kidney and liver failure and coagulopathy [4]. Therefore there are important similarities of this

46 severe disease progression to sepsis [5-8]. As such, Beltran-Garcia et al. [9] reviewed the  
47 common features of COVID-19 and sepsis and discussed the possible use of an-  
48 ti-inflammatory therapeutics in the treatment of SARS-CoV-2 disease. In a large me-  
49 ta-analysis of 24,983 patients with COVID-19, Abate et al. [10] reported that approxi-  
50 mately one third of these patients were admitted to the Intensive Care Unit (ICU); of  
51 these, 68% suffered complications, 37% were deemed to have become septic, and 39%  
52 died.

53  
54 Since the start of the COVID-19 pandemic in 2020, the disease has evolved in several  
55 waves, corresponding to the emergence of different variants of the SARS-CoV-2 virus.  
56 The original lineage-B ancestral strain (Wuhan-Hu-1) gave rise to the WHO-designated  
57 variants of concern (VOC) alpha (B.1.1.7), beta (B.1.351), gamma (P.1), delta (B.1.617.2),  
58 and finally omicron (B.1.1.529) [11]. Because of the multiplicity of mutations presented by  
59 omicron, this VOC exhibited enhanced transmissibility and immune-evasive properties  
60 [12,13]. More recently, novel recombinants have been detected, most likely arising from  
61 different variants replicating simultaneously in immunocompromised or unvaccinated  
62 individuals [14,15]. As of January 2023, the highly transmissible and immune-evasive  
63 XBB1.5 recombinant is becoming the dominant strain worldwide. In public health terms,  
64 the appearance of each new variant with immune-evasive properties is characterized by  
65 rapid and massive contamination of patients, who arrive in large numbers in the emer-  
66 gency department (ED).

67  
68 Severity of the disease is evaluated by different biological parameters such  
69 lymphopenia, blood oxygen saturation, C-reactive protein (CRP) levels and D-dimer  
70 levels [16]. In addition, chest computed tomography (CT) scan has been proposed to  
71 identify patients at risk of poor evolution [17]. Because of huge influxes of patients at the  
72 same time in the ED, laboratory and radiology departments can be overwhelmed, due to  
73 this massive activity and possibly the absence of medical workers, themselves affected by  
74 the disease, leading to a delay in the triage of the patients. Therefore, a test that would be  
75 able to determine easily and quickly the clinical severity would be welcome.

76  
77 SeptiCyte® RAPID (Immunexpress, Seattle, WA) is a gene expression assay using  
78 reverse transcription quantitative polymerase chain reaction (RT-qPCR) to measure the  
79 relative expression levels of host response genes (PLA2G7 and PLAC8) that are indicative  
80 of a dysregulated immune response during sepsis. SeptiCyte RAPID is used in conjunc-  
81 tion with clinical assessments, vital signs and laboratory findings as an aid to differenti-  
82 ate infection-positive (sepsis) from infection-negative systemic inflammation in patients  
83 suspected of sepsis [18]. SeptiCyte RAPID generates a score (SeptiScore®) that falls within  
84 discrete Interpretation bands based on the increasing likelihood of infection-positive  
85 systemic inflammation. Analysis of public next generation sequencing (NGS) datasets  
86 (e.g. [19]) suggested that SeptiCyte RAPID is able to discriminate between milder and  
87 more severe COVID-19 cases.

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89 Here, we report on a prospective study that sought to evaluate the performance of  
90 the SeptiCyte RAPID assay in patients with laboratory-confirmed COVID-19 with dif-  
91 ferent degrees of severity, and analyze whether this assay could be used as a triaging tool  
92 for patients requiring hospitalization and potentially ICU care. A preliminary version of  
93 this work has been presented in abstract form [20].

## 94 2. Materials and Methods

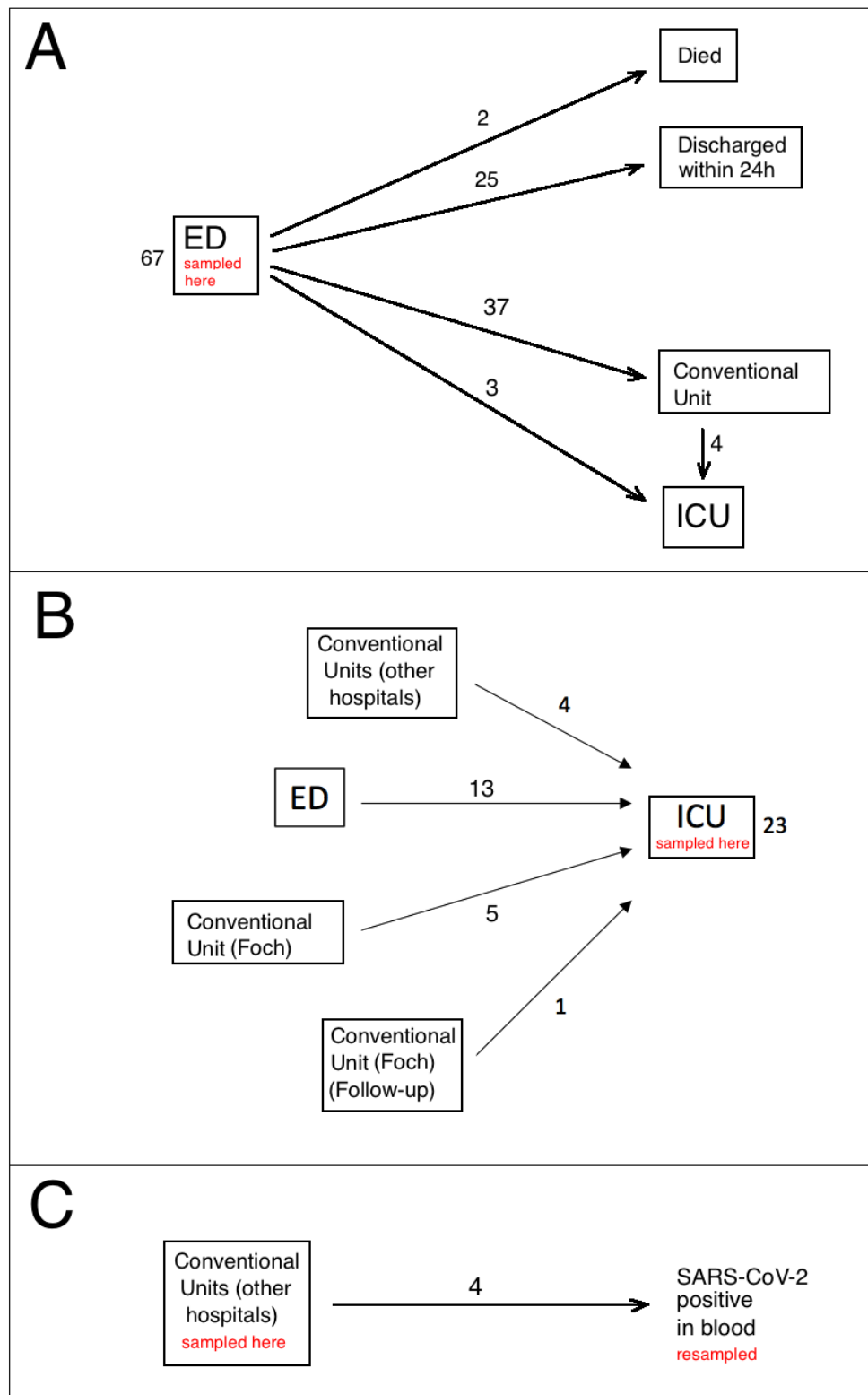
### 95 2.1 Ethic committee approval

96 This study was conducted under a blanket protocol for COVID-related research at  
97 the Foch Hospital (“Information réutilisation des données personnelles et des  
98 échantillons biologiques à des fins de recherche V2 - 9 avril 2020”), which had been es-

99 tablished and approved by the local ethics committee of the Foch Hospital (IRB00012437).  
100 The study was performed in agreement with French ethical laws, such that the patients  
101 and their families were informed that their biological samples and data used for routine  
102 care could be used in an anonymous manner unless they expressed their opposition. No  
103 patients enrolled in this study, or their families, expressed opposition to the use of their  
104 biological samples and data, which according to the French ethical laws and Foch Hos-  
105 pital ethics committee constitutes informed consent.

### 106 *2.2 Patient recruitment*

107 The study was performed between January 12 and July 15, 2021, when the  
108 SARS-CoV-2 B.1.1.7 variant was present in France. Ninety-four patients (44 females, 50  
109 males, median age 65 years) were included in the study. All enrolled patients were con-  
110 firmed by RT-qPCR to be nasopharyngeal swab positive for SARS-CoV-2 infection. Fig-  
111 ure 1 presents a diagram of the flow of the patients in this study through the ED, con-  
112 ventional units and ICU, indicating the hospital locations where blood sampling for  
113 SeptiCyte measurement occurred. A 'conventional unit' is defined as a general hospital  
114 ward, with extra precautions and isolations taken to minimize SARS-CoV-2 transmission.  
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**Figure 1.** Diagram of the flow of the patients in this study. (a) Sixty-seven patients sampled in ED. (b) Twenty-three patients sampled in ICU. (c) Four patients sampled by nasopharyngeal swab in the conventional unit, with subsequent SARS-CoV-2 (+) result from blood.

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### 2.3 Collection of blood samples

K3-EDTA whole blood samples, collected for complete blood count (CBC) and subsequently tested with SeptiCyte RAPID, were collected on admission in the ED in 67 cases (Figure 1A), in ICU for 23 patients (Figure 1B) and in conventional units for 4 patients (Figure 1C). Of the 67 patients tested in the ED, 25 were discharged within 24 hours, because they had a moderate clinical form, did not require oxygen therapy, and had no biological signs of marked inflammation. None of these 25 patients required another hospitalization for COVID-19 and all were alive at least one month after their admission to the ED. Thirty-three patients were transferred from ED to conventional units for a median time of 8 days (range 2 to 22 days), four were first transferred from ED to conventional units, but then required admission to ICU some days later (median time 6 days, range 1–12 days), and three were immediately transferred from ED to ICU. Two older patients (85 and 93 years old) died in the ED.

Twenty-three patients were tested in ICU (Figure 1B). Of these, 13 were admitted first to the ED (within 24 hours) but required high levels (>15L/min) of oxygenotherapy and therefore were transferred to ICU as soon as possible, with nasopharyngeal swab RT-qPCR and blood sample collection occurring after ICU admission. Another group of 9 patients in ICU came from conventional units, either from Foch hospital (5 cases) or from other hospitals (4 cases). Median length of stay in conventional units before ICU admission was 3 days (range 2 to 5 days). For the last case (labeled “Follow-up” in Figure 1B), the SeptiScore was determined during ICU stay, because the patient was tested during a follow-up for COVID-19 by RT-qPCR, and was still positive 35 days after the initial diagnosis of COVID-19. Of the 23 patients tested in ICU (Figure 1B), 22/23 were treated by corticotherapy and 11/23 were treated by antibiotherapy at the time of the SeptiScore determination. A blood culture was taken on the day SeptiCyte RAPID testing was performed in 20 cases, and was positive in 2 cases (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). Similarly, cytobacteriological examination of the urine was taken from 12 patients, and was positive in 2 cases (*Klebsiella pneumoniae* and *Enterobacter cloacae*). On the day of SeptiCyte RAPID testing, the SOFA score was available for 20 patients and the median value was 2 (range: 0–12). For 10 ICU patients, follow-up samples were analyzed during ICU hospitalization, spaced approximately one week apart.

Lastly (Figure 1C), for 4 patients hospitalized in conventional units, a sample was analyzed during their hospitalization (median time: 7 days, range: 3–15 days). These patients were initially hospitalized because they had a positive RT-qPCR on nasopharyngeal swab, but on the day when the SeptiScore was analyzed, SARS-CoV-2 viral sequences were also found in their blood. Testing for SARS-CoV-2 in blood samples was not performed for all the patients, but only for patients in whom the use of convalescent plasma was envisaged.

For comparison, we also quantified the SeptiScore in 8 patients from ICU with culture proven bacterial sepsis as per the Sepsis-3 criteria [2], who were SARS-CoV-2 negative.

### 2.4 Processing of samples

Diagnosis of SARS-CoV-2 infection was made using either the Alinity M SARS-CoV-2 RT-PCR assay (Abbott Molecular, Des Plaines, IL, USA) or the Xpert® Xpress SARS-CoV-2 (Cepheid Europe SAS, Maurens-Scopont, France)

Complete blood counts (CBC) were performed on a DxH 800 instrument (Beckman-Coulter, Villepinte, France) and monocyte CD16 positive quantification was performed using the CytoDiff™ reagent (Beckman-Coulter) as previously described [21].

178 Blood gas analysis, including lactate measurement, was performed on a GEM 4000 in-  
179 strument (Werfen, Le Pré-Saint-Gervais, France). CRP and D-dimer were measured on  
180 Alinity IC (Abbott Diagnostics, Rungis, France) and ACL 700 (Werfen) systems, respec-  
181 tively. All measurements were made at approximately the same time the blood samples  
182 were collected for CBC and SeptiCyte RAPID testing.

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184 SeptiCyte RAPID testing of eligible patients was performed on qualified Biocartis  
185 Idylla instruments (Biocartis, Mechelen, Belgium) installed with SeptiCyte RAPID assay  
186 software. Residual EDTA blood samples from CBC were diluted within several hours of  
187 collection with phosphate buffered saline (PBS; 1 vol blood to 2.75 vol PBS), and 900 µL of  
188 each diluted sample was put into a SeptiCyte RAPID cartridge and automatically pro-  
189 cessed.

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191 The SeptiCyte RAPID test is completely self-contained within single use cartridges  
192 that are designed to run on the Biocartis Idylla system. The entire assay process from  
193 blood sample extraction to final test result is achieved on-cartridge, with fully integrated  
194 sample preparation followed by reverse transcription and quantitative real-time poly-  
195 merase chain reaction (RT-qPCR) for the detection and quantification of changes in ex-  
196 pression of the immune host response genes *PLAC8* and *PLA2G7*.

197  
198 The test result (SeptiScore) is calculated based on the difference of cycle of quanti-  
199 fication (Cq) values in RT-qPCR for the target genes. SeptiScores range from 0-15 and  
200 higher scores are associated with increasing sepsis probabilities. A sample processing  
201 control (SPC) is included in each cartridge to confirm that adequate nucleic acid extrac-  
202 tion and amplification has occurred.

#### 203 204 *2.5 Radiologic evaluation*

205 COVID-19 disease severity was assessed as the percentage of specific lung abnor-  
206 malities or damage as observed by a radiologist on a chest CT scan: (1) peripheral, bilat-  
207 eral ground glass opacity (GGO) with or without consolidation or visible intra-lobular  
208 lines (crazy paving), or (2) multifocal GGO of rounded shape with or without consolida-  
209 tion or visible intra-lobular lines. Patients were then classified as “absence”, “mild”,  
210 “moderate”, “extensive”, “severe”, or “critical” based on 0%, present but <10%, 10-25%,  
211 25-50%, 50-75%, or >75% lung damage respectively. Three patients were indicated as  
212 having “non-specific” CT scan results, and were not considered further in the classifica-  
213 tion totals.

#### 214 215 *2.6 Statistical analyses*

216 Statistical analyses were performed using a combination of publicly available and  
217 custom scripts written in the R programming language [22]. The pROC package in R [23]  
218 was used for AUC calculations, and some calculations were verified with JLABROC4  
219 [<http://www.rad.jhmi.edu/jeng/javarad/roc/JROCFITi.html>]. The stats package in R was  
220 used for calculating p-values with Welch’s two-sided t-test or one-way ANOVA for nu-  
221 meric variables, and with Pearson’s chi-squared test for categorical variables. Summary  
222 tables were then generated using the gtsummary package in R.

223  
224 Combinatorial regression analysis, in which values of multiple clinical parameters  
225 (SeptiScore, CRP, D-dimer, lactate, monocytes, CD16-positive monocytes) were com-  
226 bined, was performed with a custom R script. Missing values in the clinical dataset were  
227 imputed prior to the combinatorial analysis, with the R package Amelia [24].

### 228 **3. Results**

229 The main characteristics of the patients in this study are summarized in Table 1.  
230 Obesity, characterized by a body mass index (BMI) > 30, was not reported in this table

231 because this parameter was available only for 46 patients. Additional risk factors (in-  
232 cluding BMI when available) and treatments of the patients during their hospitalization  
233 are indicated in Supplementary Table 1. The distribution of SARS-CoV-2 (+) patients  
234 across COVID-19 severity categories and hospital locations is indicated in Supplemen-  
235 tary Table 2.

236  
237 In the 94-patient cohort, there were 17 patients with pre-existing comorbidities in-  
238 volving lung conditions (some patients with multiple conditions): cystic fibrosis (n=1),  
239 asthma (n=4), chronic obstructive pulmonary disease (n=7), lung cancer (n=3), lung  
240 transplantation (n=4), non-specific interstitial pneumopathy (n=1). The SeptiScores for  
241 these patients as a group were compared to the SeptiScores of the remaining 77 patients  
242 in the cohort, using the Kolmogorov-Smirnov test. There was no significant difference  
243 found between the two groups ( $D = 0.293$ ,  $p = 0.243$ ).  
244



245

246 **Table 1.** Main characteristics of COVID-19 patients in this study, according to the department where the sample for SeptiScore measurement was collected and  
 247 patient follow-up occurred. Range is specified in [ ] square brackets, and percentage is specified in ( ) parentheses. Abbreviation: NA, not applicable. The  
 248 p-values for the categorical variables Sex, CT-scan performed, and Alive were calculated with Pearson's chi-squared test, while the p-values for the numeric  
 249 variables were calculated by one-way ANOVA

	Emergency Department (Figure 1a) (n=67)					ICU (Figure 1b)	Conventional unit, viremia (Figure 1c)	Bacterial Sepsis (ICU)	p-value
Follow-up	Discharged (< 24 hours in ED)	Conventional Hospitalization	Delayed ICU admission	Immediate ICU admission	Deceased in ED				
n	25	33	4	3	2	23	4	8	
Sex (F/M)	15/10	15/18	2/2	1/2	2/0	8/15	1/3	2/6	0.4
Median age (years)	60 [20 – 84]	72 [23 – 96]	66 [63 – 66]	76 [69 – 78]	89 [85 – 93]	57 [23 – 78]	56 [38 – 67]	62 [35 – 90]	0.001
Median time between COVID-19 diagnosis and SeptiCyte result, days <sup>1</sup>	0 [0 - 12]	0 [0 - 7]	0.5 [0 - 16]	0	0	3 [0 – 25]	8.5 [1 – 10]	NA	<0.001
Median time between onset of symptoms and SeptiCyte result, days (symptomatic patients)	5 (n=21) [1 – 12]	7 (n=31) [0 – 30]	6.5 (n=4) [4 – 9]	7 (n=3) [4 – 10]	NA	10.5 [4 – 42]	14 [10 – 21]	3 [1 - 10]	<0.001
Diabetes, n (%)	6 (24.0)	11 (33.3)	1 (25.0)	2 (66.7)	1 (50)	10 (43.5)	1 (25.0)	5 (62.5)	0.3
Arterial Hypertension (AHT), n (%)	9 (36.0)	13 (39.4)	2 (50.0)	3 (100)	1 (50)	14 (60.9)	0 (0)	3 (37.5)	0.2

	Emergency Department (Figure 1a) (n=67)					ICU (Figure 1b)	Conventional unit, viremia (Figure 1c)	Bacterial Sepsis (ICU)	p-value
Follow-up	Discharged (< 24 hours in ED)	Conventional Hospitalization	Delayed ICU admission	Immediate ICU admission	Deceased in ED				
Diabetes & AHT, n (%)	4 (16.0)	4 (12.1)	1 (25)	2 (66.7)	1 (50)	8 (34.8)	0 (0)	3 (37.5)	0.07
CT-scan performed, n (%)	12 (48)	30 (90.1)	4 (100)	2 (66.7)	1 (50)	15 (65.2)	4 (100)	0(0)	<0.001
Alive, n (%)	25 (100)	27 (81.8)	4 (100)	3 (100)	0 (0)	15 (65.2)	4 (100)	7 (87.5]	<0.001
Median length of hospitalization, days	0 (100)	7 [2 – 22]	21 [6 – 37]	20 [7 – 38]	1 [1-1]	24 [6 – 74]	10 [3 – 13]	10 [6 – 34]	<0.001
Median delay to death (days) <sup>2</sup>	no cases	8 [5 – 17]	no cases	no cases	1 [1 - 1]	24 [7 – 55]	no cases	7 [7 - 7]	0.005
<sup>1</sup> Positive COVID diagnosis is equated with a (+) RT-qPCR test result for SARS-CoV-2 <sup>2</sup> Time between the first day of hospitalization and death									

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### 3.1 Variations of SeptiScore and other biological markers according to patient group

Variations of the SeptiScore were analyzed in comparison to other well recognized biological markers of severity of SARS-CoV-2 infection (variations in leucocyte differential, including CD16-positive monocytes, CRP, D-dimer and lactate). As can be seen in Table 2 and Figure 2 the SeptiScore increased with the severity of the disease. In considering the entire 94 patient cohort, using samples taken at admission, SeptiScore was significantly correlated with CRP (Pearson sample correlation coefficient  $\rho = 0.513$ ;  $p < 0.0001$ ), lactate ( $\rho = 0.321$ ;  $p = 0.014$ ) lymphocyte count ( $\rho = -0.354$ ;  $p = 0.0008$ ) and monocyte count ( $\rho = -0.219$ ;  $p = 0.038$ ).

SeptiScores were significantly higher in patients requiring ICU hospitalization, compared to patients who were immediately discharged or transferred to conventional units ( $p = 0.0004$ , Kruskal-Wallis test). SeptiScores for the ICU patients were similar in value to scores for 8 SARS-CoV-2 (-) patients having culture-confirmed bacterial sepsis (Figure 2).

A receiver operating characteristic (ROC) curve analysis was performed on the different biological parameters recorded during the study, in order to identify patients requiring ICU hospitalization. An AUC  $> 0.7$  was considered to be relevant as a discriminating parameter [25]. Using data from the earliest measured values of the parameters, only two biological parameters fulfilled this criteria, SeptiScore and CRP, with an AUC of 0.78 and 0.79, and a binary cut-off of 6.6 and 100 mg/L, respectively.

276

277 **Table 2.** Comparison of the Septicyte score with conventional biological parameters and the hospital locations. Values expressed as median and [range].

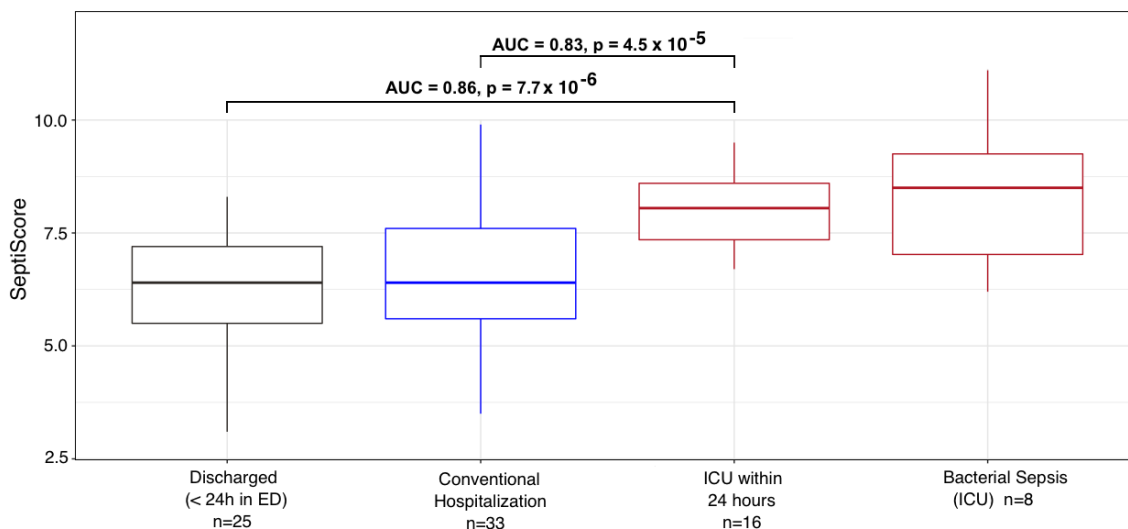
278 Abbreviations: CRP, C-reactive protein; NA, not available. p-values calculated by one-way ANOVA, which used 102 out of 131 samples.

Follow-up	Emergency Department (Figure 1a)					ICU (Figure 1b)	Conventional unit, viremia (Figure 1c)	Bacterial Sepsis (ICU)	p-value
	Discharged (< 24 hours in ED)	Conventional Hospitalization	Delayed ICU admission	Immediate ICU admission	Deceased in ED				
n	25	33	4	3	2	23	4	8	
Septicyte score (A.U)	6.4 [3.1 – 8.3]	6.4 [3.5 – 9.9]	8 [6.4 – 8.6]	8.1 [7.7 – 8.2]	7.2 [6.5 – 7.9]	7.6 [5.3 – 9.5]	6.2 [4.8 – 6.7]	8.5 [2.9 – 11.1]	0.003
White blood cell (x 10 <sup>9</sup> /L)	5 [1.9 – 5.5]	7.1 [2 – 24]	5.3 [3.5 – 8.1]	9.4 [7.5 – 10.7]	12.6 [9.8 – 15.3]	8.2 [2.2 – 39.7]	6.3 [4.8 – 7.1]	13.3 [4.4 – 24.3]	0.020
Platelets (x 10 <sup>9</sup> /L)	197 [97 – 708]	198 [86 – 588]	180 [117 – 311]	316 [136 – 421]	178 [139 – 217]	238 [68 – 433]	182 [167 – 242]	180 [17 – 330]	0.7
Neutrophils (x 10 <sup>9</sup> /L))	3.4 [1.1 – 11.6]	5.5 [1.1 – 22.8]	4.1 [2.2 – 5.7]	6.8 [6.8 – 9.5]	10.6 [8.6 – 12.7]	6.8 [1.8 – 33.4]	4.3 [3.6 – 6]	8.6 [3.1 – 18.0]	0.04
Lymphocytes (x 10 <sup>9</sup> /L)	0.9 [0.4 – 3.6]	0.8 [0.2 – 2.2]	1.1 [0.4 – 1.4]	0.7 [0.5 – 1.4]	1.1 [0.5 – 1.6]	0.65 [0.2 – 0.8]	0.8 [0.7 – 1.7]	1 [0.3 – 2.0]	0.3
Monocytes (x 10 <sup>9</sup> /L)	0.6 [0 – 1.2]	0.6 [0.1 – 1.5]	0.4 [0.2 – 1.2]	0.5 [0.2 – 1.1]	0.8 [0.6 – 0.9]	0.5 [0.1 – 2.1]	0.6 [0.3 – 0.9]	0.8 [0.1 – 1.4]	>0.9
CD16 <sup>POS</sup>	0.07	0.059	0.033	0.053	0.064	0.020	0.058	0.094	0.7

Follow-up	Emergency Department (Figure 1a)					ICU (Figure 1b)	Conventional unit, viremia (Figure 1c)	Bacterial Sepsis (ICU)	p-value
	Discharged (< 24 hours in ED)	Conventional Hospitalization	Delayed ICU admission	Immediate ICU admission	Deceased in ED				
monocytes (x 10 <sup>9</sup> /L)	[0 – 0.38]	[0.003 – 0.29]	[0.022 – 0.058]	[0.005 – 0.094]		[0.005 – 0.12]	[0.044 – 0.074]	[0.004 – 0.16]	
CRP (mg/L)	22 [1 – 279]	95 [1 – 287]	166 [84 – 181]	173 [105 – 296]	111 [86 – 137]	161 [23 – 270]	47 [2 – 154]	196 (159-233)	<0.001
D-dimer (mg/L)	0.79 [0.22 – 3.80]	0.66 [0.28 – 2.21]	1.07 [0.43 – 92.86]	1.59 [0.54 – 1.81]	6.961	0.95 [0.43 – 16.25]	1.29 [0.83 – 1.74]	NA	0.02
Lactate (mmol/L)	0.8 [0.6 – 1.2]	1.1 [0.9 – 5.3]	1.6 [1.5 – 1.7]	2.6 [1.1 – 2.6]	4.2 [3.2 – 5.1]	1.5 [0.8 - 2.5]	1.8	1.3 (0.6-8.0)	0.01

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**Figure 2.** Variation of the SeptiScore in COVID-19 patients determined within 24 hours after hospital admission according to the location of the patients. Comparison with SeptiScores of patients with culture-confirmed bacterial sepsis. COVID patients: Group 1 – Discharged within 24 hours (n = 25, Fig 1a); Group 2 – Hospitalization in conventional unit, without subsequent admission to ICU (n = 33, Fig 1a); Group 3 – transferred to ICU within 24 hours (n= 16); Group 4 – Bacterial sepsis in ICU (n = 8).

### 3.2 Variation in SeptiScore, according to lung injury observed in CT-scan

For 68 patients, a CT-scan was performed within 24 hours after admission to the hospital. Three of the patients had non-specific presentations in CT-scan, and were not considered further. Six had an absence of lung damage, while the remaining patients could be classified as having mild (n=10), moderate (n=17), extensive (n=20), severe (n=9) or critical (n=3) extents of lung damage. As can be seen in Figure 3, the SeptiScore increased with the extent of the lung injury, the highest SeptiScore values being observed in patients with severe or critical lung injuries. Following from this analysis, patients were divided in two groups: those with severe or critical ( $\geq 50\%$ ) lung injury (n = 12), vs. those with specific but  $<50\%$  lung injury (n = 53) as defined by chest CT scan. ROC curve analysis gave AUC = 0.86, with a SeptiScore cut-off of 7 providing a good discrimination between these two groups. CRP, lymphocytes, monocytes, CD16-positive monocytes and lactate were also discriminatory for patients with  $\geq 50\%$  versus  $<50\%$  lung injury (Table 3).

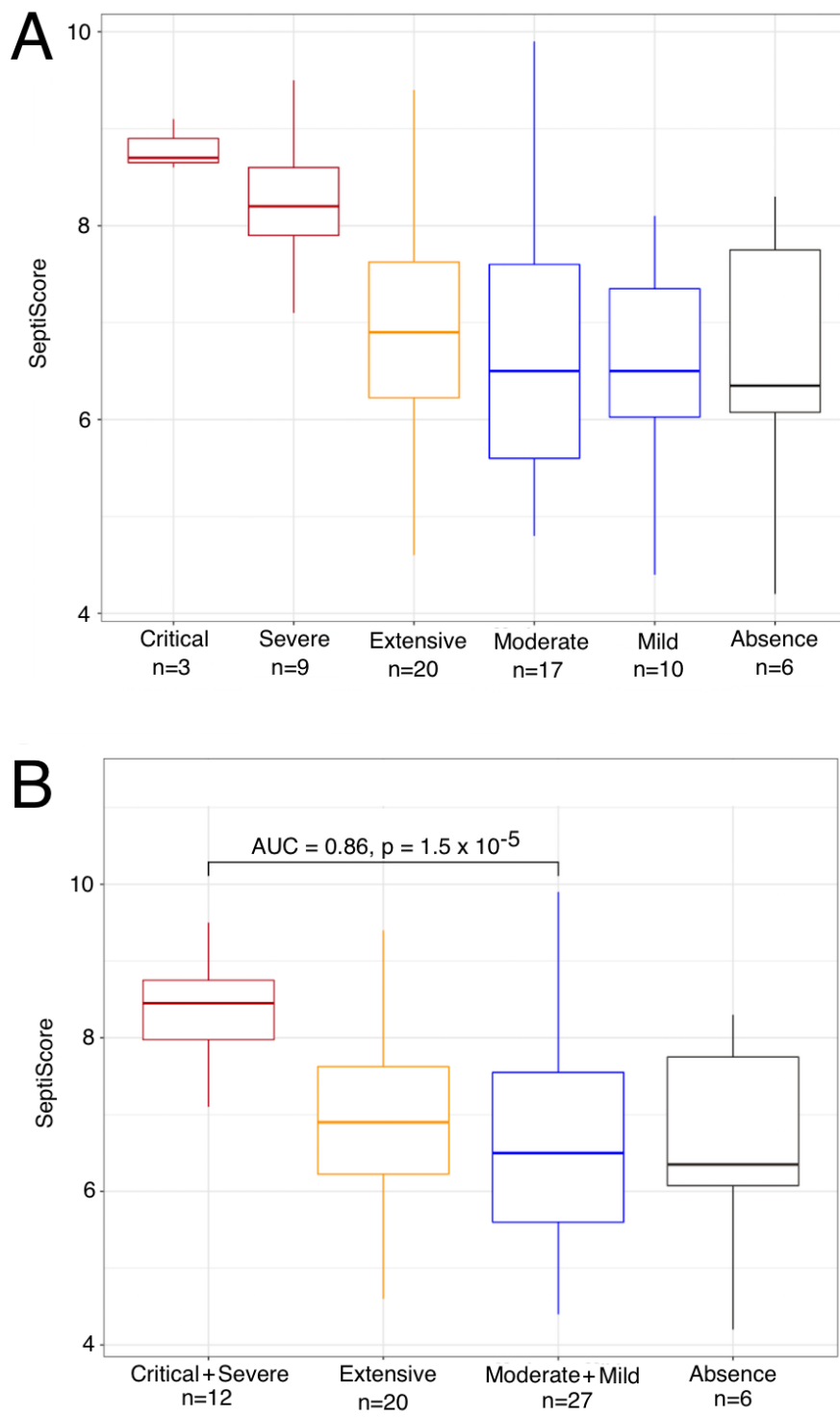
Interestingly, SeptiScores and CT scans were determined three times for two patients while they were in the ICU. Each time, both patients had lung injury  $\geq 50\%$  and their median SeptiScore was 8.4 (range 6 to 9.7), while their median CRP had decreased to 2.5 mg/L presumably because of their treatments with corticosteroids and tocilizumab.

311 **Table 3.** AUC and cut-off values of the different biological parameters tested for the discrimination of patients with lung injury <50%  
 312 (absence+mild+moderate+extensive) versus ≥50 % (severe+critical) on initial CT-scan. The first measured timepoint data were used for parameter values.  
 313 Abbreviations: AUC, area under the curve; CRP, C-reactive protein.

	<b>N, lower damage (&lt;50%)</b>	<b>N, higher damage (≥50%)</b>	<b>p-value (t-test)</b>	<b>AUC</b>	<b>Cut-off</b>	<b>Sensitivity (%)</b>	<b>Specificity (%)</b>
SeptiScore	53	12	0.00013	0.860	≥ 7	100.0	61.1
CRP (mg/L)	49	11	0.00032	0.836	> 154.1	81.8	78.4
Lymphocytes (10 <sup>9</sup> /L)	52	11	0.025	0.786	≤ 0.7	81.8	62.3
Monocytes (10 <sup>9</sup> /L)	52	11	0.016	0.758	≤ 0.5	81.8	56.6
Monocytes CD16 <sup>POS</sup> (10 <sup>9</sup> /L)	43	10	0.046	0.773	≤ 0.037	80	66.7
Lactate (mmol/L)	35	11	0.114	0.745	> 1.2	81.8	66.7

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**Figure 3.** SeptiCyte RAPID scores, stratified by COVID-19 severity as measured by chest CT scans within 24 hours of SeptiCyte RAPID testing. (A) Stratification into critical, severe, extensive, moderate, mild, and absence groups based on chest CT scans. (B) Stratification with critical+severe combined, and moderate+mild combined.

*3.3 Elevated SeptiScore is predictive of ICU admission in patients with extensive lung injury.*



328 All the patients except for one with lung injury  $\geq 50\%$  were admitted to ICU. In ad-  
329 dition, among the 17 patients with extensive lung injury (between 25 – 50 %) whose age  
330 was less than 80 years, 6 were admitted to ICU, either immediately after hospital admis-  
331 sion (3 cases) or after some days in conventional units (3 cases). For patients with exten-  
332 sive lung injury and age  $<80$  years, SeptiScores were significantly higher in those requir-  
333 ing ICU care (N=6) versus those who did not require this level of care (N=11), with me-  
334 dian SeptiScore values of 7.6 versus 6.6, respectively ( $p = 0.014$  by Welch's two sample  
335 t-test). In contrast, CRP levels were not significantly different for these patients (median  
336 levels 83 versus 114 mg/L, respectively ( $p = 0.71$  by Welch's two sample t-test). The  
337 SeptiScore AUC was 0.86, and an optimal SeptiScore cut-off for this discrimination was  
338 6.7.

#### 340 *3.4 SeptiCyte RAPID, compared to / combined with other clinical variables*

341 We conducted ROC curve analyses to compare SeptiCyte RAPID to other single  
342 biomarkers, specifically CRP, D-dimer, lactate, monocytes, and CD16-monocytes, for  
343 discriminating (critical + severe) vs. (moderate + mild) cases. These biomarkers have all  
344 been used previously by clinicians and investigators to assess the severity of COVID-19  
345 clinical trajectories [21,26-30].

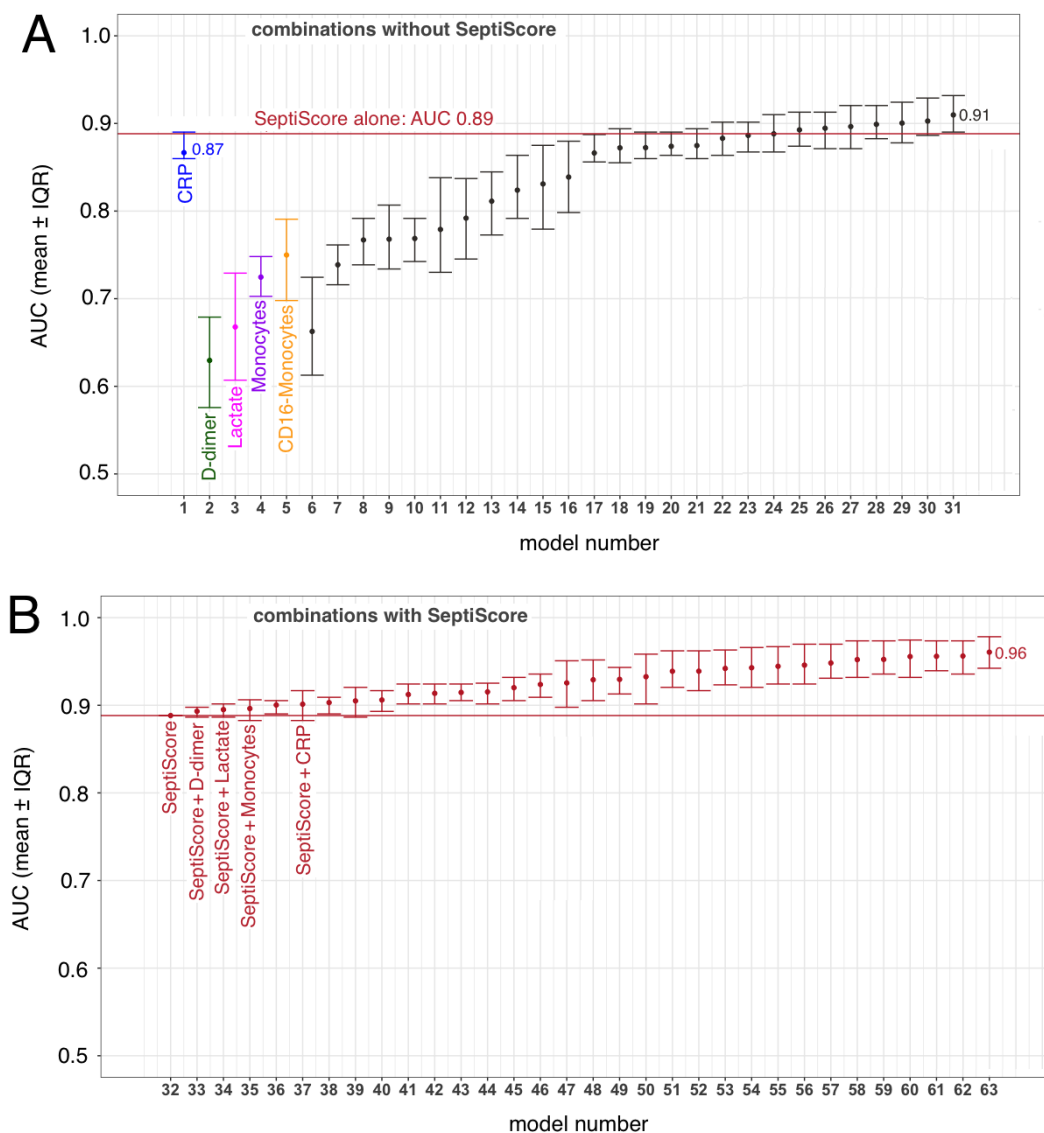
347 In these analyses, we compared critical + severe cases in ICU (n=11) versus moderate  
348 + mild cases not in ICU (n=24), with only the first SeptiCyte RAPID measurement con-  
349 sidered. The complete combinatorial analysis is presented in Supplementary Table 3. We  
350 found that the mean AUC for SeptiCyte RAPID was greater than that of any individual  
351 biomarker and of most combinations thereof (Figure 4A).

353 The single biomarker that came closest to SeptiCyte RAPID in performance was CRP  
354 (AUC 0.87, versus SeptiScore AUC 0.89). In this regard, however, we note that SeptiCyte  
355 RAPID remains discriminatory (i.e. sensitive to lung injury) during the follow-up period,  
356 when CRP has been artificially reduced due to treatment with corticosteroids and  
357 tocilizumab (see Discussion).

359 Combining the SeptiScore with one or more of these other biomarkers provided an  
360 additional improvement in AUC beyond the use of the SeptiScore alone (Figure 4B). Any  
361 of the logistic regression models containing SeptiScore with clinical parameters (Panel B)  
362 performed better than all of the singleton biomarker models, and better than most mod-  
363 els that did not contain SeptiScore (compare panels B and A).

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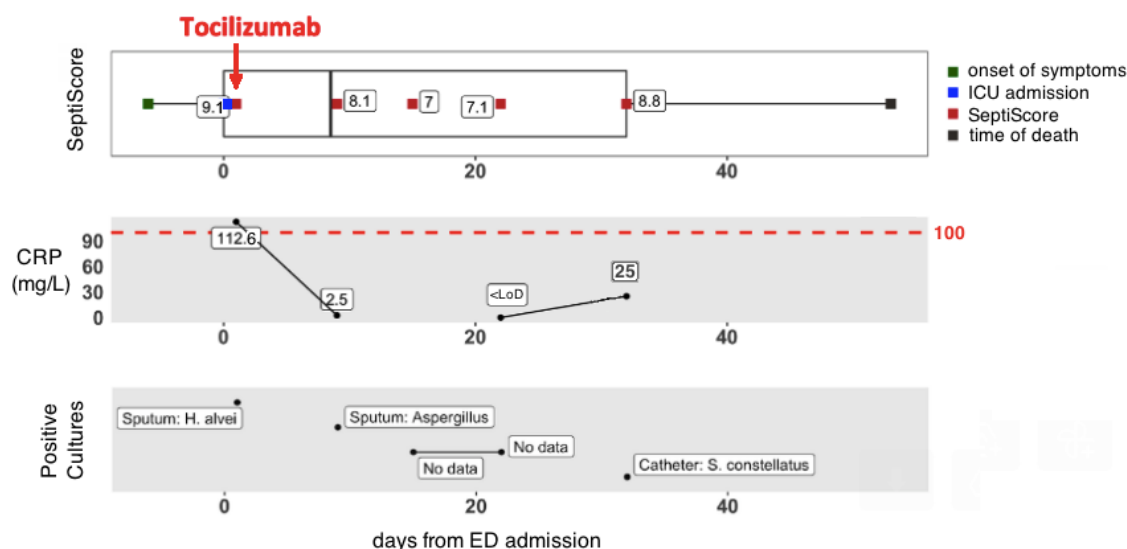


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**Figure 4.** Combining SeptiScore with other clinical variables. The variables CRP, D-dimer, lactate, monocytes, CD16-monocytes were examined alone and in combination. The patients included in this analysis were as follows: Critical + Severe in ICU (n=11) versus Moderate + Mild not in ICU (n=24), with only the first SeptiCyte RAPID measurement considered. The identities of the regression models are given in Supplementary Table 3. Error bars indicate the interquartile range of AUCs for 100 imputation replicates. (A) Combinations without SeptiScore. (B) Combinations with SeptiScore.

### 3.5 Longitudinal monitoring of patient trajectories

Finally, we consider the use of SeptiCyte RAPID, as a component of the longitudinal monitoring of COVID-19 patients during their transit through hospital. Figure 5 presents the clinical trajectory of one such patient, who presented to ED, was transferred immediately to ICU, suffered a critical level of lung damage (>75%), received intubation with mechanical ventilation, and unfortunately died. An important point to note about this patient's trajectory is that an initially high CRP level (112.5 mg/L) was greatly reduced (to 2.5 mg/L) by tocilizumab + corticosteroids. However the SeptiScore, initially 9.1, remained high throughout the patient's trajectory (8.1, 7.0, 7.1, 8.8 upon repeat testing).



**Figure 5.** Hospital trajectory of patient with “critical” lung damage (male, 65 years, angor, dyslipidemia, >75% lung injury (critical), intubated with mechanical ventilation, died). In the CRP panel, the limit of detection (LoD) = 1 mg/L.

#### 4. Discussion

Infection by SARS-CoV-2, initially described in China in 2019, rapidly spread throughout the world and has evolved in several waves, corresponding to the appearance of new variants. These waves are characterized by the influx of patients to emergency departments, which are quickly saturated. Therefore, it is important to have a rapid diagnosis which identifies patients with mild forms of the disease who can immediately be discharged, versus those who require hospitalization, or even admission to intensive care.

In addition, identification of potential risk factors that could potentially predict the disease course may be of great utility for healthcare professionals to efficiently triage patients, personalize treatment, monitor clinical progress, and allocate proper resources at all levels of care to mitigate morbidity and mortality. Different risk factors, which can be divided in demographic, clinical, hematological, biochemical, and radiographic factors, have been so far described to evaluate COVID-19 disease severity [6]. Although certain factors clearly identify patients at high risk, none can be considered a gold standard.

At this time, in addition to the clinical examination and hypoxemia, evaluation of disease severity is based on hematological tests (severity of the lymphopenia is considered as a risk factor), biochemical tests to evaluate the inflammatory state (mainly CRP), and CT-scans [31], with the patient’s age and comorbidities also taken into account. However, hospital central laboratories and imaging departments may be quickly overwhelmed by the importance of the requests, potentially leading to delays in the return of results, and consequently to emergency department overcrowding. Therefore it is important to have tests allowing a rapid assessment of the severity of the infection of patients.

In this study we have evaluated the performance of a new host immune response test, SeptiCyte RAPID, in discriminating COVID-19 severity in confirmed SARS-CoV-2 positive patients who were admitted to the ED. The study cohort reflected the real-world situation at this stage of the pandemic: it consisted of a heterogeneous group of non-acute

422 and acutely ill patients with varying phases of an inflammatory response as well as a  
423 range of comorbidities.

424  
425 SeptiCyte RAPID was found to have utility for diagnosing severe or critical  
426 COVID-19 and the subsequent risk of ICU admission in SARS-CoV-2 positive patients. At  
427 time of presentation, SeptiCyte RAPID differentiated critical+severe vs. moderate+mild  
428 COVID-19 with AUC 0.86 (Figure 3B). If the critical+severe cohort was restricted to those  
429 measured in ICU vs. moderate+mild measured outside ICU then the AUC increases to  
430 0.89 (Figure 4). In patients requiring ICU hospitalization, the SeptiScore was similar to  
431 values observed in patients with culture-confirmed bacterial sepsis.

432  
433 Additionally, the SeptiScore discriminated patients with lung injury above 50% on  
434 CT-scan (AUC = 0.88 for critical+severe vs. absence+mild+moderate+extensive), and par-  
435 ticularly could identify patients with extensive lung injury (25 – 50%) requiring ICU ad-  
436 mission. Lastly, during the follow-up of the patients, the SeptiScore remained elevated in  
437 patients with lung injury above 50%, whereas other biological markers such as CRP de-  
438 creased, likely due to anti-inflammatory treatments received by the patients (corticoster-  
439 oids, tocilizumab).

440  
441 We note that SeptiCyte RAPID was initially validated for use in patients exhibiting  
442  $\geq 2$  SIRS criteria and suspected of sepsis, with bacterial infection being the most common  
443 etiology [18]. Optimal application of the test for discriminating COVID-19 severities, or  
444 for guiding the ICU admission decision for COVID-19 patients, could involve minor ad-  
445 justment to the predefined assay cutoffs used by the SeptiCyte RAPID software. Testing  
446 of additional COVID-19 patients may provide further insight on this point.

447  
448 In comparing SeptiCyte and CRP, several points should be further emphasized. The  
449 study cohort was heavily treated with both corticosteroids (59/94 = 63%) and  
450 tocilizumab (24/94 = 26%), with some patients receiving both therapies (22/94 = 23%). It is  
451 well known that CRP is negatively modulated by both corticosteroids and tocilizumab  
452 [32-37]. Thus, in treated patients, CRP may no longer be a useful indicator of progression  
453 to more severe states, e.g. secondary bacterial infections [34,38] whereas SeptiScore val-  
454 ues were not impacted, consistent with its original function as an indicator of sepsis. The  
455 patient trajectory described in Fig. 5 illustrates this point: an initially high CRP level was  
456 greatly reduced by tocilizumab + corticosteroids, but the SeptiScore remained high  
457 throughout the patient's trajectory. In this particular case, while CRP may have been an  
458 accurate indicator of inflammatory state, it clearly was unable to indicate the underlying  
459 condition that eventually led to the patient's death. In contrast, the SeptiScore remained  
460 high and at an alert level.

461  
462 Another point to note is that CRP is an integral component of the "acute phase re-  
463 sponse" which is an early and relatively non-specific part of the innate immune response  
464 to various stressors including trauma and infection by both bacteria and viruses [39,40].  
465 The two genes of SeptiCyte RAPID (PLAC8 and PLA2G7) are not in the acute phase re-  
466 sponse pathway, so it is perhaps unsurprising that they behave differently in response to  
467 anti-inflammatory treatments.

468  
469 One strength of our study is that test results were returned quickly in real time,  
470 typically within 1-2 hours of sample collection, in a near-patient setting (even in the ED).  
471 An additional strength of the study is that it is representative of the real-world situation  
472 that occurred in our institution over the study period. However, it is clear that is also a  
473 limitation, since the patient population was quite heterogeneous in terms of pre-existing  
474 conditions and comorbidities. This study design limitation could lead to multiple varia-  
475 bles contributing to an elevation of the SeptiCyte RAPID score. A further limitation is

476 that most of the patients admitted to ICU had blood drawn after ICU admission. Con-  
477 sequently, the SeptiScore was perhaps different (possibly higher) when patients were in  
478 the ED. This question will need to be addressed in subsequent studies. A final limitation  
479 of our study is that we were unable to follow patients after discharge from hospital, to  
480 determine if they ultimately exhibited any post-acute sequelae of COVID-19 disease, i.e.  
481 signs or symptoms of “long COVID” [41,42]. It remains to be established whether  
482 SeptiCyte RAPID would have clinical utility in helping to diagnose this condition.  
483

## 484 5. Conclusions

485 This study supports the potential use of SeptiCyte RAPID in the risk stratification of  
486 COVID-19 patients, as measured by clinical severity assessment based on chest CT scans  
487 and/or ICU admission. With a quick availability from whole blood (potentially within 1  
488 hour) and with minimal user interaction, the assay may be helpful in stratifying or tri-  
489 aging patients during their transit through hospital. In contrast, hematological and bio-  
490 chemical markers as well as CT scans can take several hours, especially in the event of a  
491 massive influx of patients to the hospital. Secondly, small hospitals do not always have  
492 an imaging department available 24 hours a day, and the SeptiScore result gives good  
493 information in the event of lung damage greater than 50%. Thirdly, it can be useful for  
494 the follow-up of patients, particularly patients in the ICU, who are difficult to transport  
495 to the imaging department, and for whom the interventional treatments may have nor-  
496 malized the usual biochemical and/or hematological parameters (such as CRP). The  
497 SeptiCyte RAPID test could also constitute an approach to evaluate lung damages.  
498 Moreover, the evolution of patients with COVID-19 in intensive care is often marked by  
499 the appearance of bacterial complications, for which the SeptiScore has shown potential  
500 value (Figure 5).

501 **Abbreviations:** AHT, arterial hypertension; ARDS, acute respiratory distress syndrome; AUC, area  
502 under ROC curve; CD16, Fc receptor with low affinity for IgG, encoded by genes FCGR3A and  
503 FCGR3B; BMI, body mass index; CI, confidence interval; COVID-19, coronavirus disease 2019;  
504 CRP, C-reactive protein; CT, computed tomography; ECMO, extracorporeal membrane oxygena-  
505 tion; ED, Emergency Department; EDTA, ethylene diamine tetraacetic acid; F, female; GGO,  
506 ground glass opacity; ICU, Intensive Care Unit; M, male; NIH, United States National Institutes of  
507 Health; NGS, next-generation sequencing; NA, not available; PCT, procalcitonin; PLA2G7, Phos-  
508 pholipase A2 Group VII gene; PLAC8, Placenta Associated 8 gene; ROC, receiver operating char-  
509 acteristic [curve]; RT-qPCR, reverse transcription - quantitative polymerase chain reaction;  
510 SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SPC, sample processing control;  
511 WHO, World Health Organization.

512 **Supplementary Materials:** The following supporting information can be downloaded at:  
513 [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1), Table S1: Patient comorbidities and treatments; Table S2: Distribution of  
514 SARS-CoV-2 (+) patients across COVID-19 severity categories and hospital locations; Table S3: Lo-  
515 gistic Regression models (see Figures. 4A, 4B in main text).

516 **Author Contributions:** Conceptualization: M.V.; methodology: M.V., V.G. and J.T.K.; investiga-  
517 tion: M.V.; data acquisition, V.G., F.M., F.A., M.C.B., B.Z., F.P., T.P., E.F., and M.V.; data curation:  
518 M.V. and K.N.; custom software: K.N.; visualization: K.N., T.D.Y. and M.V.; formal analysis: M.V.,  
519 V.G., K.N., and T.D.Y.; writing—original draft preparation: M.V., and T.D.Y.; writing—review and  
520 editing: M.V., B.Z., F.M., T.P., E.F., T.D.Y., K.N. and J.T.K.; supervision: M.V.; project administra-  
521 tion: M.V.; funding acquisition: M.V. and T.D.Y. All authors have read and agreed to the published  
522 version of the manuscript.

523 **Funding:** Please add: This research was funded in part by contract # 75A50120C00125 from the  
524 DRIVE Solving Sepsis program of the Biomedical Advanced Research and Development Authority  
525 (BARDA), part of the US HHS Office of the Assistant Secretary for Preparedness and Response.

526 **Institutional Review Board Statement:** This study was conducted under a blanket protocol for  
527 COVID-related research at the Foch Hospital (“Information réutilisation des données personnelles

et des échantillons biologiques à des fins de recherche V2 - 9 avril 2020"), which had been established and approved by the local ethics committee of the Foch Hospital (IRB00012437).

**Informed Consent Statement:** The study was performed in agreement with French ethical laws, such that the patients and their families were informed that their biological samples and data used for routine care could be used in an anonymous manner unless they expressed their opposition. No patients enrolled in this study, or their families, expressed opposition to the use of their biological samples and data, which according to the French ethical laws and Foch Hospital ethics committee constitutes informed consent.

**Data Availability Statement:** Data will be provided, upon reasonable request to corresponding author.

**Acknowledgments:** We thank Biocartis for provision of the Idylla platform to the Foch Hospital site, and Julie Gaiffas and David Favvy of Biocartis for liaison support. We also thank Immunexpress for continued financial support over the course of this project. We thank Dr. Dayle Sampson for initial R coding for analysis leading to Figure 4.

**Conflicts of Interest:** T.D.Y., K.N., and J.T.K. are current or former employees and shareholders in Immunexpress. The other authors declare no competing interests. The DRIVE Solving Sepsis program of the Biomedical Advanced Research and Development Authority (BARDA) had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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