

## Early rise in circulating Endothelial Protein C Receptor correlates with poor outcome in severe sepsis

Journal:	<i>Intensive Care Medicine</i>
Manuscript ID:	ICM-2010-00494.R4
Article Type:	Original
Date Submitted by the Author:	n/a
Complete List of Authors:	<p>Guitton, Christophe; Inserm, U643, Institut de Transplantation et de Recherche en Transplantation; CHU, Service de Réanimation Médicale</p> <p>Gerard, Nathalie; Inserm, U643, Institut de Transplantation et de Recherche en Transplantation</p> <p>Sébille, Véronique; Faculté de Pharmacie, Université de Nantes, EA 4275; CHU, Plateforme de Biométrie</p> <p>Bretonnière, Cédric; CHU, Service de Réanimation Médicale</p> <p>Zambon, Olivier; CHU, Service de Réanimation Médicale</p> <p>Villers, Daniel; CHU, Service de Réanimation Médicale</p> <p>Charreau, Béatrice; Inserm, U643, Institut de Transplantation et de Recherche en Transplantation</p>
Descriptor:	SIRS/Sepsis: experimental studies, SIRS/Sepsis: clinical studies

1  
2  
3 **Early rise in circulating Endothelial Protein C Receptor correlates with poor outcome in**  
4 **severe sepsis**  
5  
6  
7

8 Christophe Guitton <sup>1,2</sup>, Nathalie Gérard <sup>1</sup>, Véronique Sébille <sup>3,4</sup>, Cédric Bretonnière <sup>2</sup>, Olivier  
9 Zambon <sup>2</sup>, Daniel Villers <sup>2</sup> and Béatrice Charreau<sup>1</sup>  
10  
11

12  
13  
14 <sup>1</sup> INSERM, U643, Nantes, F44000 France; CHU Nantes, Institut de Transplantation et de Recherche en  
15 Transplantation, ITERT, Nantes, F44000 France; UMR643 Université de Nantes, Faculté de Médecine,  
16 Nantes, F44000 France.  
17

18 <sup>2</sup> Service de Réanimation Médicale Polyvalente, CHU de Nantes, F44000 France.  
19

20 <sup>3</sup> EA 4275, Faculté de Pharmacie, Université de Nantes, Nantes, F44000 France.  
21

22 <sup>4</sup> Plateforme de Biométrie, CHU de Nantes, F44000 France.  
23  
24  
25

26 **Running title: Profiling soluble EPCR upon severe sepsis**  
27

28 Word count for text: 2987  
29

30 Word count for abstract: 248  
31

32 Figure count: 3  
33

34 Table count: 2  
35

36 Online Resource : 3  
37

38 Reference count: 36  
39  
40  
41

42 **Corresponding author:**  
43

44 Dr. Christophe GUITTON, M.D.;

45  
46 Service de Réanimation Médicale Polyvalente ; Hôtel Dieu, CHU de Nantes ; 30 Boulevard Jean Monnet ;  
47

48 44093 NANTES Cedex 1 ; FRANCE  
49

50 Phone + 33 2 40 08 73 65 ; Fax + 33 2 40 08 75 50  
51

52 E-Mail christophe.guitton@chu-nantes.fr  
53  
54  
55  
56  
57  
58  
59  
60

**Abbreviations:**

APC : activated protein C; EC : endothelial cell; EPCR : endothelial protein C receptor; ICU : intensive care unit; MPA : microscopic polyangiitis; PC : protein C; SAPS : simplified acute physiology score; mEPCR : membrane EPCR ; sEPCR : soluble EPCR; SIRS : systemic inflammatory response syndrome; SOFA : Sepsis-related Organ Failure Assessment; TF : tissue factor; TM : thrombomodulin; TNF $\alpha$  : Tumor necrosis factor-alpha; WG Wegener granulomatosis.

**Key words:** soluble EPCR, severe sepsis, gender, infection, survival analysis

## Abstract

**Purpose :** The endothelial protein C receptor (EPCR) negatively regulates the coagulopathy and inflammatory response in sepsis. Mechanisms controlling the expression of cell-bound and circulating soluble (s)EPCR are still unclear. Moreover, the clinical impact of EPCR shedding and its potential value to predict sepsis progression and outcome remain to be established.

**Methods :** We investigated the time-course of plasma sEPCR upon the 5 first days (D) of severe sepsis in 40 patients.

**Results :** Firstly, no significant difference was observed when comparing sEPCR at admission (D1) to healthy volunteers and to patients with vasculitis. We report that the kinetic profile of plasma sEPCR in patients was almost stable at the onset of sepsis with no change from D1 to D4 and then a significant decrease at D5. This pattern of release was consistently observed whatever the level of sEPCR at D1. Characteristics of patients or of infections (except Gram negative) had no or poor critical influence on sEPCR profile. However, we found that sEPCR kinetic was clearly influenced by patient's outcome (D28 survival). We demonstrate that a significant but moderate (< 15% of basal level) and transient increase in sEPCR level at Day2 associates with poor outcome at Day28.

**Conclusion :** Severe sepsis, at the onset, only triggers moderate quantitative changes in plasma sEPCR levels. Our findings suggest that in severe sepsis, an early (at D2), transient but significant increase in circulating sEPCR may be detrimental suggesting that sEPCR could provide an early biological marker of sepsis outcome.

1  
2  
3  
4  
5  
6 Severe sepsis is associated with systemic inflammation and exacerbated procoagulant  
7  
8 state mediated by the Tissue factor (TF) pathway[1-2]. The protein C (PC) anticoagulant  
9  
10 pathway is a major system that prevents blood coagulation, its impairment influence vital  
11  
12 outcome in sepsis. This pathway involves PC and protein S, and two endothelial receptors,  
13  
14 thrombomodulin (TM) and the endothelial cell protein C receptor (EPCR). Conversion of PC  
15  
16 to activated PC (APC) is initiated by thrombin bound to TM on endothelial cell (EC) surfaces.  
17  
18 Binding to EPCR increases the rate of PC activation by thrombin-TM complexes[3]. APC has  
19  
20 anticoagulant effects but also exhibits anti-inflammatory[4-5], and antiapoptotic effects[6-7].  
21  
22 EPCR also serves as a cellular binding site for Factor VII/VIIa[8-9].  
23  
24  
25  
26

27 EPCR (CD201) is a 46-kilodalton type I transmembrane protein, which is expressed  
28  
29 mainly on the luminal surface of EC from large vessels and which is homologous to major  
30  
31 histocompatibility complex class I/CD1 family proteins[10]. Disruption of the *EPCR* gene in  
32  
33 mice causes placental thrombosis and embryonic lethality confirming the key role for EPCR  
34  
35 in controlling coagulation[11].  
36  
37  
38

39 A soluble form of EPCR (sEPCR) has been described in plasma[12]. sEPCR can be  
40  
41 generated by ectodomain shedding[13] mediated by TACE/ADAM17[14] or/and, by  
42  
43 alternative mRNA splicing in haplotype-A3-carrying cells[15]. sEPCR binds both PC and  
44  
45 APC with an affinity similar to that of membrane EPCR(mEPCR)[16]. Binding of APC to  
46  
47 sEPCR interferes with binding of APC to phospholipids and inactivation of factor Va.  
48  
49 Furthermore, binding of PC to sEPCR does not enhance APC generation, suggesting a  
50  
51 procoagulant effect of sEPCR[9, 17].  
52  
53  
54  
55

56  
57 EPCR levels vary in a wide variety of pathophysiological conditions, and the consequences  
58  
59 depend partly on whether it is the membrane-associated or soluble form that is affected[12,  
60

1  
2  
3 18-19]. While the role of membrane-associated EPCR is clearly antithrombotic and anti-  
4  
5 inflammatory in physiological circumstances, the function of the circulating sEPCR remains  
6  
7 unclear.  
8  
9

10 Regulation of circulating sEPCR upon inflammatory disease has not been determined. Thus,  
11  
12 whether sEPCR levels may have a predictive value of sepsis outcome is still unknown. In the  
13  
14 present study, we investigated the kinetic of soluble EPCR levels in a cohort of 40 patients  
15  
16 with severe sepsis upon the 5 first days in our Intensive Care Unit (ICU). The first objective  
17  
18 was to compare sEPCR levels in septic patients at admission to healthy volunteers or to  
19  
20 patients with active inflammatory disease. The second aim was to investigate the time course  
21  
22 of circulating sEPCR at the onset of severe sepsis in correlation with clinical parameters and  
23  
24 outcome at D28.  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Patients, Materials and Methods

**Study population-** We recruited consecutive patients with severe sepsis in a 20 beds Medical ICU (Nantes University Hospital, France). Patients were included using the inclusion criteria according to the ACCP consensus definition for severe sepsis [20] and as described in the Electronic Supplementary Material 1 (ESM1).

The exclusion criteria were age younger than 18 years, patients without informed consent, patients not expected to survive more than 48h (moribund) or not expected to survive more than 28 days given preexisting conditions.

We collected baseline characteristics of the patients, including demographic information, severity score at admission, simplified acute physiology score (SAPS), Sepsis-related Organ Failure Assessment (SOFA) at admission and for 4 days, comorbidities, site and type of infection, and hematologic tests. Patients were followed for 28 days and/or until ICU discharge.

**Blood collection-** Blood was collected from patients within 24h of meeting the definition of severe sepsis (within the first 24 hours after the first organ dysfunction occurred) and daily in the 4 consecutive following days. For that purpose, blood samples were collected in the entrance in ICU (Day 1) and each morning using daily routine blood puncture (Day 2 to Day 5). After centrifugation, samples were immediately frozen as aliquots (-80°C).

This study also included blood samples from patients with active systemic vasculitis (n=25) including Wegener granulomatosis (WG) or microscopic polyangiitis (MPA) that we previously described[21] and blood samples from healthy adult blood donors (n=40) provided by the Etablissement Français du Sang (Nantes, France).

**Elisa assays for soluble EPCR -** Quantification of sEPCR in plasma samples was carried out using ELISA kits (Asserachrom, Stago-Diagnostica, France).

1  
2  
3 **Research ethics-** The study was approved by medical research committee (Nantes University  
4 Hospital, France). Informed consent was obtained from the patient/substitute decision-maker  
5 before inclusion in this study. All data were anonymous.  
6  
7

8  
9  
10 **Statistical Analysis –** The means and SD or the median [IQR], in case of non-normality of  
11 the distributions, are reported for continuous variables. The number of patients in each  
12 category and the corresponding percentages are given for categorical variables. Non  
13 parametric ANOVA was used for the comparison of sEPCR levels at admission between  
14 groups.  
15  
16

17  
18  
19  
20  
21  
22 Multivariate analyses were performed to study the evolution of sEPCR levels over time. To  
23 account for the correlation between measurements from the same individual, repeated  
24 measures ANOVA using linear mixed models allowing for random effects with restricted  
25 maximum-likelihood estimation were used to examine changes in sEPCR levels over time and  
26 potential interactions when appropriate (“time effect”), as well as the effect of covariates of  
27 interest (“group effect”). Several covariance structures among the repeated measurements  
28 (autoregressive, unstructured etc.) were compared using Akaike’s Information Criterion and  
29 Schwarz Bayesian Criterion. Random intercept models with autoregressive covariance  
30 structures often provided the best fit to the data. Residual analysis was used to evaluate the  
31 validity of the models assumptions including normality and homoscedasticity. An iterative  
32 stepwise selection procedure was used to select the variables that were significantly  
33 associated with the time course of sEPCR levels (variable candidates for the model were those  
34 associated with sEPCR evolution in univariate analyses with  $p < 0.20$  criterion and  
35 subsequently selected in the model using  $p < 0.05$  criterion). All measurements were  
36 transformed by calculating the natural logarithm and all analyses were adjusted on sEPCR  
37 levels at day1 in repeated measures models.  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 Multivariate analyses were subsequently performed using exact logistic regression and a  
4  
5 similar strategy was used with an iterative stepwise selection procedure to select the variables  
6  
7 that were significantly associated with day-28 survival, as assessed by the likelihood ratio  
8  
9 test. The area under the receiver operating characteristic (ROC) curve was estimated for  
10  
11 sEPCR levels at day 2.  
12  
13

14  
15 Statistical analyses were performed with SAS 9.1 statistical software (SAS Institute, Cary,  
16  
17 NC, USA). P-values <0.05 were considered to be statistically significant.  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

## Results

### Study population

Between December 2004 and September 2005, 82 patients were admitted for severe sepsis in our Medical ICU, 27 patients were not eligible because they had developed first organ dysfunction more than 24h before and 15 patients refused inclusion or delayed their response after inclusion “dead line”. Thus, the present study includes 40 consecutive adult patients with severe sepsis. Most of them (n=37, 93.5%) were in septic shock at admission but all of them at day 2. The patients ranged in age from 24 to 81 years ( $58.3 \pm 14.4$ ), and 24 (60%) were female. The mean admission SAPS II score was 51 (SD, 21), and the mean SOFA score at Day1 was 10.5 (SD, 3.4). The overall 28-day mortality rate was 22.5 %. Demographic and clinical characteristics of these patients are summarized in **Table 1**. The most common site of infection was pneumonia (27.5%). Infecting organism isolation was available for 35 patients (87.5%) with a Gram-negative bacillus for 18 patients (45%). Hemoculture was positive for 35% of patients.

### Soluble EPCR levels in patients with severe sepsis at admission (Day1): comparison with patients with inflammatory disease and healthy volunteers

Soluble EPCR was quantified concomitantly in all samples (n=182 for sepsis and n=65 for healthy controls and vasculitis) using a dedicated Elisa assay. When sEPCR levels were compared at the time of admission (D1), no significant difference (**Figure 1**) was found between patients with severe sepsis (Med [IQR]:115 [145]; Mean $\pm$ SD:  $174 \pm 117$  ng/mL) compared with healthy control subjects (156 [65];  $170 \pm 63$  ng/mL; p =0.37) and with patients with active systemic vasculitis (WG and MPA)(159[94];  $157 \pm 60$  ng/mL). As compare to the

1  
2  
3 healthy controls, sEPCR values in septic patients exhibit larger variability, especially above  
4 the median value (115 ng/mL). For further analysis, septic patients will be divided in 2 groups  
5 (High sEPCR > 115 ng/mL and Low sEPCR ≤ 115 ng/mL) according to their basal sEPCR  
6 levels at Day1.  
7  
8  
9  
10  
11

### 12 13 14 15 **Time course of soluble EPCR levels at the onset of severe sepsis**

16  
17  
18 A retrospective and concomitant determination of circulating sEPCR was performed on  
19 blood samples collected from 40 patients with severe sepsis during the 5 first days in ICU.  
20 Quantification of circulating sEPCR in the overall population of patients (**Figure 2a**) mainly  
21 showed only slight variations in the sEPCR levels at onset of sepsis. No significant change  
22 was observed from D1 to D4 while a significant decrease was found at D5 ( $p < 0.05$  versus  
23 D1, D2 and D3). Overall changes in sEPCR levels from D1 to D4 were low and remained  
24 below 10% of basal (D1) sEPCR rate. Similarly, although significant, decrease at D5 was  
25 moderate and around 15% of basal rate.  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36

37  
38 This pattern of release was consistently observed whatever the level of sEPCR in patients at  
39 D1 (**ESM2a**); higher levels (H group) were maintained during the 5-days follow-up (group  
40 effect,  $p < 0.01$ ).  
41  
42  
43  
44

45  
46 Next, uni- and multivariate statistical analysis were performed to identify factors  
47 influencing sEPCR kinetics. These parameters were selected according to their relevance to  
48 demographics and severe sepsis (early disease severity scores, disease evolution and  
49 infection). Variables analyzed include gender, age, outcome at D28, SAPS II, SOFA D1,  
50 SOFA D2, Gram staining, site of infection (pneumoniae), septicemia. First that time course of  
51 sEPCR release was clearly affected in septic patients according to patient survival at D28  
52 (**Figure 2b**). Univariate analysis showed a significant difference in sEPCR levels (Group  
53 effect “GE”,  $p = 0.029$ ), and in kinetics (Time effect “TE”  $p = 0.016$ ) between the 2 groups.  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 These results were confirmed in multivariate analysis (GE,  $p=0.003$  and TE,  $p=0.005$ ). At  
4  
5 Day 2 : patients that do not survive over D28 had significantly elevated sEPCR (Med  
6  
7 268[169], mean  $268\pm126$ ) as compared to septic patients that do survive (Med 120[92], mean  
8  
9  $143\pm92$  ng/mL;  $p<0.01$ ). Increase at D2 was only transient since sEPCR return to control  
10  
11 level at D3.  
12  
13

14  
15 A difference between males and females was observed in septic patients at D1 (respectively  
16  
17 Med 196[149], mean  $225\pm143$  versus 107[98],  $140\pm84$  ng/mL,  $p<0.05$ ) as well as in healthy  
18  
19 individuals (respectively Med 177[63], mean  $201\pm67$  versus 129[41],  $145\pm47$  ng/mL,  
20  
21  $p<0.05$ ). Nevertheless, no significant impact of gender on sEPCR profiles was found in our  
22  
23 cohort using our statistical analysis model (**ESM2b**) and similar time courses were observed  
24  
25 for males and females. Similarly, age had also no influence on sEPCR profile.  
26  
27  
28  
29

30  
31 Considering variables related to severity of sepsis, SAPS II and Mc Cabe scores had no  
32  
33 impact. Interestingly, a weak effect of SOFA score at D1 and D2 was found on sEPCR levels  
34  
35 (GE respectively  $p=0.045$  and  $0.034$ ) but with no significant impact on the kinetic from D1 to  
36  
37 D5. To further investigate these results, correlations were examined considering individual  
38  
39 time points. Interestingly, a slight correlation was only found between sEPCR D2 and SOFA  
40  
41 D2 ( $\rho=0.293$ ,  $p<0.05$ ).  
42  
43  
44

45  
46 Finally considering parameters related to infection, we found that the presence of a positive  
47  
48 blood culture (not shown) or pneumonia as septic localisation (**ESM2c**) had no effect on  
49  
50 sEPCR profiles. In contrast, a significant impact of gram negative bacterial infection was  
51  
52 observed (**ESM2d**). Univariate analysis showed higher D1-D5 plasma levels (GE  $p=0.034$ )  
53  
54 without clear effect on kinetic (TE  $p=0.376$ ) while multivariate analysis confirmed the  
55  
56 influence of gram negative on both, group level and kinetic (GE  $p=0.045$  and TE  $p=0.005$ ).  
57  
58  
59  
60

1  
2  
3 Overall, our findings show only slight variations in the course of sEPCR at the onset  
4 of sepsis. It appears that most of parameters affecting basal sEPCR rate have no incidence on  
5 the kinetic profile of sEPCR upon sepsis. However, we demonstrated that both level and  
6 kinetic of sEPCR are clearly influenced by Gram negative infection and, importantly, by D28  
7 mortality: an early significant increase in sEPCR rate at D2 being associated with a poor  
8 outcome.  
9

10  
11 Consequently, to sustain this conclusion, uni- and multi-variate analyses were performed  
12 for eleven parameters (including sEPCR at D2) relevant to D28 mortality in septic patients.  
13 These parameters include sEPCR D2, sEPCR D1 H/L, SAPS II, Gender, SOFA score at D1  
14 and D2, Age, McCabe Score, Sepsis localisation (Pneumoniae), septicemia and Gram  
15 negative infection. Major findings are reported in **Table 2**. Plasma concentration of sEPCR at  
16 D2 was the only variable found significantly associated with D28 mortality in multivariate  
17 analysis ( $p=0.0095$ ). Our data indicate that an increase in sEPCR at D2 of 100 ng/mL  
18 corresponds to an Odd Ratio of 2.44 [1.22-5.95].  
19

20  
21 We undertook a specific analysis to further investigate the accuracy of sEPCR value at D2  
22 to predict D28 mortality in our cohort. Sensitivity and specificity are presented in the ROC  
23 curve (**ESM3**). The accuracy estimated by area under the ROC curve seemed satisfactory  
24 ( $AUC = 0.819$ ; IC [0.658-0.971]). The moderate size of the samples (dead=9, alive=31) is,  
25 however, a limit for further extrapolation.  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Discussion

During sepsis, blood coagulation is activated by TF expressed on the endothelium and monocytes/macrophages in response to cytokines[22]. Inflammatory processes can shift the hemostatic balance toward thrombus formation not only by stimulating TF-dependent coagulation but also by inhibiting anticoagulant and fibrinolytic pathways[23]. EPCR is a key factor controlling coagulation through the binding of both PC and APC. Inhibition of EPCR binding of PC or APC was also found to exacerbate the septic response[24-25]. However, clinical and functional impact of its soluble counterpart remains to be established. Here, we examined for the first time the kinetic of plasma sEPCR in a cohort of patients at the onset of severe sepsis.

First, when comparing baseline levels of sEPCR in septic patients at admission we found no significant difference with healthy controls and patients with active vasculitis, although variation among the septic patients was important. In previous studies, sEPCR levels in septic patients were found significantly higher[12, 26], similar[27] or even lower[28], than in healthy volunteers. These discrepancies may reflect the distribution of the A3 haplotype (15-20%) among the cohorts since this haplotype is associated with higher baseline levels of sEPCR[18].

Moreover, we observed here that gender ratio may be another key factor affecting D1 sEPCR in septic patients. A similar difference was found in healthy donors. These results confirm our previous data indicating that gender was a critical parameter determining basal level of sEPCR [29]. Although clinical impact of gender on sepsis outcome is still controversial[30-32], whether males and females differently respond to infection is important for both clinical care and the design of clinical trials. However, probably due to the small size

1  
2  
3 of this cohort and to relative variability of basal sEPCR levels, this study was unable to  
4 demonstrate an impact of gender on the sEPCR time course. Since no DNA samples were  
5 available for genetic analysis, the respective impact of EPCR haplotype and gender was not  
6 investigated.  
7  
8  
9  
10  
11

12 Next, profiling sEPCR during the 5 first days of the disease showed no change in the  
13 level of sEPCR from D1 to D4 before a significant decrease at D5. Overall variations in  
14 sEPCR concentrations remain below 15% of baseline. Interestingly, this kinetic was found  
15 consistently in septic patients regardless of basal level and gender. Another interesting  
16 observation is that, overall, sepsis only induces a moderate variation of sEPCR plasma level  
17 as suggested by initial reports[12, 27]. Together these data suggest that level of sEPCR is  
18 tightly controlled even upon inflammation *in vivo*. This hypothesis is comforted by our results  
19 obtained using cultured vascular EC. Indeed, we found that *in vitro* inflammation  
20 downregulates the cell surface expression of EPCR without affecting the production rate and  
21 kinetic of its soluble form (Data not shown and [29]).  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35

36 All available data concerning simple coagulation disorders were noted (platelet count,  
37 Prothrombin time, activated partial thromboplastin time (aPTT) ratio patient/witness, and  
38 fibrinogen). The impact of these parameters on sEPCR was examined for each time point  
39 using a Spearman test (data not shown). No major correlation was found except for platelet  
40 count and aPTT at D4. Considering the type of infection, multivariate analysis highlighted a  
41 significant impact of gram-negative infection on both sEPCR level and kinetic. Despite data  
42 of sepsis studies large cohorts, the link between severity of coagulation disorders and type of  
43 causative microorganism had still remained controversial. [33-34] .  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54

55 We identified a statistically significant difference between D28 survivors and  
56 nonsurvivors in terms of sEPCR levels at D2 compared to baseline. This new finding suggests  
57 that sEPCR level at D2 may be an early prognostic marker of adverse outcome in septic  
58  
59  
60

1  
2  
3 patients. It could also suggest an implication of elevated sEPCR in the pathogenesis of severe  
4  
5 sepsis. While EPCR is considered as a protective molecule[24], the function of its soluble  
6  
7 form remains to be determined. Functionally, sEPCR bind PC and APC with high affinity  
8  
9 (Kd=75nM)[35] and thus may counteract (or balance) the anticoagulant action of mEPCR. On  
10  
11 another hand, sEPCR also bind the neutrophil proteinase PR3 with a similar high affinity, this  
12  
13 interaction results in the proteolytic degradation of the receptor[36]. Thus, we can speculate  
14  
15 that a significant increase in plasma sEPCR may indeed reflect uncontrolled EC activation  
16  
17 and/or irreversible EC injury. This pathogenic link remains to be examined in vitro and in  
18  
19 vivo. Whatever the mechanism, a functional impairment of the endothelial protein C  
20  
21 activation pathway reflected by an increase in sEPCR level will most probably exacerbate the  
22  
23 preexisting state of hypercoagulability in septic patients.  
24  
25  
26  
27  
28

29  
30 It is to notice that death was associated with higher sEPCR level in septic patients at  
31  
32 Day 2 but not at the time of death. Five patients died within the 5 first days of the study.  
33  
34 Although it seems difficult to draw a firm conclusion from only 5 patients, the available  
35  
36 values for the last sEPCR dosage before death showed no significant change.  
37  
38  
39  
40

41  
42 To conclude, severe sepsis, at the onset, only triggers moderate quantitative changes in  
43  
44 plasma sEPCR concentration. Our findings also suggest that in pathological conditions such  
45  
46 as severe sepsis, an early (at day2 post admission), transient but significant increase in  
47  
48 circulating sEPCR may be detrimental. These new findings suggest that measuring sEPCR at  
49  
50 Day2 could provide an early biological marker of sepsis outcome.  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



**Acknowledgments and funding:**

This work was supported by grants from l'Agence de Biomédecine, La Société de Néphrologie, l'Association pour le Développement de la Réanimation (ADR, Nantes), Lilly-France. This work was also in part supported by XENOME a EU-funded Integrated Project in Life Sciences, Genomics and Biotechnology for Health (LSHB-CT-2006-037377) and by a grant from la Fondation de l'Avenir (contrat ET8-498).

**Authorship:**

Contribution: C.G., N.G., O.Z. and C.B. performed research assays and analyzed data; V.S. performed the statistical analysis. D.V. provided critical review of the manuscript; and; B.C. and C.G. conceived and designed the study and wrote the manuscript.

**Conflict of interest disclosure:** The authors declare no competing financial interests.

## Legends of Figures

**Figure 1. Baseline sEPCR in septic patients and controls.** sEPCR was quantified in blood samples from healthy volunteers (n=40), patients with vasculitis (n=25) and in samples from 40 patients with severe sepsis harvested at admission (D1). No significant difference (p=0.37). *Individual results are shown, bars represent mean values, dashed lines represent the median value. H; high and L; low referred to sEPCR levels compare to the median value..*

**Figure 2. Time-course (from D1 to D5) analysis of sEPCR in septic patients (n=40).** sEPCR was measured daily from admission in ICU (D1) to D5. Statistical difference (p<0.05) is indicated as # for “group effect” (GE) and \$ for “time effect” (TE). (a) Overall population [TE p=0.037, § p<0.05 in comparison to D5]. Number of available samples is indicated under box plot for each time point (b) According to outcome at D28, alive (n=31), dead (n=9) [GE p=0.029; TE p=0.016; \* difference at day2, p<0.01].

## References

1. Esmon CT, (2002) Protein C pathway in sepsis. *Ann Med* 34: 598-605
2. Mackman N, (2004) Role of tissue factor in hemostasis, thrombosis, and vascular development. *Arterioscler Thromb Vasc Biol* 24: 1015-1022
3. Fukudome K, Esmon CT, (1994) Identification, cloning, and regulation of a novel endothelial cell protein C/activated protein C receptor. *J Biol Chem* 269: 26486-26491
4. Joyce DE, Gelbert L, Ciaccia A, DeHoff B, Grinnell BW, (2001) Gene expression profile of antithrombotic protein c defines new mechanisms modulating inflammation and apoptosis. *J Biol Chem* 276: 11199-11203
5. Riewald M, Petrovan RJ, Donner A, Mueller BM, Ruf W, (2002) Activation of endothelial cell protease activated receptor 1 by the protein C pathway. *Science* 296: 1880-1882
6. Feistritzer C, Schuepbach RA, Mosnier LO, Bush LA, Di Cera E, Griffin JH, Riewald M, (2006) Protective signaling by activated protein C is mechanistically linked to protein C activation on endothelial cells. *J Biol Chem* 281: 20077-20084
7. Isermann B, Vinnikov IA, Madhusudhan T, Herzog S, Kashif M, Blautzik J, Corat MA, Zeier M, Blessing E, Oh J, Gerlitz B, Berg DT, Grinnell BW, Chavakis T, Esmon CT, Weiler H, Bierhaus A, Nawroth PP, (2007) Activated protein C protects against diabetic nephropathy by inhibiting endothelial and podocyte apoptosis. *Nat Med* 13: 1349-1358
8. Ghosh S, Pendurthi UR, Steinoe A, Esmon CT, Rao LV, (2007) Endothelial cell protein C receptor acts as a cellular receptor for factor VIIa on endothelium. *J Biol Chem* 282: 11849-11857
9. Lopez-Sagaseta J, Montes R, Puy C, Diez N, Fukudome K, Hermida J, (2007) Binding of factor VIIa to the endothelial cell protein C receptor reduces its coagulant activity. *J Thromb Haemost* 5: 1817-1824
10. Laszik Z, Mitro A, Taylor FB, Jr., Ferrell G, Esmon CT, (1997) Human protein C receptor is present primarily on endothelium of large blood vessels: implications for the control of the protein C pathway. *Circulation* 96: 3633-3640
11. Gu JM, Crawley JT, Ferrell G, Zhang F, Li W, Esmon NL, Esmon CT, (2002) Disruption of the endothelial cell protein C receptor gene in mice causes placental thrombosis and early embryonic lethality. *J Biol Chem* 277: 43335-43343
12. Kurosawa S, Stearns-Kurosawa DJ, Carson CW, D'Angelo A, Della Valle P, Esmon CT, (1998) Plasma levels of endothelial cell protein C receptor are elevated in patients with sepsis and systemic lupus erythematosus: lack of correlation with thrombomodulin suggests involvement of different pathological processes. *Blood* 91: 725-727
13. Xu J, Qu D, Esmon NL, Esmon CT, (2000) Metalloproteolytic release of endothelial cell protein C receptor. *J Biol Chem* 275: 6038-6044
14. Qu D, Wang Y, Esmon NL, Esmon CT, (2007) Regulated endothelial protein C receptor shedding is mediated by tumor necrosis factor-alpha converting enzyme/ADAM17. *J Thromb Haemost* 5: 395-402
15. Saposnik B, Lesteven E, Lokajczyk A, Esmon CT, Aiach M, Gandrille S, (2008) Alternative mRNA is favored by the A3 haplotype of the EPCR gene PROCRC and generates a novel soluble form of EPCR in plasma. *Blood* 111: 3442-3451
16. Liaw PC, Neuenschwander PF, Smirnov MD, Esmon CT, (2000) Mechanisms by which soluble endothelial cell protein C receptor modulates protein C and activated protein C function. *J Biol Chem* 275: 5447-5452

17. Esmon CT, (2006) The endothelial protein C receptor. *Curr Opin Hematol* 13: 382-385
18. Saposnik B, Reny JL, Gaussem P, Emmerich J, Aiach M, Gandrille S, (2004) A haplotype of the EPCR gene is associated with increased plasma levels of sEPCR and is a candidate risk factor for thrombosis. *Blood* 103: 1311-1318
19. Simioni P, Morboeuf O, Tognin G, Gavasso S, Tormene D, Woodhams B, Pagnan A, (2006) Soluble endothelial protein C receptor (sEPCR) levels and venous thromboembolism in carriers of two dysfunctional protein C variants. *Thromb Res* 117: 523-528
20. (1992) American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 20: 864-874
21. Coupel S, Moreau A, Hamidou M, Horejsi V, Soulillou JP, Charreau B, (2007) Expression and release of soluble HLA-E is an immunoregulatory feature of endothelial cell activation. *Blood* 109: 2806-2814
22. Esmon CT, (2003) Coagulation and inflammation. *J Endotoxin Res* 9: 192-198
23. Aird WC, (2003) The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. *Blood* 101: 3765-3777
24. Taylor FB, Jr., Stearns-Kurosawa DJ, Kurosawa S, Ferrell G, Chang AC, Laszik Z, Kosanke S, Peer G, Esmon CT, (2000) The endothelial cell protein C receptor aids in host defense against *Escherichia coli* sepsis. *Blood* 95: 1680-1686
25. Taylor FB, Jr., Peer GT, Lockhart MS, Ferrell G, Esmon CT, (2001) Endothelial cell protein C receptor plays an important role in protein C activation in vivo. *Blood* 97: 1685-1688
26. Liaw PC, Esmon CT, Kahn moui K, Schmidt S, Kahn moui S, Ferrell G, Beaudin S, Julian JA, Weitz JI, Crowther M, Loeb M, Cook D, (2004) Patients with severe sepsis vary markedly in their ability to generate activated protein C. *Blood* 104: 3958-3964
27. Faust SN, Levin M, Harrison OB, Goldin RD, Lockhart MS, Kondaveeti S, Laszik Z, Esmon CT, Heyderman RS, (2001) Dysfunction of endothelial protein C activation in severe meningococcal sepsis. *N Engl J Med* 345: 408-416
28. Borgel D, Bornstain C, Reitsma PH, Lerolle N, Gandrille S, Dali-Ali F, Esmon CT, Fagon JY, Aiach M, Diehl JL, (2007) A comparative study of the protein C pathway in septic and nonseptic patients with organ failure. *Am J Respir Crit Care Med* 176: 878-885
29. Guitton C, Gérard N, Quillard T, Charreau B, (2010) Circulating EPCR: endothelial regulation and cumulative impact of gender and A3 haplotype, *In press*. *J Vasc Res* ; doi: 10.1159/000322582
30. Fine MJ, Smith MA, Carson CA, Mutha SS, Sankey SS, Weissfeld LA, Kapoor WN, (1996) Prognosis and outcomes of patients with community-acquired pneumonia. A meta-analysis. *Jama* 275: 134-141
31. Eachempati SR, Hydo L, Barie PS, (1999) Gender-based differences in outcome in patients with sepsis. *Arch Surg* 134: 1342-1347
32. Adrie C, Azoulay E, Francois A, Clec'h C, Darques L, Schwebel C, Nakache D, Jamali S, Goldgran-Toledano D, Garrouste-Orgeas M, Timsit JF, (2007) Influence of gender on the outcome of severe sepsis: a reappraisal. *Chest* 132: 1786-1793
33. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, Steingrub JS, Garber GE, Helterbrand JD, Ely EW, Fisher CJ, Jr., (2001) Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 344: 699-709

- 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10
  - 11
  - 12
  - 13
  - 14
  - 15
  - 16
  - 17
  - 18
  - 19
  - 20
  - 21
  - 22
  - 23
  - 24
  - 25
  - 26
  - 27
  - 28
  - 29
  - 30
  - 31
  - 32
  - 33
  - 34
  - 35
  - 36
  - 37
  - 38
  - 39
  - 40
  - 41
  - 42
  - 43
  - 44
  - 45
  - 46
  - 47
  - 48
  - 49
  - 50
  - 51
  - 52
  - 53
  - 54
  - 55
  - 56
  - 57
  - 58
  - 59
  - 60
34. Kinasewitz GT, Yan SB, Basson B, Comp P, Russell JA, Cariou A, Um SL, Utterback B, Laterre PF, Dhainaut JF, (2004) Universal changes in biomarkers of coagulation and inflammation occur in patients with severe sepsis, regardless of causative micro-organism [ISRCTN74215569]. *Crit Care* 8: R82-90
35. Preston RJ, Villegas-Mendez A, Sun YH, Hermida J, Simioni P, Philippou H, Dahlback B, Lane DA, (2005) Selective modulation of protein C affinity for EPCR and phospholipids by Gla domain mutation. *Febs J* 272: 97-108
36. Villegas-Mendez A, Montes R, Ambrose LR, Warrens AN, Laffan M, Lane DA, (2007) Proteolysis of the endothelial cell protein C receptor by neutrophil proteinase 3. *J Thromb Haemost* 5: 980-988

For Peer Review

**Table 1: Characteristics and outcome of patients with severe sepsis(n= 40), description of infections.**

<b>Age, y</b>	58.3 ± 14.4
<b>Gender Ratio :Female, n (%) :</b>	24 (60%)
<b>Intensive Care :Medical / Surgical, n (%):</b>	22 (55%) / 18 (45%)
<b>Mc Cabe : 1 / 2 / 3, n (%)</b>	11 (27%) / 24 (60%) / 5 (13%)
<b>Immuno depression, n (%)</b> Hematology, Cancer, Corticotherapy, HIV, Cirrhosis	23 (57%)
<b>SAPS II</b>	51 ± 21
<b>SOFA score Day 1</b>	10.5 ± 3.4
<b>Septic Shock at inclusion, n (%)</b>	37 (93.5%)
<b>Mechanical ventilation*, n(%)</b>	34 (85%)
<b>Hemodiafiltration*, n (%)</b>	12 (30%)
<b>Hydrocortisone*, n (%)</b>	30 (75%)
<b>Drotrecogin*, n (%)</b>	2 (5%)
<b>Site of Infection, n</b>	
Pneumoniae	11
Abdominal	8
Skin or Soft tissues	6
Bones or Joints	4
Urologic	4
Endocarditis	3
Septicemia	2
Meningitis	1
Pleural empyema	1
<b>Positive blood culture, n (%)</b>	14 (35%)
<b>Results of Gram's staining culture</b>	
Pure Gram-negative	14
Pure Gram-positive	17
Mixed	4
Culture negative	5
<b>ICU length of stay, d</b>	17±13
<b>Mortality, n (%) ICU</b>	12 (30%)
Day 28	9 (22.5%)

ICU: Intensive care unit; SAPS: Simplified Acute Physiology Score; SOFA: Sepsis-related Organ Failure

Assessment, \* during 5 first days of ICU hospitalisation

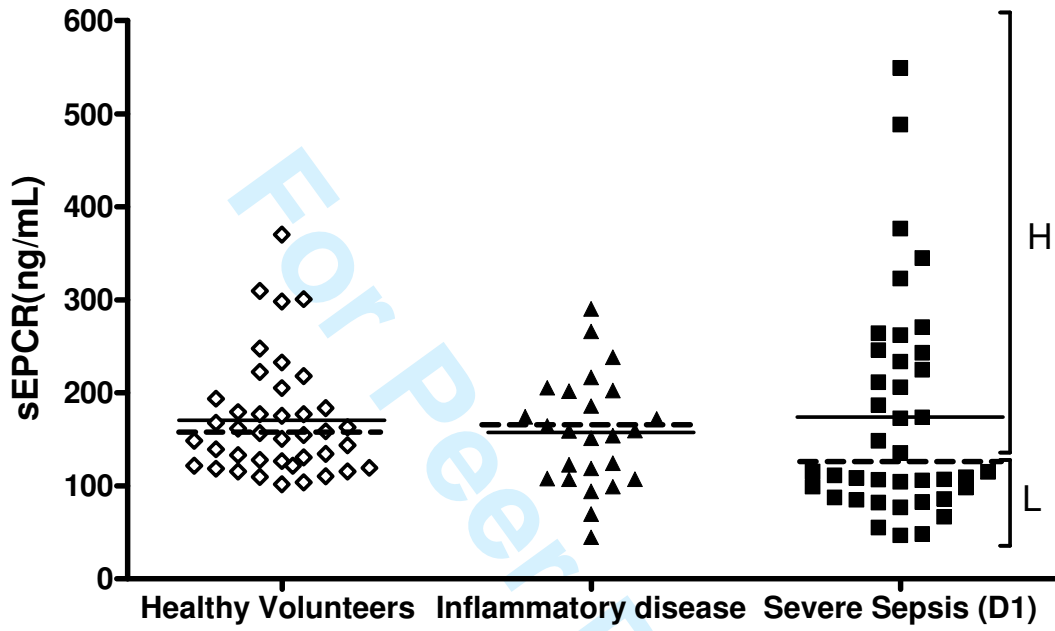
**Table 2 : Univariate and multivariate analysis of factors associated with mortality at Day 28.**

	Odd Ratio	Confidence Interval	p
<b>Univariate analysis</b>			
EPCR D2	1.009	1.002-1.018	0.0095
SAPS II	1.054	1.012-1.107	0.0087
SOFA D1	1.249	0.981-1.642	0.0740
SOFA D2	1.243	1.007-1.598	0.0417
Age	1.054	0.994-1.131	0.0845
Pneumoniae	4.948	0.800-34.047	0.0936
<b>Multivariate analysis</b>			
EPCR D2	1.009	1.002-1.018	0.0095

*The odds ratio are expressed for an increase of 1ng/ml for s EPCR at D2, 1 point for SAPS II and SOFA D1, 1 year for age. IC Interval confidence . Factors with  $p < 0.20$  are noted.*

Figure 1

Guillon C. *et al.*

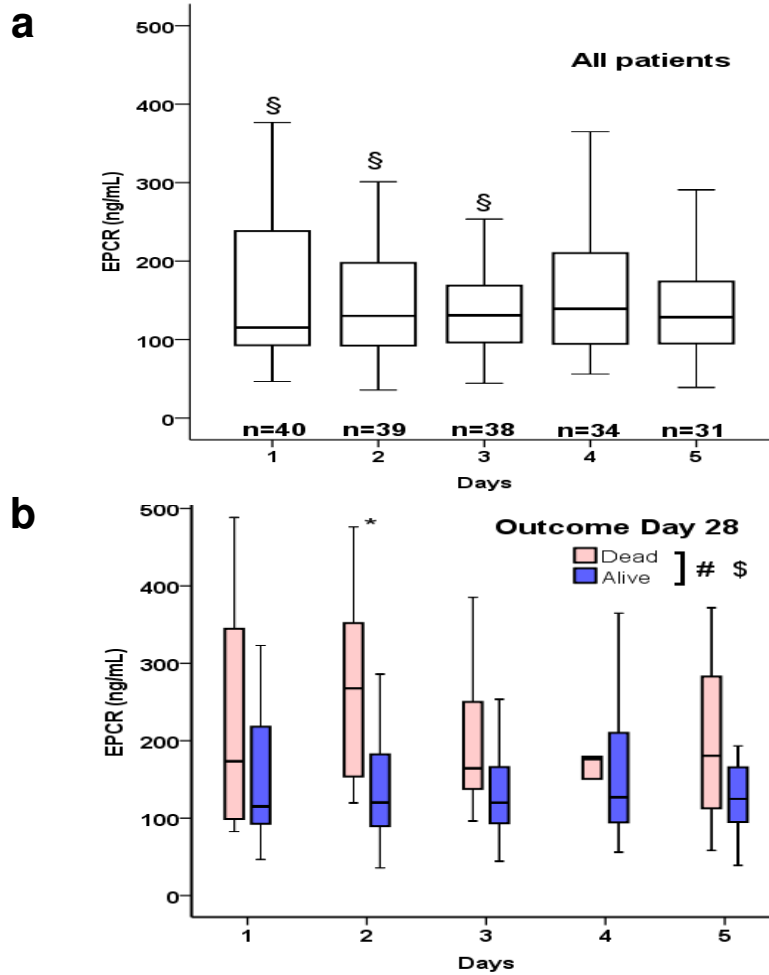


n:	40	25	40
Med (IQR):	156 (65)	159 (94)	115 (145)
Mean (SD):	170 (63)	157 (60)	174 (117)



Figure 2

Guillon C. et al.



*Electronic Supplementary Material 1*

*Guillon C. et al.*

## **Intensive Care Medicine**

### **Early rise in circulating Endothelial Protein C Receptor correlates with poor outcome in severe sepsis**

Christophe Guillon<sup>1,2</sup>, Nathalie Gérard<sup>1</sup>, Véronique Sébille<sup>3,4</sup>, Cédric Bretonnière<sup>2</sup>, Olivier Zambon<sup>2</sup>, Daniel Villers<sup>2</sup> and Béatrice Charreau

INSERM, U643, Nantes, F44000 France; CHU Nantes, Institut de Transplantation et de Recherche en Transplantation, ITERT, Nantes, F44000 France; UMR643 Université de Nantes, Faculté de Médecine, Nantes, F44000 France.

<sup>2</sup> Service de Réanimation Médicale Polyvalente, CHU de Nantes, F44000 France.

<sup>3</sup> EA 4275, Faculté de Pharmacie, Université de Nantes, Nantes, F44000 France.

<sup>4</sup> Plateforme de Biométrie, CHU de Nantes, F44000 France.

#### **Corresponding author:**

Dr. Christophe GUITTON, M.D.;

Service de Réanimation Médicale Polyvalente ; Hôtel Dieu, CHU de Nantes ; 30 Boulevard Jean Monnet ; 44093 NANTES Cedex ; FRANCE

Phone + 33 2 40 08 73 65 ; Fax + 33 2 40 08 75 50

E-Mail christophe.guillon@chu-nantes.fr

**ESM 1 :****Inclusion Criteria :**

**Patients with severe sepsis were included using the following inclusion criteria according to the ACCP consensus definition for severe sepsis**

-Suspected or proven infection- Patients had to have a known infection or a suspected infection, as evidenced by one or more of the following: white blood cells in a normally sterile body fluid; perforated viscus, radiographic evidence of pneumonia in association with the production of purulent sputum; and a syndrome associated with a high risk of infection.

- Patients had to meet at least 3 of the following 4 criteria- a core temperature of at least 38°C or below 36°C; a heart rate of at least 90 beats/min, except in patients with a medical condition known to increase the heart rate or those receiving treatment that would prevent tachycardia; a respiratory rate of at least 20 breaths/min or a PaCO<sub>2</sub> of no more than 32 mmHg or the use of mechanical ventilation for an acute respiratory process; a white blood cell count between 2000/ mm<sup>3</sup> and 40000/ mm<sup>3</sup> or a differential count showing more than 0% immature neutrophils.

- Criteria for dysfunctional organs or systems- Patients had to meet at least of the following criteria within the 24 hours period immediately preceding enrolment. The onset of the first organ dysfunction had not to precede the inclusion more than 24 hours For cardiovascular system dysfunction, arterial systolic blood pressure had to be no more than 90 mmHg or the mean arterial pressure of no more than 70 mmHg for at least h despite adequate fluid resuscitation, adequate intravascular volume status, or the use of vasopressors in an attempt to maintain a systolic blood pressure of at least 90 mmHg or a mean arterial pressure of at least 70 mmHg. For kidney dysfunction, urine output had to be less than 30 mL/ h for h and/or serum creatinin concentration more than 200 μmol/ L, despite adequate fluid resuscitation. For respiratory-system dysfunction, the ratio PaO<sub>2</sub>/FiO<sub>2</sub> had to be below 250 in the presence of other dysfunctional organs or below 200 if the lung was the only dysfunctional organ. For hematologic dysfunction, the platelet count had to be below 50000/mm<sup>3</sup>

1  
2  
3 or to have decreased by 50% in the 3 days preceding enrolment. In the case of unexplained metabolic  
4  
5 acidosis, the pH had to be no more than 7.30 and a plasma lactate level above 2 mmol/ L. For hepatic  
6  
7 dysfunction, bilirubin above 50  $\mu\text{mol/ L}$  or alcalin phosphatase more than 3-fold upper limit of the  
8  
9 normal reference range were considered. For neurologic dysfunction, coma Glasgow score had to be  
10  
11 below 3.  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

**Intensive Care Medicine****Early rise in circulating Endothelial Protein C Receptor correlates with poor outcome in severe sepsis**

Christophe Guillon <sup>1,2</sup>, Nathalie Gérard <sup>1</sup>, Véronique Sébille <sup>3,4</sup>, Cédric Bretonnière <sup>2</sup>, Olivier Zambon <sup>2</sup>, Daniel Villers <sup>2</sup> and Béatrice Charreau <sup>1</sup>

<sup>1</sup> INSERM, U643, Nantes, F44000 France; CHU Nantes, Institut de Transplantation et de Recherche en Transplantation, ITERT, Nantes, F44000 France; UMR643 Université de Nantes, Faculté de Médecine, Nantes, F44000 France.

<sup>2</sup> Service de Réanimation Médicale Polyvalente, CHU de Nantes, F44000 France.

<sup>3</sup> EA 4275, Faculté de Pharmacie, Université de Nantes, Nantes, F44000 France.

<sup>4</sup> Plateforme de Biométrie, CHU de Nantes, F44000 France.

**Corresponding author:**

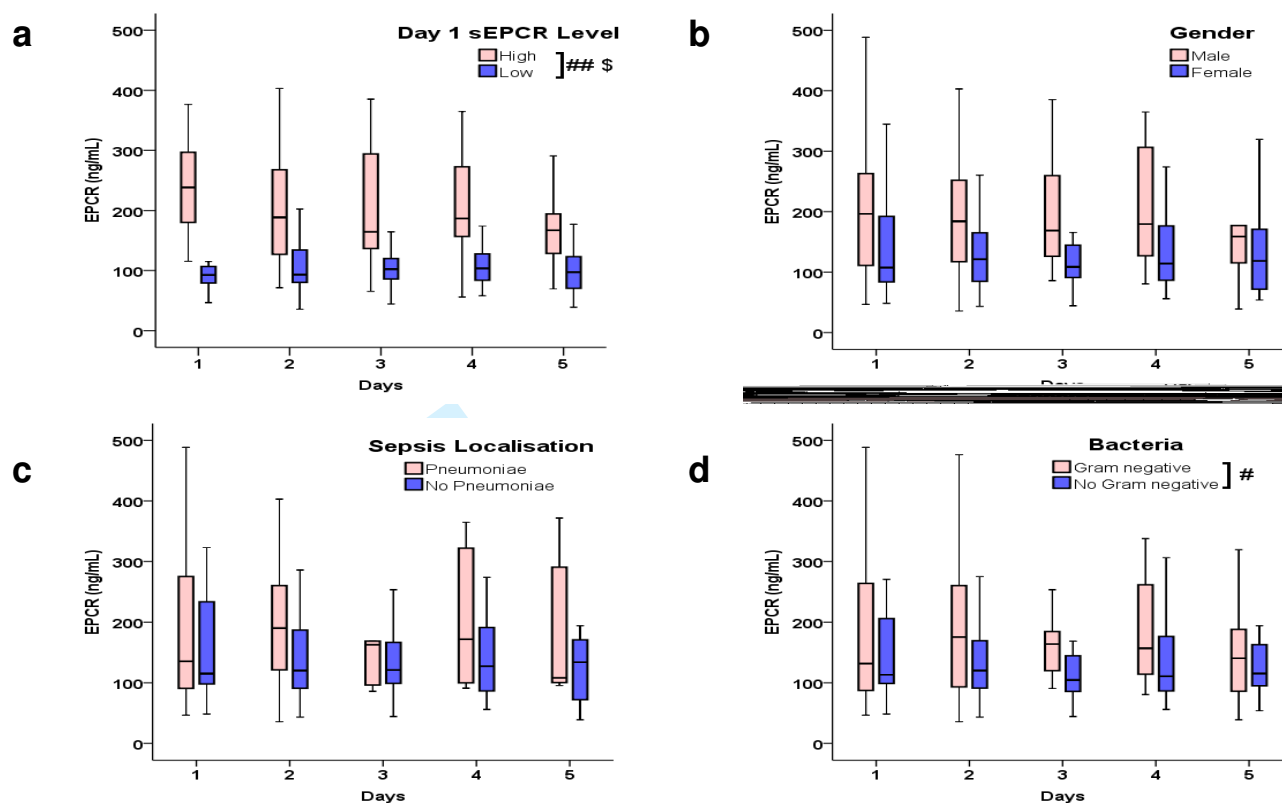
Dr. Christophe GUITTON, M.D.;

Service de Réanimation Médicale Polyvalente ; Hôtel Dieu, CHU de Nantes ; 30 Boulevard Jean Monnet ;  
44093 NANTES Cedex 1 ; FRANCE

Phone + 33 2 40 08 73 65 ; Fax + 33 2 40 08 75 50

E-Mail christophe.guillon@chu-nantes.fr

## ESM 2 : Supplemental Figure



**Time-course (from D1 to D5) analysis of sEPCR in septic patients.** sEPCR was measured daily in patients (n=40) with severe sepsis from admission in ICU (D1) to D5. For statistical analysis, repeated measures ANOVA using linear mixed models allowing for random effects with restricted maximum-likelihood estimation were used to assess changes in sEPCR levels over time [“time effect” (TE)], as well as the effect of covariates of interest [“group effect” (GE)]. P-values<0.05 were considered to be statistically significant. sEPCR levels are represented with box plot. Data presented are result from univariate analysis. For GE, statistical difference is indicated as # p<0.05 or ## p<0.01. For TE, statistical difference is indicated as \$ p<0.05. The box plots are interpreted as follows: boxes contain the middle 50% of data, line in the box indicate the median, upper and lower hinges indicate respectively the 75<sup>th</sup> and 25<sup>th</sup> percentile and “whiskers” indicate minimum and maximum data values.

**Time-course analysis of sEPCR in septic patients (a) According to sEPCR level at D1.** High level group (n=20) over median (115ng/mL) and Low level group (n=20) [GE p<0.001; TE p=0.04] **(b) According to gender,** Male (n=16), Female (n=24) [GE p=0.341; TE p=0.845] **(c) According to sepsis site,** pneumoniae (n=11) or not (n=29) [GE P=0.278; TE p=0.597] **(d) According to Gram staining bacteria,** Gram-negative (n=18) or not (n=22) [GE p=0.034; TE p=0.376].

1  
2  
3 *Electronic Supplementary Material* 3  
4  
5  
6

*Guitton C. et al.*

7  
8 **Intensive Care Medicine**  
9

10  
11 **Early rise in circulating Endothelial Protein C Receptor correlates with poor outcome in**  
12 **severe sepsis**  
13

14  
15 Christophe Guitton <sup>1,2</sup>, Nathalie Gérard <sup>1</sup>, Véronique Sébille <sup>3,4</sup>, Cédric Bretonnière <sup>2</sup>, Olivier  
16 Zambon <sup>2</sup>, Daniel Villers <sup>2</sup> and Béatrice Charreau <sup>1</sup>  
17  
18

19  
20 <sup>1</sup> INSERM, U643, Nantes, F44000 France; CHU Nantes, Institut de Transplantation et de Recherche en Transplantation, ITERT, Nantes,  
21 F44000 France; UMR643 Université de Nantes, Faculté de Médecine, Nantes, F44000 France.

22 <sup>2</sup> Service de Réanimation Médicale Polyvalente, CHU de Nantes, F44000 France.

23 <sup>3</sup> EA 4275, Faculté de Pharmacie, Université de Nantes, Nantes, F44000 France.

24 <sup>4</sup> Plateforme de Biométrie, CHU de Nantes, F44000 France.  
25

26  
27 **Corresponding author:**

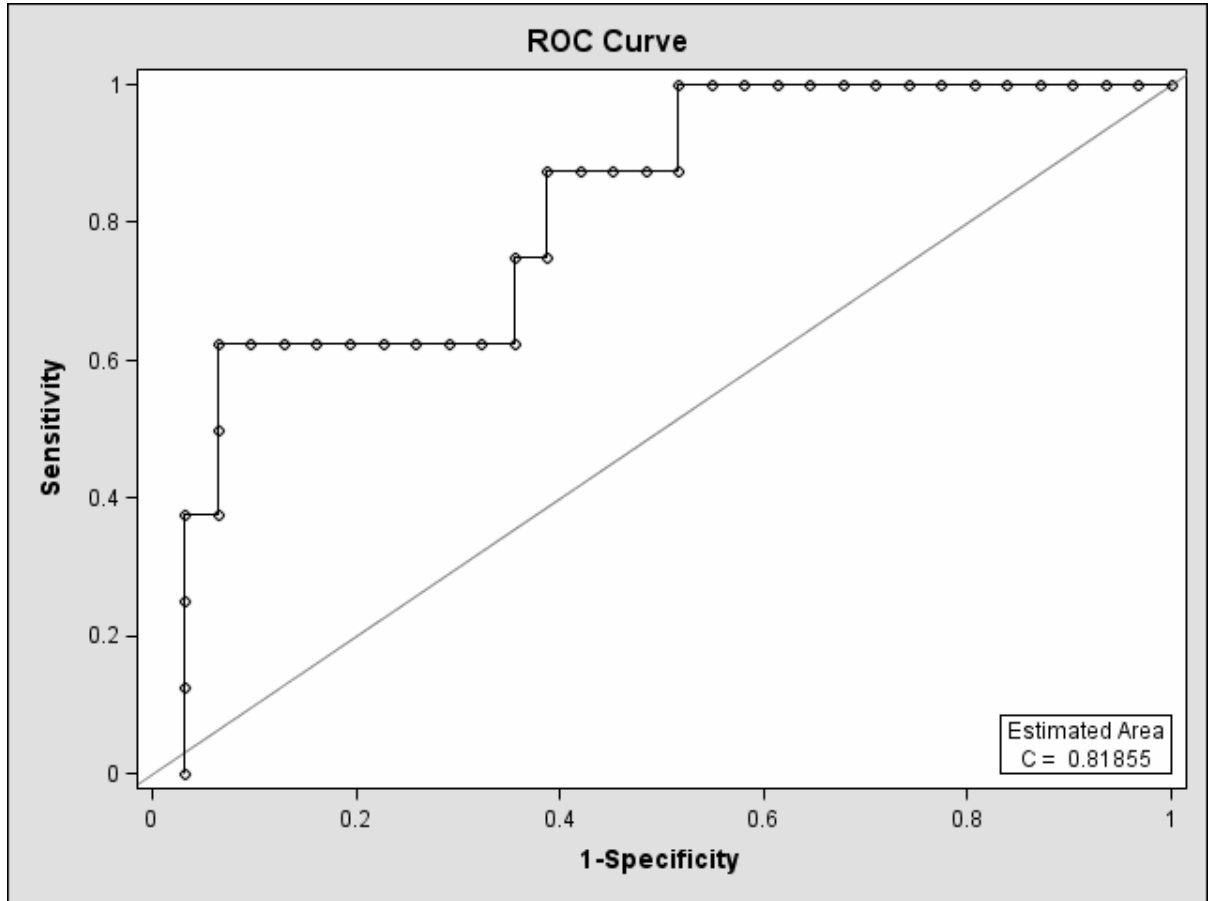
28 Dr. Christophe GUITTON, M.D.;

29  
30 Service de Réanimation Médicale Polyvalente ; Hôtel Dieu, CHU de Nantes ; 30 Boulevard Jean Monnet ;  
31 44093 NANTES Cedex 1 ; FRANCE

32 Phone + 33 2 40 08 73 65 ; Fax + 33 2 40 08 75 50

33 E-Mail christophe.guitton@chu-nantes.fr  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

ESM 3 : Supplemental Figure



The ROC curves of sEPCR Day 2 analyzed for prediction of survival on day 28

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60