Prognostic value of secretoneurin in patients with severe sepsis and septic shock: data from the ALBIOS Study

Helge Røsjø MD, PhD¹; Serge Masson, PhD², Pietro Caironi, MD^{3,4}, Mats Stridsberg MD, PhD⁵; Michela Magnoli, Stat D²; Geir Christensen MD, PhD, MHA⁶; Gabriella Moise, MD⁷; Maria Cristina Urbano, MD⁸; Luciano Gattinoni, MD, FRCP⁹; Antonio Pesenti, MD^{10,11}; Roberto Latini, MD²; Torbjørn Omland MD, PhD, MPH¹; for the ALBIOS Biomarkers Study Investigators

¹Division of Medicine, Akershus University Hospital, Lørenskog, Norway and Center for Heart Failure Research, Institute of Clinical Medicine, University of Oslo, Oslo, Norway;

²Department of Cardiovascular Research, IRCCS – Istituto di Ricerche Farmacologiche “Mario Negri”, Milan, Italy; ³Anestesia e Rianimazione, Azienda-Ospedaliero Universitaria S. Luigi Gonzaga, Orbassano, Italy; ⁴Dipartimento di Oncologia, Università degli Studi di Torino, Turin, Italy; ⁵Department of Medical Sciences, Uppsala University, Uppsala, Sweden;

⁶Institute for Experimental Medical Research, Ullevål and Center for Heart Failure Research, Institute of Clinical Medicine, University of Oslo, Oslo, Norway; ⁷Ospedale Città di Sesto San Giovanni, Sesto San Giovanni, Italy; ⁸Ospedale della Misericordia, Grosseto, Italy;

⁹Department of Anesthesiology, Emergency and Intensive Care Medicine, University of Göttingen, Germany. ¹⁰Dipartimento di Anestesia, Rianimazione ed Emergenza Urgenza, Fondazione IRCCS Ca’ Granda – Ospedale Maggiore Policlinico, Milan, Italy;

¹¹Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Università degli Studi di Milano, Milan, Italy.

Corresponding author and requests for reprints should be addressed to:

Helge Røsjø, MD, PhD, Division of Medicine, Akershus University Hospital, Sykehusveien 25, 1478 Lørenskog, Norway.

Tel: +47 915 02900 Fax: +47 67962190 e-mail: helge.rosjo@medisin.uio.no

The ALBIOS trial was funded by a grant from the Italian Medicines Agency (AIFA, grant FARM6JS3R5, 2006). This project was funded by Akershus University Hospital, the Research Council of Norway, the University of Oslo, and the South-Eastern Norway Regional Health Authority. The present study was also supported in part by funding from the Italian Ministry of Health (Ricerca Finalizzata, grant number RF-2011-02348358). Roche Diagnostics (Rotkreuz, Switzerland) provided in kind reagents for measuring N-terminal pro B-type natriuretic peptide and high sensitive cardiac troponin T.

The sponsors had no roles in any of the following: design and conduct of the study, collection, management, analysis and interpretation of the data, or preparation, review and approval of the manuscript.

HR has received personal fees from Novartis and CardiNor AS and MS has received personal fees from CardiNor AS. TO has received research grants via Akershus University Hospital from Abbott Diagnostics, Roche Diagnostics, Singulex and AstraZeneca, and personal fees from Roche Diagnostics, Abbott Diagnostics, Bayer, Novartis, and CardiNor AS.

HR, MS, GC, and TO are partners in a patent filed by the University of Oslo regarding the use of secretoneurin as a biomarker in patients with cardiovascular disease and patients with critical illness. HR, MS, GC, and TO also have financial interests in CardiNor AS, which holds the license to commercialize secretoneurin.

SM, PC, MM, GM, MCU, LG, AP and RL declare no conflicts of interest.

Key words: Sepsis, septic shock, biomarkers, secretoneurin

Short title: Secretoneurin in sepsis

Abstract: 300

Manuscript: 2999

Tables: 2

Figure: 1

ABSTRACT

Objective: Secretoneurin (SN) directly influences cardiomyocyte calcium handling and circulating SN levels seem to improve risk prediction in patients with myocardial dysfunction by integrating information on systemic stress, myocardial function, and renal function. Accordingly, in this study we hypothesized that SN would improve risk prediction in patients with sepsis, and especially in patients with septic shock as these patients are more hemodynamically unstable.

Design: Multicenter, interventional randomized clinical trial.

Setting: Multicenter, pragmatic, open-label, randomized, prospective clinical trial testing fluid administration with either 20% human albumin and crystalloids or crystalloid solutions alone in patients with severe sepsis or septic shock (ALBIOS).

Patients or Subjects: In total, 540 patients with septic shock and 418 patients with severe sepsis.

Interventions: Either 20% human albumin and crystalloids or crystalloid solutions alone.

Measurements and Main Results: We measured SN on days 1, 2 and 7 after randomization and compared the prognostic value of SN for Intensive Care Unit (ICU) and 90-day mortality with established risk indices and cardiac biomarkers in septic shock and severe sepsis. High SN levels on day 1 were associated with age and serum concentrations of lactate, bilirubin, creatinine, and N-terminal pro-B-type natriuretic peptide. Adjusting for established risk factors and cardiovascular biomarkers, SN levels on day 1 were associated with ICU (odds ratio 2.27 [95% confidence interval 1.05–4.93], $p=0.04$) and 90-day mortality (2.04 [1.02–4.10], $p=0.04$) in patients with septic shock, but not severe sepsis without shock. SN levels on day 2 were also associated with ICU (3.11 [1.34–7.20], $p=0.008$) and 90-day mortality (2.69 [1.26–5.78], $p=0.01$) in multivariate regression analyses and improved reclassification in

patients with septic shock, as assessed by the net reclassification index. Randomized albumin administration did not influence the associations between SN and outcomes.

Conclusion: SN provides early and potent prognostic information in septic patients with cardiovascular instability.

INTRODUCTION

Myocardial dysfunction is common during sepsis, especially in the subgroup of patients with shock (1-3). As untreated myocardial dysfunction is associated with a poor outcome (1-3), early identification of high-risk patients with myocardial dysfunction could reduce mortality. Accordingly, early assessable circulating biomarkers might be crucial to identify high-risk patients with myocardial dysfunction at hospital admission.

Secretoneurin (SN) is a 33-amino acid peptide belonging to the granin protein family (4). In states of myocardial dysfunction, SN production in neuroendocrine and myocardial tissues is augmented (5,6), and SN seems to regulate important processes in the myocardium (4), including cardiomyocyte Ca^{2+} handling (7). Circulating SN levels also correlate with cortisol concentrations, established biomarkers of cardiac function, and renal dysfunction in critically ill patients with infections (8). Although the pathophysiology reflected by SN as a biomarker requires additional work to be fully established, previous experimental and clinical data therefore support SN as a biomarker that could integrate information on the complex network of cardiovascular, neuroendocrine, and renal systems. All of these systems are strongly involved in the pathophysiology of sepsis and septic shock and associated with sepsis outcome. We have previously reported that SN levels at admission provide incremental prognostic information to established risk indices in critically ill patients with infections (8). Circulating SN levels also improve risk assessment in patients with acute heart failure, after ventricular arrhythmia-induced cardiac arrest, and in patients with acute respiratory failure due to cardiovascular disease, while not improving prognostication in patients with primary pulmonary-related dyspnea (7,9). Hence, SN is a promising prognostic biomarker in patients primarily with myocardial dysfunction, which could impact also the prognostic ability by SN in patients with sepsis. Accordingly, by employing bio-specimens from the largest cohort of

patients with severe sepsis or septic shock currently available, we hypothesized that SN levels would provide more additional prognostic information to established risk indices in patients with septic shock, who are hemodynamically unstable, than in patients with severe sepsis without shock.

METHODS

Additional information on Methods can be found in the online data supplement.

The ALBIOS Study

The Albumin Italian Outcome Sepsis (ALBIOS) trial was a multicenter, pragmatic, open-label, randomized, prospective clinical trial testing fluid administration with either 20% human albumin and crystalloids or crystalloid solutions alone in patients with severe sepsis or septic shock in 100 Intensive Care Units (ICUs) in Italy. The total study cohort of the ALBIOS trial included 1818 patients ≥ 18 years who met clinical criteria for severe sepsis or septic shock within 24 h from hospital admittance or anytime during the ICU stay (10).

Patients received guideline-based therapy and were randomized to either 20% albumin and crystalloids or crystalloid solutions alone from randomization until day 28 or ICU discharge, whichever came first. The study was conducted according to the Declaration of Helsinki and approved by the institutional review board of all participating hospitals. Written informed consent or deferred consent was obtained for all included patients, as granted by the review boards of its participating centres, and according to Italian legislation.

Biomarker substudy and laboratory analyses

For the current substudy, blood samples were available from 958 patients. Blood samples were collected from patients enrolled in 40 out of the 100 ICUs that agreed to participate to a

predefined biomarkers substudy. Nonetheless, these centers enrolled the majority of the patients randomized in the ALBIOS trial, therefore guaranteeing a representativeness of the disease investigated. Venous blood samples were collected after randomization on days 1, 2, and 7 (or on ICU discharge, whichever came first). SN levels were measured in EDTA plasma by an in-house radioimmunoassay in Uppsala, Sweden as previously reported (5,7-9). The SN assay has a detection limit of 50 pmol/L and a coefficient of variation of 9% in the lower range (110 pmol/L) and 4% in the upper range (380 pmol/L). The assays for N-terminal pro-B-type natriuretic peptide (NT-proBNP) and highly sensitive cardiac troponin T (hs-cTnT) measurements have previously been reported (11).

Statistical analysis

Categorical variables were presented as proportions while continuous variables as means (standard deviation) or median (Q1-Q3), as appropriate. Differences across categorical variables were assessed by the chi-square test or the Fisher's exact test, and analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis test were used for continuous variables. Correlation coefficients were calculated by Spearman rank correlation and multivariate linear regression analysis was employed to identify variables associated with increasing SN levels. The association between SN levels and mortality was tested by the log-rank test across SN tertiles in Kaplan-Meier plots and by performing univariate and multivariable logistic regression analysis. All variables associated with ICU and 90-day mortality in univariate analysis were included into the multivariable analyses (details in the Supplementary material and presented in the table). We also calculated 2 logistic models for ICU or 90-mortality with an interaction term between SN concentration on day 1 and prevalent shock. Prognostic accuracy was tested by calculating the area under the curve (AUC) with 95% confidence intervals (CI) based on receiver operating curve (ROC) statistics

and reclassification was assessed by the category-free net reclassification index (NRI), and presented as recommended for nested models (12,13). A p value <0.05 was considered statistically significant.

RESULTS

SN levels in patients with severe sepsis or septic shock

Detailed clinical characteristics of the 958 patients with SN measured on day 1 after randomization are compared to the total ALBIOS cohort in Supplementary Table 1. In general, there are no major differences between the biomarker substudy population and the total ALBIOS cohort. The median SN level on day 1 was 201 (Q1-3 161-259) pmol/L. When stratifying patients according to SN tertiles, patients with the highest SN levels were older, had higher NT-proBNP and hs-cTnT levels and higher Sequential Organ Failure Assessment (SOFA) and Simplified Acute Physiology (SAPS) II scores (Supplementary Table 2). Several other risk factors in sepsis were also more prevalent in patients with high SN levels, including chronic renal failure and history of cardiovascular disease, while use of antibiotics or appropriate antibiotic therapy did not differ across SN tertiles. SN levels on day 1 were higher in patients with septic shock (n=540) compared to patients with severe sepsis without shock (n=418): 208 (167-265) vs. 194 (158-249) pmol/L, p=0.002.

SN levels on day 1 correlated with several variables, including NT-proBNP and hs-cTnT levels, as well as SOFA and SAPS II scores (Supplementary Table 3). Increasing age and higher serum concentrations of creatinine, bilirubin, lactate and NT-proBNP levels were all independently associated with high SN levels on day 1 by multivariable linear regression analysis (Supplementary Table 4). Patients with high SN levels on day 1 were less likely to achieve hemodynamic goals during the first 6 to 24 h compared to patients with lower SN

levels, even though they received more often vasoactive drugs (Supplementary Table 5). SN levels on day 1 differed significantly across quintiles of SOFA score, including all organ-specific sub-scores but for the respiratory component (Supplementary Figure 1). There was no difference in SN levels on day 1 between patients randomized to albumin (n=474) and crystalloids (n=484): 201 (161-259) vs. 201 (163-259) pmol/L, p=0.93.

SN levels on day 1 and ICU and 90 day mortality with patients stratified according to shock

ICU mortality was 29% (159/540) among patients with septic shock and 23% (94/418) among patients with severe sepsis without shock, whereas 90-day mortality was 44% (234/536) in septic shock and 33% (135/409) in patients with severe sepsis without shock. We assessed the associations between SN levels, stratified by tertiles, and mortality in patients with septic shock and severe sepsis without shock separately: SN on day 1 was associated with ICU and 90-day mortality in patients with septic shock, and only 90-day mortality in those with severe sepsis without shock (Figure). SN levels on day 1 also remained associated with both ICU and 90-day mortality in patients with septic shock, both when adjusting for clinical variables and established risk scores (Table 1, Model #1), as well as after further addition of NT-proBNP and hs-cTnT levels to the previous models (Model #2). In contrast, the association between day 1 SN levels and 90-day mortality in patients with severe sepsis without shock was attenuated and no longer significant in multivariate analysis (Table 1, Model #2). The interaction between SN concentrations and septic shock for prognosis was also validated by logistic regression models (Supplementary Table 6). The AUROC of SN levels on day 1 to predict ICU mortality in patients with septic shock was 0.68 (0.64-0.73) and the AUC for 90-day mortality was 0.67 (0.63-0.72). The corresponding AUROCs for hs-cTnT in patients with septic shock were 0.61 (0.56-0.66) [ICU mortality] and 0.60 (0.55-0.65) [90 day mortality]

and AUROCs for NT-proBNP in septic shock were 0.66 (0.61-0.71) [ICU mortality] and 0.66 (0.61-0.70) [90 day mortality] (Table 2).

SN levels during hospitalization in patients with septic shock or severe sepsis without shock and associations with outcomes

On average, SN levels were higher in patients with septic shock compared to patients with severe sepsis without shock during the ICU stay (Supplementary Figure 2A). SN levels did not change significantly during the ICU hospitalization for the two groups of patients, neither in patients randomized to albumin and crystalloid administration nor in the group randomized to crystalloid administration alone (Supplementary Figure 2B).

SN levels on day 2 were independently associated with ICU and 90-day mortality in patients with septic shock, but not in those with severe sepsis without shock (Table 1). This remained true even after robust adjustments for clinical risk factors, with or without further addition of the circulating cardiac biomarkers NT-proBNP and hs-cTnT. In patients with shock, SN levels on day 2 also reclassified a significant proportion of subjects into their correct risk strata, both for ICU (NRI= 0.30 [0.10-0.51], $p=0.004$) and 90-day mortality (NRI=0.22 [0.05-0.42], $p=0.01$; Supplementary Figure 3). The addition of SN levels on day 2 to the basic models both down-graded risk in survivors and upgraded risk in non-survivors (Supplementary Figure 3). In patients with septic shock, SN levels on day 7 were also associated with clinical outcomes by univariate analyses, but did not add prognostic information to established risk indices and biomarkers in multivariable models (Table 1). The AUROCs for ICU and 90-day mortality of SN and established cardiac biomarkers on day 1, 2, and 7 in patients with septic shock are presented in Table 2. Changes in SN levels from day 1

to day 2 and from day 2 to day 7 were not associated with ICU or 90-day mortality (Supplementary Table 7).

DISCUSSION

The principal result of this study is that early SN measurements in patients with severe sepsis and septic shock provide additional prognostic information to established risk scores and cardiac biomarkers in patients with cardiovascular instability.

The incidence of sepsis seems to be rising in the Western world (14). Although a number of strategies have been tested (15), treatment is currently focused on early infectious control and optimal supportive therapy (16), while the mortality rate is still around 30-40%. Given that the principal causes of death in septic patients are multi-organ failure, refractive shock, and respiratory failure (17), strategies that target these pathophysiological processes could improve outcome. Moreover, early diagnosis of specific pathophysiology like myocardial and renal dysfunction will probably be of great importance due to the complex and integrated pathophysiology behind disease progression in septic patients (14,15). Pertinent to this point, current guidelines for sepsis emphasizes the early period after sepsis development as the most crucial period to start therapeutic interventions (16). However, as established risk models like SOFA and SAPS II scores incorporate different pathophysiological axes into the scoring systems (18,19), the ability of these risk models to diagnose specific pathophysiology in individual patients with sepsis and septic shock is reduced. Accordingly, there is a need to develop tools that are easy to use and can detect specific pathophysiology on ICU admission in patients with severe sepsis and septic shock.

SN is a promising novel biomarker for risk assessment in different populations (5,7-9). Recent studies have found SN levels to provide incremental prognostic information to established risk indices and biomarkers in different cohorts of patients with myocardial dysfunction (7-9). We have also found strong and independent prognostic information by SN when measured on ICU admission in two cohorts of critically ill patients with infections, including in patients with severe sepsis and septic shock from the FINNSEPSIS Study (8). The FINNSEPSIS cohort is comparable to the ALBIOS biomarker substudy cohort, but the blood samples were collected in 2005 in FINNSEPSIS, which is almost 10 years earlier than the samples collected in ALBIOS. Hence, our recent data from the ALBIOS Study validate and expand on the data from FINNSEPSIS in a modern sepsis population with treatment tailored according to the Surviving Sepsis Campaign guidelines (16). In this and other studies, the correlation coefficients between circulating SN and hs-cTnT and NT-proBNP levels have been 0.30-0.40, which support the concept of SN as a biomarker that reflects additional pathophysiology to the established cardiac biomarkers. Circulating SN concentrations are also influenced by renal dysfunction in septic patients (8), probably as a result of reduced clearance, and SN therefore provides prognostic information also by detecting kidney failure. Of relevance for septic patients, the prognostic utility of SN seems most prominent when measured early after hospital admission (7-9). We now validate and expand on these previous results by demonstrating strong and independent prognostic information by SN when measured on day 1 and day 2 after randomization in septic shock patients from the ALBIOS trial, which is the largest severe sepsis or septic shock cohort with bio-specimens. SN levels on day 2 after randomization also reclassified a significant proportion of patients into their correct risk strata when added on top of established risk scores and cardiac biomarkers, both by upgrading risk in non-survivors and by down-grading risk in survivors. Accordingly, our results support the model of SN as a biomarker with potential to improve risk assessment in

individual patients with sepsis, and especially measurements after 24 h appear to provide strong prognostic information. Still, as SN only provided incremental prognostic information in patients with septic shock, the prognostic utility of SN seems linked to the subgroup of septic patients with hemodynamic instability. This would be in line with previous data from patients hospitalized with acute dyspnea where SN provided additional prognostic information to established risk indices in patients with cardiovascular related-dyspnea, but not in patients with acute pulmonary disease (7,9). However, as we did not find the same difference in prognostic information according to the presence or absence of shock in the FINNSEPSIS Study (8), additional studies will be required to validate this concept for SN in patients with septic shock. Of note, differences in the design and execution of the FINNSEPSIS Study and the ALBIOS trial could explain some of the discrepancy for SN between the two studies. These differences include closer monitoring and reporting of shock in the interventional ALBIOS trial, which tested two strategies for fluid administration, compared to the clinical-epidemiological FINNSEPSIS Study that had no special focus on fluid administration. FINNSEPSIS also did not have centralized criteria and treatment algorithms for fluid administration, while fluid administration was conducted according to the most updated strategies for early goal-oriented therapy in ALBIOS. Hence, it is plausible that, in general, the patients that developed shock in ALBIOS may have had more severely compromised cardiovascular status compared to the septic shock patients in FINNSEPSIS. It is also conceivable that less stringent early fluid administration and other supportive therapies in FINNSEPSIS may have exposed patients with severe sepsis of that cohort to an increased risk of developing hemodynamic instability, which could explain why SN concentrations were more closely associated with mortality among severe sepsis patients in FINNSEPSIS compared to severe sepsis patients in ALBIOS trial. Still, the main conclusion of these two large, multicenter studies including patients with severe sepsis and septic shock indicates that

SN concentrations measured early after ICU admission improves risk assessment. Of note, results for AUROC demonstrate some overlap in SN levels between patients with a favorable and poor prognosis. This is not surprising as patients with septic shock die from a number of different causes, and most biomarkers are reflective only of specific pathophysiology.

This study has some limitations and strengths. Although the study represents the largest cohort with blood samples from patients with severe sepsis and septic shock currently available, it is a substudy of an interventional trial and our results may not be representative for the overall population of patients with sepsis across different ICUs. Still, as the patients with blood samples demonstrated similar clinical characteristics as the overall ALBIOS population, we believe this may not be a major problem. Mortality rates among the different groups in the ALBIOS Study were also in the same range as mortality rates previously reported in other cohorts and centers, including mortality rates in epidemiological studies like FINNSEPSIS (20). Unfortunately, cardiac imaging was not available from the patients in the ALBIOS Study and this is a limitation to the current substudy. Of note, as previous reports in patients with heart failure have not found SN to correlate with specific echocardiographic indices of myocardial function (5,7), it is unlikely that SN can be used as a surrogate marker for echocardiographic indices in patients with severe sepsis or septic shock. To the contrary; SN provides additional information also to echocardiographic indices of myocardial function (5,7), thus, potential future use of SN as risk marker in septic patients will not replace, but rather complement imaging methods of myocardial function. For this specific study we did not include an external validation cohort, but rather this report should be considered complementary to previous data of strong prognostic value of SN in two cohorts of critically ill patients with infections (8).

In conclusion, SN provides complementary information to established risk models and cardiac biomarkers in patients with septic shock, which suggests that SN primarily may be of use in septic patients with cardiovascular instability. In contrast, the prognostic utility of SN in septic patients without cardiovascular instability seems more limited.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the contribution from all patients and researchers that participated in the ALBIOS trial.

REFERENCES

1. Kakihana Y, Ito T, Nakahara M, Yamaguchi K, Yasuda T. Sepsis-induced myocardial dysfunction: pathophysiology and management. *Journal of Intensive Care* 2016;4:22.
2. Antonucci E, Fiaccadori E, Donadello K, Taccone FS, Franchi F, Scolletta S. Myocardial depression in sepsis: from pathogenesis to clinical manifestations and treatment. *Journal of Critical Care* 2014;29:500-11.
3. Latini R, Caironi P, Masson S. Cardiac dysfunction and circulating cardiac markers during sepsis. *Minerva Anesthesiol* 2016;82:697-710.
4. Bartolomucci A, Possenti R, Mahata SK, Fischer-Colbrie R, Loh YP, Salton SR. The extended granin family: structure, function, and biomedical implications. *Endocrine Reviews* 2011;32:755-97.
5. Røsjø H, Stridsberg M, Florholmen G, et al. Secretogranin II; a protein increased in the myocardium and circulation in heart failure with cardioprotective properties. *PloS ONE* 2012;7:e37401.
6. Albrecht-Schgoer K, Schgoer W, Holfeld J, et al. The angiogenic factor secretoneurin induces coronary angiogenesis in a model of myocardial infarction by stimulation of vascular endothelial growth factor signaling in endothelial cells. *Circulation* 2012;126:2491-2501.
7. Ottesen AH, Louch WE, Carlson CR, et al. Secretoneurin is a novel prognostic cardiovascular biomarker associated with cardiomyocyte calcium handling. *Journal of the American College of Cardiology* 2015;65:339-51.
8. Røsjø H, Stridsberg M, Ottesen AH, et al. Prognostic value of secretoneurin in critically ill patients with infections. *Critical Care Medicine* 2016;44:1882-90.
9. Myhre PL, Ottesen AH, Okkonen M, et al. Prognostic value of secretoneurin in patients with acute respiratory failure: data from the FINNALI Study. *Clinical Chemistry* 2016;62:1380-9.
10. Caironi P, Tognoni G, Masson S, et al. Albumin replacement in patients with severe sepsis or septic shock. *New Eng J Med* 2014;370:1412-21.
11. Masson S, Caironi P, Fanizza C, et al. Sequential N-terminal pro-B-type natriuretic peptide and high-sensitivity cardiac troponin measurements during albumin replacement in patients with severe sepsis or septic shock. *Critical Care Medicine* 2016;44:707-16.
12. Demler OV, Pencina MJ, D'Agostino RB, Sr. Misuse of DeLong test to compare AUCs for nested models. *Statistics in Medicine* 2012;31:2577-87.
13. Pencina MJ, D'Agostino RB, Sr., Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Statistics in Medicine* 2011;30:11-21.
14. Angus DC, van der Poll T. Severe sepsis and septic shock. *New Eng J Med* 2013; 369:840-51.

15. Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. *The Lancet Infectious Diseases* 2013;13:260-8.
16. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2016. *Crit Care Medicine* 2017;45:486-552.
17. Vincent JL, Nelson DR, Williams MD. Is worsening multiple organ failure the cause of death in patients with severe sepsis? *Critical Care Medicine* 2011;39:1050-55.
18. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. *JAMA* 1993;270:2957-63.
19. Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Medicine* 1996;22:707-10.
20. Karlsson S, Varpula M, Ruokonen E, et al. Incidence, treatment, and outcome of severe sepsis in ICU-treated adults in Finland: the Finnsepsis study. *Intensive Care Medicine* 2007;33:435-43.

Table 1. Association between SN levels and ICU mortality or 90-day mortality

		Univariate		Multivariable Model #1		Multivariable Model #2	
	Time of measurements, #	OR [95%CI]	p	OR [95%CI]	p	OR [95%CI]	p
Septic shock, ICU mortality	Day 1 , no./No.=159/540 (29.4%)	6.12 [3.45–10.87]	<0.0001	2.47 [1.15–5.31]	0.02	2.27 [1.05–4.93]	0.04
	Day 2 , no./No.=139/509 (27.3%)	7.10 [3.69–13.68]	<0.0001	4.02 [1.75–9.21]	0.001	3.11 [1.34–7.20]	0.008
	Day 7 , no./No.=89/412 (21.6%)	4.12 [1.94–8.79]	0.0002	2.68 [1.06–6.77]	0.04	2.31 [0.90–5.95]	0.08
Septic shock, 90-day mortality	Day 1 , no./No.=234/536 (43.7%)	5.17 [3.03–8.82]	<0.0001	2.37 [1.19–4.69]	0.01	2.04 [1.02–4.10]	0.04
	Day 2 , no./No.=213/504 (42.3%)	6.75 [3.66–12.46]	<0.0001	3.43 [1.63–7.20]	0.001	2.69 [1.26–5.78]	0.01
	Day 7 , no./No.=155/407 (38.1%)	3.35 [1.74–6.44]	0.0003	1.88 [0.80–4.40]	0.15	1.42 [0.59–3.46]	0.44
Severe sepsis without shock, ICU mortality	Day 1 , no./No.=94/418 (22.5%)	1.79 [1.03–3.13]	0.04	1.04 [0.50–2.14]	0.92	0.97 [0.46–2.02]	0.92
	Day 2 , no./No.=86/395 (21.8%)	2.09 [1.15–3.78]	0.02	1.48 [0.73–3.01]	0.28	1.27 [0.60–2.68]	0.53
	Day 7 , no./No.=65/336 (19.3%)	1.41 [0.70–2.85]	0.33	0.73 [0.28–1.92]	0.52	0.58 [0.21–1.61]	0.29
Severe sepsis without shock, 90-day mortality	Day 1 , no./No.=135/409 (33.0%)	2.26 [1.30–3.93]	0.004	0.93 [0.46–1.90]	0.84	0.83 [0.40–1.71]	0.61
	Day 2 , no./No.=128/386 (33.2%)	1.98 [1.12–3.51]	0.02	1.06 [0.52–2.15]	0.87	0.97 [0.47–1.98]	0.93
	Day 7 , no./No.=102/327 (31.2%)	1.47 [0.74–2.92]	0.28	0.87 [0.36–2.07]	0.75	0.75 [0.30–1.87]	0.54

SN was considered after natural logarithm transformation.

List of variables included in the logistic regression models (all with $p < 0.05$ at univariate analysis).

Multivariable models #1.

Septic shock, ICU mortality: Age (OR [95% CI], 1.03 [1.01-1.06]), admission to ICU for emergency surgery (0.58 [0.35-0.97]), admission to ICU for elective surgery (0.90 [0.35-2.29]), COPD (2.66 [1.45-4.89]), chronic renal failure (2.38 [0.53-10.68]), congestive or ischemic heart disease (1.52 [0.87-2.66]), SAPS II score (1.02 [1.01-1.04]), $\text{PaO}_2/\text{FiO}_2$ (0.998 [0.995-1.000]), urine output (0.999 [0.995-1.002]), serum lactate (1.09 [1.01-1.18]), creatinine (0.90 [0.74-1.08]), and bilirubin (1.06 [0.93-1.21]) concentrations, hemoglobin (0.91 [0.80-1.04]), platelet count (1.000 [0.998-1.002]), SOFA score (1.16 [0.98-1.36]), mechanical ventilation (1.13 [0.51-2.52]).

Septic shock, 90-day mortality: age (1.04 [1.02-1.06]), female sex (0.60 [0.39-0.92]), COPD (1.84 [1.02-3.33]), congestive or ischemic heart disease (2.12 [1.23-3.64]), SAPS II score (1.04 [1.02-1.05]), $\text{PaO}_2/\text{FiO}_2$ (1.000 [0.998-1.002]), urine output (0.998 [0.994-1.001]), serum lactate (1.01 [0.94-1.10]), creatinine (0.86 [0.73-1.03]), and bilirubin (1.15 [0.99-1.35]) concentrations, platelet count (1.000 [0.998-1.002]), SOFA score (1.11 [0.95-1.30]), mechanical ventilation (1.27 [0.63-2.57]).

Severe sepsis without shock, ICU mortality: age (1.02 [1.00-1.05]), chronic renal failure (1.49 [0.54-4.07]), immunodeficiency (3.36 [1.60-7.06]), SAPS II score (1.02 [0.99-1.04]), $\text{PaO}_2/\text{FiO}_2$ (0.996 [0.993-0.999]), serum creatinine concentration (1.06 [0.88-1.29]), white blood cell (0.98 [0.95-1.01]), platelet count (0.999 [0.997-1.002]), SOFA score (1.17 [1.02-1.33]), mechanical ventilation (1.76 [0.91-3.38]).

Severe sepsis without shock, 90 day mortality: age (1.03 [1.00-1.05]), BMI (0.94 [0.89-0.99]), COPD (2.45 [1.18-5.09]), chronic renal failure (2.05 [0.71-5.95]), immunodeficiency (2.29 [1.03-5.11]), congestive or ischemic heart disease (1.57 [0.81-3.03]), SAPS II score (1.03 [1.00-1.05]), PaO₂/FiO₂ (0.999 [0.996-1.002]), serum lactate (1.01 [0.90-1.14]) and creatinine (0.99 [0.82-1.21]) concentrations, white blood cell (0.97 [0.94-1.00]) and platelet counts (1.001 [0.998-1.003]), SOFA score (1.20 [1.04-1.38]), mechanical ventilation (1.50 [0.82-2.74]).

Multivariable models #2:

Further addition of hs-cTnT and NT-proBNP levels, measured on the same day as SN and entered after natural logarithm transformation, on top of models #1.

Table 2. Receiver operating statistics' area under the curve (AUROC) for SN, hs-cTnT, and NT-proBNP measurements for ICU and 90-day mortality among patients with septic shock

	SN	hs-cTnT	NT-proBNP
<i>ICU mortality</i>			
Day 1	0.68 (0.64-0.73)	0.61 (0.56-0.66)	0.66 (0.61-0.71)
Day 2	0.68 (0.63-0.73)	0.64 (0.58-0.69)	0.67 (0.62-0.72)
Day 7	0.62 (0.55-0.69)	0.63 (0.57-0.69)	0.63 (0.57-0.70)
<i>90-day mortality</i>			
Day 1	0.67 (0.63-0.72)	0.60 (0.55-0.65)	0.66 (0.61-0.70)
Day 2	0.67 (0.62-0.72)	0.61 (0.57-0.66)	0.67 (0.62-0.72)
Day 7	0.61 (0.55-0.66)	0.63 (0.57-0.68)	0.65 (0.60-0.70)

Fig. Associations between SN levels on day 1 after randomization and mortality during follow-up with patients stratified according to SN tertiles (T) and divided as patients with septic shock (A; n=540) and patients with severe sepsis without shock (B; n=418)

A

B

ONLINE DATA SUPPLEMENT

to

Prognostic value of secretoneurin in patients with severe sepsis and septic shock: data from the ALBIOS Study

Helge Røsjø MD, PhD¹; Serge Masson, PhD², Pietro Caironi, MD^{3,4}, Mats Stridsberg MD, PhD⁵; Michela Magnoli, Stat D²; Geir Christensen MD, PhD, MHA⁶; Gabriella Moise, MD⁷; Maria Cristina Urbano, MD⁸; Luciano Gattinoni, MD, FRCP⁹; Antonio Pesenti, MD^{10,11}; Roberto Latini, MD²; Torbjørn Omland MD, PhD, MPH¹; for the ALBIOS Biomarkers Study Investigators

¹Division of Medicine, Akershus University Hospital, Lørenskog, Norway and Center for Heart Failure Research, Institute of Clinical Medicine, University of Oslo, Oslo, Norway;

²Department of Cardiovascular Research, IRCCS – Istituto di Ricerche Farmacologiche “Mario Negri”, Milan, Italy; ³Anestesia e Rianimazione, Azienda-Ospedaliero Universitaria S. Luigi Gonzaga, Orbassano, Italy; ⁴Dipartimento di Oncologia, Università degli Studi di Torino, Turin, Italy; ⁵Department of Medical Sciences, Uppsala University, Uppsala, Sweden;

⁶Institute for Experimental Medical Research, Ullevål and Center for Heart Failure Research, Institute of Clinical Medicine, University of Oslo, Oslo, Norway; ⁷Ospedale Città di Sesto San Giovanni, Sesto San Giovanni, Italy; ⁸Ospedale della Misericordia, Grosseto, Italy;

⁹Department of Anesthesiology, Emergency and Intensive Care Medicine, University of Göttingen, Germany. ¹⁰Dipartimento di Anestesia, Rianimazione ed Emergenza Urgenza, Fondazione IRCCS Ca’ Granda – Ospedale Maggiore Policlinico, Milan, Italy;

¹¹Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Università degli Studi di Milano, Milan, Italy

SUPPLEMENTARY MATERIAL

ALBIOS Study

The total study cohort of ALBIOS included 1818 patients age 18 y or older who met clinical criteria for severe sepsis or septic shock within 24 h of hospital admittance or anytime during the Intensive Care Unit (ICU) stay (1). Eight patients were excluded from the analysis (2 patients in the albumin group owing to withdrawal of consent, and 5 in the albumin group and 1 in the crystalloid group owing to a randomization error). Severe sepsis was defined as proven or suspected infection, two or more signs of the systemic inflammatory response syndrome, and at least one acute sepsis-related organ dysfunction, as defined by the Sequential Organ Failure Assessment (SOFA) score (1). Shock on study inclusion was defined as a score of 3 or 4 on the SOFA cardiovascular component. SOFA scores were calculated on a daily basis and we also calculated the Simplified Acute Physiology Score (SAPS) II score (2) after 24 h in the ICU.

In the first 24 h after study inclusion, fluids were administered to both groups according to the protocol for early-goal directed therapy (3). In patients randomized to 20% albumin, albumin administration was targeted at a serum albumin level ≥ 30 g/L for. Demographic, clinical and laboratory data were collected on a daily basis and microbiological sampling was performed according to local protocols. All treatments, except fluid administration, were left to the discretion of the attending physicians in the different ICUs that participated in the ALBIOS trial, according to the international guidelines for the treatment of sepsis.

Biomarker analyses

Blood samples were centrifuged and allocated, and frozen plasma samples were shipped to a central biorepository and stored at -70° C. We used the Elecsys proBNP assay (Roche Diagnostics, Penzberg, Germany) for N-terminal pro-B-type natriuretic peptide (NT-proBNP) measurements and the Elecsys TNT hs STAT assay (Roche Diagnostics) for high-sensitivity cardiac troponin T (hs-cTnT) measurements (4).

Statistical analysis

Differences in clinical characteristics and hemodynamic goals across tertiles of SN concentration were compared by chi-square test of Fisher's exact test for categorical variables; for continuous variables, we used analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis test. We calculated Spearman rank correlation coefficients between continuous variables and SN levels on day 1. Moreover, to identify variables related to high SN levels on day 1, considered on a natural logarithm scale, a stepwise multivariable linear regression model was performed including all baseline characteristics significantly associated with secretoneurin at a p level <0.05 . Non-parametric analysis of variance on ranks of SN concentrations was used to assess (1) the influence of prevalent shock over time in the overall population and (2) the effect of the randomized treatments (albumin vs. crystalloids) over time separately in patients with or without septic shock. Only patients with SN measurements at all three time points were considered for this analysis.

The relationship between SN concentrations at each time point (days 1, 2 and 7) with ICU mortality and 90-day mortality was first described by median SN levels (Q1-Q3) and compared with Kruskal-Wallis test. Then, a univariate logistic regression model was done to evaluate the prognostic value of SN, considered as a continuous variable after natural logarithm transformation. Finally, multivariable logistic regression models were performed to evaluate the independent prognostic value of SN, adjusting for the variables associated with the outcome of interest. In addition to clinical variables, we included hs-cTnT and NT-proBNP levels into multivariable models as both of these established cardiac biomarkers also were associated with clinical outcomes. We assessed the prognostic utility of SN separately in the subgroups of patients with severe sepsis with or without septic shock at study entry.

Measures of discrimination ability were calculated, in patients with septic shock, as (1) the area under the ROC curve (AUROC) independently for each circulating marker (SN, NT-proBNP and hs-cTnT), and (2) the continuous Net Reclassification Improvement (cNRI), obtained after adding SN concentrations to the multivariate models described above. Kaplan-Meier survival analysis was performed by tertiles of SN concentrations on day 1 in the subgroups of patients with severe sepsis with or without shock. Normally distributed changes in SN concentrations on day 2 from day 1, and on day 7 from day 2, were expressed as the difference in log-transformed levels and analysed with univariate and multivariate logistic regression model to assess the relationship with ICU mortality and 90-day mortality.

A two-sided p value of <0.05 was deemed statistically significant. All statistical analyses were performed with SAS software (SAS Institute, Cary, NC, USA) version 9.4.

REFERENCES

1. Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Medicine* 1996;22:707-10.
2. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. *JAMA* 1993;270:2957-63.
3. Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Medicine* 2013;39:165-228.
4. Masson S, Caironi P, Fanizza C, et al. Sequential N-terminal pro-B-type natriuretic peptide and high-sensitivity cardiac troponin measurements during albumin replacement in patients with severe sepsis or septic shock. *Critical Care Medicine* 2016;44:707-16.

Supplementary Table 1. Comparison between patients with SN measured on day 1 (n=958) and the total ALBIOS study cohort (n=1810).

	<i>ALBIOS population (n=1810)</i>		<i>Present study (SN population) (n =958)</i>	
Characteristics	<i>Albumin (n=903)</i>	<i>Crystalloids (n=907)</i>	<i>Albumin (n=474)</i>	<i>Crystalloids (n=484)</i>
Age – year	70 [57-77]	69 [59-77]	70 [57–78]	70 [59–78]
Female sex – No. (%)	360 (39.9)	357 (39.4)	198 (41.8)	197 (40.7)
Reason for admission to ICU- No. (%)				
Medical	511 (56.6)	518 (57.1)	273 (57.6)	267 (55.2)
Elective surgery	69 (7.6)	58 (6.4)	37 (7.8)	30 (6.2)
Emergency surgery	323 (35.8)	331 (36.5)	164 (34.6)	187 (38.6)
Preexisting conditions – No. (%)				
Liver disease	13 (1.4)	14 (1.5)	7 (1.5)	7 (1.5)
COPD	113 (12.5)	108 (11.9)	63 (13.3)	57 (11.8)
Chronic renal failure	44 (4.9)	32 (3.5)	25 (5.3)	17 (3.5)
Immunodeficiency	115 (12.7)	128 (14.1)	60 (12.7)	64 (13.2)
Congestive or ischemic heart disease	149 (16.5)	165 (18.2)	77 (16.2)	87 (18.0)
SAPS II score	48 [37–59]	48 [37–60]	46 [36-55]	46 [35-57]
Physiological /Laboratory variables				
Heart rate – beats/min	105±22	106±20	103±20	105± 21
Mean arterial pressure – mmHg	74±16	73±15	75±16	74±14
Central venous pressure – mmHg	10.0±4.9	9.8±4.7	10.1±4.7	9.8±4.6
Urine output – ml/hr	50 [20–100]	50 [25–100]	60 [27–100]	60 [30–100]
Lactate – mmol/liter	2.3 [1.4–4.2]	2.5 [1.6–4.3]	2.3 [1.4–4.0]	2.3 [1.5–4.0]
Central venous oxygen saturation	73 [65–79]	73 [68–80]	74 [66–79]	74 [69–80]
SOFA score [at baseline]	8 [6–10]	8 [5–10]	7 [5-10]	7 [5-9]
Shock – No. (%)	565 (62.6)	570 (62.8)	273 (57.6)	267 (55.2)
Mechanical ventilation – No. (%)	709 (78.5)	737 (81.3)	371 (78.3)	389 (80.4)
Death at 28 days – no./total No. (%)	285/895 (31.8)	288/900 (32.0)	135/468 (28.9)	128/481 (26.6)
Death at 90 days – no./total No. (%)	365/888 (41.1)	389/893 (43.6)	185/467 (39.6)	184/478 (38.5)

Supplementary Table 2. Characteristics of the ALBIOS biomarker substudy with patients stratified according to SN tertiles measured on day 1 after randomization

Characteristics	SN on day 1 (pmol/L)				
	<i>All</i>	<i>T1</i> (11.1-174.0)	<i>T2</i> (175.0-236.0)	<i>T3</i> (237.0-2791.0)	<i>P</i>
No. (%)	958 (100)	319 (33.3)	319 (33.3)	320 (33.4)	
Age – year	70 [58–78]	66 [49–76]	71 [61–78]	71 [62–80]	< 0.0001
Female sex – no. (%)	395 (41.2)	132 (41.4)	143 (44.8)	120 (37.5)	0.17
Body Mass Index – kg/m ²	26.43±5.57	26.33±6.03	26.73±5.59	26.23±5.04	0.48
<i>Reason for admission to ICU - no. (%)</i>					0.16
Medical	540 (56.4)	175 (54.9)	167 (52.4)	198 (61.9)	
Elective surgery	67 (7.0)	23 (7.2)	26 (8.2)	18 (5.6)	
Emergency surgery	351 (36.6)	121 (37.9)	126 (39.5)	104 (32.5)	
<i>Preexisting conditions – no. (%)</i>					
Liver disease	14 (1.5)	6 (1.9)	3 (0.9)	5 (1.6)	0.60
COPD	120 (12.5)	35 (11.0)	42 (13.2)	43 (13.4)	0.59
Chronic renal failure	42 (4.4)	4 (1.3)	9 (2.8)	29 (9.1)	<.0001
Immunodeficiency	124 (12.9)	43 (13.5)	37 (11.6)	44 (13.8)	0.68
Congestive or ischemic heart disease	164 (17.1)	39 (12.2)	56 (17.6)	69 (21.6)	0.01
SAPS II score	46.7±15.6	40.5±13.4	45.9±14.5	53.9±9	<0.0001
<i>Physiological /Laboratory variables</i>					
Heart rate – beats/min	104±21	102±19	104±22	105±21	0.11
Mean arterial pressure – mmHg	75±15	76±14	76±16	72±15	0.0004
Mean arterial pressure after 6 hrs – mmHg	78±13	80±13	79±14	76±14	0.0007
Central venous pressure – mmHg	9.95±4.64	9.50±4.15	9.90±4.61	10.46±5.09	0.04
PaO ₂ /FiO ₂	190 [130–270]	190 [130–270]	190 [130–270]	200 [130–280]	0.51
Urine output – ml/hr	60 [30–100]	70 [50–100]	60 [32–100]	50 [10–90]	<0.0001
Lactate – mmol/liter	2.3 [1.4–4.0]	2.0 [1.3–3.5]	2.5 [2.2–1.4]	2.7 [1.7–5.1]	<0.0001
Serum albumin – g/liter	24.44±6.25	24.54±6.35	24.54±5.98	24.21±6.42	0.78
Hemoglobin – g/dl	10.92±1.97	11.18±1.95	10.91±2.00	10.66±1.92	0.004
Serum creatinine – mg/dL	1.5 [0.9–2.5]	1.0 [0.7–1.5]	1.4 [0.9–2.2]	2.6 [1.6–3.8]	<0.0001
White blood cells – 10 ³ /mm ³	12.0 [5.7–18.6]	11.7 [5.5–18.7]	12.1 [6.6–18.8]	12.1 [5.6–17.8]	0.60
Central venous oxygen saturation	72.60±9.80	74.03±8.99	72.08±9.71	71.71±10.49	0.01
Serum bilirubin (mg/dL)	0.8 [0.5–1.5]	0.8 [0.5–1.3]	0.8 [0.5–1.6]	0.9 [0.6–1.8]	0.03
Platelet count (10 ⁹ /L)	166 [102–237]	173 [113–240]	170 [106–241]	145 [83–233]	0.007
NT-proBNP – median [Q1-Q3]	4399 [1313-13837]	2063 [738-5724]	3928 [1449-10371]	10734 [3791-26838]	< 0.0001
hs-cTnT – median [Q1-Q3]	50.4 [21.7-133.5]	31.5 [14.0-86.0]	44.8 [21.0-112.7]	88.4 [41.4-217.8]	< 0.0001
SOFA score [at baseline]	7 [5-10]	6 [4-8]	7 [5-9]	9 [7-11]	<0.0001
Shock – no. (%)	540 (56.4)	161 (50.5)	179 (56.1)	200 (62.5)	0.01

Mechanical ventilation – no. (%)	760 (79.3)	249 (78.1)	258 (80.9)	253 (79.1)	0.67
Randomized to Albumin arm – no. (%)	474 (49.5)	157 (49.2)	154 (48.3)	163 (50.9)	0.79
Antibiotics at the time of randomization – no (%)	898 (93.7)	298 (93.4)	297 (93.1)	303 (94.7)	0.68
Antibiotics at 6 hours after randomization – no (%)	954 (99.6)	318 (99.7)	317 (99.4)	319 (99.7)	0.85
Appropriateness of antibiotic therapy at Day 1 after randomization according to site culture – no (%)	422/558 (75.6)	131/171 (76.6)	138/186 (74.2)	153/201 (76.1)	0.85
Appropriateness of antibiotic therapy at Day 1 after randomization according to blood culture – no (%)	211/259 (81.5)	53/69 (76.8)	72/85 (84.7)	86/105 (81.9)	0.45

ANOVA or Kruskal-Wallis test for continuous variables, chi-square or Fisher tests for categorical variables.

Supplementary Table 3. Correlation coefficients between SN levels measured on day 1 after randomization and continuous laboratory or clinical variables

Variables	rho	P
Serum creatinine – mg/dL	0.51	<0.0001
NT-proBNP – ng/L	0.40	<0.0001
SAPS II score	0.37	<0.0001
SOFA score [at baseline]	0.35	<0.0001
hs-cTnT – ng/L	0.31	<0.0001
Urine output – ml/hr	-0.22	<0.0001
Lactate – mmol/liter	0.17	<0.0001
Age – year	0.16	<0.0001
Mean arterial pressure after 6 hrs – mmHg	-0.16	<0.0001
Mean arterial pressure – mmHg	-0.14	<0.0001
Serum bilirubin (mg/dL)	0.11	0.001
Platelet count ($10^9/L$)	-0.11	0.001
Hemoglobin – g/dL	-0.10	0.002
Central venous pressure – mmHg	0.09	0.009
Central venous oxygen saturation (%)	-0.09	0.01
Heart rate – beats/min	0.07	0.02
Serum albumin – g/liter	-0.03	0.33
PaO ₂ /FiO ₂	0.02	0.49
Body-mass index – kg/m ²	0.008	0.82
White blood cells – $10^3/mm^3$	-0.002	0.94

Supplementary Table 4. Variables associated with increasing SN levels on day 1

Variable	Parameter Estimate	Standard Error	t value	P
Serum creatinine (mg/dl)	0.080	0.009	9.28	<0.0001
NT-proBNP (ng/L)	0.000006	0.000001	4.42	<0.0001
Serum bilirubin (mg/dL)	0.024	0.009	2.70	0.007
Serum lactate (mmol/L)	0.014	0.005	2.68	0.008
Age (year)	0.0018	0.0009	2.05	0.04

Linear regression model in which the dependent variables is the natural logarithm transformed SN levels and the following covariates: age, chronic renal failure, congestive or ischemic heart disease, mean arterial pressure, central venous pressure, urine output, serum lactate, hemoglobin, serum creatinine, central venous oxygen saturation, serum bilirubin, platelets count, shock, NT-proBNP and hs-cTnT on day 1.

Supplementary Table 5. Achievement of hemodynamic goals during the first 6-24 h and day 1 concentrations of SN

Characteristics	SN on day 1				
	<i>All</i>	<i>T1 (11-174)</i>	<i>T2 (175-236)</i>	<i>T3 (237-2791)</i>	<i>P</i>
Central venous pressure after 6 hours – no. (%)					0.17
< 8 mmHg	205/907 (22.6)	82/308 (26.6)	62/301 (20.6)	61/298 (20.5)	
8-12 mmHg	398/907 (43.9)	130/308 (42.2)	142/301 (47.2)	126/298 (42.3)	
> 12 mmHg	304/907 (33.5)	96/308 (31.2)	97/301 (32.2)	111/298 (37.3)	
Mean arterial pressure after 6 hours $\geq$ 65 mmHg – no. (%)	822/958 (85.8)	286/319 (89.7)	277/319 (86.8)	259/320 (80.9)	0.006
Central venous oxygen saturation after 6 hours $\geq$ 70% - no. (%)	612/811 (75.5)	229/275 (83.3)	188/268 (70.2)	195/268 (72.8)	0.0008
Lactate at day 1 < 2 mmol/liter – no. (%)	562/923 (60.9)	218/307 (71.0)	194/306 (63.4)	150/310 (48.4)	<.0001
Vasoactive drugs at day 1 – no. (%)					
Dopamine	234/948 (24.7)	71/318 (22.3)	77/316 (24.4)	86/314 (27.4)	0.33
Norepinephrine	514/948 (54.2)	149/318 (46.9)	163/316 (51.6)	202/314 (64.3)	<.0001
Epinephrine	57/948 (6.0)	10/318 (3.1)	15/316 (4.8)	32/314 (10.2)	0.0005
Dobutamine	134/948 (14.1)	29/318 (9.1)	43/316 (13.6)	62/314 (19.8)	0.0006
Vasopressin	9/948 (0.9)	4/318 (1.3)	1/316 (0.3)	4/314 (1.3)	0.36
Two or more vasoactive drugs at day 1 – no. (%)	265/948 (28.0)	58/318 (18.2)	81/316 (25.6)	126/314 (40.1)	<.0001
Doses of vasoactive drugs at day 1 - μ g/kg/min					
Dopamine	7.44 $\pm$ 3.94	6.72 $\pm$ 3.29	7.91 $\pm$ 4.46	7.61 $\pm$ 3.92	0.17
Norepinephrine	0.28 $\pm$ 0.30	0.24 $\pm$ 0.26	0.28 $\pm$ 0.28	0.30 $\pm$ 0.35	0.21
Epinephrine	0.13 $\pm$ 0.12	0.11 $\pm$ 0.07	0.09 $\pm$ 0.06	0.16 $\pm$ 0.15	0.16
Inotropic score on day 1	8 [0–21]	5 [0–18]	8 [0–21]	10 [0–29]	<.0001
Mean arterial pressure at day 1 $\geq$ 65 mmHg – no. (%)	852/948 (89.9)	298/318 (93.7)	294/316 (93.0)	260/314 (82.8)	<.0001

The inotropic score was calculated as dopamine dose on day 1 + (epinephrine dose at day 1)*100 + (norepinephrine dose at day 1)*100 (7). ANOVA for continuous variables, Chi-square test for categorical variables.

Supplementary Table 6. Logistic regression models to assess interaction between SN concentrations on day 1 and septic shock for ICU and 90-day mortality

ICU mortality: analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
SN on day 1 (ln transformed)	1	1.1985	0.2040	34.5160	<.0001
Prevalent shock	1	-3.1568	1.1023	8.2011	0.004
Interaction between shock and SN on day 1	1	0.6138	0.2040	9.0522	0.003

90-day mortality: analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
SN on day 1 (ln transformed)	1	1.2290	0.1964	39.1707	<.0001
Prevalent shock	1	-2.0185	1.0526	3.6775	0.06
Interaction between shock and SN on day 1	1	0.4132	0.1964	4.4281	0.04

Supplementary Table 7. Associations between changes in SN levels during ICU stay and clinical outcomes

Outcome	Patients	Changes from day 1 to day 2					Changes from day 2 to day 7				
		No. events / no. patients (%)	Univariate		Multivariable		No. events / no. patients (%)	Univariate		Multivariable	
			OR [95%CI]	p	OR [95%CI]	p		OR [95%CI]	p	OR [95%CI]	p
ICU mortality	Severe sepsis	84/384 (21.9)	0.99 [0.49-2.00]	0.97	1.18 [0.51-2.71]	0.69	61/320 (19.1)	0.50 [0.21-1.21]	0.12	0.51 [0.19-1.41]	0.20
	Shock	132/486 (27.2)	1.40 [0.71-2.74]	0.33	2.09 [0.93-4.67]	0.07	87/395 (22.0)	0.61 [0.27-1.39]	0.24	0.61 [0.24-1.53]	0.29
90 day mortality	Severe sepsis	123/375 (32.8)	0.71 [0.37-1.36]	0.30	0.90 [0.40-1.99]	0.78	98/311 (31.5)	0.66 [0.31-1.40]	0.28	1.00 [0.40-2.49]	0.99
	Shock	202/483 (41.8)	1.56 [0.84-2.88]	0.16	1.73 [0.84-3.53]	0.14	151/390 (38.7)	0.51 [0.25-1.05]	0.07	0.50 [0.21-1.19]	0.12

Changes from day 1 to day 2 or 7 were considered as difference in natural logarithm concentration from day x to day y [$\ln(\text{SN}_{\text{day y}}) - \ln(\text{SN}_{\text{day x}})$].

Covariates included in the multivariable analyses were the same as in the multivariate models #1 reported below Table 2.

Supplementary Figure 1. Secretoneurin concentration on day 1 by quintiles of overall SOFA score and by organ-specific SOFA sub-scores on day 1

For the overall SOFA score: Q1= 0–4, Q2= 5–6, Q3= 7–8, Q4= 9–10, Q5= 11–18.

Supplementary Figure 2. Time-course of SN concentration by shock and randomized treatment

SN concentrations on days 1, 2 and 7 are reported in patients with the three time-points available, 311 patients with severe sepsis without shock (151 randomized to albumin, 160 to crystalloids) and 377 with septic shock (190 randomized to albumin, 187 to crystalloids). Data shown as median [Q1-Q3] after stratification for sepsis severity (A) or sepsis severity and randomized treatment (B). Non-parametric analysis of variance were done on ranks of SN concentrations. In all patients: time $p=1.00$, shock $p=0.0004$, interaction time by shock $p=0.62$; in patients with severe sepsis without shock: time $p=1.00$, treatment $p=0.47$, interaction time by treatment $p=0.79$; in patients with septic shock: time $p=1.00$, treatment $p=0.24$, interaction time by treatment $p=0.91$. * $P<0.05$ vs. severe sepsis at corresponding time point by Kruskal-Wallis test.

Supplementary Figure 3. Reclassification of mortality by SN levels on day 2 in patients with septic shock

SN levels on day 2 reclassified a significant proportion of patients with septic shock into their correct risk strata when added on top of the basic risk model (Model #1 in Table 2), both for ICU mortality (top panels: NRI 0.30 [95% CI 0.10-0.51], $p=0.004$; NRI events= 12%, $p=0.21$; NRI non-events= 18%, $p=0.0006$) and 90 day mortality (lower panels: NRI 0.22 [0.05-0.42], $p=0.01$; NRI events = 10%, $p=0.13$; NRI non-events= 12%, $p=0.05$).