Circulating Biologically Active Adrenomedullin (bio-ADM) Predicts Hemodynamic Support Requirement and Mortality During Sepsis

Circulating biologically active adrenomedullin (bio-ADM) predicts hemodynamic support requirement and long-term survival during sepsis.

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Abstract

Background: The biological role of adrenomedullin, a hormone involved in hemodynamic homeostasis, is controversial in sepsis, since administration of either the peptide or an antibody against it may be beneficial.

Objectives: In a large cohort of patients with sepsis or septic shock, we evaluated the clinical and prognostic significance of early and sequential bio-ADM assessment.

Methods: Plasma bio-ADM was assessed on days 1, 2 and 7 after randomization of 956 patients to albumin or crystalloids for fluid resuscitation in the multicenter Albumin Italian Outcome Sepsis (ALBIOS) trial. We tested the association of bio-ADM and its time-dependent variation with fluid therapy, vasopressor administration, organ failures and long-term survival.

Results: Plasma bio-ADM on day 1 (median [Q1-Q3], 110 [59-198] pg/mL) was higher in patients with septic shock, and was associated with multiple organ failures and the average extent of hemodynamic support therapy (fluids and vasopressors) and serum lactate time-course over the first week. Moreover, it predicted incident cardiovascular dysfunction in patients without shock at enrollment (OR [95% CI] 1.9 [1.4-2.5], $p<0.0001$, for an increase of 1 interquartile range of bio-ADM concentration). Bio-ADM trajectory during the first week of treatment clearly predicted 90-day survival, after adjustment for clinically relevant covariates (HR [95% CI] 1.3 [1.2-1.4], $p<0.0001$), and its reduction below 110 pg/mL at day 7 was associated to a marked reduction in 90-day mortality. Changes over the first 7 days of bio-ADM concentrations were not dependent on albumin treatment.

Conclusions: In patients with sepsis, the circulating, biologically active form of adrenomedullin may help individualizing hemodynamic support therapy, while avoiding harmful effects. Its possible pathophysiologic role makes bio-ADM a potential candidate for future targeted therapies.

Key words: adrenomedullin – biomarker – sepsis – septic shock – fluid requirement – prognosis

Abbreviations list

ADM = adrenomedullin

ALBIOS = Albumin Italian Outcome Sepsis

ARDS = acute respiratory distress syndrome

bio-ADM = bioactive adrenomedullin

ED = emergency department

HR = hazard ratio

ICU = intensive care unit

IQR = interquartile range

RIFLE = risk, injury, failure, loss, and end-stage kidney injury

SAPS = simplified acute physiology score

SIRS = systemic inflammatory response syndrome

SOFA = sequential organ failure assessment

Introduction

Since its discovery in 1993 (1), the 52-aminoacid peptide adrenomedullin has been implicated in the pathobiology of several diseases, including cardiovascular disorders (2). Adrenomedullin (ADM) is indeed up-regulated in different tissues, as well as in several conditions, in association, among others, with myocardial injury, systemic inflammatory response syndrome (SIRS), shock, cellular hypoxia, and oxidative stress. In addition, remarkably high circulating levels of ADM have been reported in sepsis (3), where its role remains controversial (4). On one hand, in fact, during sepsis, ADM can exert beneficial effects by reducing endothelial hyperpermeability and vascular leakage, as shown in cell culture models (5-7). Indeed, exogenous administration of ADM appears to be protective in animal models of endotoxin (8) and α-toxin infusion (6), or acute respiratory distress syndrome (ARDS) (9). On the other hand, it is well known that higher circulating levels of ADM are invariably associated with worse outcomes (10-13). Moreover, administration of anti-ADM antibodies can attenuate sepsis-induced multiple organ failures in murine models (14-16), and a humanized monoclonal anti-ADM antibody is currently under clinical development (17). Several factors may probably be at the interplay of such apparent paradox, such as differences in the circulating levels of ADM, the specific type of experimental model employed, the stage of the disease, and the underlying co-morbidities and co-interventions in clinical settings.

The interpretation of ADM as a biomarker is difficult since several fragments of the precursor prohormone circulate in blood, and do not necessarily reflect the activity of ADM. Recently, a sandwich immunoassay for specifically measuring the biologically active (amidated) form of ADM (named bioactive ADM or bio-ADM) has been developed (18). By employing these reagents, bio-ADM levels in patients being evaluated at the Emergency Department (ED) for acute heart failure were shown to be predictive of clinically relevant 30-day outcomes (19). In a monocentric study performed on 101 consecutive patients presenting at the ED with the suspicion of sepsis, Marino *et al.* observed that bio-ADM levels were associated with short-term mortality and vasopressor requirement (18).

In the present study, we aimed at testing, for the first time, this biomarker in a large and well-defined cohort of patients with severe sepsis or septic shock, enrolled in the biomarker substudy of the Albumin Italian Outcome Sepsis (ALBIOS) trial (20). Given the biological role of ADM in fluid homeostasis and systemic circulation, and the profound pathophysiologic perturbations seen in the early phase of sepsis and during its treatment, we were particularly interested in describing the relation between bio-ADM and hemodynamic support requirements during the first days of treatment. In addition, we described bio-ADM trajectory over time and its prognostic value for long-term survival.

Material and Methods

Study design

ALBIOS was a multicenter, pragmatic, open-label, randomized trial that enrolled 1818 patients with severe sepsis or septic shock admitted to 100 intensive care units (ICUs). Study design, inclusion and exclusion criteria, and main results have been published elsewhere (20). Briefly, patients aged 18 years or older who met clinical criteria for severe sepsis or septic shock within the previous 24 hours and at any time during the ICU stay were enrolled. Patients were randomly assigned to receive either 20% albumin and crystalloids or crystalloids alone, from randomization until day 28 or discharge from the ICU, whichever came first. In the albumin group, 20% albumin was administered to achieve a daily serum albumin level equal to or greater than 30 g/L. In the crystalloid group, patients received only crystalloids according to the clinical needs. In both groups, synthetic colloid solutions were not allowed. During the early phase of volume resuscitation (first 24 hours after enrolment), fluids were administered in both groups according to the “early goal-directed therapy” protocol (21).

Demographic, clinical, and laboratory data were collected on a daily basis. Microbiological sampling and routine surveillance cultures were obtained according to standard guidelines operating at each center. All treatments, with the exception of fluid management, were according to standard guidelines (22) under the responsibility of the attending physician, who usually was not the local investigator of the trial (20).

The study complied with the 1975 Declaration of Helsinki as revised in 2008, and approved by the Institutional Review Board of each center. Written informed consent or deferred consent was obtained from each participant, according to the Italian legislation.

A detailed description of clinical definitions employed can be found in the Online Appendix.

Sample collection and circulating biomarker measurements

In a subset of 956 patients recruited from 40 centers participating in a predefined biomarker sub-study, venous blood samples were serially collected 1, 2 and 7 days after enrolment (or at ICU discharge, whichever came first) and centrifuged; plasma was then shipped on dry ice to a central repository and stored at -70°C until assayed. Bioactive adrenomedullin (bio-ADM) was measured using a novel chemiluminescence immunoassay provided by Sphingotec GmbH (Hennigsdorf, Germany), as previously described (18). In brief, in a one-step sandwich chemiluminescence immunoassay, based on Acridinium NHS-ester labeling for the detection of bioactive ADM in unprocessed, neat plasma, it uses two mouse monoclonal antibodies, one directed against the midregion (*solid phase*), and the other directed against the amidated C-terminal moiety of ADM (*labelled antibody*). The assay utilizes 50 µL of plasma samples/calibrators and 200 µL of labelled detection antibody. The analytical assay sensitivity is 2 pg/mL. In prior work (18), the median bio-ADM concentration of 200 healthy adults equaled 20.7 pg/mL, with a 99th percentile of 43.0 pg/mL.

Statistical methods

Values are expressed as means and standard deviations, medians and interquartile ranges (IQR), or percentages as appropriate. Group comparisons of continuous variables were performed using Kruskal-Wallis test. Biomarker data were log-transformed, if necessary. Categorical data were compared using the χ^2-test.

Cox proportional-hazards regression was employed to analyze the effect of risk factors on survival in uni- and multi-variable analyses. The assumptions of proportional hazard were tested for all variables. To asses which transformation was appropriate for bio-ADM, the biomarker was modelled as transformed by

using restricted cubic splines to assess for non-linear relationships (23). Five knots at quartiles of the predictors were used in our analysis. To test if bio-ADM provided additional predictive information in multivariable models, we used the likelihood ratio chi-square test for nested models to assess. For continuous variables, hazard ratios (HR) with 95% confidence intervals were standardized to describe the HR for a biomarker change of one IQR. The predictive value of each model was assessed by the model likelihood ratio chi-square statistic. Survival curves plotted by the Kaplan-Meier method were used for illustrative purposes. Logistic regression or Cox-regression analysis were employed to evaluate bio-ADM for the prediction of incident shock, as appropriate.

All statistical tests were two-tailed and a two-sided p-value of 0.05 was considered for significance. The statistical analyses were performed using R version 2.5.1 (http://www.r-project.org, library Design, Hmisc, ROCR) and Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Variables associated with bio-ADM levels

Median concentration of bio-ADM on day 1 was 110 [59-198] pg/mL (n=956). **Table 1** shows patients' demographic and baseline characteristics according to tertiles of bio-ADM concentrations on the same day. By multiple linear regression analysis, the variables most strongly associated with higher bio-ADM concentrations, were, by decreasing order of r^2, higher SOFA score ($F=31$, $p<0.0001$), ICU admission after emergency surgery ($F=15$, $p<0.0001$), higher heart rate ($F=21$, $p<0.0001$), higher serum creatinine levels ($F=17$, $p<0.0001$), higher central venous pressure ($F=16$, $p=0.0001$), lower serum albumin concentration ($F=14$, $p=0.0002$), positive blood culture ($F=7$, $p=0.001$), lower mean arterial pressure ($F=13$, $p=0.0003$), higher BMI ($F=12$, $p=0.0005$), and immunodeficiency ($F=11$, $p=0.001$).

Bio-ADM, hemodynamic goals and support requirements

Higher concentrations of bio-ADM on day 1 were associated with less frequent achievement of hemodynamic goals during the first 6 to 24 hours after randomization, in terms of central venous pressure and oxygen saturation, mean arterial pressure and serum lactate concentrations (**Table 2**). Patients in the upper tertile of bio-ADM received more frequently two or more vasoactive drugs, and showed higher values of inotropic score and vasopressor dependency index, as compared to the middle and the lower tertiles (**Table 2**). As shown in **Figure 1**, the inotropic score over the first 7 days of treatment in patients alive at that time was greater in those with higher levels of bio-ADM at day 1 as compared to those with lower levels. In parallel, serum lactate levels were significantly higher in patients in the upper tertile (**Central Illustration, panel A**). Moreover, patients in the upper tertile of bio-ADM showed also greater need of fluids during the first week of treatment, as shown by the average fluid administered, the average amount of albumin or crystalloids received, as well as the average and the cumulative fluid balance observed over the first 7 days (**Table 2**).

Bio-ADM and organ failures

Prevalent and incident organ failures, defined as organ-specific SOFA scores of 3 or 4 for the respiratory, coagulation, hepatic and renal systems, were more frequent at higher concentrations of bio-ADM on day 1 (**Figure S1**). Accounting for multiple organ failures and death, we found conclusive evidence of elevated bio-ADM levels in patients with multiple prevalent or multiple incident organ failures, as well as in those who died during the follow up period.

Bio-ADM and septic shock

Patients presenting with septic shock at the time of enrollment had significantly higher concentrations than those with severe sepsis only ($p<0.0001$), and showed a greater reduction over the first 7 days, as compared to patients without shock (p for interaction time x shock <0.0001, **Figure S2**).

Among patients without shock at study entry, there was a progressive increase in the likelihood of having incident shock with higher concentrations of bio-ADM (**Figure S3**), that remained significant after adjustments for the SAPS II score or for clinically relevant variables (**Table S1**).

Bio-ADM and 90-day survival

Over 90 days of follow-up, 369 patients (38.6%) died. Main clinical characteristics of survivors and non-survivors are shown in **Table S2**. **Figure 2** shows the Kaplan-Meier survival at 90 days by quartiles of bio-ADM concentrations on day 1, with a clear, stepwise increment of the risk of mortality with increasing bio-ADM levels. The progressive relationship between bio-ADM concentrations on day 1 and 90-day mortality, was confirmed by restrictive spline curves (**Figure S4)**.

In Cox proportional hazard models adjusted for clinically-relevant covariates, bio-ADM concentrations on day 1 remained independently associated with 90-day mortality in the overall cohort **(Table 3**).

Time course of bio-ADM and 90-day survival

To evaluate the association between the variation over time of bio-ADM and short-term survival, we analyzed bio-ADM time course over the first 7 days by survival after this period. Bio-ADM decreased over the first 7 days after randomization in the subgroup of patients surviving the first week of treatment, while it remained stable in those dying (p for interaction between time and survival<0.0001, **Figure S5**).

Central Illustration (panel B) shows the Kaplan-Meier survival curves for 90-day mortality according to the evolution of bio-ADM levels from day 1 to day 7, stratified according to the median concentration of bio-ADM on day 1 (110 pg/mL). Patients with bio-ADM concentrations decreasing from above the median value on day 1 to below the median value on day 7 showed a 90-day mortality rate close to those with bio-ADM remaining below the median value at both time points (33.3 vs. 26.7%, **Central Illustration, panel B**). In contrast, outcome was markedly worse and comparable both in patients with increasing bio-ADM concentrations over time (66.7%) and in those with higher bio-ADM levels at both time points (64.6%). In time-dependent Cox regression models adjusted for clinically relevant covariates, evolution of bio-ADM concentrations over the first 7 days was independently associated with 90-day mortality (**Figure 3**).

Bio-ADM and other clinical outcomes

Finally, higher concentrations of bio-ADM on day 1 were associated with further clinically relevant outcomes (**Table S3**). Of note, after adjustments, patients with higher levels of bio-ADM at day 1 showed longer length of hospital stay and a higher incidence of renal replacement therapy during the study period ($p < 0.01$, for both), as compared to patients with lower levels of bio-ADM.

Randomized treatment (albumin or crystalloids vs. crystalloids alone) had no effect on bio-ADM concentrations over time (**Table S4**).

Discussion

In a large cohort of well-characterized patients with severe sepsis or septic shock enrolled in a multicenter, randomized trial, we observed: (1) plasma concentration of bio-ADM was high, especially in patients with septic shock; (2) higher bio-ADM levels on day 1 predicted the intensity of the hemodynamic support requirement over the first week of treatment; (3) bio-ADM predicted incident organ-specific failure and shock; (4) the trajectory of bio-ADM over the first week was strongly and independently associated with long-term survival.

The hormone adrenomedullin exerts multiple effects with possible clinical importance, of which the most prominent appear to be vasodilation, diuresis and natriuresis (24). Although evidence suggests its importance in the pathophysiology of sepsis (25), experimental findings seem controversial, since two apparently opposite interventions, such as exogenous administration of the peptide or modulation with antibodies directed against the same peptide, produce benefits in preclinical sepsis models (4). The present study design, i.e. the analysis in a large-scale clinical trial of this peptide as a biomarker, is not ideally suited to provide fully mechanistic insights into the role of adrenomedullin in sepsis. Conversely, it is a unique opportunity to evaluate the clinical potential of this biomarker in a reliable and accurate manner. Indeed, as initial and basic finding, we confirm that the biologically active adrenomedullin circulates in elevated

concentrations during severe sepsis and septic shock. In previous studies, adrenomedullin levels have been estimated either with a competitive radioimmunoassay characterized by technical limitations (10), or with an assay of unknown analytical performance (13), or, further, by measuring an inactive, stable fragment of pro-adrenomedullin (11,12). Since the activity of adrenomedullin depends on the conversion of the C-terminal glycine residue to an amide, and since amidation rate differs amongst pathological states (26,27), the levels measured in the present study are very likely to better reflect the biological relevance of adrenomedullin in sepsis.

Aggressive fluid therapy and hemodynamic support to restore peripheral organ perfusion based upon pre-defined hemodynamic targets are the cornerstone of the management of patients with severe sepsis and septic shock (22). Despite these standardized procedures, several experimental and clinical evidence clearly suggest that this approach may have harmful effects, and should be individualized (28-30). Several clinical studies have indeed shown an independent association between an increasingly positive fluid balance and reduced survival in patients with sepsis (31,32). Since instrumental and laboratory measurements (such as chest radiograph, central venous pressure or venous oxygen saturation, ultrasonography, serum lactate measurements) (33-36) may have incomplete value in guiding individual hemodynamic support requirement, we sought to investigate whether a simple, objective, measurement of a circulating biomarker, with potential vasodilating and hyper-permeability effects, early in the clinical course of sepsis, could predict the subsequent need for fluids or vasopressors administration. In our study, we indeed observed that higher bio-ADM levels on day 1 predicted more positive fluid balance, as well as higher doses of vasopressor agents administered during the first 7 days of treatment, independently on the overall patient severity. In the same cohort of patients, we have previously shown that plasma concentrations of a natriuretic peptide were extremely elevated in patients with severe sepsis or septic shock, up to levels normally encountered during acute heart failure (37). We speculate that these levels may reflect excessive fluid administration in the early phase of the treatment of sepsis, resulting in high atrial and ventricular filling pressures, which are the main determinants of cardiac release of natriuretic peptides (38). The present study confirms that circulating biomarkers of endocrine systems controlling fluid homeostasis and vasodilation are strikingly up-regulated during severe sepsis and septic shock, likely in relation to fluid overloading and the intensity of

hemodynamic support applied. Appropriately designed, prospective clinical studies are warranted to determine whether these biomarkers will help in accurately tailoring the hemodynamic support and fluid management of septic patient to reduce the negative consequences of their liberal administration.

Higher levels of bio-ADM on day 1 predicted incident shock in patients with sepsis at the time of enrollment without overt signs of cardiovascular failure. Moreover, patients having early elevated levels of bio-ADM showed a higher proportion of organ dysfunction or failures, for each organ component included in the SOFA score (liver, kidney, coagulation, and respiration). Of note, after correction for competing risks between the co-presence of multi-organ failures or the subsequent development of death, we found conclusive evidence indicating an independent associations between bio-ADM and multiple prevalent or incident organ failures, as well as with the subsequent development of death during follow up. Taken together, these results clearly highlight the role of bio-ADM as an early bio-indicator of an increased risk of sepsis-related overall morbidity and mortality. Our findings also support two clinically relevant aspects that merit further discussion. First, due to its predictive power for the subsequent development of shock, early bio-ADM monitoring may guide towards a timely implementation and monitoring of an adequate hemodynamic support in those patients who are likely to develop subsequently overt cardiovascular dysfunction. Indeed, similarly to what has been observed for the initiation of antimicrobial therapy (39,40), recent evidence has shown an increased risk of death for each hour delay in the initiation of vasopressor therapy during septic shock (41). Second, our findings also support the primacy of the impairment of systemic and regional circulation over other pathophysiologic mechanisms responsible for the development of sepsis-associated organ dysfunctions. Therefore, bio-ADM may be also considered as a potential novel candidate for a targeted therapy of sepsis, aimed at blunting its excessive effects.

We also observed that the trajectory of bio-ADM over the first 7 days after the diagnosis of severe sepsis or septic shock was a strong indicator of subsequent and long-term mortality. Concordant evidence were obtained when the previously published cut-off of 70 pg/mL (18) was applied instead of the median concentration of bio-ADM *(data not shown)*. The association between time-dependent bio-ADM variation and outcome was independent of the more robust risk factors commonly observed in septic patients, and

already tested in the original ALBIOS trial (20), i.e. age, total SOFA score, serum lactate concentration and central venous oxygen saturation. Consequently, bio-ADM may be considered a good candidate for early risk stratification in patients with sepsis or septic shock. Moreover, our data suggest that the sequential assessment of bio-ADM may be an important tool to monitor the efficacy of the hemodynamic treatment applied, which may be targeted to obtain a reduction of bio-ADM levels (i.e., to a level lower than 110 pg/mL).

The present study has some limitations. First, it did not include all the study population originally enrolled in the ALBIOS trial, as only a subgroup of the participating centers was involved in the biomarker sub-study. Nonetheless, these centers enrolled in the ancillary biomarker study the majority of the patients enrolled in the main trial, guaranteeing therefore the representativeness of the disease and the entire cohort investigated. Second, as ALBIOS was a pragmatic trial, circulating cardiac biomarkers were not assessed at enrollment, since the first measurement was obtained the subsequent morning. Therefore, bio-ADM levels on day 1 might have been affected by early study treatment. Nonetheless, the availability of plasma collections at further time points (over the first week of treatment) allows the evaluation of the time-course of the biomarkers with a reasonable accuracy during the first critical phase of the treatment of sepsis. Third, since only a relative small percentage of patients initiated vasopressor therapy after day 1, the present study can only provide definitive indication on the potential of bio-ADM as an early predictor of fluid requirement, or as an indicator of the response to vasopressor administration. Further studies, in large cohorts of patients with the suspicion of sepsis and without shock, are necessary to further elucidate this hypothesis.

Conclusions

We showed in a large and representative cohort of acutely-ill patients with severe sepsis or septic shock that the circulating, biologically active form of adrenomedullin is extremely elevated, and may be valuable for clinical monitoring of fluid and vasopressor requirements, in order to individualize an adequate hemodynamic support therapy and avoid potentially harmful effects. The strict association between early bio-ADM levels and subsequent development of multiple organ dysfunctions and death, in addition to hemodynamic support requirement denotes an important role of bio-ADM in the pathophysiology of sepsis

and septic shock. Efforts are ongoing to evaluate if bio-ADM may become an actionable target during septic shock, by modulating its effects through a treatment including a humanized monoclonal anti-adrenomedullin antibody (17).

Perspectives

Competency in Medical Knowledge 1: Aggressive intravenous fluid administration and hemodynamic support to restore peripheral organ perfusion are the cornerstone of the management of patients with severe sepsis and septic shock.

Competency in Medical Knowledge 2: The biological role of adrenomedullin, a hormone involved in hemodynamic homeostasis, is controversial during sepsis, since administration of either the peptide or an antibody against it may be beneficial.

Translational Outlook: We showed in a large cohort of acutely-ill patients with sepsis that the circulating, biologically active form of adrenomedullin is strictly associated with the extent of hemodynamic support requirement, predicting incident shock, the response to the therapy applied, and outcomes, and it may be, therefore, an optimal candidate molecule for personalizing treatments and targeted therapies.

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Table 1. Patients' demographic and baseline physiologic characteristics according to tertiles of bio-ADM concentrations on day 1.

Characteristics	T1 (<73 pg/mL)	T2 (73-153 pg/mL)	T3 (≥154 pg/mL)	p
No. (%)	319 (33.4)	318 (33.3)	319 (33.4)	
Age (year)	68 [53-76]	71 [60-78]	71 [62-78]	0.004
Males (No. (%))	194 (60.8)	193 (60.7)	174 (54.5)	0.19
Body Mass Index (kg/m^2)	24.8 [23.2-27.7]	25.5 [23.2-28.6]	26.1 [23.8-29.4]	0.004
Reason for admission to ICU (No. (%))				<0.0001
Elective surgery	28 (8.8)	16 (5)	23 (7.2)	
Emergency surgery	86 (27)	115 (36.2)	150 (47)	
Medical	205 (64.3)	187 (58.8)	146 (45.8)	
Pre-existing conditions (No. (%))				
Liver disease	3 (0.9)	5 (1.6)	6 (1.9)	0.60
COPD	37 (11.6)	29 (9.1)	52 (16.3)	0.02
Chronic renal failure	10 (3.1)	11 (3.5)	21 (6.6)	0.06
Immunodeficiency	27 (8.5)	40 (12.6)	57 (17.9)	0.002
Congestive or ischemic heart disease	53 (16.6)	50 (15.7)	61 (19.1)	0.50
Any previous conditions	105 (32.9)	110 (34.6)	150 (47)	<0.0001
SAPS II score	38 [30-50]	45 [35-55]	53 [43-63]	<0.0001
SOFA score	5 [3-7]	7 [5-10]	10 [8-12]	<0.0001
Physiological variables				
Heart rate (beats/min)	90 [76-101.5]	95 [80-110]	104 [89-117]	<0.0001
Mean arterial pressure (mmHg)	85 [77-93]	80 [72-88]	75 [67-83]	<0.0001
Central venous pressure (mmHg)	10 [7-12]	11 [8-13.75]	12 [9-15]	<0.0001
Central venous oxygen saturation (%)	75 [70-80]	75 [70-80]	76 [70-80]	0.886
PaO_2 /FiO_2	218 [160-284]	228 [163-284]	200 [138-272]	0.008
Urine output (mL)*	2050 [1310-3003]	1970 [1115-3015]	1285 [568-2200]	<0.0001
Laboratory variables				
Serum albumin (g/L)	28 [24-31]	27 [23-31]	25 [21-29]	<0.0001
Serum lactate (mmol/L)	1.3 [0.9-1.8]	1.6 [1.2-2.42]	2.3 [1.6-4.2]	<0.0001
Hemoglobin (g/dL)	10.6 [9.6-11.8]	10.5 [9.5-11.6]	10.5 [9.5-11.5]	0.47
Platelet count (10^9/L)	178.0 [119.5-240.5]	140.0 [80.0-218.5]	113.5 [63.0-185.0]	<0.0001
Serum creatinine – mg/dL	0.9 [0.6-1.3]	1.3 [0.9-2.2]	2.0 [1.3-3.2]	<0.0001
White blood cells (10^3/mm^3)	12.1 [8.6-16.7]	12.5 [7.5-19.8]	11.7 [6.9-17.5]	0.26
Serum bilirubin (mg/dL)	0.7 [0.4-1.1]	0.9 [0.5-1.6]	1.3 [0.7-2.4]	<0.0001
Positive blood culture (No. events /No. patients (%))	59/280 (21.1)	100/288 (34.7)	112/282 (39.1)	<0.0001
Septic shock (No. (%))	139 (43.6)	194 (61)	206 (64.6)	<0.0001
Randomized to albumin (No. (%))	161 (51)	159 (50)	153 (48)	0.80
Antibiotics at randomization (No. (%))	294 (92.2)	300 (94.3)	302 (94.7)	0.37
Antibiotics 6 hours after randomization (No. (%))	319 (100)	315 (99.1)	318 (99.7)	0.17
90-day mortality (no. events /No. patients (%))	87/312 (27.9)	117/316 (37.0)	165/315 (52.4)	<0.0001

COPD denotes patients with chronic obstructive pulmonary disease, SAPS II score the Simplified Acute Physiology Score II (27), SOFA score the Sequential Organ Failure Assessment score (25), and PaO_2/FiO_2 the ratio between arterial partial pressure of oxygen and inspired oxygen fraction. Urinary output indicates total urinary output from the time of enrollment to day 1.

Table 2. Bio-ADM levels and hemodynamic goals during the first 6 to 24 hours

Hemodynamic goal	***T1***	***T2***	***T3***	**P**
No. (%)	319 (33.4)	318 (33.3)	319 (33.4)	
Central venous pressure after 6 hours – No. (%)				<0.001
< 8 mmHg	112 (37.1)	80 (26.7)	60 (19.7)	
8-12 mmHg	113 (37.4)	108 (36)	107 (35.2)	
> 12 mmHg	77 (25.5)	112 (37.3)	137 (45.1)	
Central venous oxygen saturation after 6 hours ≥ 70% - No. (%)	203 (76.6)	216 (79.4)	192 (70.3)	0.04
Mean arterial pressure on day 1 ≥ 65 mmHg – No. (%)	302 (95.9)	288 (91.4)	260 (82.3)	<0.001
Serum lactate concentration on day 1 < 2 mmol/liter – No. (%)	244 (79.2)	193 (63.7)	121 (39)	<0.001
Doses of vasoactive drugs on day 1 - median (IQR) µg/kg/min				
Dopamine	5 [4-8]	7 [5-9]	6.75 [4.65-10]	0.04
Norepinephrine	0.14 [0.1-0.28]	0.2 [0.1-0.32]	0.2 [0.1-0.34]	0.04
Epinephrine	0.1 [0.05-0.14]	0.09 [0.05-0.2]	0.1 [0.06-0.2]	0.80
Two or more vasoactive drugs at day 1 – No. (%)	42 (13.2)	82 (25.8)	140 (43.9)	<0.001
Inotropic score at day 1	0 [0-12]	7.3 [0-20]	16 [4.5-33]	<0.001
Vasopressor dependency index at day 1 - $mmHg^{-1}$	0 [0-0.13]	0.1 [0-0.26]	0.22 [0.06-0.49]	<0.001
Administered fluids from day 1 to day 7 (mL)				
Daily average fluids	3551 [2920-4165]	3568 [2979-4271]	3716 [3165-4469]	0.02
Daily average albumin	29 [0-100]	43 [0-100]	57 [0-143]	0.004
Daily average crystalloids	1721 [1256-2309]	1761 [1228-2500]	1897 [1391-2587]	0.07
Daily average fluid balance	-463 [-992-289]	-370 [-1036-385]	20 [-690-719]	<0.001
Cumulative fluid balance	-1642 [-4721-1039]	-1465 [-5101-1693]	0 [-3373-3336]	<0.001

The inotropic score was calculated as follows (30): (dopamine dose x 1) + (adrenaline dose x100) + (noradrenaline dose x100), wherein all doses are expressed as µg/kg/min. The vasopressor dependency index represents the ratio between inotropic score and the associated mean arterial pressure. See Methods for further details.

Table 3. Relation of bio-ADM with 90-day mortality

	No. events/No. patients at risk	Chi^2/ ΔChi^2	P-value	HR (95% CI)
All patients				
Univariate	369/956	64.9	<0.0001	1.38 (1.29-1.47)
Adjusted, model 1	369/956	18.6	<0.0001	1.21 (1.12-1.31)
Adjusted, model 2	351/918	11.6	0.0007	1.17 (1.08-1.28)
Adjusted, model 3	290/788	5.9	0.015	1.14 (1.03-1.27)
Patients with severe sepsis				
Univariate	135/417	27.9	<0.0001	1.50 (1.31-1.71)
Adjusted, model 1	135/417	9.5	0.0021	1.29 (1.11-1.50)
Adjusted, model 2	130/400	8.7	0.003	1.31 (1.11-1.55)
Adjusted, model 3	106/342	6.0	0.01	1.28 (1.06-1.53)
Patients with septic shock				
Univariate	234/539	34.0	<0.0001	1.35 (1.24-1.47)
Adjusted, model 1	234/539	10.1	0.0015	1.19 (1.08-1.32)
Adjusted, model 2	221/518	5.1	0.02	1.14 (1.02-1.28)
Adjusted, model 3	184/446	2.0	0.16	1.10 (0.97-1.25)

Bio-ADM concentration on day 1 was entered as continuous variable. Model 1 includes bio-ADM and SAPS II score; model 2 includes bio-ADM and SOFA score; model 3 includes age, SOFA score, serum lactate concentration and central venous oxygen saturation at baseline (20). Hazard ratio interval for an increase of one interquartile range of bio-ADM concentration on day 1. The ΔChi^2 refers to an incremental value of bio-ADM compared to the model with clinical variables as described alone.

Figure legends

Central Illustration: Time-course of serum lactate concentration according to early bio-ADM levels and survival according to the evolution of bio-ADM concentrations over 7 days

Panel A: Evolution of the serum lactate concentrations over the first 7 days post-enrolment in patients alive at 7 days, according to tertiles of bio-ADM concentrations on day 1. * $p < 0.01$ across tertiles of bio-ADM by linear regression analysis; after adjustment for the SAPS II score, $p \leq 0.01$ from day 1 to day 7. **Panel B:** Survival according to the evolution of bio-ADM concentrations over 7 days. Patients were divided into four groups according to bio-ADM concentrations on day 1 and day 7, according to the median value of bio-ADM on day 1 (110 pg/mL). Kaplan-Meier survival curves at 90 days are shown for these four groups.

Figure 1. Time-course of inotropic score according to early bio-ADM levels

Evolution of the inotropic score over the first 7 days post-enrolment in patients alive at 7 days, according to tertiles of bio-ADM concentrations on day 1. The inotropic score was calculated each day as follows (30): (dopamine dose x 1) + (adrenaline dose x100) + (noradrenaline dose x100), wherein all doses are expressed as µg/kg/min. See Methods for further details. * $p < 0.01$ across tertiles of bio-ADM by linear regression analysis; after adjustment for the SAPS II score, $p<0.05$ at day 1 and day 2.

Figure 2. Kaplan-Meier survival curves by bio-ADM levels on day 1

Ninety-day mortality according to quartiles of bio-ADM concentrations on day 1: Q1 <59 pg/mL, total 61 events/239 patients at risk (25.5%); Q2 59-109 pg/mL, 79/239 (33.1%); Q3 110-198 pg/mL, 90/239 (37.7%); Q4 >198 pg/mL, 139/239 (58.2%). P for chi-square test <0.0001.

Figure 3. Time-dependent bio-ADM concentration predicts 90-day survival

Association between time-dependent bio-ADM concentration from day 1 to day 7 and 90-day survival in the entire study population, and by the presence or absence of shock at the time of enrollment. Time-dependent bio-ADM concentration from day 1 to day 7 was entered as a linear variable in univariate (square symbol) or

multivariable (circle) models. The model included time-dependent bio-ADM, age, SOFA score, serum lactate concentration and central venous oxygen saturation at baseline (20). Hazard ratio interval for an increase of one interquartile range of time-dependent bio-ADM concentration.

Central Illustration

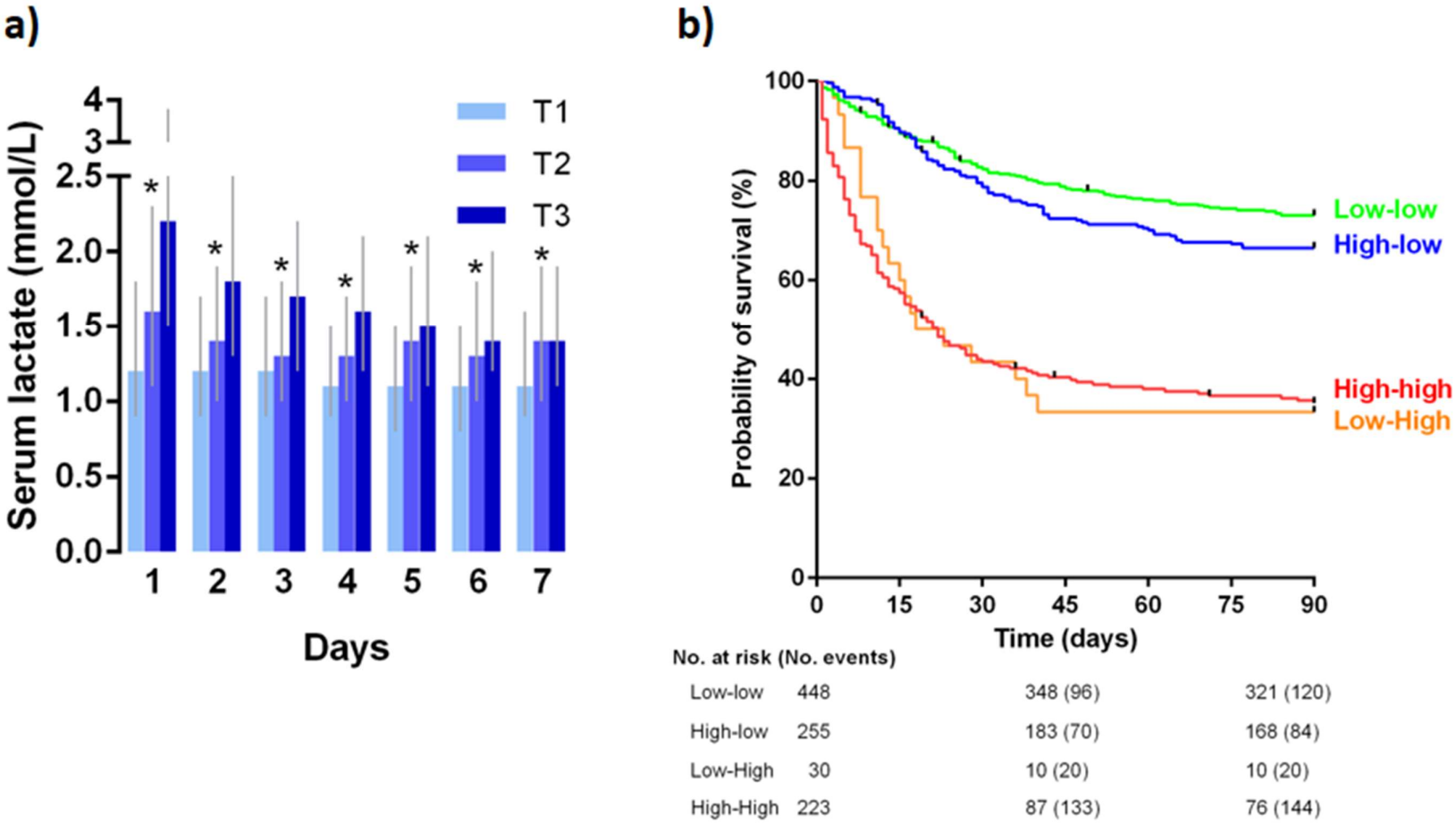


Figure 1

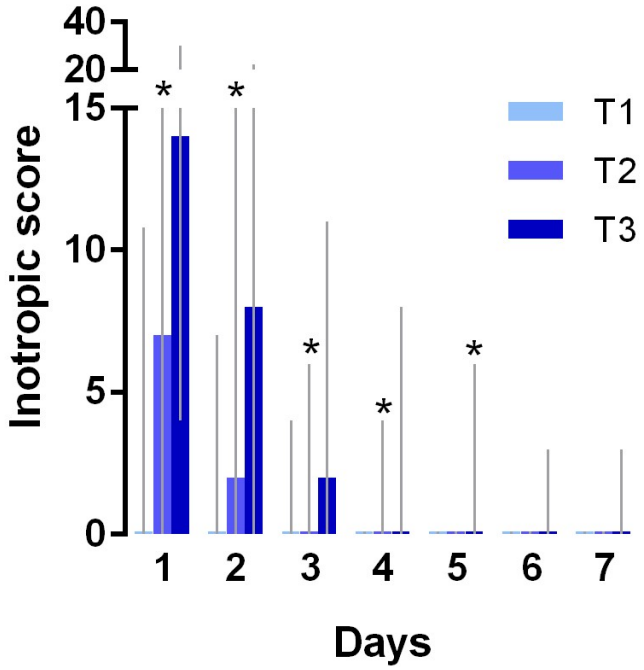
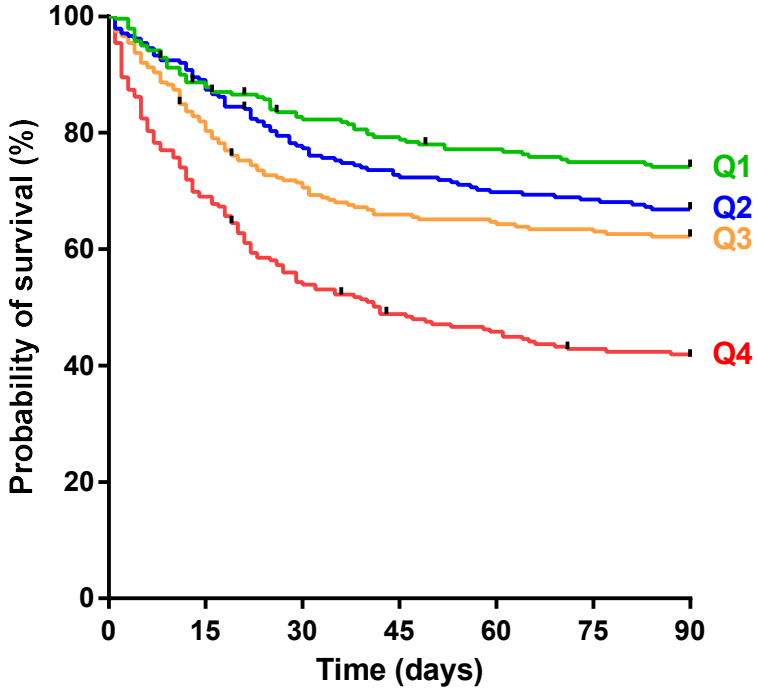


Figure 2



No. at risk (No. events)			
Q1	239	185 (50)	172 (61)
Q2	239	173 (66)	159 (79)
Q3	239	156 (81)	147 (90)
Q4	239	114 (122)	97 (139)

Figure 3