

Activated Protein C has No Effect on Pulmonary Capillary Endothelial Function in Septic Patients with Acute Respiratory Distress Syndrome: Association of Endothelial Dysfunction with Mortality

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ABSTRACT

Introduction: Pulmonary capillary endothelium-bound (PCEB) angiotensin-converting enzyme (ACE) activity is a direct and quantifiable index of pulmonary endothelial function that decreases early in acute respiratory distress syndrome (ARDS) and correlates with its

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severity. Endothelial dysfunction is a major pathophysiology that underlies sepsis-related ARDS. Recombinant human activated protein C (rhAPC), now withdrawn from the market, has been used in the recent past as an endothelial-protective treatment in patients with septic organ dysfunction.

Methods: We investigated the effect of rhAPC on pulmonary endothelial function in 19 septic patients suffering from ARDS. Applying indicator-dilution type techniques, we measured single-pass transpulmonary percent metabolism (%M) and hydrolysis (v) of the synthetic, biologically inactive, and highly specific for ACE substrate, ^3H -benzoyl-Phe-Ala-Pro (BPAP), under first-order reaction conditions, and calculated lung functional capillary surface area before and after treatment with rhAPC.

Results: Pulmonary endothelium ACE activity was severely impaired in septic patients with ARDS, and was not affected by rhAPC treatment. Additionally, poor outcome was related to a more profound decrease in PCEB-ACE activity. Angiotensin-converting enzyme-substrate utilization was statistically significantly lower in non-survivors as compared to survivors, with no changes over time within each group: BPAP %M: $32.7 \pm 3.4\%$ at baseline to $25.6 \pm 2.9\%$ at day 7 in survivors versus 20.8 ± 2.8 to $15.5 \pm 5\%$, respectively, in non-survivors ($p = 0.044$), while hydrolysis (v): 0.41 ± 0.06 at baseline to 0.30 ± 0.04 at day 7 in survivors compared to 0.24 ± 0.04 to

0.18 ± 0.06 , respectively, in non-survivors ($p = 0.049$).

Conclusion: rhAPC administration in septic patients with ARDS did not improve PCEB-ACE activity indices. However, these indices might be useful in the early recognition of septic patients with ARDS at high risk of mortality.

Keywords: ACE; ARDS; Prognosis; rhAPC; Sepsis

INTRODUCTION

The pulmonary microvascular endothelium possesses numerous metabolic properties, many of which are catalyzed by ectoenzymes expressed on the luminal pulmonary endothelial surface [1]. Angiotensin-converting enzyme (ACE) is the ectoenzyme that catalyzes the conversion of angiotensin I to angiotensin II, as well as the degradation of bradykinin, thus regulating the vascular tone. Given the strategic location of the lungs, and the extent of the pulmonary capillary endothelial surface, the pulmonary vasculature is the main site of ACE activity [1].

Pulmonary capillary endothelium-bound (PCEB) ACE activity has been widely studied in animals and humans by indicator-dilution techniques and has been shown to provide a quantifiable, direct and sensitive measurement of endothelial function under normal conditions and pulmonary diseases [1, 2].

In acute respiratory distress syndrome (ARDS), most commonly occurring during sepsis, pulmonary capillary endothelial cells appear to be among the first lung cells to be insulted, leading to impaired metabolic functionality [3, 4]. In this respect, PCEB-ACE activity has been shown to decrease early during ARDS and to correlate with its severity [5].

Activated protein C (APC) system dysregulation plays an important role in the pathogenesis of sepsis-associated organ dysfunction [6]. In 2004, the use of recombinant human APC (rhAPC; *drotrecogin alfa* (activated); Xigris; Eli Lilly, Indianapolis, IN, USA) in patients with sepsis-induced organ failure was recommended based on the results of the PROWESS study

[7, 8]. We hypothesized that rhAPC administration in septic patients with ARDS would improve pulmonary endothelial function, as assessed by PCEB-ACE activity indices.

It should be noted that the drug was withdrawn from the market after the negative results of the PROWESS-SHOCK study [9]. However, our study on the effect of rhAPC on pulmonary endothelial function of septic patients with ARDS was performed during the period that the drug was in use in patients with sepsis-induced organ dysfunction.

METHODS

Compliance with Ethics Guidelines

The study was approved by the Evangelismos Hospital Research Ethics Committee (94/9-9-2003). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, also as revised in 2013. Informed written consent was obtained from all patients' next-of-kin prior to any study procedure. Our study was conducted and completed in 2004, before the International Committee of Medical Journal Editors initiated the policy requiring investigators to deposit information about trial designs. Consequently, our study was not registered.

Study Population and Measurements

In 2004, 19 critically-ill septic patients with ARDS treated with rhAPC were enrolled in the study. All subjects were hospitalized in a general intensive care unit (ICU), were mechanically ventilated, and had at least two organ dysfunctions consequent to infection, one of which was sepsis-associated respiratory dysfunction. All patients suffered from severe sepsis or septic shock and, furthermore, all patients had ARDS except three who had acute lung injury, according to the respective definitions at the time of the study [10, 11]; based on the

current definitions, they are all considered to have suffered from sepsis or septic shock and ARDS [12, 13]. The decision to treat with rhAPC was taken by the patients' attending physicians, in compliance with the administration criteria valid at that time [8]. They all received rhAPC at 24 µg/kg/h for 96 h in a continuous IV drip. All the precautions were taken regarding the risk of bleeding before and after major or minor surgical procedures. Major bleeding events were not noticed. After the completion of the study, patients were followed up and complete clinical and laboratory data were recorded for 28 days or until their discharge from the ICU. At the end of the study, the subjects were grouped according to their outcome (28 days survival or prior discharge from the ICU) into survivors and non-survivors.

PCEB-ACE activity was measured prior to initiation of rhAPC treatment (measurement #1 at baseline), during the 96-h infusion (measurement #2 at 24 h; measurement #3 at 48 h; measurement #4 at 96 h) and 3 days after the end of the rhAPC infusion (measurement #5, day 7). No patient was under ACE inhibitors. Each patient's baseline measurement served as control of him/herself.

A detailed description of the methodology used is given elsewhere [2]. Trace amounts of the radiolabelled synthetic ACE substrate ^3H benzoyl-Phe-Ala-Pro (BPAP) were injected as a rapid bolus into a (jugular or subclavian) central vein, and arterial blood was withdrawn into a fraction collector for analysis.

Single transpulmonary pass substrate utilization is expressed as percent metabolism (%M) and transpulmonary hydrolysis ($v = [E] \times t_c \times k_{\text{cat}}/K_m$), where $[E]$ equals the enzyme concentration available for reaction, t_c the capillary transit time, k_{cat} the catalytic rate constant, and K_m the Michaelis–Menten constant. Functional capillary surface area (FCSA) was additionally calculated as hydrolysis \times pulmonary plasma flow and depends on the total enzyme mass that is available for reaction, and the constants k_{cat} and K_m . Both v and %M are indices that reflect ACE activity per capillary, while FCSA is a reflection of ACE activity per vascular bed [1, 2, 14].

Statistical Analysis

Data are presented as mean \pm SE, or median (interquartile range) when data distribution was skewed. Comparisons between groups were made by Student's t test or Mann–Whitney test, when appropriate, for continuous data, and Chi square test for qualitative data. Differences within time were examined by the Friedman test. Mixed effect models were fitted to examine the effect of time, outcome and their interaction. The Tukey–Kramer test was used for multiple comparisons adjustment. All p values are two-sided. Differences were considered significant at $p < 0.05$.

RESULTS

Characteristics of the Study Population

Patient baseline demographic and clinical data are shown in Table 1. Patients were grouped according to their clinical outcome (28 days survival) as survivors ($n = 9$) and non-survivors ($n = 10$). There were no statistically significant differences between the two groups in baseline characteristics.

The Effect of Recombinant Human APC on Pulmonary Endothelial ACE Activity and FCSA

In all patients, BPAP %M did not significantly change during the study period: from $26.4 \pm 2.6\%$ (baseline) to $24.4 \pm 2.9\%$ at the end of the 96-h rhAPC infusion, and to 22.5 ± 2.8 at the end of the 7 days study (Fig. 1a; $p = 0.541$). In a similar pattern a non-significant fluctuation of hydrolysis (v) was observed in all patients from 0.32 ± 0.04 at baseline to 0.29 ± 0.04 at day 4 and to 0.27 ± 0.04 at day 7 (Fig. 1b; $p = 0.641$). FCSA at baseline was 1550 ± 212 ml/min, while similar values were obtained at day 4 (1549 ± 276 ml/min) and at day 7 (1561 ± 292 ml/min) (Fig. 1c; $p = 0.652$).

Table 1 Demographic and clinical data of the 19 patients enrolled in the study

	All patients (19)	Survivors (9)	Non-survivors (10)	<i>P</i> value
Age (years), mean \pm SE	64 \pm 3.3	61.8 \pm 4.9	66.3 \pm 4.5	0.503
Male sex, <i>n</i> (%)	15 (79)	7 (78)	8 (80)	0.906
Days in ICU, median (IQR)	4 (2–8)	3 (1.5–6)	6 (2.75–17)	0.09
Days on MV, median (IQR)	4 (2–9)	3 (1.5–6)	6 (2.75–11.75)	0.119
Cause of ARDS, <i>n</i> (%)				0.809
Direct	9 (47)	4 (44)	5 (50)	
Indirect	10 (53)	5 (66)	5 (50)	

Data are expressed as number of patients (*n*), mean \pm SE, or median (25–75% interquartile range)

ARDS acute respiratory distress syndrome, ICU intensive care unit, IQR interquartile range, MV mechanical ventilation

PCEB-ACE Activity and Outcome (28 Days Survival)

Neither %M nor hydrolysis changed significantly with time in either group. In survivors, BPAP %M tended to decrease from $32.7 \pm 3.4\%$ at baseline to $25.6 \pm 2.9\%$ at day 7, and in non-survivors from $20.8 \pm 2.8\%$ at baseline to $15.5 \pm 5\%$ at day 7. Likewise, ν tended to decrease from 0.41 ± 0.06 at baseline to 0.30 ± 0.04 at day 7 in survivors, and 0.24 ± 0.04 to 0.18 ± 0.06 in non-survivors (Fig. 2a, b; $p = 0.489$ and $p = 0.499$, respectively). Nonetheless, ACE substrate utilization was significantly lower in non-survivors as compared to survivors (Fig. 2a, b; $p = 0.044$ and $p = 0.049$).

Functional Capillary Surface Area and Outcome (28-day Survival)

FCSA increased slightly, from 1760 ± 327 ml/min at baseline to 1890 ± 338 ml/min at day 7 in survivors, while in the non-survivors FCSA dropped from 1362 ± 276 to 821 ± 402 ml/min. It tended to be higher in survivors, without, however, reaching significance (Fig. 2c, $p = 0.075$). It should be noted that the obtained values might be influenced by differences in cardiac output per patient, observed during the study (data not shown), since they affect FCSA measurements (see “Methods”).

Disease Severity and Lung Injury Scores

Patients' PO_2/FiO_2 did not significantly change with time (Fig. 3a; $p = 0.458$). However, when patients were divided into survivors and non-survivors, the former revealed significantly higher values than the latter (Fig. 3b; $p = 0.032$). Similar patterns were observed for lung injury scores (LIS) (Fig. 3c; $p = 0.726$). Again, survivors had significantly lower LIS than non-survivors (Fig. 3d; $p = 0.025$). SOFA scores decreased with time in all patients (Fig. 4a; $p = 0.007$). The same pattern (i.e. decrease with time) was observed in both survivors and non-survivors, and were lower in survivors than in non-survivors (Fig. 4b; $p = 0.001$ and $p = 0.04$, respectively). Patients' APACHE II scores also decreased with time in all patients (Fig. 4c; $p = 0.033$). The same pattern (i.e. decrease with time) was observed in both survivors and non-survivors (Fig. 4d; $p = 0.045$). However no differences were noted between the two groups (Fig. 4d; $p = 0.189$).

It is worth mentioning that PCEB-ACE activity indices were the only significantly different parameters between survivors and non-survivors at baseline; no other disease or lung injury severity score was significantly different at baseline (data not shown). More specifically, in survivors, BPAP %M at baseline was $32.7 \pm 3.4\%$ compared to $20.8 \pm 2.8\%$ in non-survivors (Mann–Whitney, $p = 0.017$). Baseline

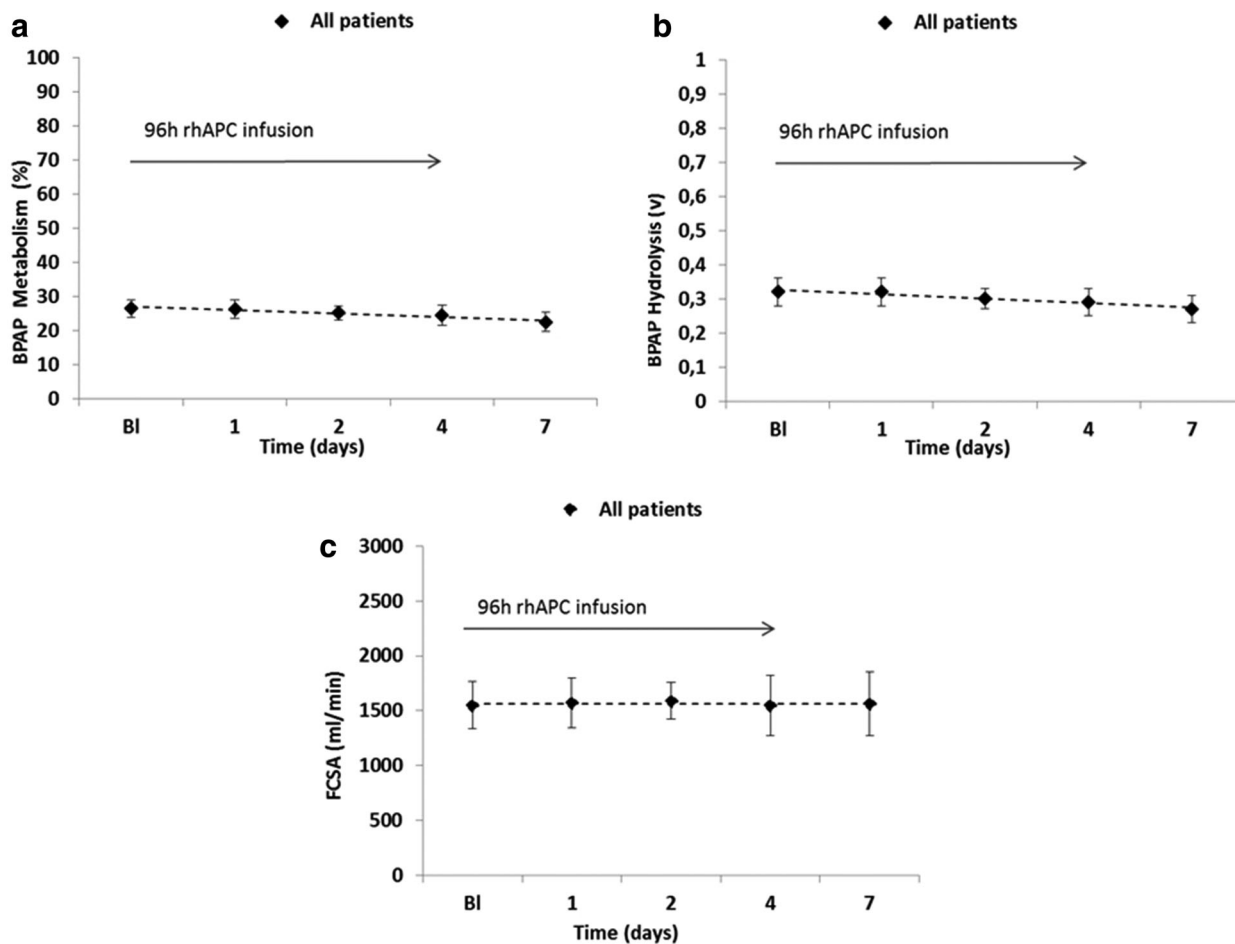


Fig. 1 The effect of recombinant human APC on pulmonary endothelial ACE activity and FCSA. PCEB-ACE activity was measured prior to initiation of treatment with rhAPC (measurement #1 at baseline, *Bl*), during the 96 h infusion (measurement #2 at 24 h, measurement #3 at 48 h, measurement #4 at 96 h) and 3 days after the end of the rhAPC infusion (measurement #5 at day 7). Substrate (BPAP) percent metabolism (%*M*) (a), BPAP hydrolysis

(*v*) (b) and functional capillary surface area (FCSA) (c) were estimated in all patients over time. Data are presented as mean ± SE. The Friedman test was used, $p > 0.05$. *BPAP* $^3\text{[H]}$ benzoyl-Phe-Ala-Pro, *FCSA* functional capillary surface area, *PCEB-ACE* pulmonary capillary endothelium-bound angiotensin converting enzyme, *rhAPC* recombinant human activated protein C

hydrolysis (*v*) was 0.41 ± 0.06 in survivors and 0.24 ± 0.04 in non-survivors (Mann–Whitney, $p = 0.017$).

DISCUSSION

In sepsis, the dysregulated interaction between inflammation and coagulation underlies organ dysfunction [6]. On this basis, it was suggested that natural anticoagulants, that are down-

regulated in sepsis, could be used as a salvage therapy in septic organ failure. Recombinant human APC was the only anticoagulant initially shown to be effective in decreasing mortality in severely septic patients [7]. Furthermore, several experimental studies have documented the beneficial effects of the treatment with rhAPC (intravenous or inhaled) on inflammation-induced lung injury [15–19].

Pulmonary capillary ACE activity has been proved to be a reliable index of endothelial

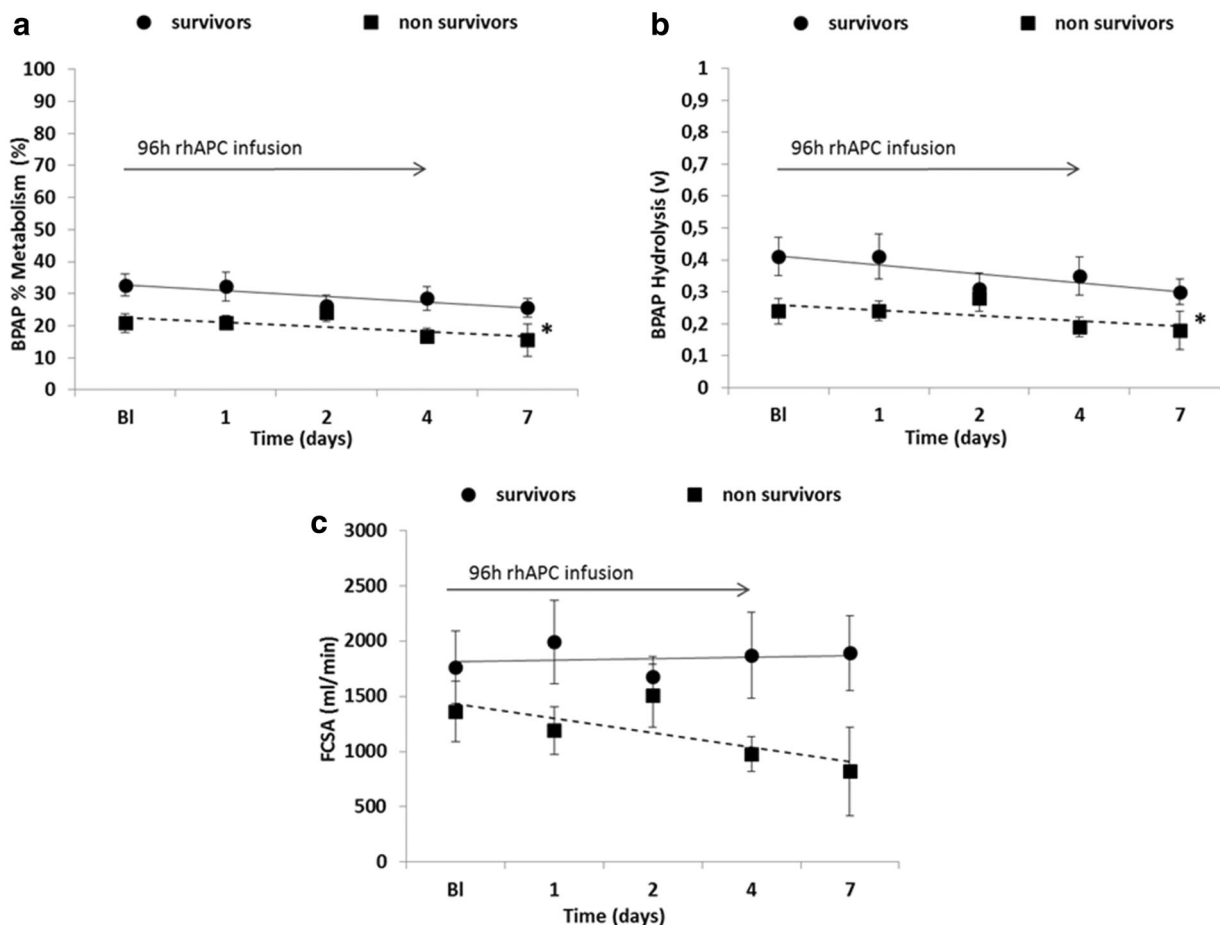


Fig. 2 PCEB-ACE activity and outcome (28-day survival). PCEB-ACE activity was measured prior to initiation of treatment with rhAPC (measurement #1 at baseline, *Bl*), during the 96-h infusion (measurement #2 at 24 h, measurement #3 at 48 h, measurement #4 at 96 h) and 3 days after the end of the rhAPC infusion (measurement #5 at day 7). Patients were grouped according to their clinical outcome (28-day survival) as survivors ($n = 9$) and non-survivors ($n = 10$). Substrate (BPAP) percent

metabolism (%*M*) (**a**), BPAP hydrolysis (*v*) (**b**) and functional capillary surface area (FCSA) (**c**) were estimated in all patients with time. Data are presented as mean \pm SE. The mixed effect model was used, $*p < 0.05$. BPAP 3 [H] benzoyl-Phe-Ala-Pro, FCSA functional capillary surface area, PCEB-ACE pulmonary capillary endothelium-bound angiotensin converting enzyme, rhAPC recombinant human activated protein C

dysfunction in variable pulmonary diseases [1, 5, 20]. Contrary to other surrogate markers of endothelial injury, such as circulating adhesion molecules and other soluble endothelial-derived proteins, its measurement by means of indicator-dilution type techniques provides a direct and sensitive quantification of pulmonary endothelial function at the patients' bedside. Both substrate transpulmonary hydrolysis (*v*) and FCSA have been shown to

decrease early during the ARDS continuum and to be inversely related to LIS [5].

In this study we evaluated baseline PCEB-ACE activity in a homogenous, septic population with ARDS, in the sense that they all fulfilled the criteria for rhAPC administration. We rationalized that a beneficial effect of rhAPC on pulmonary microcirculation would be depicted as an amelioration of the PCEB-ACE activity indices. Our hypothesis was not confirmed:

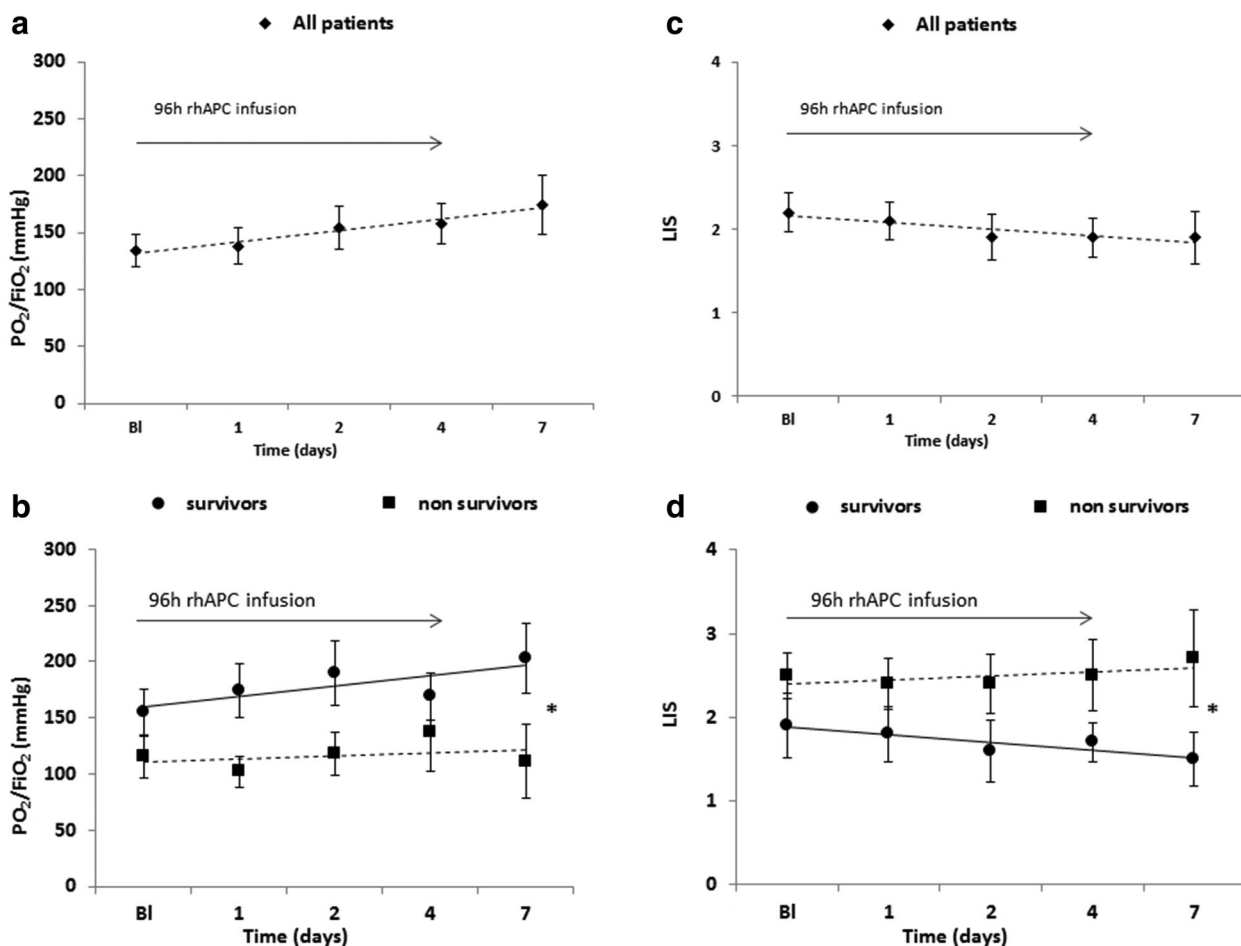


Fig. 3 Lung disease severity scores (PO₂/FiO₂ and LIS). PO₂/FiO₂ was measured prior to initiation of treatment with rhAPC (measurement #1 at baseline, *Bl*), during the 96-h infusion (measurement #2 at 24 h, measurement #3 at 48 h, measurement #4 at 96 h) and 3 days after the end of the rhAPC infusion (measurement #5 at day 7). Results are given for the whole cohort (a) and for survivors versus non-survivors (b). Lung injury scores (*LIS*) were measured prior to initiation of treatment with rhAPC (measurement

#1 at baseline, *Bl*), during the 96-h infusion (measurement #2 at 24 h, measurement #3 at 48 h, measurement #4 at 96 h) and 3 days after the end of the rhAPC infusion (measurement #5 at day 7). Results are given for the whole cohort (c) and for survivors versus non-survivors (d). Data are presented as mean ± SE. a, c The Friedman test was used, *p* > 0.05. b, d The mixed effect model was used, **p* < 0.05. *rhAPC* recombinant human activated protein C

rhAPC treatment had no significant effect on pulmonary endothelial function as estimated by this method. Our results coincided with the uncertainty that emerged about the efficacy and the concerns regarding the use of rhAPC in the treatment of sepsis. The PROWESS-SHOCK study that was conducted for the re-examination of the risk/benefit profile of rhAPC failed to document a mortality benefit [9], leading to the withdrawal of the product from the market. Despite rhAPC withdrawal, related research has

been recently published, driving us to present these previously obtained data. In this context, a study using the pulmonary leak index of ⁶⁷Gallium transferrin technique to measure alveolo-capillary permeability in patients with ARDS treated with rhAPC also failed to reveal a beneficial effect of the treatment [21].

In our study population, the estimated BPAP percent metabolism and hydrolysis were considerably decreased compared to the values of normal volunteers with no lung pathologies

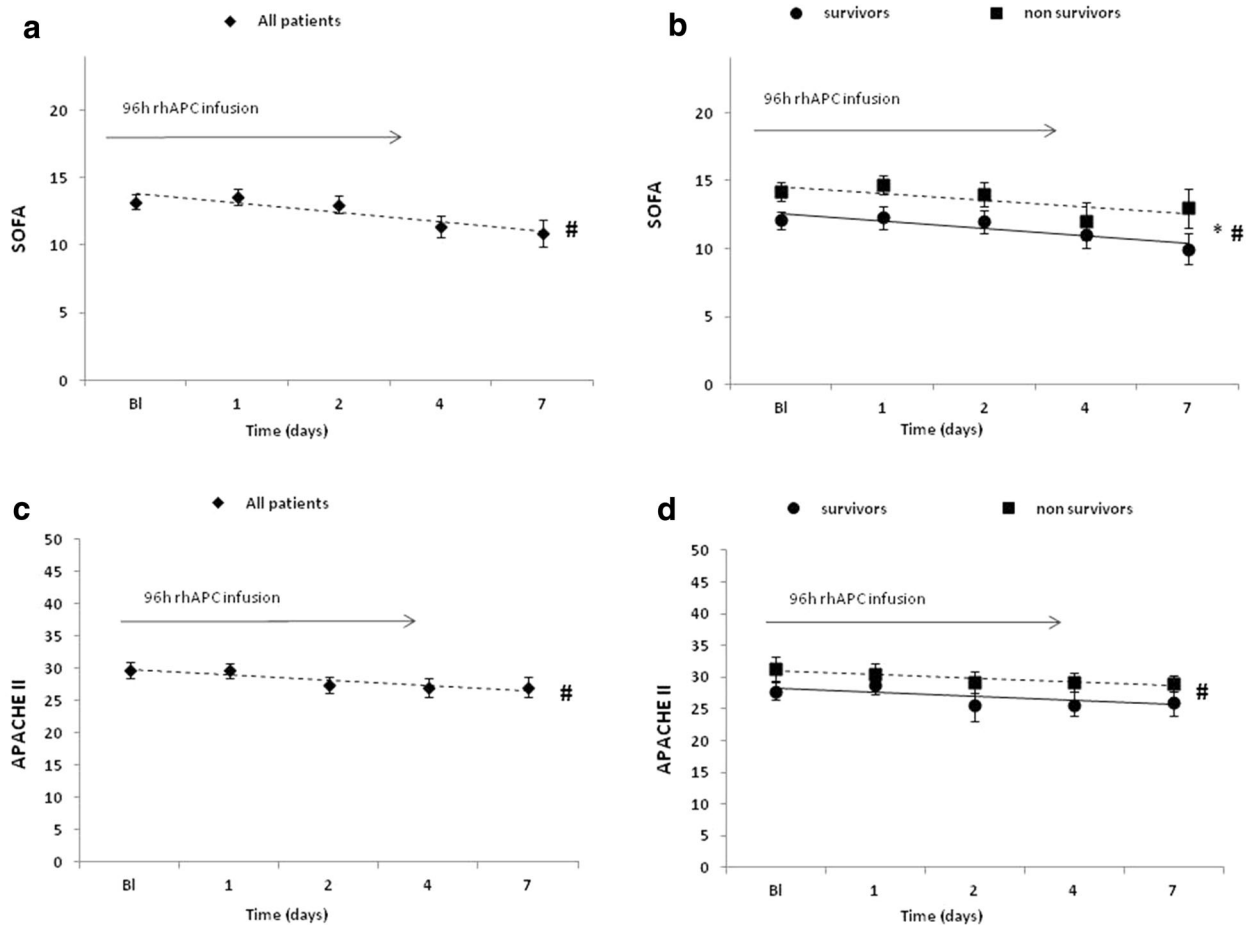


Fig. 4 Disease severity scores. SOFA scores were measured in all patients prior to initiation of treatment with rhAPC (measurement #1 at baseline, *Bl*), during the 96-h infusion (measurement #2 at 24 h, measurement #3 at 48 h, measurement #4 at 96 h) and 3 days after the end of the rhAPC infusion (measurement #5 at day 7). Results are given for the whole cohort (**a**) and for survivors versus non-survivors (**b**). APACHE II scores were measured in all patients prior to initiation of treatment with rhAPC (measurement #1 at baseline, *Bl*), during the 96-h infusion (measurement #2 at 24 h, measurement #3 at 48 h,

measurement #4 at 96 h) and 3 days after the end of the rhAPC infusion (measurement #5 at day 7). Results are given for the whole cohort (**c**) and for survivors versus non-survivors (**d**). Data are presented as mean \pm SE. **a**, **c** The Friedman test was used, $^{\#}p < 0.05$ with time. **b**, **d** The mixed effect model was used, $^{\#}p < 0.05$ with time and $^*p < 0.05$ between groups. *APACHE* Acute physiology and chronic health evaluation, *rhAPC* recombinant human activated protein C, *SOFA* sequential organ failure assessment

[1, 2, 22]. Further analysis of our results attempted to examine a possible relation of PCEB-ACE activity with patient outcome. Indeed, poor outcome (defined as mortality) was associated with more prominent pulmonary capillary endothelial dysfunction, as expressed by the significantly lower values of PCEB-ACE activity indices (i.e. %*M* and ν). These results were in accordance with the

estimations of disease and respiratory severity indices and scores: when mixed effect models were fitted, non-survivors were found to express worse PO_2/FiO_2 , as well as LIS and SOFA scores. However, it is worth mentioning that the values of %*M* and hydrolysis (ν) were statistically lower in the non-survivors group from the very first measurement (baseline measurement) compared to survivors, in contrast to all other

measured disease severity indices, which showed no significant difference between the two groups at baseline (data not shown). This may denote a superiority of the PCEB-ACE activity indices in providing early information on outcome.

The complex pathophysiology of sepsis and the heterogeneity of septic patients are perhaps reasons why clinicians are still lacking specific treatment options for this syndrome. Our study population consisted of septic patients with ARDS and similar clinical severity, with the limitation of the small size. Nevertheless, our results support a potential implementation of direct markers of endothelial dysfunction in everyday clinical practice. Such markers accompanied by clinical severity scores and other surrogate indices might serve the need for better risk stratification and personalized treatment in sepsis.

CONCLUSION

In our cohort of septic patients with ARDS, pulmonary endothelium ACE activity was severely impaired, denoting pulmonary endothelial dysfunction. Our results showed that rhAPC treatment had no effect on pulmonary endothelium function. However, we were able to demonstrate that PCEB-ACE activity indices quantitatively expressed the endothelial pathophysiology that underlies sepsis-associated ARDS. Furthermore, they seem to represent early and possibly more sensitive markers of outcome compared to other well-established clinical severity indices and scores. It should be noted that the indicator-dilution techniques are arduous procedures, not easily performed in the everyday clinical practice. Future studies using “less demanding” techniques at the bedside are needed in an effort to develop clinically useful tests [23].

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Disclosures. At the time of the study, Katerina Kaziani’s affiliation was First Department of Critical Care Medicine and Pulmonary Services, GP Livanos and M Simou Laboratories, Medical School of the National and Kapodistrian University of Athens, Evangelismos Hospital, Athens, Greece. Her current affiliation is 5th Department of Internal Medicine and Infectious Diseases Unit, General Hospital “Evangelismos”, Athens, Greece. Stylianos E. Orfanos has received an unrestricted grant and speaker honoraria from Pharmaserve-Lilly. Katerina Kaziani, Alice G. Vassiliou, Anastasia Kotanidou, Chariclea Athanasiou, Ioanna Korovesi, and Konstantinos Glynos have nothing to disclose.

Compliance with Ethics Guidelines. The study was approved by the Evangelismos Hospital Research Ethics Committee (94/9-9-2003). All procedures followed were in

accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013. Informed written consent was obtained from all patients' next-of-kin prior to any study procedure.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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