

Decreasing mortality with drotrecogin alfa in high risk septic patients A meta-analysis of randomized trials in adult patients with multiple organ failure and mortality >40%

MASSIMILIANO GRECO • GIOVANNI LANDONI • LEDA NOBILE •
GIACOMO MONTI • LAURA PASIN • CAETANO NIGRO •
LUCA CABRINI • ALBERTO ZANGRILLO

GIOVANNI LANDONI (✉) •
MASSIMILIANO GRECO •
LEDA NOBILE •
GIACOMO MONTI •
LAURA PASIN •
LUCA CABRINI •
ALBERTO ZANGRILLO
Department of Anesthesia
and Intensive Care
IRCCS San Raffaele Scientific Institute
Via Olgettina 60 Milan 20132 Italy
Phone: +390226436154
Fax: +390226436152
E-mail: landoni.giovanni@hsr.it

CAETANO NIGRO
Dante Pazzanese Institute of Cardiology
São Paulo, Brasil

ABSTRACT

Objective. Sepsis is a complex inflammatory disease, rising in response to infection. Drotrecogin alfa, approved in 2001 for severe sepsis, has been withdrawn from the market. The aim of this study was to assess if drotrecogin alfa-activated can reduce mortality in the more severe septic patients.

Methods. We searched PubMed, Embase, Scopus, BioMedCentral, and in Clinicaltrials.gov databases to identify every randomized study performed on drotrecogin alfa-activated in any clinical setting in humans, without restrictions on dose or time of administration. Our primary end-point was mortality rate in high risk patients. Secondary endpoints were mortality in all patients, in patients with an Acute Physiology and Chronic Health Evaluation (APACHE) 2 score ≥ 25 and in those with an APACHE 2 score ≤ 25 .

Results. Five trials were identified and included in the analysis. They randomized 3196 patients to drotrecogin alfa and 3111 to the control group. Drotrecogin alfa was associated with a reduction in mortality (99/263 [37.6%] vs 115/244 [47.1%], risk ratios (RR) = 0.80[0.65; 0.98], $p = 0.03$) in patients with multiple organ failure and a mortality risk in the control group of >40%, but not in the overall population or in lower risk populations.

Conclusions. In high risk populations of patients with multiple organ failure and a mortality of >40% in the control group, Drotrecogin alfa may still have a role as a lifesaving treatment. No beneficial effect in low risk patients was found. An individual patient meta-analysis including all randomized controlled trial on sepsis is warranted, along with new studies on similar drugs such as protein C zymogen.

Key words: sepsis, shock, intensive care, critically ill, mortality, drotrecogin alfa, recombinant human activated protein C

Introduction

Sepsis is a complex systemic inflammatory syndrome rising in response to severe infection, and is a leading cause of mortality and morbidity worldwide. Eighteen million sepsis cases

have been estimated to occur each year, with a mortality rate of nearly 30%. (1) Sepsis is a multifaceted syndrome involving endothelial dysfunction, inflammatory response, immunity, deregulation of intercellular signaling, and cytokine storms. (2,3) Sepsis is classified as severe when organ dysfunction ensues.

Recombinant human activated protein C (drotrecogin alfa-activated) was

approved in 2001 by FDA for severe septic patients at high risk of death, with an Acute Physiology and Chronic Health Evaluation (APACHE) II score higher than 25, while the European marketing authorization was guaranteed for patients with severe sepsis and two or more organ failures. Recently, the drug was voluntarily withdrawn from the market by the pharmaceutical company, in response to the findings of a large

randomized trial that questioned its risk/benefit ratio in patients with septic shock. (4)

Protein C is the plasmatic zymogen, and it is activated in the presence of thrombin - thrombomodulin complex, thus limiting thrombin production (through inactivation of factors Va and VIIIa) while also promoting fibrinolysis. Moreover, it may inhibit cytokine production, neutrophil activation and leukocyte adhesion. (5–8) Protein C shares the same benefits of its activated form (drotrecogin alfa) in septic shock, where it exerts a complex role between immunity, coagulation and inflammation.

Protein C seems safer than drotrecogin alfa, with no reported increase in bleeding episodes. However high quality randomized evidence on Protein C is lacking. The finding of a significant positive effect of drotrecogin alfa on patients' mortality could be relevant even if the drug is not commercialized anymore, as a similar but safer and much less studied drug exists: Protein C.

We therefore performed a meta-analysis of randomized control trials (RCTs) to assess if drotrecogin alfa-activated can reduce mortality in the more severe septic patients, defined as those with multiple organ failure and a high mortality rate (> 40%).

Materials and methods

Search Strategy

We searched PubMed, Embase, Scopus, BioMedCentral, and the Clinicaltrials.gov database (updated September 1st 2012) for relevant studies. Search was independently conducted by four trained investigators. The search strategy (9) included any randomized study ever performed on drotrecogin alfa-activated (Xigris[®]; Eli Lilly and Company, Indianapolis, IN, USA) in any clinical setting in humans, and is available in the Appendix. Moreover, pertinent references of retrieved articles and reviews were retrieved to identify more articles. No language restriction was enforced.

Study Selection

Results from database and literature queries were first independently exami-

ned at a title/abstract level by the four investigators, with divergences resolved by consensus. Pertinent articles were retrieved as full text and analyzed adopting the following inclusion criteria: random allocation to treatment and comparison of drotrecogin alfa-activated versus control. There were no restrictions on dose or time of administration.

The exclusion criteria were: duplicate data, and non-adult studies. Two investigators independently assessed compliance with selection criteria and selected studies for the final analysis, with divergences finally resolved by consensus.

Data Abstraction and Study Characteristics

Two authors extracted study end-points and main outcomes, study design, population, clinical setting, and treatment duration.

The primary end-point of the present investigation was the mortality rate in high risk patients, identified as having multiple organ dysfunction, and a high mortality rate in controls. A 40% mortality rate was arbitrarily chosen as the cut-off to define high mortality rate.

Secondary endpoints were mortality in all patients, mortality in patients with an APACHE 2 score ≥ 25 and in those with an APACHE 2 score ≤ 25 .

Internal Validity and Risk of Bias Assessment

The internal validity and risk of bias of included trials was appraised by two independent reviewers according to Cochrane Collaboration methods, (10) with divergences resolved by consensus. Publication bias was assessed by visual inspection of funnel plots.

Data Analysis and Synthesis

Computations were performed with RevMan 5. (11) Statistical heterogeneity and inconsistency were measured using I^2 . Binary outcomes from individual studies were analyzed in order to compute individual and pooled risk ratios (RR) with pertinent 95% confidence intervals (CI, with equivalence set at 1, odds ratio (OR) <1 favoring the first treatment, and OR>1 favoring the second treatment), by means of

Mantel-Haenszel method and with random effect model (to better account for clinical and statistical variations). No continuous variables were included in analyses.

Statistical significance was set at the two-tailed 0.05 level for hypothesis testing and at 0.10 for heterogeneity testing. Unadjusted P values are reported throughout. This study was performed in compliance with The Cochrane Collaboration and PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. (12)

Results

Database searches and scanning of article bibliographies yielded a total of 214 results. Excluding 188 non-pertinent titles or abstracts, we retrieved in complete form, and assessed according to the selection criteria, 16 studies (figure 1). Six studies were further excluded because they were not randomized. (1,13–17) Two studies were excluded because they involved a pediatric setting. (18,19) Two were further excluded because they were conducted in other settings, like pancreatitis and pulmonary embolism. (20,21) One was excluded due to the administration of a study drug in controls. (22) Ultimately, therefore, we identified 5 eligible randomized clinical trials for inclusion in the analysis. (4,8,23–25)

Study Characteristics

The 5 included trials randomized 6307 patients (3196 to drotrecogin alfa and 3111 to the control group) (table 1). All these studies reported data on mortality and were multicentre. Study quality appraisal indicated that studies were of variable quality (table 2) and that 3 of them had low risk of bias.

Quantitative Data Synthesis

Drotrecogin alfa was associated with a reduction in mortality (99/263 [37.6%] vs 115/244 [47.1%], RR=0.80[0.65; 0.98], p for effect=0.03, p for heterogeneity=0.77, $I^2=0\%$ with 507 patients and two studies included) (4,23) (figure 2) in high risk patients, but not in the overall population or in other populations at lower risk (table 3). A trend was noted towards an excess in mortality in

Table 1. Description of included studies.

First author	Journal	Year	Multicentric	Follow up	Case	Controls	Dose	Comparator
Bernard (8)	Crit Care Med	2001	yes	28 days	90	41	From 1 to 30 ug/kg/min	Placebo
Bernard (23)	New Engl J Med	2001	yes	28 days	850	840	24 ug/kg/min	Placebo
Abraham (24)	New Engl J Med	2005	yes	1 year	1316	1297	24 ug/kg/min	Placebo
Dhainaut (25)	Intensive Care Med	2009	yes	90 days	94	99	24 ug/kg/min	Placebo
Ranieri (4)	New Engl J Med	2012	yes	90 days	846	834	24 ug/kg/min	Placebo

Table 2. Risk of bias assessment of included studies.

	Adequate Sequence Generation?	Allocation Concealment?	Blinding?	Concurrent Therapies Similar?	Incomplete Outcome Data Addressed?	Uniform and Explicit Outcome Definitions?	Free From Selective Outcome Reporting?	Free From Other Bias?	Overall Risk of Bias?
Bernard (8)	Unclear	Unclear	Yes	Yes	Unclear	Yes	Yes	Unclear	Intermediate
Bernard (23)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low
Abraham (24)	Yes	yes	Yes	Yes	Yes	Yes	Yes	Yes	Low
Dhainaut (25)	Unclear	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Intermediate
Ranieri (4)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low

Table 3. Results of pooled estimates in different population of patients.

Populations	Mortality Cases	Mortality controls	RR	95% CI	p for effect	p for heterogeneity	I ²
High risk	354/995 (35.6%)	390/975 (40%)	0.93	0.69-1.24	0.6	0.004	82%
Low risk	472/2010 (23.5%)	358/1980 (18%)	1.29	0.82-2.04	0.28	0.001	92%
Overall	739/3196 (23.1%)	726/3111 (23.4%)	1.00	0.84-1.19	1	0.02	65%

the treatment group when low risk patients were considered (table 3). Visual inspection of funnel plots did not identify a skewed or asymmetrical shape for mortality.

Discussion

The most important result of this study is that drotrecogin alfa reduces mortality in high risk septic patients (identified in this study as those with multiple organ failure and mortality in the control group higher than 40%). While another systematic review (26) on the effects of drotrecogin alfa included randomized and non randomized studies, this meta-analysis was based solely on high quality randomized controlled trials. The preset study also confirms that drotrecogin alfa does not reduce mortality in the overall population of septic patients,

as previously described. (23,27) Moreover, in low risk patients with an APACHE II score ≤ 25 , no significantly higher risk of death was found. This finding is reassuring when confronted with the increased risk for severe complications (bleeding) described in the ADDRESS study.

The Prospective Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) trial (23) and its following subgroup analyses (28,29) found a consistent reduction in mortality in patients at higher risk of death, as defined by multi-organ failure or APACHE II scores higher than 25. Early enthusiasm left room for a more cautious interpretation after publication of the Administration of drotrecogin Alfa (Activated) in Early Stage Severe Sepsis (ADDRESS) trial, (24,30) conducted

on patients with severe sepsis and low risk of death. This trial was terminated earlier for safety concerns by the monitoring committee. No difference was shown on 28th day or in-hospital mortality between drotrecogin alfa and placebo, while an increase in serious bleeding in the drotrecogin alfa group was detected.

The subsequent PROWESS-SHOCK trial was recently published by Ranieri et al. (4) This multicenter randomized controlled trial was conducted to test the hypothesis that drotrecogin alfa could reduce mortality in patients with septic shock. This population of patients was identified from the previous published trials as potentially benefiting from protein C activated. However, no significant reduction in mortality was found at 28 days (26.4% in drotreco-

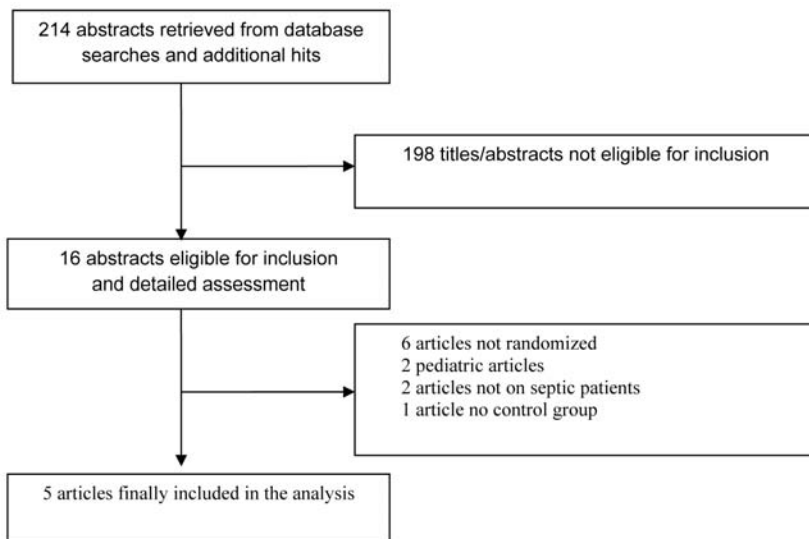
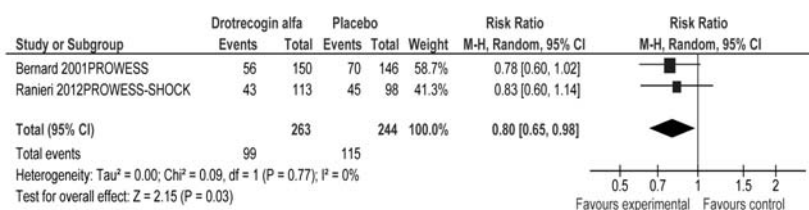


Figure 1. Flow diagram for selection of articles.



CI, confidence interval; RR, risk ratio.

Figure 2. Forest plot for the risk of mortality in very high risk patients.

gin alfa group vs 24.2% in controls, $p=0.31$) or 90 days (34.1% in drotrecogin alfa group vs 32.7% in controls), nor in subgroups of patients defined by organ failure or APACHE II scores. Drotrecogin alfa was on the market for ten years before being withdrawn. In this decade sepsis treatment has dramatically improved, with the development of new therapeutic strategies: the introduction of sepsis bundles, (31) including early goal directed therapy, (32) the use of protective lung ventilation, (33) and other interventions which have been more recently questioned, like glycemic control (34) and corticosteroid therapy. (35) These new strategies have reduced mortality up to 50% (36,37) in septic patients, a finding confirmed in recent trials. (38–40) For this reason the comparison of earlier studies on drotrecogin

alfa with more recent studies may be misleading, due to the differences in treatment. The consistent beneficial effect of drotrecogin alfa on mortality was indeed clearly demonstrated only in the first studies (PROWESS), when mortality in the standard treatment group was around 40%. The APACHE II score was developed by Knaus et al. (41) to graduate critically ill patients' prognosis. However, with improvement in sepsis treatment, the reliability of APACHE II scores in predicting mortality is reduced or at least modified compared with studies published several years ago. Consequently, to reduce confounding due to higher survival rate in more recent studies, in this study we defined a subgroup of very high risk patients. We considered not only the APACHE 2 score or the number of organs involved, but

we adopted the control group mortality rate as a stable index of poor prognosis among decades. Despite the secular trend of improved survival, in this population of high risk patients drotrecogin alfa treatment was associated with a significant reduction in mortality. The new therapeutic strategies for sepsis focus on timely interventions in order to limit decline of organ function. Early intervention limits the subsequent cascade of endothelial and cellular dysfunction that leads to an ominous prognosis. Drotrecogin alfa (activated) may still be beneficial, thanks to its pro-fibrinolytic anti-inflammatory action, in patients with very poor prognosis where both full blown endothelial dysfunction and compromise of inflammatory and coagulation cascade are present. The PROWESS-SHOCK (4) showed the same trend in mortality in this high risk subpopulation of patients (RR 0.83 [95% CI 0.6-1.14]), but was underpowered to reach statistical significance. Moreover, no increase in serious bleeding was detected ($p=0.81$), a reassuring finding that counterbalance the results of Abraham et al. (24)

Our results are consistent with those from Kalil et al. that found a beneficial effect of drotrecogin alfa in septic patients through a meta-analysis that included high and low quality studies, like case series and non-randomized controlled trial and reporting drotrecogin effect on the overall population of septic patients. (42) Given these results, even if drotrecogin alfa has been withdrawn from the market, the commercial availability of its twin molecule Protein C zymogen is an exciting alternative. (43-45) High risk septic patients may still benefit from protein C action, and while further analysis should be conducted on drotrecogin alfa, at least with an individual patient meta-analysis, the effect of Protein C zymogen should be evaluated in further trials. Limitations The limitations of this study are those specifically related to meta-analysis, including suboptimal quality of the included studies. Moreover, in this

study we identified high risk patients through an ex-post analysis that identified the highest risk patient despite improved patient prognosis. A cut-off of 40% mortality was arbitrarily chosen to identify this high risk population of patients despite the secular trend of improved survival. As this population of patients was identified by an ex-post

analysis, it cannot be translated as is in the clinical setting without the development of new predictive scores.

Conclusion

In high risk patients with multiple organ failure and high mortality rate in the control group, drotrecogin alfa may still have a role as a life saving treatment.

No beneficial effect in lower risk patients was found. Since no new large RCT will probably be conducted on this drug, now withdrawn from the market, an individual patient meta-analysis including all randomized controlled trials on sepsis is warranted, along with new studies on similar drugs such as protein C zymogen.

APPENDIX 1

("Drotrecogin alfa" "protein C activated" OR "xigris") AND (sepsis OR "septic shock") AND (randomised controlled trial[pt] OR controlled clinical trial[pt] OR randomised controlled trials[mh] OR random allocation[mh] OR double-blind method[mh] OR single-blind method[mh] OR clinical trial[pt] OR clinical trials[mh] OR (clinical trial[tw] OR ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind[tw]))) OR (latin square[tw]) OR placebos[mh] OR placebo*[tw] OR random*[tw] OR research design[mh:noexp] OR comparative study[tw] OR follow-up studies[mh] OR prospective studies[mh] OR cross-over studies[mh] OR control[tw] OR controls[tw] OR controlled[tw] OR prospectiv*[tw] OR volunteer*[tw]) NOT (animal[mh] NOT human[mh]) NOT (comment[pt] OR editorial[pt] OR meta-analysis[pt] OR practice-guideline[pt] OR review[pt])).

REFERENCES

1. Slade E, Tamber PS, Vincent J-L. The Surviving Sepsis Campaign: raising awareness to reduce mortality. *Crit Care* 2003;7:1-2.
2. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. *New Engl J Med* 2003;348:138-50.
3. Trzeciak S, Cinel I, Dellinger RP, Shapiro NI, Arnold RC, Parrillo JE, et al. Resuscitating the Microcirculation in Sepsis: The Central Role of Nitric Oxide, Emerging Concepts for Novel Therapies, and Challenges for Clinical Trials. *Acad Emerg Med* 2008;15:399-413.
4. Ranieri VM, Thompson BT, Barie PS, Dhainaut JF, Douglas IS, Finfer S, et al. Drotrecogin alfa (activated) in adults with septic shock. *N Engl J Med* 31 2012;366(22):2055-64.
5. Rosenberg RD, Aird WC. Vascular-bed-specific hemostasis and hypercoagulable states. *N Engl J Med* 1999;340:1555-64.
6. Murakami K, Okajima K, Uchiba M, Johno M, Nakagaki T, Okabe H, et al. Activated protein C attenuates endotoxin-induced pulmonary vascular injury by inhibiting activated leukocytes in rats. *Blood* 1996;87:642-7.
7. Esmon CT. The endothelial cell protein C receptor. *Thromb Haemost* 2000;83:639-43.
8. Bernard GR, Ely E, Wright TJ, Fraiz J, Stasek JE Jr, Russell JA, et al. Safety and dose relationship of recombinant human activated protein C for coagulopathy in severe sepsis. *Crit care med* 2001;29:2051.
9. Biondi-Zoccai GGL, Agostoni P, Abbate A, Testa L, Burzotta F. A simple hint to improve Robinson and Dickersin's highly sensitive PubMed search strategy for controlled clinical trials. *Int J Epidemiol* 2005;34:224-5.
10. Cochrane Handbook for Systematic Reviews of Interventions (v 5.0.2). Available on-line at: <http://www.cochrane-handbook.org>. Higgins JPT, Green S (editors). *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.
11. Review Manager (RevMan) [Computer program] Version 5.0. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008. Available from <http://ims.cochrane.org/revman>.
12. Liberati A, Altman DG, Tetzlaff J, Mulrow G, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009;339:b2700.
13. De Pont AC, Bakhtiari K, Hutten B, de Jonge E, Vroom MB, Meijers JC, et al. Recombinant human activated protein C resets thrombin generation in patients with severe sepsis—a case control study. *Critical Care* 2005;9:R490-7.
14. Bertolini G, Rossi C, Anghileri A, Livigni S, Addis A, Poole D. Use of Drotrecogin alfa (activated) in Italian intensive care units: the results of a nationwide survey. *Intensive Care Med* 2007;33:426-34.
15. Benefield RJ, Drevets DA, Huycke MM, Gentry CA. A multicenter evaluation of the safety of drotrecogin alfa (activated) in patients with baseline bleeding precautions. *Curr Drug Saf* 2012;7:3-7.
16. Spapen H, Nguyen DN, Troubleyn J, Huyghens L, Schiettecatte J. Drotrecogin alfa (activated) may attenuate severe sepsis-associated encephalopathy in clinical septic shock. *Crit Care* 2010;14:R54.
17. Kanji S, Perreault MM, Chant C, Williamson D, Burry L. Evaluating the use of Drotrecogin alfa (activated) in adult severe sepsis: a Canadian multicenter observational study. *Intensive Care Med* 2007;33:517-23.

18. Nadel S, Goldstein B, Williams MD, Dalton H, Peters M, Macias WL, et al. Drotrecogin alfa (activated) in children with severe sepsis: a multicentre phase III randomised controlled trial. *Lancet* 2007;369(9564):836–43.
19. Barton P, Kalil AC, Nadel S, Goldstein B, Okhuysen-Cawley R, Brill R, et al. Safety, pharmacokinetics, and pharmacodynamics of drotrecogin alfa (activated) in children with severe sepsis. *Pediatrics* 2004;113:7–17.
20. Dempfle CEH, Elmas E, Link A, Suvajac N, Liebe V, Janes J, et al. Endogenous plasma activated protein C levels and the effect of enoxaparin and drotrecogin alfa (activated) on markers of coagulation activation and fibrinolysis in pulmonary embolism. *Crit Care* 2011;15:R23.
21. Kyhälä L, Mentula P, Kylänpää L, Moilanen E, Puolakkainen P, Pettilä V, et al. Activated Protein C Does Not Alleviate the Course of Systemic Inflammation in the APCAP Trial. *Int J Inflam* 2012;2012:1–8.
22. Shorr AF, Janes JM, Artigas A, Tenhunen J, Wyncoll DL, Mercier E, et al. Randomized trial evaluating serial protein C levels in severe sepsis patients treated with variable doses of drotrecogin alfa (activated). *Crit Care* 2010;14:R229.
23. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001;344:699–709.
24. Abraham E, Laterre P-F, Garg R, Levy H, Talwar D, Trzaskoma BL, et al. Drotrecogin alfa (activated) for adults with severe sepsis and a low risk of death. *N Engl J Med* 2005;353:1332–41.
25. Dhainaut J-F, Antonelli M, Wright P, Desachy A, Reignier J, Lavoue S, et al. Extended drotrecogin alfa (activated) treatment in patients with prolonged septic shock. *Intensive Care Med* 2009;35:1187–95.
26. Costa V, Brophy JM. Drotrecogin alfa (activated) in severe sepsis: a systematic review and new cost-effectiveness analysis. *BMC Anesthesiol* 2007;7:5.
27. Martí-Carvajal AJ, Solà I, Gluud C, Lathyris D, Cardona AF. Human recombinant protein C for severe sepsis and septic shock in adult and paediatric patients. *Cochrane Database Syst Rev*. 2012;12:CD00438829.
28. Ely E, Laterre PF, Angus DC, Helterbrand JD, Levy H, Dhainaut JF, et al. Drotrecogin alfa (activated) administration across clinically important subgroups of patients with severe sepsis. *Critical care med* 2003;31:12.
29. Vincent J-L, Angus DC, Artigas A, Kalil A, Basson BR, Jamal HH, et al. Effects of drotrecogin alfa (activated) on organ dysfunction in the PROWESS trial. *Crit Care Med* 2003;31:834–40.
30. Laterre P-F, Abraham E, Janes JM, Trzaskoma BL, Correll NL, Booth FV. ADDRESS (ADMinistration of DRotrecogin alfa ŠactivatedĆ in Early stage Severe Sepsis) long-term follow-up: One-year safety and efficacy evaluation. *Crit Care Med* 2007;35:1457–63.
31. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al. Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock: 2008. *Crit Care Med* 2008;36:296–327.
32. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *New Engl J Med* 2001;345:1368–77.
33. Sevransky JE, Levy MM, Marini JJ. Mechanical ventilation in sepsis-induced acute lung injury/acute respiratory distress syndrome: an evidence-based review. *Crit Care Med* 2004;32:S548–53.
34. Van Den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, et al. Intensive insulin therapy in critically ill patients. *New Engl J Med* 2001;345:1359–67.
35. Annane D. Corticosteroids for severe sepsis: An evidence-based guide for physicians. *Ann Intensive Care* 2011;1:1–7.
36. Castellanos-Ortega A, Suberviola B, García-Astudillo LA, Holanda MS, Ortiz F, Llorca J, et al. Impact of the Surviving Sepsis Campaign protocols on hospital length of stay and mortality in septic shock patients: results of a three-year follow-up quasi-experimental study. *Crit Care Med* 2010;38:1036–43.
37. Girardis M, Rinaldi L, Donno L, Marietta M, Codeluppi M, Marchegiano P, et al. Effects on management and outcome of severe sepsis and septic shock patients admitted to the intensive care unit after implementation of a sepsis program: a pilot study. *Crit Care* 2009;13:R143.
38. Rice TW, Wheeler AP, Bernard GR, Vincent JL, Angus DC, Aikawa N, et al. A randomized, double-blind, placebo-controlled trial of TAK-242 for the treatment of severe sepsis. *Crit Care Med* 2010;38:1685–94.
39. ARISE; ANZICS APD Management Committee. The outcome of patients with sepsis and septic shock presenting to emergency departments in Australia and New Zealand. *Crit Care Resusc* 2007;9:8–18.
40. Levy MM, Dellinger RP, Townsend SR, Linde-Zwirble WT, Marshall JC, Bion J, et al. The Surviving Sepsis Campaign: results of an international guideline-based performance improvement program targeting severe sepsis. *Intensive Care Med* 2010;36:222–31.
41. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: A severity of disease classification system. *Crit Care Med* 1985;13:818–29.
42. Kalil AC, Larosa SP. Effectiveness and safety of drotrecogin alfa (activated) for severe sepsis: a meta-analysis and metaregression. *Lancet Infect Dis* 2012;12:678–86.
43. Silveti S, Crivellari M, Mucchetti M, Taddeo D, Franco A, Landoni G, Zangrillo A. Administration of protein C concentrates in patients without congenital deficit: a systematic review of the literature. *SIGNA VITAE* 2013;8:15-9.
44. Crivellari M, Della Valle P, Landoni G, Pappalardo F, Gerli C, Bignami E, et al. Human protein C zymogen concentrate in patients with severe sepsis and multiple organ failure after adult cardiac surgery. *Intensive Care Med* 2009;35:1959-63.
45. Crivellari M, Silveti S, Gerli C, Landoni G, Franco A, Bove T, et al. Protein C zymogen in adults with severe sepsis or septic shock. *Med Intensiva* 2013 doi: 10.1016/j.medin.2013.04.005. [Epub ahead of print]