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Human recombinant protein C for severe sepsis and septic shock in adult and paediatric patients (Review)

Martí-Carvajal AJ, Solà I, Gluud C, Lathyris D, Anand V

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[Intervention Review]

Human recombinant protein C for severe sepsis and septic shock in adult and paediatric patients

Arturo J Martí-Carvajal[1], Ivan Solà[2], Christian Gluud[3], Dimitrios Lathyris[4], Vidhu Anand[5]

[1]Iberoamerican Cochrane Network, Valencia, Venezuela. [2]Iberoamerican Cochrane Centre, Biomedical Research Institute Sant Pau (IIB Sant Pau), CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain. [3]Cochrane Hepato-Biliary Group, Copenhagen Trial Unit, Centre for Clinical Intervention Research, Department 7812, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark. [4]"George Gennimatas" General Hospital, Thessaloniki, Greece. [5]Department of Medicine, University of Minnesota, Minneapolis, MN, USA

Contact: Arturo J Martí-Carvajal, Iberoamerican Cochrane Network, Valencia, Venezuela. arturo.marti.carvajal@gmail.com.

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ABSTRACT

Background

Sepsis is a common and frequently fatal condition. Human recombinant activated protein C (APC) has been introduced to reduce the high risk of death associated with severe sepsis or septic shock. This systematic review is an update of a Cochrane review originally published in 2007.

Objectives

We assessed the benefits and harms of APC for patients with severe sepsis or septic shock.

Search methods

We searched CENTRAL (*The Cochrane Library* 2013, Issue 5); MEDLINE (June 2012 to May 2013); EMBASE (June 2012 to May 2013); BIOSIS (June 2012 to May 2013); CINAHL (June 2012 to May 2013) and LILACS (June 2012 to May 2013). There was no language restriction.

Selection criteria

We included randomized clinical trials assessing the effects of APC for severe sepsis or septic shock in adults and children. We excluded studies on neonates. We considered all-cause mortality at day 28 and at the end of study follow up, and hospital mortality as the primary outcomes.

Data collection and analysis

We independently performed trial selection, risk of bias assessment, and data extraction in duplicate. We estimated relative risks (RR) for dichotomous outcomes. We measured statistical heterogeneity using the I^2 statistic. We used a random-effects model.

Main results

We identified one new randomized clinical trial in this update which includes six randomized clinical trials involving 6781 participants in total, five randomized clinical trials in adult (N = 6307) and one randomized clinical trial in paediatric (N = 474) participants. All trials had high risk of bias and were sponsored by the pharmaceutical industry. APC compared with placebo did not significantly affect all-cause mortality at day 28 compared with placebo (856/3643 (23.49%) versus 837/3549 (23.58%); RR 1.00, 95% confidence interval (CI) 0.88 to 1.14; I^2 = 49%). APC did not significantly affect in-hospital mortality (393/1767 (22.2%) versus 379/1710 (22.1%); RR 1.01, 95% CI 0.87 to 1.16; I^2 = 20%). APC was associated with an increased risk of serious bleeding (113/3424 (3.3%) versus 74/3343 (2.2%); RR 1.45, 95% CI 1.08 to 1.94;

I^2 = 0%). APC did not significantly affect serious adverse events (463/3334 (13.9%) versus 439/3302 (13.2%); RR 1.04, 95% CI 0.92 to 1.18; I^2 = 0%). Trial sequential analyses showed that more trials do not seem to be needed for reliable conclusions regarding these outcomes.

Authors' conclusions

This updated review found no evidence suggesting that APC should be used for treating patients with severe sepsis or septic shock. APC seems to be associated with a higher risk of bleeding. The drug company behind APC, Eli Lilly, has announced the discontinuation of all ongoing clinical trials using this drug for treating patients with severe sepsis or septic shock. APC should not be used for sepsis or septic shock outside randomized clinical trials.

PLAIN LANGUAGE SUMMARY

Human recombinant activated protein C for severe sepsis and septic shock in adult and paediatric patients

Sepsis and septic shock are major causes of death. Sepsis is a complex syndrome resulting from a presumed or known infection, and its pathogenesis involves interactions between inflammation and blood clotting pathways. This serious medical condition is characterized by an inflammatory response to an infection which can affect the whole body. Patients with sepsis may have developed the inflammatory response because of microbes in their blood, urine, lungs, skin, or other tissues. Severe sepsis can lead to multiple organ failure due to blood clotting in the finer blood vessels. This reduces the amount of blood reaching the organs and septic shock ensues. Protein C reduces the clotting process and a lack of protein C can lead to an exaggeration of blood clotting. Sepsis and septic shock decrease protein C levels in the body. It has been suggested that human recombinant activated protein C (APC) will increase the levels of protein C and ameliorate or prevent multiple organ failure. In this updated Cochrane review we searched the databases until June 2012. We included six randomized clinical trials which involved 6781 people (6307 adult and 474 paediatric participants) with either a high or low risk of death. All trials had high risk of bias and were sponsored by the pharmaceutical industry (Eli Lilly). We found no evidence suggesting that APC reduced the risk of death in adults or children with severe sepsis or septic shock. On the contrary, APC increased the risk of serious bleeding.

On 25th October 2011, the European Medicines Agency issued a press release on the worldwide withdrawal of Xigris® (human recombinant activated protein C) from the market by Eli Lilly due to lack of beneficial effect on 28-day mortality in the PROWESS-SHOCK trial. Furthermore, Eli Lily has announced the discontinuation of all ongoing clinical trials. APC should not be used for sepsis or septic shock outside randomized clinical trials.

Current evidence does not support the use of human recombinant activated protein C in adults or children with severe sepsis or septic shock; moreover, there is an increased risk of bleeding associated with its use.

SUMMARY OF FINDINGS

Summary of findings for the main comparison. Human recombinant activated protein C compared to placebo for severe sepsis or septic shock

Human recombinant activated protein C compared to placebo for severe sepsis or septic shock

Patient or population: patients with severe sepsis or septic shock
Settings:
Intervention: Human recombinant activated protein C
Comparison: placebo

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo	Human recombinant activated protein C				
28-Day all-cause mortality (adult and paediatric patients) Follow up: 28 days	**Study population**		**RR 1.00** (0.86 to 1.16)	6781 (6 studies)	⊕⊕⊕⊖ **moderate**[1,2]	
	229 per 1000	**229 per 1000** (197 to 266)				
	Moderate					
In-hospital mortality (adult and paediatric patients) Follow up: 28 days	**Study population**		**RR 1.04** (0.94 to 1.15)	4307 (5 studies)	⊕⊕⊕⊖ **moderate**[1,3]	
	272 per 1000	**283 per 1000** (256 to 313)				
	Moderate					
Serious bleeding events in adult and paediatric patients (days 0 to 28) Follow up: 28 days	**Study population**		**RR 1.45** (1.08 to 1.94)	6767 (6 studies)	⊕⊕⊕⊖ **moderate**[1,4]	
	22 per 1000	**32 per 1000** (24 to 43)				
	Moderate					

Serious adverse events (adult and paediatric patients)	**Study population**		**RR 1.04** (0.92 to 1.18)	6636 (5 studies)	⊕⊕⊕⊖ **moderate**[1,4]
	133 per 1000	**138 per 1000** (122 to 157)			
	Moderate				

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Limitations related to the completion of the original trial protocols in most of the trials (ADDRESS, RESOLVE, PROWESS and Dhainaut 2009). All RCTs were sponsored by a drug company (Eli Lilly).
[2] I^2: 56%.
[3] I^2: 11%.
[4] I^2: 0%.

BACKGROUND

Description of the condition

Definition of sepsis and its nature

Sepsis is a Greek word that means 'decomposition of animal or vegetable organic matter in the presence of bacteria' (Geroulanos 2006). It is defined as clinical evidence of infection plus a systemic inflammatory response to infection that is associated with more than one disturbance of systems and control mechanisms that maintain relatively constant body conditions (homeostasis). These disturbances manifest as abnormal body temperature, elevated heart rate, excessive rapidity of breathing (tachypnoea) or hyperventilation, and altered white blood cell count (ACCP-SCCM 1992; Bone 1992). Sepsis is severe when it is accompanied by acute organ dysfunction, decreased blood flow in an organ (hypoperfusion), or abnormally low blood pressure (hypotension). Septic shock occurs when severe sepsis persists despite adequate fluid resuscitation (ACCP-SCCM 1992; Annane 2005; Bone 1992). Septic shock is the most common form of shock among patients in the intensive care unit (Vincent 2013).

Epidemiology and burden of sepsis

Severe sepsis is a common and frequently fatal condition with high associated costs (Guidet 2005). The US Centers of Disease Control and Prevention estimated an increase in incidence from 73.6 per 100,000 people in 1979 to 175.9 per 100,000 people in 1989 (Angus 2001a). The incidence and mortality from sepsis varies according to the season, country, ethnic group, anatomical sites, number of organ dysfunctions, the population studied, hospital site and period. The mortality rates vary from 20% to 80% (Angus 2001b; Barnato 2008; Brun-Buisson 1995; Brun-Buisson 2006; Danai 2007; Das 2000; Khwannimit 2009; Rangel-Frausto 1995).

Average costs per patient with sepsis in the USA are USD 22,100, with annual total national costs of USD 16.7 billion (Angus 2001b). Annual overall costs have been estimated in Spain to be EUR 70 million (Iñigo 2006). In China, the mean hospital cost was estimated at USD 11,390 per patient and USD 502 per patient per day (Cheng 2007).

Approaches for treating sepsis

Sepsis involves a high heterogeneity of patients and types of infections (Carlet 2008; Holmes 2003; Marshall 2008; Matsuda 2007; Remick 2007; Sipahi 2006; Treacher 2009). Current treatment of severe sepsis involves treatment of the infection with appropriate early antibiotic therapy against the identified or presumed organisms, surgical drainage where necessary, and supportive treatment according to the patient's symptoms and signs (Bochud 2001; Bochud 2004; Cohen 2004; Cunneen 2004; Dellinger 2008; Garnacho 2003; Garnacho 2006; Girbes 2008; Marshall 2004; Schuerholz 2008).

However, antibiotic, surgical and supportive care are not always enough, and there is an urgent need for new therapies to reduce the mortality associated with severe sepsis (Paul 2009; Vincent 2002). Clinical practice guidelines (Dellinger 2008; Vincent 2004) include recommendations about adjunctive therapies that have been developed in an effort to reduce mortality from severe sepsis, such as supportive care (early goal-directed therapy, vasopressor, haemofiltration); target bacterial virulence factors (antiendotoxin antibodies); target host response factors (activated protein C, moderate doses of steroids, and blockage of cytokines); and immunotherapy (Azevedo 2008; Boussekey 2008; Cohen 2009; Cunnington 2008; Dellinger 2008; Hotchkiss 2010; Iannaro 2009; Kumar 2004; Leon 2008; Leone 2010; Moine 2007; Parrish 2008; Rhodes 2004; Rigato 2006; Sandrock 2010; Sibila 2008; Vincent 2002; Wang 2009a; Wang 2009b; Wesche-Soldato 2007; Wittebole 2008). A number of these recommendations have been insufficiently studied (Perner 2012).

Description of the intervention

Sepsis is associated with alterations in blood coagulation, the fibrinolytic systems, and inflammatory pathways (Cinel 2009; King 2013; Levi 2008; Rittirsch 2008; Sriskandan 2008). This leads to disorders of tissue perfusion that generate multiple organ system failure with depletion of platelets and coagulation factors, and activation of natural inhibitors of coagulation (King 2013).

One of the natural inhibitors of coagulation is protein C (Esmon 2006). In patients with sepsis, protein C is depleted and the production of activated protein C (APC) is impaired, shifting the balance towards more intravascular coagulation and organ failure. The administration of APC, therefore, has theoretical advantages (Healy 2002; Hinds 2001; Mann 2002). However, APC has a short biological half life and in severe sepsis the pool of circulating protein C is rapidly depleted (Mann 2002; Mann 2009; Yan 2001), which means that protein C levels may serve as a useful prognostic indicator of outcome in sepsis and related diseases (Fisher 2000; Shorr 2006). In addition, protein C levels have been considered as a possible tool for monitoring treatment with APC (Shorr 2008).

In November 2001, the US Food and Drug Administration (FDA) approved recombinant human activated protein C (drotrecogin alfa activated, marketed as Xigris®) for use in people with severe sepsis based on their APACHE II score (see Appendix 1). Eli Lilly and Company's license depended on the results of only one randomized clinical trial (PROWESS, recombinant human activated protein C worldwide evaluation in severe sepsis) (PROWESS 2001). In August 2002, the European Agency for Evaluation of Medicinal Products approved the addition of the drug to best standard care based on the sepsis-related multiple organ failure (SOFA) score (EMEA 2005) as a measure of disease activity (see Appendix 2).

How the intervention might work

APC exerts antithrombotic, anticoagulant, anti-inflammatory, and pro-fibrinolytic effects by complex molecular pathways which have recently been reviewed (Christiaans 2013). It is therefore thought that APC may ameliorate or prevent coagulation processes during infection thereby leading to less organ failure and fewer deaths.

Why it is important to do this review

This is the third update of this Cochrane review, which had been performed for the following reasons.

1. The search strategy has been updated (June 2012) leading to the identification of a new randomized clinical trial on APC in patients with septic shock (PROWESS-SHOCK 2012).
2. The trials have undergone updated assessment of risk of bias.
3. The trials have been subjected to trial sequential analyses in order to assess the risk of random errors and the potential need for further trials.

OBJECTIVES

We assessed the benefits and harms of activated protein C (APC) for the treatment of patients with severe sepsis or septic shock.

METHODS

Criteria for considering studies for this review

Types of studies

We included randomized clinical trials with parallel or cross-over designs, irrespective of publication status or language. For cross-over trials, we planned to include only data from the first intervention period.

Types of participants

We included patients with severe sepsis or septic shock, irrespective of the aetiology or their ages. We excluded neonates (< 28 days old, at any gestational age or birth weight) because another Cochrane review addresses this question (Kylat 2012).

We sought trials that defined severe sepsis according to standardised international criteria, such as the American College of Chest Physicians and the Society of Critical Care Medicine Consensus Conference international guidelines (ACCP-SCCM 1992).

Types of interventions

Intervention

Recombinant human APC delivered intravenously plus conventional care

Control

Placebo plus conventional care or no intervention plus conventional care

We accepted the definitions of conventional care provided by the trialists, defining it as an integrative approach to sepsis care including sepsis identification and control, antibiotic therapy, fluid therapy, blood product administration, and mechanical ventilation with sepsis-induced acute lung injury (Dellinger 2008).

Types of outcome measures

Primary outcomes

1. All-cause mortality at day 28, and at the end of study follow up
2. Hospital mortality
3. Adverse events: number and type of adverse events defined as patients with any untoward medical occurrence not necessarily having a causal relationship with the treatment. We reported on adverse events that led to treatment discontinuation and those that did not lead to treatment discontinuation separately

We have defined serious adverse events according to the International Conference on Harmonisation (ICH) Guidelines (ICH-GCP1997) as any event that at any dose results in death, is life-threatening, requires in-patient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability, or is a congenital anomaly or birth defect, and any important medical event which may have jeopardised the patient or required intervention to prevent it. All other adverse events were considered non-serious.

Secondary outcomes

1. Bleeding
2. Thrombotic event
3. Quality of life measures (based on any item from a validated scale)
4. Time to discharge from intensive care unit
5. Development of organ failure

Search methods for identification of studies

Electronic searches

We updated the search of our previous review (Martí-Carvajal 2011) by searching the Cochrane Central Register of Controlled Trials (CENTRAL) (*The Cochrane Library* 2013, Issue 5), see Appendix 3; Ovid MEDLINE (June 2012 to May 2013), see Appendix 4; Ovid EMBASE (June 2012 to May 2013), see Appendix 5; LILACS (June 2012 to May 2013), see Appendix 6; BIOSIS via Ovid (June 2012 to May 2013), see Appendix 7; and CINAHL via EBSCOhost (June 2012 to May 2013), see Appendix 8. We used the specific search terms listed below in combination with the Cochrane highly sensitive search strategy for identifying trials (Higgins 2011).

Our search terms were:

1. anticoagulants;
2. protein C;
3. recombinant protein*;
4. blood coagulation factor inhibitor*;
5. disseminated intravascular coagulation;
6. APC alfa;
7. APC;
8. rh APC;
9. recombinant human activated protein C;
10. sepsis;
11. septic shock;
12. shock;
13. septic;
14. sepsis syndrome;
15. septicemia;
16. septicaemia.

Searching other resources

We also searched in:

1. the references of review articles;
2. books related to sepsis treatment and critical care.

We did not search databases of ongoing trial registers due to the fact that on 25 October 2011 Eli Lilly announced the discontinuation of all ongoing clinical trials. We included all relevant identified studies, regardless of language or publication status (published, unpublished, in press and in progress). We checked the citations in the reports of the trials that were identified by the above methods.

Data collection and analysis

Selection of studies

Arturo Martí-Carvajal (AMC) and Andrés Felipe Cardona (AFC) independently screened the results of the search strategy for potentially relevant trials, and independently assessed them for inclusion based on the inclusion criteria. We resolved disagreements through discussion with Dimitrios Lathyris (DL) until a consensus was reached.

Data extraction and management

Two authors carried out data extraction (AMC and AFC) using a pre-designed data extraction form that contained publication details, patient population, randomization, allocation concealment, details of blinding measures, description of interventions, and results (Zavala 2006). We resolved discrepancies through discussion. We involved a third author (Ivan Solà (IS)) to check for accuracy the data entered into the Review Manager software (RevMan 5.1).

Assessment of risk of bias in included studies

All authors independently assessed the risk of bias of the trials according to the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).

We assessed the following domains, using the following definitions.

Generation of the allocation sequence

- Low risk of bias, if the allocation sequence was generated by a computer or random number table, drawing of lots, tossing of a coin, shuffling of cards, or throwing dice.
- Unclear, if the trial was described as randomized but the method used for the allocation sequence generation was not described.
- High risk of bias, if a system involving dates, names, or admittance numbers was used for the allocation of patients. These studies are known as quasi-randomized and were excluded from the present review when assessing beneficial effects.

Allocation concealment

- Low risk of bias, if the allocation of patients involved a central independent unit, on-site locked computer, identical-appearing numbered drug bottles or containers prepared by an independent pharmacist or investigator, or sealed envelopes.
- Unclear, if the trial was described as randomized but the method used to conceal the allocation was not described.
- High risk of bias, if the allocation sequence was known to the investigators who assigned participants or if the study was quasi-randomized. The latter was excluded from the present review when assessing beneficial effects.

Blinding (or masking)

We assessed each trial (as 'Low', 'Unclear', or 'High risk') with regard to the following levels of blinding:

- blinding of clinician (person delivering treatment) to treatment allocation;
- blinding of participant to treatment allocation;
- blinding of outcome assessor to treatment allocation.

Incomplete outcome data

- Low risk of bias, if the numbers and reasons for dropouts and withdrawals in all intervention groups were described or it was specified that there were no dropouts or withdrawals.
- Unclear, if the report gave the impression that there had been no dropouts or withdrawals but this was not specifically stated.
- High risk of bias, if the number or reasons for dropouts and withdrawals were not described.

We further examined the percentage of dropouts overall in each trial and per randomization arm and we evaluated whether intention-to-treat analysis was performed or could be performed from the published information.

Selective outcome reporting

- Low risk of bias, if pre-defined or clinically relevant and reasonably expected outcomes were reported on.
- Unclear, if not all pre-defined or clinically relevant and reasonably expected outcomes were reported on or were not reported on fully, or it was unclear whether data on these outcomes were recorded or not.
- High risk of bias, if one or more clinically relevant and reasonably expected outcomes were not reported on; and data on these outcomes were likely to have been recorded.

Other bias

- Low risk of bias, the trial appeared to be free of other components that could put it at risk of bias.
- Unclear, the trial may or may not be free of other components that could put it at risk of bias.
- High risk of bias, there were other factors in the trial that could put it at risk of bias.

We considered low risk of bias trials to be those that adequately generated their allocation sequence; had adequate allocation concealment, adequate blinding, adequate handling of incomplete outcome data; were free of selective outcome reporting; and were free of other bias.

We considered trials in which we could assess one of the domains as high risk of bias or unclear risk of bias as trials with high risk of bias.

AMC and IS assessed the included studies and registered the information in tables; see 'Characteristics of included studies'. AFC and DL checked the entered data.

Measures of treatment effect

We pooled the relative risk (RR) with 95% confidence interval (CI) for the following binary outcomes: all-cause mortality at day 28, and at the end of study follow up; hospital mortality; bleeding; a thrombotic event; development of organ failure; and adverse events.

For continuous outcomes (quality of life and time to discharge from the intensive care unit) the standardised mean difference with 95% CI was calculated, as recommended by Higgins 2011.

Dealing with missing data

For all included trials, we registered the levels of attrition. We contacted the main author of PROWESS 2001 and researchers of

ADDRESS 2005 for missing data. We used data gathered from the FDA website (FDA 2001a; FDA 2001b) to manage unpublished data from PROWESS 2001.

Assessment of heterogeneity

We quantified the statistical heterogeneity using the I^2statistic, which describes the percentage of total variation across studies that is due to heterogeneity rather than sampling error (Higgins 2003). When heterogeneity was detected (I^2 > 50%) we attempted to identify the possible causes of the heterogeneity.

Assessment of reporting biases

We did not assess publication bias by a funnel plot because we included only six randomized clinical trials.

Data synthesis

We pooled the results from the trials using the Review Manager software (RevMan 5.1). We summarised the findings using a random-effects model.

Subgroup analysis and investigation of heterogeneity

We devoted further efforts to identifying possible causes of heterogeneity. We explored the impact of the included trials' risk of bias and the condition of the individuals by subgroup analyses. We anticipated clinical heterogeneity for the following participant and intervention characteristics.

1. Participants' severity of disease (APACHE score, dichotomized at 25).
2. Participants' age.
3. Level of protein C content in the blood.
4. Site of infection.
5. Number of organs showing dysfunction.
6. Positive blood culture.

These different variables justified subgroup analyses. We performed subgroup analysis only for the primary outcomes.

Trial sequential analysis

Meta-analysis of cumulative data may run the risk of random errors ('play of chance') due to sparse data and repetitive analyses of the same data (Brok 2008; Brok 2009; Thorlund 2010; Thorlund 2011; Wetterslev 2008; Wetterslev 2009). In order to assess the risks of random errors in our cumulative meta-analyses, we conducted diversity-adjusted trial sequential analyses based upon the proportion with the outcome in the control group; an a priori set relative risk reduction of 20%; an alpha of 5%, a beta of 20%; and the diversity in the meta-analysis (CTU 2011; Thorlund 2009; Thorlund 2011). We conducted sensitivity analysis of the trial sequential analysis to estimate the potential need for further trials.

Sensitivity analysis

Our search strategy found two FDA reports (FDA 2001a; FDA 2001b) which made it clear that the PROWESS 2001 contained two substudies: one with 720 participants where the trial was carried out following the initial protocol; and another trial with 970 participants where the original protocol had been amended. We used this information to perform a sensitivity analysis. See Appendix 9.

Summary of findings tables

We used the GRADE proposals (Guyatt 2011) to assess the quality of the body of evidence associated with the following outcomes: 28-day all-cause mortality in adult and paediatric patients, in-hospital mortality, and serious bleeding events (days 0 to 28). We constructed a 'Summary of findings for the main comparison (SoF) using the GRADEPro software (http://ims.cochrane.org/revman/other-resources/gradepro). GRADE classifies the quality of a body of evidence based on the extent to which one can be confident that an estimate of effect or association reflects the outcome being assessed (Balshem 2011; Guyatt 2011a; Guyatt 2011b; Guyatt 2011c; Guyatt 2011d; Guyatt 2011e; Guyatt 2011f; Guyatt 2011g).

RESULTS

Description of studies

Results of the search

In this updated version we detected 519 references of potential interest after duplicates were removed (Figure 1). We identified and retrieved 10 potentially relevant articles (see table 'Characteristics of excluded studies'). From these 529 references we excluded 491 as they did not meet our inclusion criteria. We included 38 references, including the FDA's reports developed during APC approval (FDA 2001a; FDA 2001b), to six randomized clinical trials.

Figure 1. Study flow diagram from June 2012 to May 2013.

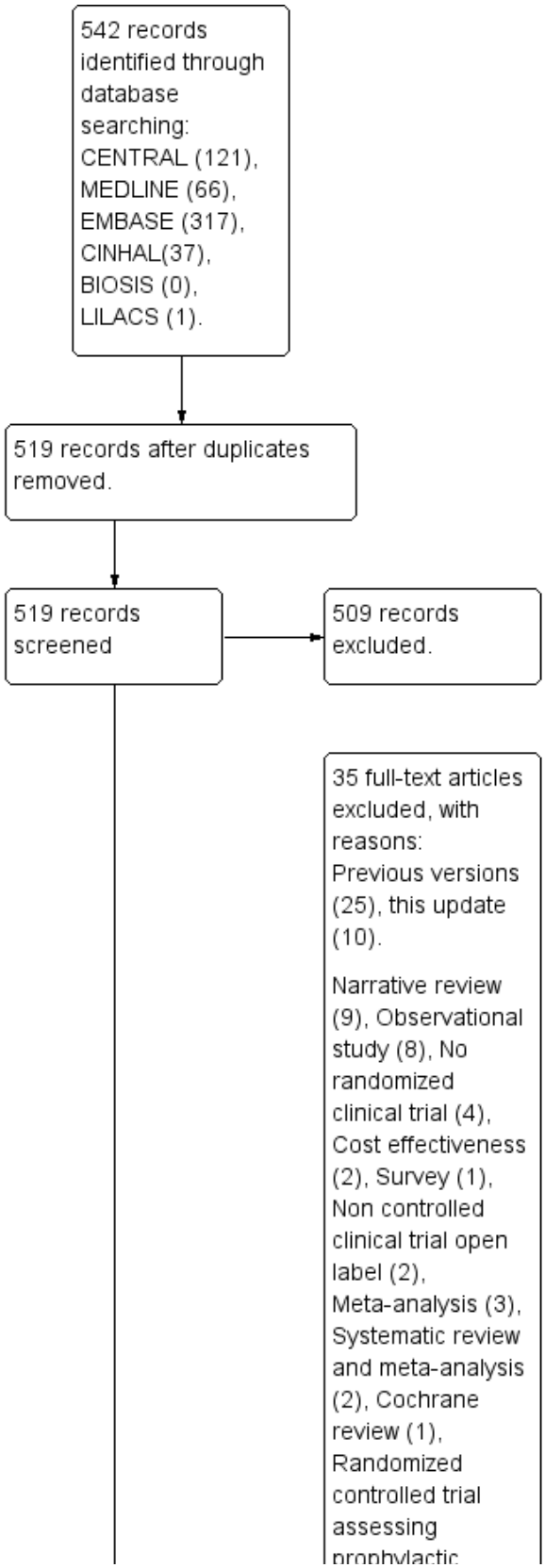
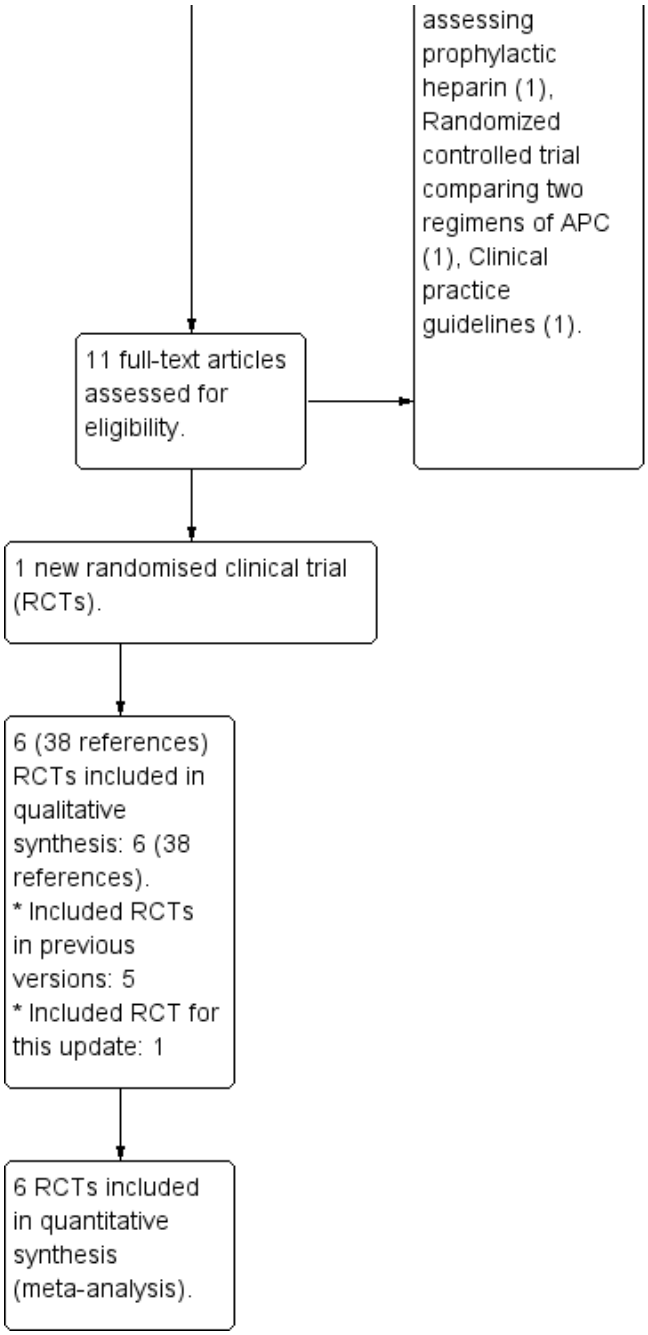


Figure 1. (Continued)



Included studies

All the trials included in this review were funded by Eli Lilly and Company and involved authors that were stockholders, had served as consultants, or were employed by the company.

This update adds a new randomized clinical trial (PROWESS-SHOCK 2012) to the five included in our previous version of the review. These randomized clinical trials included 6781 participants, 6307 adults (ADDRESS 2005; Dhainaut 2009; PROWESS 2001; PROWESS-SHOCK 2012; rhAPC Sepsis Study 2001) and 474 paediatric patients (RESOLVE 2007). All included studies compared APC with placebo. The first trial on the topic was a randomized phase II dose-ranging trial (rhAPC Sepsis Study 2001), whereas the rest were phase III clinical trials to assess the efficacy and safety of APC (ADDRESS 2005; Dhainaut 2009; PROWESS 2001; PROWESS-SHOCK 2012; RESOLVE 2007). Five randomized clinical trials were conducted using a dose of 24 µg/kg/hr intravenously for a total duration of 96 hours (ADDRESS 2005; PROWESS 2001; PROWESS-SHOCK 2012; RESOLVE 2007; rhAPC Sepsis Study 2001). The Dhainaut 2009 trial was carried out but with the same dose of drug but with a total duration of 168 hours.

With the exception of the rhAPC Sepsis Study 2001 that took place in the United States and Canada, the rest were international trials in: 164 centres in 11 countries (PROWESS 2001); 516 centres

from 34 countries (ADDRESS 2005); 64 centres in nine countries (Dhainaut 2009); 104 centres in 18 countries (RESOLVE 2007); and 208 centres in Europe, North and South America, Australia, New Zealand, and India (PROWESS-SHOCK 2012). Five trials enrolled adult participants (ADDRESS 2005; Dhainaut 2009; PROWESS 2001; PROWESS-SHOCK 2012; rhAPC Sepsis Study 2001) and the remaining trial enrolled children (N = 79 > 1 month to < 18 years old) and neonates (N = 3 < 1 month old) (RESOLVE 2007). We could not discriminate the outcomes in the two subgroups of paediatric patients so we included all. Data from the rhAPC Sepsis Study 2001 and PROWESS 2001 trials were used in the regulatory process by the FDA.

We provide an outline of the characteristics of the included trials as follows. These are described in full in the 'Characteristics of included studies' table.

The rhAPC Sepsis Study 2001 assessed the safety and effect on coagulopathy of a range of doses of APC (in 90 patients with a mean APACHE II score of 16.8 (SD 5.2)) compared with a saline placebo (in 41 patients with a mean APACHE II score of 18.4 (SD 6.9)). Up to 70% of patients were in septic shock and needed mechanical support on the day prior to infusion; with an underlying medical cause of sepsis in most of them. One third of patients had multiple organ failure and the rest had single organ failure, it was the cardiovascular and respiratory systems that most frequently failed. Decisions regarding the use of antimicrobial agents, intravenous fluids, cardiovascular and respiratory support, and surgical intervention were left to the treating physician and were not pre-specified in the protocol. The intervention could be provided for between 48 and 96 hours but the study did not report the administration time.

The PROWESS study was the first phase III trial. It set out to assess the reduction in rate of death from all causes at 28 days with APC (drotrecogin alfa activated) compared with a placebo consisting of 0.9% saline and the addition of 0.1% human serum albumin (PROWESS 2001). The trial established the standard dose of 24 µg/kg/hr intravenously for a total duration of 96 hrs, assessed in the rest of the included trials. Despite the information reported in the main publication of the PROWESS study, the data from two FDA reports (FDA 2001a; FDA 2001b) made it clear that the trial contained two different protocols, one with 720 participants following the initial protocol and a subsequent phase with 970 participants where the registered protocol had been changed. The study defined sepsis as an infection with at least three signs of systemic inflammatory response syndrome and one sepsis-induced organ dysfunction. A sample size calculation was detailed only in the FDA reports, which included up to 27 subgroups analyses. The FDA report stated that the study planned to enrol 2280 patients with two interim analyses, after 760 and 1520 patients had been enrolled. The journal publication reported the results for 1690 randomized patients (850 receiving APC and 840 receiving placebo) because recruitment was recommended to be suspended after the second interim analysis due to a statistically significant reduction in 28-day mortality.

Data gathered from the FDA reports showed that the trial was split into two different protocols (FDA 2001a; FDA 2001b), an issue that was not reported in the main PROWESS publication. After the enrolment of 720 participants under the first protocol (from July 1998 to July 1999), a second protocol was approved (on 5 March 1999) seven months before the first interim analysis, after which an additional 970 participants were enrolled (from June 1999 to June 2000). The amendment was approved 'prior to the first unblinding of the external statistical services organization statistician, who prepared analyses for the prospectively defined first interim analysis' (FDA 2001a). Some baseline characteristics, by the first and second protocols, are shown in Appendix 10 (FDA 2001a; FDA 2001b).

The main changes from the first protocol were the addition of new exclusion criteria and the addition of 0.1% human serum albumin to the initial 0.9% sodium chloride placebo. Furthermore, in August 1999 the sponsor introduced a change in the manufacturing of the drug (FDA 2001b). The original manufactured drug was referred to as 'Bulk Drug Substance' (BDS) BDS2 and the newly manufactured drug as BDS2+. A number of extensive analyses were conducted. No differences were detected between the two manufactured products. Given the complexity of the molecule, however, one cannot exclude the possibility of undetected differences (FDA 2001b). Detailed changes to the PROWESS protocol are shown in Appendix 9 and Appendix 11. The exclusion criteria of PROWESS are shown in Appendix 12.

After the FDA approval of APC, the ADDRESS 2005 study was designed to assess the effects of drotrecogin alfa activated in adults with severe sepsis and a low risk of death. Although the sample size was calculated for 11,444 patients, the data monitoring committee recommended the early termination of enrolment for futility after an interim analysis when the recruitment reached 1500 patients. At the time of trial termination, 2640 had enrolled in the study but data were analysed for 2613: 1316 receiving drotrecogin alfa activated and 1297 receiving 0.9% sodium chloride. Despite its objective, the trial included 12% of patients at high risk of death.

The RESOLVE study was the only included trial that recruited paediatric patients (between 38 weeks and 17 years of age) with severe sepsis and organ dysfunction (RESOLVE 2007). The trial compared results of 240 children receiving drotrecogin alfa activated with 237 children receiving 0.9% saline. The sample size was calculated to show differences regarding a composite time to complete organ failure resolution (CTCOFR) score of three organ systems, cardiovascular, respiratory, and renal, based on The International Paediatric Sepsis Conference (Goldstein 2005). After two years, enrolment was suspended when the second planned interim analysis suggested that there was little chance of reaching the efficacy endpoint by completion of the trial (FDA 2005). Appendix 13 shows the RESOLVE trial resolution organ dysfunction definitions.

Dhainaut 2009 assessed an extended dose of drotrecogin alfa activated (during 72 additional hours after 96 hours of APC) in 94 patients with vasopressor requirements and persistent septic shock compared with 99 patients that received the sodium chloride placebo. APACHE scores were not part of the inclusion criteria but at baseline participants had a mean score of 28.1 (SD 8.1) and a mean of 2.9 (SD 1.0) organ failures. Baseline data also showed an imbalance in protein C levels. The study based its sample size calculation on the PROWESS trial, planning the recruitment of 270 patients. This had to be reduced to 200 patients.

PROWESS-SHOCK 2012 was designed as "Whilst mindful of the results of the PROWESS, ADDRESS and RESOLVE studies, and Lilly's obligations to drug registration agencies, the primary goal of the trial is to provide clinicians with robust evidence regarding the efficacy and safety of DAA in a clearly defined and

clinically important patient population" (Finfer 2008). This trial was conducted to assess the effects of APC in adults patients with septic shock. This randomized clinical trials involved 1680 patients and found that human recombinant activated protein C (rhAPC) failed to reduce 28-day all-cause mortality when compared with placebo (Mitka 2011). On 25 October 2011, the European Medicines Agency issued a press release on the worldwide withdrawal of Xigris® (rhAPC) from the market by Eli Lilly due to its lack of beneficial effect on 28-day mortality in the PROWESS-SHOCK study (EMEA 2011).

Excluded studies

This updated review excluded a total of 35 studies which did not meet the inclusion criteria (Barton 2004; Bearden 2002; Bernard 2004; Bertolini 2007; Casey 2002; Costa 2007; Decruyenaere 2009; Ferrer 2009; Freeman 2003; Goldstein 2006; Green 2005; Heslet 2004; Houston 2009; Kanji 2007; Levi 2008; Lucioni 2002; Marraro 2009; McCoy 2003; McCoy 2004; Neilson 2003; van Doorn 2008; Vincent 2005; Wheeler 2008; Wiedermann 2005). See the 'Characteristics of excluded studies' table.

Ongoing studies

Eli Lilly has announced the discontinuation of all ongoing clinical trials (EMEA 2011).

Risk of bias in included studies

The risk of bias was evaluated in each of the included randomized clinical trials. Full details are shown in Risk of bias in included studies, Figure 2, and Figure 3.

Figure 2. Methodological quality graph: review authors' judgements about each methodological quality item presented as percentages across all included studies.

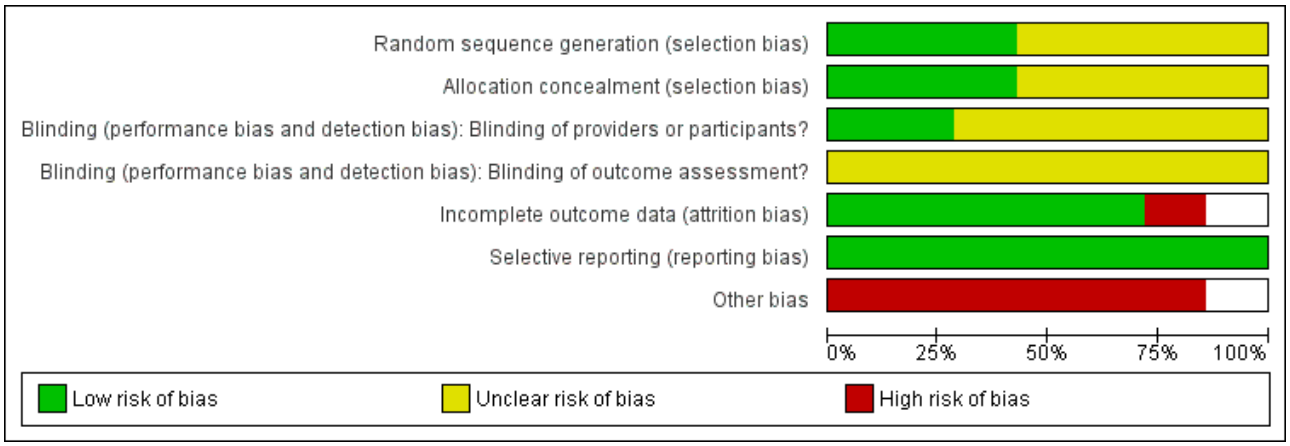
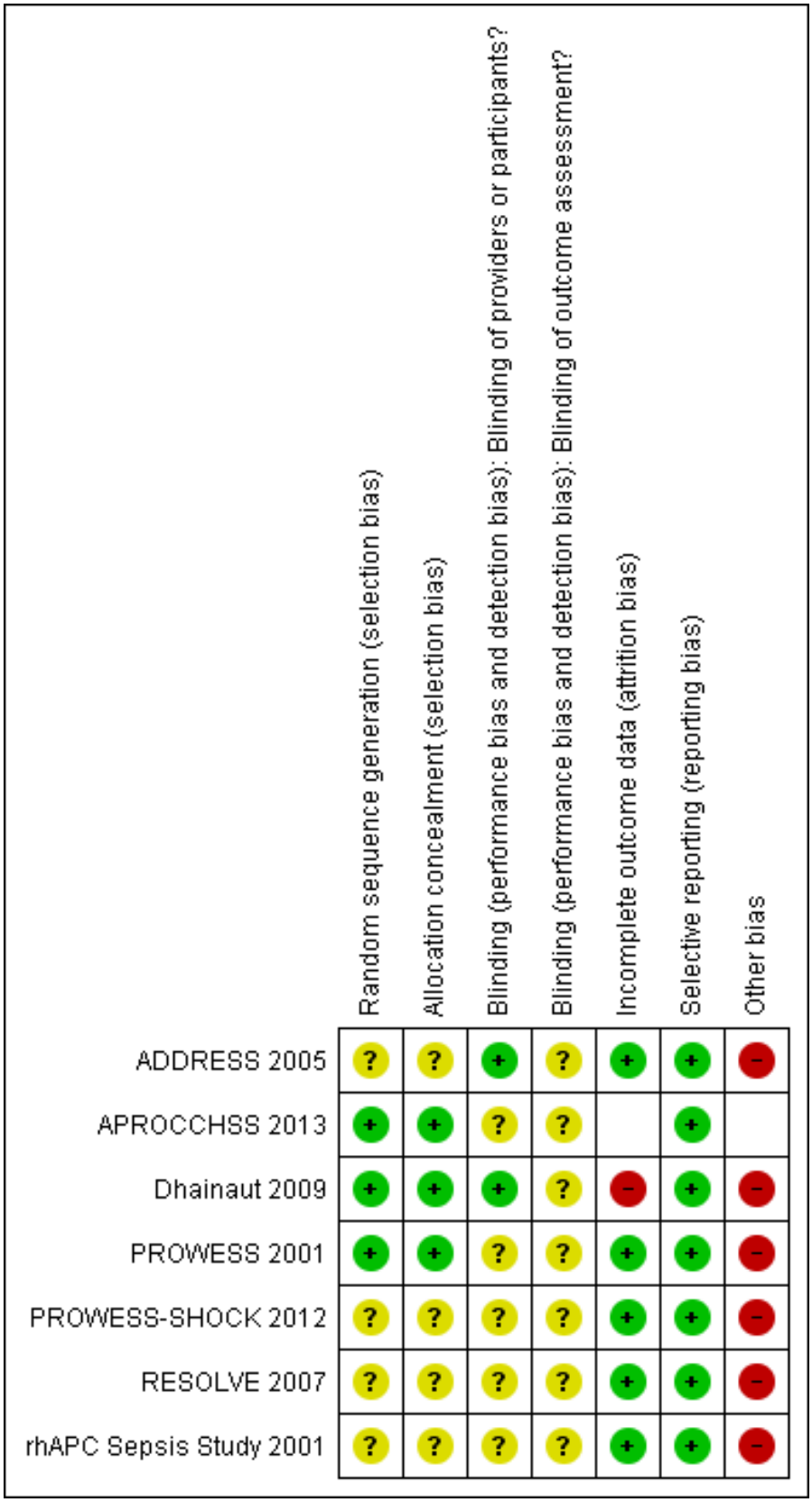


Figure 3. Methodological quality summary: review authors' judgements about each methodological quality item for each included study.



Allocation

Risk of bias arising from the method of generation of the allocation sequence was low in two trials (Dhainaut 2009; PROWESS 2001). It was unclear in the remainder of trials (ADDRESS 2005; Dhainaut 2009; PROWESS-SHOCK 2012; RESOLVE 2007; rhAPC Sepsis Study 2001).

Risk of bias arising from the method of allocation concealment was rated as low in one trial (PROWESS 2001). Allocation concealment was not reported in the remaining trials (ADDRESS 2005; PROWESS-SHOCK 2012; RESOLVE 2007; rhAPC Sepsis Study 2001).

Blinding

Risk of bias due to blinding of participants and personnel was rated as low in two trials because the drug preparations were covered to make them indistinguishable (ADDRESS 2005; Dhainaut 2009). The risk of bias was unclear in the remainder of trials (PROWESS 2001; PROWESS-SHOCK 2012; RESOLVE 2007; rhAPC Sepsis Study 2001).

Blinding of outcome assessors (detection bias)

Risk of bias arising from lack of blinding of outcome assessment was rated as unclear in all included trials (ADDRESS 2005; Dhainaut 2009; PROWESS 2001; PROWESS-SHOCK 2012; RESOLVE 2007; rhAPC Sepsis Study 2001). From FDA 2001a we obtained the information that all deaths and serious adverse events in PROWESS 2001 were reviewed by the sponsor (Eli Lilly) in a blinded manner.

Incomplete outcome data

Risk of attrition bias was rated as low in five trials (ADDRESS 2005; PROWESS 2001; PROWESS-SHOCK 2012; RESOLVE 2007; rhAPC Sepsis Study 2001). Risk of attrition bias was rated as high in Dhainaut 2009.

Selective reporting

Risk of reporting bias was rated as low in all trials (ADDRESS 2005; Dhainaut 2009; PROWESS 2001; PROWESS-SHOCK 2012; RESOLVE 2007; rhAPC Sepsis Study 2001).

Other potential sources of bias

All the trials included in this review were funded by Eli Lilly and Company and involved authors that were stockholders, had served as consultants, or were employed by the company (ADDRESS 2005; Dhainaut 2009; PROWESS 2001; PROWESS-SHOCK 2012; RESOLVE 2007; rhAPC Sepsis Study 2001).

There is some concern regarding the completion of the included trials as they were designed based on their original protocols. PROWESS 2001 modified its inclusion criteria and amended the original protocol after the inclusion of 700 patients, and prematurely stopped the recruitment for benefit. Both ADDRESS 2005 and RESOLVE 2007 terminated the enrolment early for futility.

ADDRESS 2005 showed a discrepancy in the report with the total number of participants who completed the study and those who were analysed.

Effects of interventions

See: **Summary of findings for the main comparison** Human recombinant activated protein C compared to placebo for severe sepsis or septic shock

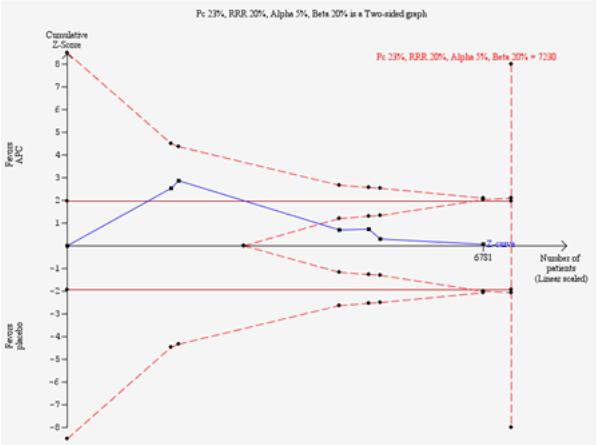
Results were based on 6781 participants (Summary of findings for the main comparison).

Primary outcomes

All-cause mortality at day 28, and at the end of study follow up

Pooled estimates of the five trials including adult patients (ADDRESS 2005; Dhainaut 2009; PROWESS 2001; PROWESS-SHOCK 2012; rhAPC Sepsis Study 2001) and one trial including paediatric patients (RESOLVE 2007) did not show differences in 28-day mortality between APC and placebo (1547 events in 6781 patients; RR 1.00, 95% CI 0.86 to 1.16; $I^2 = 56\%$) (Analysis 1.1). Trial sequential analysis for 28-day all-cause mortality suggested that no more trials may be needed for disproving an intervention effect of 20% relative risk reduction. Smaller risk reductions might still require further trials (Figure 4).

Figure 4. Trial sequential analysis of human recombinant activated protein C (APC) versus placebo on all-cause mortality at 28-days based on the diversity-adjusted required information size (DARIS) of 7230 patients. This DARIS was calculated based upon a proportion of patients dying within 28 days out of 23.3% in the control group; a RRR of 20% in the experimental intervention group; an alpha (α) of 5%; a beta (β) of 20%; and a diversity of 71%. The cumulative Z-curve (blue line) crosses temporally the conventional alpha of 5%, but reverts to insignificant values. The cumulative Z-curve never crosses the trial sequential alpha-spending monitoring boundaries. After the third trial, the cumulative Z-curve crosses the trial sequential beta-spending monitoring boundary, showing that the area of futility has been reached. This suggests that no more trials may be needed for disproving an intervention effect of 20% relative risk reduction. Smaller risk reductions might still require further trials.



We found a high statistical heterogeneity (I^2 = 56%). Therefore, we performed a subgroup analysis by age (adult patients compared to paediatric patients) and by treatment duration (96 hours compared to > 96 hours) (Dhainaut 2009).

Subgroup analysis by age (adult patients compared to paediatric patients)

Meta-analysis of five trials with adult patients (N = 6307 participants, 1465 events) did not show a difference in 28-day mortality between APC and placebo (pooled RR 1.00, 95% CI 0.84 to 1.19; I^2 = 65%) (ADDRESS 2005; Dhainaut 2009; PROWESS 2001; PROWESS-SHOCK 2012; rhAPC Sepsis Study 2001). The paediatric trial did not show a difference in 28-day mortality between APC and placebo (RR 0.98, 95% CI 0.66 to 1.46; P = 0.93) (RESOLVE 2007). The test for subgroups did not show differences between the groups (I^2 = 0%, P = 0.94). See Analysis 1.3.

Subgroup analysis by treatment duration comparing 96 hours to more than 96 hours

Meta-analysis of five trials conducted over the conventional treatment duration (96 hours) and including adult and paediatric patients (N = 6588 participants, 1479 events) did not show a difference in 28-day mortality between APC and placebo (pooled RR 0.97, 95% CI 0.83 to 1.14; I^2 = 59%) (ADDRESS 2005; PROWESS 2001; PROWESS-SHOCK 2012; RESOLVE 2007; rhAPC Sepsis Study 2001). The extended treatment duration (> 96 hours) trial did not show a difference in 28-day mortality between APC and placebo (RR 1.26, 95% CI 0.86 to 1.85; P = 0.24) (Dhainaut 2009). The test for subgroups did not show differences between the groups (I^2 = 32.7%, P = 0.22). See Analysis 1.4.

Sensitivity analysis by PROWESS 2001 first protocol compared to second protocol

According to the FDA data for the PROWESS 2001 trial (FDA 2001a; FDA 2001b), APC did not show a reduction in mortality when the trial maintained the original protocol (RR 0.94, 95% CI 0.75 to 1.1) and only had a beneficial effect in the second half of the study (RR 0.71, 95% CI 0.57 to 0.87) (Analysis 1.5). The test for subgroups showed significant differences between the groups (I^2 = 68.4%, P = 0.08).

We therefore performed a sensitivity analysis including data from all trials (ADDRESS 2005; Dhainaut 2009; PROWESS 2001; PROWESS-SHOCK 2012; RESOLVE 2007; rhAPC Sepsis Study 2001) compared with data from ADDRESS 2005; Dhainaut 2009; PROWESS-SHOCK 2012; RESOLVE 2007; rhAPC Sepsis Study 2001 and the PROWESS 2001 modified protocol. Meta-analysis of six trials did not show differences in 28-day mortality between APC and placebo (RR 1.00, 95% CI 0.86 to 1.16; I^2 = 56%). Meta-analysis of six trials but excluding the data of the PROWESS 2001 first protocol did not show a differences in 28-day mortality between APC and placebo and found a higher statistical heterogeneity (RR 0.93, 95% CI 0.70 to 1.24; I^2 = 87%). The test for subgroups did not show differences between the groups (I^2 = 0%, P = 0.66) (Analysis 1.6).

Subgroup analysis by severity of disease (APACHE II scores lower compared to higher than 25)

Three included trials (ADDRESS 2005; PROWESS 2001; PROWESS-SHOCK 2012) allowed a subgroup analysis by severity of disease (APACHE II score lower compared to higher than 25). APC did not show a reduction in the patients with a APACHE II score below 25 (low risk of death) (RR 1.05, 95% CI 0.92 to 1.2; I^2 = 0%), nor for patients with a high risk of death (RR 0.96, 95% CI 0.68 to 1.35; I^2 = 86%). The test for subgroups did not show differences between the groups (I^2 = 0%, P = 0.62) (Analysis 1.7).

Subgroup analysis of patients according to protein C deficiency class

Two trials reported the effects of APC according to the protein C content in the blood of the patients (PROWESS 2001; PROWESS-SHOCK 2012). The effect of APC versus placebo on mortality was not significant in patients having a protein C deficiency (less than 80% of normal value) (700 events in 2613 patients; RR 0.91, 95% CI 0.70 to 1.17; I^2 = 75%). Data from PROWESS 2001 and PROWESS-SHOCK 2012 did not show a difference in the patients not having a protein C deficiency (more than 80% of normal value) on 28-day mortality (62 events in 290 patients; RR 0.65, 95% CI 0.41 to 1.04; I^2 = 0%). Data from PROWESS 2001 did not show a difference between APC and placebo in patients with an unknown protein C concentration on 28-day mortality (30 events in 116 patients; RR 1.12, 95% CI 0.60 to 2.07; P = 0.73). The test for subgroups did not show differences between the groups (I^2 = 8.6%, P = 0.33) (Analysis 1.8).

Subgroup analysis of patients according to the baseline organ dysfunction category (single organ dysfunction (< 2) compared to multiple organ dysfunction (≥ 2)

Two trials reported the effects of APC according to single organ dysfunction (< 2) in patients with low and high risk of bias (ADDRESS 2005; PROWESS-SHOCK 2012). The effect of APC versus placebo on mortality was not significant in these patients (575 events in 1778 patients; RR 1.17, 95% CI 0.95 to 1.45; I^2 = 0%). Two trials reported the effects of APC according to multiple organ dysfunction (in patients with an unknown protein C concentration ≥ 2) in patients with low and high risk of bias (ADDRESS 2005; PROWESS-SHOCK 2012). The effect of APC versus placebo on mortality was not significant in these patients (602 events in 2503 patients; RR 1.04, 95% CI 0.91 to 1.20; I^2 = 0%). The test for subgroups did not show differences between the groups (I^2 = 0%, P = 0.38) (Analysis 1.9).

Subgroup analysis of patients according to the baseline organ dysfunction category (baseline organ dysfunction category (< 3) compared to multiple organ dysfunction (≥ 3)

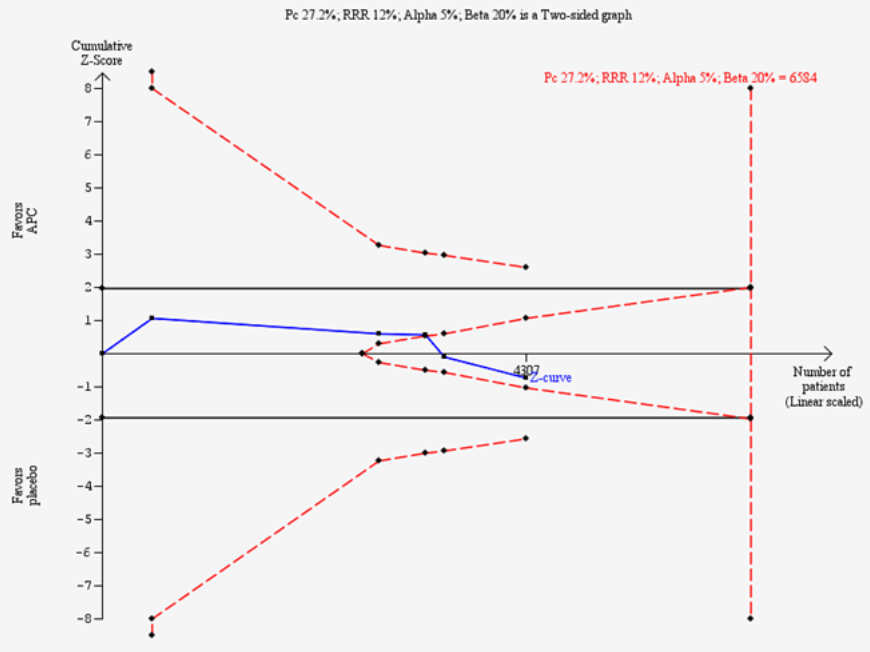
Two trials reported the effects of APC according to the baseline organ dysfunction category (< 3) (PROWESS 2001; PROWESS-SHOCK 2012). The effect of APC versus placebo on mortality was not significant in these patients (255 events in 1224 patients; RR 1.05, 95% CI 0.60 to 1.86; I^2 = 73%). Two trials reported the effects of APC according to multiple organ dysfunction (≥ 3) (PROWESS 2001; PROWESS-SHOCK 2012). The effect of APC versus placebo on mortality was not significant in these patients (637 events in 2145 patients; RR 0.90, 95% CI 0.67 to 1.22; I^2 = 81%). The test for subgroups did not show differences between the groups (I^2 = 0%, P = 0.64) (Analysis 1.10).

Hospital mortality

Estimates of five trials (ADDRESS 2005; Dhainaut 2009; PROWESS 2001; PROWESS-SHOCK 2012; RESOLVE 2007) did not show differences in hospital mortality between APC and placebo (1187 events in 4307 patients; RR 1.04, 95% CI 0.94 to 1.15; I^2 = 11%) with marginal heterogeneity (Analysis 1.11).

Trial sequential analysis for in-hospital mortality suggested that no more trials may be needed for disproving an intervention effect of 14% relative risk reduction. Smaller risk reductions might still require further trials (Figure 5).

Figure 5. Trial sequential analysis of human recombinant activated protein C (APC) versus placebo in-hospital mortality based on the diversity-adjusted required information size (DARIS) of 6584 patients. This DARIS was calculated based upon a proportion of patients dying in-hospital out of 27.2% in the control group; a RRR of 12% in the experimental intervention group; an alpha (α) of 5%; a beta (β) of 20%; and a diversity of 15%. The cumulative Z-curve (blue line) crossed the trial sequential beta-spending monitoring boundary, showing that the area of futility has been reached. This suggests that no more trials may be needed for disproving an intervention effect of 12% relative risk reduction. Smaller risk reductions might still require further trials.

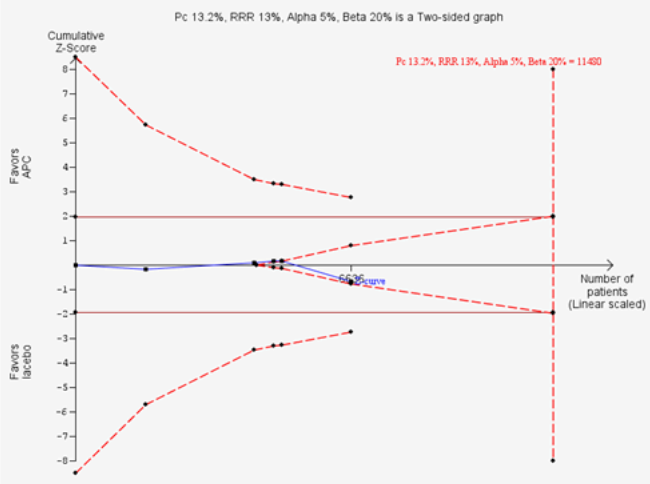


Adverse events

Adverse events (including bleeding and non-bleeding events such as skin rash, hypertension, abnormal healing, hallucinations) were reported in five studies (ADDRESS 2005; APROCCHSS 2013; Dhainaut 2009; PROWESS 2001; RESOLVE 2007). The RR for serious adverse events did not show a difference when APC and placebo were compared (575/3542 (16.23%) versus 550/3505 (15.69%); RR 1.02, 95% CI 0.93 to 1.13; I^2 = 0%) (see Analysis 1.12).

Trial sequential analysis for adverse events suggested that no more trials may be needed for disproving an intervention effect of 13% relative risk reduction (Figure 6).

Figure 6. Trial sequential analysis of human recombinant activated protein C (APC) versus placebo on adverse events based on the diversity-adjusted required information size (DARIS) of 11480 patients. This DARIS was calculated based upon a proportion of patients developing adverse events out of 13.2% in the control group; a RRR of 13% in the experimental intervention group; an alpha an alpha (α) of 5%; a beta (β) of 20%; and a diversity of 0%. The cumulative Z-curve (blue line) crossed the trials sequential beta-spending monitoring boundary, showing that the area of futility has been reached. This suggest that no more trials are needed for disproving and intervention effect of 13% relative risk reduction.



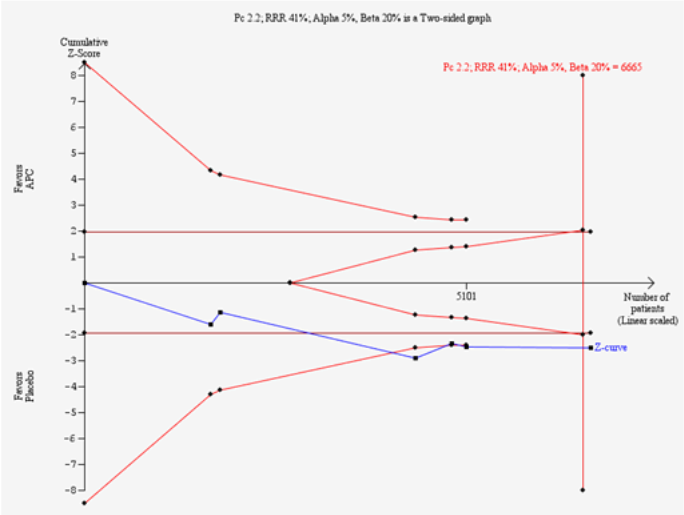
Secondary outcomes

Bleeding

APC showed no an increased risk of serious bleeding when compared with placebo (139/3632 (3.82%) versus 103/3546 (2.90%); RR 1.26, 95% CI 0.94 to 1.67; I^2 = 16%) (Analysis 1.13).

Trial sequential analysis for serious bleeding suggested that no more trials may be needed for disproving an intervention effect of 41% relative risk reduction (Figure 7).

Figure 7. Trial sequential analysis of human recombinant activated protein C (APC) versus placebo on serious bleeding based on the diversity-adjusted required information size (DARIS) of 6665 patients. This DARIS was calculated based upon a proportion of patients with serious bleeding out of 2.2% in the control group; a RRR of 41% in the experimental intervention group; an alpha (α) of 5%; a beta (β) of 20%; and a diversity of 0%. The cumulative Z-curve (blue line) crossed the lower conventional alpha of 5% and the lower trial sequential alpha-spending monitoring boundaries, showing that we have robust data for significant harm.



The RR for serious bleeding during infusion did not show a difference when APC and placebo were compared (RR 1.80, 95% CI 0.96 to 3.40; I^2 = 16%). See Analysis 1.14. The RR for central nervous system bleeding, including paediatric and adult patients, did not show a differences between the APC and placebo groups (RR 1.72, 95% CI 0.72 to 4.12; I^2 = 16%) (PROWESS-SHOCK 2012; RESOLVE 2007). See Analysis 1.15.

Quality of life

This outcome was not evaluated in the included trials.

Development of organ failure

This outcome was not evaluated in the included trials.

Time to discharge from an intensive care unit

PROWESS 2001 evaluated the number of days from the start of APC infusion to intensive care unit discharge. APC and placebo-treated patients showed the same median number of days until discharge for hospital survivor patients (nine days, P = 0.7) and for hospital non-survivor patients (eight days, P = 0.58). The interquartile ranges were not reported for any group.

Thrombotic events

One trial (PROWESS 2001) reported a similar proportion of thrombotic events in both intervention groups: 2% in APC the group versus 3% in the placebo group.

DISCUSSION

Summary of main results

Meta-analysis of six trials showed that activated protein C (APC) did not have a statistically significant effect on 28-day mortality when compared with placebo in patients with severe sepsis or septic shock. In addition, our results showed an increased risk of bleeding events, which does not support the use of this drug (Summary of findings for the main comparison).

Overall completeness and applicability of evidence

The results in this review are based on data from trials that included a broad range of patients with different co-morbidities who received different treatment approaches. Although these aspects could be considered as a threat to applicability, the consistency in the results derived from our analyses shows that the included trials may represent a broad picture of patients with severe sepsis or septic shock.

Quality of the evidence

The main source of bias in the included trials was the lack of detail in describing the generation of the randomization sequence or the allocation concealment (ADDRESS 2005; Dhainaut 2009; RESOLVE 2007; rhAPC Sepsis Study 2001). Apart from that the trials were apparently adequately blinded and were free of outcome reporting biases. However, if the trials had problems with randomization this may also affect blinding to the intervention.

There are two issues that are not negligible and could have an effect on the confidence in the overall estimates shown in this review. All the included trials were sponsored by Eli Lilly and included authors that were stockholders or were employed by this company. A large number of studies show that drug-industry sponsorship is more likely to be associated with statistically significant findings (Bekelman 2003; Bhandari 2004; Jørgensen 2006; Khan 2008; Lexchin 2003; Lexchin 2005; Melander 2003; Sismondo 2008a, Sismondo 2008b). The pharmaceutical industry has invested in developing new treatments for common clinical conditions, such as severe sepsis with mortality that exceeds 30%, but it should be mandatory that the parties with a special interest conduct randomized clinical trials according to the highest standards and subject to public scrutiny by independent parties.

Most of the included trials had limitations related to the completion of the originally designed protocols. Both ADDRESS 2005 and RESOLVE 2007 terminated their recruitment for futility. Dhainaut 2009 had to modify its recruitment rules due to difficulties in enrolling patients, and reduced the planned trial by 25%. The modification of the planned sample size and high attrition could lead to a false negative conclusion in this trial. The PROWESS 2001 turned out to be composed of two different trials after we got access to data from FDA. The first protocol with 720 participants had a focus on protein C deficient patients, which was removed in the second protocol. The second protocol also introduced changes to the manufacturing of the drug. We found no differences between the two manufactured products in our test of interaction analysis. Given the complexity of the molecule, however, one cannot exclude the possibility of undetected differences (FDA 2001b). Furthemore, the sponsor objective excluded patients with non-sepsis related diseases (FDA 2001b). See Appendix 9; Appendix 10; Appendix 11; Appendix 12 for details of changes between the PROWESS 2001 first and second protocol.

See Summary of findings for the main comparison for details.

Potential biases in the review process

In the process for performing a systematic review, there is a group of biases called 'significance-chasing biases' (Ioannidis 2010). This group includes publication bias, selective outcome reporting bias, selective analysis reporting bias, and fabrication bias (Ioannidis 2010). Publication bias represents a major threat to the validity of systematic reviews, particularly in reviews that include small trials. However, this Cochrane review has a low risk of publication bias due to the thorough trial search process. Selective outcome reporting bias operates through suppression of information on specific outcomes and has similarities to study publication bias in that 'negative' results remain unpublished (Ioannidis 2010). All included randomized clinical trials in this Cochrane review had a low risk of selective outcome reporting bias.

Agreements and disagreements with other studies or reviews

Our review provides evidence derived from six trials conducted in different populations and using different drug dosages and durations of treatment. Our results for the main outcome, 28-day mortality, are similar to the findings from other systematic reviews (Costa 2007; Wiedermann 2005) and similarly for remarks made that the risks could exceed the benefits.

A recent meta-analysis showed a significant reduction in hospital mortality and increased rates of bleeding in patients with severe sepsis (Kalil 2012). This meta-analysis included both randomized clinical trials and analytical, controlled, and single-group studies (prospective matched controlled cohort studies and prospective and retrospective single group studies). This meta-analysis did not report the assessment of risk of bias. Therefore, it contains a high risk of bias (systematic errors). This is a likely explanation why their meta-analysis and our systematic review reach contradicting conclusions (Deeks 2003).

One Cochrane review assessing the benefits and harms of APC for neonates with sepsis found insufficient data to support or reject APC (Kylat 2012). Furthermore, due to our present results in adults with sepsis, neonates with sepsis should not be exposed to APC. Further trials in adults that show positive findings should be conducted before use in neonates are considered (Kylat 2012).

AUTHORS' CONCLUSIONS

Implications for practice

This updated Cochrane review provides evidence to show that APC seems to lack clinical benefits and is associated with an increased risk of bleeding in patients suffering from severe sepsis or septic shock. Therefore, APC should not be used for patients with sepsis outside randomized clinical trials.

Implications for research

The European Medicines Agency issued a press release on the worldwide withdrawal of Xigris (activated protein C (APC) or drotrecogin alfa) from the market by Eli Lilly due to lack of beneficial effect on 28-day mortality in the PROWESS-SHOCK study. Furthermore, Eli Lily has announced the discontinuation of all other ongoing clinical trials (EMEA 2011).

ACKNOWLEDGEMENTS

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We would like to thank the following.

Dr Peter C Gotzsche for his comments on our original published review (Martí-Carvajal 2007).

Dr Djillali Annane for sending us information about the ongoing trial NCT00625209 (Activated Protein C and Corticosteroids for Human Septic Shock (APROCCHS)).

Dr Georgia Salanti for her participation in the original published reviews (Martí-Carvajal 2007; Martí-Carvajal 2008).

Dr Nicola Petrucci, Prof Nathan Pace, Dr John Fraser, Prof Leonard Leibovici, Mark Edward and Kathie Godfrey for their help and editorial advice during the preparation of the original review (Martí-Carvajal 2007).

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

ADDRESS 2005

Methods	Design: parallel-design (2 arms). Multicentre study: 516 centres. International: 34 countries. Follow-up period: 28 days.
Participants	N = 2640 enrolled and randomized patients (1333 patients APC group/1307 placebo group). 2610 patients received one of two interventions (1301 patients APC group/1293 placebo group). 2613 patients completed study and analysed (1316 patients APC group/1297 placebo group). 1. Age (years) C group: 58.8±16.8 Placebo group: 58.6±16.7 Total group: not reported Range: not reported 2. Sex APC group: 58.5% men Placebo group: 58.5% men Total group: 1516 men (57.4%) 3. Mean APACHE II score APC group: 18.2±5.8 Placebo group:18.2±5.9 4. APACHE II score < 25 APC group: 1168 (87.6) Placebo group: 1147 (87.8)

ADDRESS 2005 *(Continued)*

	5. Inclusion criteria: • patients had severe sepsis, defined as the presence of a suspected or known infection and sepsis-induced dysfunction of at least one organ (cardiovascular, renal, respiratory, haematologic; or unexplained metabolic acidosis) and a low risk of death. 6. Exclusion criteria: • APC contraindicated according to the applicable label in the country in which patients enrolled; • increased risk of bleeding; • moribund state, not expected to survive for 28 days; • disease progressed; • investigator determined, in the best interest of patient, to initiate treatment with commercial APC, treatment assignment was unblinded and the study drug was discontinued but follow up of the patient continued; • unblinding required to permit investigator to treat the patient appropriately for the indicated duration of therapy (i.e., 96 hrs).
Interventions	1. Activated protein C (APC) (Eli Lilly): APC (manufactured from clones of a single cell) was delivered at dose 24 µg/kg/h intravenously for a total duration of 96 hrs. 2. Placebo (0.9% sodium chloride): delivered at dose of 24 µg/kg/h intravenously for a total duration of 96 hrs. Co-intervention: all other patient care was at the discretion of the investigators and was not specified in the study protocol. Patients had to begin treatment with the study drug within 48 hrs of documentation of the first organ dysfunction. Treatment duration: 96 hrs.
Outcomes	Primary Death from any cause, assessed 28 days after the start of the infusion. Secondary In-hospital mortality within 90 days after the start of the infusion.
Notes	1. RCT also included high risk patients (12.2% placebo group, 12.4% in APC group). 2. 1307 patients, placebo group: 1293 received drug/14 did not = 1293 patients (98.9%). 3. 1333 patients, APC group: 1317 received drug/16 did not = 1317 patients (98.7%). 4. A priori sample size estimation: yes. 5. Eli Lilly (sponsor) designed trials together with external executives and steering committees of the Administration of Drotrecogin Alfa (Activated) in Early Stage Severe Sepsis (ADDRESS) study group. Sponsor collected and analysed the data. Some authors employees and stockholders of Eli Lilly. One author received lecture fees and research grant from Lilly, Germany. Two authors reported having served as paid consultants for Eli Lilly. Data collected and analysed by the sponsor. 6. There is a misunderstanding with the final number in the flow chart of this included study (Page 1335). 7. Study required by the FDA to assess the effects of APC in patients with severe sepsis and low risk of death (APACHE II lower than 25 or single organ failure). 8. Early termination for futility.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote: "We used block randomization stratified according to investigative site and within a site in terms of whether the patient received or was intended to

ADDRESS 2005 *(Continued)*

		receive low-dose heparin for prophylaxis against deep-vein thrombosis at the start of infusion of the study drug". Comment: Insufficient information to permit judgment of 'Low risk' or 'High risk'.
Allocation concealment (selection bias)	Unclear risk	Insufficient information to permit judgment of 'Low risk' or 'High risk'.
Blinding (performance bias and detection bias) Blinding of providers or participants?	Low risk	Quote "Patients, investigators, and all others involved in conducting the study remained blinded to the treatment assignments for the duration of the study" (Page 1333). Quote: 'All delivery systems for the study drug were covered to ensure blinding' (Page 1333).
Blinding (performance bias and detection bias) Blinding of outcome assessment?	Unclear risk	Data were not provided to judge if outcome measurement was blinded.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Quote: "was analysed in the intention-to-treat population, defined as all patients who were randomly assigned to treatment, even if the patient did not receive the assigned or correct treatment, did not follow the protocol, or received commercial DrotAA as a result of the investigator's decision". Data for primary outcome available for 98.97% of the randomized sample, with balanced reasons for withdrawals or losses to follow up.
Selective reporting (reporting bias)	Low risk	No protocol available. All the outcomes listed in the method section described in results.
Other bias	High risk	Sponsor bias. Bias in the presentation of data (Porta 2008). Comment: this trial has a misunderstanding with the final number in the flow chart of this included study.

APROCCHSS 2013

Methods	Design: 2x2 factorial design Multicentre study: 24 hospitals. Country: France. Follow-up period: 180 days Phase: III
Participants	Inclusion criteria • Were included, patients of both sex, who have reached legal age and with 1) indisputable orprobable septic shock for less than 24 hours, 2) one or more clinically or microbiologicallydocumented outbreaks, 3) at least 2 organ failures, each defined by a SOFA score ≥ 3 for atleast 6 consecutive hours (E1), 4) treated with catecholamines for at least 6 h and less than 24hours (dopamine ≥15 µg/kg /min, or epinephrine or norepinephrine at a rate ≥0.25 µg/kg/minor at least equal to 1mg/h), or any other vasoconstrictor to maintain a systolic blood pressure1≥ 90 mmHg or mean arterial pressure ≥ 65 mmHg despite adequate fluid resuscitation,6)giving a free, informed and written consent. Otherwise, the consent will be obtained fromthe person of trust or, if not, from a family member, if present. In the event that neither theperson of trust or a family member would be present on the day of inclusion, the

APROCCHSS 2013 *(Continued)*

	patient maybe still included. It will be informed and its consent will be asked for further possiblere-search and use of their data (Law 2004-806 of August 9, 2004, Article L1122-1-2) Exclusion criteria 1.- Any previous episode of severe sepsis or septic shock during the current hospitalization 2- Pregnant or breastfeeding women 3- Decision of withholding or withdrawing care, 4- Severe underlying disease with a life-expectancy of one month or less, 5- Formal indication for corticosteroids (prednisone 30 mg or equivalent, for one month or more), 6- Any surgical procedure within the past 72 hours, or any surgery associated with a high risk of bleeding, or a surgical procedure scheduled for the next 24 h, 7- Gastro-duodenal bleeding in the past 6 weeks. 8- Chronic liver disease, i.e. Child C. 9- Recent trauma, 10- Head trauma in the past 3 months 11- Stroke in the past 3 months 12- Any intra-cranial process 13- Platelets count < 30.000 /mm3 14- Any formal indication for anticoagulant except prophylactic heparin 15- Any other risk of bleeding as assessed by patient's physician 16- Known hypersensitivity to drotrecogin alfa activated 17- No affiliation to a French Social Security
Interventions	• Placebo Comparator: 1 placebo of hydrocortisone, placebo of fludrocortisone and placebo of activated protein CIntervention: Drug: placebos • Active Comparator: 2 Hydrocortisone plus fludrocortisone and a placebo of activated protein CIntervention: Drug: hydrocortisone and fludrocortisone and placebo • Active Comparator: 3 placebo of hydrocortisone, placebo of fludrocortisone and activated protein CIntervention: Drug: recombinant human activated protein C and placebos • Active Comparator: 4 hydrocortisone plus fludrocortisone plus activated protein CIntervention: Drug: recombinant human activated protein C and hydrocortisone and fludrocortisone http://clinicaltrials.gov/ct2/show/record/NCT00625209 From APROCCHSS 2013: Marketed DAA was infused at a constant rate of 24 µg/kg/hour for 96 hours. Control: placebo (0.9% saline). Infusion was interrupted 2 hours before any percutaneous procedure or major surgery and was resumed 1 hour or 12 hours later. Patients, investigators, and sponsor were blinded to treatment. Co-intervention: The use of antibiotics, fluid management, vasopressor therapy and ventilatory support had to follow the Surviving Sepsis Campaign guidelines (E2). Compliance to these guidelines was checked regularly at each investigator meeting.
Outcomes	Primary: 1. 90-day mortality. Secondary 1. Mortality rates at day-28 and 180, and at ICU and hospital discharge; 2. Organ failure-free (SOFA <6) days; 3. Vasopressor- and mechanical ventilation-free days; and 4. Serious adverse events (intracranial hemorrhage, any bleeding requiring surgical haemostasis, or transfusion of more than 2 red blood cell concentrates).

APROCCHSS 2013 *(Continued)*

Notes	Clinical trial registration: NCT00625209. Official Title: Activated Protein C and Corticosteroids for Human Septic Shock (APROCCHSS). Brief Title: Activated Protein C and Corticosteroids for Human Septic Shock. Conducted: September 2, 2008, to October 25, 2011 Sample size Stopped: On October 25 2011, the trial was suspended following the withdrawal from the market of APC. Sponsor: University of Versailles Role of sponsor: Assistance Publique – Hôpitaux de Paris was the study sponsor and took full administrative responsibility. The study sponsor did not take part to trial design, patients' recruitment, data management, analysis, interpretation, and reporting. All contributors remained independent from the sponsor. This trial will remain the only industry-independent trial on drotrecogin alfa activated in adults with septic shock. This trial was performed mainly in centers which used routinely XIGRIS since the drug was available on the French market, and has enrolled adults with septic shock and a baseline risk of death of almost twice of the one observed in PROWESS SHOCK. This trial demonstrated that the drug provided no survival benefit in these sick patients admitted to intensive care units in which physicians had experience with XIGRIS use.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote "Randomization was centralized, performed through a secured Website, and stratified according to centre, using permutation blocks."
Allocation concealment (selection bias)	Low risk	Quote "Randomization was centralized, performed through a secured Website..."
Blinding (performance bias and detection bias) Blinding of providers or participants?	Unclear risk	Patients, investigators, and sponsor were blinded to treatment. (ask how?)
Blinding (performance bias and detection bias) Blinding of outcome assessment?	Unclear risk	Patients, investigators, and sponsor were blinded to treatment.(ask how)
Selective reporting (reporting bias)	Low risk	

Dhainaut 2009

Methods	Design: parallel-design (2 arms). Multicentre study: 64 centres. International: nine countries. Follow-up period: 24 days after initiation of the study drug and 28 days after standard drug initiation.

Dhainaut 2009 *(Continued)*

Participants	Planned sample size: 270 (135 by arm). Sample size reduced to 200 ("ensure that the study completed in an acceptable timeframe"). Entered to study: 201 participants. Randomized: 199 participants (73% of the planned sample size). Lost post-randomization: 6 participants (3%, 6/199). • Experimental arm: 4 ("owing to entry criteria exclusion"). • Placebo arm: 2 ("owing to entry criteria exclusion and death"). Received study drug, 193 participants (71.5%, 193/270): 94 (70%) experimental arm versus 99 (73.3%) placebo arm. 1. Age (years) Mean (± SD): 62.0 ±13.4 (APC arm) versus 62.7 ±13.0 (placebo arm). 2. Sex (male): 61.7% (APC arm) versus 59.6% (placebo arm). 3. Inclusion criteria: • age: ≥18 years; • severe sepsis; • continued to require vasopressor support (having been treated with at least 84 hrs of a planned 96 hr infusion of standard DAA). 4. Exclusion criteria: • required extensive or multiple surgical procedures within the next 3 days; • platelet count <30,000/mm^3; • receiving therapeutic heparin (>15,000 IU/day of unfractionated heparin or larger doses of low molecular weight heparin than used for prophylaxis of deep venous thrombosis, or >15 IU/kg/hr for renal replacement purposes); • not expected to survive 24 days given their pre-existing uncorrectable medical condition; • had received treatment within the last 30 days with any drug that had not received regulatory approval; • pregnant or breastfeeding; • contraindicated for treatment with APC; • not given written informed consent; • were no longer vasopressor dependent; • patients whose family or primary physician had not committed to aggressive management of the patients.
Interventions	Experimental APC 24 µg/kg/hr. Maximum of 72hr extended infusion. "There was to be no time interval between the standard and study infusions; however, a maximum of 2 hr was allowed in case of unforeseen circumstances. Interruptions were acceptable as long as infusions were restarted within 24 hr and within the 72-hr treatment period. If a patient resolved their need for vasopressor support for 12 continuous hours before completion of treatment, the infusion was discontinued and not restarted". Placebo Sterile 0.9% sodium chloride.
Outcomes	Primary Time to resolution of vasopressor-dependent hypotension (dopamine ≥5 µg/kg/min; or epinephrine, phenylephrine, vasopressin, or norepinephrine at any dose) within 72 hrs. Secondary 1. 28-day all-cause mortality. 2. 90-day in-hospital mortality.

Dhainaut 2009 *(Continued)*

	3. Organ function (sequential organ failure assessment, SOFA) and biomarker evaluations (protein C, D-dimers, prothrombin time). Safety: serious adverse events and adverse events.
Notes	A priori sample size estimation: 270 patients (power: 81% "to detect a difference between treatment groups using the log rank statistic with a two sided significance level of 0.1 if the true hazard ratio was 0.63. This corresponds to a difference of about 16.5% in time to resolution of vasopressor-dependent cardiovascular organ failure") (Page 1189). Official title: 'A Phase IIIb Study to Determine Efficacy and Safety of Extended DrotrecoginAlfa (Activated) Therapy in Patients With Persistent Requirement for Vasopressor Support After 96 Hour Infusion With Commercial Drotrecogin Alfa (Activated)'. ClinicalTrials.gov identifier: NCT00190788. Sponsors and Collaborators: Eli Lilly and Company. Role: not described. First received: September 12, 2005. Last updated: October 10, 2007. Conducted between 2004 and 2007. Sample size reduced by 25% due to the slow recruitment.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: '[...] pharmacist or designee who obtained treatment assignments from an interactive voice response system [...]'.
Allocation concealment (selection bias)	Low risk	Quote: '[...] pharmacist or designee who obtained treatment assignments from an interactive voice response system [...]'.
Blinding (performance bias and detection bias) Blinding of providers or participants?	Low risk	Quote: 'Patients and study personnel remained blinded to treatment throughout the study, apart from a pharmacist [...] prepared the drug (covered to maintain the blinding)'.
Blinding (performance bias and detection bias) Blinding of outcome assessment?	Unclear risk	Data were not provided to judge if outcome measurement was blinded. IInsufficient information to permit judgment of 'Low risk' or 'High risk'.
Incomplete outcome data (attrition bias) All outcomes	High risk	Quote: "Analyses used the intention to treat (ITT) population". Data for primary outcome available for 96.01% of the randomized sample but reasons for withdrawals or losses to follow up remain unclear. 27.5% of patients had protocol violations due to drug discontinuation.
Selective reporting (reporting bias)	Low risk	No protocol available. All the outcomes listed in the method section described in results.
Other bias	High risk	Sponsor bias. Design bias (Porta 2008). Comment: The trial has a considerable number of imbalances at baseline: lower protein C levels, more admissions to acute care hospitals, more cancer histories, higher SOFA cardiovascular scores for the intervention group, and more myocardial infarction histories in the placebo group.

PROWESS 2001

Methods	Design: parallel-design (2 arms). Multicentre study: 164 centres. International: 11 countries. Follow-up period: 28 days.
Participants	Planned sample size: 2280. N = 1728 randomized patients; 1690 patients (APC 850/placebo group 840) for the efficacy and safety analysis. 1. Age: APC group 60.5 ±17.2 yrs; placebo group 60.6±16.5 yrs. Range: 18-96. 2. Sex APC group: 56.1% men; placebo group 58.0% men. 3. Plasma protein C activity (median level) APC group (n = 799): 47%; placebo group (n= 775) 50%. Protein C deficiency (Yes): APC group 83.4%; placebo group 79.8%. 4. Inclusion criteria: • patients with a known or suspected infection on the basis of clinical data at the time of screening; • to meet the following criteria within a 24-hour period: 3 or more signs of systematic inflammation; and sepsis-induced dysfunction of at least one organ system that lasted no longer than 24 hrs. 5. Exclusion criteria: • pregnancy or breastfeeding; • age <18 yr or weight >135 kg; • platelet count <30,000/mm^3; • conditions that increased the risk of bleeding: surgery requiring general or spinal anesthesia within 12 hours before the infusion, the potential need for such surgery during the infusion, or evidence of active bleeding postoperatively; a history of severe head trauma requiring hospitalization, intracranial surgery, or stroke within 3 months before the study or any history of intracerebral arteriovenous malformation, cerebral aneurysm, or mass lesions of the central nervous system; a history of congenital bleeding diatheses; gastrointestinal bleeding within 6 weeks before the study unless corrective surgery had been performed; and trauma considered to increase the risk of bleeding; • known hypercoagulable condition, including resistance to activated protein C; hereditary deficiency; • of protein C, protein S, or antithrombin III; presence of anticardiolipin antibody, antiphospholipid antibody, lupus anticoagulant, or homocysteinemia; or recently documented (within 3 months before the study) or highly suspected deep-vein thrombosis or pulmonary embolism; • patient's family, physician, or both, not in favour of aggressive treatment of patient; or presence of an • advanced directive to withhold life-sustaining treatment; • patient not expected to survive 28 days because of uncorrectable medical condition; such as poorly • controlled neoplasm or other end-stage disease; • moribund state in which death was perceived to be imminent; • human immunodeficiency virus infection in association with a last known CD4 count of ≤50/mm^3; • history of bone marrow, lung, liver, pancreas, or small-bowel transplantation; • chronic renal failure requiring haemodialysis or peritoneal dialysis; • known or suspected portosystemic hypertension, chronic jaundice, cirrhosis, or chronic ascites; • acute pancreatitis with no established source of infection; • participation in another investigational study within 30 days before the current study; • use of any of the following medications or treatment regimens: unfractionated heparin to treat an active thrombotic event within 8 hours before the infusion†; low-molecular-weight heparin at a higher dose than recommended for prophylactic use (as specified in the package insert) within 12 hours

PROWESS 2001 *(Continued)*

	before the infusion; warfarin (if used within 7 days before study entry and if the prothrombin time exceeded the upper limit of the normal range for the institution); acetylsalicylic acid at a dose of more than 650 mg/day within 3 days before the study; thrombolytic therapy within 3 days before the study‡; glycoprotein IIb/IIIa antagonists within 7 days before study entry; antithrombin III at a dose of more than 10,000 U within 12 hours before the study; or protein C within 24 hours before the study. Notes • Acute renal failure was not an exclusion criterion. • Prophylactic treatment with a dose of unfractionated heparin of up to 15,000 U per day was permitted. • Thrombolytic agents were permitted for the treatment of thromboses within a catheter.
Interventions	1. Activated protein C (APC) (Xigris, Eli Lilly, Indianapolis). APC (product from an established mammalian cell line into which the complementary DNA for human protein C was delivered) at doses of 24 µg per kilogram of body weight per hour intravenously at a constant rate from foil-wrapped bags for a total duration of 96 hrs. 2. Placebo (0.9% saline with or without 0.1% human serum albumin). Placebo was delivered at doses of 24 µg per kilogram of body weight per hour intravenously at a constant rate from foil-wrapped bags for a total duration of 96 hrs. Co-intervention: not standardised approach to critical care (e.g., antibiotics, fluids, vasopressors, or ventilatory support). The infusion was stopped 1 hour before any percutaneous procedure or major surgery and was resumed 1 hour and 12 hrs later, respectively, in the absence of bleeding complications. Treatment duration: 96 hrs.
Outcomes	Death from any cause.
Notes	1. A priori sample size estimation Parameters: "Assuming that the true placebo 28-day all-cause mortality rate in the primary analysis population was 30%, this planned sample size was sufficient to ensure greater than 80% power to conclude efficacy if drotrecogin alfa (activated), in truth, was associated with an 18% relative risk reduction in 28-day all-cause mortality" (FDA 2001a). 17/38 (placebo group) and 21/38 (APC group) never received study drug. APC group: 14/21 met at least one exclusion criterion, 4/21 became moribund before the infusion could be started, 3/21 consent was withdrawn before start of infusion. Placebo group: 15/17 did not meet the entry criteria, and 2/17 became moribund before start of infusion. Eli Lilly supplied intervention, employed three of the trial authors, and five trial authors have served as consultants. Eli Lilly supplied intervention, employed two persons for helping in analysis and interpretation of the data, and three clinical research associates.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Block randomization stratified according to site was used, and all assignments were made through a central randomization centre".
Allocation concealment (selection bias)	Low risk	"An unblinded pharmacist at each site will obtain the patient's treatment code from the central randomization centre and prepare the study medication according to the pharmacist's instruction brochure. There are very few anticipated reasons for breaking the randomization code. Given that there is no known treatment to increase the clearance or counter the effects of APC, incidences where unblinding could be beneficial will be extremely rare. The investigator must contact the VCC prior to unblinding a patient's therapy assignment."

PROWESS 2001 *(Continued)*

		Information supplied by Bridget Swindell, RN, Clinical Trials Manager, Vanderbilt Coordinating Center (bridget.swindell@Vanderbilt.Edu). Quote from FDA 2001a: "The patient treatment assignments were provided to investigators by Covance, a contract research organization, via a central randomization centre. The code for the treatment assignment was retained by Covance".
Blinding (performance bias and detection bias) Blinding of providers or participants?	Unclear risk	"The patients, investigators, VCC and Lilly (with the possible exception of study drug coordination and Global Safety Monitoring personnel) were blinded to the study therapy until all patients have completed the protocol and the study has ended." Information supplied by Bridget Swindell, RN, Clinical Trials Manager, Vanderbilt Coordinating Center (bridget.swindell@Vanderbilt.Edu). Comments: trial did not report the appearance of the drug preparations.
Blinding (performance bias and detection bias) Blinding of outcome assessment?	Unclear risk	Quote: "All measurements were performed by a central laboratory". Quote from FDA 2001a: 'All deaths and serious adverse events were reviewed by Lilly in a blinded manner'.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete data for primary outcome available. 38 patients did not receive the allocated drug, with balanced reasons between groups. One patient allocated to the intervention did not receive the drug and was imputed as a negative outcome.
Selective reporting (reporting bias)	Low risk	FDA data available. All outcomes available in the trial publication and reported in results.
Other bias	High risk	Sponsor bias. Design bias (Porta 2008). Trial was stopped early for benefit. Quote: "At the time of the second interim analysis of data from 1520 patients, enrolment was suspended because the differences in the mortality rate between the two groups exceeded the a priori guideline for stopping the trial". (Page 701). This trial was not supplied the stopping criteria.

PROWESS-SHOCK 2012

Methods	Design: parallel-design (2 arms). Multicentre study: 104 centres. International: 18 countries. Follow-up period: 28 days. Intention-to-treat analysis: yes (Quote ''Intention-to-treat'' conducted by Independent Academic Statistical Center and Sponsor) (Finfer 2008).
Participants	Randomized: 1697 patients (852 patients APC group/845 placebo group). Received APC: 1666 patients (833 (97.8%) APC group/ 833 (98.6%) (placebo group). 1. Median age (interquartile range): APC group 2.3 (0.7-7.8) months; placebo group 2.8 (0.7-9.4) months. Total group: 38 weeks corrected gestational age and 17 years. 2. Sex: APC group, male 59.6%; placebo group, male 48.5%.

PROWESS-SHOCK 2012 *(Continued)*

	3. Inclusion criteria (Finfer 2008): Inclusion criteria to obtain informed consent: • aged C 18 years old; • must have an infection requiring intravenous antimicrobial therapy; • must meet at least two of the four systemic inflammatory response syndrome (SIRS) criteria; • must have septic shock, defined as: (a) The patient must have received intravenous fluid resuscitation of ≥ 30 mL/kg administered within the time period spanning the 4 hours before and 4 hours after initiation of vasopressor therapy, (b) The patient must have a continuous requirement for at least one of the vasopressors listed below at the dose shown below for at least four hours: norepinephrine ≥ 5 mcg/min, dopamine ≥ 10 mcg/kg/min, phenylephrine ≥ 25 mcg/min, epinephrine ≥ 5 mcg/min, vasopressin ≥ 0.03 units/min, and (c) The patient must meet at least 1 of the following criteria consistent with hypoperfusion during the 36 hours prior to study entry: metabolic acidosis: base deficit ≥ 5.0 mmol/L or venous bicarbonate\18 mmol/L or lactate <2.5 mMol/L; urine output <0.5 mL/kg h-1 for 1 hour or a 50% increase in creatinine from a known baseline level, acute hepatic dysfunction: AST or ALT> 500 IU/dL or bilirubin >2 g/dL. Inclusion criterion to proceed to randomization • Patients must remain vasopressor dependent throughout the pretreatment period and through the time of randomization with the goal of maintaining a systolic blood pressure of approximately 90 mm Hg or higher or a mean arterial pressure of 65 mm Hg or higher with reasonable attempts made to wean the patient from vasopressor support, if applicable. (Note: dopamine at doses\5 mcg/kg/min does not fulfil the criteria for vasopressor dependency.) 4. Exclusion criteria: • high risk of intracranial bleeding; • expected to die before the end of the 28 days of the study from pre-existing conditions; • end-stage renal or liver disease; • patients with co-existing illnesses with a high risk of death (e.g., metastatic cancer).
Interventions	1. Activated protein C (APC) (Eli Lilly, Indianapolis) 24 µg/kg/hr intravenously for 96 hrs. 2. Placebo: 0.9% saline solution intravenously. Treatment duration: 96 hrs. All other treatments were at the discretion of treating clinicians.
Outcomes	Primary Death from any cause 28 days. Secondary 1. 28-day mortality in patients with severe protein C deficiency (plasma concentration, ≤50% of the lower limit of the normal range). 2. 90-day mortality. 3. Measures of organ dysfunction. 4. Safety.
Notes	ClinicalTrials.gov number, NCT00604214 A priori sample size estimation: yes. Quote: "We determined that the planned enrolment of 1500 patients would provide a power of 80% at a significance level of 0.05 to detect an absolute difference of 7 percentage points (20% relative risk reduction) in the primary outcome of 28-day mortality from the placebo rate of 35%. An independent data and safety monitoring board conducted interim analyses, as described previously.* The protocol specified an increase in sample size if the 28-day mortality for 750 patients was less than 30%." Page 2057 from PROWESS-SHOCK 2012 and * page 1941 from Finfer 2008. Sponsor: Eli Lilly.

PROWESS-SHOCK 2012 *(Continued)*

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org and the protocol of this trial (Finfer 2008).

We contacted to the main author for asking data on 28-day all-cause mortality by APACHE II score, 28-day all-cause mortality by protein C deficiency class, 28-Day all-cause mortality by number of organ dysfunction, and In-hospital mortality. This information was sent by PROWESS-SHOCK 2012 Steering Committee (12 July 2012).

This trial was not stopped for futility. This information was supplied by the main author, 27 June 2012.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote " A centralized system randomly assigned..." (Page 2056). Insufficient information to permit judgment of 'Low risk' or 'High risk'.
Allocation concealment (selection bias)	Unclear risk	Quote " A centralized system randomly assigned..." Insufficient information to permit judgment of 'Low risk' or 'High risk'.
Blinding (performance bias and detection bias) Blinding of providers or participants?	Unclear risk	Quote " patients, care-givers, data collectors, statisticians, the study steering committee, and the clinical coordinating centre will be blinded" Comment: trial did not report the appearance of the drug preparations.
Blinding (performance bias and detection bias) Blinding of outcome assessment?	Unclear risk	Data were not provided to judge if outcome measurement was blinded.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Data for primary outcome available for the 98.9% of the randomized sample, with balanced reasons for withdrawals or losses to follow up.
Selective reporting (reporting bias)	Low risk	All outcomes available in the trial publication and reported in results.
Other bias	High risk	Sponsor bias.

RESOLVE 2007

Methods	Design: parallel-design (2 arms). Multicentre study: 104 centres. International: 18 countries. Follow-up period: 28 days.
Participants	N = 477 patients (240 patients APC group/237 placebo group). Median age (interquartile range): APC group 2.3 (0.7-7.8) months; placebo group 2.8 (0.7-9.4) months. Total group: 38 weeks corrected gestational age and 17 years. 1. Age (years), median (IQR) APC group: 2.3 (0.7 and 7.8) Placebo group: 2·8 (0.7 and 9.4) 2. Sex

RESOLVE 2007 *(Continued)*

	APC group, male 59.6% Placebo group, male 48.5% 3. Inclusion criteria: • suspected or proven infection, and systemic inflammation; • sepsis-induced cardiovascular; • respiratory organ dysfunction within 12 hrs before entering the study. 4. Exclusion criteria: • high risk of intracranial bleeding; • expected to die before the end of the 28 days of the study from pre-existing conditions; • end-stage renal or liver disease; • (see web panel 1 for full inclusion criteria and web panel 2 for full exclusion criteria).
Interventions	1. Activated protein C (APC) (Eli Lilly, Indianapolis) 24 µg/kg/hr intravenously for 96 hrs. 2. Placebo: 0·9% saline. Treatment duration: 96 hrs. The study drug was to be started within 42 hrs of the first documented organ dysfunction and within 24 hrs of dual cardiovascular and respiratory organ dysfunction. Standard of care was at the discretion of the primary physician and not dictated by the trial protocol.
Outcomes	Primary Composite time to complete organ failure resolution (CTCOFR) score. Secondary 1. 28-day mortality. 2. Major amputations. 3. Safety.
Notes	NCT00049764. Date for conducting trial: Enrolment of the patients was between November 2002 and March 2005. Follow up of the enrolled patients (N: 477) was until 3 April 2005. A priori sample size estimation: Yes. Quote "We calculated that a sample size of 600 patients would provide 80% power for a clinically relevant 1·2-day reduction" (Page 839). RoI of founding sources: This trial was funded by Eli Lilly, and the authors from the funding company were involved in all aspects of the study. The decision to stop the study, which was made by the sponsors at the recommendation of the Data Safety Monitoring Board (Page 840) Early termination for futility. Quote "... enrolment was suspended after the second planned interim analysis suggested there was little chance of reaching the efficacy endpoint by completion of the trial." (Page 840). Data from April 21, 2005 re: Discontinuation of Study F1K-MC-EVBP, Investigation of the Efficacy and Safety of Drotrecogin Alfa (Activated) in paediatric Severe Sepsis (http://www.fda.gov/medwatch/safety/2005/xigris_dearHCP_4-21-05.htm).

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote: "block randomization (block size of 4)." (Page 837).

RESOLVE 2007 *(Continued)*

		Insufficient information to permit judgment of 'Low risk' or 'High risk'.
Allocation concealment (selection bias)	Unclear risk	Insufficient information to permit judgment of 'Low risk' or 'High risk'.
Blinding (performance bias and detection bias) Blinding of providers or participants?	Unclear risk	Quote: "All study personnel, except the pharmacist responsible for dispensing study drug, remained unaware of treatment assignment". Comments: trial did not report the appearance of the drug preparations.
Blinding (performance bias and detection bias) Blinding of outcome assessment?	Unclear risk	Data were not provided to judge if outcome measurement was blinded.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Quote: "Analysis was by intention to treat". Data for primary outcome available for the 96.16% of the randomized sample, with balanced reasons for withdrawals or losses to follow up.
Selective reporting (reporting bias)	Low risk	Registration data available [NCT00049764]. All outcomes available in the trial publication and reported in results.
Other bias	High risk	Sponsor bias.

rhAPC Sepsis Study 2001

Methods	Design: Parallel-design (2 arms). Phase: II International multicentre study: 40 centres (USA and Canada). Follow-up period: 28 days.
Participants	N = 135 randomized patients; 131 patients (90 APC group/41 placebo group) for the efficacy and safety analysis. 1. Age APC group 58 ±14 yrs Placebo group 62±16 yrs Total group: 60±15 yrs Range: 19-89 yrs 2. Sex (men) APC group: 63% Placebo group: 66% Total group: 64% 3. Plasma protein C activity (mean level) APC group (N = 88): 43±21% Placebo group (N= 37): 36±17% Total group (N= 125): 41±20% % lower than 40% protein C activity APC group: 47% placebo group: 60% Total group: 50% 131 patients who received study drug (FDA 2001a): 90 received APC (Stage 1: 46 patients; Stage 2: 44 patients); 41 received placebo (Stage 1: 26 patients; Stage 2: 15 patients).

rhAPC Sepsis Study 2001 *(Continued)*

	Post-randomization loss: 4 (one withdrew consent, and three did not meet entry criteria). Inclusion criteria: • ≥18 years; • severe sepsis; • known or suspected site of infection; • evidence of infection: modified definition of systematic inflammatory response syndrome - SIRS (ACCP/SCCM consensus conference), and cardiovascular, renal, or respiratory organ failure; • no more than 24 hrs between meeting SIRS and organ failure entry criteria; • no more than 36 hrs between meeting entry criteria and study drug infusion. Exclusion criteria: • activated partial thromboplastin time (APTT) more than 2 times the upper limit of references range for that institution or platelet count lower than 30,000/mm^3; • active bleeding likely to become life threatening or an increased risk for bleeding; • therapeutic doses of heparin within the previous 8 hrs, acetylsalicylic acid (ASA) doses higher than 650 mg/day within the previous 7 days, or warfarin or thrombolytic treatment within the past month; • anticipated requirement for therapeutic heparin, warfarin or ASA higher than 650 mg/day during the infusion period; • not expected to live more than 6 hr, or not expected to survive 28 days given their pre-existing; medical condition, or known or suspected to have sustained irreversible cessation of all brain function; • end-stage renal disease, receiving either haemodialysis or peritoneal dialysis.
Interventions	1. Activated protein C (APC) (Eli Lilly, Indianapolis). APC (from an established mammalian cell culture) was delivered at two stages. Stage 1: 4 dose tiers of 48 hrs duration infusion, as a continuous intravenous infusion; 18 patients enrolled in each tier. Doses studied in this stage: 12, 18, 24, and 30 µg/kg/hr. Stage 2: 3 dose tiers of 96 hrs duration of infusion, as continuous intravenous infusion; 20 patients in each tier. Doses studied in this stage: 12, 18, and 24 µg/kg/hr. 2. Placebo (saline) Stage 1: 4 dose tiers of 48 hrs duration infusion, as a continuous intravenous infusion; 18 patients enrolled in each tier. Doses studied in this stage: 12, 18, 24, and 30 µg/kg/hr. Stage 2: 3 dose tiers of 96 hrs duration of infusion, as continuous intravenous infusion; 20 patients in each tier. Doses studied in this stage: 12, 18, and 24 µg/kg/hr. 3. Co-intervention: use of antimicrobial agents, intravenous fluids, cardiovascular and respiratory support and surgical intervention left up to treating physician, not prespecified in the protocol. Appropriateness of antimicrobial therapy: not assessed. Infusion rates for individual patients could be decreased from 25% to 75% when bedside whole blood APTT measurements were consistently 95 sec. Treatment duration: 48 hrs (Stage 1); 96 hr (Stage 2). 1. Activated protein C (APC) (Xigris, Eli Lilly, Indianapolis). APC (from an established mammalian cell line using complementary DNA for human protein C) at doses of 24 µg/kg/hr intravenously at a constant rate from foil-wrapped bags for a total duration of 96 hrs. 2. Placebo (0.9% saline with or without 0.1% human serum albumin). Placebo delivered at doses of 24 µg/kg/hr intravenously at a constant rate from foil-wrapped bags for a total duration of 96 hrs. Co-intervention: non standardized approach to critical care (e.g. antibiotics, fluids, vasopressors, or ventilatory support). The infusion was stopped 1 hr before any percutaneous procedure or major surgery and was resumed 1 hr and 12 hrs later, respectively, in the absence of bleeding complications. Treatment duration: 96 hrs.
Outcomes	• Primary 1. Safety (serious adverse events, serious bleeding events). 2. Assessment of antiAPC antibody response.

rhAPC Sepsis Study 2001 *(Continued)*

	• Secondary 1. IL-6 blood levels. 2. Organ failure-free and other. 3. All-cause 28-day mortality.
Notes	1. It did not distinguish APC from endogenous plasma APC. 2. A priori sample estimation: no. 3. This study was funded by Eli Lilly and Company. 4. Disclosure: not given.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient information to permit judgment of 'Low risk' or 'High risk'.
Allocation concealment (selection bias)	Unclear risk	Insufficient information to permit judgment of 'Low risk' or 'High risk'.
Blinding (performance bias and detection bias) Blinding of providers or participants?	Unclear risk	Double blind infusion with the use of a saline. Comment: trial did not report the appearance of the drug preparations.
Blinding (performance bias and detection bias) Blinding of outcome assessment?	Unclear risk	Data were not provided to judge if outcome measurement was blinded. Insufficient information to permit judgment of 'Low risk' or 'High risk'.
Incomplete outcome data (attrition bias) All outcomes	Low risk	135 patients randomized, 131 analysed (1 consent withdrawn, 3 did not fit inclusion criteria).
Selective reporting (reporting bias)	Low risk	No protocol available. All the outcomes listed in the method section described in results.
Other bias	High risk	Sponsor bias.

Dr Gordon Bernard supplied some data from PROWESS 2001 (February 2, 2003).
Dr Jonathan Janes from Ely Liily supplied some data from ADDRESS 2005 (December 8, 2005).
Dr Jonathan Janes from Ely Lilly supplied some data from ADDRESS 2005 (April 16, 2007).

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Alaniz 2010	Narrative review.
Barie 2011a	Observational study.
Barton 2004	Not randomized clinical trial.

Study	Reason for exclusion
Bearden 2002	Narrative review.
Bernard 2004	Not randomized clinical trial.
Bertolini 2007	Survey.
Boyle 2012	Observational study.
Casey 2002	Narrative review.
Costa 2007	Cost-effectiveness study.
Decruyenaere 2009	Non-controlled clinical trial open-label.
Ferrer 2009	Observational study.
Freeman 2003	Meta-analysis.
Goldstein 2006	Non-controlled clinical trial open-label.
Green 2005	Systematic review.
Heslet 2004	Clinical practice guidelines.
Houston 2009	Narrative review.
Kalil 2010a	Meta-analysis.
Kanji 2007	Observational study.
Khan 2010	Systematic review.
Kylat 2012	Cochrane review.
Levi 2007	Randomized controlled trial assessing prophylactic heparin.
Lopez 2010	Observational study.
Lucioni 2002	Cost-effectiveness analysis.
Marraro 2009	Narrative review.
McCoy 2003	Narrative review.
McCoy 2004	Narrative review.
Neilson 2003	Not randomized clinical trial.
Sadique 2011	Observational study.
Sanchez 2010	Observational study.
Sanchez-Garcia 2011	Observational study.
Shorr 2010	Randomized clinical trial comparing two regimens of APC.

Study	Reason for exclusion
van Doorn 2008	Observational study.
Vincent 2005	Not randomized clinical trial.
Wheeler 2008	Observational study.
Wiedermann 2005	Meta-analysis.

DATA AND ANALYSES

Comparison 1. APC versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 28-Day all-cause mortality (paediatric and adult patients)	7	7192	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.88, 1.14]
2 Any-Day all cause mortality (paediatric and adults patients)	7		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.1 28-Day all-cause mortality	7	7192	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.88, 1.14]
2.2 90-Day all-cause mortality	1	411	Risk Ratio (M-H, Random, 95% CI)	1.03 [0.84, 1.26]
3 28-Day all-cause mortality by age of the participants	7	7192	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.88, 1.14]
3.1 28-Day all-cause mortality (paediatric patients)	1	474	Risk Ratio (M-H, Random, 95% CI)	0.98 [0.66, 1.46]
3.2 28-Day all-cause mortality (adult patients)	6	6718	Risk Ratio (M-H, Random, 95% CI)	1.01 [0.87, 1.16]
4 28-Day all-cause mortality by treatment duration	6	6781	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.86, 1.16]
4.1 96 hours	5	6588	Risk Ratio (M-H, Random, 95% CI)	0.97 [0.83, 1.14]
4.2 >96 hours	1	193	Risk Ratio (M-H, Random, 95% CI)	1.26 [0.86, 1.85]
5 28-Day all-cause mortality (according to PROWESS first and second protocol)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
5.1 First protocol	1	720	Risk Ratio (M-H, Random, 95% CI)	0.94 [0.75, 1.17]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
5.2 Second protocol	1	970	Risk Ratio (M-H, Random, 95% CI)	0.71 [0.57, 0.87]
6 28-Day all-cause mortality all trials compared to all trials excluding PROWESS first protocol)	7		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
6.1 Adult and paediatric patients	7	7192	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.88, 1.14]
6.2 Adult and paediatric patients (excluding PROWESS 2001 first protocol)	7	6352	Risk Ratio (M-H, Random, 95% CI)	0.95 [0.74, 1.21]
7 28-Day all-cause mortality by APACHE II score	3		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
7.1 APACHE < 25	3	3999	Risk Ratio (M-H, Random, 95% CI)	1.05 [0.92, 1.20]
7.2 APACHE >= 25	3	1978	Risk Ratio (M-H, Random, 95% CI)	0.96 [0.68, 1.35]
8 28-Day all-cause mortality by protein C deficiency class	2		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
8.1 Protein C deficiency (less than 80% of normal value)	2	2613	Risk Ratio (M-H, Random, 95% CI)	0.91 [0.70, 1.17]
8.2 No protein C deficiency (more than 80% of normal value)	2	290	Risk Ratio (M-H, Random, 95% CI)	0.65 [0.41, 1.04]
8.3 Unknown protein C concentration	1	116	Risk Ratio (M-H, Random, 95% CI)	1.12 [0.60, 2.07]
9 28-Day all-cause mortality, subgroup analysis by baseline organ dysfunction category	2		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
9.1 Single organ dysfunction (<2) in patients with low and high risk of death	2	1778	Risk Ratio (M-H, Random, 95% CI)	1.17 [0.95, 1.45]
9.2 Multiple organ dysfunction (=>2) in patients with low and high risk of death	2	2503	Risk Ratio (M-H, Random, 95% CI)	1.04 [0.91, 1.20]
10 28-Day all-cause mortality, subgroup analysis by baseline organ dysfunction category	2		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
10.1 < 3 dysfuntional organs	2	1224	Risk Ratio (M-H, Random, 95% CI)	1.05 [0.60, 1.86]
10.2 >=3 dysfuntional organs	2	2145	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.67, 1.22]

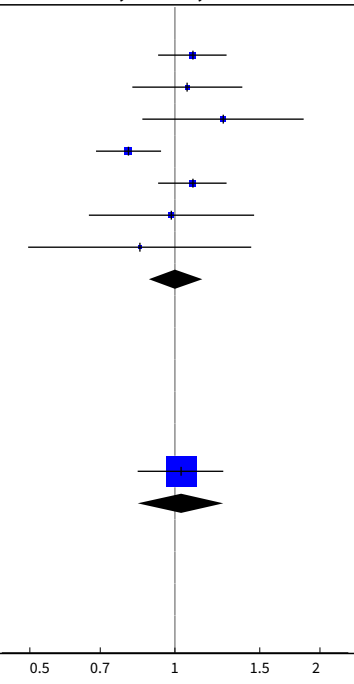
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
11 In-hospital mortality	5	4307	Risk Ratio (M-H, Random, 95% CI)	1.04 [0.94, 1.15]
11.1 PROWESS data	1	510	Risk Ratio (M-H, Random, 95% CI)	0.85 [0.66, 1.10]
11.2 Patients with low risk of death	1	2300	Risk Ratio (M-H, Random, 95% CI)	1.01 [0.85, 1.20]
11.3 Paediatric patients	1	474	Risk Ratio (M-H, Random, 95% CI)	0.98 [0.66, 1.46]
11.4 Patients with prolonged septic shock	1	193	Risk Ratio (M-H, Random, 95% CI)	1.25 [0.93, 1.67]
11.5 Patients with high risk of death	1	830	Risk Ratio (M-H, Random, 95% CI)	1.09 [0.95, 1.24]
12 Serious adverse events	6	7047	Risk Ratio (M-H, Random, 95% CI)	1.02 [0.93, 1.13]
12.1 Adult patients	5	6570	Risk Ratio (M-H, Random, 95% CI)	1.03 [0.93, 1.14]
12.2 Paediatric patients	1	477	Risk Ratio (M-H, Random, 95% CI)	0.97 [0.66, 1.40]
13 Serious bleeding events (days 0 to 90)	7	7178	Risk Ratio (M-H, Random, 95% CI)	1.26 [0.94, 1.67]
13.1 Paediatric patients	1	477	Risk Ratio (M-H, Random, 95% CI)	0.99 [0.51, 1.93]
13.2 Adult patients	6	6701	Risk Ratio (M-H, Random, 95% CI)	1.30 [0.94, 1.82]
14 Serious bleeding during infusion	4	3676	Risk Ratio (M-H, Random, 95% CI)	1.80 [0.96, 3.40]
14.1 Adult patients	3	3277	Risk Ratio (M-H, Random, 95% CI)	2.32 [0.92, 5.83]
14.2 Paediatric patients	1	399	Risk Ratio (M-H, Random, 95% CI)	1.13 [0.42, 3.05]
15 Central nervous system bleeding events in pediatric and adult patients	2	2143	Risk Ratio (M-H, Random, 95% CI)	1.72 [0.72, 4.12]

Analysis 1.1. Comparison 1 APC versus placebo, Outcome 1 28-Day all-cause mortality (paediatric and adult patients).

Study or subgroup	APC n/N	placebo n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
ADDRESS 2005	243/1316	220/1297		21.24%	1.09[0.92,1.28]
APROCCHSS 2013	76/208	70/203		13.95%	1.06[0.82,1.38]
Dhainaut 2009	37/94	31/99		8.33%	1.26[0.86,1.85]
PROWESS 2001	210/850	259/840		22.19%	0.8[0.69,0.94]
PROWESS-SHOCK 2012	223/846	202/834		21.32%	1.09[0.92,1.28]
RESOLVE 2007	41/239	41/235		8.05%	0.98[0.66,1.46]
rhAPC Sepsis Study 2001	26/90	14/41		4.92%	0.85[0.5,1.44]
Total (95% CI)	**3643**	**3549**		**100%**	**1[0.88,1.14]**
Total events: 856 (APC), 837 (placebo)					
Heterogeneity: Tau^2=0.01; Chi^2=11.76, df=6(P=0.07); I^2=48.98%					
Test for overall effect: Z=0.07(P=0.95)					

Analysis 1.2. Comparison 1 APC versus placebo, Outcome 2 Any-Day all cause mortality (paediatric and adults patients).

Study or subgroup	APC n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.2.1 28-Day all-cause mortality					
ADDRESS 2005	243/1316	220/1297		21.24%	1.09[0.92,1.28]
APROCCHSS 2013	76/208	70/203		13.95%	1.06[0.82,1.38]
Dhainaut 2009	37/94	31/99		8.33%	1.26[0.86,1.85]
PROWESS 2001	210/850	259/840		22.19%	0.8[0.69,0.94]
PROWESS-SHOCK 2012	223/846	202/834		21.32%	1.09[0.92,1.28]
RESOLVE 2007	41/239	41/235		8.05%	0.98[0.66,1.46]
rhAPC Sepsis Study 2001	26/90	14/41		4.92%	0.85[0.5,1.44]
Subtotal (95% CI)	**3643**	**3549**		**100%**	**1[0.88,1.14]**
Total events: 856 (APC), 837 (Control)					
Heterogeneity: Tau^2=0.01; Chi^2=11.76, df=6(P=0.07); I^2=48.98%					
Test for overall effect: Z=0.07(P=0.95)					
1.2.2 90-Day all-cause mortality					
APROCCHSS 2013	99/208	94/203		100%	1.03[0.84,1.26]
Subtotal (95% CI)	**208**	**203**		**100%**	**1.03[0.84,1.26]**
Total events: 99 (APC), 94 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.26(P=0.79)					
Test for subgroup differences: Chi^2=0.03, df=1 (P=0.85), I^2=0%					

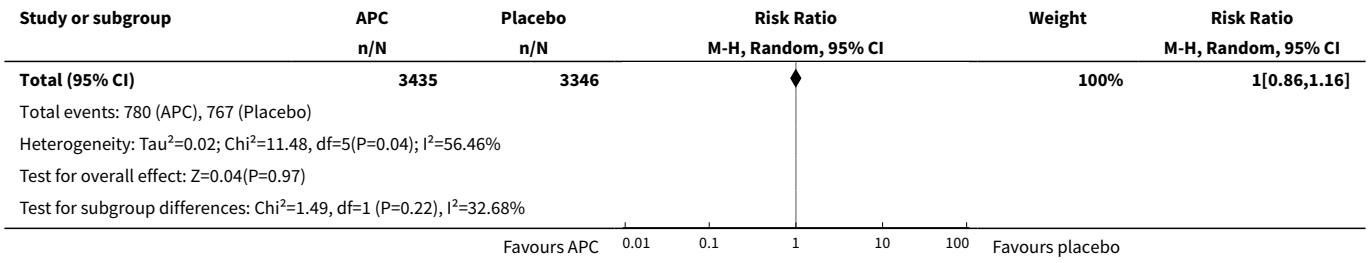


Analysis 1.3. Comparison 1 APC versus placebo, Outcome 3 28-Day all-cause mortality by age of the participants.

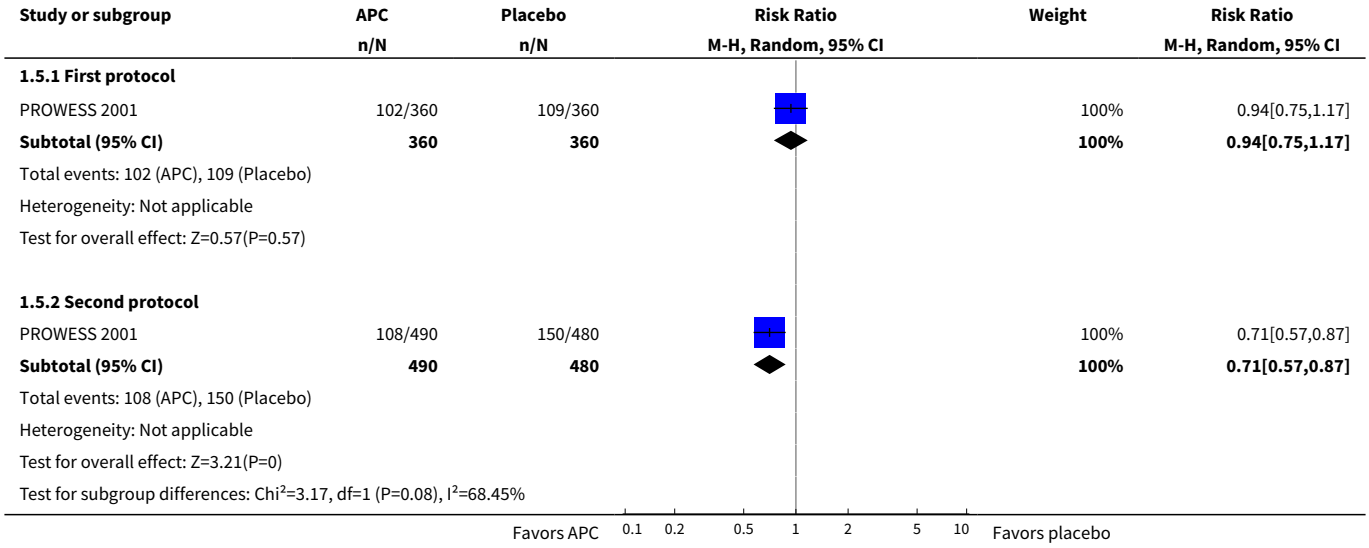
Study or subgroup	APC n/N	Placebo n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.3.1 28-Day all-cause mortality (paediatric patients)					
RESOLVE 2007	41/239	41/235		8.05%	0.98[0.66,1.46]
Subtotal (95% CI)	**239**	**235**		**8.05%**	**0.98[0.66,1.46]**
Total events: 41 (APC), 41 (Placebo)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.08(P=0.93)					
1.3.2 28-Day all-cause mortality (adult patients)					
ADDRESS 2005	243/1316	220/1297		21.24%	1.09[0.92,1.28]
APROCCHSS 2013	76/208	70/203		13.95%	1.06[0.82,1.38]
Dhainaut 2009	37/94	31/99		8.33%	1.26[0.86,1.85]
PROWESS 2001	210/850	259/840		22.19%	0.8[0.69,0.94]
PROWESS-SHOCK 2012	223/846	202/834		21.32%	1.09[0.92,1.28]
rhAPC Sepsis Study 2001	26/90	14/41		4.92%	0.85[0.5,1.44]
Subtotal (95% CI)	**3404**	**3314**		**91.95%**	**1.01[0.87,1.16]**
Total events: 815 (APC), 796 (Placebo)					
Heterogeneity: Tau^2=0.02; Chi^2=11.76, df=5(P=0.04); I^2=57.48%					
Test for overall effect: Z=0.11(P=0.92)					
Total (95% CI)	**3643**	**3549**		**100%**	**1[0.88,1.14]**
Total events: 856 (APC), 837 (Placebo)					
Heterogeneity: Tau^2=0.01; Chi^2=11.76, df=6(P=0.07); I^2=48.98%					
Test for overall effect: Z=0.07(P=0.95)					
Test for subgroup differences: Chi^2=0.01, df=1 (P=0.91), I^2=0%					

Analysis 1.4. Comparison 1 APC versus placebo, Outcome 4 28-Day all-cause mortality by treatment duration.

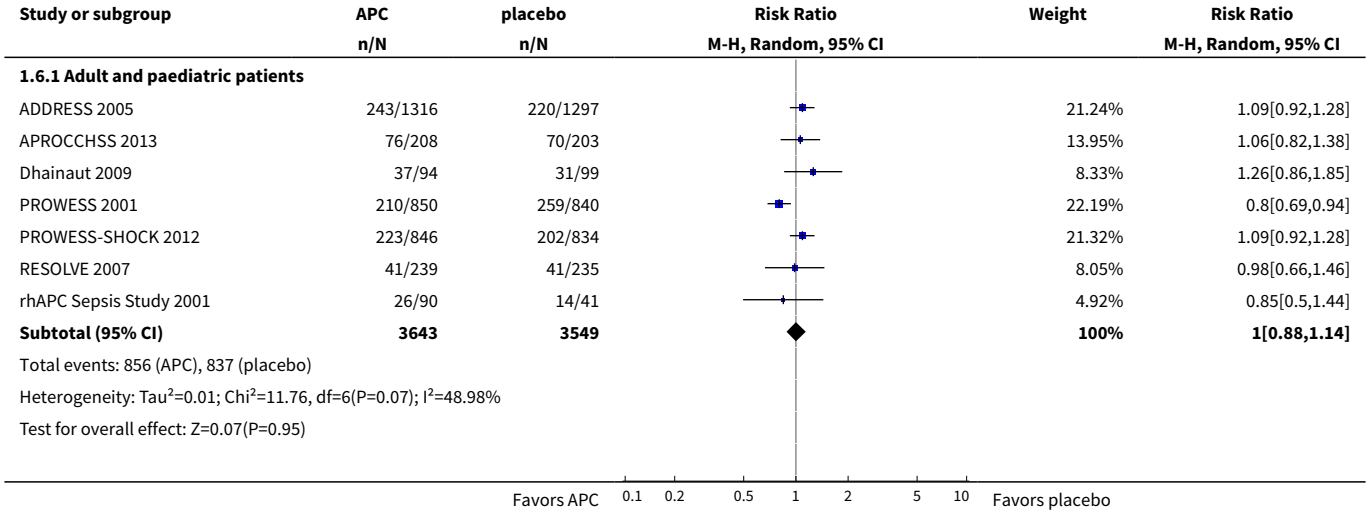
Study or subgroup	APC n/N	Placebo n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.4.1 96 hours					
ADDRESS 2005	243/1316	220/1297		23.99%	1.09[0.92,1.28]
PROWESS 2001	210/850	259/840		24.88%	0.8[0.69,0.94]
PROWESS-SHOCK 2012	223/846	202/834		24.06%	1.09[0.92,1.28]
RESOLVE 2007	41/239	41/235		10.17%	0.98[0.66,1.46]
rhAPC Sepsis Study 2001	26/90	14/41		6.4%	0.85[0.5,1.44]
Subtotal (95% CI)	**3341**	**3247**		**89.5%**	**0.97[0.83,1.14]**
Total events: 743 (APC), 736 (Placebo)					
Heterogeneity: Tau^2=0.02; Chi^2=9.85, df=4(P=0.04); I^2=59.39%					
Test for overall effect: Z=0.37(P=0.71)					
1.4.2 >96 hours					
Dhainaut 2009	37/94	31/99		10.5%	1.26[0.86,1.85]
Subtotal (95% CI)	**94**	**99**		**10.5%**	**1.26[0.86,1.85]**
Total events: 37 (APC), 31 (Placebo)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.17(P=0.24)					

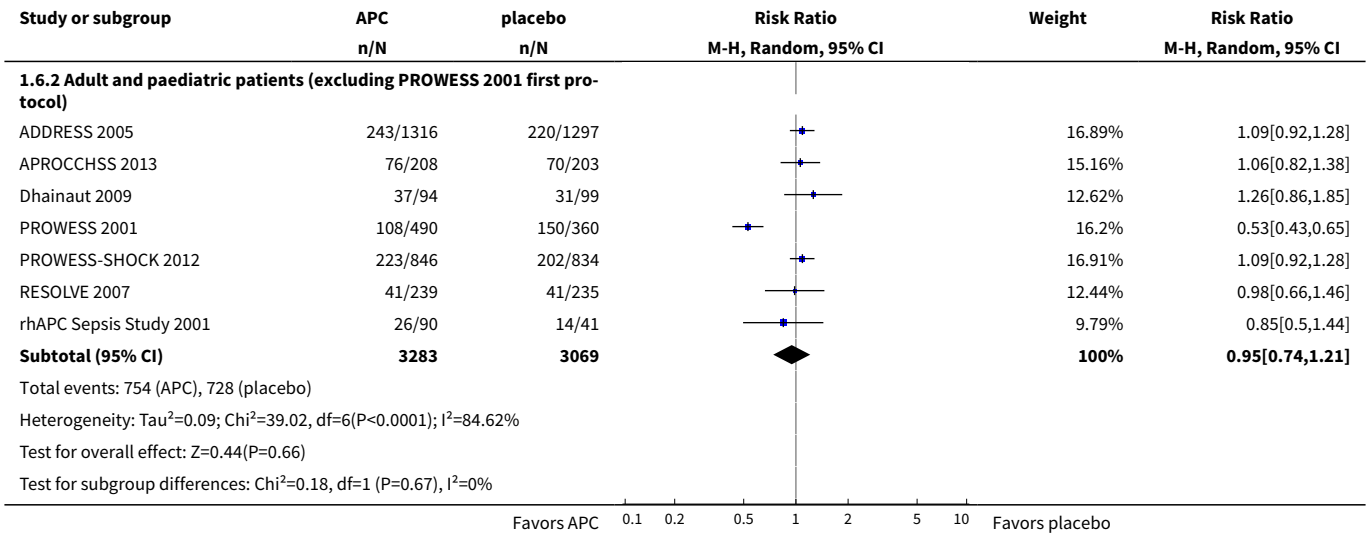


Analysis 1.5. Comparison 1 APC versus placebo, Outcome 5 28-Day all-cause mortality (according to PROWESS first and second protocol).

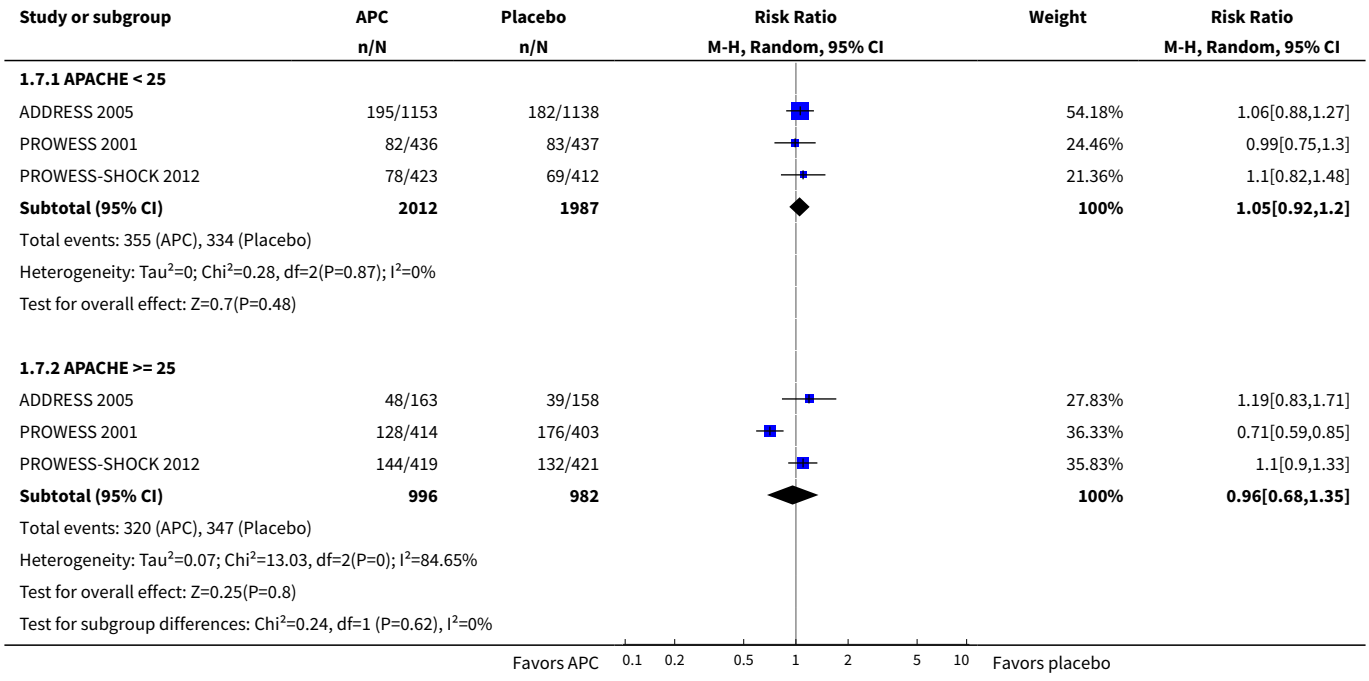


Analysis 1.6. Comparison 1 APC versus placebo, Outcome 6 28-Day all-cause mortality all trials compared to all trials excluding PROWESS first protocol).

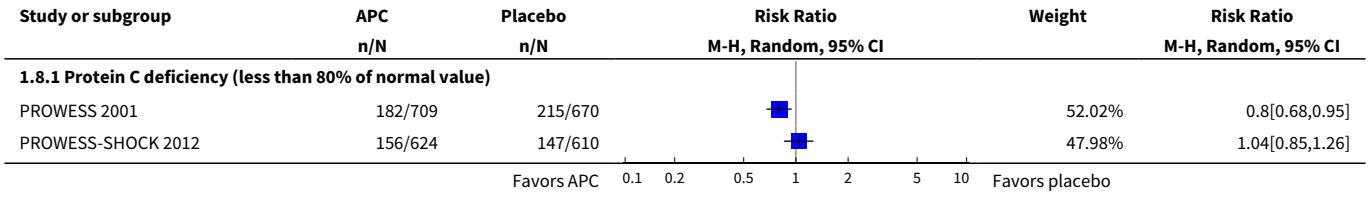


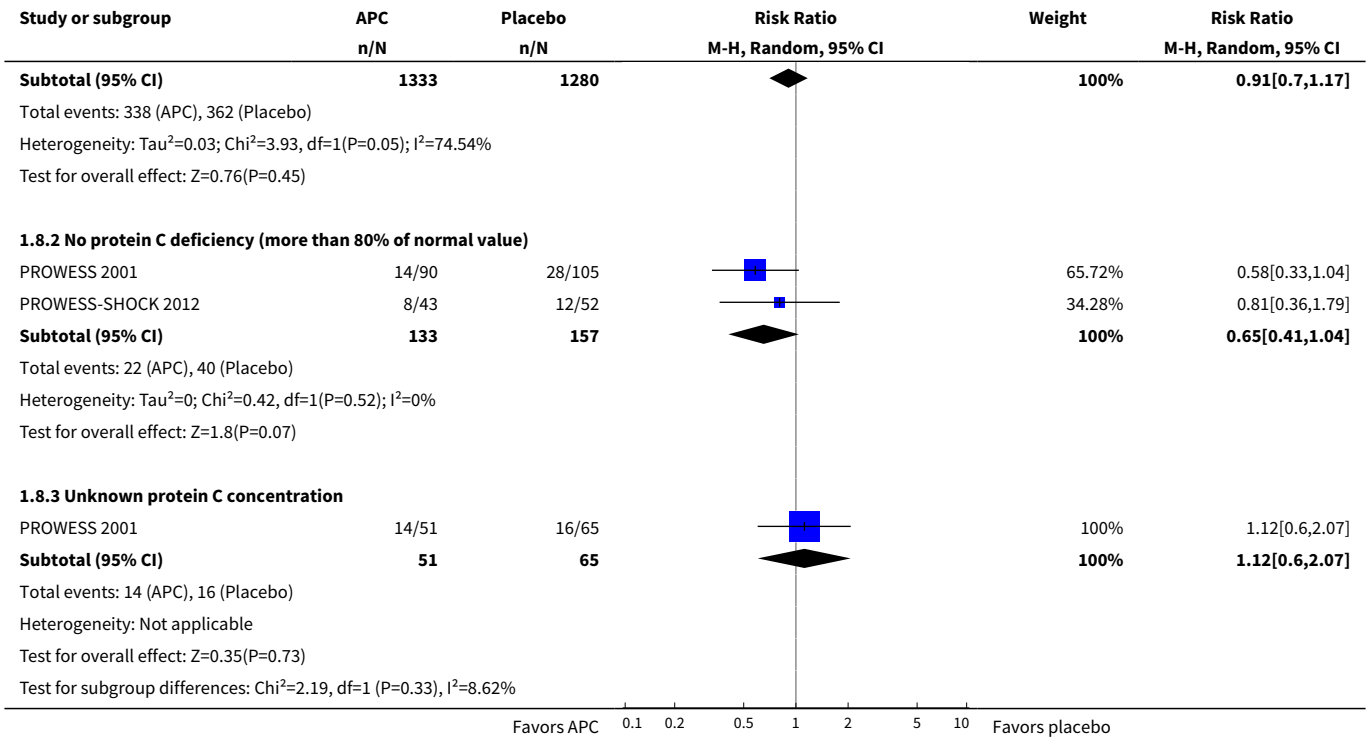


Analysis 1.7. Comparison 1 APC versus placebo, Outcome 7 28-Day all-cause mortality by APACHE II score.

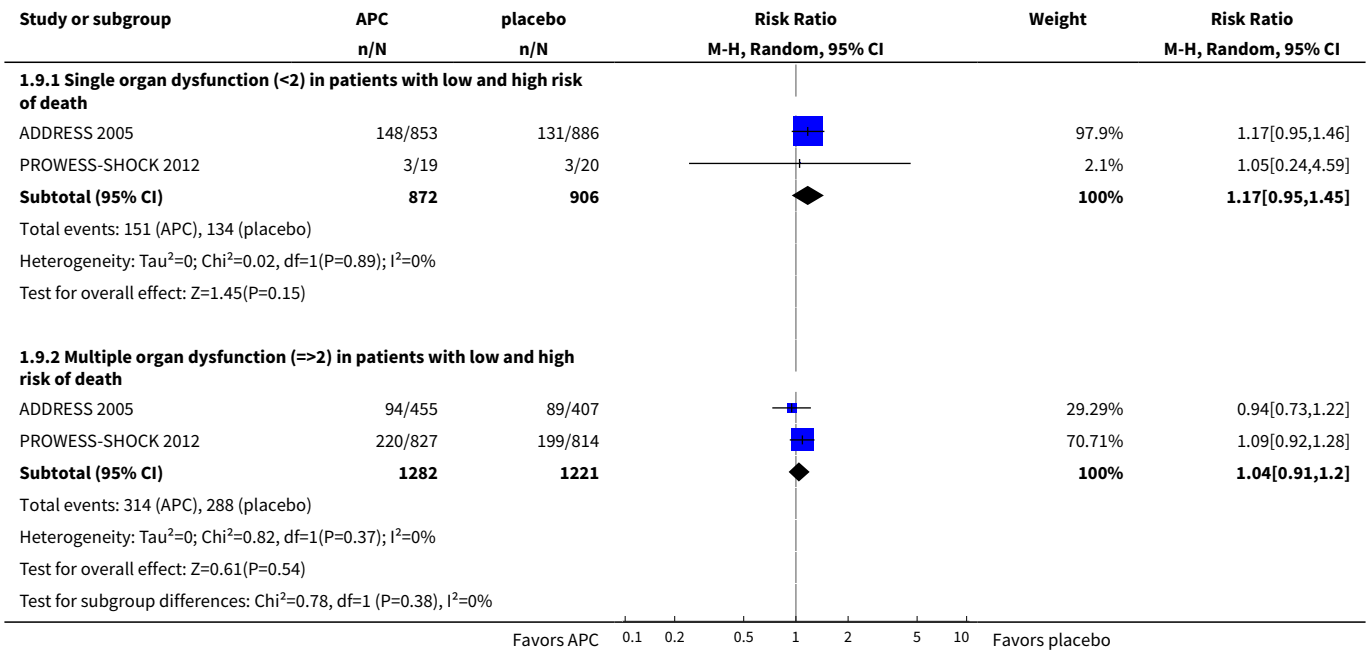


Analysis 1.8. Comparison 1 APC versus placebo, Outcome 8 28-Day all-cause mortality by protein C deficiency class.





Analysis 1.9. Comparison 1 APC versus placebo, Outcome 9 28-Day all-cause mortality, subgroup analysis by baseline organ dysfunction category.

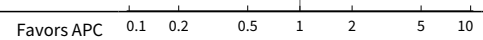


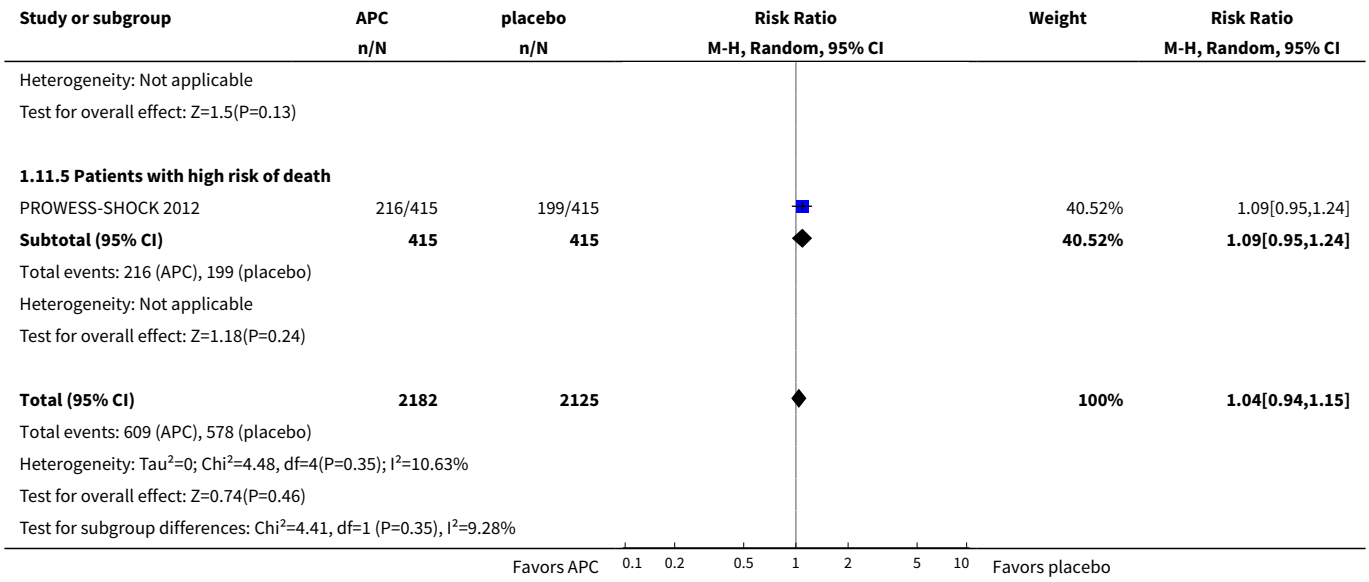
Analysis 1.10. Comparison 1 APC versus placebo, Outcome 10 28-Day all-cause mortality, subgroup analysis by baseline organ dysfunction category.

Study or subgroup	APC n/N	Placebo n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.10.1 < 3 dysfuntional organs					
PROWESS 2001	96/485	114/476		58.99%	0.83[0.65,1.05]
PROWESS-SHOCK 2012	27/132	18/131		41.01%	1.49[0.86,2.57]
Subtotal (95% CI)	**617**	**607**		**100%**	**1.05[0.6,1.86]**
Total events: 123 (APC), 132 (Placebo)					
Heterogeneity: Tau^2=0.13; Chi^2=3.75, df=1(P=0.05); I^2=73.34%					
Test for overall effect: Z=0.18(P=0.86)					
1.10.2 >=3 dysfuntional organs					
PROWESS 2001	112/364	145/364		48.61%	0.77[0.63,0.94]
PROWESS-SHOCK 2012	196/714	184/703		51.39%	1.05[0.88,1.25]
Subtotal (95% CI)	**1078**	**1067**		**100%**	**0.9[0.67,1.22]**
Total events: 308 (APC), 329 (Placebo)					
Heterogeneity: Tau^2=0.04; Chi^2=5.2, df=1(P=0.02); I^2=80.77%					
Test for overall effect: Z=0.66(P=0.51)					
Test for subgroup differences: Chi^2=0.21, df=1 (P=0.64), I^2=0%					

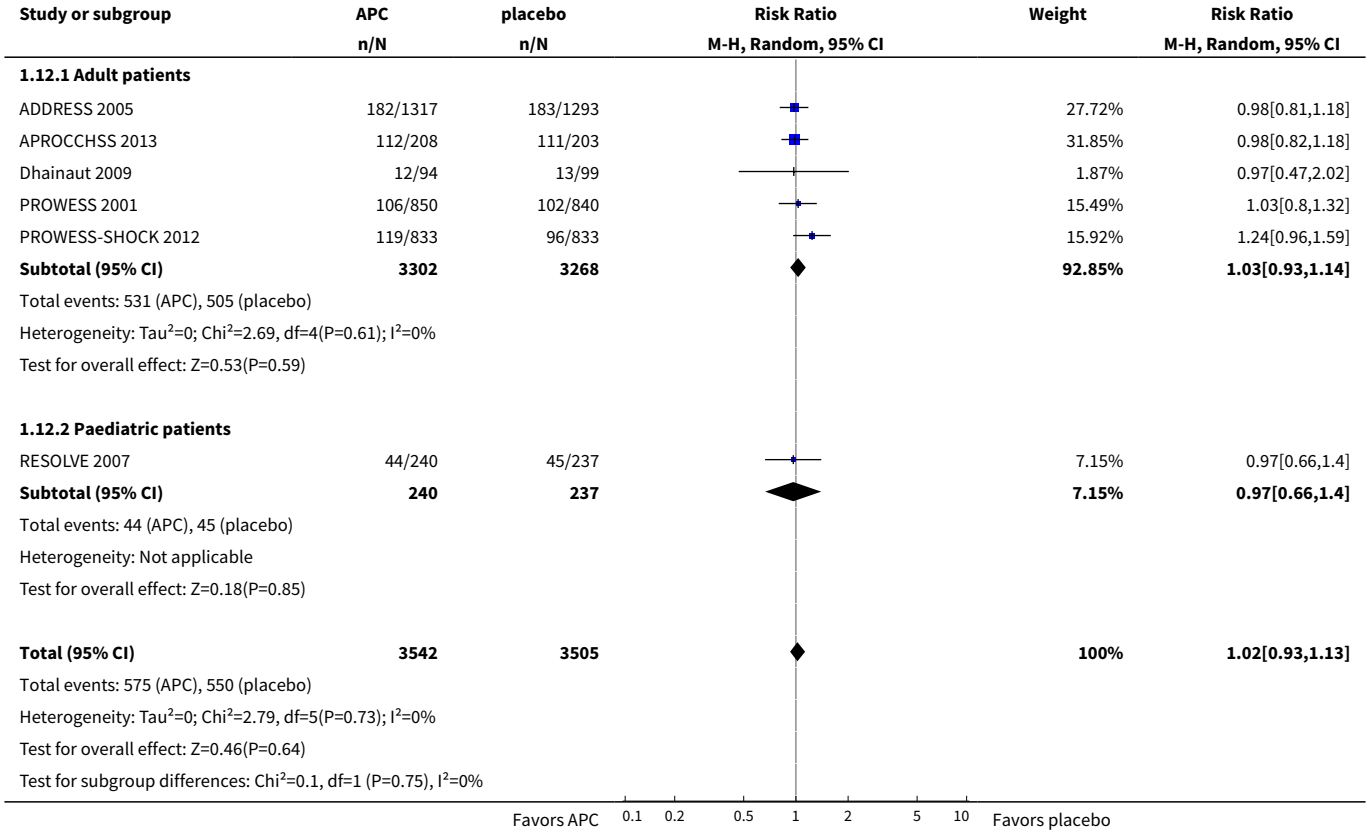
Analysis 1.11. Comparison 1 APC versus placebo, Outcome 11 In-hospital mortality.

Study or subgroup	APC n/N	placebo n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.11.1 PROWESS data					
PROWESS 2001	82/275	82/235		14.19%	0.85[0.66,1.1]
Subtotal (95% CI)	**275**	**235**		**14.19%**	**0.85[0.66,1.1]**
Total events: 82 (APC), 82 (placebo)					
Heterogeneity: Tau^2=0; Chi^2=0, df=0(P<0.0001); I^2=100%					
Test for overall effect: Z=1.22(P=0.22)					
1.11.2 Patients with low risk of death					
ADDRESS 2005	219/1159	213/1141		28.39%	1.01[0.85,1.2]
Subtotal (95% CI)	**1159**	**1141**		**28.39%**	**1.01[0.85,1.2]**
Total events: 219 (APC), 213 (placebo)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.14(P=0.89)					
1.11.3 Paediatric patients					
RESOLVE 2007	41/239	41/235		6.09%	0.98[0.66,1.46]
Subtotal (95% CI)	**239**	**235**		**6.09%**	**0.98[0.66,1.46]**
Total events: 41 (APC), 41 (placebo)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.08(P=0.93)					
1.11.4 Patients with prolonged septic shock					
Dhainaut 2009	51/94	43/99		10.8%	1.25[0.93,1.67]
Subtotal (95% CI)	**94**	**99**		**10.8%**	**1.25[0.93,1.67]**
Total events: 51 (APC), 43 (placebo)					



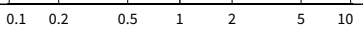


Analysis 1.12. Comparison 1 APC versus placebo, Outcome 12 Serious adverse events.



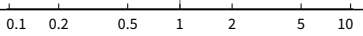
Analysis 1.13. Comparison 1 APC versus placebo, Outcome 13 Serious bleeding events (days 0 to 90).

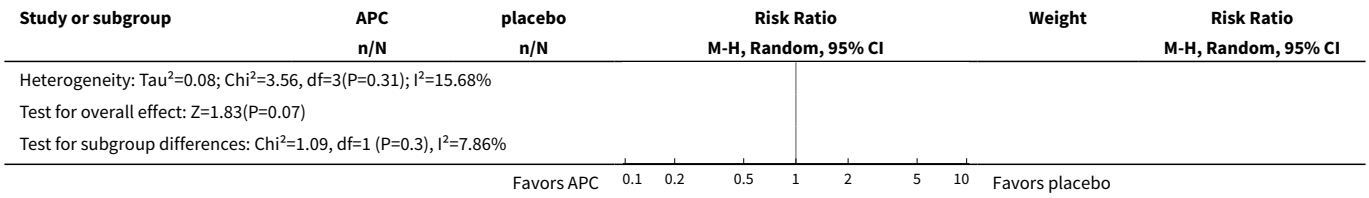
Study or subgroup	APC n/N	placebo n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.13.1 Paediatric patients					
RESOLVE 2007	16/240	16/237		15.15%	0.99[0.51,1.93]
Subtotal (95% CI)	**240**	**237**		**15.15%**	**0.99[0.51,1.93]**
Total events: 16 (APC), 16 (placebo)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.04(P=0.97)					
1.13.2 Adult patients					
ADDRESS 2005	51/1317	28/1293		27.5%	1.79[1.14,2.82]
APROCCHSS 2013	26/208	29/203		24.51%	0.88[0.53,1.43]
Dhainaut 2009	1/94	2/99		1.41%	0.53[0.05,5.71]
PROWESS 2001	30/850	17/840		18.74%	1.74[0.97,3.14]
PROWESS-SHOCK 2012	10/833	8/833		8.62%	1.25[0.5,3.15]
rhAPC Sepsis Study 2001	5/90	3/41		4.07%	0.76[0.19,3.03]
Subtotal (95% CI)	**3392**	**3309**		**84.85%**	**1.3[0.94,1.82]**
Total events: 123 (APC), 87 (placebo)					
Heterogeneity: Tau^2=0.04; Chi^2=6.47, df=5(P=0.26); I^2=22.73%					
Test for overall effect: Z=1.57(P=0.12)					
Total (95% CI)	**3632**	**3546**		**100%**	**1.26[0.94,1.67]**
Total events: 139 (APC), 103 (placebo)					
Heterogeneity: Tau^2=0.02; Chi^2=7.11, df=6(P=0.31); I^2=15.67%					
Test for overall effect: Z=1.57(P=0.12)					
Test for subgroup differences: Chi^2=0.53, df=1 (P=0.47), I^2=0%					



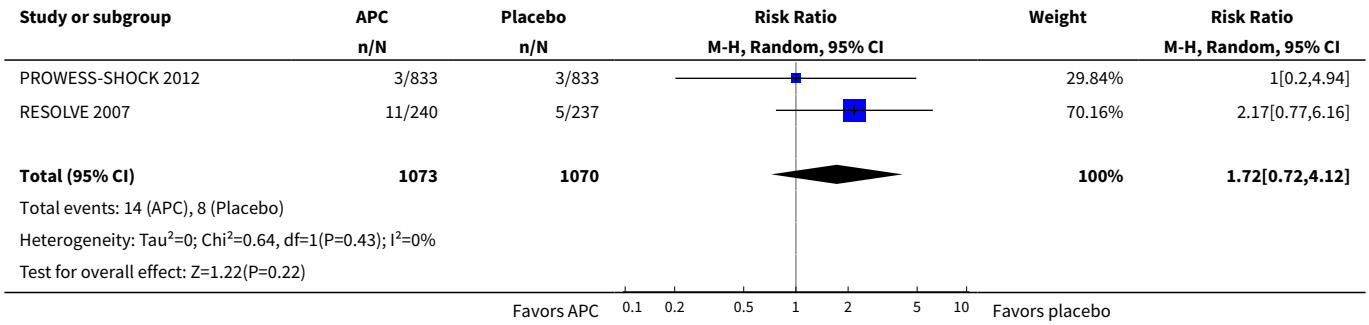
Analysis 1.14. Comparison 1 APC versus placebo, Outcome 14 Serious bleeding during infusion.

Study or subgroup	APC n/N	placebo n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.14.1 Adult patients					
ADDRESS 2005	31/1317	15/1293		59.25%	2.03[1.1,3.74]
Dhainaut 2009	1/94	1/99		5.06%	1.05[0.07,16.6]
PROWESS 2001	7/228	0/246		4.73%	16.18[0.93,281.68]
Subtotal (95% CI)	**1639**	**1638**		**69.03%**	**2.32[0.92,5.83]**
Total events: 39 (APC), 16 (placebo)					
Heterogeneity: Tau^2=0.18; Chi^2=2.33, df=2(P=0.31); I^2=14.08%					
Test for overall effect: Z=1.78(P=0.07)					
1.14.2 Paediatric patients					
RESOLVE 2007	8/201	7/198		30.97%	1.13[0.42,3.05]
Subtotal (95% CI)	**201**	**198**		**30.97%**	**1.13[0.42,3.05]**
Total events: 8 (APC), 7 (placebo)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.23(P=0.82)					
Total (95% CI)	**1840**	**1836**		**100%**	**1.8[0.96,3.4]**
Total events: 47 (APC), 23 (placebo)					





Analysis 1.15. Comparison 1 APC versus placebo, Outcome 15 Central nervous system bleeding events in pediatric and adult patients.



APPENDICES

Appendix 1. Acute Physiology And Chronic Health Evaluation (APACHE)

Definition	APACHE I	APACHE II	Score interpretation
Scale for measuring the severity of disease of hospitalized patients. It was created by Dr William A Knaus in June 1978.	System original with 33 physiologic measurements. (June 1978).	Simplified version with 12 physiologic measurements and more precisely represented the complex interactions of diseases and severity of prognosis (released 1985).	Score interpretation 0-4 ˜4% death rate; 5-9 ˜8% death rate;10-14 ˜15% death rate; 15-19 ˜25% death rate; 20-24 ˜40% death rate; 25-29 ˜55% death rate; 30-34 ˜75% death rate; over 34 ˜85% death rate. http://www.emedicine.com/splash/etools_xml.pl?file=apache_ii_score_for_adults&prog=edecision

Appendix 2. Sepsis-related organ failure (SOFA) score

The SOFA score is composed of scores from six organ systems: respiratory, cardiovascular, hepatic, coagulation, renal, and neurological graded from 0 to 4 according to the degree of dysfunction or failure.	1.Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, 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. Janssens U, Dujardin R, Graf J, Lepper W, J Ortlepp, Merx M, et al . Value of SOFA (Sequential Organ Failure Assessment) score and total maximum SOFA score in 812 patients with acute cardiovascular disorders. Critical Care 2001; 5 Suppl:225.

Appendix 3. Search strategy for CENTRAL, *The Cochrane Library*

#1 MeSH descriptor Anticoagulants explode all trees
#2 anticoagulant*
#3 MeSH descriptor Protein C explode all trees
#4 protein next C
#5 MeSH descriptor Recombinant Proteins explode all trees
#6 recombinant near protein*
#7 MeSH descriptor Blood Coagulation Factor Inhibitors explode all trees
#8 blood coagulation factor inhibitor*
#9 MeSH descriptor Disseminated Intravascular Coagulation explode all trees
#10 disseminated intravascular coagulation
#11 drotrecogin near alfa
#12 apc or rh?APC or rhAPC
#13 recombinant human activated protein c
#14 (#1 OR #2 OR #3 OR #4 OR #5 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13)
#15 MeSH descriptor Sepsis explode all trees
#16 MeSH descriptor Shock, Septic explode all trees
#17 MeSH descriptor Systemic Inflammatory Response Syndrome explode all trees
#18 Septicemia:ab,ti
#19 seps* or (sept* near shock*) or (sepsis next syndrome) or septic?em*
#20 (#15 OR #16 OR #17 OR #18 OR #19)
#21 (#14 AND #20)

Appendix 4. Search strategy for MEDLINE (OvidSP)

1. exp Anticoagulants/ or exp Protein-C/ or exp Blood-Coagulation-Factor-Inhibitors/ or exp Recombinant-Proteins/ or exp Disseminated-Intravascular-Coagulation/
2. anticoagulant*.ti,ab. or (APC alfa or APC or rh?APC or rhAPC or protein C or recombinant protein* or blood coagulation factor inhibitor* or disseminated intravascular coagulation or recombinant human activated protein C).mp.
3. 1 or 2
4. (((seps* or sept*) adj3 shock*) or sepsis syndrome or septic?em*).mp.
5. exp Sepsis-Syndrome/ or Sepsis/ or exp SHOCK SEPTIC/ or exp SEPSIS SYNDROME/ or exp SEPTICEMIA/
6. 4 or 5
7. 6 and 3
8. ((randomised controlled trial or controlled clinical trial).pt. or randomised.ab. or placebo.ab. or clinical trials as topic.sh. or randomly.ab. or trial.ti.) not (animals not (humans and animals)).sh.
9. 8 and 7

Appendix 5. Search strategy for EMBASE (OvidSP)

1. exp anticoagulant-agent/ or exp activated-protein-C/ or exp protein-C/ or exp blood-clotting-inhibitor/ or exp disseminated-intravascular-clotting/
2. (Protein?C or recombinant protein* or blood coagulation factor inhibitor* or disseminated intravascular coagulat* or drotrecogin alfa or APC or rh?APC or rhAPC or recombinant human activated protein C).mp. or anticoagulant*.ti,ab.
3. 1 or 2
4. sepsis/ or exp septic-shock/ or exp septicemia/
5. (((seps* or sept*) adj3 shock*) or sepsis syndrome or septic?em*).mp.
6. 4 or 5
7. 6 and 3
8. (placebo.sh. or controlled study.ab. or random*.ti,ab. or trial*.ti,ab. or ((singl* or doubl* or tripl*) adj3 (blind* or mask*)).mp.) not (animals not (humans and animals)).sh.
9. 8 and 7

Appendix 6. Search strategy for LILACS (accessed through Biblioteca Virtual em Saúde)

1. Sepsis OR septic shock
2.- Drotrecogin
3.- 1 AND 2

Appendix 7. Search strategy for BIOSIS Previews (OvidSP)

1. anticoagulant*.ti,ab. or (APC alfa or APC or rh?APC or rhAPC or protein C or recombinant protein* or blood coagulation factor inhibitor* or disseminated intravascular coagulation or recombinant human activated protein C).mp.
2. (((seps* or sept*) adj3 shock*) or sepsis syndrome or septic?em*).mp. or (seps* or sept*).ti,ab.
3. 1 and 2
4. (controlled study.ab. or random*.ti,ab. or trial*.ti,ab. or ((singl* or doubl* or tripl*) adj3 (blind* or mask*)).mp.) not (animals not (humans and animals)).mp.
5. 4 and 3

Appendix 8. Search strategy for CINAHL (EBSCOhost)

S1 (MH "Anticoagulants")
S2 (MM "C-Reactive Protein")
S3 (MH "Blood Coagulation Factors")
S4 (MH "Recombinant Proteins")
S5 (MM "Disseminated Intravascular Coagulation")
S6 TI anticoagulant* or AB anticoagulant* or TX (APC alfa or APC or rh?APC or rhAPC) or TX ((protein C) or (recombinant protein*) or (blood coagulation factor inhibitor*) or (disseminated intravascular coagulation) or (recombinant human activated protein C))
S7 S1 or S2 or S3 or S4 or S5 or S6
S8 (MM "Systemic Inflammatory Response Syndrome")
S9 (MH "Sepsis")
S10 (MH "Shock, Septic+")
S11 TX ((seps* or sept*) and shock*) or AB sepsis syndrome or AB septic?em*
S12 S8 or S9 or S10 or S11
S13 S7 and S12
S14 TI random* or AB random* or TI trial* or TX ((single or double or triple) and (blind* or mask*)) or TX (multicenter or crossover) or AB placebo*
S15 S13 and S14

Appendix 9. Secondary objectives first and the second protocol PROWESS 2001

First protocol (FDA 2001a; FDA 2001b)	Second protocol (FDA 2001a; FDA 2001b)
1.To evaluate the effects of APC on organ function (cardiovascular (shock), respiratory, renal, haematologic, and hepatic).	1. "Simplify the primary objective to clarify that there would be a single primary analysis that included all patients meeting the diagnosis of severe sepsis. To this end, references to the protein C deficient subpopulation and the shock subpopulation were removed".
2.To evaluate the health economic impact of APC administration in patients with severe sepsis and/or septic shock.	2. "Clarify exclusion criteria for patients with oesophageal varices"
3.To further characterize pharmacokinetics of APC administration.	3. "Add exclusion criteria for patients having undergone bone marrow, lung, liver, pancreas, or small bowel transplantation".
	4. "Add exclusion criteria for patients who were considered moribund and where death was imminent (within 24 hours)".
	5. "Add exclusion criteria for patients whose family had not committed to aggressive management of the patient".
	6. "Add exclusion criteria for patients with acute pancreatitis without known infection".
	7. "Clarify exclusion criteria for patients with a history of malignancy".

(Continued)

	8. "Add exclusion criteria for patients having organ failure for greater than 24 hours at the time of meeting all inclusion criteria".
	9. "Change placebo from normal saline to 0.1% human serum albumin".
	10. "Replace “septic shock status” with “Protein C activity class” as a covariate for the primary analysis".

Appendix 10. Baseline characteristics by PROWESS 2001 first protocol compared second protocol

Characteristics (FDA 2001b)	First (N = 720)	Second (N= 970)
Patients with > 1 condition	321 (45%)	260 (27%)
Patients with no condition	399 (55%)	710 (73%)
History of allergic reaction	80 (11%)	1 (0%)
History of pneumonia	46 (6%)	17 (2%)
1st induced organ failure multiple	105 (15%)	87 (9%)
1st induced organ failure acidosis	87 (12%)	40 (4%)
28-day all-cause mortality	211 (29.3%)	258 (26.5%)

Appendix 11. Primary objectives according to first and the second protocol of PROWESS 2001

First protocol (FDA 2001a; FDA 2001b)	Second protocol (FDA 2001a; FDA 2001b)
1.To demonstrate that APC reduces 28-day mortality in patients with severe sepsis and/or septic shock.	1.To demonstrate that APC reduces 28-day mortality in patients with severe sepsis
2.To demonstrate that APC reduces 28-day mortality in protein C deficient patients with severe sepsis and/or septic shock.	-

Appendix 12. Exclusion criteria of the patients in the PROWESS trial from PROWESS 2001

Reason
1. Pregnancy or breastfeeding.

(Continued)

2. Age <18 yr or weight >135 kg.
3. Platelet count <30,000/mm3.
4. Patient with conditions that increased the risk of bleeding.
5. Patient with known hypercoagulable condition.
6. Patient's family, physician, or both not in favour of aggressive treatment of patient or presence of an advanced directive to withhold life-sustaining treatment.
Patient not expected to survive 28 days because of uncorrectable medical condition, such as poorly controlled neoplasm or other end-stage disease.
7. Moribund state in which death was perceived to be imminent.
8. Human immunodeficiency virus infection in association with a last known CD4 count of 50/mm3 or more.
9. History of bone marrow, lung, liver, pancreas, or small-bowel transplantation.
10. Chronic renal failure requiring haemodialysis or peritoneal dialysis (acute renal failure was not an exclusion criterion).
11. Known or suspected systemic hypertension, chronic jaundice, cirrhosis, or chronic ascites.
12. Acute pancreatitis with no established source of infection.
13. Participation in another investigational study within 30 days before the current study.
14. Use of any of the following medications or treatment regimens: warfarin, acetylsalicylic acid, glycoprotein IIb/IIIa antagonists, and low-molecular weight heparin at a higher dose than recommended for prophylactic use.

Appendix 13. RESOLVE trial resolution organ dysfunction definitions

General	Cardiovascular	Respiratory	Renal
The last day the patient needed vasoactive agents, invasive mechanical ventilation, or renal replacement therapy.	Requiring less than 5 µg/kg/min dopamine or dobutamine, or cessation of epinephrine, norepinephrine, phenylephrine, or any other vasoactive agent used for haemodynamic support.	Cessation of invasive mechanical ventilation (i.e., not requiring intermittent positive pressure) including continuous positive airway pressure or bimodal positive airway pressure.	Cessation of renal replacement therapy (peritoneal dialysis, haemodialysis, ultrafiltration, or haemofiltration).

FEEDBACK

Comment from Dr Peter Gøtzsche, 10 August 2007

Summary

I wish to congratulate the authors for a very fine review. The authors conclude that activated protein C (APC) should not be used in sepsis with an APACHE II score of less than 25 or in pediatric patients and that there is very weak evidence supporting APC use in patients with severe sepsis and at high-risk of death.

I believe the conclusion should be stronger than this. This drug should not be used at all. The division after a score of 25 was not preplanned, and the overall result, including all randomized patients, was not statistically significant. In such circumstances, subgroup analyses are inappropriate and dangerously misleading, as the authors also point out. I have compared the results for patients below and above 25 and got P = 0.59, confirming the lack of a difference between the effect in these two subgroups.
We have been fooled so often in the past into believing that various, very expensive drugs for the treatment of sepsis were effective because of their presumed beneficial effects on the coagulation processes. Sadly, the only thing that has been effective with APC was its marketing.

Submitter agrees with default conflict of interest statement:

I certify that I have no affiliations with or involvement in any organization or entity with a financial interest in the subject matter of my feedback

Reply

Thank you for your comments about our Cochrane review "Human recombinant activated protein C for severe sepsis" [1].

Overall, we agree with your comments. The exaggeration of the effectiveness of protein C is further highlighted in the discussion of our review, regarding the methodological concerns and the poorly documented amendment of the protocol of the PROWESS study [2].

In summary, this review found no evidence suggesting that APC should be used for treating patients with severe sepsis or septic shock. Additionally, APC seems to be associated with a higher risk of bleeding. Unless additional RCTs provide evidence of a treatment effect, policy-makers, clinicians and academics should not promote the use of APC.

References
1. Martí-Carvajal 2007

2. Bernard 2001

Contributors

Summary: Dr Peter Gøtzsche 10th August 2007.

Reply: Prof Arturo Martí-Carvajal, on behalf of the authors, 10th September 2007.

WHAT'S NEW

Date	Event	Description
13 December 2018	Amended	Editorial team changed to Cochrane Emergency and Critical Care
6 February 2017	Review declared as stable	No update planned due to intervention no longer being available on the market

HISTORY

Protocol first published: Issue 3, 2003
Review first published: Issue 3, 2007

Date	Event	Description
12 July 2012	New citation required but conclusions have not changed	One new author (Christian Gluud) has joined the review team
11 July 2012	New search has been performed	1. This systematic review has been updated with results of the one new randomized clinical trial (PROWESS-SHOCK 2012). 2. We reran the searches until June 2012 3. We included trial sequential analysis 4. Adverse events was considered as primary outcome.

Date	Event	Description
17 April 2012	Amended	Contact details updated.
15 February 2012	New citation required but conclusions have not changed	Important note (warning) added to the systematic review's abstract, plain language summary, main text conclusion and Published notes in response to the withdrawal of the drug Xigris
15 February 2012	Amended	This systematic review will be updated when results of the PROWESS-SHOCK or other trials are published.
14 March 2011	New citation required but conclusions have not changed	1. This review is an update of the previous Cochrane systematic review (Martí-Carvajal 2008) that included four RCTs, excluded 19 studies and had two ongoing studies. 2. In the previous version (Martí-Carvajal 2008) we searched the databases until 2005. In this updated version we reran the searches until June 16th 2010. 3. This new updated version now includes five RCTs in total. The previous ongoing study NCT00190788 2005 has now been completed. We have assessed it and it meets our inclusion criteria (Dhainaut 2009). This study strengthens but does not change the updated review's conclusions. 4. Change in authors: Georgia Salanti (co-author in Martí-Carvajal 2008) has left the review team. Two new authors (Mr Ivan Solà and Dr Dimitrios Lathyris) have joined the review team of this updated version.
14 March 2011	New search has been performed	1. We found 19 new excluded studies (Bearden 2002; Bertolini 2007; Costa 2007; Decruyenaere 2009; Ferrer 2009; Goldstein 2006; Gullo 2005; Higgins 2005; Hodder 2009; Houston 2009; Kubler 2006; Levi 2008; Lucioni 2002; Mackenzie 2006; Marraro 2009; Riou 2006; van Doorn 2008; Wheeler 2008; Wiedermann 2005. 2. We included three new ongoing studies NCT00625209; NCT00604214; NCT00067730. 3. We amended the plain language summary. 4. We included new subheadings in the background, methods and discussion sections. 5. We incorporated risk of bias (ROB) and summary of findings tables (SOF).
31 March 2008	Amended	Converted to new review format.
12 November 2007	New citation required but conclusions have not changed	Substantive amendment. The conclusion of the updated review (in the abstract and the text) was amended in response to Dr Peter C. Gotzsche's comments. The updated review found no evidence suggesting that APC should be used for treating patients with severe sepsis or septic shock

CONTRIBUTIONS OF AUTHORS

Conceiving the review: Arturo Martí-Carvajal (AMC)
Co-ordinating the review: AMC, Andrés Felipe Cardona (AFC), Dimitrios Lathyris (DL)
Undertaking manual searches: AMC, AFC
Screening search results: AMC, AFC
Organizing retrieval of papers: AMC
Screening retrieved papers against inclusion criteria: AMC, AFC

Appraising quality of papers: AMC, Ivan Solà (IS), DL, AFC
Abstracting data from papers: AMC, AFC, IS
Writing to authors of papers for additional information: AMC
Providing additional data about papers: AMC
Obtaining and screening data on unpublished studies: AMC
Data management for the review: AMC, AFC, IS
Entering data into Review Manager (RevMan 5.1): AMC
RevMan statistical data: AMC, DL, Christian Gluud (CG)
Other statistical analysis not using RevMan: AMC, CG
Double entry of data: data entered by person one, AFC; data entered by person two, AMC
Interpretation of data: AMC, IS, CG, AFC, DL
Statistical inferences: ACM, CG, DL
Writing the review: ACM, IS, AFC, DL, CG

Amended the review during the editorial process: ACM, IS
Securing funding for the review: AMC
Guarantor for the review (one author): AMC
Person responsible for reading and checking the review before submission: AMC, IS, DL, AFC, CG

DECLARATIONS OF INTEREST

In 2004, Arturo Martí-Carvajal was employed by Eli Lilly to run a four-hour workshop on 'How to critically appraise clinical trials on osteoporosis and how to teach this'. This activity was not related to his work with The Cochrane Collaboration or any Cochrane review.

In 2007, Arturo Martí-Carvajal was employed by Merck to run a four-hour workshop on 'How to critically appraise clinical trials and how to teach this'. This activity was not related to his work with The Cochrane Collaboration or any Cochrane review.

Ivan Solà: none known.

Christian Gluud: none known.

Dimitrios Lathyris: none known.

Andrés Felipe: none known

Cardona: none known.

SOURCES OF SUPPORT

Internal sources

- Cochrane Hepato Biliary Group, Denmark.

  Academic.

External sources

- Iberoamerican Cochrane Centre, Spain.

  Academic support
- Cochrane Anaesthesia Review Group, Denmark.

  Academic support

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

1. We have inserted a statement at the end of the 'Sensitivity analysis' section of the 'Summary of findings' tables.
2. We have performed a trial sequential analyses using Trial Sequential Analysis software (CTU 2011).
3. For this update we included 'Adverse events' as a primary outcome according to the *Cochrane Handbook for Systematic Reviews of Interventions*.

NOTES

February 2012

Warning: On October 25th 2011, the European Medicines Agency issued a press release on the worldwide withdrawal of Xigris (activated protein C/drotrecogin alfa) from the market by Eli Lilly due to lack of beneficial effect on 28-day mortality in the PROWESS-SHOCK study. Furthermore, Eli Lily has announced the discontinuation of all other ongoing clinical trials. The final results of the PROWESS-SHOCK study are expected to be published in 2012. This systematic review will be updated when results of the PROWESS-SHOCK or other trials are published.

INDEX TERMS

Medical Subject Headings (MeSH)

Age Factors; Anti-Infective Agents [adverse effects] [*therapeutic use]; Cause of Death; Drug Recalls; Early Termination of Clinical Trials; Hospital Mortality; Protein C [adverse effects] [*therapeutic use]; Randomized Controlled Trials as Topic; Recombinant Proteins [adverse effects] [therapeutic use]; Sepsis [*drug therapy] [mortality]; Shock, Septic [drug therapy] [mortality]

MeSH check words

Adult; Child; Humans