Supplementary Material

Contents

Supplementary Material

Polymyxin B-immobilized hemoperfusion and mortality in critically ill adult patients with sepsis/septic shock: a systematic review with meta-analysis and trial sequential analysis

Appendix: Search Strategy

eMethod 1. Search strategies

A. Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) 1946 to Present

Date Searched: 31 Oct 2017
Records Retrieved: 152

1. exp Sepsis/
2. Sepsis-Associated Encephalopathy/
3. Systemic Inflammatory Response Syndrome/
4. Vasoplegia/
5. bacill?emia*.tw,kf.
6. bacter* shock.tw,kf.
7. bacter?emia*.tw,kf.
8. (blood adj2 poison*).tw,kf.
9. candid?emia*.tw,kf.
10. endotox?emia*.tw,kf.
11. endotoxi* shock.tw,kf.
12. fung?emia*.tw,kf.
13. parasit?emia*.tw,kf.
14. (py?emia* or pyohemia*).tw,kf.
15. sepsis.tw,kf.
16. septic.tw,kf.
17. septic?emia*.tw,kf.
18. SIRS.tw,kf.
19. systemic inflammatory response syndrome*.tw,kf.
20. toxic shock.tw,kf.
21. vasopl?egia*.tw,kf.
22. vir?emia*.tw,kf.
23. or/1-22 [Combined MeSH & text words for sepsis]
24. Polymyxin B/
25. aerosporin.tw,kf,nm.
26. PMX*.tw,kf,nm.
27. polymyxin*.tw,kf,nm. or 1404-26-8.rn.
28. Poly RX.tw,kf,nm.
29. toraymyxin*.tw,kf,nm.
30. or/24-29 [Combined MeSH & text words for polymixin]
31. Blood Component Removal/
32. Endotoxins/bl [Blood]
33. exp Hemofiltration/
34. Hemoperfusion/
35. apheres?s.tw,kf.
36. blood component removal*.tw,kf.
37. DHP-PMX.tw,kf.
38. (endotoxin* adj3 (a?sor* or eliminat* or remov*)).tw,kf.
39. h?emadsor*.tw,kf.
40. (h?emo-filtrat* or h?emofiltrat*).tw,kf.
41. (h?emo-diafiltrat* or h?emodiafiltrat*).tw,kf.
42. (h?emo-dialysis or h?emodialysis).tw,kf.
43. (h?emo-perfus* or h?emoperfus*).tw,kf.
44. (h?emo-sor* or h?emosor*).tw,kf.
45. PMX-?HP*.tw,kf.
46. pheres?s.tw,kf.

47. or/31-46 [Combined MeSH & textwords for hemoperfusion]
48. and/30,47 [Combined concepts for PMX hemoperfusion]
49. and/23,48 [Combined searches for sepsis & PMX hemoperfusion]
50. controlled clinical trial.pt.
51. randomized controlled trial.pt.
52. drug therapy.fs.
53. groups.ab.
54. placebo.ab.
55. random*.ab.
56. trial.ab.
57. or/50-56
58. exp animals/ not humans.sh.
59. 57 not 58
60. and/49,59 [Cochrane highly sensitive RCT filter - modified]
61. remove duplicates from 60

B. Database: Ovid Embase 1974 to 2016 Week 22

Date Searched: 31 May 2016
Records Retrieved: 128

1. exp bacteremia/
2. exp fungemia/
3. sepsis/
4. sepsis associated encephalopathy/
5. septic shock/
6. septicemia/
7. systemic inflammatory response syndrome/
8. vasoplegia/
9. bacill?emia*.tw,kw.
10. bacter* shock.tw,kw.
11. bacter?emia*.tw,kw.
12. (blood adj2 poison*).tw,kw.
13. candid?emia*.tw,kw.
14. endotox?emia*.tw,kw.
15. endotoxi* shock.tw,kw.
16. fung?emia*.tw,kw.
17. parasit?emia*.tw,kw.
18. (py?emia* or pyohemia*).tw,kw.
19. sepsis.tw,kw.
20. septic.tw,kw.
21. septic?emia*.tw,kw.
22. SIRS.tw,kw.
23. systemic inflammatory response syndrome*.tw,kw.
24. toxic shock.tw,kw.
25. vasopl?egia*.tw,kw.
26. vir?emia*.tw,kw.
27. or/1-26 [Combined Emtree & text words for sepsis]
28. polymyxin B/
29. aerosporin.tw,kw,tn.
30. PMX*.tw,kw,tn.
31. polymyxin*.tw,kw,tn. or 1404-26-8.rn.

32. Poly RX.tw,kw,tn.
33. toraymyxin*.tw,kw,tn.
34. or/28-33 [Combined Emtree & text words for polymixin]
35. apheresis/
36. endotoxin/
37. hemofiltration/
38. hemoperfusion/
39. apheres?s.tw,kw.
40. blood component removal*.tw,kw.
41. DHP-PMX.tw,kw.
42. (endotoxin* adj3 (a?sor* or eliminat* or remov*)).tw,kw.
43. h?emadsor*.tw,kw.
44. (h?emo-filtrat* or h?emofiltrat*).tw,kw.
45. (h?emo-diafiltrat* or h?emodiafiltrat*).tw,kw.
46. (h?emo-dialysis or h?emodialysis).tw,kw.
47. (h?emo-perfus* or h?emoperfus*).tw,kw.
48. (h?emo-sor* or h?emosor*).tw,kw.
49. PMX-?HP*.tw,kw.
50. pheres?s.tw,kw.
51. or/35-50 [Combined Emtree & textwords for hemoperfusion]
52. and/34,51 [Combined concepts for PMX hemoperfusion]
53. and/27,52 [Combined searches for sepsis & PMX hemoperfusion]
54. clinical trial/
55. crossover procedure/
56. double blind procedure/
57. placebo/
58. prospective study/
59. randomization/
60. randomized controlled trial/
61. single blind procedure/
62. (allocat* adj2 random*).tw.
63. (blind* adj (treble or triple)).tw.
64. double blind*.tw.
65. placebo*.tw.
66. randomi?ed controlled trial*.tw.
67. RCT.tw.
68. single blind*.tw.
69. or/54-68
70. abstract report/
71. case study/
72. case report/
73. letter/
74. or/70-73
75. 69 not 74
76. animals/ not (animals/ and humans/)
77. 75 not 76 [modified SIGN RCT filter: http://www.sign.ac.uk/methodology/filters.html#random]
78. and/53,77 [RCT filter applied to combined searches for sepsis & PMX hemoperfusion]
79. remove duplicates from 78

C. Database: Cochrane Library via Wiley

Date Searched: 31 May 2016

Appendix: Search Strategy

Records Retrieved: 43

ID	Search
#1	[mh Sepsis]
#2	[mh ^"Sepsis-Associated Encephalopathy"]
#3	[mh ^"Systemic Inflammatory Response Syndrome"]
#4	[mh ^Vasoplegia]
#5	bacill*mia*:ti,ab,kw
#6	"bacter* shock":ti,ab,kw
#7	bacter*mia*:ti,ab,kw
#8	(blood near/2 poison*):ti,ab,kw
#9	candid*mia*:ti,ab,kw
#10	endotox*mia*:ti,ab,kw
#11	"endotoxi* shock":ti,ab,kw
#12	fung*mia*:ti,ab,kw
#13	parasit*mia*:ti,ab,kw
#14	(py*mia* or pyohemia*):ti,ab,kw
#15	sepsis:ti,ab,kw
#16	septic:ti,ab,kw
#17	septic*mia*:ti,ab,kw
#18	SIRS:ti,ab,kw
#19	"systemic inflammatory response syndrome*":ti,ab,kw
#20	"toxic shock":ti,ab,kw
#21	vasopl*gia*:ti,ab,kw
#22	vir*mia*:ti,ab,kw
#23	{or #1-#22}
#24	[mh ^"Polymyxin B"]
#25	aerosporin:ti,ab,kw
#26	PMX*:ti,ab,kw
#27	polymyxin*:ti,ab,kw
#28	"Poly RX":ti,ab,kw
#29	toraymyxin*:ti,ab,kw
#30	{or #24-#29}
#31	[mh ^"Blood Component Removal"]
#32	[mh ^Endotoxins/BL]
#33	[mh Hemofiltration]
#34	[mh ^Hemoperfusion]
#35	apheres*s:ti,ab,kw
#36	"blood component removal*":ti,ab,kw
#37	DHP-PMX:ti,ab,kw
#38	(endotoxin* near/3 (a?sor* or eliminat* or remov*)):ti,ab,kw
#39	h*madsor*:ti,ab,kw
#40	(h*mo-filtrat* or h*mofiltrat*):ti,ab,kw
#41	(h*mo-diafiltrat* or h*modiafiltrat*):ti,ab,kw
#42	(h*mo-dialysis or h*modialysis):ti,ab,kw
#43	(h*mo-perfus* or h*moperfus*):ti,ab,kw
#44	(h*mo-sor* or h*mosor*):ti,ab,kw
#45	PMX-?HP*:ti,ab,kw
#46	pheres?s:ti,ab,kw
#47	{or #31-#46}
#48	#30 and #47
#49	#23 and #48

Appendix: Search Strategy

D. Database: CINAHL via EBSCOhost (1937 to current)
Date Searched: 31 May 2016
Records Retrieved: 16

S1. (MH "Bacteremia")
S2. (MH "Fungemia+")
S3. (MH "Sepsis")
S4. (MH "Shock, Septic")
S5. (MH "Systemic Inflammatory Response Syndrome")
S6. "bacter* shock"
S7. bacter#emia*
S8. (blood N2 poison*)
S9. candid#emia*
S10. endotox#emia*
S11. "endotoxi* shock"
S12. fung#emia*
S13. parasit#emia*
S14. (pyaemia* or pyemia* or pyohemia*)
S15. sepsis
S16. septic
S17. septic#emia*
S18. SIRS
S19. "systemic inflammatory response syndrome*"
S20. "toxic shock"
S21. vasopl#egia*
S22. vir#emia*
S23. S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22
S24. (MH "Polymyxin B")
S25. aerosporin
S26. PMX*
S27. polymyxin*
S28. "Poly RX"
S29. toraymyxin*
S30. S24 OR S25 OR S26 OR S27 OR S28 OR S29
S31. (MH "Blood Component Removal")
S32. (MH "Endotoxins/BL")
S33. (MH "Hemofiltration+")
S34. (MH "Hemoperfusion")
S35. apheres?s
S36. "blood component removal*"
S37. DHP-PMX
S38. (endotoxin* n/3 (a?sor* or eliminat* or remov*))
S39. (haemadsor* or hemadsor*)
S40. (haemo-filtrat* or hemo-filtrat* or haemofiltrat* or hemofiltrat*)
S41. (haemo-diafiltrat* or hemo-diafiltrat* or haemodiafiltrat* or hemodiafiltrat*)
S42. (haemo-dialysis or haemodialysis or hemo-dialysis or hemodialysis)
S43. (haemo-perfus* or haemoperfus* or hemo-perfus* or hemoperfus*)
S44. PMX-?HP*
S45. pheres?s

S46. S31 OR S32 OR S33 OR S34 OR S35 OR S36 OR S37 OR S38 OR S39 OR S40 OR S41 OR S42 OR S43 OR S44 OR S45
S47. S30 AND S46
S48. S23 AND S47
S49. (MH "Clinical Trials+")
S50. (MH "Placebos")
S51. (MH "Quantitative Studies")
S52. (MH "Random Assignment")
S53. PT Clinical trial
S54. TX allocat* random*
S55. TX clinic* n1 trial*
S56. TX placebo*
S57. TX randomi* control* trial*
S58. TX ((singl* n1 blind*) or (singl* n1 mask*)) or TX ((doubl* n1 blind*) or (doubl* n1 mask*)) or TX ((tripl* n1 blind*) or (tripl* n1 mask*)) or TX ((trebl* n1 blind*) or (trebl* n1 mask*))
S59. S49 OR S50 OR S51 OR S52 OR S53 OR S54 OR S55 OR S56 OR S57 OR S58
S60. S48 AND S59
S61. ((MH "Vertebrates+") NOT MH Human)
S62. S60 NOT S61

E. Database: PubMed via NCBI Entrez
Date Searched: 1 June 2016
Records Retrieved: 134

((("Sepsis"[Mesh] OR "Sepsis-Associated Encephalopathy"[Mesh] OR "Systemic Inflammatory Response Syndrome"[Mesh:noexp] OR "Vasoplegia"[Mesh] OR bacillaemia[tiab] OR bacillemia[tiab] OR "bacterial shock"[tiab] OR bacteraemia[tiab] OR bacteraemias[tiab] OR bacteremia[tiab] OR bacteremias[tiab] OR "blood poisoning"[tiab] OR candidaemia[tiab] OR candidaemias[tiab] OR candidemia[tiab] OR candidemias[tiab] OR endotoxaemia[tiab] OR endotoxaemias[tiab] OR endotoxemia[tiab] OR endotoxemias[tiab] OR "endotoxic shock"[tiab] OR fungaemia[tiab] OR fungaemias[tiab] OR fungemia[tiab] OR fungemias[tiab] OR parasitaemia[tiab] OR parasitaemias[tiab] OR parasitemia[tiab] OR parasitemias[tiab] OR pyaemia[tiab] OR pyohemia[tiab] OR pyemia[tiab] OR pyemias[tiab] OR sepsis[tiab] OR septic[tiab] OR septicaemia[tiab] OR septicaemias[tiab] OR septicemia[tiab] OR septicemias[tiab] OR SIRS[tiab] OR "systemic inflammatory response syndrome"[tiab] OR "toxic shock"[tiab] OR vasoplegia[tiab] OR vasoplegias[tiab] OR viraemia[tiab] OR viraemias[tiab] OR viremia[tiab] OR viremias[tiab]) AND (("Polymyxin B"[Mesh] OR aerosporin[tiab] OR PMX[tiab] OR "PMX-B"[tiab] OR polymyxin[tiab] OR polymyxins[tiab] OR "Poly RX"[tiab] OR "1404-26-8"[EC/RN Number]) AND ("Blood Component Removal"[Mesh:noexp] OR "Endotoxins/blood"[Mesh:noexp] OR "Hemofiltration"[Mesh] OR "Hemoperfusion"[Mesh] OR aphereses[tiab] OR apheresis[tiab] OR "blood component removal"[tiab] OR "DHP-PMX"[tiab] OR ((endotoxin[tiab] OR endotoxins[tiab]) AND (absorb[tiab] OR absorbed[tiab] OR absorbing[tiab] OR absorbs[tiab] OR absorption[tiab] OR adsorption[tiab] OR eliminate[tiab] OR eliminated[tiab] OR eliminates[tiab] OR eliminating[tiab] OR removal[tiab] OR remove[tiab] OR removed[tiab] OR removes[tiab] OR removing[tiab])) OR haemadsorption[tiab] OR hemadsorption[tiab] OR "haemo-filtration"[tiab] OR haemofiltrate[tiab] OR haemofiltration[tiab] OR "hemo-filtrate"[tiab] OR "hemo-filtration"[tiab] OR hemofiltrate[tiab] OR hemofiltration[tiab] OR "haemo-diafiltration"[tiab] OR haemodiafiltrate[tiab] OR haemodiafiltration[tiab] OR "hemo-diafiltration"[tiab] OR hemodiafiltrate[tiab] OR hemodiafiltration[tiab] OR "haemo-dialysis"[tiab] OR haemodialysis[tiab] OR "hemo-dialysis"[tiab] OR hemodialysis[tiab] OR "haemo-perfusion"[tiab] OR haemoperfuse[tiab] OR haemoperfusion[tiab] OR "hemo-perfusion"[tiab] OR hemoperfusion[tiab] OR phereses[tiab] OR pheresis[tiab]))) AND ("controlled clinical trial"[Publication Type] OR "randomized controlled trial"[Publication Type] OR "drug therapy"[Subheading] OR groups[tiab] OR placebo[tiab] OR random[tiab] OR randomisation[tiab] OR randomised[tiab] OR randomization[tiab] OR randomized[tiab] OR randomly[tiab] OR trial[tiab])) NOT (((Animals[MESH] OR Animal Experimentation[MESH] OR "Models, Animal"[MESH] OR

Vertebrates[MESH]) NOT (Humans[MESH] OR Human experimentation[MESH])) OR (((animals[tiab] OR animal model[tiab] OR rat[tiab] OR rats[tiab] OR mouse[tiab] OR mice[tiab] OR rabbit[tiab] OR rabbits[tiab] OR pig[tiab] OR pigs[tiab] OR porcine[tiab] OR swine[tiab] OR dog[tiab] OR dogs[tiab] OR hamster[tiab] OR hamsters[tiab] OR chicken[tiab] OR chickens[tiab] OR sheep[tiab]) AND (publisher[sb] OR inprocess[sb] OR pubmednotmedline[sb])) NOT (human[ti] OR humans[ti] OR people[ti] OR children[ti] OR adults[ti] OR seniors[ti] OR patient[ti] OR patients[ti])))

F. Database: Ichushi-Web (Inception to Present)
Date Searched: 25 April 2016
Records Retrieved: 1420

#1 敗血症/TH
#2 敗血症/TA
#3 菌血症/TA
#4 #1 or #2 or #3 [Combined thesaurus & text words for sepsis]
#5 "Polymyxin B"/TH
#6 Polymyxins/TH
#7 polymyxin/TA
#8 polymixin/TA
#9 ポリミキシン/TA
#10 ポリミクシン/TA
#11 PMX/TA
#12 #5 or #6 or #7 or #8 or #9 or #10 or #11 [Combined thesaurus & text words for polymixin]
#13 #12 and (PT=症例報告除く)

G. Trial Registry: ClinicalTrials.gov - https://clinicaltrials.gov/
Date Searched: 1 June 2016
Records Selected: 13

Advanced Search >
Recruitment: All Studies
Study Results: All Studies
Study Type: Interventional Studies
Conditions: "Bacteremia" OR "Candidemia" OR "Endotoxemia" OR "Fungemia" OR "Parasitemia" OR "Sepsis" OR "Sepsis-Associated Encephalopathy" OR "Shock, Septic" OR "Systemic Inflammatory Response Syndrome" OR "Viremia"
Interventions: "Polymyxin B" OR "Polymyxins"

H. Trial Registry: WHO International Clinical Trials Registry Platform (ICTRP) - http://apps.who.int/trialsearch/
Date Searched: 1 June 2016
Records Selected: 14

Advanced Search >
Condition: bacteraemia OR bacteremia OR candidaemia OR candidemia OR endotoxaemia OR endotoxemia OR fungaemia OR fungemia OR parasitaemia OR parasitemia OR sepsis OR septic OR SIRS OR systemic inflammatory response syndrome OR viraemia OR viremia
AND
Intervention: aerosporin OR polymyxin OR polymixins OR PMX OR PMX-B OR PMX-HP OR Poly RX OR toramyxin

Appendix: Search Strategy

Recruitment status: ALL

I. Trial Registry: UM UMIN- CTR (Inception to Present) - http://www.umin.ac.jp/ctr/
Date Searched: 25 April 2016
Records Selected: 3

#1 ポリミキシン
#2 PMX
#3 polymixin
#4 #1 or #2 or #3

eMethod 2. Changes from the protocol.

Primary outcomes
PROTOCOL: We defined serious adverse events as hypotension and massive bleeding
AMENDMENT: We collected the number of patients with at least one serious adverse event. A serious adverse event was defined by authors of the original trials or defined as an adverse event that required emergent treatment.

Secondary outcomes
AMENDMENT: As related total mortality was reported in all studies, we added total mortality defined as 28 day mortality or any follow-up duration in each study to secondary outcomes.

Sensitivity analyses
PROTOCOL: We planned to exclude trials for which missing data are imputed.
AMENDMENT: We imputed missing data on 28-day mortality in two ways: imputation with data of death in PMX-HP group, and with data of alive in control group (worst case scenario); and imputation with data of alive in PMX-HP group, and with data of dead in control group (best case scenario).

eTables

eTable 1. Domains for assessment of the risk of bias.

1.	Random sequence generation (selection bias).
2.	Allocation concealment (selection bias).
3.	Blinding of participants and personnel (performance bias).
4.	Blinding of outcome assessment (assessment bias).
5.	Incomplete outcome data reporting (attrition bias).
6.	Selective outcome reporting (reporting bias).
7.	Sponsorship bias.
8.	Other biases (Co-intervention imbalance).

eTable 2. Eligible studies excluded from quantitative synthesis. References in the upper row and countries, study sites, sponsors, study sizes, patient status, and estimated primary completion date are shown in the lower row.

Ongoing

1. Chinese Clinical Trial Register [Internet]. Chengdu (Sichuan): Ministry of Health (China). 2007 Jun 27—. Identifier ChiCTR-IOR-14005248, Efficacy and Safety of Polymyxin B Hemoperfusion (TORAYMYXIN) in Gram-negative Abdominal Sepsis-A prospective, multicenter, randomized controlled trial; 2014 Sep 16; [1 page]. Available from: http://www.chictr.org.cn					
China	Multicenter	Industry sponsored	60	Gram-negative abdominal sepsis	NA
2. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000 Feb 29—. Identifier NCT01756755, Endotoxin Adsorber Hemoperfusion and Microcirculation; 2012 Dec 20; [about 6 screens]. Available from: https://clinicaltrials.gov/ct2/show/NCT01756755					
Taiwan	Multicenter	Investigator initiated	40	Abdominal or High EAA sepsis/ septic shock	Jan 2017
3. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000 Feb 29—. Identifier NCT02413541, The Pilot Study of the Efficacy of Polymyxin-B Hemoperfusion in Critically Ill Patients With Severe Sepsis; 2015 Mar 31; [about 4 screens]. Available from: https://clinicaltrials.gov/ct2/show/NCT02413541					
Thailand	NA	Investigator initiated	90	EAA measured Sepsis	NA
4. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000 Feb 29—. Identifier NCT01948778, The Effects of a Polyethyleneimine-coated Membrane (oXiris™) for Hemofiltration Versus Polymyxin B- Immobilized Fibre Column (Toraymyxin™) for Hemoperfusion on Endotoxin Activity and Inflammatory Conditions in Septic Shock- A Randomized Controlled Pilot Study (ENDoX-study); 2013 Aug 30; [about 5 screens]. Available from: https://clinicaltrials.gov/ct2/show/NCT01948778					
Switzerland	NA	Investigator initiated	30	High EAA septic shock	NA

Trials with inadequate data

5. Nakamura T, Kawagoe Y, Matsuda T, Ueda Y, Koide H. Effects of polymyxin B immobilized fiber on urinary N-acetyl-beta-glucosaminidase in patients with severe sepsis. ASAIO J. 2004;50(6):563-7.					
Japan	NA	NA	120	Sepsis	—

6. Pavlovic G, Bonhomme F, Frei A, Dunn-Siegrist I, Gisselbaeck M, Pugin J. Impact of early per-operative use of polymyxin-B hemoperfusion in septic patients undergoing emergency abdominal surgery [abstract]. In: Proceedings of Euroanaesthesia 2015; 2015 May 30- Jun 2; Berlin, Germany: ESA; 2015. Abstract #10AP5-8.

Switzerland	Single center	NA	28	Abdominal sepsis/ septic shock	—

eTable 3. Summary of risk of bias assessment

a. 28-day mortality

Reference	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Sponsorship bias	Other biases
Vincent, 2005[14]	Low	Low	High	Low	Low	Low	High	Low
Cantaluppi, 2008[31]	Low	Low	High	Low	Low	Low	Low	Low
Cruz, 2009[15]	Low	Low	High	Low	Low	Low	Low	Low
Payen, 2015[17]	Low	Low	High	Low	Low	Low	Low	Low
EUPHRATES, 2017[18]	Low	Low	Low	Low	Low	Low	High	Low

b. The number of patients with at least one serious adverse event

Reference	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Sponsorship bias	Other biases
Vincent, 2005[14]	Low	Low	High	High	Low	Low	High	Low
Payen, 2015[17]	Low	Low	High	High	Low	Low	Low	Low
EUPHRATES, 2017[18]	Low	Low	Low	Low	Low	Low	High	Low

c. Organ dysfunction scores

Reference	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Sponsorship bias	Other biases
Vincent, 2005[14]	Low	Low	High	Low	Low	Low	High	Low
Cantaluppi, 2008[31]	Low	Low	High	Low	Unclear	Low	Low	Low
Cruz, 2009[15]	Low	Low	High	Low	Low	Low	Low	Low
Payen, 2015[17]	Low	Low	High	Low	Low	Low	Low	Low
EUPHRATES, 2017[18]	Low	Low	Low	Low	Low	Low	High	Low

eTable 4. Reported numbers of any adverse events and device-related adverse events.

Study		PMX	Standard
Nakamura, 2003[31]	Number of patients in the population	35	25
	Erythema	1	1
Vincent, 2005[14]	Number of patients in the population	17	18
	Fever	1	0
	Pleural effusions	3	5
	Anemia	4	3
	Fluid overload	3	3
Payen, 2015[17]	Number of patients in the population	119	113
	Severe adverse events	6	3
	Hemorrhagic adverse events	20	20
*EUPHRATES, 2017[18]	Number of patients in the population	212	220
	Infections	45	46
	Cardiac disorders	22	26
	Respiratory, thoracic and mediastinal disorders	23	21
	General disorders and administration site conditions	25	16
	Gastrointestinal disorders	18	7
	Blood and lymphatic system disorders	10	10
	Hepatobiliary disorders	12	7
	Vascular disorders	11	8
	Nervous system disorders	10	8
	Renal and urinary disorders	6	8
	Metabolism and nutrition disorders	8	4
	Injury, poisoning and procedural complications	5	4
	Neoplasms benign, malignant and unspecified	4	3
	Psychitric disorders	3	2
	Musculoskeletal and connective tissue disorders	4	0
	Endocrine disorders	1	0
	Eye disorders	0	1
	Investigations	1	0

Device-related adverse events	Cruz, 2009[15]	Vincent, 2005[14]	Payen, 2015[17]	*EUPHRATES, 2017[18]
Number of patients in the PMX group	34	17	119	212
Cartilage clotting	4	4	25	17
Hypotension	1		2	
Tachycardia	2			
Catheter dysfunction			2	

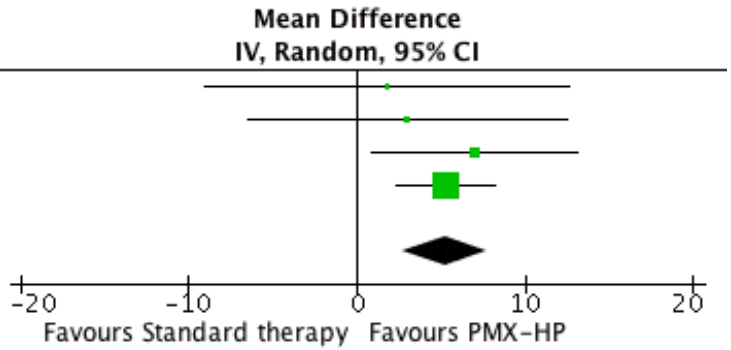
*Data of the safety populations (N=432)

eFigure 1. Mean arterial pressure over 24-72 hours after treatment

Study or Subgroup	PMX-HP Mean	PMX-HP SD	PMX-HP Total	Standard therapy Mean	Standard therapy SD	Standard therapy Total	Weight	Mean Difference IV, Random, 95% CI	Year
Vincent 2005	10.8	13.65	17	9	18.87	18	5.2%	1.80 [-9.07, 12.67]	2005
Cantaluppi 2008	72	10.8	8	69	8.3	8	6.9%	3.00 [-6.44, 12.44]	2008
Cruz 2009	84	11.5	34	77	13.4	30	16.3%	7.00 [0.84, 13.16]	2009
EUPHRATES 2017	9.4	17.3	224	4.1	14.4	226	71.5%	5.30 [2.36, 8.24]	2017
Total (95% CI)			**283**			**282**	**100.0%**	**5.23 [2.75, 7.72]**	

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.92$, df = 3 ($P = 0.82$); $I^2 = 0\%$

Test for overall effect: $Z = 4.12$ ($P < 0.0001$)

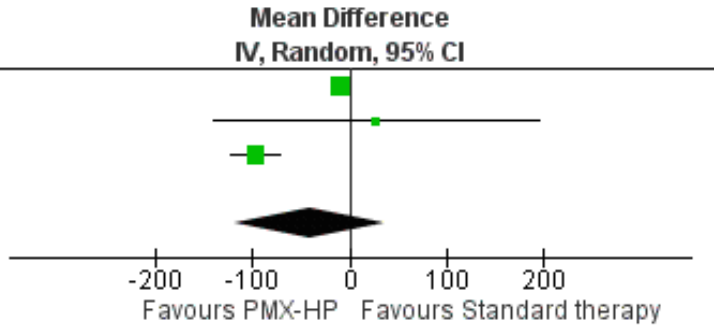


eFigure 2. Endotoxin levels measured by LAL assay over 24-72 hours after treatment

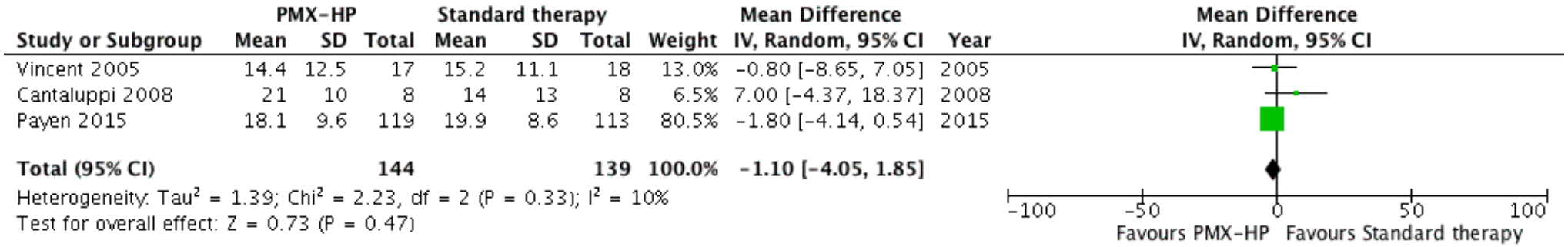
Study or Subgroup	PMX-HP Mean	SD	Total	Standard therapy Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI	Year
Nakamura 2003	4	2.1	35	13.4	3.8	25	43.7%	-9.40 [-11.04, -7.76]	2003
Vincent 2005	120.3	324	16	93.3	114.2	17	14.4%	27.00 [-140.78, 194.78]	2005
Cantaluppi 2008	87.74	19.45	8	184.63	30.56	8	41.8%	-96.89 [-121.99, -71.79]	2008
Total (95% CI)			**59**			**50**	**100.0%**	**-40.77 [-118.53, 36.99]**	

Heterogeneity: Tau² = 3597.21; Chi² = 46.65, df = 2 (P < 0.00001); I² = 96%

Test for overall effect: Z = 1.03 (P = 0.30)



eFigure 3. Vasopressor free days at 28 day

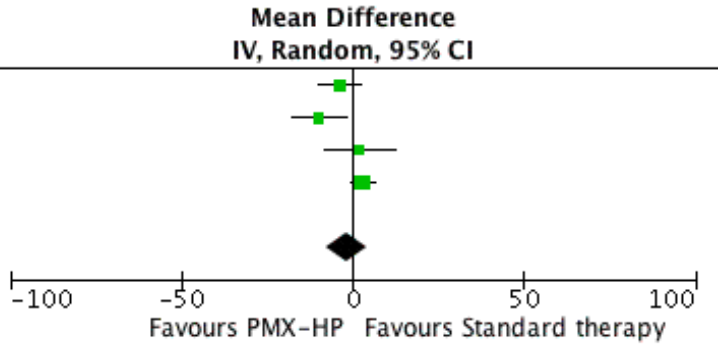


eFigure 4. ICU length of stay

Study or Subgroup	PMX-HP Mean	PMX-HP SD	PMX-HP Total	Standard therapy Mean	Standard therapy SD	Standard therapy Total	Weight	Mean Difference IV, Random, 95% CI	Year
Vincent 2005	13.2	9.4	17	17	9.4	18	26.7%	-3.80 [-10.03, 2.43]	2005
Cantaluppi 2008	15	10	8	25	6	8	22.3%	-10.00 [-18.08, -1.92]	2008
Cruz 2009	20.3	15.2	34	18.3	25.4	30	17.5%	2.00 [-8.43, 12.43]	2009
Payen 2015	16.4	15.9	119	13.6	10.6	113	33.5%	2.80 [-0.66, 6.26]	2015
Total (95% CI)			**178**			**169**	**100.0%**	**-1.95 [-7.91, 4.00]**	

Heterogeneity: $Tau^2 = 24.41$; $Chi^2 = 9.97$, df = 3 (P = 0.02); $I^2 = 70\%$

Test for overall effect: Z = 0.64 (P = 0.52)

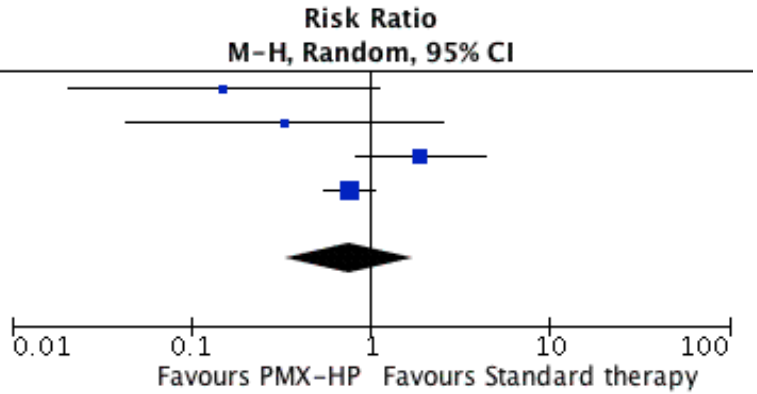


eFigure 5. The need for RRT

Study or Subgroup	PMX-HP Events	PMX-HP Total	Standard therapy Events	Standard therapy Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Vincent 2005	1	17	7	18	12.5%	0.15 [0.02, 1.10]	2005
Cantaluppi 2008	1	8	3	8	12.0%	0.33 [0.04, 2.56]	2008
Cruz 2009	13	34	6	30	31.7%	1.91 [0.83, 4.40]	2009
EUPHRATES 2017	47	224	62	226	43.9%	0.76 [0.55, 1.06]	2017
Total (95% CI)		**283**		**282**	**100.0%**	**0.76 [0.33, 1.71]**	
Total events	62		78				

Heterogeneity: $Tau^2 = 0.37$; $Chi^2 = 7.70$, df = 3 (P = 0.05); $I^2 = 61\%$

Test for overall effect: Z = 0.67 (P = 0.50)

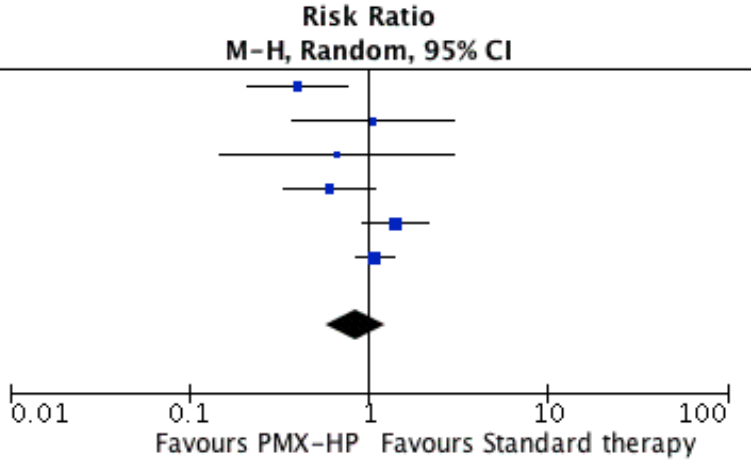


eFigure 6. Mortality for 28day or any follow-up duration

Study or Subgroup	PMX-HP Events	PMX-HP Total	Standard therapy Events	Standard therapy Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Nakamura 2003	9	35	16	25	16.9%	0.40 [0.21, 0.76]	2003
Vincent 2005	5	17	5	18	9.6%	1.06 [0.37, 3.02]	2005
Cantaluppi 2008	2	8	3	8	5.6%	0.67 [0.15, 2.98]	2008
Cruz 2009	11	34	16	30	18.0%	0.61 [0.34, 1.09]	2009
Payen 2015	40	119	27	113	22.7%	1.41 [0.93, 2.13]	2015
EUPHRATES 2017	84	224	78	226	27.2%	1.09 [0.85, 1.39]	2017
Total (95% CI)		**437**		**420**	**100.0%**	**0.85 [0.58, 1.26]**	
Total events	151		145				

Heterogeneity: $Tau^2 = 0.13$; $Chi^2 = 13.99$, df = 5 (P = 0.02); $I^2 = 64\%$

Test for overall effect: Z = 0.81 (P = 0.42)

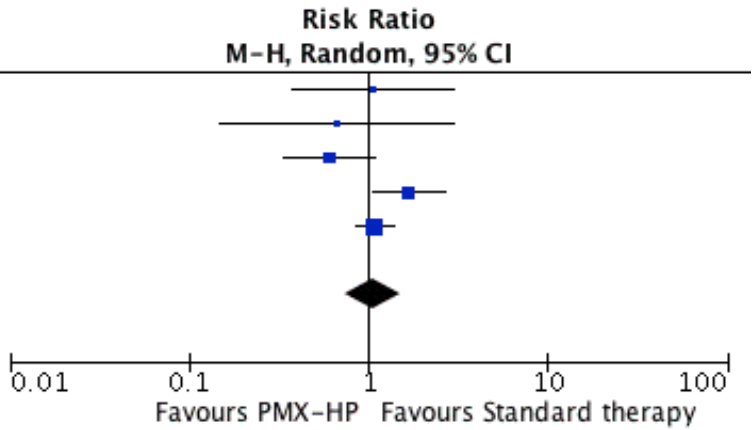


eFigure 7a. Sensitivity analysis: Imputed missing data with worst case scenario

Study or Subgroup	PMX-HP Events	PMX-HP Total	Standard therapy Events	Standard therapy Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Vincent 2005	5	17	5	18	9.3%	1.06 [0.37, 3.02]	2005
Cantaluppi 2008	2	8	3	8	5.0%	0.67 [0.15, 2.98]	2008
Cruz 2009	11	34	16	30	20.6%	0.61 [0.34, 1.09]	2009
Payen 2015	39	125	22	118	26.5%	1.67 [1.06, 2.65]	2015
EUPHRATES 2017	84	224	78	226	38.6%	1.09 [0.85, 1.39]	2017
Total (95% CI)		**408**		**400**	**100.0%**	**1.05 [0.74, 1.50]**	
Total events	141		124				

Heterogeneity: $Tau^2 = 0.07$; $Chi^2 = 7.62$, df = 4 (P = 0.11); $I^2 = 47\%$

Test for overall effect: Z = 0.28 (P = 0.78)

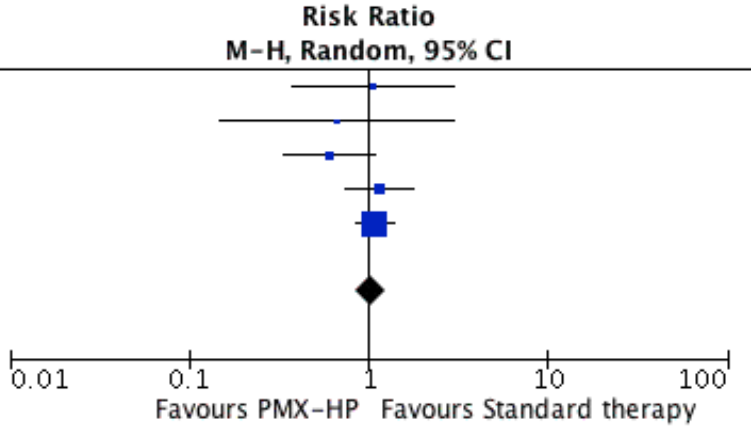


eFigure 7b. Sensitivity analysis: Imputed missing data with best case scenario

Study or Subgroup	PMX-HP Events	PMX-HP Total	Standard therapy Events	Standard therapy Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Vincent 2005	5	17	5	18	3.5%	1.06 [0.37, 3.02]	2005
Cantaluppi 2008	2	8	3	8	1.7%	0.67 [0.15, 2.98]	2008
Cruz 2009	11	34	16	30	11.1%	0.61 [0.34, 1.09]	2009
Payen 2015	33	125	27	118	19.8%	1.15 [0.74, 1.80]	2015
EUPHRATES 2017	84	224	78	226	63.8%	1.09 [0.85, 1.39]	2017
Total (95% CI)		**408**		**400**	**100.0%**	**1.02 [0.84, 1.24]**	
Total events	135		129				

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 3.85$, df = 4 (P = 0.43); $I^2 = 0\%$

Test for overall effect: Z = 0.21 (P = 0.84)

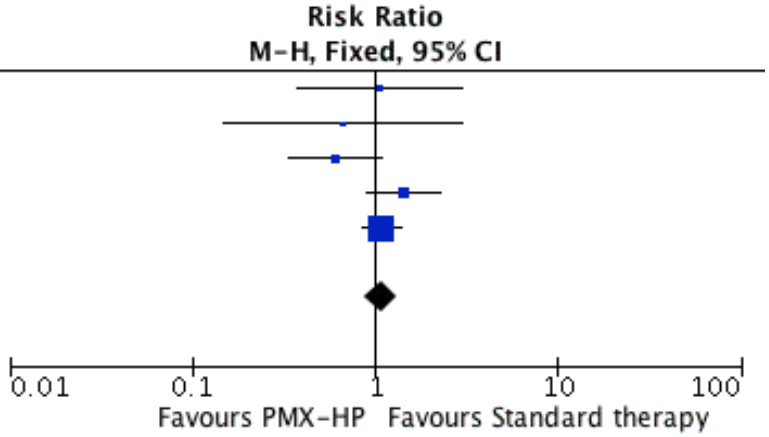


eFigure 8. Sensitivity analysis: Fixed effect model

Study or Subgroup	PMX-HP Events	PMX-HP Total	Standard therapy Events	Standard therapy Total	Weight	Risk Ratio M-H, Fixed, 95% CI	Year
Vincent 2005	5	17	5	18	3.9%	1.06 [0.37, 3.02]	2005
Cantaluppi 2008	2	8	3	8	2.4%	0.67 [0.15, 2.98]	2008
Cruz 2009	11	34	16	30	13.6%	0.61 [0.34, 1.09]	2009
Payen 2015	33	119	22	113	18.0%	1.42 [0.89, 2.29]	2015
EUPHRATES 2017	84	224	78	226	62.1%	1.09 [0.85, 1.39]	2017
Total (95% CI)		**402**		**395**	**100.0%**	**1.07 [0.88, 1.31]**	
Total events	135		124				

Heterogeneity: $Chi^2 = 5.35$, df = 4 (P = 0.25); $I^2 = 25\%$

Test for overall effect: Z = 0.68 (P = 0.50)

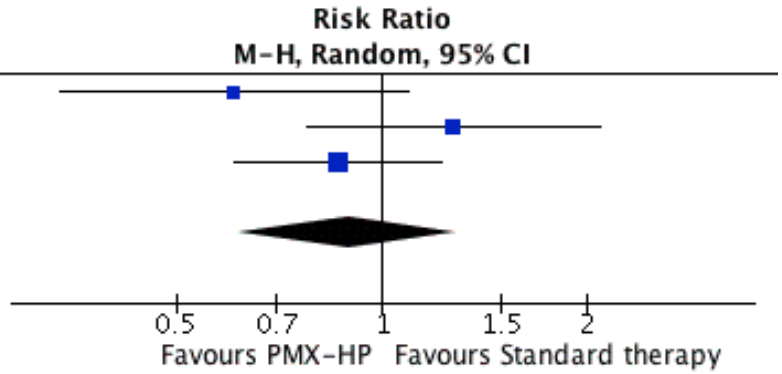


eFigure 9. Sensitivity analysis: per protocol population

Study or Subgroup	PMX-HP Events	PMX-HP Total	Standard Events	Standard Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Cruz 2009	11	34	16	30	25.2%	0.61 [0.34, 1.09]	2009
Payen 2015	28	113	22	113	31.3%	1.27 [0.78, 2.09]	2015
EUPHRATES 2017	36	113	48	130	43.5%	0.86 [0.61, 1.23]	2017
Total (95% CI)		**260**		**273**	**100.0%**	**0.89 [0.62, 1.29]**	
Total events	75		86				

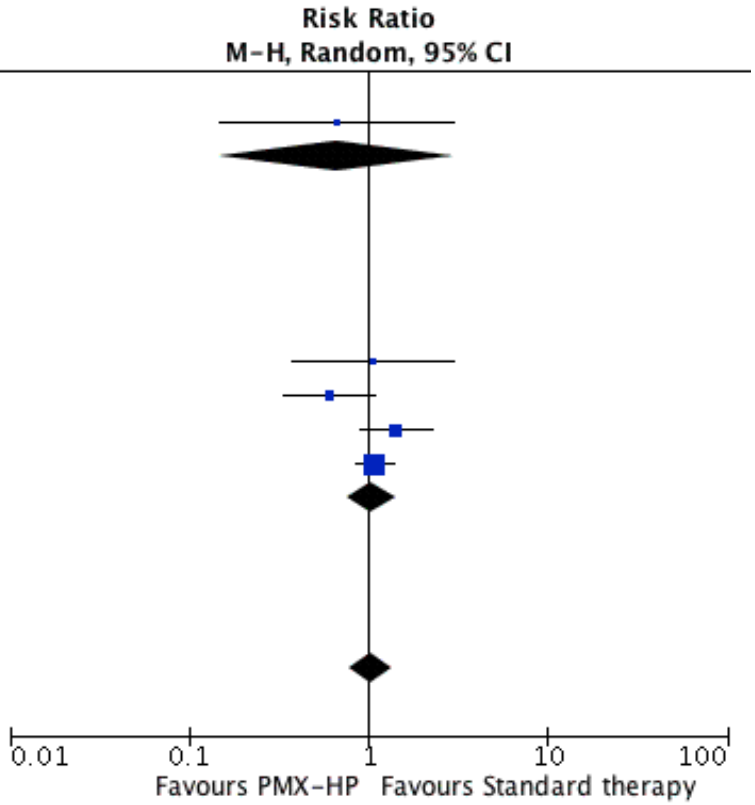
Heterogeneity: $Tau^2 = 0.05$; $Chi^2 = 3.70$, df = 2 (P = 0.16); $I^2 = 46\%$

Test for overall effect: Z = 0.61 (P = 0.54)



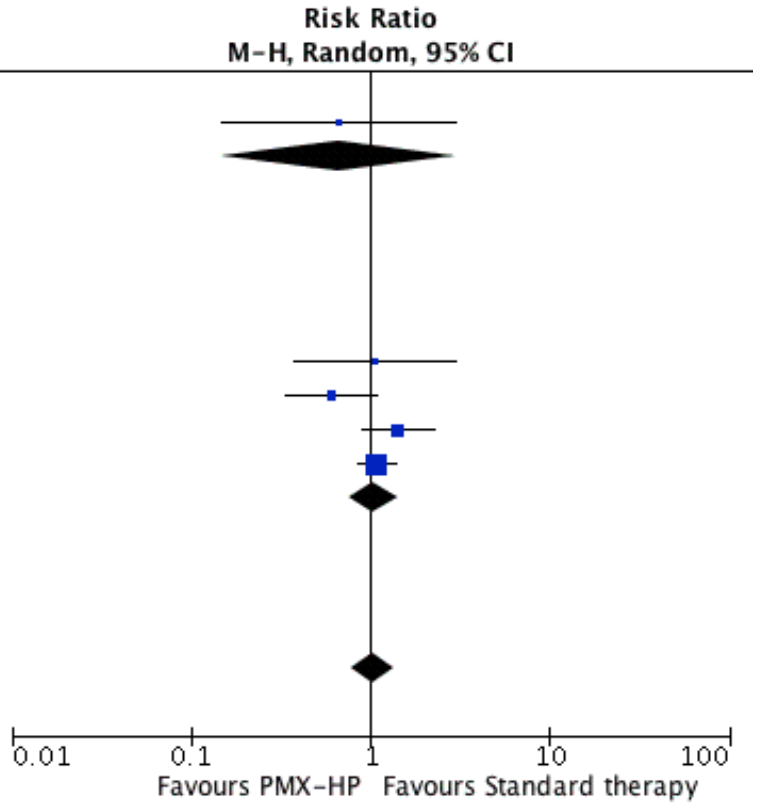
eFigure 10. Subgroup analysis: culture positive patients vs mixed or not confirmed patients

Study or Subgroup	PMX-HP Events	PMX-HP Total	Standard therapy Events	Standard therapy Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
2.1.1 Culture positive sepsis							
Cantaluppi 2008	2	8	3	8	3.4%	0.67 [0.15, 2.98]	2008
Subtotal (95% CI)		**8**		**8**	**3.4%**	**0.67 [0.15, 2.98]**	
Total events	2		3				
Heterogeneity: Not applicable							
Test for overall effect: Z = 0.53 (P = 0.60)							
2.1.2 Mixed or not confirmed							
Vincent 2005	5	17	5	18	6.6%	1.06 [0.37, 3.02]	2005
Cruz 2009	11	34	16	30	17.5%	0.61 [0.34, 1.09]	2009
Payen 2015	33	119	22	113	24.1%	1.42 [0.89, 2.29]	2015
EUPHRATES 2017	84	224	78	226	48.4%	1.09 [0.85, 1.39]	2017
Subtotal (95% CI)		**394**		**387**	**96.6%**	**1.04 [0.76, 1.42]**	
Total events	133		121				
Heterogeneity: Tau^2 = 0.04; Chi^2 = 4.99, df = 3 (P = 0.17); I^2 = 40%							
Test for overall effect: Z = 0.22 (P = 0.82)							
Total (95% CI)		**402**		**395**	**100.0%**	**1.03 [0.78, 1.36]**	
Total events	135		124				
Heterogeneity: Tau^2 = 0.03; Chi^2 = 5.35, df = 4 (P = 0.25); I^2 = 25%							
Test for overall effect: Z = 0.20 (P = 0.84)							
Test for subgroup differences: Chi^2 = 0.32, df = 1 (P = 0.57), I^2 = 0%							

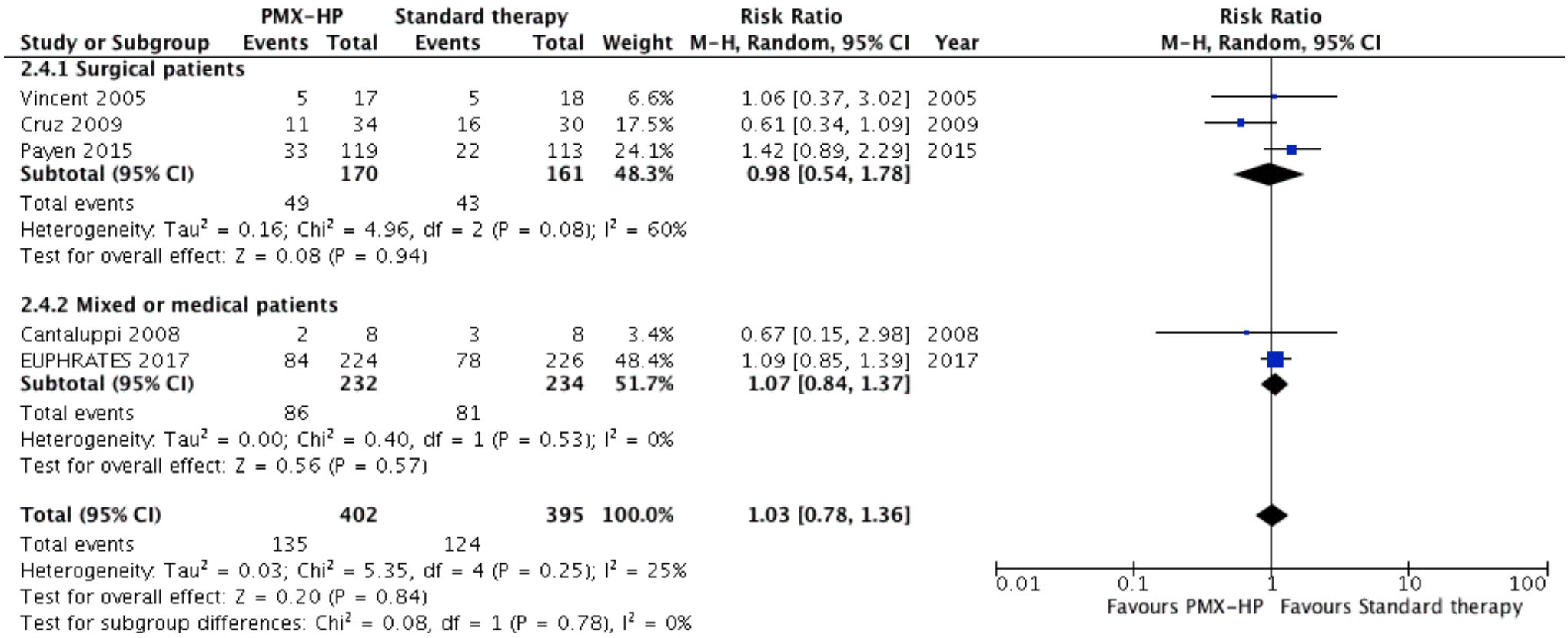


eFigure 11. Subgroup analysis: Patients with confirmed gram negative sepsis vs other studies

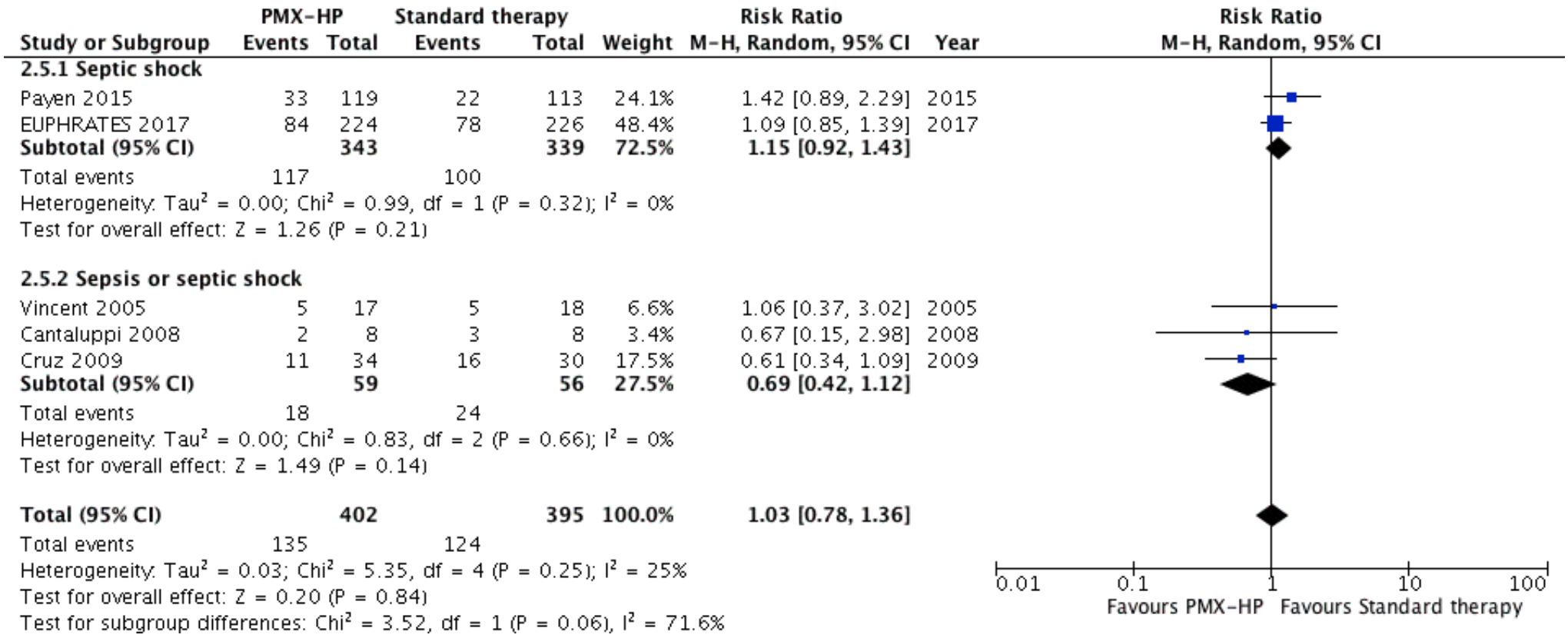
Study or Subgroup	PMX-HP Events	PMX-HP Total	Standard therapy Events	Standard therapy Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
2.2.1 Confirmed Gram Negative Sepsis							
Cantaluppi 2008	2	8	3	8	3.4%	0.67 [0.15, 2.98]	2008
Subtotal (95% CI)		**8**		**8**	**3.4%**	**0.67 [0.15, 2.98]**	
Total events	2		3				
Heterogeneity: Not applicable							
Test for overall effect: Z = 0.53 (P = 0.60)							
2.2.2 Other studies							
Vincent 2005	5	17	5	18	6.6%	1.06 [0.37, 3.02]	2005
Cruz 2009	11	34	16	30	17.5%	0.61 [0.34, 1.09]	2009
Payen 2015	33	119	22	113	24.1%	1.42 [0.89, 2.29]	2015
EUPHRATES 2017	84	224	78	226	48.4%	1.09 [0.85, 1.39]	2017
Subtotal (95% CI)		**394**		**387**	**96.6%**	**1.04 [0.76, 1.42]**	
Total events	133		121				
Heterogeneity: Tau^2 = 0.04; Chi^2 = 4.99, df = 3 (P = 0.17); I^2 = 40%							
Test for overall effect: Z = 0.22 (P = 0.82)							
Total (95% CI)		**402**		**395**	**100.0%**	**1.03 [0.78, 1.36]**	
Total events	135		124				
Heterogeneity: Tau^2 = 0.03; Chi^2 = 5.35, df = 4 (P = 0.25); I^2 = 25%							
Test for overall effect: Z = 0.20 (P = 0.84)							
Test for subgroup differences: Chi^2 = 0.32, df = 1 (P = 0.57), I^2 = 0%							



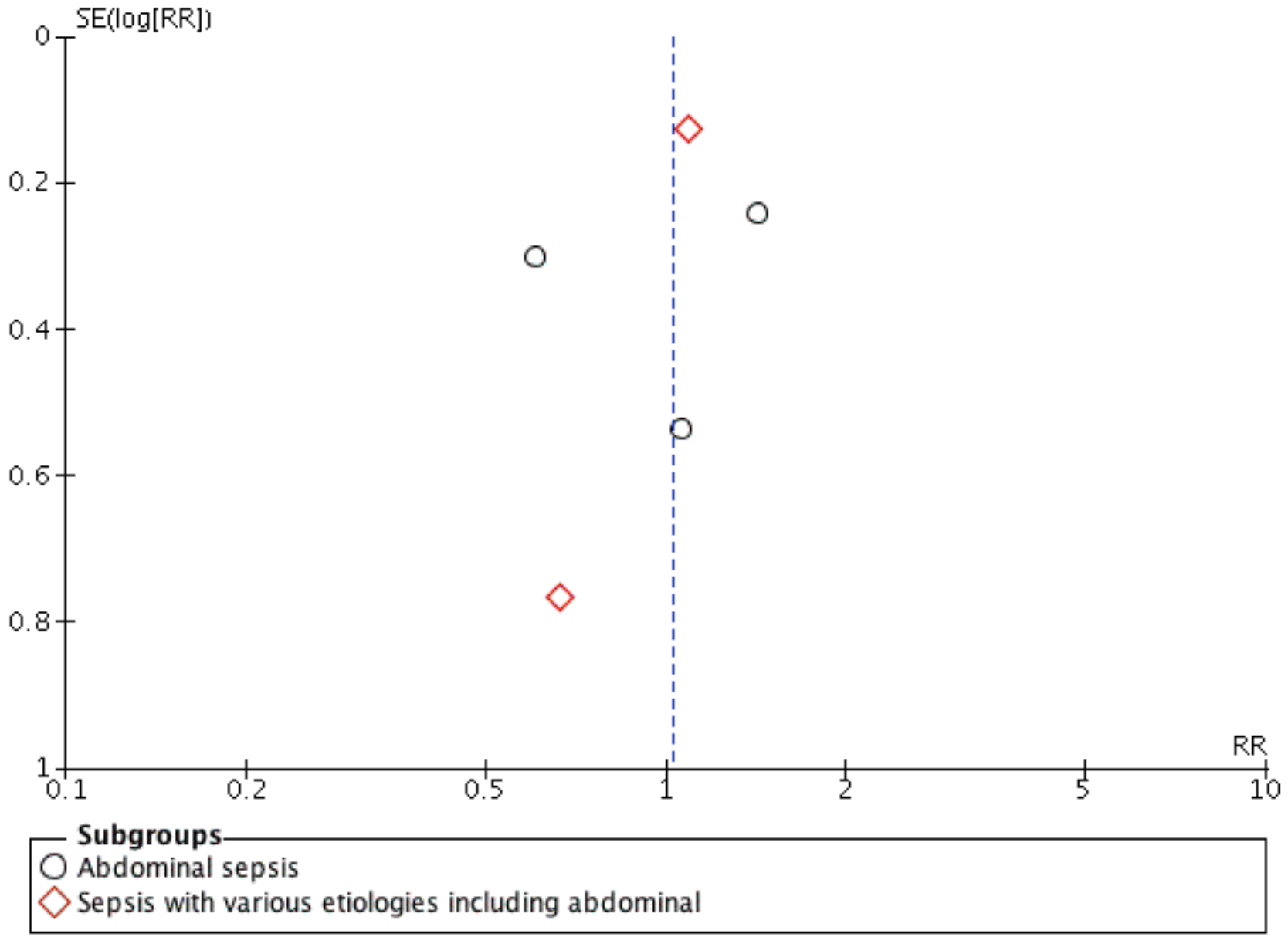
eFigure 12. Subgroup analysis: Surgical patients vs mixed or medical patients



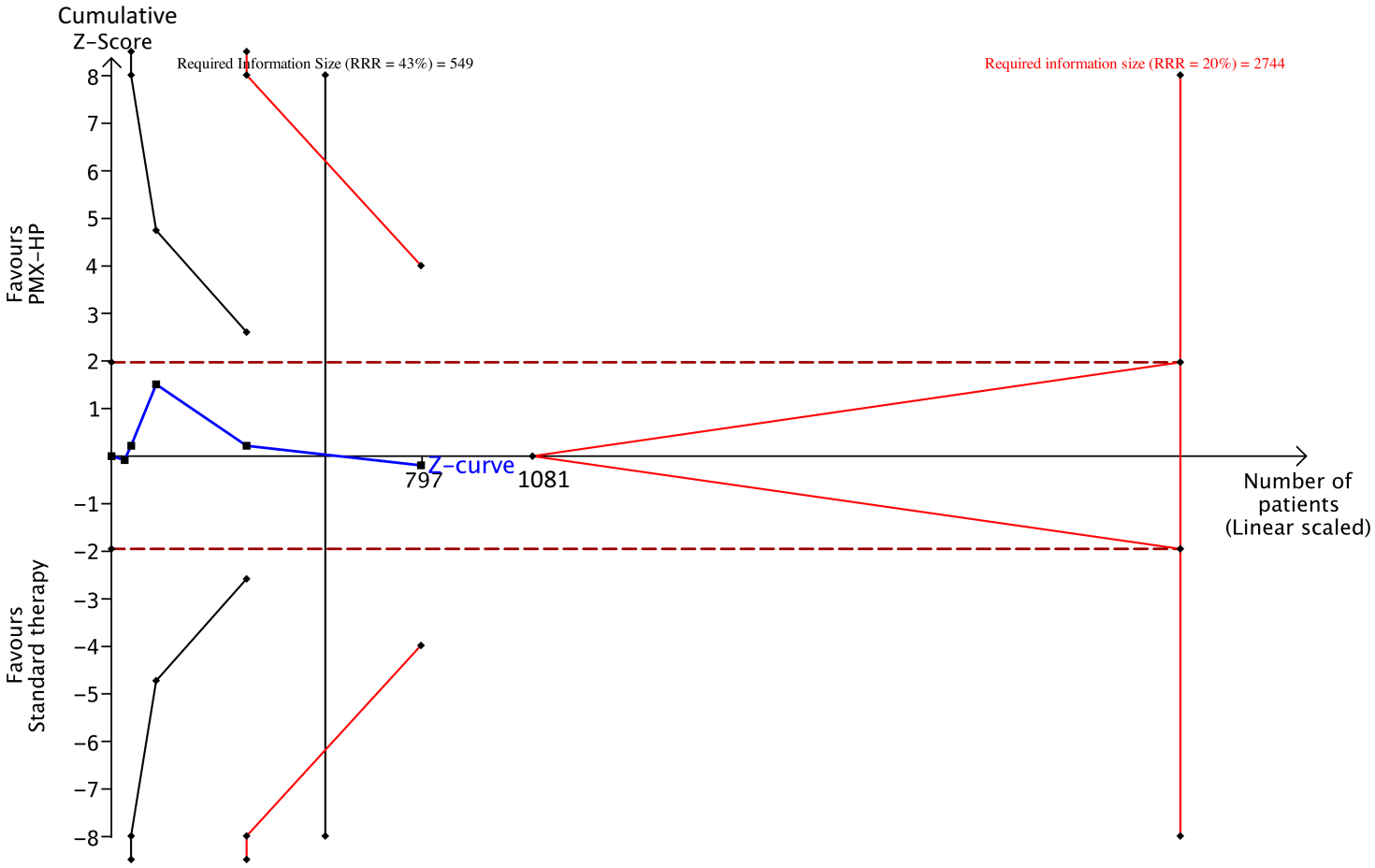
eFigure 13. Subgroup analysis: patients with septic shock vs severity-mixed sepsis



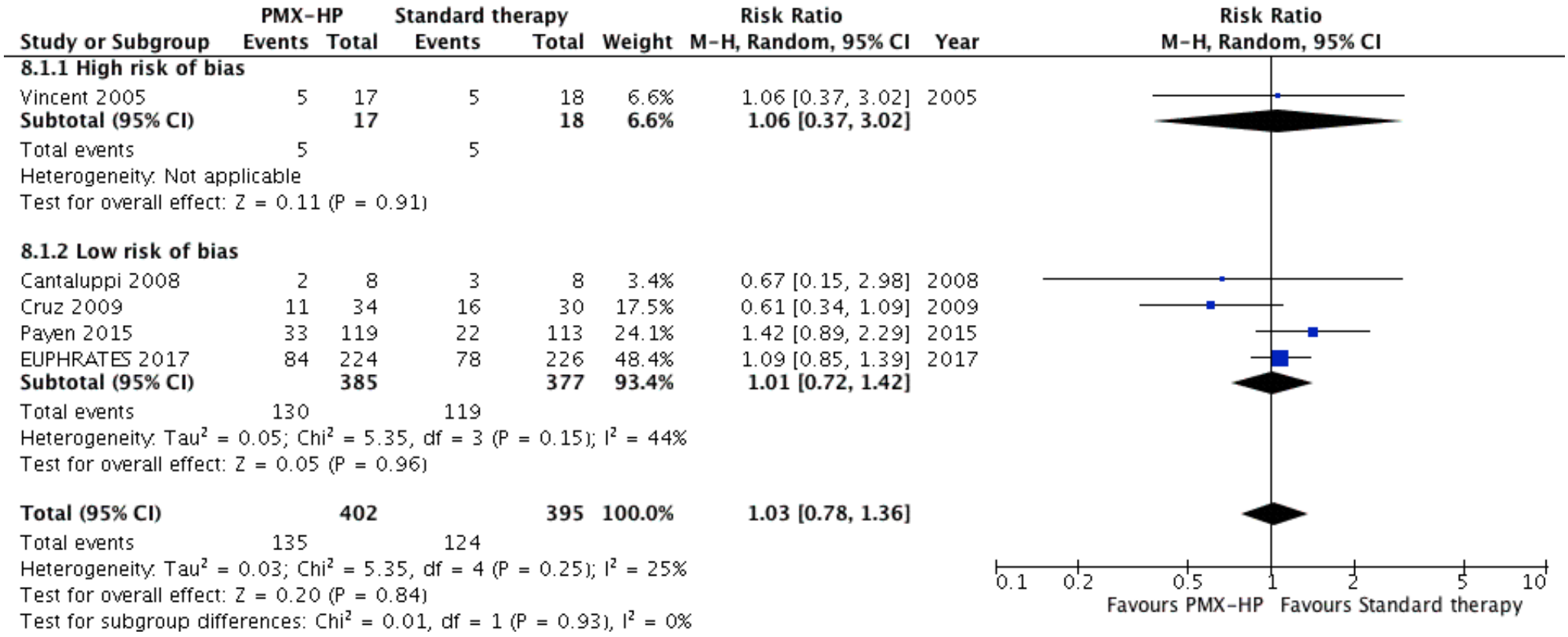
eFigure 14. Funnel plot for 28-day mortality



eFigure 15. Sensitivity analysis: trial sequential analysis of trials reporting 28 day mortality. The cumulative z curve (blue line) was constructed using a random effect model. Etched dark red line shows conventional test boundary. Two trial sequential monitoring boundaries (TSMB) of two different settings were constructed. Futility boundaries (inner boundaries) were also constructed for each setting. One TSMB (red full line) based on a diversity adjusted information size of 2744 patients, which was calculated using alfa = 0.05 (two sided), beta = 0.20 (power 80%), an anticipated relative risk reduction of 20.0%, and a control event rate of 35.0%. The other TSMB (black line) based on a diversity adjusted information size of 549 patients, which was calculated using alfa = 0.05 (two sided), beta = 0.20 (power 80%), an anticipated relative risk reduction of 43.0%, and a control event rate of 35.0%.

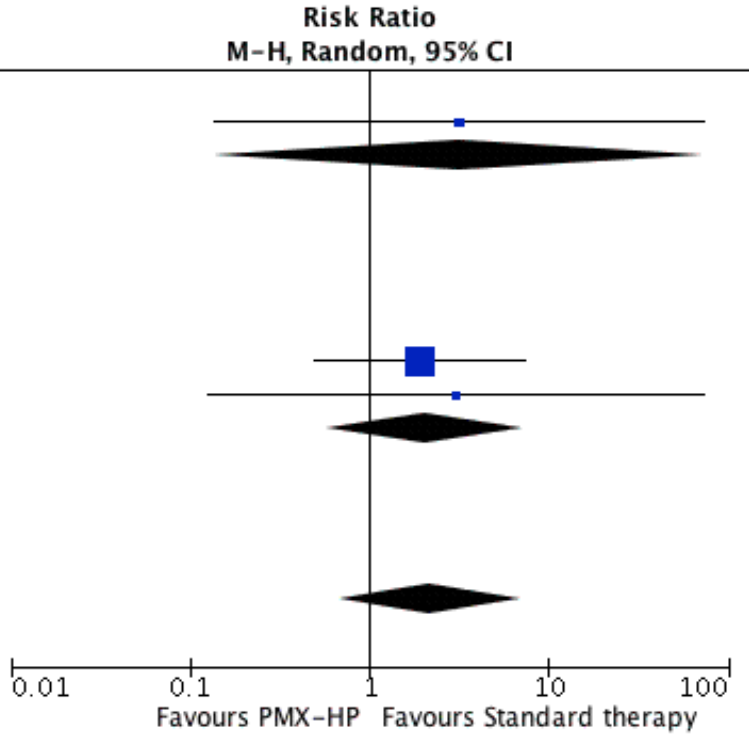


eFigure 16. Subgroup analysis: trials with high risk of bias in sponsorship bias and either or both of blinding of participants and personnel and/or blinding of outcome assessment, were considered to be the high risk of bias overall. Outcome, 28-day mortality. One trial was at high risk of bias in the blinding and sponsorship domains. The direction of the effect estimates coincided with other studies, and the total estimates were based mostly on the data from low risk of bias studies (> 93% of the weight)

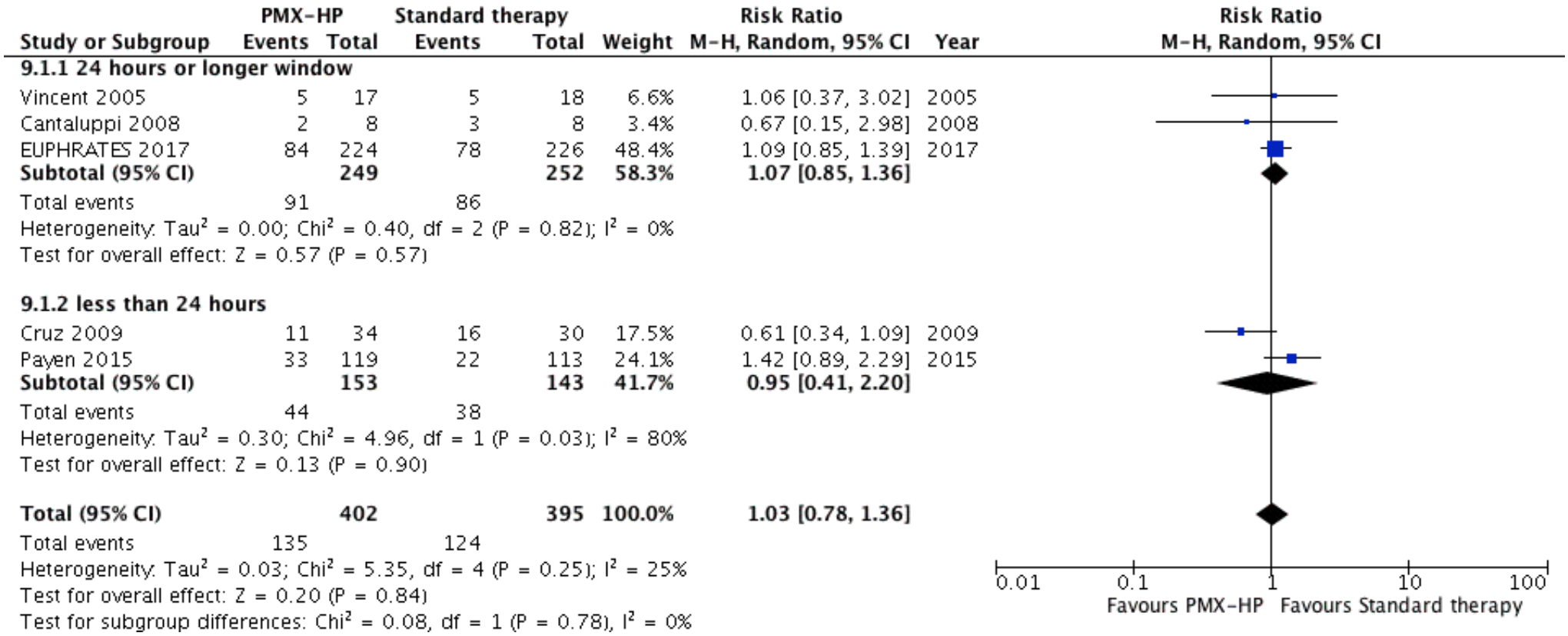


eFigure 17. Subgroup analysis: trials with high risk of bias in sponsorship bias and either or both of blinding of participants and personnel and/or blinding of outcome assessment were considered to be the high risk of bias overall. Outcome, the number of patients with at least one serious adverse event. One trial was at high risk of bias in the blinding and sponsorship domains. The direction of the effect estimates coincided with other studies, and the total estimates were based mostly on the data from low risk of bias studies (> 86% of the weight)

Study or Subgroup	PMX-HP Events	PMX-HP Total	Standard therapy Events	Standard therapy Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
8.2.1 High ROB							
Vincent 2005	1	17	0	18	13.8%	3.17 [0.14, 72.80]	2005
Subtotal (95% CI)		**17**		**18**	**13.8%**	**3.17 [0.14, 72.80]**	
Total events	1		0				
Heterogeneity: Not applicable							
Test for overall effect: Z = 0.72 (P = 0.47)							
8.2.2 Low ROB							
Payen 2015	6	119	3	113	73.0%	1.90 [0.49, 7.41]	2015
EUPHRATES 2017	1	224	0	226	13.3%	3.03 [0.12, 73.90]	2017
Subtotal (95% CI)		**343**		**339**	**86.2%**	**2.04 [0.58, 7.14]**	
Total events	7		3				
Heterogeneity: Tau^2 = 0.00; Chi^2 = 0.07, df = 1 (P = 0.79); I^2 = 0%							
Test for overall effect: Z = 1.12 (P = 0.26)							
Total (95% CI)		**360**		**357**	**100.0%**	**2.17 [0.68, 6.94]**	
Total events	8		3				
Heterogeneity: Tau^2 = 0.00; Chi^2 = 0.13, df = 2 (P = 0.93); I^2 = 0%							
Test for overall effect: Z = 1.30 (P = 0.19)							
Test for subgroup differences: Chi^2 = 0.07, df = 1 (P = 0.80), I^2 = 0%							



eFigure 18. Subgroup analysis: studies of early initiation of the therapy versus late initiation of the therapy. Outcome, 28-day mortality.



Supplementary Material

Rationalizing antimicrobial therapy in the ICU: a narrative review

Table E1. Multi-marker diagnostic panels for sepsis

Marker [Refs]	Biomarkers	Domain	Clinical performance studies	AUC	Sensitivity	Specificity
Sepsis score [1]	PCT, C3a	ICU	None, discovery cohort only (n = 33)	(0.93)	(0.90)	(0.80)
Sepsis score [2]	PCT, WBC, temperature, heart rate, blood pressure	ICU	None, discovery cohort only (n = 78)	(0.94)	-	-
Composite six-marker test [3]	suPAR, sTREM-1, MIF, CRP, PCT, neutrophils	ID ward	None, discovery cohort only (n = 151)	(0.88)	(0.88)	(0.78)
Sepsis bioscore [4]	PCT, sTREM-1, polymorpho-nuclear CD64 index	ICU	1 pragmatic single-center cohort (n = 79)	0.95	-	-
FAIM3/PLAC8 ratio [5]	FAIM3, PLAC8	CAP in ICU	1 internal validation data set (n = 134)	0.78	0.97	0.28
sNIP score [6]	NLRP1, IDNK, PLAC8	cIAI in ICU	1 internal validation data set (n = 73) 2 independent explanatory data sets (total n = 86)	0.91 0.86-0.98	0.95 -	0.79 -
Gene expression score [7]	TLR5, CD59,CLU, FGL2, IL7R, HLA-DPA1,CPVL	ICU	1 pragmatic multicenter cohort (n = 246)	0.81	0.80	0.59
Sepsis Meta Score [8,9]	CAECAM1, ZDHHC19, C9orf95, GNA15, BATF, C3AR1, KIAA1370, TGFBI, MTCH1, RPGRIP1, HLA-DPB1	ICU	4 independent mixed data sets (total n = 218) 3 independent mixed data sets (total n = 213)	0.73-0.89 0.92-0.93	- 0.95	- 0.60
SeptiCyte LAB [10,11,12,13]	CAECAM4, LAMP1, PLAC8, PLA2G7	ICU	5 explanatory multicenter cohorts (total n = 345) 1 explanatory single-center cohort (n = 70) 1 pragmatic multicenter cohort (n = 467) 1 pragmatic multicenter cohort (n = 447)	0.77-0.95 0.95-0.99 [1] 0.73 0.82-0.89 [2]	0.79-1.00 - 0.96 0.92-0.97[#]	0.33-0.91 - 0.19 0.34-0.65[#]

Table E1 footnote

Test characteristics refer to estimates as reported in validation (rather than discovery) samples, if available. Explanatory cohorts exploit an artificial contrast between sepsis cases and SIRS controls whereas pragmatic cohorts include consecutively enrolled patients (thus better reflecting an intended use population with a true diagnostic dilemma).

ICU, intensive care unit; ID, infectious diseases; CAP, community-acquired pneumonia; cIAI, complicated intra-abdominal infection

[1] Estimates varied depending on the RNA sequencing method used; [2] Estimates varied depending on the definition of the reference diagnosis

References for Table E1

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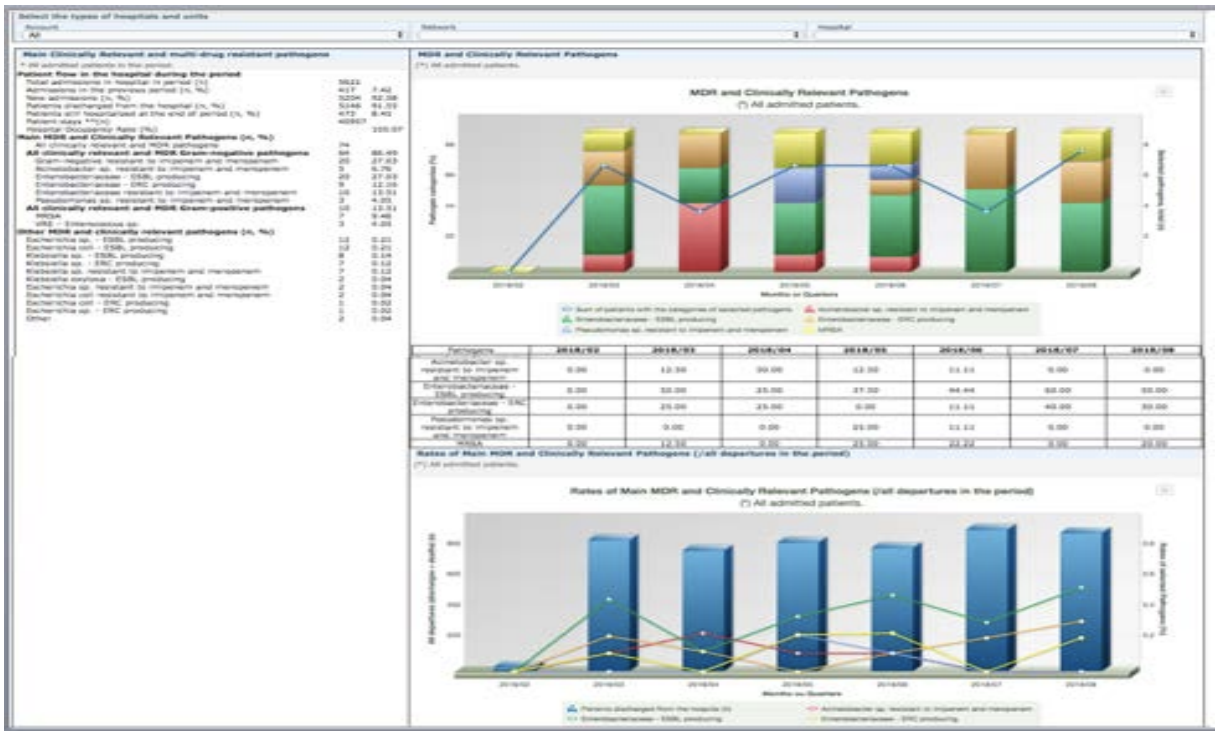
Sampson D, Rothwell V, Newman R, Bhide S, Fox BA, Kirk JT, Navalkar K, Davis RF, Brandon RA, Brandon RB. Validation of a Host Response Assay, SeptiCyte LAB, for Discriminating Sepsis from Systemic Inflammatory Response Syndrome in the ICU. *Am J Respir Crit Care Med* 2018; 198(7): 903-913

Figure E1.


MDR and Clinically Relevant Pathogens
(*) All admitted patients.
Rates of Main MDR and Clinically Relevant Pathogens (/all departures in the period)
(*) All admitted patients.

Supplementary Material

Cardiovascular clusters in septic shock combining clinical and echocardiographic parameters: a post hoc analysis

Cardiovascular clusters in septic shock combining clinical and echocardiographic parameters: A post-hoc analysis of the Hemopred and Hemosepsis studies.

Guillaume Geri, Philippe Vignon, Alix Aubry, Anne-Laure Fedou, Cyril Charron, Stein Silva, Xavier Repessé, Antoine Vieillard-Baron

Supplementary material

Figure S1. Internal validity and stability of the clustering partition. The connectivity (Panel A) indicates the degree of connectedness of the clusters and should be minimized. The Dunn index (Panel B) reflects compactness and separation of clusters using the ratio of the smallest distance between observations not in the same cluster to the largest intra-cluster distance. It should be maximized.

The average proportion of non-overlap (APN) (Panel C) measures the average proportion of observations not placed in the same cluster by clustering based on the full data and clustering based on the data with a single column removed. The average distance (AD)(Panel D) computes the average distance between observations placed in the same cluster by clustering based on the full data and clustering based on the data with a single column removed. The average distance between means (ADM)(Panel E) computes the average distance between cluster centers for observations placed in the same cluster by clustering based on the full data and clustering based on the data with a single column removed. All these measures should be minimized.

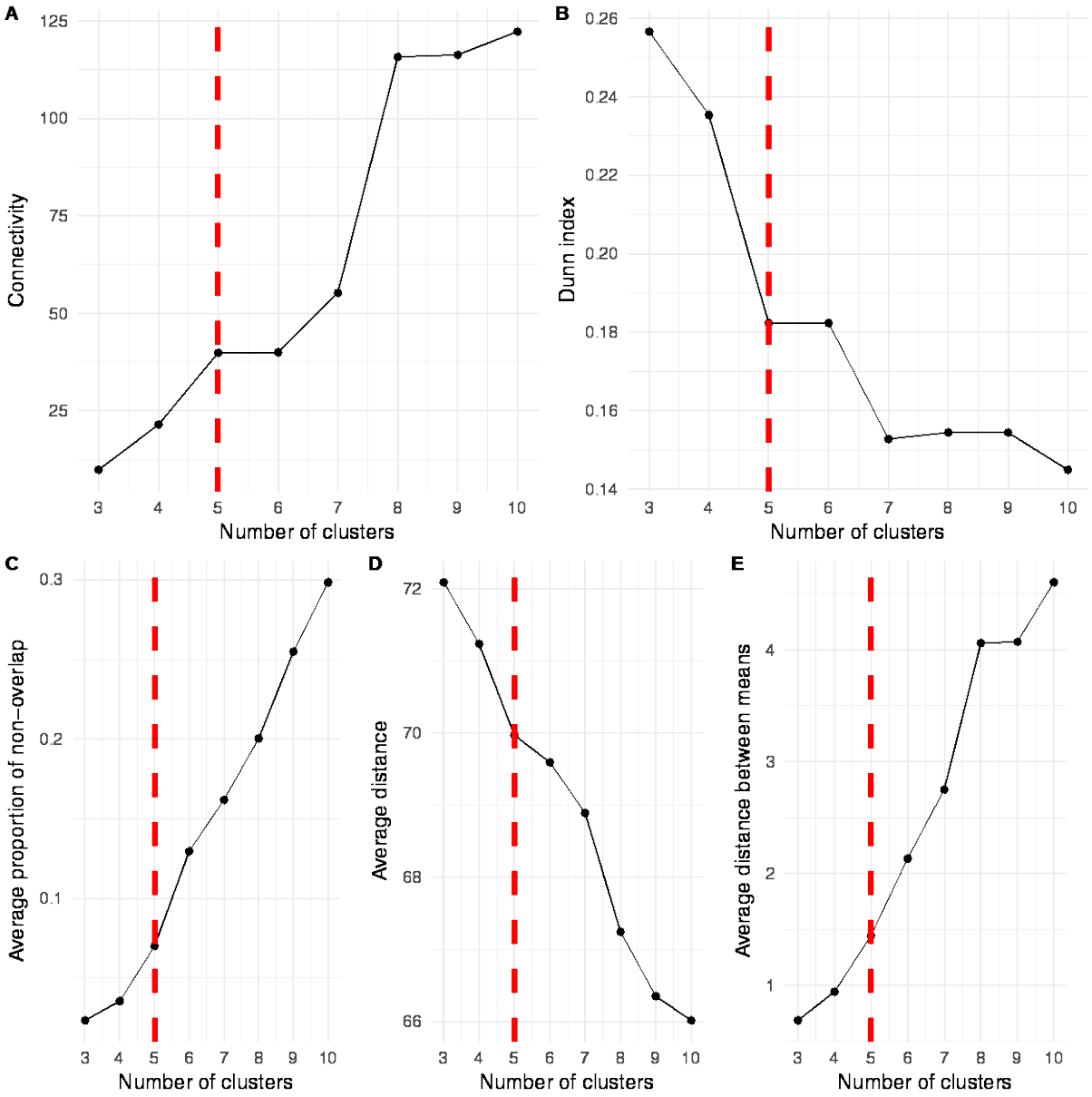

A
Connectivity
125
100
75
50
25
3 4 5 6 7 8 9 10
Number of clusters
B
Dunn index
0.26
0.24
0.22
0.20
0.18
0.16
0.14
3 4 5 6 7 8 9 10
Number of clusters
C
Average proportion of non−overlap
0.3
0.2
0.1
3 4 5 6 7 8 9 10
Number of clusters
D
Average distance
72
70
68
66
3 4 5 6 7 8 9 10
Number of clusters
E
Average distance between means
4
3
2
1
3 4 5 6 7 8 9 10
Number of clusters

Figure S2: Principal component analysis plan showing the uncertainty related to the missingness after multiple imputation using iterative PCA and 1,000 bootstrap replications.
dAP: diastolic arterial pressure, sAP: systolic arterial pressure, Ao VTI: aortic blood flow velocity time integral, LVEF: left ventricular ejection fraction, LVFAC: left ventricular fractional area contraction, RV/LV EDA: right to left ventricular en-diastolic area ratio, ΔSVC: superior vena cava collapsibility index, Norepi: norepinephrine, H. rate: heart rate, Epi: epinephrine.

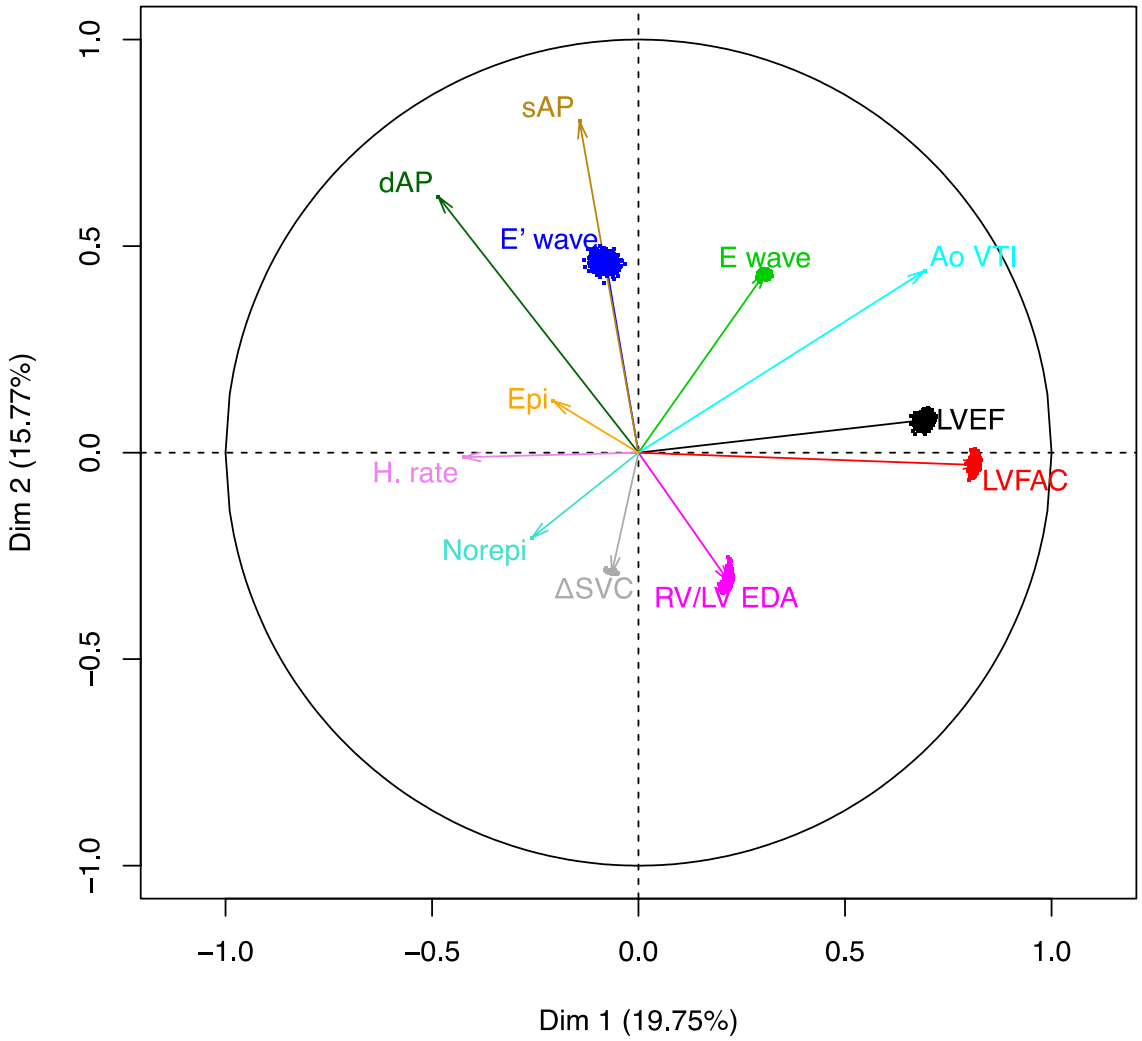


Figure S3. Flow chart of the study

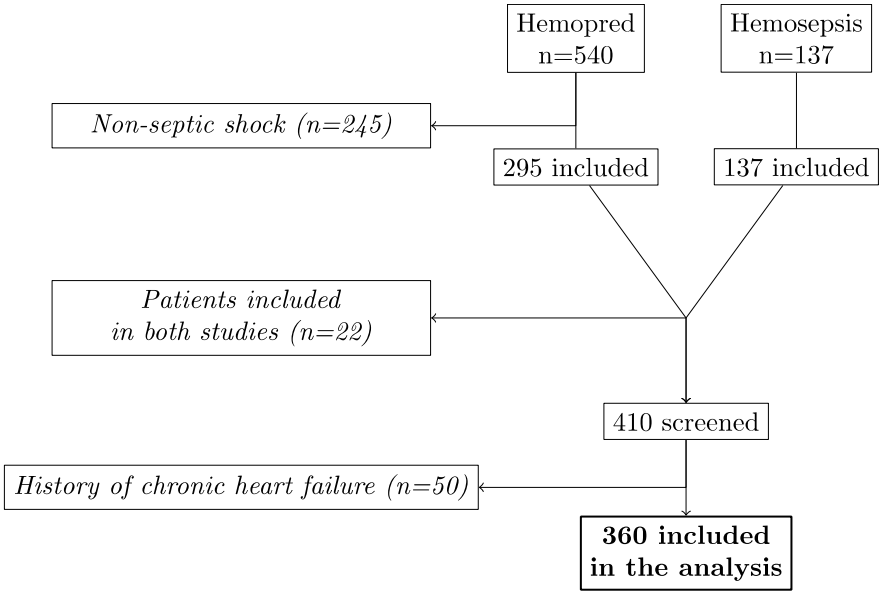


Figure S4. Missing values and missing patterns. At the top of the figure are shown the number of missing values for each variable used in the clustering analysis. In rows are shown the missing patterns. For instance, E' wave had 31 missing values and was the sole variable with missing values in 24 cases while E' and E waves were simultaneously missing in 2 cases

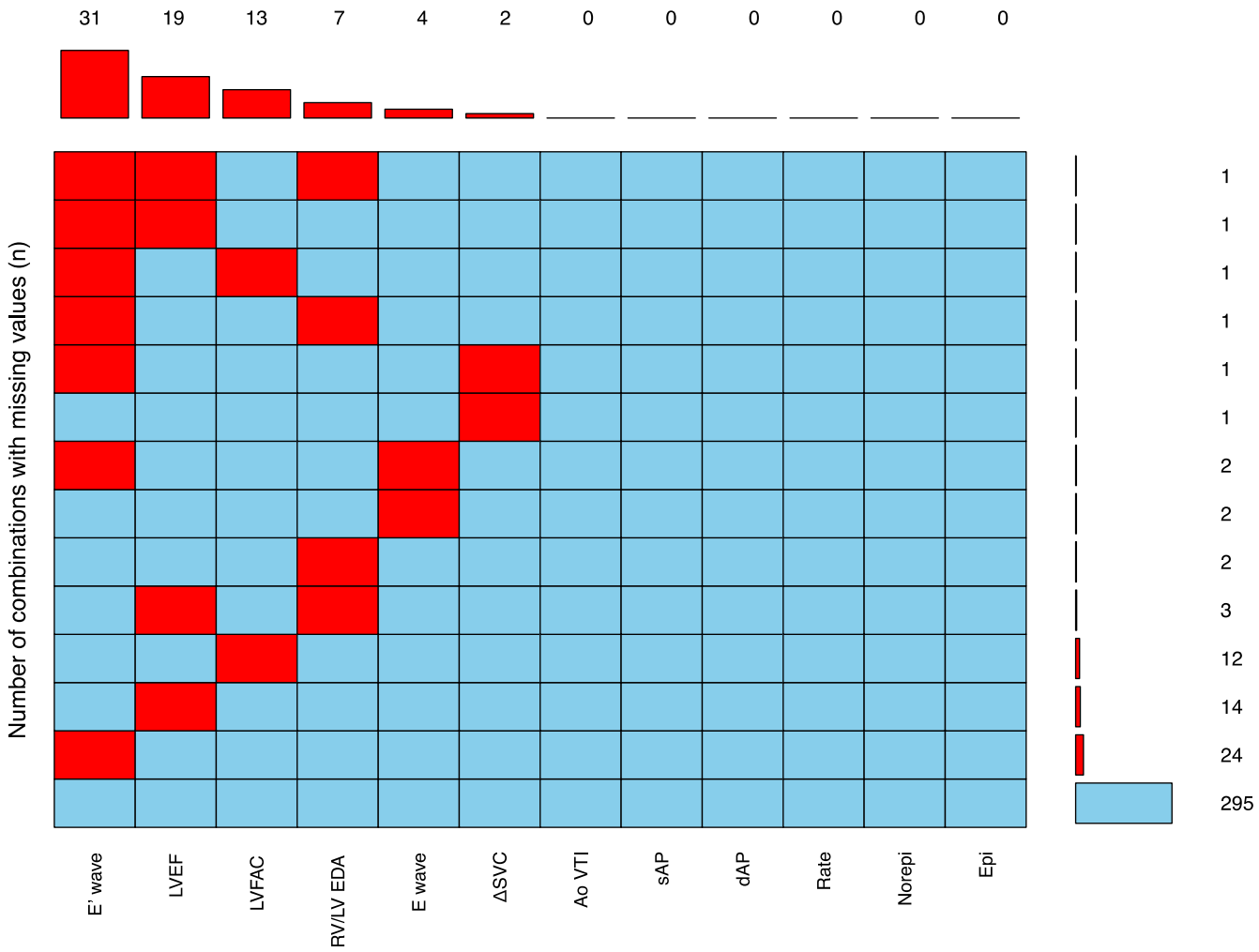


Table S1: Comparison of baseline characteristics according to the study the patients have been included in.

	Hemopred N=247	**Hemosepsis N=113**	**P value**
Age, median [iqr], years	65.0 [56.5;75.0]	62.0 [52.0;72.0]	0.035
Male gender, n(%)	175 (70.9)	58 (51.3)	0.001
SAPS II, median [iqr]	57.0 [42.0;70.0]	55.5 [46.0;70.0]	0.672
SOFA score, median [iqr]	10.0 [7.0;12.0]	10.0 [7.0;12.0]	0.579
Arterial blood lactate, median [iqr], mmol/L	2.2 [1.5; 4.0]	3.2 [2.0; 4.7]	<0.001
PaO_2/FiO_2 ratio, median [iqr]	190.0 [114.0;264.4]	163.0 [109.5;259.0]	0.423
Site of infection (%)			0.014
Lung	122 (49.4)	47 (41.6)	
Urinary tract	10 (4.0)	15 (13.3)	
GI tract	74 (30.0)	35 (31.0)	
Skin	15 (6.1)	9 (8.0)	
Others	26 (10.5)	7 (6.2)	

Supplementary Material

Fever control in critically ill adults. An individual patient data meta-analysis of randomised controlled trials

FEVER CONTROL IN CRITICALLY ILL ADULTS.

An Individual Patient Data Meta-Analysis of Randomised Controlled Trials

Electronic Supplementary Material

SUPPLEMENTARY METHODS

Corresponding authors for all studies were emailed to ask if they would be prepared to share data for this analysis. Two follow-up emails were sent if the author did not respond.

Because the APACHE-II score was not available for the Schortgen et al study, we performed a sensitivity analysis in which the SAPS-3 score was rescaled to give it the same range as the APACHE-II score. As the APACHE II score ranges from 0 to 71 and the SAPS-3 score ranges from 0 to 217, we multiplied the SAPS-3 by 71/217 to obtain usable illness severity data. All analyses performed with the APACHE-II score were performed using these additional data.

We conducted a post-hoc analysis comparing hospital-free days and ICU-free days to day 28 by treatment group. All patients who died during follow-up were assigned the worst possible outcome of zero free days. Free-days were compared between treatment groups using the Wilcoxon rank-based test with the estimate of difference along with corresponding 95% CI calculated using the Hodges-Lehman estimator method.

SUPPLEMENTARY RESULTS

DETAILS OF STUDIES

Table S1. Details of studies

Haupt MT, Jastremski MS, Clemmer TP, Metz CA, Goris GB. Effect of ibuprofen in patients with severe sepsis: a randomized, double-blind, multicenter study. The Ibuprofen Study Group. *Crit Care Med.* 1991 Nov;19(11):1339-47.

- Multicentre pharmaceutical company trial in ICU patients with severe sepsis conducted in the USA (n=29)

DATA WERE NOT ABLE TO BE OBTAINED FOR INCLUSION IN THE IPDMA (no response to email inviting participation)

Participant characteristics		
Variable	Intervention (n=16)	Control (n=13)
Age - yr	48±16	55±14
Male sex – no. (%)	10/16 (62.5)	6/13 (46.2)
Invasively ventilated – no. (%)	13/16 (81.3)	11/13 (84.6)
On vasopressors – no. (%)	not reported	not reported
Proven infection – no. (%)	6/16 (37.5)	6/13 (46.2)
Diagnosis of acute brain pathology	not reported	not reported
Intervention details		
Nature of intervention	ibuprofen	placebo
Duration of study treatment - days	1	1
Outcomes		
Mortality at last reported time point (in-hospital mortality)	9/16 (56.3)	4/13 (30.8)
ICU length of stay	not reported	not reported
Hospital length of stay	not reported	not reported
Body temperature at 12 hours (°C)	37.1±0.9 (n=16)	38.1±0.7 (n=12)

Bernard GR, Wheeler AP, Russell JA, Schein R, Summer WR, Steinberg KP, Fulkerson WJ, Wright PE, Christman BW, Dupont WD, Higgins SB, Swindell BB. The effects of ibuprofen on the physiology and survival of patients with sepsis. The Ibuprofen in Sepsis Study Group. *N Engl J Med.* 1997 Mar 27;336(13):912-8.

- Multicentre investigator-initiated trial in ICU patients with sepsis conducted in the USA and Canada (n=455)

DATA WERE INCLUDED IN THE IPDMA

Participant characteristics		
Variable	Intervention (n=224)	Control (n=231)
Age - yr	54±18	56±16
Male sex – no. (%)	93/224 (41.5)	79/231 (34.2)
Invasively ventilated – no. (%)	175/224 (78.1)	176/231 (76.2)
On vasopressors – no. (%)	not reported	not reported
Proven infection – no. (%)	168/224 (75.0)	176/231 (76.2)
Diagnosis of acute brain pathology	not reported	not reported

Intervention details		
Nature of intervention	ibuprofen	placebo
Duration of study treatment - days	2	2
Outcomes		
Mortality at last reported time point (day 30 mortality)	83/224 (37.1)	92/231 (39.8)
ICU length of stay	not reported	not reported
Hospital length of stay	not reported	not reported
Body temperature at 12 hours (°C)	36.9±1.1 (n=224)	37.6±1.2 (n=231)

Gozzoli V, Schöttker P, Suter PM, Ricou B. Is it worth treating fever in intensive care unit patients? Preliminary results from a randomized trial of the effect of external cooling. *Arch Intern Med.* 2001 Jan 8;161(1):121-3.

- Single centre trial* in surgical ICU patients with fever and SIRS conducted in Switzerland (n=38)

DATA WERE NOT ABLE TO BE OBTAINED FOR INCLUSION IN THE IPDMA (Corresponding author replied to email indicating that data were no longer available as the study was 'too old'.)

Participant characteristics		
Variable	Intervention (n=18)	Control (n=20)
Age - yr	54±13	53±19
Male sex – no. (%)	14/18 (77.8)	16/20 (80.0)
Invasively ventilated – no. (%)	not reported	not reported
On vasopressors – no. (%)	not reported	not reported
Proven infection – no. (%)	9/18 (50.0)	10/20 (50.0)
Diagnosis of acute brain pathology	not reported	not reported
Intervention details		
Nature of intervention	external cooling started if temp>38.5°C and stopped if <37.5°C	No antipyretic treatment
Duration of study treatment - days	until ICU discharge	until ICU discharge
Outcomes		
Mortality at last reported time point (ICU mortality)	2/18 (11.1)	3/20 (15.0)
ICU length of stay	11±13	9±10
Hospital length of stay	28±22	31±24
Body temperature at 12 hours (°C)	not reported	not reported

Memi D, Karamanlio lu B, Turan A, Koyuncu O, Pamukçu Z. Effects of lornoxica on the physiology of severe sepsis. *Crit Care.* 2004 Dec;8(6):R474-82.

- Single centre trial* in ICU patients with severe sepsis conducted in Turkey (n=40)

DATA WERE NOT ABLE TO BE OBTAINED FOR INCLUSION IN THE IPDMA (no response to email inviting participation)

Participant characteristics		
Variable	Intervention (n=20)	Control (n=20)

Age - yr	49 (SD not reported)	51 (SD not reported)
Male sex – no. (%)	13/20 (65)	9/20 (45)
Invasively ventilated – no. (%)	20/20 (100)	20/20 (100)
On vasopressors – no. (%)	not reported	not reported
Proven infection – no. (%)	20/20 (100)	20/20 (100)
Diagnosis of acute brain pathology	not reported	not reported
Intervention details		
Nature of intervention	lornoxicam	placebo
Duration of study treatment - days	3	3
Outcomes		
Mortality at last reported time point (ICU mortality)	7/20 (35)	8/20 (40)
ICU length of stay -days	10.2±7.1 (survivors only; n=13)	9.2±8.4 (survivors only; n=12)
Hospital length of stay	not reported	not reported
Body temperature at 12 hours (°C)	not reported	not reported

Schulman CI, Namias N, Doherty J, Manning RJ, Li P, Elhaddad A, Lasko D, Amortegui J, Dy CJ, Dlugasch L, Baracco G, Cohn SM. The effect of antipyretic therapy upon outcomes in critically ill patients: a randomized, prospective study. *Surg Infect (Larchmt).* 2005 Winter;6(4):369-75.

- Single centre trial* in trauma ICU patients with fever conducted in the USA (n=82)

DATA WERE NOT ABLE TO BE OBTAINED FOR INCLUSION IN THE IPDMA (no response to email inviting participation)

Participant characteristics		
Variable	Intervention (n=44)	Control (n=38)
Age - yr	47±20	47±20
Male sex – no. (%)	30/44 (68.2)	29/38 (76.3)
Invasively ventilated – no. (%)	not reported	not reported
On vasopressors – no. (%)	not reported	not reported
Proven infection – no. (%)	not reported	not reported
Diagnosis of acute brain pathology – no. (%)	0/44 (0)	0/38 (0)
Intervention details		
Nature of intervention	paracetamol if temp>38.5°C with addition of a cooling blanket if temp>39.5°C	paracetamol and a cooling blanket if temp>40°C
Duration of study treatment	until ICU discharge	until ICU discharge
Outcomes		
Mortality at last reported time point (ICU mortality)	7/44 (15.9)	1/38 (2.6)
ICU length of stay -days	22±30	20±14
Hospital length of stay	not reported	not reported
Body temperature at 12 hours (°C)	not reported	not reported

Morris PE, Promes JT, Guntupalli KK, Wright PE, Arons MM. A multi-center, randomized, double-blind, parallel, placebo-controlled trial to evaluate the efficacy, safety, and pharmacokinetics of intravenous ibuprofen for the treatment of fever in critically ill and non-critically ill adults. *Crit Care.*2010;14(3):R125.

- Multicentre pharmaceutical company trial in hospitalized patients with fever (some of whom were critically ill) conducted in USA, Thailand, and Australia (n=53 critically ill patients)

DATA WERE NOT ABLE TO BE OBTAINED FOR INCLUSION IN THE IPDMA (no response to email inviting participation)

Participant characteristics		
Variable	Intervention (n=40)	Control (n=13)
Age - yr	not reported for the critically ill patients specifically	not reported for the critically ill patients specifically
Male sex – no. (%)	not reported for the critically ill patients specifically	not reported for the critically ill patients specifically
Invasively ventilated – no. (%)	40/40 (100)	13/13 (100)
On vasopressors – no. (%)	4/40 (10)	0/13 (0)
Proven infection – no. (%)	not reported for the critically ill patients specifically	not reported for the critically ill patients specifically
Diagnosis of acute brain pathology – no. (%)	not reported	not reported
Intervention details		
Nature of intervention	ibuprofen	placebo
Duration of study treatment - days	1	1
Outcomes		
Mortality at last reported time point (ICU mortality)	5/40 (12.5)	1/13 (7.7)
ICU length of stay -days	not reported for the critically ill patients specifically	not reported for the critically ill patients specifically
Hospital length of stay	not reported for the critically ill patients specifically	not reported for the critically ill patients specifically
Body temperature at 12 hours (°C)	38.0†	38.5±0.8

Honarmand H, Abdollahi M, Ahmadi A, Javadi MR, Khoshayand MR, Tabeefar H, Mousavi S, Mahmoudi L, Radfar M, Najafi A, Mojtahedzadeh M. Randomized trial of the effect of intravenous paracetamol on inflammatory biomarkers and outcome in febrile critically ill adults. *Daru.* 2012 Aug 28;20(1):12.

- Single centre pharmaceutical company trial in ICU patients with fever and SIRS conducted in Iran (n=20)

DATA WERE NOT ABLE TO BE OBTAINED FOR INCLUSION IN THE IPDMA (no response to email inviting participation)

Participant characteristics		
Variable	Intervention (n=10)	Control (n=10)

Age - yr	49.5±17.0	45.4±21.1
Male sex – no. (%)	8/10 (80)	6/10 (60)
Invasively ventilated – no. (%)	10/10 (100)	10/10 (100)
On vasopressors – no. (%)	not reported	not reported
Proven infection – no. (%)	4/10 (40)	5/10 (50)
Diagnosis of acute brain pathology – no. (%)	0/10 (0)	0/10(0)
Intervention details		
Nature of intervention	paracetamol if temp >38.3°C	antipyretic only if >40°C (treatment not explicitly described)
Duration of study treatment - days	10	10
Outcomes		
Mortality at last reported time point (ICU mortality)	2/10 (20)	3/10 (30)
ICU length of stay -days	23.6±13.7	22.1±10.9
Hospital length of stay	not reported	not reported
Body temperature at 12 hours (°C)	37.7±0.9	37.4±0.6

Schortgen F, Clabault K, Katsahian S, Devaquet J, Mercat A, Deye N, Dellamonica J, Bouadma L, Cook F, Beji O, Brun-Buisson C, Lemaire F, Brochard L.
Fever control using external cooling in septic shock: a randomized controlled trial. *Am J Respir Crit Care Med.* 2012 May 15;185(10):1088-95.

- Multicentre investigator-initiated trial in sedated, mechanically ventilated adults in ICU with septic shock conducted in France and Switzerland (n=200)

DATA WERE INCLUDED IN THE IPDMA

Participant characteristics		
Variable	Intervention (n=101)	Control (n=99)
Age – yr; median [IQR]	62 [51-70]	61 [49-70]
Male sex – no. (%)	75/101 (74.3)	67/99 (67.8)
Invasively ventilated – no. (%)	101/101 (100)	99/99 (100)
On vasopressors – no. (%)	101/101 (100)	99/99 (100)
Proven infection – no. (%)	78/101 (77.2)	72/99 (72.7)
Diagnosis of acute brain pathology – no. (%)	not reported	not reported
Intervention details		
Nature of intervention	external cooling to normothermia (36.5-37°C)	no external cooling
Duration of study treatment - days	2	2
Outcomes		
Mortality at last reported time point (hospital discharge)	43/101 (42.6)	48/99 (48.5)
ICU length of stay -days	17±14	16±17
Hospital length of stay	36±40	28±31
Body temperature at 12 hours (°C)	36.8±0.7	38.4±1.1

Niven DJ, Stelfox HT, Léger C, Kubes P, Laupland KB. Assessment of the safety and feasibility of administering antipyretic therapy in critically ill adults: a pilot randomized clinical trial. *J Crit Care*. 2013 Jun;28(3):296-302.

- Multicentre investigator-initiated trial ICU patients with fever conducted in Canada (n=26)

DATA WERE INCLUDED IN THE IPDMA

Participant characteristics		
Variable	Intervention (n=14)	Control (n=12)
Age - yr; median [IQR]	53 [43-67]	58 [49-69]
Male sex – no. (%)	8/14 (57.1)	8/12 (66.7)
Invasively ventilated – no. (%)	14/14 (100)	12/12 (100)
On vasopressors – no. (%)	3/14 (21.4)	7/12 (58.3)
Proven infection – no. (%)	14/14 (100)	9/12 (75)
Diagnosis of acute brain pathology – no. (%)	0/14 (0)	0/12(0)
Intervention details		
Nature of intervention	paracetamol if temp 38.3 C; physical cooling if temp 39.5 C	paracetamol if temp 40.0 C; physical cooling if temp 40.5 C
Duration of study treatment - days	until ICU discharge	until ICU discharge
Outcomes		
Mortality at last reported time point (28 day mortality)	3/14 (21.4)	2/12 (16.7)
ICU length of stay -days	not reported	not reported
Hospital length of stay	not reported	not reported
Body temperature at 12 hours (°C)	not reported	not reported

Yang YL, Liu DW, Wang XT, Long Y, Zhou X, Chai WZ. Body temperature control in patients with refractory septic shock: too much may be harmful. *Chin Med J (Engl).* 2013;126(10):1809-13.

- Single centre trial* in ICU patients with refractory septic shock conducted in China (n=65)

DATA WERE NOT ABLE TO BE OBTAINED FOR INCLUSION IN THE IPDMA (no response to email inviting participation)

Participant characteristics		
Variable	Intervention (n=34)	Control (n=31)
Age - yr	68.8±18.0	66.6±13.0
Male sex – no. (%)	18/34 (53)	16/31 (52)
Invasively ventilated – no. (%)	34/34 (100)	31/31 (100)
On vasopressors – no. (%)	34/34 (100)	31/31 (100)
Proven infection – no. (%)	29/34 (85)	28/31 (90)
Diagnosis of acute brain pathology – no. (%)	0/34 (0)	0/31(0)
Intervention details		
Nature of intervention	physical cooling to maintain	physical cooling to maintain

	temp 36.0-37.5°C	temp 37.5-38.3°C
Duration of study treatment - days	3	3
Outcomes		
Mortality at last reported time point (28 day mortality)	21/34 (61.8)	8/31 (25.8)
ICU length of stay -days	not reported	not reported
Hospital length of stay	not reported	not reported
Body temperature at 12 hours (°C)	36.4±1.2	37.8±1.6

Janz DR, Bastarache JA, Rice TW, Bernard GR, Warren MA, Wickersham N, Sills G, Oates JA, Roberts LJ 2nd, Ware LB; Acetaminophen for the Reduction of Oxidative Injury in Severe Sepsis Study Group. Randomized, placebo-controlled trial of acetaminophen for the reduction of oxidative injury in severe sepsis: the Acetaminophen for the Reduction of Oxidative Injury in Severe Sepsis trial. *Crit Care Med.* 2015 Mar;43(3):534-41.

- Single centre investigator-initiated trial in ICU patients with severe sepsis conducted in the USA (n=40)

DATA WERE NOT ABLE TO BE OBTAINED FOR INCLUSION IN THE IPDMA (no response to email inviting participation)

Participant characteristics		
Variable	Intervention (n=18)	Control (n=22)
Age - yr; median [IQR]	50 [41-64]	58 [47-63]
Male sex – no. (%)	9/18 (50)	12/22 (54.5)
Invasively ventilated – no. (%)	8/18 (44.4)	6/22 (27.3)
On vasopressors – no. (%)	8/18 (44.4)	10/22 (45.5)
Proven infection – no. (%)	not reported	not reported
Diagnosis of acute brain pathology – no. (%)	not reported	not reported
Intervention details		
Nature of intervention	paracetamol	Placebo
Duration of study treatment - days	3	3
Outcomes		
Mortality at last reported time point (ICU mortality)	1/18 (5.6)	4/22 (18.2)
ICU length of stay -days	not reported	not reported
Hospital length of stay	not reported	not reported
Body temperature at 12 hours (°C)	not reported	not reported

Young P, Saxena M, Bellomo R, Freebairn R, Hammond N, van Haren F, Holliday M, Henderson S, Mackle D, McArthur C, McGuinness S, Myburgh J, Weatherall M, Webb S, Beasley R; HEAT Investigators; Australian and New Zealand Intensive Care Society Clinical Trials Group. Acetaminophen for Fever in Critically Ill Patients with

- Suspected Infection. N Engl J Med. 2015 Dec 3;373(23):2215-24. Multicentre investigator-initiated trial in ICU patients with fever and suspected in infection conducted in Australia and New Zealand (n=690)

DATA WERE INCLUDED IN THE IPDMA

Participant characteristics		
Variable	Intervention (n=347)	Control (n=344)
Age - yr	59.1±16.9	57.9±17.4
Male sex – no. (%)	224/347 (64.6)	225/344 (65.4)
Invasively ventilated – no. (%)	176/347 (50.7)	182/344 (52.0)
On vasopressors – no. (%)	174/347 (50.1)	181/344 (52.6)
Proven infection – no. (%)	217/347 (62.5)	214/344 (62.2)
Diagnosis of acute brain pathology – no. (%)	0/347 (0)	0/344 (0)
Intervention details		
Nature of intervention	paracetamol	placebo
Duration of study treatment - days	up to 28	up to 28
Outcomes		
Mortality at last reported time point (90 day mortality)	55/345	57/344
ICU length of stay -days	7.2±9.3	7.9±12.2
Hospital length of stay -days	19.3±19.1	21.0±23.8
Body temperature at 12 hours (°C)	37.1±0.9	37.7±0.9

Saxena MK, Taylor C, Billot L, Bompoint S, Gowardman J, Roberts JA, Lipman J, Myburgh J. The Effect of Paracetamol on Core Body Temperature in Acute Traumatic Brain Injury: A Randomised, Controlled Clinical Trial. PLoS One. 2015 Dec 17;10(12):e0144740.

- Multicentre investigator-initiated trial in ICU patients with acute traumatic brain injury in Australia (n=41)

DATA WERE INCLUDED IN THE IPDMA

Participant characteristics		
Variable	Intervention (n=21)	Control (n=20)
Age - yr; median [IQR]	33±16	33±18
Male sex – no. (%)	18/21 (85.7)	15/20 (75.0)
Invasively ventilated – no. (%)	21/21 (100)	20/20 (100)
On vasopressors – no. (%)	5/21 (24)	6/20 (30)
Proven infection – no. (%)	not reported	not reported
Diagnosis of acute brain pathology – no. (%)	21/21 (100)	20/20 (100)
Intervention details		
Nature of intervention	paracetamol	placebo
Duration of study treatment - days	3	3
Outcomes		
Mortality at last reported time point (hospital mortality)	3/21 (14.3)	1/20 (5.0)
ICU length of stay –days; median [IQR]	13.0 [7.0-15.0]	12.0 [6.0-15.0]
Hospital length of stay; median [IQR]	36.5 [23.0-48.0]	29.0 [20.0-41.0]
Body temperature at 12 hours (°C)	37.2±0.6	37.6±0.8

Plus-minus values are means±SD.
Abbreviations: ICU: Intensive Care Unit; IPDMA: Individual Patient Data Meta-Analysis; IQR: Interquartile range; SD: standard deviation.

SURVIVAL ANALYSES

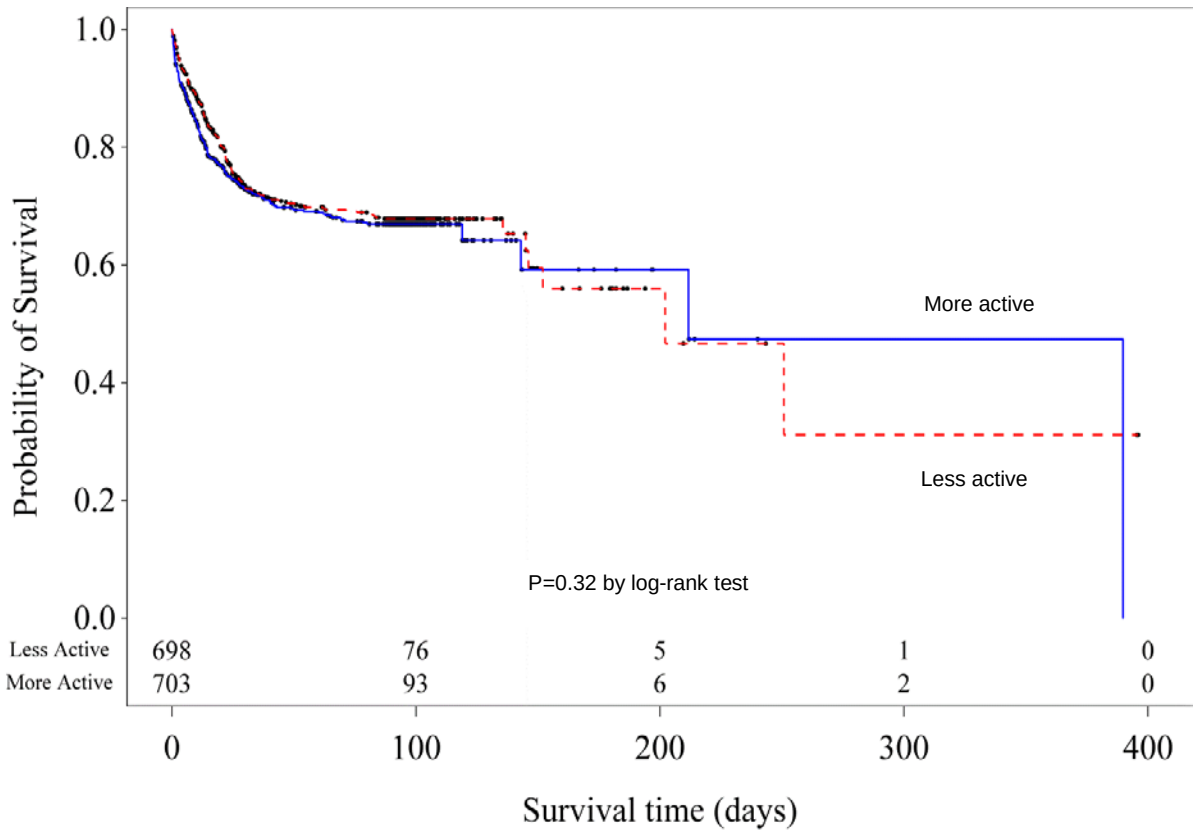


Fig. S1. Extended Kaplan-Meier survival estimates of the probably of survival

More active fever management did not result in a statistically significant difference in survival time compared with *less* active fever management in sensitivity analyses: hazard ratio (adjusted for age, sex, and study); 0.90 (95% CI, 0.74 to 1.09), P=0.28; hazard ratio (adjusted for age, sex, study, & APACHE-II score incorporating rescaled SAP-3 data from the Schortgen et al study); 0.89 (95% CI, 0.73 to 1.08), P=0.71.

MORTALITY

More active fever management did not result in a statistically significant difference in ICU compared with *less* active fever management in sensitivity analyses: odds ratio (adjusted for age, sex, and study); 0.75 (95% CI, 0.55 to 1.01), P=0.06; odds ratio (adjusted for age, sex, study, & APACHE-II score incorporating rescaled SAP-3 data from the Schortgen et al study); 0.74 (95% CI, 0.54 to 1.01), P=0.06.

LENGTH OF STAY DATA

Compared with *less* active fever management, *more* active fever management was associated with longer hospital length of stay in patients who died in ICU; ratio of geometric means 1.86 (95% CI, 1.36 to 2.53), and with shorter hospital length of stay patients who survived ICU; ratio of geometric means 0.93 (95% CI, 0.83 to 1.06); P<0.001 for interaction.

Compared with *less* active fever management, *more* active fever management was associated with longer hospital length of stay in patients who died in ICU; ratio of geometric means 1.92 (95% CI, 1.43 to 2.56), and with shorter hospital length of stay patients who survived ICU; ratio of geometric means 0.93 (95% CI, 0.83 to 1.06); P<0.001 for interaction.

FREE DAYS

ICU-free days to day 28 were similar by treatment group: median of 0 days (IQR 0 to 15 days) for *less* active temperature management vs. median of 0 days (IQR 0 to 16 days) for *more* active temperature management; estimate

of difference 0 days (95% CI, 0 to 0 days); P=0.16. Hospital-free days to day 28 were similar by treatment group: median of 17 days (IQR 0 to 24 days) for *less* active temperature management vs. median of 18 days (IQR 0 to 25 days) for *more* active temperature management; estimate of difference 0 days (95% CI, 0 to 0 days); P=0.37.

Subgroup	No. of individuals: More active	No. of individuals: Less active	Ratio of geometric means (95% CI)	*P* Value for Interaction
Invasively ventilated				
Yes	498	500	1.13 (0.97 to 1.31)	0.37
No	209	206	1.00 (0.79 to 1.26)	
Receiving inotropes and/or vasopressor				
Yes	358	377	1.27 (1.07 to 1.51)	0.007
No	349	329	0.90 (0.76 to 1.08)	
Ventilated & receiving inotropes and/or vasopressors				
Yes	291	304	1.32 (1.11 to 1.60)	0.006
No	416	402	0.94 (0.79 to 1.11)	
Infection present at baseline				
Yes	672	673	1.07 (0.95 to 1.22)	0.64
No	35	33	1.25 (0.68 to 2.32)	
Temperature 39.5 C				
Yes	89	67	1.35 (0.92 to 1.95)	0.25
No	614	639	1.06 (0.93 to 1.21)	
Age 75 years				
Yes	104	106	1.31 (0.91 to 1.73)	0.34
No	614	599	1.06 (0.92 to 1.21)	
APACHE-II score 25				
Yes	110	99	1.01 (0.74 to 1.38)	0.95
No	495	508	1.02 (0.89 to 1.16)	
Intervention includes physical cooling				
Yes	101	99	1.63 (1.17 to 2.25)	0.008
No	606	607	1.01 (0.89 to 1.16)	
Overall	707	706	1.08 (0.89 to 1.14)*	

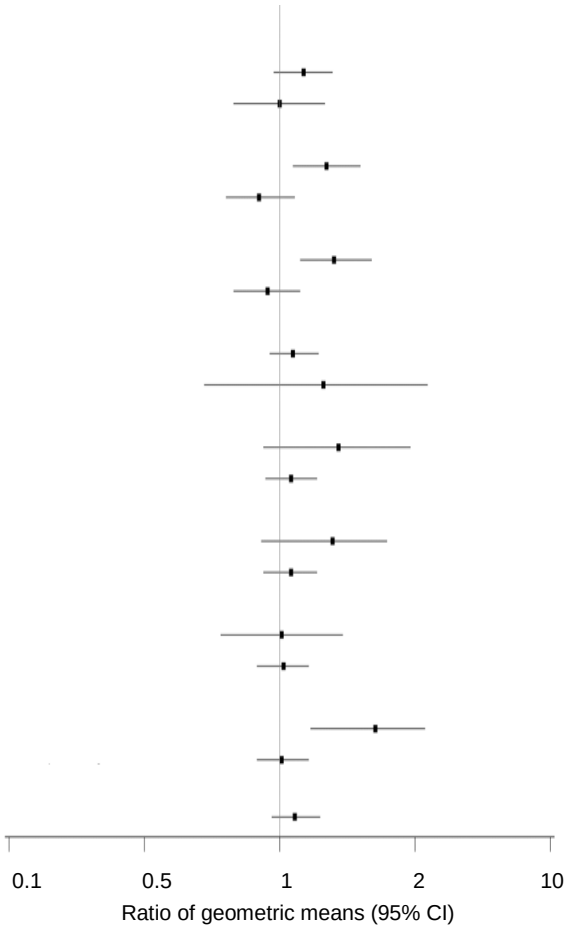


Fig. S2. Hospital length of stay with *more* active fever management vs. *less* active fever management by subgroup

* Unadjusted P value for overall comparison, 0.19; P value adjusted for age, sex, study, & APACHE-II score (excluding Schortgen et al trial), 0.82; P value adjusted for age, sex, study, & APACHE-II score (including Schortgen et al trial with SAPS III data from that trial rescaled to give the same range as APACHE-II data), 0.12; P value adjusted for age, sex, & study, 0.22.

Supplementary Material

Early PREdiction of sepsis using leukocyte surface biomarkers: the ExPRES-sepsis cohort study

Online only supplement

Shankar-Hari M et al. Early PREdiction of Sepsis using leucocyte cell surface biomarkers: The (ExPRES-Sepsis) cohort study

eMethods

eMethods-1: Detailed study cohort description[1] and description of gating with rationale
eMethods-2: Reliability and optimisation

eTables

eTable-1: Leukocyte biomarkers evaluated
MFI = mean fluorescence index; CD = Cluster of differentiation;

eTable-2: Table of Intraclass correlation coefficients (ICC) from the intra- and inter-rater reliability studies for each of the 47 biomarkers. Biomarkers rejected on intra-rater reliability testing are shown in red. Biomarkers rejected on inter-rater reliability testing are shown in blue

eTable-3: Rationale for Biomarkers selected for discriminant analysis in cross cohort comparison

eTable-4: Members of the independent expert review group who reviewed the provisional data from reliability, cross cohort comparisons, and discriminant analysis for the primary and secondary outcomes.

eTable-5: Comparison of recent leukocyte biomarker studies using multi-site flow cytometry with standardisation for illness trajectory prediction [2-4]

eFigures

eFigure-1: Flow diagram showing the decision analysis for assessing intra- and inter-rater reliability for the 47 biomarkers, and selecting biomarkers considered reliable for evaluation in cross cohort comparisons, as reported in the published protocol.

eFigure-2: Extreme phenotype derivation algorithm

eFigure-3: Cross cohort comparison of significant markers taken forward for further evaluation

eFigure-4: Comparison of biomarkers between sick phenotype, and well phenotypes
Shows significant differences in neutrophil CD279 between sick and well phenotype. MFI = Median Fluorescence intensity, reported on log10 scale. Statistical significance was determined using the Bonferroni-Dunn method to correct for multiple testing, with alpha = 0.05.

eMethods-1: Detailed study cohort description

This was reported in the published protocol paper and is replicated here for completion[1].
We recruited three distinct patient cohorts:
Cohort 1: Patients presenting to hospital with suspected infection with a systemic inflammation (discovery cohort).
Cohort 2: hospitalised patients with community-acquired severe sepsis requiring treatment in critical care (true- positive cohort).
Cohort 3: patients presenting with no suspicion of infection or systemic inflammation, needing hospitalisation ('non-sepsis comparison population').

Inclusion criteria

Cohort 1
Age ≥16 years (≥18 years in England), (2) SIRS criteria met, (3) clinical suspicion of sepsis (blood cultures and/ or other samples taken for microbial culture, or antibiotics started by clinical team), (4) no clinical suspicion of severe sepsis or septic shock at the time of enrolment and (5) enrolled within 12hours of hospital (ED) presentation.
Cohort 2
Age ≥16 years (≥18 years in England), (2) SIRS criteria met, (3) clinical suspicion of sepsis (blood cultures and/ or other samples taken for microbial culture, or antibiotics started by clinical team), (4) severity of sepsis requiring critical care admission (based on decision of caring clinical teams), (5) enrolled within 72 hours of hospital admission and (6) not enrolled into cohort 1 of ExPRES-Sepsis.
Cohort 3
Age ≥16 years (≥18 years in England), (2) does not meet SIRS criteria, (3) no clinical suspicion of sepsis (blood cultures and/or other samples NOT taken for microbial culture, and antibiotics NOT started by clinical team), (4) patient expected to be admitted to hospital, (5) patient NOT expected to die during hospital admission.

Exclusion criteria (for all cohorts)

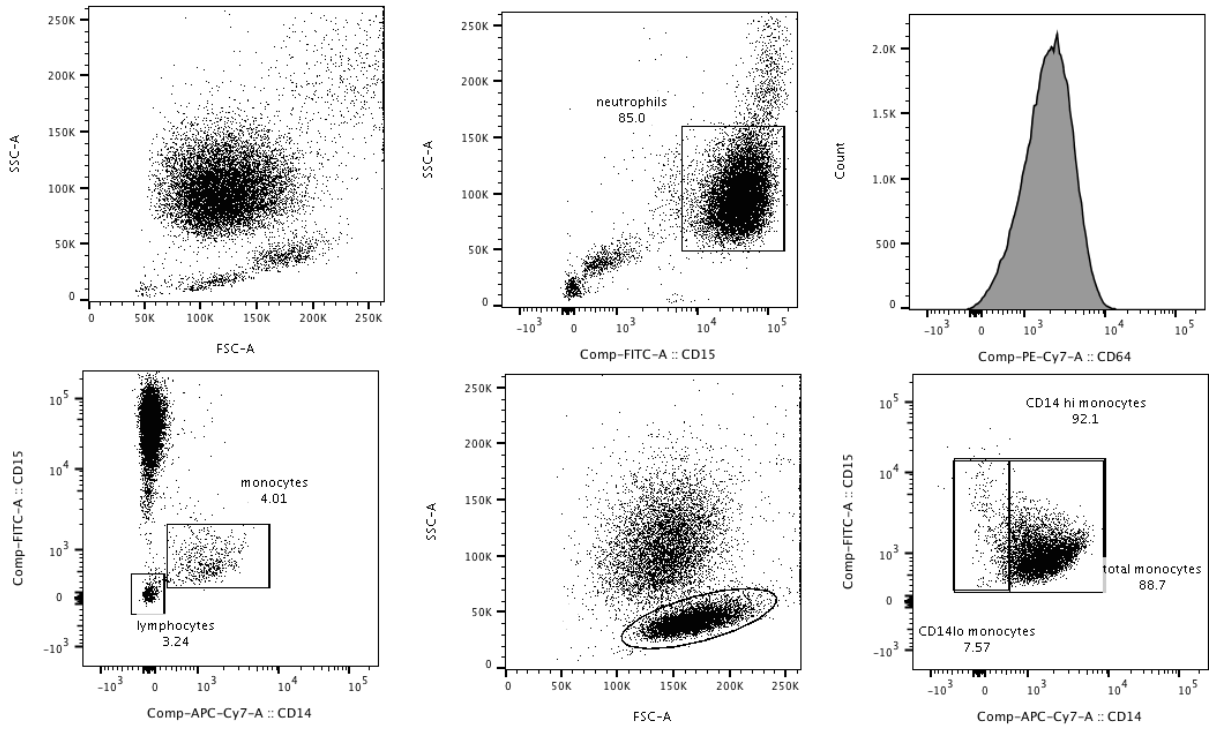
Exclusions were chosen to ensure conditions that provoke a sterile inflammatory response or lead to immune dysfunction did not act as confounders during flow cytometry analysis. Patients who would not be actively treated were also excluded.
The exclusion criteria are any of: (1) acute pancreatitis, (2) haematological malignancy, (3) recent chemo- therapy (past 2 weeks), (4) myelodysplastic syndromes, (5) known neutropenia, (6) HIV infection, (7) viral hepatitis infection, (8) pregnancy, (9) blood transfusion >4units in past week, (10) oral corticosteroids for >24hours prior to enrolment, (11) decision not for active therapy/for palliative care at admission and (12) inability to consent the patient.

eMethods-1: Description of gating and rationale

An a priori standard operating gating procedure was developed to identify other leukocyte biomarkers using the raw flow cytometry data. This involved: (1) initial strategy, based on pre-existing data, (2) expert learning and strategy refinement, to ensure ideal identification of leukocyte subtypes, and (3) expert consensus and finalisation of the gating strategy. These stages were undertaken iteratively by expert flow cytometrists (at least 2 years of flow cytometry experience) with cycles of testing and re-testing until a final procedure for each biomarker was agreed. We designed five separate panels.

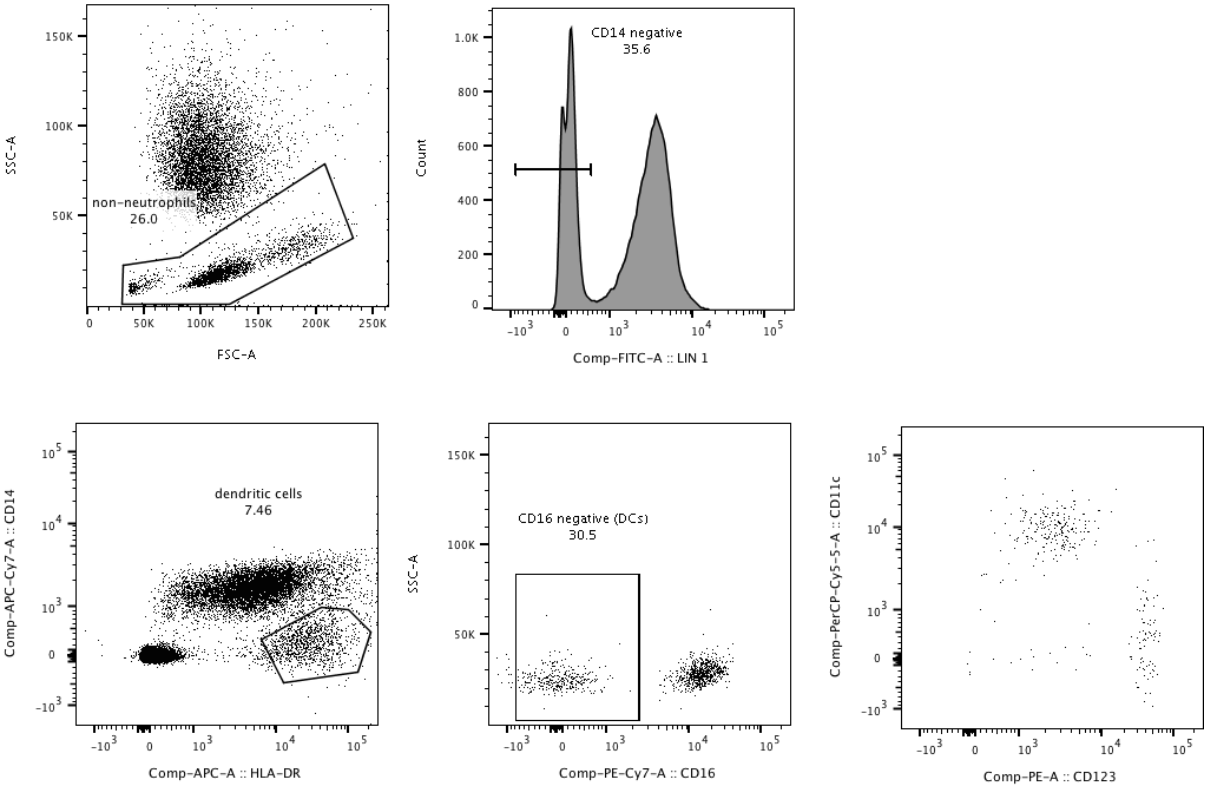
Panel-A

Marker	Fluorophore	Clone	Rationale/justification for use in the study
CD14	APC-H7	MΦP9	CD14 and CD15 have been chosen to help isolate monocytes and neutrophils. CD24, CD35, CD64 and CD312 have been chosen as markers of sepsis. Neutrophil CD64 is increased in infections. CD24 has been noted to be up regulated in in-vitro models of sepsis and blocking this pathway has been suggested to ameliorate sepsis. An increased CD312 (EMR2) expression on neutrophils has been liked with SIRS. An increase in CD35 expression has been linked to bacterial infection as compared to viral infection.
CD15	FITC	W6D3	
CD24	PerCP-Cy5.5	ML5	
CD35	PE	E11	
CD64	PE-Cy7	10.1	
CD312	AF647 (APC)	2A1 Serotec	



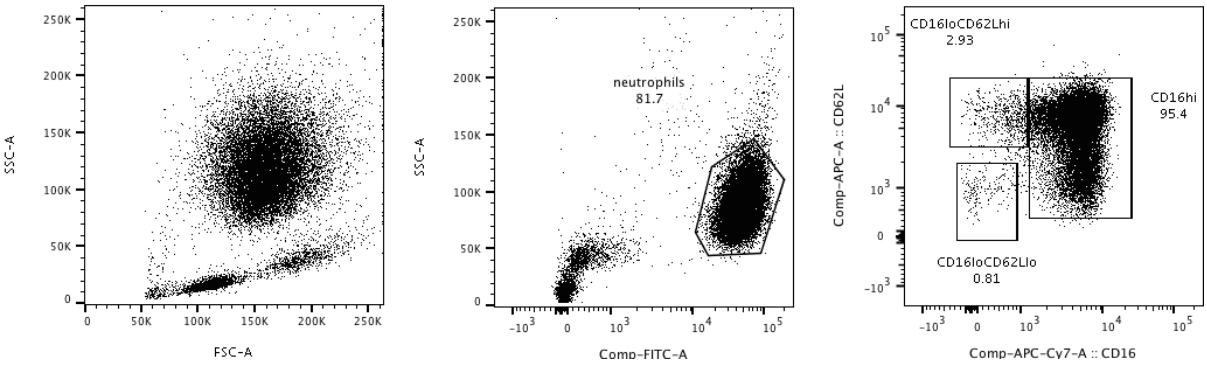
Panel B

Marker	Fluorophore	Clone	Rationale/justification for use in the study
CD3	FITC	SK7	The strategy at looking at myeloid and plasmacytoid dendritic cells is based on previous studies and a commercial assay developed by BD Biosciences. CD3, CD19 and CD56 have been chosen, all on the FITC channel, as lineage selection markers to help gate for dendritic cells. CD11c and CD123 have been chosen to allow differentiation between dendritic cell subtypes. CD14 and CD16 have been chosen to detect monocyte subtypes. Low monocyte HLA-DR has been associated with poor outcome in sepsis.
CD11c	PerCP-Cy5.5	S.HCL-3	
CD14	APC-H7	MΦP9	
CD16	PE-Cy7	B73.1	
CD19	FITC	4G7	
CD56	FITC	NCAM16.2	
CD123	PE	9F5	
HLA-DR	APC	G46-6	



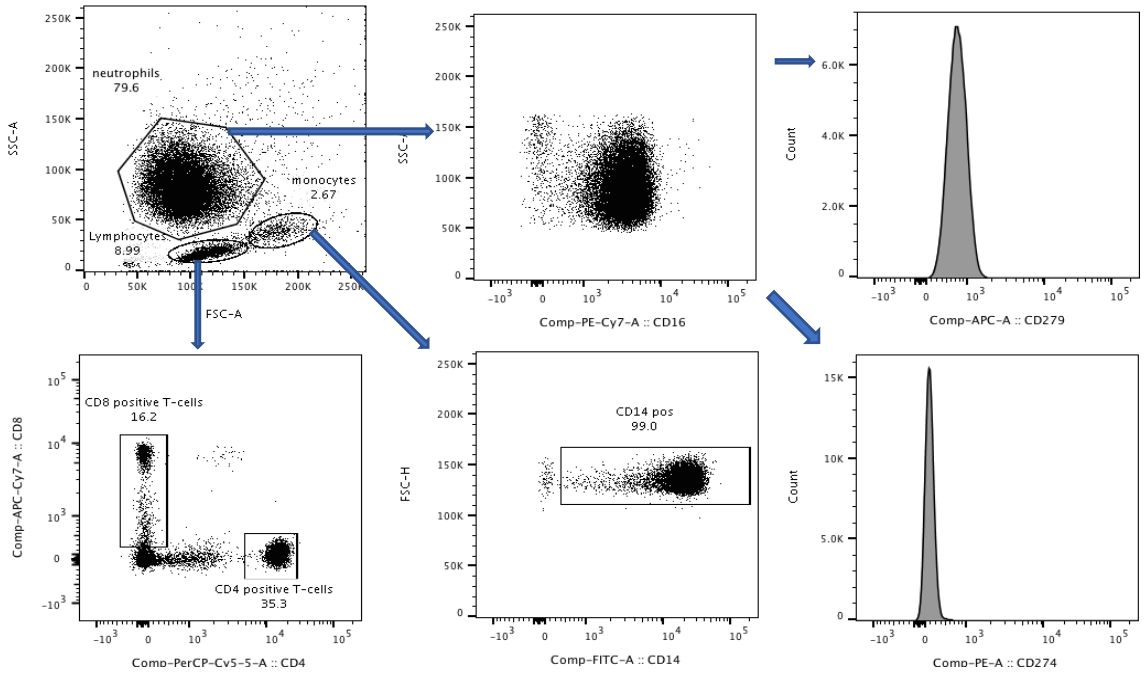
Panel-C

Marker	Fluorophore	Clone	Rationale/justification for use in the study
CD9	PE	M-L13	CD11b and CD62L have been chosen to be investigated as markers of sepsis. CD11b expression is enhanced in neonatal sepsis. CD9, CD15 and CD16 have been chosen to explore neutrophil progenitors, as immature neutrophils are associated with worse outcomes in sepsis patients.
CD11b	PE-Cy7	ICRF 44	
CD15	FITC	W6D3	
CD16	APC-H7	3G8	
CD62L	APC	DREG-56	



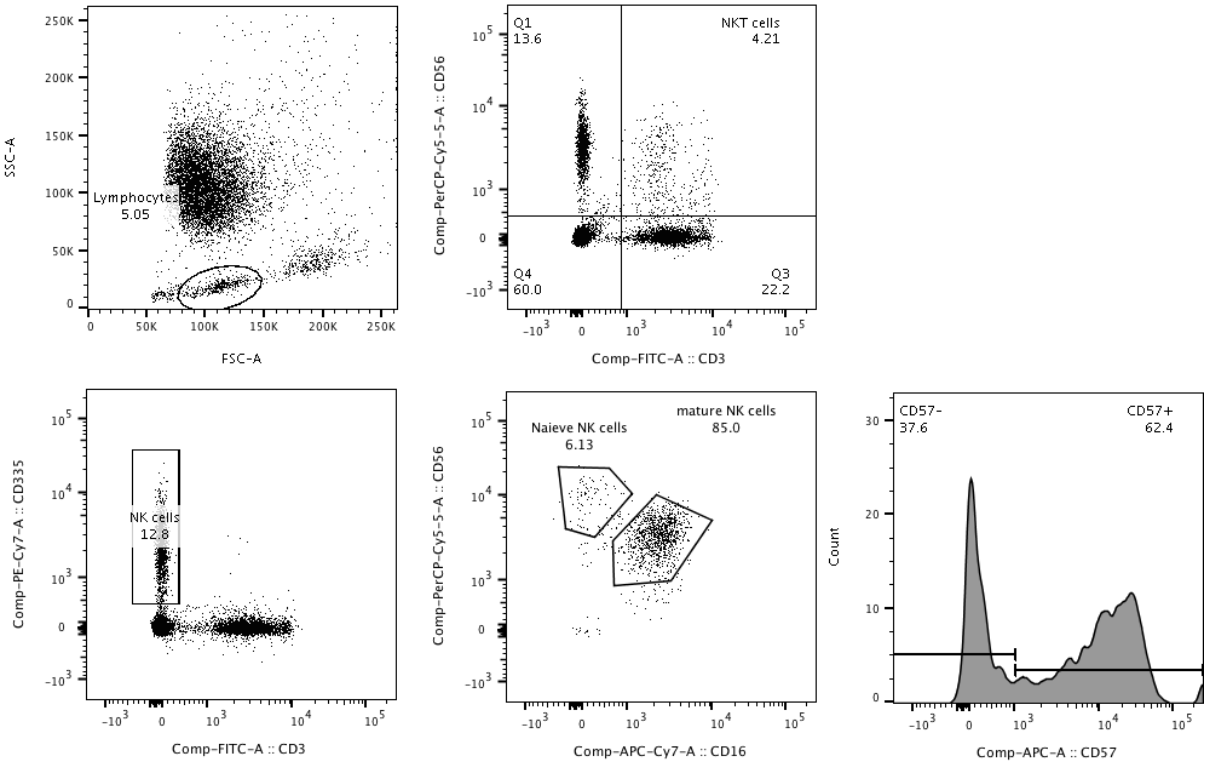
Panel-D

Marker	Fluorophore	Clone	Rationale/justification for use in the study
CD4	PerCp-Cy5.5	SK3	CD4 and CD8 have been chosen to differentiate between T-helper and cytotoxic T cells. CD14 and CD16 have been chosen to detect monocyte subtypes. CD274 (PD-L1) and CD279 (PD-1) are being assessed as potential markers of predicting sepsis, as increased expression is associated with worse outcomes in critically ill sepsis cohorts.
CD8	APC-H7	SK1	
CD14	FITC	MΦP9	
CD16	PE-Cy7	B73.1	
CD274	PE	MIH1	
CD279	APC	MIH4	



Panel-E

Marker	Fluorophore	Clone	Rationale/justification for use in the study
CD3	FITC	SK7	CD3 detects lymphocyte, NK and NKT cell populations. CD56 has been chosen to detect NKT cells. CD335 has been chosen to detect NK cells. CD16, CD56 and CD57 have been chosen to detect NK cell subsets.
CD16	APC-H7	3G8	
CD56	PerCP-Cy5.5	B159	
CD57	APC	NK-1	
CD284	PE	T901	
CD335	PE-Cy7	p9E2/NKp46	



Gating procedure

We evaluated stability of sample acquisition assessment by plotting time versus forward scatter and eliminated sections of poor flow. We then excluded cell aggregates using flow height versus area on the forward scatter. Further artefacts (debris) were eliminated by creating an “all cells” gate on the forward versus side scatter. Bi-exponential scaling was used for displaying and gating of flow cytometry data.

Neutrophil biomarkers

1. Display CD15 vs SSC on the ‘all cells’ gate for granulocytes
2. Gate tightly on $CD15^{hi}$ for neutrophils
3. Determine MFI for true neutrophil population for CD14, CD15, CD24, CD35, CD64, and CD312

Monocyte biomarkers

1. Display CD14 vs CD15 on all cells gate
2. Gate lymphocytes as $CD14^{lo}$ / $CD15^{lo}$
3. Display FSC-A vs SSC-A on all cells gate
4. Gate for presumed monocytes by light scatter properties
5. Calculate true monocyte gate as “presumed monocytes” NOT lymphocytes NOT neutrophils
6. Display CD14 vs CD15 on true monocyte gate

7. Plot 3 gates: total monocytes will be the largest rectangular gate, and 2 further gates: $CD14^{hi}$ monocytes and $CD14^{lo}$ monocytes
8. Determine MFI for CD14, CD15, CD24, CD35, CD64, and CD312 markers on each monocyte population

Dendritic cells

1. Display Lin^- (FITC-A) histogram on non-neutrophil gate (Note when referring to Lin- (lineage negative) we are referring to the $CD3^{negative}$ / $CD19^{negative}$ / $CD56^{negative}$ population.
2. Gate for Lin^- population using the marker tool - take the upper marker to the base of the Lin^+ population
3. Display HLA-DR vs CD14 on the above Lin^- subpopulation
4. Gate $HLA\text{-}DR^{positive}$ / $CD14^{negative}$ sub-population using a square or polygon gate
5. Display sub-population as CD16 vs SSC
6. Gate for total dendritic cells (DCs) as $CD16^{negative}$ using a square gate
7. Report total DCs as % of non-granulocyte cells
8. Report MFI of HLA-DR on total DCs

Dendritic cell subsets

1. Display CD123 vs CD11c on the above total DCs
2. Gate for myeloid dendritic cells as $CD11c^{high}$ / $CD123^{low}$
3. Gate for plasmacytoid dendritic cells as $CD11c^{low}$ / $CD123^{high}$
4. Gate for non-specific dendritic cells as $CD11c^{low}$ / $CD123^{low}$
5. Report DC subtypes as % of total DCs
6. Report mDC as % of non-granulocytes

Neutrophil progenitors

1. Display neutrophil population for CD16 vs CD62L
2. Gate the following neutrophil sub-types:
    a. $CD16^{hi}$ as mature neutrophils
    b. $CD62L^{hi}$ / $CD16^{mid}$ (as presumed late immature neutrophils)
    c. $CD62L^{lo}$ / $CD16^{lo}$ (as early immature neutrophils)
3. Record each gate as percentage of total granulocytes

Natural Killer T cells

1. Display CD3 vs CD56, on total lymphocytes
2. Report NKT cells (CD3+/CD56+) as % of total lymphocytes

Natural Killer cells and subsets

1. Display CD3 vs CD335, on total lymphocytes
2. Gate NK cells as $CD3^-/CD335^+$
3. Report total NK cells as % of total lymphocytes
4. NK subsets
    a. Display CD16 vs CD56 on total NK cells
    b. Gate naïve NK subset as $CD56^{++}$ / $CD16^-$; report as % of total NK cells
    c. Gate mature NK subset as $CD56^+$ / $CD16^+$; report as % of total NK cells
    d. Cytotoxic NK subset
        i. Display histogram of CD57 on $CD56^+$ / CD16++ mature NK subset (from previous step)
        ii. Report $CD57^{high}$ cytotoxic NK subset as % of mature NK cells (i.e. parent population)

eMethods-2: Reliability and optimisation

Sample size

Based on published recommendations[5], a sample size of 50 files was selected for the measurement of inter-observer agreement, and 13 files were selected for intra-observer agreement. For intra-observer agreement each expert observer re-analysed a different set of 13 files. For inter-observer agreement three different readers analysed the same 50 files. All 47 biomarkers were read from each file. For the intra-observer agreement, the files were presented in random order to readers by an independent individual.

Statistical testing of reproducibility

Each panel biomarker was assessed separately for intra- followed by inter-rater reliability. Reliability was assessed using intra-class correlation (ICC) coefficients and Bland-Altman plots. These measures were generated for both intra- and inter- observer agreement. ICC is expressed on a scale from 0 to 1: an ICC of 1.0 is interpreted as no variance between each observer, the ideal situation where observers can be considered interchangeable. An ICC cut-off of 0.9 was selected as the threshold for selection on the basis of previous literature. ICCs below this were judged to have inadequate reliability.

Analysis of each laboratory marker generated 3 intra-observer ICC coefficient statistics, describing the repeatability of reading by each observer. For inter-rater reliability each marker had 1 ICC coefficient statistic, describing the repeatability of the gating strategy across the 3 observers. Further assessment of each reader and each marker was performed using descriptive summary statistics and Bland-Altman statistics as required. Logarithmic transformation of data was carried out for further analyses if any data was not normally distributed. Bland-Altman plots were displayed as means vs differences, with the mean of differences referred to as bias, and upper and lower limits of agreements (U-LoA and L-LoA respectively) also generated.

Interpretation of reliability statistics

A protocol and rules-based system was created *a priori* to interpret the results of the reliability study (Figure 1). Intra-observer reliability was assessed first; the rationale being that intra-rater reliability was essential before any comparison between readers was likely to have clinical utility in discriminant analysis. If intra-rater reliability was established, the analysis proceeded to inter-observer reliability analysis. We required biomarkers to demonstrate both intra- and inter-rater reliability in order to be taken to the cross-cohort comparisons.

The interpretation strategy was designed to allow re-examination of markers which might potentially be falsely excluded due to any single data points which might be outliers.

Protocol for reliability assessment

Intra-observer reliability

Step 1

a. Is the intra-observer ICC for all 3 observers is greater than or equal to 0.9?
b. If yes, the outcome will be classified as having high reliability, and biomarker taken forward to the inter-observer interpretation stage
c. If no, proceed to step 2

Step 2

a. Do 2 out of 3 observers have an intra-observer ICC of greater than or equal to 0.9?
b. If no, outcome classified as having low reliability, and biomarker not be taken forward for primary analysis
c. If yes, proceed to step 3

Step 3

a. Data further analysed with a Bland-Altman plot and summary statistics. Qualitative and quantitative assessment of systematic bias, limits of agreement, and note if there are any points felt to be outliers.
b. Expert decision whether biomarker should be re-classified as having high reliability?
c. If yes, biomarker taken forward to the inter-observer interpretation stage
d. If no, biomarker not taken forward

Inter-observer reliability data

Step 1

a. Is the overall inter-observer ICC greater than or equal to 0.9?
b. If yes, the biomarker classified as having high reliability.
c. If no, proceed to step 2.

Step 2

a. Was poor agreement caused by a small number of outlier comparisons from one reader?
b. Was poor agreement attributable due to a single reader with a systematic bias?
c. Were limits of agreement consistent with acceptable precision?
d. Based on a-c assessment biomarker classified as having adequate reliability or rejected from further evaluation.

Results from statistical assessment

The results from the intra- and inter-rater reliability studies is shown in etable-2.

eTable-1: Leukocyte biomarkers evaluated

Leukocyte subset	Biomarker measurement description
Neutrophil biomarkers (N = 12 biomarkers)	MFI for CD14, CD15, CD24, CD35, CD64, CD312, CD11b, CD274, CD279
	CD62L low CD16 low neutrophil subset as proportion of total neutrophils
	CD16 high neutrophil subset as proportion of total neutrophils
	CD62L high CD16 mid neutrophil subset as proportion of total neutrophils
Monocyte biomarkers (N = 22 biomarkers)	MFI for CD14, CD15, CD24, CD35, CD64, CD312, CD11b
	MFI for HLA-DR as measured on total monocytes, classical monocyte subset, non-classical monocyte subset, and intermediate monocyte subset
	MFI for CD274 as measured on total monocytes, classical monocyte subset, non-classical monocyte subset, and intermediate monocyte subset
	MFI for CD279 as measured on total monocytes, classical monocyte subset, non-classical monocyte subset, and intermediate monocyte subset
	Classical, intermediate and non-classical monocyte subsets, all 3 as proportion of total monocytes
Dendritic cell biomarkers (N = 6 biomarkers)	MFI for HLA-DR measured on total dendritic cells
	Total dendritic cells, and myeloid dendritic cells, both as proportion of non-granulocyte cells
	Myeloid, plasmacytoid, and non-specific dendritic cell subtypes, as proportion of total dendritic cells
Lymphocyte biomarkers (N = 2 biomarkers)	MFI for CD274 and CD279 measured on CD8 lymphocytes
Natural killer cell biomarkers (N = 5 biomarkers)	Natural Killer cells and NKT cells, both measured as proportion of total lymphocytes
	Naïve, mature and cytotoxic NK subsets, measured as proportion of total Natural Killer cells

eTable-2: Table of Intraclass correlation coefficients (ICC) from the intra- and inter-rater reliability studies for each of the 47 biomarkers. Biomarkers rejected on intra-rater reliability testing are shown in red. Biomarkers rejected on inter-rater reliability testing are shown in blue

Marker	Inter-observer ICC	Intra-observer ICC		
		Edinburgh	Newcastle	London
Neutrophil CD14	1	1	1	1
Neutrophil CD15	1	1	1	1
Neutrophil CD24	1	1	1	1
Neutrophil CD35	1	1	1	0.9999
Neutrophil CD64	1	1	1	1
Neutrophil CD312	1	1	1	0.9995
Monocyte CD14	0.9998	1	1	0.9993
Monocyte CD15	0.9998	1	0.9998	0.9997
Monocyte CD24	0.999	0.9986	0.9996	0.999
Monocyte CD35	0.9992	0.9997	0.9997	0.9994
Monocyte CD64	0.9997	0.9998	0.9996	0.9993
Monocyte CD312	0.9943	0.9998	0.9997	0.9985
HLA-Dr expression on all dendritic cells (DCs)	0.7785	0.9557	0.9675	0.935
Myeloid DC as % of parent cell	0.829	0.8373	0.9942	0.7764
Non-specific DC as % of parent cell	0.864	0.6017	0.9873	0.9364
Plasmacytoid DC as % of parent cell	0.7611	0.9704	0.9796	0.9434
Total DC as % of non-granulocyte cells	0.7484	0.967	0.9582	0.9266
Myeloid DC as % of non-granulocyte cells	0.8208	0.9305	0.9961	0.8775
HLA-Dr expression on all monocytes	0.9994	0.9996	0.9994	0.9997
HLA-Dr expression on classical monocytes	0.9989	0.9997	0.9995	0.9997
HLA-Dr expression on non-classical monocytes	0.9299	0.9762	0.9933	0.9962
HLA-Dr expression on intermediate monocytes	0.9962	0.9976	0.9976	0.989
Classical monocytes, as % of all monocytes	0.929	0.9985	0.9938	0.9928
Non-classical monocytes, as % of all monocytes	0.9744	0.9887	0.9716	0.9907
Intermediate monocytes, as % of all monocytes	0.959	0.9929	0.9673	0.9678
Neutrophil CD11b.mfi	0.9999	1	0.9999	0.9999
Monocyte CD11b.mfi	0.9968	0.9978	0.9991	0.9995
Neutrophil CD16hi	0.7295	0.9843	0.8097	0.915
Neutrophil CD62Lhi/CD16mid	0.798	0.9844	0.802	0.9383
Neutrophil CD62Llow/CD16low	0.9493	0.9962	0.9929	0.9827
CD274 expression on CD8 lymphocytes	0.812	0.5054	0.9903	0.9771
CD274 expression on classical monocytes	0.9997	0.9998	0.9999	0.9453
CD274 expression on non-classical monocytes	0.9855	0.6597	0.997	0.9182
CD274 expression intermediate monocytes	0.9793	0.9919	0.9963	0.9594
CD274 expression on neutrophils	0.9998	0.998	1	0.9949
CD274 expression on all monocyte	0.9997	0.9998	0.9997	0.9997
CD279 expression on CD8 lymphocytes	0.914	0.9937	0.9682	0.9925
CD279 expression on classical monocytes	0.9943	0.975	0.9993	0.9874
CD279 expression on non-classical monocytes	0.1717	-0.1038	0.9924	0.974
CD279 expression on intermediate monocytes	0.9242	0.9189	0.9908	0.9689
CD279 expression on neutrophils	0.9978	0.9977	0.9989	0.9966
CD279 expression on all monocytes	0.9935	0.9703	0.9989	0.9863
NK measured as % of total lymphocytes	0.9985	0.9998	0.9989	0.9963
NKT measured as % of total lymphocytes	0.9883	0.9986	0.9958	0.9979
Cytotoxic NK cells, measured as % of NK cells	0.951	0.9888	0.9602	0.8446
Mature NK cells, measured as % of NK cells	0.8212	0.9765	0.9882	0.916
Naïve NK cells, measured as % of NK cells	0.7978	0.9681	0.986	0.9348

eTable-3: Rationale for Biomarkers selected for discriminant analysis in cross cohort comparison

Biomarker	Biological role	Cross cohort Kruskal Wallis test	Expert assessment	Selection
Neutrophil biomarkers				
CD14	LPS receptor with TLR4	P < 0.0001	Values cross cohorts mainly within 0-100 MFI range so limited variability. Cell numbers high. Differences mainly between cohort 1 and cohorts 2&3 which had similar values and range. Overall values lower in cohort 1.	NO
CD15	Carbohydrate adhesion module	P < 0.0001	Values for MFI range widely to maximum >8000. Cell numbers high. Clear cross cohort differences: highest cohort 3; lowest cohort 2; intermediate cohort 1.	YES
CD24	Cell adhesion glycoprotein; mediates cell apoptosis. Neutrophil expression	P < 0.001	Values for MFI cross cohort range widely to >50000. Cell numbers high. Clear cross cohort differences: highest cohort 1&2 with wide variability; lowest in cohort 1. Potential biological significance	YES
CD35	Complement receptor (type 1).	P < 0.0001	Values for MFI range widely to maximum >60000. Cell numbers high. Clear cross cohort differences: highest cohort 2; lowest cohort 3; intermediate cohort 1.	YES
CD64	Fc-gamma receptor 1	P < 0.0001	Values for MFI range widely to maximum >20000. Cell numbers high. Clear cross cohort differences: highest cohort 2; lowest cohort 3; intermediate cohort 1.	YES
CD312	G-protein coupled molecule	P < 0.0001	Values for MFI range to maximum >3000. Cell numbers high. Clear cross cohort differences: highest cohort 2; lowest cohort 1; intermediate cohort 2.	YES
CD11b	Complement receptor 3	P < 0.0001	Values for MFI range widely to maximum >60000. Cell numbers high. Clear cross cohort differences: highest cohort 1; lower and similar in cohorts 2&3.	YES
CD62L low CD16 low as % total neutrophils	Exploratory group	P < 0.0001	Values for percent mostly <2% with small number of outliers. Percent in small numbers ranged to >5%. Cell numbers very low. Cross cohort differences lack potential for discrimination.	NO
CD274	PD1 ligand	P < 0.0001	Values for MFI range to maximum >1000. Cell numbers high. Clear cross cohort differences: highest cohorts 1&2; lowest 3.	YES
CD279	PD1	P < 0.0001	Values for MFI range to maximum ≈1000. Cell numbers high. Clear cross cohort differences: wide range of values in cohorts 1&2 with lower values than cohort 3. Values consistently high in cohort 3.	YES
Monocyte biomarkers				
CD14	LPS receptor with TLR4	P = 0.277	No cross cohort differences. Biomarker was mainly selected as monocyte selection marker	NO

CD15	Carbohydrate adhesion module	P = 0.036	Minimal cross cohort differences	NO
CD24	Cell adhesion glycoprotein; mediates cell apoptosis. Neutrophil expression	P < 0.017	Minimal cross cohort differences. Lacks biological plausibility for monocytes.	NO
CD35	Complement receptor (type 1).	P < 0.0001	Values for MFI range widely to maximum >40000. Cell numbers moderate. Clear cross cohort differences: highest cohorts 1&2; lowest cohort 3.	YES
CD64	Fc-gamma receptor 1	P < 0.0001	Values for MFI range widely to maximum >100000 (in cohort 1). Cell numbers intermediate. Clear cross cohort differences: highest cohorts 1&2; lowest cohort 3.	YES
CD312	EMR2 cell surface marker on monocytes	P = 0.009	Values for MFI range to maximum >6000. Cell numbers intermediate. Some cross-cohort differences: highest cohort 2; lower in cohorts 1&3.	YES
CD11b	Complement receptor 3	P < 0.0001	Values for MFI range widely to maximum >80000. Cell numbers intermediate. Some cross cohort differences: highest cohorts 1&2; lower cohorts 3.	YES
CD274 total monocytes	PD1 ligand - check point inhibitors family	P < 0.0001	Values for MFI range to maximum >1000. Cell numbers intermediate. Some cross cohort differences: highest cohorts 1&2; lowest 3.	YES
CD279 total monocytes	PD1 – check point inhibitors family	P < 0.0001	Values for MFI range to maximum ≈500. Cell numbers intermediate. Clear cross cohort differences: wide range of values in cohorts 1&2 with lower values than cohort 3. Values consistently high in cohort 3.	YES
HLA-DR total monocytes	Antigen presentation	P < 0.0001	Values for MFI range to >30000. Cell numbers high. Clear cross cohort differences with highest levels in cohort 3, lowest in cohort 2, and intermediate with wide range in cohort 1.	YES
Percent classical monocytes		P<0.0003	Although differences apparent across the three groups, the cell numbers for non-classical and intermediate monocyte groups low. General patterns suggest lower percentages of classical monocytes in cohort 2 relative to cohorts 1&3; lower percent non-classical monocytes in cohort 1 compared to cohorts 2&3; lower percent of intermediate monocytes in cohort 3 compared to cohorts 1&2. Likely mathematical linkage and collinearity between these biomarkers, which would be problematic in discriminant and multivariable analysis.	NO
Percent non-classical monocytes		P < 0.0001		
Percent intermediate monocytes		P < 0.0001		
CD274 on classical monocytes	PD1 ligand - check point inhibitors family	P < 0.0001	Although differences across groups exist, cell numbers small and absolute differences in MFI values between groups small. Likely mathematical linkage and collinearity with CD274 on total monocytes, which would be problematic in discriminant and multivariable analysis	NO
CD274 on intermediate monocytes	PD1 ligand - check point inhibitors family	P < 0.0001		
CD279 on classical monocytes	PD1 – check point inhibitors family	P < 0.0001	Although differences across groups exist, cell numbers small and absolute differences in MFI values between groups small. Likely mathematical linkage and collinearity with CD274 on total monocytes, which would be problematic in discriminant and multivariable analysis	NO
CD279 on intermediate monocytes	PD1 – check point inhibitors family	P < 0.0001		
HLA-DR on classical monocytes		P < 0.0001	Patterns all mirror differences across cohorts for HLA-DR on total monocytes. Cell numbers for non-classical and intermediate monocytes very low. Likely mathematical linkage and collinearity with HLA-DR on total monocytes, which would be problematic in discriminant and multivariable analysis	NO
HLA-DR on non-classical monocytes		P < 0.0001		

HLA-DR on intermediate monocytes		P < 0.0001		
Lymphocyte biomarkers				
CD279 on CD8 T cells	PD1 – check point inhibitors family	P < 0.0001	Cell numbers intermediate. Some cross cohort differences: highest cohorts 1 and 2.	YES

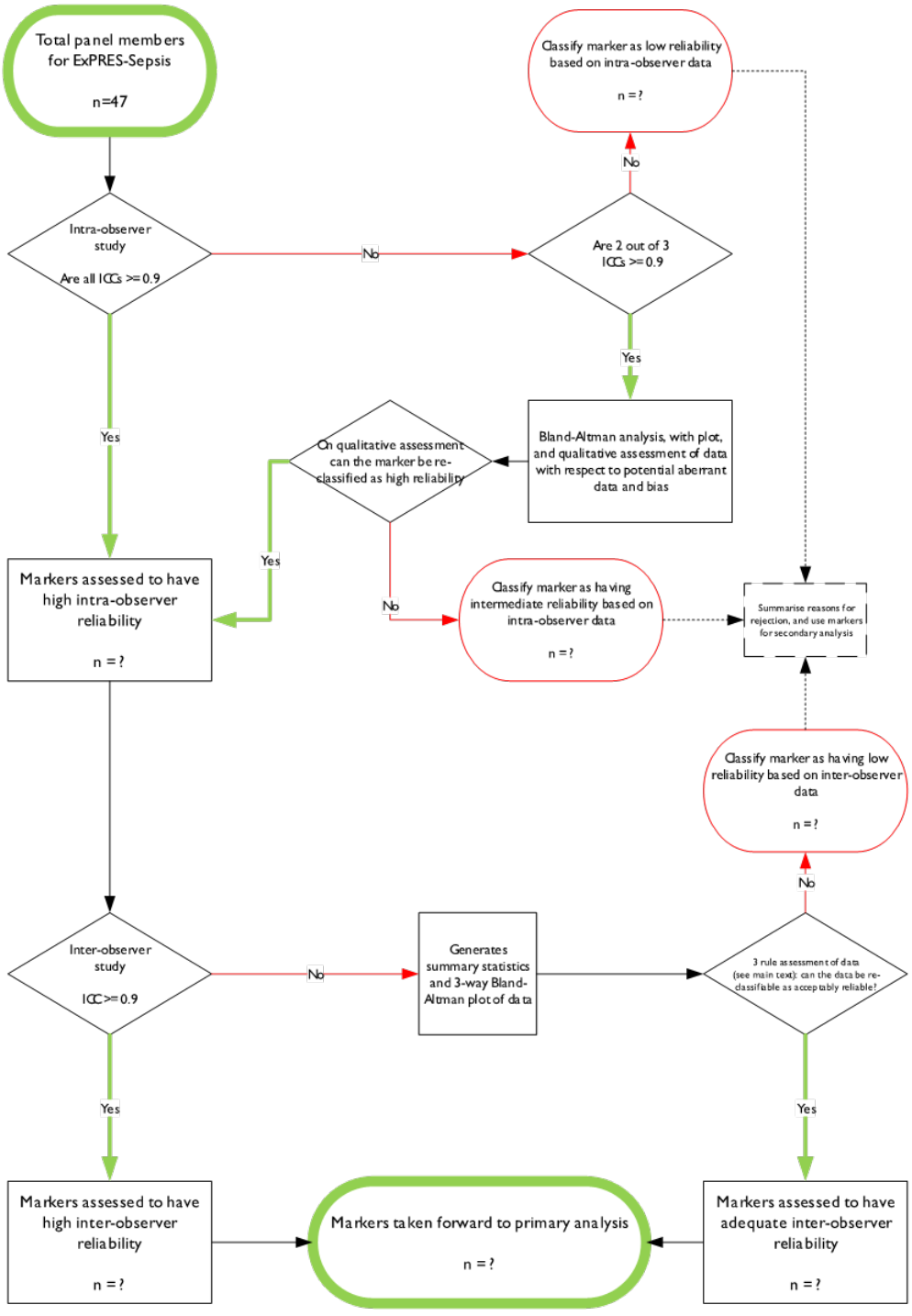
eTable-4: Members of the independent expert review group who reviewed the provisional data from reliability, cross cohort comparisons, and discriminant analysis for the primary and secondary outcomes. This group recommended the post hoc extreme phenotype analysis to further explore differences between patients who subsequently recovered quickly versus progressed to severe sepsis.

Name	**Position, Institution**
Mervyn Singer	Professor of Intensive Care Medicine, University College, London, UK
Jean-Daniel Chiche	Professor of Critical Care Medicine, Hospital Cochin, Paris, France
Paul Dark	Professor of Critical Care Medicine, University of Manchester, Manchester, UK

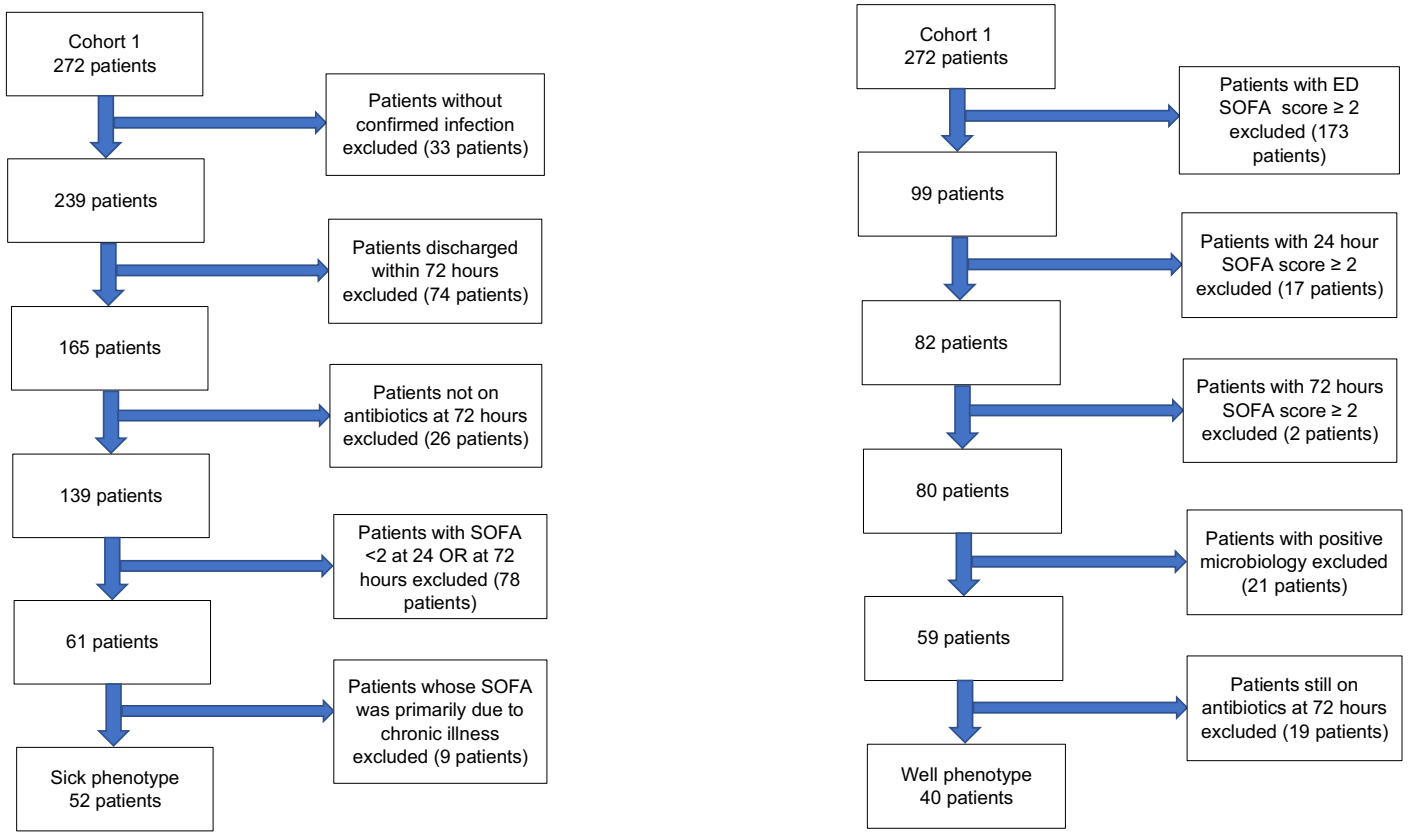
eTable-5: Comparison of recent leukocyte biomarker studies using multi-site flow cytometry with standardisation for illness trajectory prediction [2-4]

Study characteristics	Current study (EXPRESS) N=259	Guerin E at al [4] N=177	Daix T el al[2] N=781	Conway-Morris A et al [3] N=138
Primary objective	Predict deterioration to develop sepsis sepsis-3 sepsis within 24 or 72 hours	Predict early evolution (deterioration or stability/improvement) of sepsis at 48 hours	Predict early evolution (deterioration or stability/improvement) of sepsis	Validate[6] cellular markers of immune dysfunction to stratify risk of secondary infection
Case definition at sampling	Patients with suspected infection attending ED	Patients with sepsis, severe sepsis or septic shock	Patients with sepsis, severe sepsis or septic shock	Critically ill patients predicted to remain in ICU for >=48 hours
Sites (N)	4	1	11	4
Number of leukocyte biomarkers evaluated	47 leukocyte biomarkers including leukocyte subsets (see eTable-1) assessed for reliability, discriminant value, followed by best subsets logistic regression	24 markers and 23 leukocyte subsets CD36; CD2; CD294; CD19; CD16; CD45; CD11b; CD16; CD8; CD64; CD11c; CD10; CD24; CD34; CD123; CD138; CD4; CD38; CD25; CD56; CD127; CD3; CD116; HLA-DR	CD64; CD10; CD3; CD24; CD11b; CD16; CD45	Neutrophil Cd88; Monocyte HLA-DR; proportion of regulatory T cell subsets
Key findings	Optimum biomarker combination of increased neutrophil CD24 and neutrophil CD279, and reduced monocyte HLA-DR expression to predict subsequent deterioration to sepsis	Immature granulocytes (CD10dim CD16dim) predicted clinical deterioration	Immature granulocytes associated with clinical worsening, when associated with T cell lymphopenia	Confirmed our previous findings[6]
Comparison of key findings of other studies with our EXPRESS study		CD16low subset did not have cross cohort discrimination in our EXPRESS study	CD64 MFI had univariate association, which disappeared with best-subsets logistic regression. Lymphopenia did not have cross cohort discrimination; CD24 expression in neutrophils was associated with clinical deterioration in our EXPRESS study	Different study population; HLA-DR was associated with clinical deterioration to sepsis in our EXPRESS study
Biological relevance of key markers reported in each study	CD24 expressed on mature granulocytes and B cells; down-regulated on neutrophils in sepsis, induces neutrophil apoptosis which is impaired in sepsis. CD279 and HLA-DR are markers of associated with sepsis related immunosuppression consistently reported in literature	Myeloid derived immature granulocytes appear cytotoxic towards T lymphocytes	CD64 is a Fc gamma receptor expressed on leukocytes; consistently reported as a diagnostic marker for sepsis	

eFigure-1: Flow diagram showing the decision analysis for assessing intra- and inter-rater reliability for the 47 biomarkers, and selecting biomarkers considered reliable for evaluation in cross cohort comparisons. This algorithm is replicated as presented in the protocol manuscript [1].

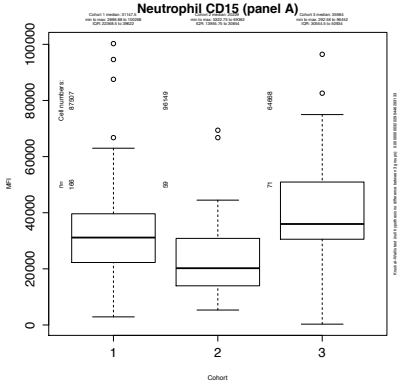


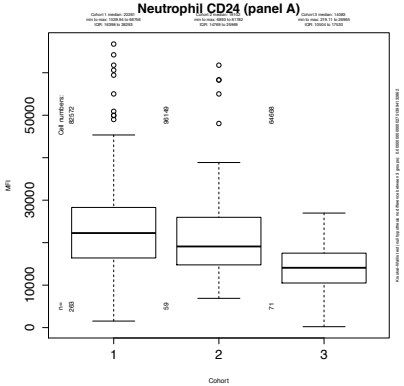
eFigure-2: Extreme phenotype derivation

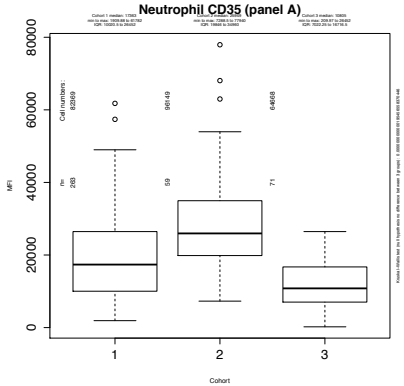


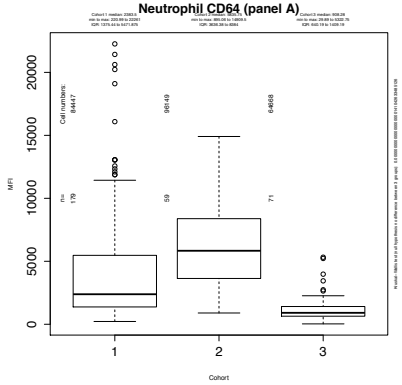
eFIgure-3: Cross cohort comparison of significant markers taken forward for further evaluation

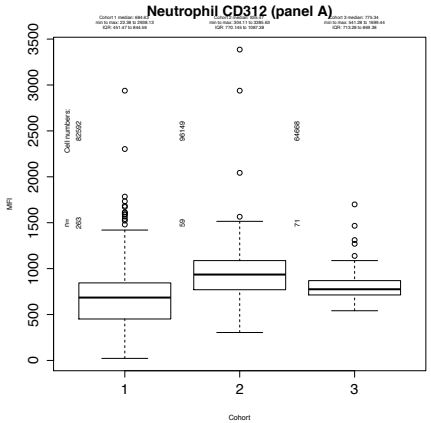
eFIgure-3a: Neutrophil markers

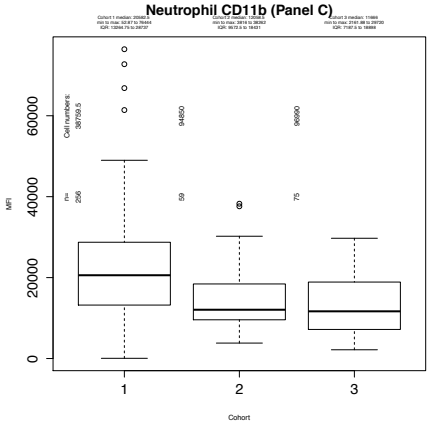


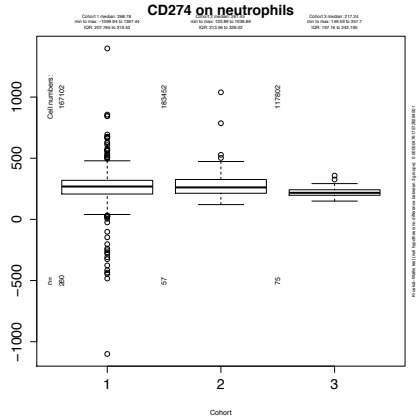


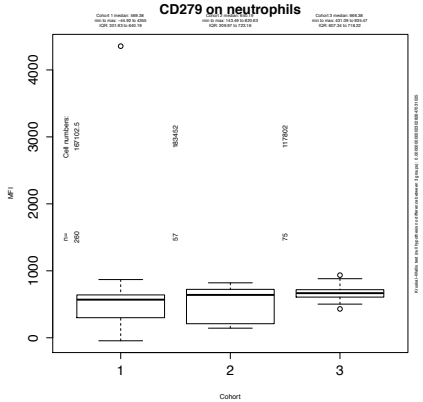






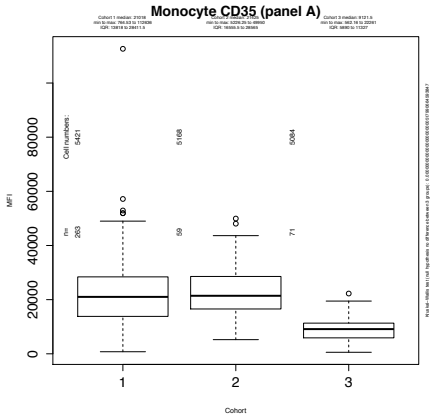


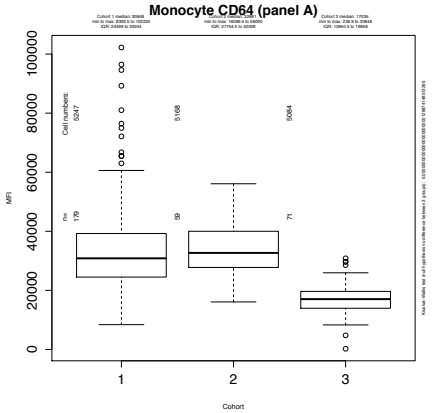


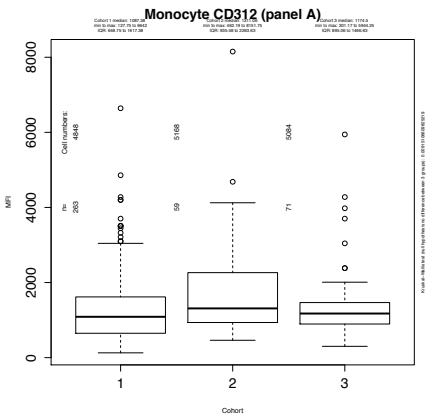


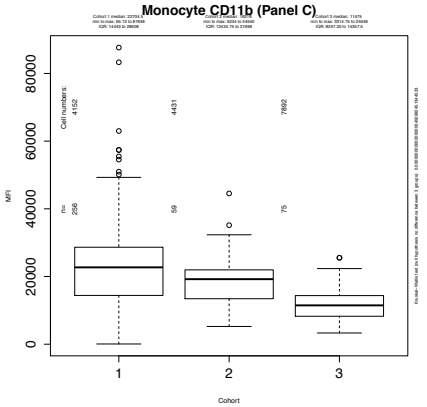
eFIgure-3: Cross cohort comparison of significant markers taken forward for further evaluation

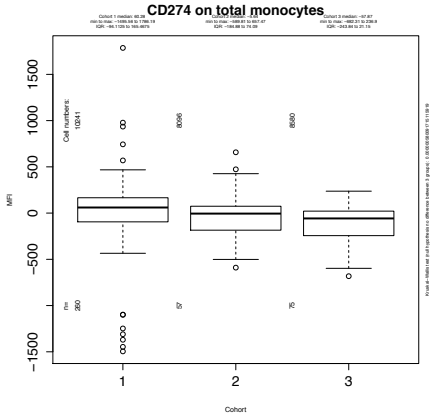
eFIgure-3b: monocyte biomarkers and CD279 on CD8-T cells

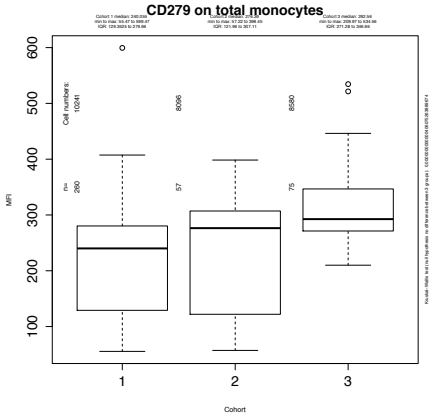


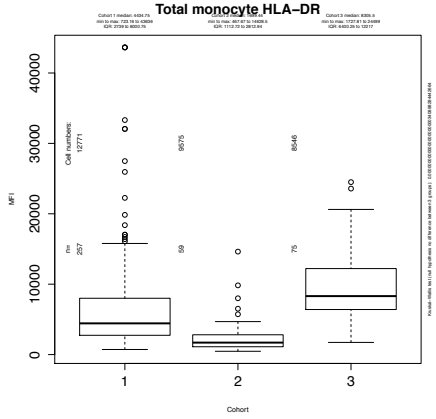


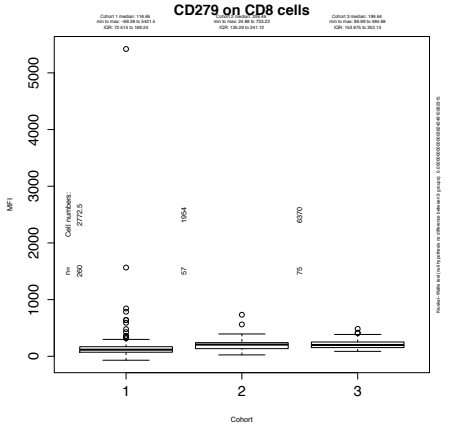




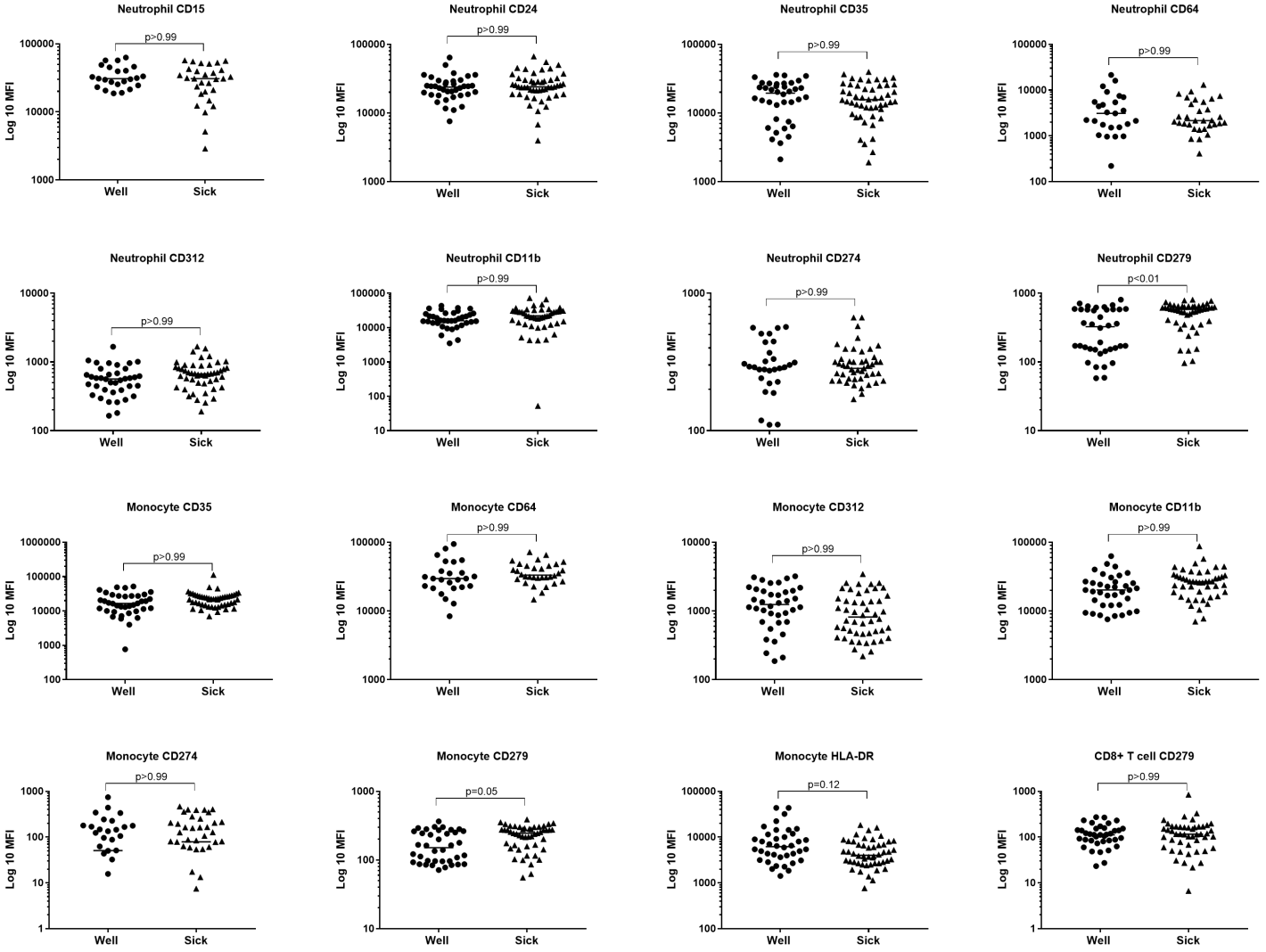








eFigure-4: Biomarker profile differences in the well versus sick extreme phenotype



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1. Datta D, Conway Morris A, Antonelli J, et al. Early PREdiction of Severe Sepsis (ExPRES-Sepsis) study: protocol for an observational derivation study to discover potential leucocyte cell surface biomarkers. BMJ Open. 2016;6(8):e011335.
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3. Conway Morris A, Datta D, Shankar-Hari M, et al. Cell-surface signatures of immune dysfunction risk-stratify critically ill patients: INFECT study. Intensive Care Med. 2018;44(5):627-635.
4. Guerin E, Orabona M, Raquil MA, et al. Circulating immature granulocytes with T-cell killing functions predict sepsis deterioration*. Crit Care Med. 2014;42(9):2007-2018.
5. Altman DG. Practical statistics for medical research. London: Chapman & Hall 2010.
6. Conway Morris A, Anderson N, Brittan M, et al. Combined dysfunctions of immune cells predict nosocomial infection in critically ill patients. Br J Anaesth. 2013;111(5):778-787.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Manuscript reference; page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	**Title; Page-1**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	**Abstract; Page-5 and Page-6**
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	**Introduction; Page-7 and Page-8**
Objectives	3	State specific objectives, including any prespecified hypotheses	**Introduction Page-7 and Page-8; Parah-3**
Methods			
Study design	4	Present key elements of study design early in the paper	**Methods: Page-8, 9, 10, 11, 12, and Page-13 Electronic supplement; Page 2, 3, 4, 5, 6, 7 and Page-8**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	**Methods: Page-8, 9, 10, 11, 12, and Page-13 Electronic supplement**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	**Methods: Page-8 Online only supplement; Page-2,3,**
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed	**Not applicable**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	**Methods: Page-8, 9, 10, 11, 12, and Page-13 Online only supplement; Page-4,5, 6, 7 and Page-8 Statistics Page-6**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	***n/a***
Bias	9	Describe any efforts to address potential sources of bias	**Discussion; Page 18**
Study size	10	Explain how the study size was arrived at	**Methods Page 10**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	**Statistics; Page 10, 11, 12 and Page-13**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	**Statistics; Page 10, 11, 12 and Page-13**
		(*b*) Describe any methods used to examine subgroups and interactions	**Statistics; Page 10, 11, 12 and Page-13**
		(*c*) Explain how missing data were addressed	**Statistics; Page 10, 11, 12 and Page-13**
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	**Statistics; Page 10, 11, 12 and Page-13**
		(*e*) Describe any sensitivity analyses	**N/a**

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	**Page-13 and Page-14**
		(b) Give reasons for non-participation at each stage	**n/a**
		(c) Consider use of a flow diagram	**n/a**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	**Table-1**
		(b) Indicate number of participants with missing data for each variable of interest	**As above**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	**Table-1**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	**Table-1; Figure-1; eTable-2; Table-3**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	**Table-3**
		(*b*) Report category boundaries when continuous variables were categorized	**Table-3**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	**AUROC and Odds ration with each SD increase in biomarker value**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	**n/a**
Discussion			
Key results	18	Summarise key results with reference to study objectives	**Page 16; Parah-2**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	**Page 18**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**Discussion: Pages 16, 17, 18, and Page-19**
Generalisability	21	Discuss the generalisability (external validity) of the study results	**Discussion: Page Page-16 paragraph-; Page-19**
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	**Page-3**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies. **Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Supplementary Material

Using multiple 'omics strategies for novel therapies in sepsis

SUPPLEMENT

VASST was approved by the research ethics boards of all participating institutions. Patients, next of kin or surrogate decision maker gave written informed consent.