World Sepsis Day 2019

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sepsis
save
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Foreword by:
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MDR Gram-negative Bacilli Infections

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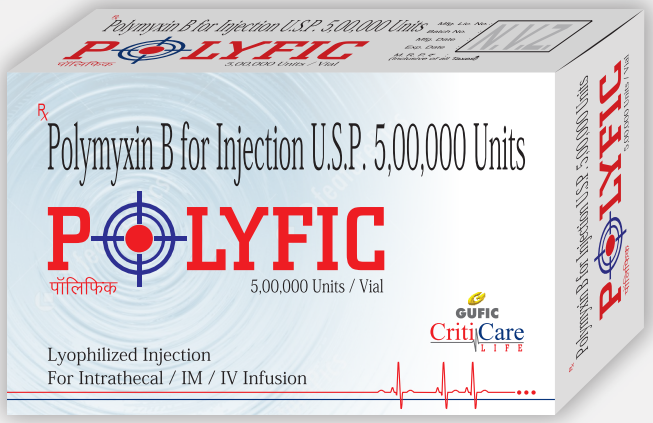
Inj. Polymyxin B Sulphate 5,00,000 Units

Reconsider to treat Gram-negative infections

Indian manufacturer of Polymyxin-B

API imported from Xellia pharmaceutical's-Denmark

Micronized particle size



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Foreword

Sepsis is a life-threatening condition associated with high mortality rates and considerable long-term morbidity with sepsis survivors often facing persistent physical or psychological sequelae many years after discharge. Mortality rates in patients who survive sepsis also remain elevated for several years after the initial insult. Early diagnosis, enabling rapid appropriate, and effective management to be started is essential if the global burden of sepsis is to be reduced.

Caused by a dysregulated host response to infection, sepsis can be difficult to diagnose accurately and rapidly. Multiple biomarkers of sepsis have been proposed, but none is specific for sepsis and few are widely available. Once sepsis is diagnosed, the patient should be managed by a sepsis team wherever possible, with treatment focusing on the three key arms: infection control, hemodynamic stabilization, and modulation of the sepsis response. Diagnosis of infection using microbiological cultures is time-consuming, and can delay the start of appropriate antibiotics. Initial antibiotics should therefore, be chosen to cover all possible bacteria according to likely site and source of infection and local microbiological patterns. Once culture results are available, the antibiotic prescription can be altered to reduce risks of unwanted adverse effects from unnecessary antibiotics and limit the development of antibiotic resistance. Newer molecular methods that can identify infection without the need for culture processing are beginning to become available and will help speed the use of appropriate antibiotics. Any infectious source, e.g., infected line or intraperitoneal abscess, must be removed as a matter of urgency.

Hemodynamic status should be stabilized using fluids and vasopressor agents according to the four SOSD stages: Salvage, Optimization, Stabilization, and De-escalation, respectively. Targets for fluid and vasopressor administration should be adapted to individual patient requirements as much as is possible as, for example, a cardiac index or mean arterial pressure that is adequate for one patient may not be suitable for another. Improved techniques to monitor the microcirculation should help identify ongoing need for fluids and/or vasoactive agents but remain experimental at present. Other organ support, e.g., mechanical ventilation and renal replacement therapy should be started as required, when available. International guidelines on sepsis management and hemodynamic support are available, but must be adapted to local conditions and available resources.

No drugs are currently available that modulate the sepsis response, with the possible exception of corticosteroids in patients with severe septic shock. However, many potential agents are being studied. New techniques, including those based on omics technologies, are being developed to help characterize specific clinical and biological phenotypes of individual patients with sepsis, which will help in the identification of effective treatments and in the appropriate targeting of treatment choices in the future.

In this booklet, published in conjunction with the World Sepsis Day 2019 and aimed at advanced critical care intensivists in India, some key published articles about various aspects of the management of sepsis have been reproduced. Written by International experts in the field, these provide an up-date on some of the latest developments in the field of sepsis, and I am sure will be of interest to all those involved in the treatment of patients with sepsis.

Happy reading!

Prof. Jean-Louis Vincent

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Critical Appraisal

SYSTEMATIC REVIEW

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

Tomoko Fujii[1,2], Riki Ganeko[3], Yuki Kataoka[4], Toshi A. Furukawa[5], Robin Featherstone[6], Kent Doi[7], Jean-Louis Vincent[8], Daniela Pasero[9], René Robert[10], Claudio Ronco[11] and Sean M. Bagshaw[12*]

Abstract

Purpose: Polymyxin B-immobilized hemoperfusion (PMX-HP) is an adjuvant therapy for sepsis or septic shock that clears circulating endotoxin. Prior trials have shown that PMX-HP improves surrogate endpoints. We aimed to conduct an evidence synthesis to evaluate the efficacy and safety of PMX-HP in critically ill adult patients with sepsis or septic shock.

Methods: We searched for randomized controlled trials (RCTs) in MEDLINE, EMBASE, the Cochrane Library, the Health Technology Assessment Database, CINAHL, "Igaku Chuo Zasshi", the National Institute of Health Clinical Trials Register, the World Health Organization International Clinical Trials Registry Platform, the University Hospital Medical Information Network Clinical Trials Registry, the reference lists of retrieved articles, and publications by manufacturers of PMX-HP. The primary outcomes were 28-day all-cause mortality, the number of patients with at least one serious adverse event, and organ dysfunction scores. The GRADE methodology for the certainty of evidence was used.

Results: Six trials (857 participants; weighted mean age 62.5 years) proved eligible. Patient-oriented primary outcomes were assessed. The pooled risk ratio (RR) for 28-day mortality associated with PMX-HP was 1.03 [95% confidence interval (CI) 0.78–1.36; $I^2 = 25\%$; $n = 797$]. The pooled RR for adverse events was 2.17 (95% CI 0.68–6.94; $I^2 = 0\%$; $n = 717$). Organ dysfunction scores over 24–72 h after PMX-HP treatment did not change significantly (standardized mean difference − 0.26; 95% CI − 0.64 to 0.12; $I^2 = 78\%$; $n = 797$). The certainty of the body of evidence was judged as low for both benefit and harm using the GRADE methodology.

Conclusions: There is currently insufficient evidence to support the routine use of PMX-HP to treat patients with sepsis or septic shock.

Registration: PROSPERO International Prospective Register of Systematic Reviews (CRD42016038356).

Keywords: Sepsis, Septic shock, Polymyxin B-immobilized hemoperfusion, Systematic review, Meta-analysis

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Introduction

Sepsis remains desperately fatal and septic shock has a hospital mortality rate as high as 20–50% worldwide [1–5]. Many interventions have been evaluated to improve the prognosis of sepsis, but large multi-centered trials of various therapies have failed to demonstrate consistent benefit [6]. As fundamental elements of sepsis treatment, including timely and appropriate antimicrobial therapies, adequate fluids, and vasopressors, have not changed for decades [7, 8], there currently is dire need for new and effective therapies.

Endotoxin, a principal component of the outer membrane of Gram-negative bacteria, is recognized as a potent mediator of the host response to infection and development of sepsis [9]. Studies measuring endotoxin levels in patients with septic shock have found that high levels of endotoxin activity correlated with worse clinical outcomes [10, 11]. Polymyxin B (PMX) is a cyclic cationic polypeptide antibiotic with high affinity for endotoxin. A novel strategy whereby PMX is bound and immobilized to polystyrene fibers in a hemoperfusion device was developed in Japan [12, 13]. The suggested mechanism of PMX hemoperfusion (PMX-HP) is to remove circulating endotoxin by adsorption, which modulates and limits the maladaptive host response to infection and the progression of the organ injury cascade of sepsis.

Selected clinical trials have suggested PMX-HP can improve the physiological profile of patients with sepsis [14–16]; however, it remains uncertain whether PMX-HP can reproducibly improve patient outcomes, as the trials have largely focused on surrogate endpoints or have been underpowered to detect effects on clinically important outcomes [16]. Additional studies have recently been completed evaluating PMX-HP, including two large multi-centre randomized controlled trials (RCTs) [17, 18].

We therefore conducted an up-to-date systematic review and evidence synthesis evaluating the impact of PMX-HP as an adjuvant therapy for critically ill adult patients with sepsis or septic shock on clinical outcomes and health services utilization. We hypothesized that use of PMX-HP would improve survival among adult critically ill patients with sepsis or septic shock.

Methods

Protocol and registration

This systematic review was conducted using guidelines in the Cochrane Collaboration and Centre for Reviews and Dissemination and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guideline [19]. The protocol has been registered with the PROSPERO International Prospective Register of Systematic Reviews; registration number CRD42016038356, and published in full elsewhere [20].

Eligibility criteria

All relevant RCTs that investigated the effect of PMX-HP for patients with sepsis or septic shock were included. The primary research question was "what is the efficacy, effectiveness and potential harm of PMX-HP compared with standard therapy?" We obtained all relevant studies irrespective of language or publication status. Adults aged 18 years or older with sepsis, severe sepsis or septic shock were included. The diagnosis of sepsis was based on clinically suspected or documented systemic infection with any signs of systemic inflammatory response syndrome. Septic shock was classically defined as hypotension resistant to fluid administration and requiring norepinephrine or other vasopressors [21]. The intervention was use of the PMX-HP for the adjuvant treatment of sepsis or septic shock. The comparison was standard treatment only or sham hemoperfusion. Primary outcomes were 28-day all-cause mortality, the number of patients with at least one serious adverse event, and organ dysfunction scores [22] over 24–72 h after the treatment. Secondary outcomes included 90-day all-cause mortality, mean arterial blood pressure over 24–72 h after the treatment, endotoxin levels over 24–72 h after the treatment, duration of vasopressor therapy or vasopressor-free days, the receipt of renal replacement therapy (RRT), costs related to health services, and total mortality defined as mortality at 28 days or any follow-up duration when available.

Information sources

The search strategy was developed in collaboration with an experienced health research librarian. We searched MEDLINE (from the inception to Oct 2017), EMBASE, the Cochrane Library, the Health Technology Assessment Database, Cumulative Index to Nursing and Allied Health Literature, Pubmed, and "Igaku Chuo Zasshi" of the Japan Medical Abstract Society (from the inception to June 2016). The search strategies for MEDLINE were developed and were modified for searching all the other databases (eMethod 1). The search strategies were further peer-reviewed by a second research librarian [23]. For ongoing trials, we searched the National Institute of Health Clinical Trials Register, the World Health Organization International Clinical Trials Registry Platform, and the University Hospital Medical Information Network Clinical Trials Registry. We also searched citations from all included studies. We contacted experts in the field of critical care nephrology and selected commercial entities

that develop or license PMX-HP to identify additional unpublished and/or on-going trials.

Study selection

Two authors independently screened titles and abstracts of all trial reports we identified by the search to code them as 'retrieve' (eligible or potentially eligible/unclear) or 'do not retrieve'. The full texts of reports classified as 'retrieve' were reviewed independently according to predetermined eligibility criteria. Discrepancies were resolved through discussion with a third reviewer, as required. We identified and excluded duplicates of the same study.

Data collection process

Two reviewers independently extracted data using standardized and piloted data extraction sheets. We abstracted the following information: study characteristics, patient characteristics, sample size, interventions, comparators, potential biases in the conduct of the trial, outcomes, methods of statistical analysis, and funding support. Agreement between the two reviewers concerning the primary outcome and the risk of bias for the primary outcome was reported as percentage agreement with an intra-class correlation coefficient, and percentage agreement with a weighted kappa, respectively.

Assessment of risk of bias in individual studies

Two reviewers independently assessed the risk of bias of the included studies using the tool described in the Cochrane Handbook for Systematic Reviews of Interventions [24], which consists of eight domains (eTable 1). The risk of bias assessment was done at the outcome level for the primary outcomes. When the original reports provided insufficient details, we made direct inquiry of the study authors. When the assessors disagreed, the final rating was decided through discussion or with the involvement of another member of the review group, if necessary. The key domain of risk of bias for 28-day mortality was allocation concealment. The overall risk of bias was also summarized in further subgroup analyses. More details of assessment of risk of bias is provided in the protocol [20].

Summary measures

As the measure of treatment effect for dichotomous outcomes, we used the risk ratio (RR) and its 95% confidence interval (CI). Continuous outcomes were pooled by calculating the mean difference (MD) with a 95% CI except for organ dysfunction scores. As the data for the organ dysfunction scores were available in the sepsis-related organ failure assessment (SOFA) score [25] or multiple organ dysfunction score [26] (MODS), we pooled standardized mean differences (SMDs) with a 95% CI [27].

Synthesis of results

We analyzed data from the included studies using Review Manager [28]. The proportion of treatment failure was calculated according to the intention-to-treat (ITT) principle. All randomized patients for whom outcome data were not available were assumed as no events. The effect of imputation was explored by a sensitivity analysis. Given the clinical heterogeneity including variability in the etiologies of sepsis in the population of interest, we used a random-effects model in all analyses [29]. We assessed overall heterogeneity by visual inspection of the forest plots, and statistical heterogeneity using the I^2 statistic and Chi-squared test. I^2 values above 50% were considered to represent substantial statistical heterogeneity. To assess reporting bias, we constructed funnel plots, and visual inspection was performed to investigate the asymmetry. Certainty of the body of evidence was assessed using the grading of recommendations assessment, development and evaluation (GRADE) framework [30]. The GRADE framework characterizes the certainty of a body of evidence on the basis of study limitations, imprecision, inconsistency, indirectness, and other considerations. The starting point for certainty in each estimate is high, but is downgraded according to the assessments of these five domains if there are serious concerns. When the effect estimates were affected substantially by the risk of bias of included studies, then we downgraded the certainty of the evidence in a domain of risk of bias.

Additional analyses

To test the robustness of the effect estimates of PMX-HP, and to explain heterogeneity, we used sensitivity analyses and subgroup analyses. We planned the following sensitivity analyses for 28-day mortality: (1) risk of bias; we included only trials with low risk of bias in allocation concealment; (2) imputed missing data; we imputed missing data on 28-day mortality in two ways: assuming the missing outcomes as events (death) in the PMX-HP group, and as no event in the control group (worst-case scenario); and assuming the missing outcomes as no event in the PMX-HP group, and as event in the control group (best-case scenario); (3) per protocol; and (4) statistical method; we used a fixed-effect model. We performed a priori subgroup analyses for the participant group and the intervention if sufficient detail was present in the eligible studies with the following hypotheses: (1) participants with abdominal sepsis, culture-confirmed sepsis, gram-negative infections, surgery, acute kidney injury (AKI), or septic shock will show greater treatment effect than patients without those conditions; and (2)

greater dose of intervention (i.e., longer duration; more than one treatment) will show greater treatment effect. eMethod 2 explains changes from the protocol [20].

Post hoc analyses

Trial sequential analysis (TSA) was done with a diversity-adjusted information size calculated using a two-sided alfa of 0.05, a power of 80%, an anticipated relative risk reduction of 20.0%, and a control event rate of 35.0%. TSA viewer version 0.9.5.10 Beta (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Copenhagen, DE. 2016) was used. An additional sensitivity analysis including zero total event studies using continuity correction was done using R version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria. 2017). We added post hoc subgroup analysis for the overall risk of bias with a different criterion of assessment in the included studies, and the maximum time window from the onset of sepsis/septic shock or surgery to the first therapy.

Results

Of the 1700 citations identified from electronic and hand searches, 12 reports were identified for the review, and after exclusion of ongoing trials or inadequate reports (eTable 2), we included 6 unique trials [14, 15, 17, 18, 31, 32] in the meta-analysis (Fig. 1). The agreement of eligibility between the two reviewers was 90% [Cohen's weighted kappa: 0.79 (95% CI 0.62–0.97)]. Table 1 shows the characteristics of the trials included in the meta-analysis. All the trials used a PMX B-immobilized hemoperfusion device (Toraymyxin 20R). The number of participants across trials ranged between 16 and 450. The weighted mean age of study participants was 62.5 years (range 56.0–69.7). Sixty-one percent were male. Agreement for the primary outcome and the risk of bias items between the two reviewers was 100% (intra-class correlation coefficient: 1).

Primary outcomes

For 401 patients involved in the 5 studies [14, 15, 17, 18, 31] and contributing to 28-day mortality data (representing 83% of the included participants), the pooled RR was 1.03 (95% CI 0.78–1.36; $I^2 = 25\%$; $n = 797$; Table 2, Fig. 2a). All five trials were adjudicated as low risk of bias for the outcome (eTable 3a). The number of patients with at least one serious adverse event was reported in three studies [14, 17, 18]. The pooled RR was 2.17 (95% CI 0.68–6.94; $I^2 = 0\%$; $n = 717$; Fig. 2b). Cruz et al. [15] reported only device-related adverse events in the PMX-HP group (eTable 4). Five studies [14, 15, 17, 18, 31] reported either organ dysfunction scores at 24–72 h after the treatment or their changes over 24–72 h after the treatment. Only the EUPHRATES trial [18] reported MODS, and the others [14, 15, 17, 31] reported SOFA score. For 797 patients in the 5 studies [14, 15, 17, 18, 31], the SMD for the organ dysfunction scores was − 0.26 (95% CI − 0.64 to 0.12; Fig. 2c). The heterogeneity between the 5 trials was high ($I^2 = 78\%$).

Secondary outcomes

Among 232 patients in the ABDO-MIX trial [17] that reported 90-day mortality, PMX-HP did not reduce 90-day mortality (RR, 1.41: 95% CI 0.93–2.13; Table 2). Four trials [14, 15, 18, 31] reported mean arterial pressure. Pooled results found a statistically significant relationship between PMX-HP and increase in mean arterial pressure (MD, 5.23; 95% CI 2.75–7.72; $I^2 = 0\%$; $n = 565$; Table 2, eFigure 1). Three trials [14, 31, 32] involving 109 patients reported endotoxin levels (pg/mL) over 24–72 h after treatment measured by limulus amebocyte lysate assay (MD, − 40.77: 95% CI − 118.53 to 36.99; $I^2 = 96\%$; Table 2, eFigure 2). There were no differences in vasopressor-free days at 28 days ($n = 283$, 3 trials, eFigure 3), ICU length of stay ($n = 347$, 4 trials, eFigure 4), or the receipt of RRT ($n = 565$, 4 trials, eFigure 5; Table 2). Two studies [15, 18] provided data for duration of RRT (5.2 days for PMX-HP vs. 5.6 days for standard; $p = 0.03$) [15] and RRT-free days to day 28 (14.7 vs. 15.0 days; $p = 0.81$) [18].

One study [33] performed an economic analysis using data collected in the EUPHAS trial involving 64 patients [15] and suggested PMX-HP was cost-effective. They adopted the Italian healthcare provider's perspective and showed a mean incremental cost-effective ratio of EUR 2558 per incremental undiscounted life-year gained and EUR 3864 per incremental discounted life-year gained [33]. For 856 patients involved in the 6 studies [14, 15, 17, 18, 31, 32] contributing mortality data at 28 days or any follow-up duration, the pooled RR for death among patients treated with PMX-HP was 0.85 (95% CI 0.58–1.26; $I^2 = 64\%$; Table 2, eFigure 6).

Sensitivity and subgroup analyses

None of the 5 studies contributing to 28-day mortality [14, 15, 17, 18, 31] was rated at high risk of bias in allocation concealment. Sensitivity analyses with imputation of missing data with the worst-case scenario (pooled RR, 1.05: 95% CI 0.74–1.50; $I^2 = 47\%$, eFigure 7a) and with the best-case scenario (pooled RR, 1.02: 95% CI 0.84–1.24; $I^2 = 0\%$, eFigure 7b), and sensitivity analysis using a fixed-effect model (pooled RR, 1.07: 95% CI 0.88–1.31; $I^2 = 25\%$, eFigure 8), and per-protocol mortality (pooled RR, 0.89: 95% CI 0.62–1.29; $I^2 = 46\%$, eFigure 9) attested to the robustness of the primary analysis.

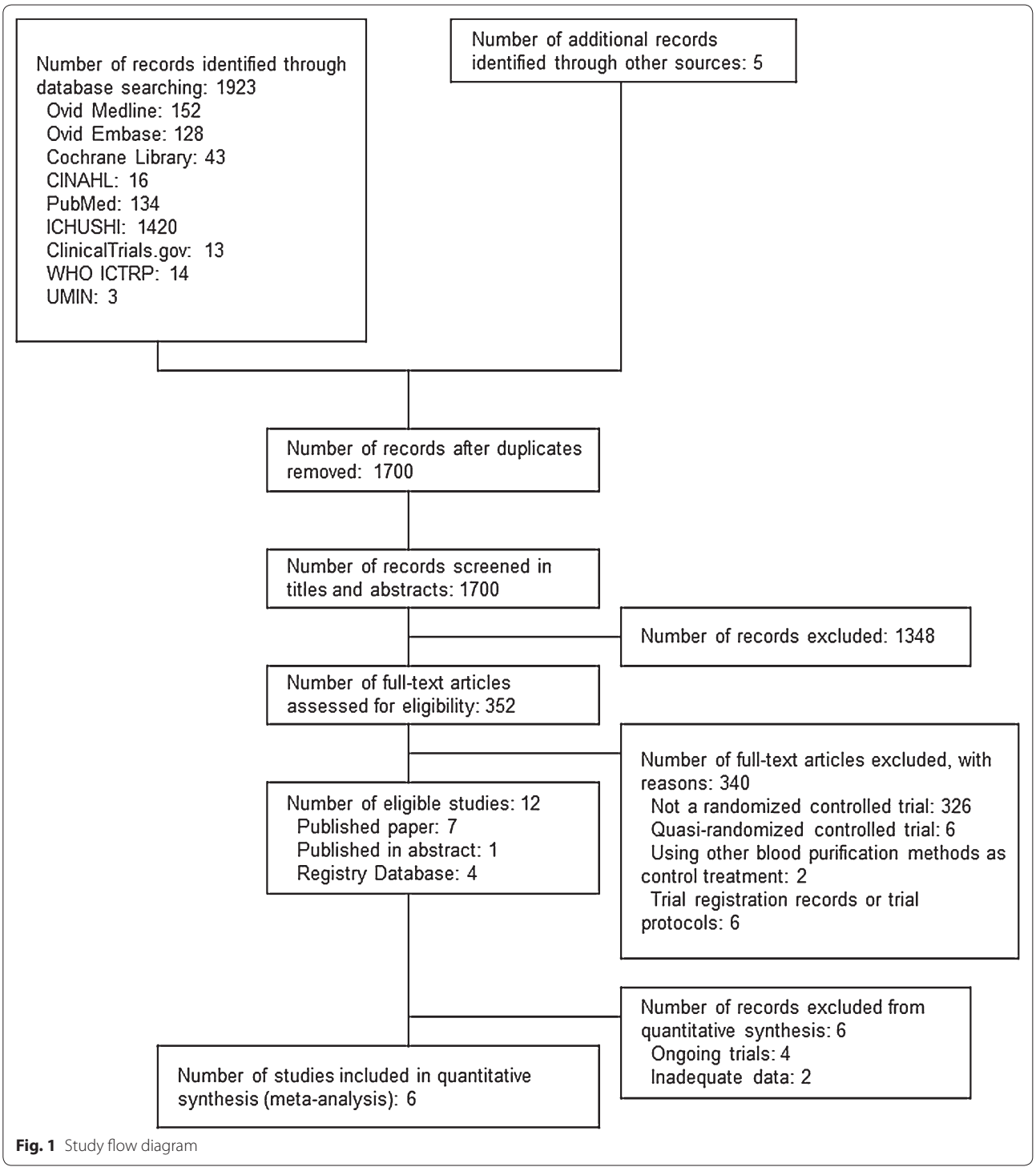

Fig. 1 Study flow diagram

Subgroup analyses for 5 studies reporting 28-day mortality [14, 15, 17, 18, 31] by trial participants with different sepsis etiologies (abdominal only vs. various etiologies including abdominal), trial participants with sepsis confirmed by culture (culture-confirmed vs. mixed or not confirmed), trial participants with gram-negative infections (culture-confirmed vs. others), trial participants with surgery (surgical vs. mixed or medical), or severity of trial participants (septic shock only vs. sepsis or septic shock) did not show any subgroup interaction

Table 1 Characteristics of the trials included in the meta-analysis

Source	Country	Study cites	Funding	Total no. of patients	Exclusion from ITT analysis	Age, mean (SD), years	Sex, male, female, *n*	Patient status	Duration, no. of sessions	Primary outcome
Nakamura 2003 [32]	Japan	NA	Investigator-initiated	60	0	56 (NA)	40, 20	Culture-positive sepsis	2 h, twice	Unclear[b]
Vincent 2005 [14]	Six countries in Europe	Multicenter	Industry-sponsored	35	0	57.7 (15.6)[a]	22, 13	Abdominal sepsis, surgical	2 h, once	The SOFA score
Cantaluppi 2008 [31]	Italy	Two centers	Investigator-initiated	16	0	60 (11.3)	12, 4	Confirmed gram-negative sepsis	2 h, twice	Viability of renal cell cultures
Cruz 2009 [15], Berto 2011 [33]	Italy	Multicenter	Industry-sponsored	64	0	63.8 (14.2)[a]	42, 22	Abdominal sepsis, surgical	2 h, twice	MAP and vasopressor requirement
Payen 2015 [17]	France	Multicenter	Industry-sponsored	243	11	69.7 (11.6)	134, 98	Abdominal sepsis, surgical, septic shock	2 h, twice	28-day mortality
EUPHRATES 2017 [18], Klein 2014 [34]	USA, Canada	Multicenter	Industry-sponsored	450	0	59.8 (14.9)[a]	273, 177[a]	Septic shock, high endotoxin activity assay	2 h, twice	28-day mortality

ITT Intention to treat, *SD* standard deviation, *NA* not available, *SOFA* sequential organ failure assessment, *MAP* mean arterial pressure

[a] Data provided by the study authors

[b] Reported endotoxin levels after treatment, survival at unknown follow-up period, adverse events

Table 2 Outcome measures

	Studies	Study reference no.	PMX-HP	Standard	Effect estimate (95% CI)	I^2 (%)
Primary outcomes						
28-day mortality	5[a]	14, 15, 17, 18, 31	135/402	124/395	Pooled RR, 1.03 (0.78, 1.36)	25
Number of patients with at least one serious adverse event	3[a]	14, 17, 18	8/360	3/357	Pooled RR, 2.17 (0.68, 6.94)	0
Change in organ dysfunction scores over 24–72 h after treatment	5[a]	14, 15, 17, 18, 31			SMD, − 0.26 (− 0.64, 0.12)	78
Secondary outcomes						
90-day all-cause mortality	1	17	40/119	27/113	RR, 1.41 (0.93, 2.13)	NA
Change in mean arterial blood pressure over 24–72 h after the treatment	4[a]	14, 15, 18, 31			MD, 5.23 (2.75, 7.72)	0
Endotoxin levels measured by LAL assay over 24–72 h after the treatment	3[a]	14, 31, 32			MD, − 40.77 (− 118.53, 36.99)	96
28-day vasopressor-free days	3[a]	14, 17, 31			MD, − 1.10 (− 4.05, 1.85)	10
ICU length of stay	4[a]	14, 15, 17, 31			MD, − 1.95 (− 7.91, 4.00)	70
The need for RRT	4[a]	14, 15, 18, 31			Pooled RR, 0.76 (0.33, 1.71)	61
Mortality at 28 days or any follow-up duration	6[a]	14, 15, 17, 18, 31, 32	144/436	140/420	Pooled RR, 0.85 (0.58, 1.26)	64

CI Confidence interval, *RR* risk ratio, *NA* not available, *SMD* standardized mean difference, *MD* mean difference, *ICU* intensive care unit

[a] Includes data provided from the study authors

(Table 3, Fig. 2a, eFigure 10–13). The subgroup of patients with AKI was not reported for their 28-day mortality in any of the included studies.

Certainty of evidence

The visual inspection of the funnel plot for the 28-day mortality suggested no apparent publication bias, but there were few studies to assess for asymmetry (eFigure 14). The certainty of evidence for the three primary outcomes was downgraded by one level each for risk of bias and imprecision, and were all considered low (Table 4).

Post hoc analyses

TSA showed the adjusted CI for 28-day mortality was 0.58–1.82 ($I^2 = 25\%$; $n = 797$). The required information size to show a relative riskreduction (RRR) of 20% was 2744 (eFigure 15), and to show 2-point reduction of organ dysfunction scores was 895. An additional analysis including zero total event studies using continuity correction showed the pooled RR of the number of patients with at least one serious adverse event as 2.03 (95% CI 0.67–6.17; $I^2 = 0\%$; $n = 733$). Post hoc subgroup analyses did not show subgroup interaction for the efficacy and safety outcomes. Assessment of overall risk of bias with different criteria did not affect our results (eFigures 16–18).

Discussion

Summary of key findings

The current systematic review showed that PMX-HP did not reduce 28-day mortality or organ dysfunction scores of adult sepsis or septic shock patients, and did not appear to significantly increase the risk of adverse events. PMX-HP did not reduce 90-day mortality, or significantly reduce the utilization of health resources.

Context with prior literature

Early experimental or clinical studies evaluating blood purification in sepsis have largely focused on methods of hemofiltration [35–37]. Meanwhile, large multi-centered clinical trials have found intensity of RRT beyond conventionally recommended doses does not improve survival of patients with AKI and sepsis [37–41]. Moreover, the early application of continuous venovenous hemofiltration (CVVH) was implied to worsen the severity of organ dysfunction in severe sepsis [41]. These observations suggest that alternative strategies to better target blood purification and improved survival in sepsis are necessary.

Previous systematic reviews implied that the use of PMX-HP was associated with a survival benefit, improvements in hemodynamics, and reduction in circulating endotoxin levels [16, 42, 43]. In our up-to-date systematic review, we excluded six studies where randomization was not rigorous (e.g., in five studies, allocation was alternating; and in one study, allocation was performed through discussion with patients) following direct inquiry with the study authors [44–49]. Five out of the six excluded studies [45–49] that showed large beneficial effect with relatively small sample sizes were included in previous systematic reviews [16, 42, 43]. We included two newly completed high-profile randomized trials [17, 18], and found no apparent benefit on survival.

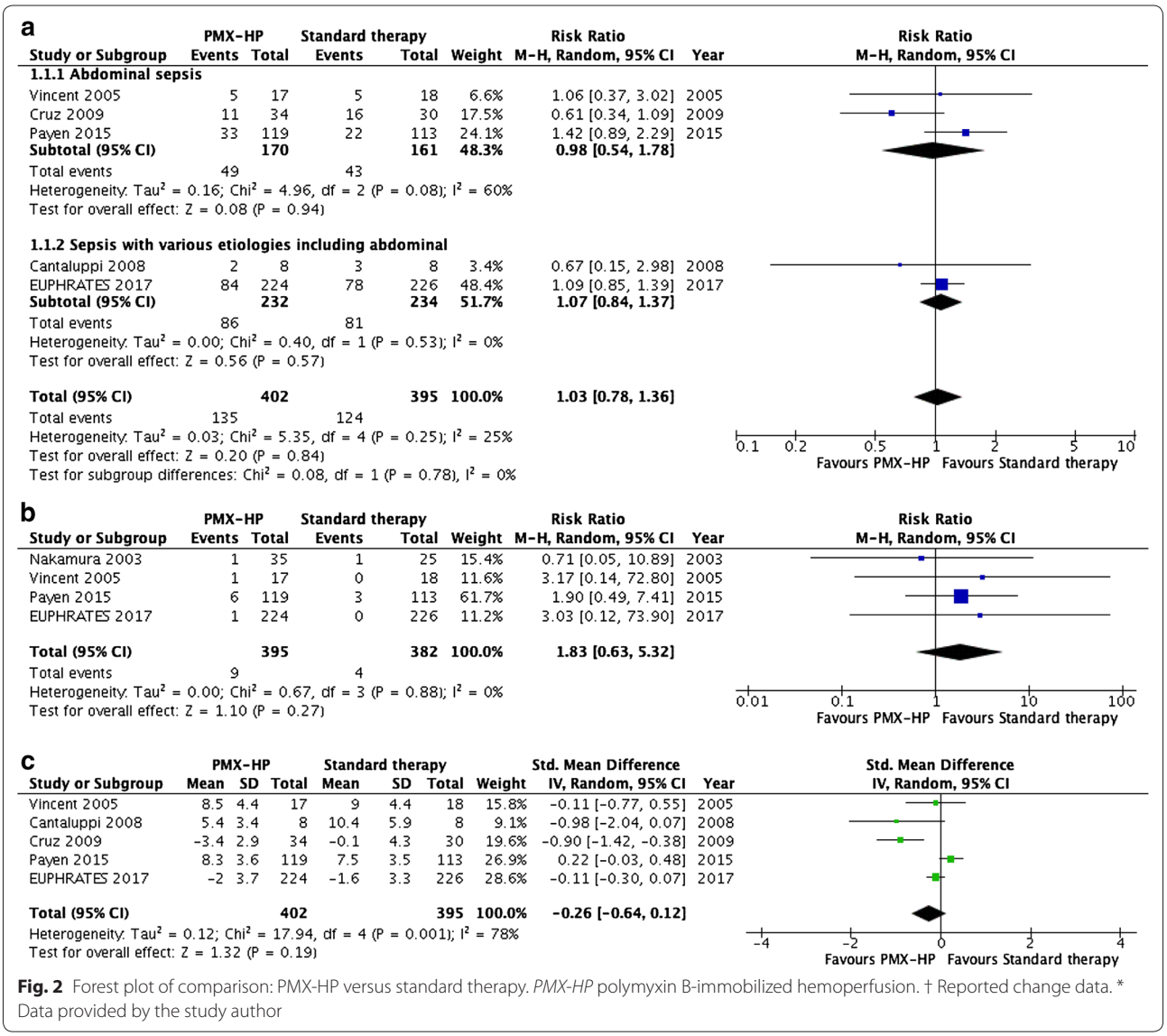

Fig. 2 Forest plot of comparison: PMX-HP versus standard therapy. *PMX-HP* polymyxin B-immobilized hemoperfusion. † Reported change data. * Data provided by the study author

There might be several possible explanations for our findings. First, there may be no beneficial effect of using PMX-HP in patients with sepsis or septic shock. Vincent et al. [14] and a post hoc analysis of ABDO-MIX study [50] measured cytokines after completion of PMX-HP treatment, including TNF-alfa, interleukins, and IFN-gamma, and no significant differences were found [50]. These results imply PMX-HP treatment may not significantly remove endotoxin and/or suppress inflammatory cytokines sufficiently to modify the course of organ dysfunction and risk of death.

Second, the pooled analysis with 797 patients may still lack sufficient statistical power to detect small but clinically meaningful effects of PMX-HP treatment, as shown in the TSA. If we assume an absolute risk reduction of 15% (i.e., 43% RRR) with an estimated baseline mortality of 35%, as adopted in the EUPHRATES trial [34], we have already accumulated sufficient information to conclude a null effect. Kaukonen et al. showed mortality in patients with severe sepsis has declined considerably over the last decade [1], likely in part implying substantial temporal progress in the overarching care provided to critically ill patients. The effect of PMX-HP, if any, could be heterogeneous and much smaller than expected, possibly due largely to PMX-HP being only an adjuvant therapy in the context of multiple interventions used to manage adult critically ill patients with sepsis.

Third, patient selection and case mix may have influenced the expected outcomes. Biologically targeted therapy is sensible to enrich the trial population with patients most likely to derive benefit from the intervention; however, the EUPHRATES trial, the only study that adopted

Table 3 Subgroup analyses of 28-day mortality related to polymyxin B-immobilized hemoperfusion

Subgroup	Studies	Patients	Pooled risk ratio	I^2 (%)	*p* value
Participants: culture	5	797		0	0.57
Culture-positive sepsis	1[a]	16	0.67 [0.15, 2.98]		
Not confirmed	4[a]	781	1.04 [0.76, 1.42]		
Participants: gram-negative infection	5	797		0	0.57
Confirmed gram-negative infection	1[a]	16	0.67 [0.15, 2.98]		
Not confirmed gram-negative	4[a]	781	1.04 [0.76, 1.42]		
Participants: surgical	5	797		0	0.78
Surgical	3	331	0.98 [0.54, 1.78]		
Mixed or medical	2[a]	466	1.07 [0.84, 1.37]		
Participants: severity of sepsis	5	797		72	0.06
Septic shock	2[a]	682	1.15 [0.92, 1.43]		
Sepsis or septic shock	3[a]	115	0.69 [0.42, 1.12]		
Intervention: no. of sessions	5	797		0	0.93
Single session	1	35	1.06 [0.37, 3.02]		
Two sessions	4[a]	762	1.01 [0.72, 1.42]		
More than two sessions	0	0	NA		

NA Not available

[a] Includes data provided from the study authors

endotoxin activity as an eligibility criterion, may still have been too small to detect a small but clinically important difference. There are three ongoing trials measuring endotoxin activity at the inclusion of the trials (eTable 2); however, the sample sizes of these trials are also likely to be too small to likely detect a clinically important effect of PMX-HP or to change the overall conclusions of sequential meta-analyses.

Strengths and limitations

We have conducted a rigorous peer-reviewed literature search to identify relevant randomized trials, including a database in Japan where the PMX-HP filter was developed. Furthermore, we have directly contacted all the study authors to assess the eligibility and the quality of each trial to minimize bias in our effect estimation. The inclusion of two new and larger studies [17, 18] has empowered the pooled analysis and enabled the up to date evidence synthesis. We have also performed several predefined sensitivity analyses to confirm the robustness of the findings. However, there are several limitations for this review. Limited numbers of studies did not allow detailed analysis and interpretation to address the issue of heterogeneity in the case mix and in treatment effect in response to PMX-HP. Second, as we conducted meta-analysis with aggregated data, we could not classify participants involved in the studies into complementary subgroups at each patient level. Third, we observed considerable heterogeneity in several analyses. Practice variation across the included studies may have contributed to heterogeneity. Similarly, there may be a biological basis for responsiveness to PMX-HP among a heterogeneous population of patients with sepsis that is incompletely understood. Fourth, as we have performed multiple analyses in this systematic review, we recommend caution when interpreting significant findings, such as the modest increase of mean arterial pressure. Finally, we have made some changes from our original protocol, due largely to the availability of the data.

Implications for clinicians, policy, and future research

Our review would suggest there is no definitive evidence to support the routine use of PMX-HP for adult critically ill patients with sepsis or septic shock. While there was no significant difference in risk shown in our review, the potential risk of serious adverse events with use of PMX-HP should be considered. The available evidence did not prove its efficacy for improved survival, and as such, performing an economic evaluation may not be justified.

The imprecision of the results does not preclude further trials to assess the efficacy of PMX-HP. In the EUPHRATES trial, post hoc exploratory per-protocol analyses showed a beneficial effect among adult patients with a MODS greater than 9 [18], a finding warranting further verification. Future clinical trials should aim to explore specific patient populations with adequate sample size, for example, those with elevated blood endotoxin level, or high organ dysfunction scores, if any clinical effect of PMX-HP is to be detected.

Table 4 Evidence table of the systematic review

Quality assessment							No. of patients		Effect		Certainty
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	PMX-HP	Standard therapy	Relative (95% CI)	Absolute (95% CI)	
28-day mortality											
5	Randomized trials	Serious[a]	Not serious	Not serious	Serious[b]	None	135/402 (33.6%)	124/395 (31.4%)	RR 1.03 (0.78–1.36)	9 more per 1000 (from 69 fewer to 113 more)	⨁⨁◯◯ LOW
The number of patients with at least one serious adverse event											
3	Randomized trials	Serious[a]	Not serious	Not serious	Serious[b]	None	8/360 (2.2%)	3/357 (0.8%)	RR 2.17 (0.68–6.94)	10 more per 1000 (from 3 fewer to 50 more)	⨁⨁◯◯ LOW
Organ dysfunction score over 24–72 h after treatment											
5	Randomized trials	Serious[a]	Not serious	Not serious	Serious[b]	None	402	395	–	SMD 0.26 lower (0.64 lower to 0.12 higher)	⨁⨁◯◯ LOW

CI Confidence interval, *RR* risk ratio, *SMD* standardized mean difference

[a] One trial was at high risk of bias in the blinding and sponsorship domains

[b] Because of the wide confidence interval

Conclusions

Among adult patients with sepsis or septic shock, use of PMX-HP compared with standard therapy alone was not proven to reduce 28-day mortality or to reduce organ dysfunction scores, or significantly increase the risk of serious adverse events. Considering the certainty of the body of evidence was low for both benefit and harm, to date, there is no strong evidence to support the routine use of PMX-HP as an adjuvant therapy in critically ill adult patients with sepsis or septic shock.

Electronic supplementary material

The online version of this article (https://doi.org/10.1007/s00134-017-5004-9) contains supplementary material, which is available to authorized users.

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Author contributions

Drs. TF and SMB had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: TF, SMB, RG, YK, RF, and TAF. Acquisition of data: TF, SMW, KD, RG, YK, RF, JV, DP, RR, and CR. Analysis and interpretation of data: TF, SMB, YK, TAF, JV, DP, RR, and CR. Drafting of the manuscript: TF, TAF, SMB, and KD. Critical revision of the manuscript for important intellectual content: RF, RG, YK, JV, DP, RR, CR. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of this work.

Compliance with ethical standards

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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REVIEW

Rationalizing antimicrobial therapy in the ICU: a narrative review

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Abstract

The massive consumption of antibiotics in the ICU is responsible for substantial ecological side effects that promote the dissemination of multidrug-resistant bacteria (MDRB) in this environment. Strikingly, up to half of ICU patients receiving empirical antibiotic therapy have no definitively confirmed infection, while de-escalation and shortened treatment duration are insufficiently considered in those with documented sepsis, highlighting the potential benefit of implementing antibiotic stewardship programs (ASP) and other quality improvement initiatives. The objective of this narrative review is to summarize the available evidence, emerging options, and unsolved controversies for the optimization of antibiotic therapy in the ICU. Published data notably support the need for better identification of patients at risk of MDRB infection, more accurate diagnostic tools enabling a rule-in/rule-out approach for bacterial sepsis, an individualized reasoning for the selection of single-drug or combination empirical regimen, the use of adequate dosing and administration schemes to ensure the attainment of pharmacokinetics/pharmacodynamics targets, concomitant source control when appropriate, and a systematic reappraisal of initial therapy in an attempt to minimize collateral damage on commensal ecosystems through de-escalation and treatment-shortening whenever conceivable. This narrative review also aims at compiling arguments for the elaboration of actionable ASP in the ICU, including improved patient outcomes and a reduction in antibiotic-related selection pressure that may help to control the dissemination of MDRB in this healthcare setting.

Keywords: Antibiotic stewardship, Antimicrobial resistance, Empirical therapy, Critical illness, Carbapenem, Outcome, Sepsis

Introduction

Antibiotics are massively used in ICUs around the world [1]. While the adequacy and the early implementation of empirical coverage are pivotal to cure patients with community- and hospital-acquired sepsis, antimicrobial therapy is not always targeted and, in more than one out of two cases, may be prescribed in patients without confirmed infections. Moreover, antibiotic de-escalation is insufficiently considered. The resulting selection pressure together with the incomplete control of cross-colonization with multidrug-resistant bacteria (MDRB) makes the ICU an important determinant of the spread of these pathogens in the hospital. As instrumental contributors of antimicrobial stewardship programs (ASP), intensivists should be on the leading edge of conception, optimization, and promotion of therapeutic schemes for severe infections and sepsis, including the limitation of antimicrobial overuse.

In this narrative review based on a literature search (MEDLINE database) completed in September 2018,

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we sought to summarize recent advances and emerging perspectives for the optimization of antibiotic therapy in the ICU, notably better identification of patients at risk of MDRB infection, more accurate diagnostic tools enabling a rule-in/rule-out approach for bacterial sepsis, an individualized reasoning for the selection of single-drug or combination empirical regimen, the use of adequate dosing and administration schemes to ensure the attainment of pharmacokinetics/pharmacodynamics targets, concomitant source control when appropriate, and a systematic reappraisal of initial therapy in an attempt to minimize collateral damage on commensal ecosystems through de-escalation and treatment-shortening whenever conceivable. We also aimed to compile arguments for the elaboration of actionable ASP in the ICU, including improved patient outcomes and a reduction in antibiotic-related selection pressure that may help to control the dissemination of MDRB in this healthcare setting.

How antimicrobial therapy influences bacterial resistance

The burden of infections due to extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E) and MDR *Pseudomonas aeruginosa* is rising steadily, carbapenem-resistant *Acinetobacter baumannii* and carbapenemase-producing *Enterobacteriaceae* (CRE) are spreading globally, while methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci generate major issues in several geographical areas [2–14]. These trends now apply for both ICU-acquired infections and imported bacterial sepsis as a result of the successful dissemination of MDRB in hospital wards and other healthcare environments (Fig. 1).

Up to 70% of ICU patients receive empirical or definite antimicrobial therapy on a given day [1]. The average volume of antibiotic consumption in this population has been recently estimated as 1563 defined daily doses (DDD) per 1000 patient-days (95% confidence interval 1472–1653)—that is, almost three times higher than in ward patients, with marked disparities for broad-spectrum agents such as third-generation cephalosporins [15]. Whilst most of the underlying mechanisms ensue from a succession of sporadic genetic events that are not directly induced by antibiotics, the selection pressure exerted by these drugs stands as a potent driver of bacterial resistance (Fig. 2) [16, 17].

At the patient level, antimicrobial exposure allows the overgrowth of pathogens with intrinsic or acquired resistance to the administered drug within commensal ecosystems or, to a lesser extent, at the site of infection. Of note, some mechanisms may confer resistance to various classes, notably the overexpression of efflux pumps in non-fermenting Gram-negative bacteria, thereby resulting in the selection of MDR mutants following only a single-drug exposure [18]. At the ICU scale, consumption volumes of a given class correlate with resistance rates in clinical isolates, including for carbapenems or polymyxins [19–26], although this may fluctuate depending on bacterial species and settings [27, 28].

Take-home message

This narrative review summarizes the available evidence, emerging options, and unsolved controversies for the optimization of antibiotic therapy in the ICU. The potential benefit of antibiotic stewardship programs to improve patient outcomes and reduce the ecological side effects of these drugs is also discussed.

Yet, in addition to its clinical spectrum, anti-anaerobic properties should be considered when appraising the ecological impact of each antibiotic [29]. Indeed, acquisition of MDR Gram-negative bacteria through in situ selection, cross-transmission, or environmental reservoirs may be eased by antimicrobial-related alterations of the normal gut microbiota—primarily resident anaerobes—and the colonization resistance that it confers [30]. A prior course of anti-anaerobic drugs may notably predispose to colonization with ESBL-E [31], AmpC-hyperproducing *Enterobacteriaceae* [32], or CRE [33, 34]. The degree of biliary excretion of the drug appears as another key factor to appraise its potential impact on intestinal commensals [35–37].

Risk factors for multidrug-resistant pathogens

The clinical value of identifying risk factors for MDRB infection is to guide empirical therapy before the availability of culture results—that is, pathogen identification and antimicrobial susceptibility testing (AST). However, no single algorithm may be used to predict a MDRB infection given the complex interplay between the host, the environment, and the pathogen, thus requiring an individualized probabilistic approach for the selection of empirical drugs (Table 1).

Colonization markedly amplifies the risk of subsequent infection with a given MDRB. However, the positive predictive value of this risk factor never exceeds 50% whatever the colonizer is [2, 38–40]. For instance, ESBL-E infections occur during the ICU stay in only 10–25% of ESBL-E carriers [41]. Whether an MDRB carrier becomes infected is related to a further series of factors that may or not be related to those associated with the risk of acquired colonization [2, 38, 39]. Overall, the presence or absence of documented carriage should not

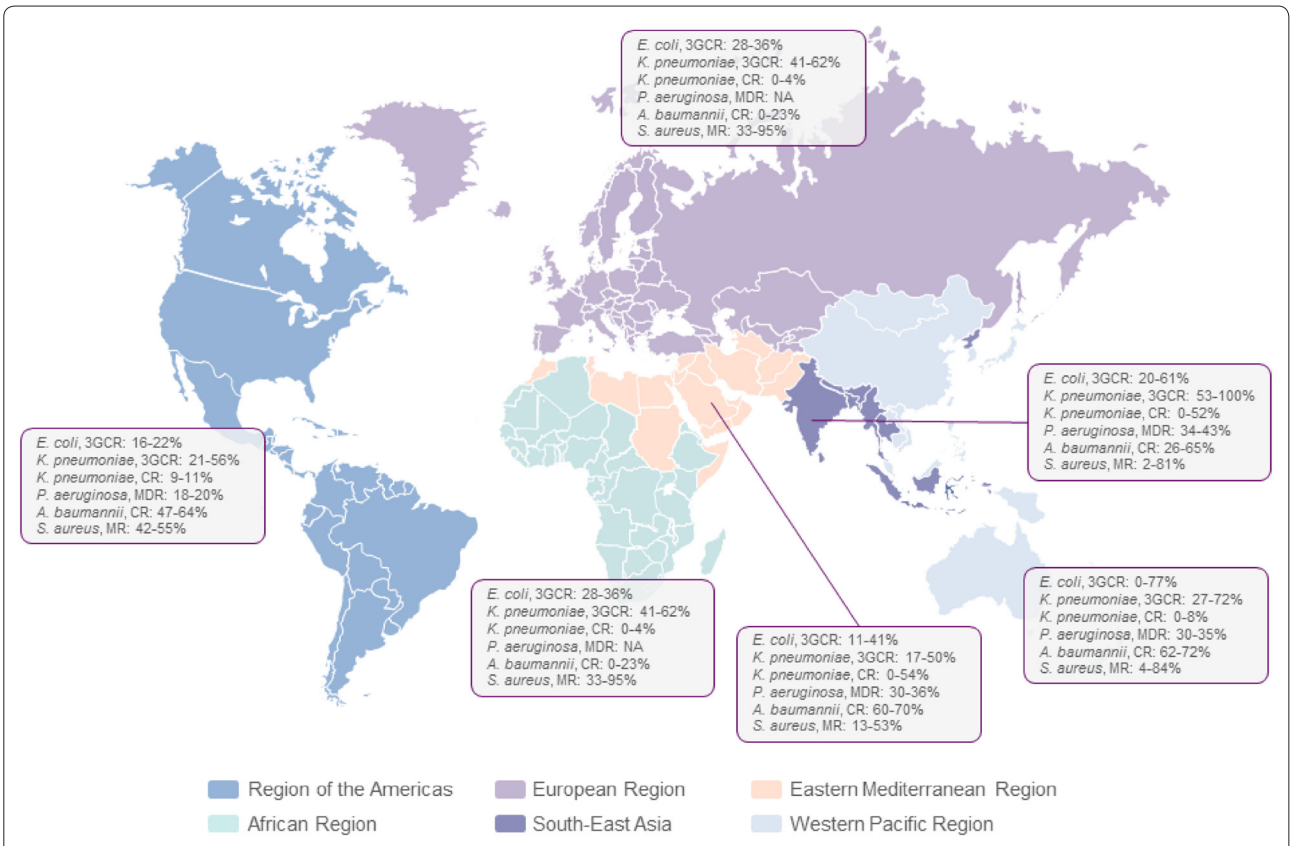

Fig. 1 Current resistance rates in major pathogens responsible for hospital-acquired infections according to World Health Organization (WHO) regions. 3GCR third-generation cephalosporin-resistant, CR carbapenem-resistant, MDR multidrug-resistant, MR methicillin-resistant. Data were extracted from the WHO Antimicrobial Resistance Global Report 2014 [171], National Healthcare Safety Network/Centers for Disease Control and Prevention Report 2011–2014 [11], European Antimicrobial Resistance Surveillance Network Annual Report 2016 [172], International Nosocomial Infection Control Consortium Report 2010–2015 [10], CHINET Surveillance Network Report 2014 [14], and other references [5, 12, 13]. Available resistance rates in the specific context of ICU-acquired infections are in the upper ranges of reported values for all geographical areas

be considered as the unique requisite for the choice of empirical therapy.

Patients with advanced co-morbid illnesses, prolonged hospital stays, use of invasive procedures, and prior antibiotic exposure are at increased risk of MDRB infections [42, 43].

The patient location is another determinant of risk as there are vast differences in the epidemiology of MDRB globally, regionally, and even within hospitals in the same city [2, 44]. Reasons for these discrepancies may include socioeconomic factors as well as variations in case-mix, antimicrobial consumption, and hygiene practices.

When not to start antimicrobials in the ICU

Although mixed [45], the available evidence supports a beneficial effect of prompt antibiotic administration on survival rates in sepsis and septic shock, irrespective of the number of organ dysfunctions [46–49]. However, the clinical diagnosis of sepsis is challenging in critically ill patients having multiple concurrent disease processes, with up to 50% of febrile episodes being of non-infectious origin [50]. Furthermore, collection of microbiological evidence for infection is typically slow, and previous antibiotic exposure may render results unreliable. Indeed, cultures remain negative in 30–80% of patients clinically considered infected [51, 52]. Uncertainty regarding antibiotic initiation in patients with suspected lower respiratory tract infection is further complicated by the fact that as many as one-third of pneumonia cases requiring ICU admission are actually viral [53, 54].

In 2016, the Sepsis-3 task force introduced the quick sepsis-related organ failure assessment score (qSOFA), a bedside clinical tool for early sepsis detection [55]. Although the predictive value of qSOFA for in-hospital mortality has been the focus of several external validation studies [56], it remains to be investigated whether this new score may help to rationalize antibiotic use in patients with suspected infection. Yet, published data suggest that qSOFA may lack sensitivity for early

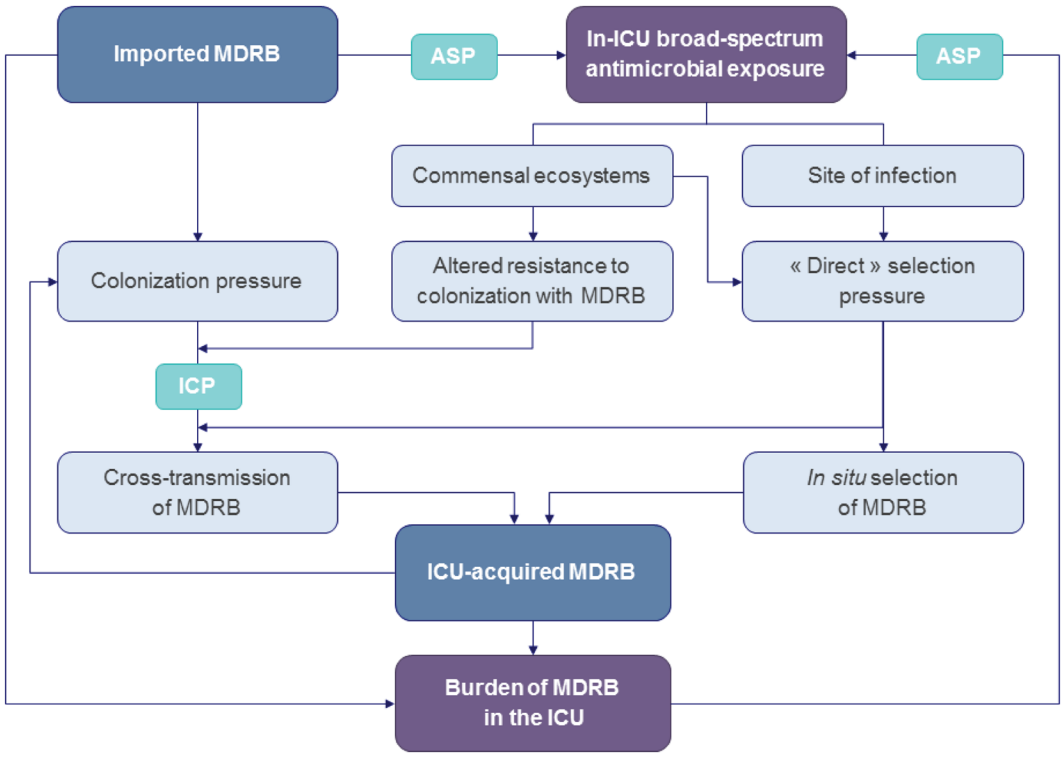

Fig. 2 Drivers of antimicrobial resistance in the ICU. MDRB multidrug-resistant bacteria, ASP antimicrobial stewardship programs, ICP infection control programs. "Direct" selection pressure indicates the selection of a pathogen with resistance to the administered drug. Green vignettes indicate the positioning of countermeasures. ASP may notably encompass every intervention aimed at limiting the ecological impact of antimicrobials agents, including rationalized empirical initiation, choice of appropriate drugs with the narrowest spectrum of activity (especially against resident intestinal anaerobes) and minimal bowel bioavailability, and reduced treatment duration [173]. ICP may include educational interventions to ensure a high level of compliance to hand hygiene and other standard precautions, targeted contact precautions in MDRB carriers (e.g., carbapenemase-producing *Enterobacteriaceae*), appropriate handling of excreta, and environment disinfection [167]

Table 1 Determinants of increased risk of MDRB infection at ICU admission and during the ICU stay

Predictors of MDRB infection	At ICU admission	During the ICU stay
Patient features	Co-morbid illness/immunosuppression/recent hospital and/or ICU stay	Higher severity of acute illness/Invasive interventions
Type of infection	Hospital-acquired > healthcare-associated > community-acquired	ICU-acquired > others
Antimicrobial selection pressure	Prior antibiotics*/antifungals	Antibiotics*/antifungals in the ICU
Colonization status	Previously documented colonization with MDRB	In-ICU acquisition of MDRB
Local epidemiology	Epidemiology of MDRB in community/hospital/areas recently traveled to	Local epidemiology of MDRB in the ICU
Infection prevention measures	Poor hygiene practices in hospital	Poor hygiene practices in the ICU

MDRB multidrug-resistant bacteria, *ICU* intensive care unit

*Especially if agents with broad-spectrum and/or potent activity against intestinal anaerobes

identification of patients meeting the Sepsis-3 criteria for sepsis [57].

Hence, antibiotics are mostly used empirically in ICU patients [58]. A provocative before–after study, however, suggested that aggressive empirical antibiotic use might be harmful in this population [59]. In fact, a conservative approach—with antimicrobials started only after confirmed infection—was associated with a more than 50% reduction in adjusted mortality as well as higher rates of appropriate initial therapy and shorter treatment durations.

Biomarkers may help to identify or—perhaps more importantly—rule out bacterial infections in this setting, thus limiting unnecessary antibiotic use and

encouraging clinicians to search for alternative diagnoses. Many cytokines, cell surface markers, soluble receptors, complement factors, coagulation factors, and acute phase reactants have been evaluated for sepsis diagnosis [60], yet most offer only poor discrimination [61]. Procalcitonin (PCT) levels are high in bacterial sepsis but remain fairly low in viral infections and most cases of non-infectious systemic inflammatory response syndrome (SIRS). However, a PCT-based algorithm for initiation (or escalation) of antibiotic therapy in ICU patients neither decreases overall antimicrobial consumption nor shortens time to adequate therapy or improves patient outcomes [62]. Thus, PCT is currently not recommended as part of the decision-making process for antibiotic initiation in ICU patients [49].

Considering the complexity of the host response and biomarker kinetics, a combined approach which integrates the clinical pretest probability of infection could facilitate the discrimination between bacterial sepsis and non-infectious SIRS in emergency departments and probably also in critically ill patients [63]. Given their high sensitivity, such multi-marker panels may be primarily used to rule out sepsis, albeit only in a subset of patients as a result of their suboptimal specificity (Table E1). In contrast, novel molecular assays for rapid pathogen detection in clinical samples show good specificity, yet poor sensitivity, thus providing a primarily rule-in method for infection (see below). For the foreseeable future, however, physicians will remain confronted with considerable diagnostic uncertainty and, in many cases, still have to rely on their clinical judgment for decisions to withhold or postpone antimicrobial therapy.

Impact of immune status

The host immune status is a key factor for the initial choice of antimicrobial therapy in the ICU [64]. Solid organ transplant recipients receiving immunosuppressive medications to prevent allograft rejection can present with sepsis or septic shock and very few or even no typical warning signs such as fever or leukocytosis. The level of required immunosuppression and the site of infections vary according to the allograft type; the timing of infection from original transplant surgery delineates the occurrence of nosocomial sepsis and opportunistic infections (Table 2) [65]. In hematological of solid cancer patients receiving cytoablative chemotherapy, the duration and level of neutropenia will be essential factors for the choice of empirical therapy [66, 67]. HIV-infected patients are not only susceptible to community-acquired infections but also to a vast panel of opportunist infections depending on CD4 cell count [68]. Other host immune profiles encompass immunoglobulin deficiencies and iatrogenic immunosuppression (Table 2) [69]. Because immunocompromised patients may have multiple concomitant dysfunctional immune pathways, co-infections (bacterial and/or viral and/or fungal) are possible and, when suspected, required several antimicrobials as part of empirical therapy. Of note, ageing has been associated with impairments in both innate and adaptive immunity that may predispose to severe bacterial infections; yet, the impact of immunosenescence on the management of ICU patients warrants further investigation [70, 71].

Early microbiological diagnosis: from empirical to immediate adequate therapy

The concepts of empirical therapy and de-escalation originate from the timeframe of routine bacteriological diagnosis. With culture-based methods, the turnaround time from sampling to AST results necessitates 48 h or more, leaving much uncertainty about the adequacy of empirical coverage at the acute phase of sepsis. Molecular diagnostic solutions have therefore been developed to accelerate the process without losing performance in terms of sensitivity and specificity.

A wide array of automated PCR-based systems targeting selected pathogens and certain resistance markers have recently been introduced (Table 3). Several panels are now widely available in clinical laboratories for specific clinical contexts (e.g., suspected bloodstream infections, pneumonia, or meningoencephalitis), offering a "syndromic approach" to microbiological diagnosis [72, 73]. Syndromic tests can be run with minimal hands-on time and identify pathogens faster than conventional methods (i.e., 1.5–6 h), especially when implemented as point-of-care systems. However, these tests remain expensive (> 100 USD per test) and must be performed alongside conventional cultures, which they cannot entirely replace. They also provide partial information about antibiotic susceptibility since only a limited number of acquired resistance genes are screened (e.g., those encoding ESBL or carbapenemase). Overall, further investigations are warranted to fully appraise their potential impact on patient outcomes [72].

A next step will be the daily use of clinical metagenomics—that is, the sequencing of nucleic acids extracted directly from a given clinical sample for the identification of all bacterial pathogens and their resistance determinants [74]. Fast sequencers such as the Nanopore (Oxford Nanopore Technologies, Oxford, UK) allow turnaround times of 6–8 h at similar costs to that of syndromic tests [75, 76]. This approach can also assess the host response

Table 2 Spectrum of empirical antimicrobial therapy in immunocompromised patients

Type of immune deficiency	Infection risk to guide antimicrobial rationale	Antimicrobial empirical coverage
Solid organ transplant	Timing from transplant surgery 0–2 months: high risk of HAI 2–6 months: high risk of both HAI and CAI 6–12 months: low risk of HAI, moderate risk of HAI and OI > 12 months: low risk of HAI, moderate risk of CAI and OI	*Pseudomonas* spp., *S. aureus*, *Candida* spp., *Aspergillus* spp., *Cryptococcus* spp. *Nocardia* spp., endemic mycoses, CMV PCP, tuberculosis, *S. pneumoniae*
Neutropenia	Absolute neutrophil count, duration, and comorbidities > 500 cells/μL, anticipated to last < 7 days < 100 cells/μL, anticipated to last > 7 days Shock, mucositis, diarrhea, central line	Low risk *Pseudomonas* spp., *S. aureus, S. viridans*, molds *Pseudomonas* spp., *S. aureus, S. viridans*, *Candida* spp.
HIV	CD4 cell count 200–500 cells/μL: low risk of OI 50–200 cells/μL: high risk of OI < 50 cells/μL: very high risk of OI HIV-induced humoral immunodeficiency at any CD4 level HIV and intravenous drug abuse	Tuberculosis Tuberculosis, PCP Cryptococcosis, toxoplasmosis, CMV *S. pneumoniae* S. aureus
Immunoglobulin deficiency	Common variable immunodeficiency Chronic lymphocytic leukemia Multiple myeloma Chronic granulomatous disease	Encapsulated bacteria[a] Encapsulated bacteria[a], *S. aureus* Encapsulated bacteria[a] *S. aureus*, *Burkholderia cepacia*, *Aspergillus* spp.
Iatrogenic immunosuppression	Steroids (prednisone > 20 mg/day) Inhibitors of TNF, IL-1, IL-6, IL-17, IL-12/23 Anti-CD20 monoclonal antibodies Anti-CD52 monoclonal antibodies	*Candida* spp., PCP, *Nocardia* spp. Tuberculosis, *S. aureus*, *Listeria* spp., *Legionella* Low risk *Aspergillus* spp., *Mucor*, *Listeria* spp.

HAI hospital-acquired infection, *CAI* community-acquired infection, *OI* opportunistic infection, *CMV* cytomegalovirus, *PCP Pneumocystis jirovecii* pneumonia

[a] Encapsulated bacteria: *S. pneumoniae, H. influenza, N. meningitidis, Salmonella* spp., *Klebsiella* spp.

Table 3 New diagnostic tools for bacterial infection in critically ill patients

Method	Based on	Available	Pros	Cons
Direct AST	Culture	Yes	Cheap Decreases TAT by 24 h	Lacks standardization Does not work for polymicrobial infection
Accelerate Pheno™	Culture	Yes	Faster than conventional methods Automatized 1 h for identification, 6–8 h for AST	Expensive Low throughput For positive blood cultures only
Lab automation	Culture	Yes	Real-time culturing decreasing TAT	Integration with stewardship Cost Exploitation of results outside working hours
Syndromic tests	PCR	Yes	Fast (TAT 1–8 h) Minimal hands-on time	Expensive Not exhaustive Minimal information on antibiotic resistance
Clinical metagenomics	NGS	In development	Exhaustive Potentially fast Host response	Experimental Interpretation of results Expensive

AST antimicrobial susceptibility testing, *TAT* turnaround time, *NGS* next-generation sequencing

at the infection site by sequencing the retro-transcribed RNA, possibly adding to its diagnostic yield [77]. Nonetheless, significant improvements in nucleic extraction rates, antibiotic susceptibility inference, and the exploitation of results into actionable data must be made before clinical metagenomics can be part of routine diagnostic algorithms.

Besides new-generation tools, rapidly applicable information can still be obtained from culture-based methods such as direct AST on lower respiratory tract samples (time from sample collection ca. 24 h) [78] or lab automation with real-time imaging of growing colonies—for instance, the Accelerate Pheno™ system (Accelerate Diagnostics, Tucson, AZ) provides AST results in 6–8 h from a positive blood culture [79].

To be effective, all these tests must be integrated into the clinical workflow, thereby raising other organizational challenges and requiring the implementation of ASP [80].

The right molecule(s) but avoid the wrong dose

Key features to appraise the optimal dozing of a given antibiotic include the minimum inhibitory concentration (MIC) of the pathogen and the site of infection. Still, for most cases, clear guidance on how to adapt the dose on the basis of such characteristics is lacking, leaving much uncertainty on this issue. Defining the right dose in patients with culture-negative sepsis is a further challenge, although targeting potential pathogens with the highest MICs may appear to be a reasonable approach.

Underdosing of antibiotics is frequent in critically ill patients. Indeed, up to one out of six patients receiving beta-lactams does not reach the minimal concentration target (i.e., free antibiotic concentrations above the MIC of the pathogen during more than 50% of the dosing interval), and many more do not reach the target associated with maximal bacterial killing (i.e., concentrations above $4 \times$ MIC during 100% of the dosing interval) [81]. This is particularly worrisome in the first hours of therapy when a maximal effect is highly desirable. Unfortunately, no standard remedy for this problem is available, and the solution depends on the physicochemical properties of the drug (e.g., hydrophilic versus lipophilic), patient characteristics, administration scheme, and the use of organ support (e.g., renal replacement therapy or extracorporeal membrane oxygenation) [82].

The volume of distribution—an important determinant of adequate antibiotic concentrations—is not measurable in critically ill patients. Yet, those with evidence for increased volume of distribution (e.g., positive fluid balance) require a higher loading dose to rapidly ensure adequate tissue concentrations, particularly for hydrophilic antibiotics, and for both intermittent and continuous infusion schemes [83]. This first dose must not be adapted to the renal function for antibiotics with predominant or exclusive renal clearance.

Many antibiotics used in the ICU are cleared by the kidneys; so, dosing adaptation for subsequent infusions must be considered in case of acute kidney injury (AKI) or augmented renal clearance (i.e., a measured creatinine clearance of 130 mL/min/1.73 m^2 or higher). This latter situation is associated with lower antibiotic exposure [84] and implies higher maintenance doses to keep concentrations at the targeted level, yet therapeutic drug monitoring (TDM) appears necessary to avoid overdosing.

These features can be integrated into pharmacokinetics (PK)/pharmacodynamics (PD) optimized dosing which can be considered a three-step process (Fig. 3). PK models can be used when selecting the dose for each of these steps [85] even if these predictions are estimations only with still important intra- and inter-individual variations. These are nowadays available in several stand-alone software packages, and integration in prescription drug monitoring systems (PDMS) will be the next step. TDM can be used to further refine therapy for many antibiotics [86].

Is there a role for routine therapeutic drug monitoring?

TDM may be employed to minimize the risk of antimicrobial toxicity and maximize drug efficacy through optimized PK, especially for aminoglycosides and glycopeptides. Indeed, high peak levels of aminoglycosides over the pathogen MIC appear beneficial in patients with ventilator-associated pneumonia or other life-threatening MDRB infections [87–89], while adequate trough vancomycin concentrations improve the clinical response in those with bloodstream infection due to MRSA [90].

However, the role of routine TDM in optimizing beta-lactam dosing remains controversial. The main issues that nowadays prevent the implementation of such a strategy in clinical practice are (1) the lack of a standardized method to reliably measure beta-lactam concentrations with a high intercenter reproducibility, (2) the delayed results of TDM for clinicians (i.e., the lack of a "point-of-care" for beta-lactam TDM in most of hospitals), (3) the optimal timing and number of samples to adequately describe the time course of drug concentrations, (4) the fact that the association between insufficient beta-lactam concentrations and the increased risk of therapeutic failure or impaired outcome is based only on retrospective studies, (5) the absence of clinical data showing a potential role of adequate beta-lactam levels in the emergence of resistant strains, (6) the poor characterization of the optimal duration of beta-lactam levels exceeding the MIC of the infective pathogen, when available, or of the optimal PK target in case of empirical therapy, and (7) the time needed to obtain the MIC of the infective pathogen, which precludes an adequate targeted therapy using PK principles [81, 91]. It is therefore possible that epidemiological cutoff (ECOFF) values are an acceptable option [92], but further studies are needed before the routine TDM of beta-lactams becomes available in most ICUs. Interestingly, high beta-lactam concentrations may result in drug-related neurotoxicity, which represents another potential role for TDM in critically ill patients [93, 94].

Key questions about antimicrobials

New and long-established antimicrobials

Polymyxins are considered the cornerstone of therapy for infections due to extremely drug-resistant (XDR) Gram-negative bacteria, including carbapenem-resistant *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae*. Of note, recent studies indicate that colistin and polymyxin B are associated with less renal and neurological

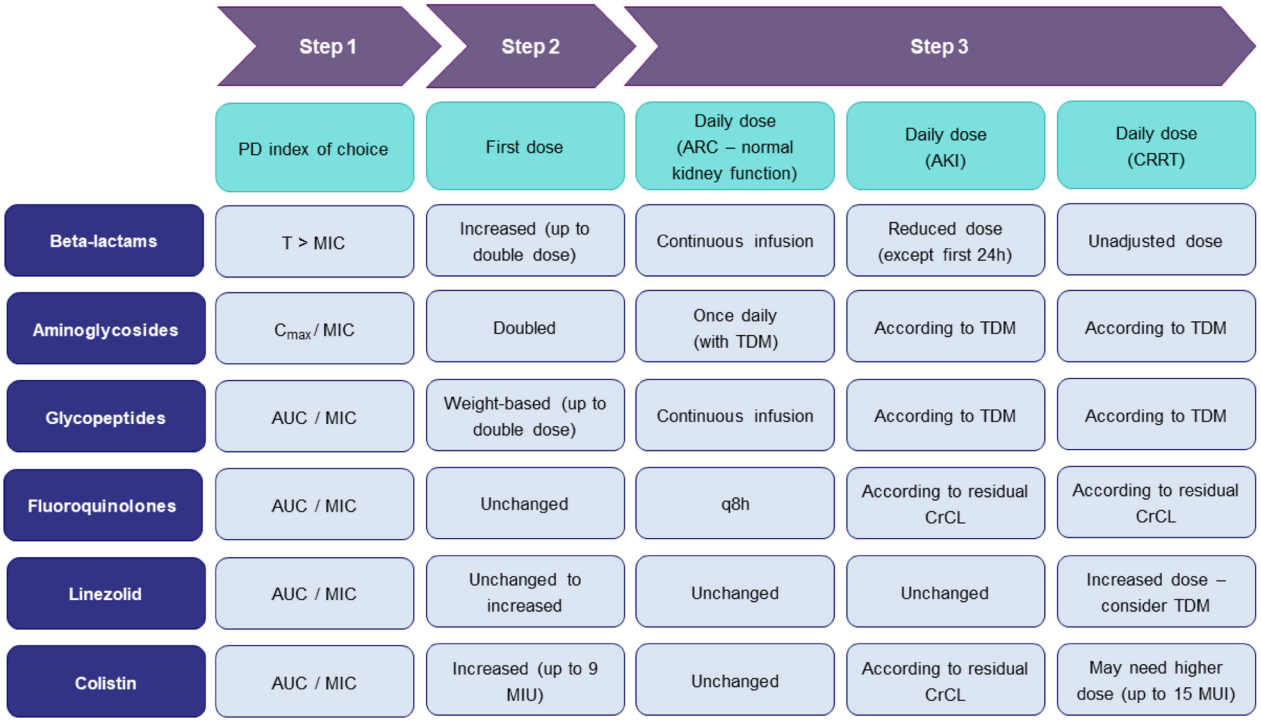

Fig. 3 Sequential optimization of antimicrobial pharmacokinetics in critically ill patients. In obese patients, dosing regimen should be adapted on the basis of lean body weight or adjusted body weight for hydrophilic drugs (e.g., beta-lactams or aminoglycosides) and on the basis of lean body weight for lipophilic drugs (e.g., fluoroquinolones or glycylcyclines)—see Ref. [174] for details. Dosing regimens for the first antibiotic dose (unchanged, increased, or doubled) are proposed by comparison with those usually prescribed in non-critically ill patients. PD pharmacodynamics, MIC minimal inhibitory concentration, AUC area under the curve, ARC augmented renal clearance, TDM therapeutic drug monitoring, AKI acute kidney injury, CRRT continuous renal replacement therapy, CrCL creatinine clearance

toxicity than previously reported. Several questions remain incompletely addressed, including the need and type of combination therapies, optimal dosing regimen, ways to prevent the emergence of resistance, and role of aerosolized therapy. Fosfomycin may also have a role in these infections.

Drugs newly approved or in late development phase mainly include ceftolozane–tazobactam, ceftazidime–avibactam, ceftaroline–avibactam, aztreonam–avibactam, carbapenems combined with new beta-lactamase inhibitors (e.g., vaborbactam, relebactam), cefiderocol, plazomicin, and eravacycline (Table 4). These drugs have mainly been tested in complicated urinary tract infection, complicated intra-abdominal infections (cIAI), or skin and soft tissue infection (SSTI). Limited data are currently available in ICU patients [95], notably for dosing optimization in severe MDRB infections. Piperacillin–tazobactam appears less effective than carbapenems in bloodstream infections caused by ESBL-E [96, 97]; however, ceftolozane–tazobactam and ceftazidime–avibactam might be considered as carbapenem-sparing options for treatment of such infections in areas with high prevalence of CRE. The actual question is should we still save carbapenems instead of saving new antibiotics?

In addition to glycopeptides, long-established antibiotics with activity against MRSA mainly include daptomycin (e.g., for bloodstream infections) and linezolid (e.g., for hospital-acquired pneumonia, HAP) [98, 99]. These alternatives may be preferred in patients with risk factors for AKI. Daptomycin appears safe even at high doses and in prolonged regimens, with rhabdomyolysis representing a rare, reversible side effect. Conversely, linezolid has been linked with several adverse events most often associated with specific risk factors (e.g., renal impairment, underlying hematological disease, or extended therapy duration), suggesting a role for TDM in patients at high risk of toxicity. Next, "new-generation" cephalosporins such as ceftaroline and ceftobiprole have been approved for the treatment of MRSA infections and seem promising in overcoming the limitations associated with the older compounds. Other new agents with activity against MRSA include lipoglycopeptides (dalbavancin, oritavancin, and telavancin), fluoroquinolones (delafloxacin, nemonoxacin, and zabofloxacin), an oxazolidinone (tedizolid), a dihydrofolate reductase inhibitor (iclaprim), and

Table 4 Indications and doses of new and long-established antibiotics for treating MDR bacteria

Drug	Usual dosing regimen for serious infections	Indication	Status
Recent release or late development phases			
Ceftaroline	600 mg q12 h, IV	BSI, CAP, cSSTI	Approved
Ceftobiprole	500 mg q8 h IV	BSI, HAP	Approved
Ceftazidime/avibactam	2.5 g q8 h IV	BSI, HAP, VAP, cIAI, UTI	Approved
Ceftolozane/tazobactam	1.5 g q8 h/3 g q8 h (VAP) IV	BSI, UTI, cIAI, HAP, VAP	Approved for cIAI and UTI Phase 3 for HAP and VAP
Aztreonam/avibactam	6500 mg ATM/2167 mg AVI q24 h on day 1 followed by 6000 mg ATM/2000 mg AVI q24 h, IV	HAP, VAP, BSI, UTI	Phase 3
Meropenem/vaborbactam	2 g/2 g q8 h IV	BSI, UTI, cIAI, HAP/VAP	Approved (FDA)
Cefiderocol	2 g q8 h IV	BSI, HAP, VAP, cIAI, UTI	Phase 3
Imipenem/relebactam	500 mg/250–125 mg q6 h IV	BSI, HAP, VAP, cIAI, UTI	Phase 3
Eravacycline	1 mg/kg q12 h IV	cIAI	Under evaluation (EMA and FDA)
Plazomicin	15 mg/kg q24 h IV	In combination for BSI, UTI, HAP, VAP	Approved
Tedizolid	200 mg q24 h IV, oral	cSSTI, HAP/VAP	Approved for cSSTI, phase 3 for HAP and VAP
Long-established antibiotics			
Piperacillin/tazobactam	4.5 g every 6 h CI	BSI, HAP, VAP, UTI, cIAI	Approved
Ceftazidime	6 g every 24 h CI	BSI, HAP, VAP, UTI	Approved
Cefepime	2 g every 8 h or CI	BSI, HAP, VAP, UTI	Approved
Aztreonam	1 g (2 g) every 8 h	BSI, HAP, VAP, UTI, SSTI	Approved
Imipenem/cilastatin	500 mg (1 g) every 6 h	BSI, HAP, VAP, UTI, cIAI	Approved
Meropenem	1 g (2 g) every 8 h or CI	BSI, HAP, VAP, UTI, cIAI	Approved
Tigecycline	100–200 mg loading those, then 50–100 mg every 12 h	cIAI	Approved
"Old"antibiotics			
Gentamicin	7 mg/kg/day every 24 h	In combination for BSI, UTI, c HAP, cIAI, VAP	Approved
Amikacin	25–30 mg/kg/day every 24 h	In combination for BSI, UTI,VA HAP, VAP	Approved
Colistin	9 MU loading dose, 4.5 MU every 8–12 h	In combination for BSI, UTI, HAP, VAP	Approved
Fosfomycin	4–6 g every 6 h CI	In combination for BSI, UTI, HAP, VAP	Approved
Vancomycin	15–30 mg/kg loading dose, 30–60 mg/kg every 12 h, 6 h or CI	BSI, HAP, VAP	Approved
Linezolid	600 mg every 12 h	BSI, HAP, VAP, SSTI	Approved

BSI bloodstream infection, *HAP* hospital-acquired pneumonia, *VAP* ventilator-associated pneumonia, *cIAI* complicated intra-abdominal infection, *UTI* urinary tract infection, *CI* continuous infusion, *FDA* US Food and Drug Administration, *EMA* European Medicines Agency

a tetracycline (omadacycline); yet, the yield of these new options remains to be investigated in critically ill patients with severe MRSA infection [100].

Single-drug or combination regimen

The question of whether antibiotic combinations provide a beneficial effect beyond the empirical treatment period remains unsettled. Meta-analyses of randomized controlled trials (RCTs) comparing beta-lactams vs. beta-lactams combined with another agent demonstrate no difference in clinical outcomes in a variety of infections caused by Gram-negative pathogens; however, patients with sepsis or septic shock were underrepresented [101, 102]. In contrast, a meta-analysis of randomized and observational studies focused on sepsis or septic shock showed that combination therapy is beneficial in high-risk patients (i.e., projected mortality rate greater than 25%) [103]. This positive impact may be especially pronounced in neutropenic patients and when a pathogen with reduced antimicrobial susceptibility is involved (e.g., *P. aeruginosa*) [104].

To date, there is no RCT to examine whether combination therapy is superior to monotherapy for CRE infections. Observational studies suggest that the benefit of

combination therapy is mainly observed in patients with serious underlying diseases or high pretreatment probability of death (e.g., septic shock) [105–109]. The most effective regimen is challenging to define, as only one of the aforementioned studies reported survival benefit with a specific drug combination (colistin plus tigecycline plus meropenem) after adjustment for potential confounders [109].

Although there have been five RCTs and several meta-analyses for the treatment of carbapenem-resistant *A. baumannii* infections, the optimal treatment regimen has not yet been determined [110–115]. Notably, none of the RCTs demonstrated a survival benefit with combination therapy, although one study showed a better clinical response with colistin plus high-dose ampicillin/sulbactam and three studies reported faster microbiological clearance when combining colistin with rifampin or fosfomycin. A recent meta-analysis, however, demonstrated survival benefit in bacteremic patients who were receiving high doses of colistin (more than 6 MIU per day) in combination with another agent [116].

Continuous prolonged or intermittent administration of beta-lactams and other time-dependent antimicrobials

The proportion of the interdose interval with drug concentration above the pathogen MIC is predictive of efficacy for time-dependent antibiotics, including beta-lactams. This parameter may be increased by reducing the interdose interval and/or by using extended infusions (EI) over 3–4 h or continuous infusion (CI). Stochastic models show that prolonged beta-lactam infusions increase the probability of target attainment against isolates with borderline MIC, especially in patients with ARC or increased volume of distribution [117].

Most RCTs comparing intermittent versus prolonged beta-lactam infusions could not find significant differences in outcomes. However, in a recent meta-analysis of RCTs comparing prolonged (EI or CI) and intermittent infusions of antipseudomonal beta-lactams in patients with sepsis, prolonged infusion was associated with improved survival, including when carbapenems or beta-lactam/beta-lactam inhibitor combinations were analyzed separately [118]. Prolonged infusions might only be needed in some patients—e.g., those with beta-lactam underdosing using intermittent administration schemes, or infections caused by isolates with elevated MICs. Because these features cannot be anticipated, it seems reasonable to consider the use of prolonged infusions of sufficiently stable antipseudomonal beta-lactams in all patients with sepsis.

For some other drugs such as vancomycin, the ratio area under the curve/MIC is considered the PK/PD parameter predictive of efficacy (Fig. 3). A recent meta-analysis suggested that continuous vancomycin infusion is associated with lower nephrotoxicity but not better cure or lower mortality than intermittent infusions [119]; nevertheless, included studies had many limitations and further investigations are needed to address this issue.

De-escalation: impact in practice

Conceptually, de-escalation is a strategy whereby the provision of effective antibiotic treatment is achieved, while minimizing unnecessary exposure to broad-spectrum agents that would promote the development of resistance. Practically, it consists in the reappraisal of antimicrobial therapy as soon as AST results are available. However, no clear consensus on de-escalation components exists and various definitions have been used (e.g., changing the "pivotal" agent for a drug with a narrower spectrum and/or lower ecological effects on microbiota, or discontinuing an antimicrobial combination), resulting in equivocal interpretation of the available evidence [120, 121].

De-escalation is applied in only 40–50% of inpatients with bacterial infection [121]. This reflects physician reluctance to narrow the covered spectrum when caring for severely ill patients with culture-negative sepsis and/or MDRB carriage [120]. Importantly, the available evidence does not suggest a detrimental impact of de-escalation on outcomes [120, 122], including in high-risk patients such as those with bloodstream infections, severe sepsis, VAP, and neutropenia [123, 124]. However, further well-designed RCTs are needed to definitely solve this issue.

Increasing physician confidence and compliance with de-escalation has become a cornerstone of ASP. Paradoxically, there is a lack of clinical data regarding the impact of de-escalation on antimicrobial consumption and emergence of resistance [120]. While this strategy has been associated with reduced use of certain antimicrobial classes [125, 126], no study demonstrated that it may allow a decrease in overall antimicrobial consumption, and an increase in antibiotic exposure has even been observed [123, 125, 127]. Similarly, the few studies that addressed this point reported no impact—or only a marginal effect—of de-escalation on the individual hazard of MDRB acquisition or local prevalence of MDRB [125–127].

In light of these uncertainties, efforts should focus on microbiological documentation to increase ADE rates in patients with sepsis. New diagnostic tools should be exploited to hasten pathogen identification and AST availability. Lastly, human data on the specific impact of each antimicrobial on commensal ecosystems and the risk of MDRB acquisition are needed to optimize

antibiotic streamlining and further support de-escalation strategies [37, 128].

Duration of antibiotic therapy and antibiotic resistance

Prolonged durations of antibiotic therapy have been associated with the emergence of antimicrobial resistance [129]. Yet, short-course antibiotic therapy has been shown to be effective and safe in a number of infections, including community-acquired pneumonia, VAP, urinary tract infections, cIAI, and even some types of bacteremia [130–136]. The shortening of antibiotic durations on the basis of PCT kinetics has also been shown to be safe, including in patients with sepsis [51, 52]. However, the recent ProACT trial failed to confirm the ability of PCT to reduce the duration of antibiotic exposure compared to usual care in suspected lower respiratory tract infections [137]. Given the importance of overruling in available RCTs and the relatively long duration of therapy in control groups, the question remains unresolved. In particular, the efficacy and costs of PCT if an active ASP is in place remain to be evaluated.

Many national and international guidelines encourage physicians to shorten the overall durations of antibiotic therapy for a number of infections. Shorter courses are now recommended for pneumonia, urinary tract infections, and cIAI with source control [49, 138–142]. However, despite the presence of these recommendations, recent studies suggest that excessive durations of antibiotics are still being administered, thereby offering further opportunities for ASP [143, 144]. However, clinicians should also be aware that, under some circumstances, short-course therapy may be detrimental to patient outcomes, especially in case of prolonged neutropenia, lack of adequate source control, infection due to XDR Gram-negative bacteria, and endovascular or foreign body infections [130, 145].

Source control

Source control to eliminate infectious foci follows principles of drainage, debridement, device removal, compartment decompression, and often deferred definitive restoration of anatomy and function [146]. If required, source control is a major determinant of outcome, more so than early adequate antimicrobial therapy [147–149], and should never be considered as "covered" by broad-spectrum agents. Therefore, surgical and radiological options for intervention must be systematically discussed, especially in patients with cIAI or SSTI. The efficacy of source control is time-dependent [150–153] and adequate procedures should therefore be performed as rapidly as possible in patients with septic shock [49], while longer delays may be acceptable in closely monitored stable patients. Failure of source control should be considered in cases of persistent or new organ failure despite resuscitation and appropriate antimicrobial therapy, and requires (re)imaging and repeated or alternative intervention. Importantly, source control procedures should include microbiological sampling whenever possible to facilitate ADE initiatives.

Antibiotic stewardship programs in the ICU

Implementing ASP in the ICU improves antimicrobial utilization and reduces broad-spectrum antimicrobial use, incidence of infections and colonization with MDRB, antimicrobial-related adverse events, and healthcare-associated costs, all without increase in mortality [26, 154, 155]. According to the ESCMID Study Group for Antimicrobial Stewardship, ASP should be approached as "a coherent set of actions which promote using antimicrobials in ways that ensure sustainable access to effective therapy for all who need them" [156]. Therefore, ASP should be viewed as a quality improvement initiative, requiring (1) an evidence-based, ideally bundled, change package, (2) a clear definition of goals, indicators, and targets, (3) a dynamic measurement and data collection system with feedback to prescribers, (4) a strategy for building capacity, and (5) a plan to identify and approach areas for improvement and solve quality gaps. This necessarily implies the appointment of a member of the ICU staff as a leader with expertise in the field of antimicrobial therapy and prespecified functions for the implementation of the local ASP.

Three main kinds of interventions may be used in ASP [157–159]:

- Restrictive, in which one tries to reduce the number of opportunities for bad behavior, such as formulary restrictions, pre-approval by senior ASP doctor (either an external infectious disease specialist or a specified expert in the ICU team), and automatic stop orders
- Collaborative or enhancement, in which one tries to increase the number of opportunities and decrease barriers for good behavior, such as education of prescribers, implementation of treatment guidelines, promotion of ADE, use of PK/PD concepts, and prospective audit and feedback to providers
- Structural, which may include the use of computerized antibiotic decision support systems, faster diagnostic methods for antimicrobial resistance, antibiotic consumption surveillance systems, ICU leadership commitment, staff involvement, and daily collaboration between ICU staff, pharmacists, infection control units, and microbiologists

The implementation of ASP should take into account the need for a quick answer from the system in case of severe infections (e.g., regarding as unacceptable the delay in the first antimicrobial delivery due to too restrictive pharmacy-driven prescription policies).

An ASP should consensually rest on multifaceted interventions to achieve its fundamental goals (Table 5), namely improving outcomes and decreasing antimicrobial-related collateral damage in infected patients. Yet, the weight of each component must be customized according to the context and culture of every single ICU in terms of habits for antibiotic prescription, MDRB prevalence, local organizational aspects, and available resources. For this purpose, concepts of implementation science should be applied—that is, identifying barriers and facilitators that impact the staff's compliance to guidelines in order to design and execute a structured plan for improvement [160].

The appropriate dashboard in the ICU

The availability of constantly updated information is pivotal to improve decision-making processes in the ICU [161, 162]. As the epidemiology of MDRB is continuously evolving, close monitoring of local resistance patterns may help to rationalize the empirical use of broad-spectrum antibiotics in this setting. With the expanding utilization of electronic medical records and applications specifically developed for the ICU, streaming analytics can provide dashboards containing real-time and easily accessible data for intensivists [162, 163]. Such dashboards should capture data from medical records and microbiology systems, display an intuitive and user-friendly interface, and be available on both ICU computers and mobile devices to allow easy access to actionable data at the bedside. Finally, a complete dashboard should include information not only on dynamics of resistance patterns but also on local antimicrobial consumption, adherence to protocols of care and antibiotic guidelines, healthcare-associated infections (e.g., source, type, severity), and general patient characteristics (e.g., comorbidities, severity of illness, main diagnosis, and length of the ICU stay) (Fig. 4). Although studies demonstrating the efficacy of such dashboards in reducing resistance have not been published so far, these tools could

Table 5 Implementation and objectives of antibiotic stewardship programs in the ICU

Implementation of ASP	
Pre-requisites	Evidence-based, ideally bundled change package Dynamic data collection systems with feedback to prescribers Strategy for building capacity, including the appointment of an ICU staff member as ASP leader
Pre-implementation phase	Identification of determinants for antibiotic prescription and opportunities for improvement at the ICU level
Implementation phase	Building of a customized plan to solve quality gaps, based on educational and behavioral interventions Continuous collaboration between ICU staff members, microbiologists, pharmacists, and infection control units Clear definition of goals and indicators
Pragmatic objectives of ASP	
Stewardship of empirical antibiotic therapy	Distinction between bacterial infections, non-bacterial infections, and non-infectious inflammatory syndromes Early identification of sepsis (*antibiotic initiation might be delayed pending microbiological data in certain patients without new or worsening organ failure*) Consideration of local resistance patterns and patient's individual risk factors for MDRB for the choice of empirical drugs Efforts to obtain early microbiological documentation (including rapid diagnostic tools, conventional cultures, and source control when appropriate) Optimization of PK/PD Promotion of single-drug regimen whenever possible for patients without septic shock and/or risk factors for MDRB Restricted use of broad-spectrum, costly, and/or potentially toxic antibiotics
Stewardship of definite antibiotic therapy	Reappraisal of the diagnosis of bacterial infection at day 2–3 (microbiological and radiological data, clinical evolution) Early antibiotic cessation in patients without confirmed infection In patients with likely or confirmed infection: dosing adaptation when appropriate (e.g., if changes in Vd and/or renal clearance), routine discussion for de-escalation (e.g., spectrum narrowing and switching from combination to single-drug regimen) and shortening of treatment duration (e.g., PCT-based algorithms, adequate source control, favorable clinical evolution)
Overall objectives	Improvement in patient outcomes Reduction in ecological and non-ecological (e.g., toxicity or allergy) side effects of antibiotics Reduction of antibiotic- and resistance-related costs

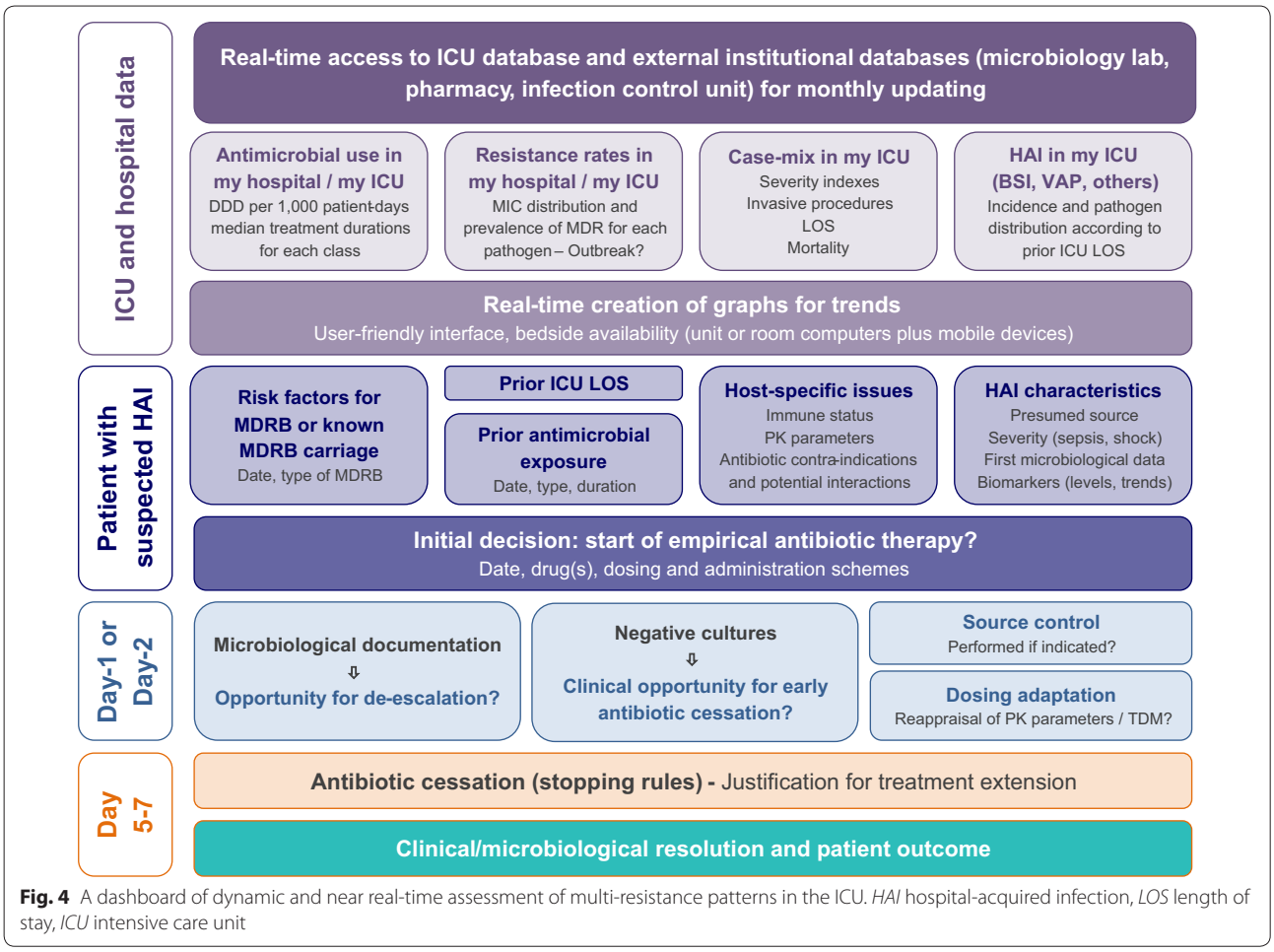

Fig. 4 A dashboard of dynamic and near real-time assessment of multi-resistance patterns in the ICU. *HAI* hospital-acquired infection, *LOS* length of stay, *ICU* intensive care unit

allow a structured audit-feedback approach that is one of the cornerstones of ASP implementation in the ICU [164–166].

Concluding remarks

Both the poor outcomes associated with bacterial sepsis and the current epidemiology of MDRB urge the need for improving the management of antibiotic therapy in ICU patients. Well-designed studies remain warranted to definitely address several aspects of this issue, notably the clinical input of rapid diagnostic tools and TDM, the potential benefit of combination versus single-drug therapies, the optimal dosing regimens before the availability of AST results or for patients with culture-negative sepsis, and the prognostic yield of ASP. Although beyond the scope of this review, the exploitation of other research axes may further help to control the spread of MDRB in the ICU setting, including optimization of infection control policies [167], a comparative appraisal of the impact of broad-spectrum antibiotics on the gut microbiota through novel metagenomics approaches [168], and the evaluation of emerging options such as orally administered antimicrobial-adsorbing charcoals, probiotics, or fecal microbiota transplantation to protect or restore the commensal ecosystems of ICU patients [29, 169, 170].

Electronic supplementary material
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Compliance with ethical standards

Conflicts of interests

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Research Update

ORIGINAL

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

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Abstract

Purpose: Mechanisms of circulatory failure are complex and frequently intricate in septic shock. Better characterization could help to optimize hemodynamic support.

Methods: Two published prospective databases from 12 different ICUs including echocardiographic monitoring performed by a transesophageal route at the initial phase of septic shock were merged for post hoc analysis. Hierarchical clustering in a principal components approach was used to define cardiovascular phenotypes using clinical and echocardiographic parameters. Missing data were imputed.

Findings: A total of 360 patients (median age 64 [55; 74]) were included in the analysis. Five different clusters were defined: patients well resuscitated (cluster 1, $n = 61$, 16.9%) without left ventricular (LV) systolic dysfunction, right ventricular (RV) failure or fluid responsiveness, patients with LV systolic dysfunction (cluster 2, $n = 64$, 17.7%), patients with hyperkinetic profile (cluster 3, $n = 84$, 23.3%), patients with RV failure (cluster 4, $n = 81$, 22.5%) and patients with persistent hypovolemia (cluster 5, $n = 70$, 19.4%). Day 7 mortality was 9.8%, 32.8%, 8.3%, 27.2%, and 23.2%, while ICU mortality was 21.3%, 50.0%, 23.8%, 42.0%, and 38.6% in clusters 1, 2, 3, 4, and 5, respectively ($p < 0.001$ for both).

Conclusion: Our clustering approach on a large population of septic shock patients, based on clinical and echocardiographic parameters, was able to characterize five different cardiovascular phenotypes. How this could help physicians to optimize hemodynamic support should be evaluated in the future.

Keywords: Septic shock, Hemodynamic failure, Cluster

Introduction

In the past, it was considered that hemodynamic alterations in septic shock occur in different phases, an early phase with a low flow state related to hypovolemia, a second phase after the initial resuscitation with a hyperdynamic state, and finally a third phase with cardiac failure leading to multiorgan failure and death [1]. Since the landmark study by Parker et al. [2], it has been progressively accepted that depressed left ventricular (LV) systolic function may develop at the early phase of septic shock. For a long time, the assessment of hemodynamic instability in septic shock was based on right heart catheterization, while alternatives are now well recognized, including critical care echocardiography (CCE) [3]. The high incidence of early myocardial alterations has been confirmed by echocardiographic studies [4, 5] and one found an incidence of LV systolic dysfunction of 39% during the first day [6]. There is then an urgent need for better characterization of cardiovascular phenotypes

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in order to propose targeted/personalized medicine for hemodynamic support [7], as for the adequate need for fluids [8, 9] or inotropic support. Although CCE may detect combined mechanisms of circulatory failure in septic shock (i.e., vasoplegia, hypovolemia, LV systolic dysfunction, and right ventricular (RV) failure) [10], it failed to extend such an integrative and individualized approach in daily clinical practice. Cardiovascular phenotypes are insufficiently characterized and rely on too simplistic and inadequate definitions, as reflected by the binary approach mostly based on the value of LV ejection fraction (EF) to identify sepsis-induced LV systolic dysfunction [11].

We therefore hypothesized that the application of a clustering approach to a large database of septic shock patients monitored by CCE could help to better characterize the different cardiovascular phenotypes.

Materials and methods

Study design

The databases of two recently published prospective, observational, multicenter studies using CCE during early resuscitation of patients with septic shock were merged. The Hemosepsis study (inclusion January 2011–December 2013) compared the identification of hemodynamic profiles using both CCE and transpulmonary thermodilution in septic shock patients in sinus rhythm [12]. The Hemopred study included patients (November 2012–November 2014) with shock of any origin, mostly related to sepsis, to compare the accuracy of the different parameters of fluid responsiveness [13]. In both cohorts, we excluded patients with a history of chronic heart failure. Overall, the patients included came from 12 different ICUs. Septic shock was not defined by the Sepsis-3 definition as Hemopred and Hemosepsis were designed before its publication. Diagnosis was based on a suspected infection responsible for sustained hypotension despite adequate fluid loading that required vasopressors, with associated clinical signs of tissue hypoperfusion (mottled skin, encephalopathy, oliguria for more than 2 h) that were biologically confirmed (pH < 7.38 and base deficit > − 5 mmol/L or lactate > 2 mmol/L or central venous oxygen saturation < 70%).

CCE

CCE was performed using a transesophageal route (TEE) in all patients during the first 12 h following the diagnosis of septic shock, after initial fluid resuscitation and vasopressor infusion. Patients were all intubated, sedated, and perfectly adapted to the respirator, as no spontaneous effort was observed during the echo procedure. Views, recorded parameters, and measurements were extensively described previously following the same prospective procedure [12, 13]. Echo parameters are all well recognized in the literature as good/adequate parameters of cardiovascular status in sepsis. Briefly, we systematically measured parameters of LV systolic function, i.e., LVEF and LV fractional area change (FAC) [14], and of LV diastolic function, i.e., maximal mitral Doppler *E* wave velocity and maximal tissue Doppler velocity of the lateral aspect of the mitral annulus at early diastole (*E*′) [15]. RV function was evaluated by the RV/LV end-diastolic area (EDA) ratio [16]. Fluid responsiveness was assessed using the superior vena cava collapsibility index (ΔSVC) [17]. We measured the velocity time integral (VTI) in the LV outflow tract and the diameter of the aortic annulus, which allowed us to calculate LV stroke volume and cardiac index (CI) [18]. The ultrasound systems used in the two cohorts were the same in each participating center as a result of the relatively narrow period of time encompassing the two studies. Images were all obtained and interpreted by intensivists trained in advanced level CCE, as mentioned in the two original studies. Images were not "validated" by independent experts to increase the external validity of our results which correspond to hemodynamic data obtained during daily echocardiographic assessment on clinical grounds. Importantly, no a priori criteria of "abnormality" were applied for the different parameters to best take into account inter-individual variabilities, allowing better characterization of cardiovascular phenotypes.

Take-home message

Using a clustering approach including clinical and echocardiographic parameters, 5 different hemodynamic phenotypes were identified in 360 septic shock patients, left ventricular (LV) systolic dysfunction, LV hyperkinesia, still hypovolemia, right ventricular failure and well-resuscitated phenotype.

Patient characteristics and clinical hemodynamic evaluation

We calculated the sequential organ failure assessment (SOFA) score and the simplified acute physiology score (SAPS II). Mortality at day 7 and in the ICU was also recorded, as was the origin of infection.

In each patient, several clinical hemodynamic parameters were prospectively recorded at the time of the CCE: heart rate, invasive systolic (SAP), diastolic (DAP), and mean arterial pressure (MAP); central venous pressure (CVP) and central venous oxygen saturation ($ScVO_2$) were measured through a catheter placed in the internal jugular or subclavian vein; serum lactate level, volume of initial filling, presence of epinephrine, dobutamine, or norepinephrine and if so respective doses were also

recorded. We also recorded blood gas analysis and respiratory settings.

Statistical analysis

Baseline characteristics were reported as median [interquartile range] and *n* (%) for quantitative and qualitative variables, respectively. Quantitative variables were compared using nonparametric tests, the Mann–Whitney test or the Kruskal–Wallis test, as appropriate. Qualitative variables were compared using Pearson's Chi-square test or Fisher's exact test, as appropriate.

A two-step clustering approach was used to (1) reduce the dimensionality of the dataset and (2) to perform hierarchical clustering. This approach, so-called hierarchical clustering on principal components (HCPC), was performed using the factoMineR package in R [19]. We first performed a principal component analysis including hemodynamic parameters (i.e., LVEF, LVFAC, mitral Doppler *E* wave velocity, lateral mitral tissue Doppler *E*′ velocity, aortic VTI, RV/LV EDA, ΔSVC, systolic arterial blood pressure, diastolic arterial blood pressure, heart rate, norepinephrine and epinephrine infusion doses). Variables were standardized as they were measured in different units. The HCPC procedure allows one, after the hierarchical clustering step is performed, to choose the number of clusters based on the hierarchical tree and to perform a K-means clustering to improve the initial partition obtained from the hierarchical clustering [20]. Agglomerative hierarchical clustering used the Ward's criterion and an Euclidean metric. Data on the internal validity and stability of the analysis are shown in the supplementary material (Fig. S1). Missing data were imputed using iterative principal component analysis (implemented in the imputePCA R function), as previously described. Briefly, this method starts using a mean imputation, performs principal component analysis on the completed dataset, and missing values are then updated by the fitted values using a predefined number of dimensions [21]. Multiple imputations were also performed to visualize the variability related to the imputation process (Fig. S2).

We then compared variables (hemodynamic and non-hemodynamic-related variables) according to clusters. We chose not to include hemodynamic variables whose proportion of missing values was higher than 10%.

Last, we evaluated the diagnostic performance of the three most important variables for each cluster using two methods: (1) evaluation of the area under the receiver operating curve (AUROC) of a multivariable logistic regression with a binary variable of being in each cluster (yes/no) as the dependent variable and these three variables as independent variables and (2) calculation of sensitivity, specificity, and negative and positive predictive values for the combination of these three variables above or below the thresholds. These thresholds were picked up from the description of the hemodynamic parameters we described across clusters as follows: the first interquartile (Q1) when the mean of the cluster was lower than the overall mean and the third interquartile (Q3) when the mean of the cluster was higher than the overall mean; 95% confidence intervals were calculated for all these results.

A p value lower than 0.05 was considered significant. All statistical analyses were performed using RStudio (Version 1.1.414—2009–2018 RStudio, Inc.).

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Results

Among 432 patients from both cohorts, 360 were analyzed (Fig. S3). We excluded from the analysis 50 patients with a history of chronic heart failure, as it was not at all characterized. In most patients (82%), there was no missing data (Fig. S4). Characteristics of the population and mortality are reported in Table 1. Median age was 64 [interquartile 55; 74], SOFA score 10 [7; 12], and SAPS II 57 [45; 70]. No difference was observed between patients initially included in the Hemosepsis or Hemopred cohort regarding severity scores (Table S1). Twenty-one patients (5.8%) were in atrial fibrillation at the time of CCE and 42 patients (11.7%) already received inotropic drug (dobutamine, $n = 17$ or epinephrine, $n = 25$). Day 7 and in-ICU mortality were 20.1% and 35%, respectively.

Analysis in clusters characterized five distinct cardiovascular phenotypes (Tables 1, 2; Fig. 1). Sixty-one patients (16.9%) could be considered as "well resuscitated" (cluster 1), since we observed neither LV systolic dysfunction and RV failure nor fluid responsiveness. Both CI and $ScvO_2$ were within the normal range. Sixty-four patients (17.7%) had an "LV systolic dysfunction" phenotype (cluster 2). These patients exhibited low LVEF, LVFAC, and CI (29% [22; 40], 26% [18; 33], and 2.2 L/min/m^2 [1.7; 2.5], respectively), had higher lactate level, required a higher dose of norepinephrine, and were not fluid responders. Only nine of these patients had a LVEF higher than 45%. $ScVO_2$ remained within the normal range. Similarly, LV filling pressure as reflected by E/E' ratio remained non-elevated despite cardiac failure. Eighty-four patients (23.3%) had a phenotype reflecting a

Table 1 Baseline characteristics of the patients included in the analysis according to cluster partition

	All patients	Cluster					*p* value
		1	2	3	4	5	
	N = 360	*n* = 61	*n* = 64	*n* = 84	*n* = 81	*n* = 70	
Demographics							
Age, years	64 [55; 74]	59.0 [50.0; 68.0]	64.0 [54.0; 75.0]	66.0 [59.0; 75.0]	63.0 [55.0; 73.0]	64.0 [55.0; 76.0]	0.011
Male gender	233 (64.7)	35 (57.4)	40 (62.5)	62 (73.8)	54 (66.7)	42 (60.0)	0.245
Chronic respiratory failure	52 (14.4)	5 (1.4)	12 (3.3)	12 (3.3)	12 (3.3)	11 (3.1)	0.561
Atrial fibrillation at time of CCE	21 (5.8)	4 (1.1)	6 (1.7)	4 (1.1)	3 (0.8)	4 (1.1)	0.670**
SAPS II	57 [45; 70]	50.0 [39.0; 63.0]	62.0 [51.0; 74.0]	55.0 [42.0; 67.0]	59.5 [48.0; 72.0]	56.5 [44.0; 70.0]	0.004
SOFA score	10 [7, 12]	10.0 [8.0; 12.0]	10.0 [8.0; 12.5]	10.0 [7.0; 11.0]	11.0 [7.5; 13.0]	9.0 [7.0; 11.0]	0.089
Arterial blood lactate level, mmol/L	2.5 [1.5; 4.3]	2.7 [1.6; 4.1]	3.1 [2.1; 6.6]	1.9 [1.3; 3.0]	2.6 [1.6; 4.0]	2.7 [1.6; 4.7]	< 0.001
Non-hemodynamic parameters							
$PaCO_2$, mmHg	40 [34; 47]	42.0 [35.9; 49.0]	40.0 [33.5; 44.5]	40.3 [35.0; 48.0]	40.0 [33.5; 47.3]	38.5 [34.0; 43.0]	0.376
PaO_2/FiO_2, mmHg	184 [113; 262]	170.5 [111.9; 258.8]	174.0 [92.9; 241.8]	195.5 [120.3; 269.4]	153.8 [105.5; 231.5]	204.0 [125.0; 296.0]	0.099
PaO_2/FiO_2 ratio Berlin classification*							0.628
> 300 mmHg, *n* (%)	57 (15.9)	12 (20.0)	8 (12.5)	13 (15.5)	9 (11.2)	15 (21.4)	
200–300 mmHg, *n* (%)	100 (27.9)	14 (23.3)	20 (31.2)	26 (31.0)	20 (25.0)	20 (28.6)	
< 200 mmHg, *n* (%)	201 (56.1)	34 (56.7)	36 (56.2)	45 (53.6)	51 (63.8)	35 (50.0)	
Site of infection							0.001
Lung, *n* (%)	169 (46.9)	22 (36.1)	23 (35.9)	43 (51.2)	48 (59.3)	33 (47.1)	
Urinary tract, *n* (%)	25 (6.9)	3 (4.9)	7 (10.9)	7 (8.3)	4 (4.9)	4 (5.7)	
GI tract, *n* (%)	109 (30.3)	20 (32.8)	26 (40.6)	21 (25.0)	13 (16.0)	29 (41.4)	
Skin, *n* (%)	24 (6.7)	8 (13.1)	4 (6.2)	7 (8.3)	2 (2.5)	3 (4.3)	
Others, *n* (%)	33 (9.2)	8 (13.1)	4 (6.2)	6 (7.1)	14 (17.3)	1 (1.4)	
Outcome							
Day 7 mortality, *n* (%)	72 (20.1)	6 (9.8)	21 (32.8)	7 (8.3)	22 (27.2)	16 (23.2)	< 0.001
ICU mortality, *n* (%)	126 (35.0)	13 (21.3)	32 (50.0)	20 (23.8)	34 (42.0)	27 (38.6)	0.001

*Two missing FiO_2

**Exact Fisher's test

"hyperkinetic" state (cluster 3). In these patients, LV systolic function (LVEF 60% [52.5; 66]) and CI (3.3 L/min/m^2 [2.3; 4.3]) were increased compared to other clusters, and patients exhibited no sign of fluid responsiveness. Eighty-one patients (22.5%) had a hemodynamic profile consistent with underlying "RV failure" (cluster 4). These patients exhibited a markedly high RV/LV EDA ratio (0.8 [0.6; 0.9]) with a normal or supranormal LV systolic function (LVEF 57% [46; 64]) and no more fluid responsiveness. In this subset of patients, more patients had a PaO_2/FiO_2 lower than 200 mmHg. Finally, 70 patients (19.4%) belonged to the last cardiovascular phenotype which could be named "still hypovolemic" (cluster 5). These patients exhibited a low CI (2.6 L/min/m^2 [1.9; 3.1]) despite an increased LV systolic function (LVEF 58% [50; 65]) due to sustained fluid responsiveness, as reflected by markedly elevated ΔSVC (39% [31; 54]), and low preload (CVP 8 mmHg [5; 12]). Interestingly, patients in this cluster received significantly more fluids before CCE than the others, 2762 mL [2500; 4000] and 2000 mL [1000; 3433],

Table 2 Hemodynamic data of the 360 patients included in the study according to the cluster partition

	All patients	Cluster					*p* value
		1	2	3	4	5	
	N = 360	*n* = 61	*n* = 64	*n* = 84	*n* = 81	*n* = 70	
Hemodynamic parameters							
Heart rate, per minute	107 [90; 124]	125.0 [115.0; 140.0]	111.0 [98.0; 127.5]	93.0 [79.5; 106.0]	103.0 [85.0; 116.0]	111.0 [99.0; 125.0]	< 0.001
Systolic arterial blood pressure, mmHg	112 [96; 129]	129.0 [115.0; 141.0]	111.5 [101.5; 124.0]	127.0 [114.0; 139.0]	90.0 [80.0; 100.0]	105.0 [95.0; 120.0]	< 0.001
Diastolic arterial blood pressure, mmHg	59 [51; 70]	67.0 [58.0; 76.0]	70.0 [59.5; 76.0]	63.0 [54.0; 70.5]	47.0 [40.0; 51.0]	59.0 [53.0; 68.0]	< 0.001
Mean arterial blood pressure, mmHg	77 [67; 88]	84.0 [77.0; 97.0]	81.5 [72.5; 92.0]	84.5 [75.0; 91.0]	61.0 [52.0; 67.0]	74.0 [68.0; 82.0]	< 0.001
Cardiac index, L/min/m^2	2.9 [2.1; 3.8]	3.8 [3.0; 4.4]	2.2 [1.7; 2.5]	3.3 [2.3; 4.3]	3.2 [2.4; 3.9]	2.6 [1.9; 3.1]	< 0.001
$ScvO_2$, %	79 [71; 85]	80.0 [72.1; 86.2]	78.0 [66.0; 84.1]	82.3 [75.0; 85.0]	77.7 [69.1; 84.0]	77.0 [69.4; 84.0]	0.038
Central venous pressure, mmHg	10 [7, 13]	11.0 [8.0; 13.5]	10.0 [9.0; 14.0]	10.0 [7.5; 12.0]	9.0 [6.0; 13.0]	8.5 [5.5; 12.0]	0.041
Fluid expansion before CCE, mL	2000 [1000; 3500]	2000 [1228; 3433]	2000 [1000; 3500]	2000 [1500; 3000]	2000 [1000; 3289]	2762 [2500; 4000]	0.175
Hemodynamic treatments							
Epinephrine infusion, *n* (%)	25 (6.9)	7 (11.5)	11 (17.2)	6 (7.1)	0 (0.0)	1 (1.4)	< 0.001
Epinephrine infusion rate, mg/h	0.0 [0.0; 0.0]	0.0 [0.0; 1.0]	0.0 [0.0; 0.0]	0.0 [0.0; 0.0]	0.0 [0.0; 0.0]	0.0 [0.0; 0.0]	< 0.001
Norepinephrine infusion, *n* (%)	309 (85.8)	58 (95.1)	56 (87.5)	64 (76.2)	70 (86.4)	61 (87.1)	0.027
Norepinephrine infusion rate, mg/h	1.9 [0.6; 4.0]	2.2 [1.0; 4.0]	3.0 [1.5; 5.5]	1.0 [0.2; 2.3]	2.1 [0.7; 5.0]	1.8 [0.8; 3.5]	< 0.001
Dobutamine infusion, *n* (%)	17 (4.7)	1 (0.3)	6 (1.7)	2 (0.6)	5 (1.4)	3 (0.8)	0.246*
Dobutamine infusion rate, µg/kg/min	5 [5; 7.5]	5**	5 [5; 6.9]	6 [5.5; 6.5]	8 [5; 10]	5 [3.7; 5,5]	0.551
Echocardiographic parameters							
LVEF, %	54 [40; 64]	51.0 [40.0; 60.0]	29.5 [22.0; 40.5]	60.0 [52.5; 66.0]	57.0 [46.0; 64.0]	57.9 [50.0; 65.0]	< 0.001
LVFAC, %	46 [33; 58]	39.0 [29.0; 47.0]	26.0 [18.5; 33.4]	58.0 [46.9; 64.6]	53.0 [42.0; 63.0]	50.0 [43.0; 60.0]	< 0.001
Mitral *E* wave, cm/s	68 [54; 87]	90.0 [78.0; 105.0]	56.5 [44.0; 64.5]	77.0 [64.0; 89.5]	68.0 [56.0; 88.0]	51.0 [44.5; 67.0]	< 0.001
Mitral *E′* wave, cm/s	10 [7.5; 13.6]	14.9 [13.0; 18.0]	8.9 [6.0; 10.9]	10.2 [8.3; 12.9]	8.7 [7.0; 11.4]	9.0 [7.0; 12.0]	< 0.001
E/*E′* ratio	6.8 [5.3; 9.3]	6.0 [4.6; 7.4]	6.6 [5.3; 9.4]	7.3 [5.5; 9.3]	8.4 [5.2; 11.1]	6.3 [4.3; 8.8]	0.003
Aortic VTI, cm	15.4 [12.8, 19]	16.0 [14.0; 18.6]	10.9 [8.7; 14.2]	19.6 [17.6; 23.8]	17.0 [13.9; 19.8]	13.2 [11.1; 15.7]	< 0.001
RV/LV EDA	0.6 [0.5; 0.8]	0.6 [0.5; 0.6]	0.6 [0.5; 0.7]	0.6 [0.5; 0.7]	0.8 [0.6; 0.9]	0.6 [0.5; 0.7]	< 0.001
ΔSVC, %	13.2 [6; 29.2]	12.5 [5.3; 22.0]	10.0 [5.4; 17.3]	10.8 [5.3; 17.8]	9.5 [3.1; 20.0]	39.4 [30.8; 54.0]	< 0.001

*Exact Fisher's test

**Only one patient received dobutamine

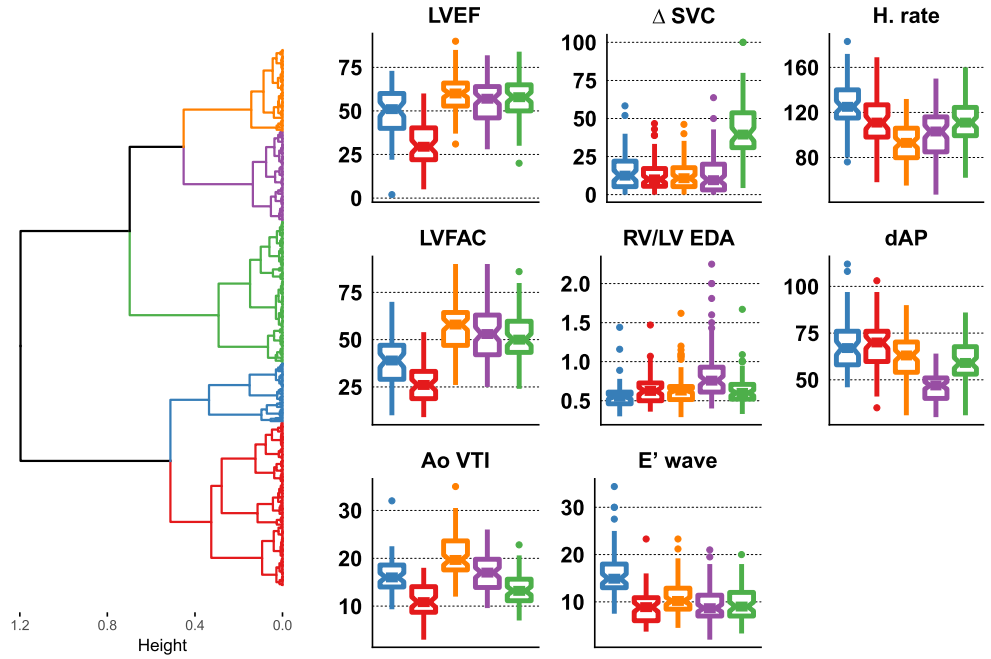


Fig. 1 Cardiovascular phenotypes. The left panel shows the dendrogram generated using the hierarchical clustering approach. The right panel shows the main hemodynamic parameters characterizing these cardiovascular phenotypes. LVEF left ventricular ejection fraction, LVFAC left ventricular fractional area change, Ao VTI aortic blood flow velocity time integral, ΔSVC superior vena cava collapsibility index, RV right ventricle, LV left ventricle, EDA end-diastolic area, H. rate heart rate, dAP diastolic arterial pressure

respectively, $p < 0.001$. ICU mortality was 21.3 [95% CI 13.0; 33.1], 50.0 [38.1; 61.9], 23.8 [16.0; 33.9], 42 [31.8; 52.8], and 38.6 [28.0; 50.3] % in clusters 1, 2, 3, 4, and 5, respectively ($p < 0.001$). Figure 2 and Table 3 report the distribution of parameters which characterize clusters as well as the respective importance of each of them in the partition process. Figure 3 reports the overall good performance of the three most important variables in each cluster, with a very high specificity but a quite low sensitivity.

Discussion

The clustering approach combining echocardiographic parameters (LVEF, LVFAC, aortic VTI, RV/LV EDA, ΔSVC, mitral *E* wave velocity, and *E′* wave velocity) and clinical parameters (heart rate, blood pressure, type and dose of catecholamine) allowed us to characterize five distinct cardiovascular phenotypes, the hemodynamic profiles of which correspond to "well-resuscitated" patients (16.9%, cluster 1), patients with LV systolic dysfunction (17.7%, cluster 2), hyperkinetic profile (23.3%, cluster 3), RV failure (22.5%, cluster 4), and sustained hypovolemia (19.4%, cluster 5).

This approach in clustering without any a priori criteria was able to distinguish different phenotypes between all the expected alterations of the macrocirculation. In the LV systolic dysfunction phenotype (cluster 2), patients had a higher serum lactate level, a lower CI, and a higher dose of norepinephrine. While differences in lactate and CI probably reflect the severity of underlying septic cardiomyopathy, the higher dose of norepinephrine could have participated in the development of LV failure. It has been reported that in animal models alteration in LV intrinsic contraction is constant in sepsis [22] and that the level of LV afterload especially alters LVEF in this abnormal heart [23]. Accordingly, the increased dose of norepinephrine administered in this subset of patients could have unmasked, or even participated in, the observed LV systolic dysfunction by increasing afterload. Jardin et al. and Parker et al. reported higher systemic vascular resistance in patients with LV systolic dysfunction [2, 24]. Boissier et al. [25] recently reported a negative correlation between echocardiographic parameters of LV systolic function and those of LV afterload. Patients with this cardiovascular phenotype also failed to exhibit increased *E*/*E′*, which is widely considered as a surrogate of LV filling pressure [26]. The absence of elevation in LV filling pressure has been reported as a specific characteristic of this hemodynamic profile, not only when evaluated by the *E*/*E′* but also when measured in the past using a pulmonary artery catheter [2, 24]. It was suggested to be related to an increase in LV compliance

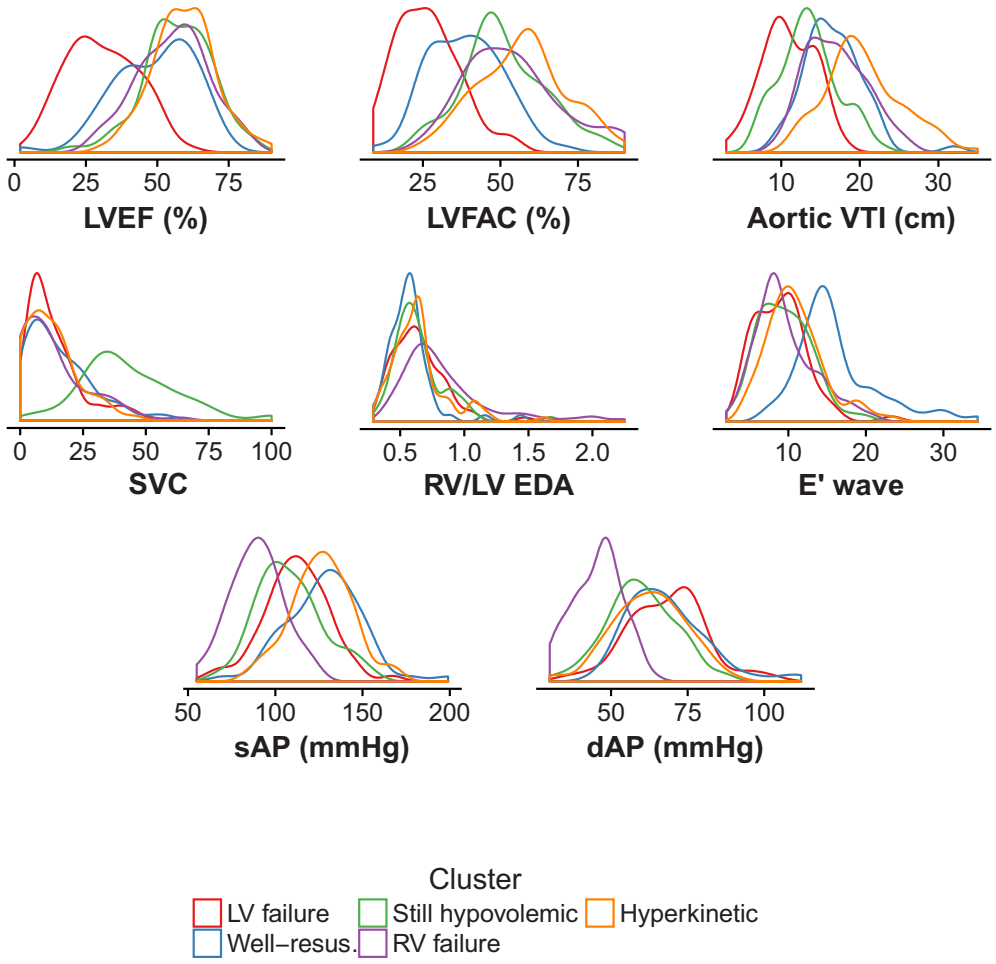


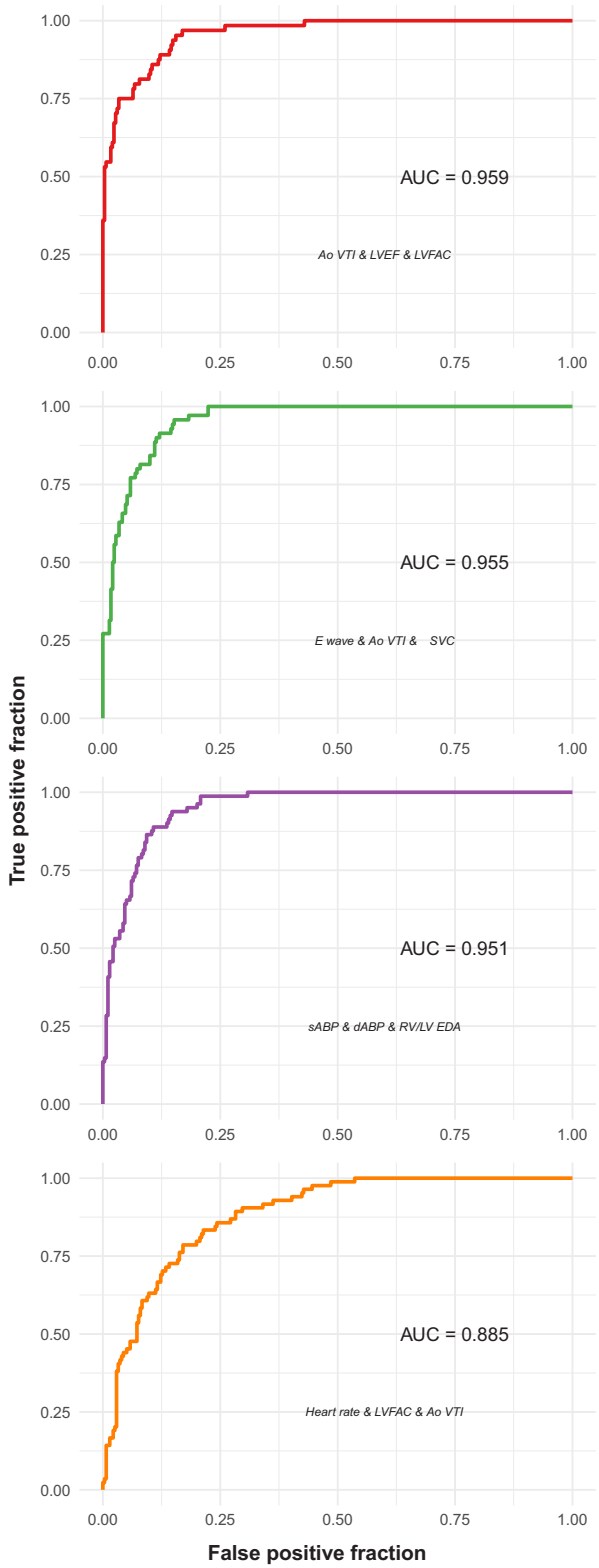
Fig. 2 Distribution of the factors contributing most in the clustering approach in the five cardiovascular phenotypes. LVEF left ventricular ejection fraction, LVFAC left ventricular fractional area change, Ao VTI aortic blood flow velocity time integral, ΔSVC superior vena cava collapsibility, RV right ventricle, LV left ventricle, EDA end-diastolic area, dAP diastolic arterial pressure, sAP systolic arterial pressure

Table 3 Variables that significantly contributed to the cluster

Variable	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
LVEF (%)	**–**	**31/51 (4×10^{-30})**	59/51 (1×10^{-7})	56/51 (2×10^{-3})	57/51 (2×10^{-4})
LVFAC (%)	39/47 (10×10^{-5})	**26/47 (9×10^{-25})**	**57/47 (4×10^{-10})**	54/47 (2×10^{-5})	51/47 (1×10^{-2})
Aortic VTI (cm)	**–**	**11/16 (4×10^{-18})**	**20/16 (5×10^{-20})**	–	**14/16 (8×10^{-6})**
Mitral *E* wave (cm/s)	**92/71 (2×10^{-14})**	58/71 (1×10^{-6})	78/71 (2×10^{-3})	–	**55/71 (3×10^{-10})**
E′ wave (cm/s)	**16/11 (5×10^{-22})**	9/11 (4×10^{-4})	–	10/11 (4×10^{-3})	10/11 (7×10^{-3})
RV/LV EDA	0.57/0.68 (8×10^{-4})	–	–	**0.85/0.68 (2×10^{-10})**	–
Δ SVC (%)	–	13/19 (2×10^{-3})	12/19 (8×10^{-5})	13/19 (2×10^{-3})	**43/19 (2×10^{-35})**
sBP (mmHg)	128/112 (4×10^{-9})	–	126/112 (3×10^{-10})	**89/112 (1×10^{-23})**	–
dBP (mmHg)	68/60 (9×10^{-7})	68/60 (5×10^{-7})	–	**45/60 (1×10^{-26})**	–
Heart rate (/min)	**127/108 (2×10^{-11})**	–	**93/108 (2×10^{-10})**	101/108 (9×10^{-3})	–
Norepinephrine infusion rate (mg/h)	–	3.8/2.6 (7×10^{-5})	1.6/2.6 (7×10^{-5})	3.1/2.6 (3×10^{-2})	–
Epinephrine infusion rate (mg/h)	–	0.3/0.1 (1×10^{-2})	–	0/0.1 (2×10^{-2})	–

Bold numbers show the three most important variables for each cluster. Values shown in each cell are mean in cluster/overall mean (*p* value)

LVEF left ventricular ejection fraction, *LVFAC* left ventricular fractional area change, *Ao VTI* aortic blood flow velocity time integral, Δ*SVC* superior vena cava collapsibility, *RV* right ventricle, *LV* left ventricle, *EDA* end-diastolic area, *dAP* diastolic arterial pressure, *sAP* systolic arterial pressure



LVEF <40% & Ao VTI <14cm & LVFAC <33%

LV failure	
n	35/64
Sensibility	54.7 [42.5;66.9]
Specificity	97.6 [95.9;99.4]
Pos. pred. value	83.3 [72.1;94.6]
Neg. pred. value	90.9 [87.7;94]

Ao VTI < 16 cm & E wave < 67 cm/s & SVC > 39 %

Hypovolemic	
n	18/70
Sensibility	25.7 [15.5;36]
Specificity	99.3 [98.4;100.3]
Pos. pred. value	90 [76.9;103.1]
Neg. pred. value	84.7 [80.9;88.5]

RV/LV EDA >0.8 & sABP <100mmHg & dABP <51mmHg

RV failure	
n	24/81
Sensibility	29.6 [19.7;39.6]
Specificity	98.9 [97.7;100.1]
Pos. pred. value	88.9 [77;100.7]
Neg. pred. value	82.9 [78.8;86.9]

Ao VTI >20cm & Heart rate <106bpm & LVFAC >58%

Hyperkinesia	
n	15/84
Sensibility	17.9 [9.7;26]
Specificity	98.2 [96.6;99.8]
Pos. pred. value	75 [56;94]
Neg. pred. value	79.7 [75.4;84]

Fig. 3 Performance of the combination of the three most important variables in the clustering approach for each cluster. The ROC curves on the left of the figure are drawn from a multivariable logistic regression with being in a cluster (binary variable yes/no) as the dependent variable and the three most important variables as independent variables. The areas under the curve and the three variables are listed on the plot. On the right of the figure are presented the diagnostic performance of the combination of the three variables for each cluster

due to sepsis [27]. Our clustering approach was also able to differentiate the "still hypovolemic" cardiovascular phenotype (cluster 5) from the "hyperkinetic" (cluster 3), both conditions being characterized by the presence of global LV hyperkinesia. Nevertheless, CI, aortic VTI (a surrogate of LV stroke volume), and CVP were lower in patients with persistent hypovolemia when compared to their counterparts, and ΔSVC was consistent with fluid responsiveness, as opposed to patients with a hyperkinetic hemodynamic profile. Patients who had a cardiovascular phenotype consistent with RV failure (cluster 4) were the only ones to exhibit a large increase in RV/LV EDA, which was recently considered to define RV failure [16] It is noteworthy that these patients had a higher proportion of PaO_2/FiO_2 level lower than 200 mmHg, suggesting that RV failure was potentially secondary to the development of ARDS-related septic shock. Finally, we failed to identify a cardiovascular phenotype corresponding to patients with isolated LV diastolic dysfunction, as previously reported [15]. In contrast, as indicated by the median E' maximal velocity, LV diastolic dysfunction was uniformly distributed in all phenotypes, with the exception of the well resuscitated patients (cluster 1).

Our approach may have potential interest for optimizing hemodynamic support using CCE after the very early resuscitation phase since personalized medicine has gained more and more importance [7]. A recent pilot study suggested that early vasopressor infusion could restrict fluid volume without detrimental effect on prognosis [9]. If our approach is able to better characterize patients who really need more fluids after the early phase, this could significantly change management and prognosis. While fluid overload is suggested to be detrimental [28], inappropriate use of vasopressors in still hypovolemic situations may lead to increase in tissue hypoperfusion and severe ischemia of vital organs [29]. The need for inotropic infusion in septic shock is still an issue of controversy. Indeed no study ever reported a relationship between LV systolic dysfunction and mortality and a beneficial effect of inotrope infusion. In a recent single-center randomized controlled trial, infusion of levosimendan did not modify prognosis but has deleterious cardiovascular effects [14]. However, the authors did not select their population at all on the basis of a pre-existing LV systolic dysfunction phenotype [14]. Our clustering approach, defining LV systolic dysfunction without a binary and simplistic threshold value of LVEF but by combining the usual echocardiographic and clinical hemodynamic variables as done in daily practice, could allow better characterization of these patients. How these patients could benefit from dobutamine infusion should be evaluated in the future. Finally, it is now well recognized that the right ventricle may fail in septic shock, especially when associated with ARDS, and that it may induce low flow state [30–32]. It was suggested that this profile may induce false positive pulse pressure variations [33, 34], a parameter frequently used to manage fluids. Accurately diagnosing RV failure could also help physicians to adequately optimize hemodynamic and respiratory support [30].

Our study suffers from limitations. First, even if one patient could only be statistically classified into one cardiovascular phenotype, interquartiles show a substantial between-cluster overlap for most studied parameters. This was expected since clusters were more defined by a specific association of parameters than by the presence of a single specific one. A soft clustering approach that addresses the overlapping issues might be of interest in future research [35]. Moreover, application of clustering in clinical practice is therefore not so obvious, even though we report in Fig. 3 and Table 3 the most valuable parameters that counted in the determination of each cluster and their value distribution. Last, the explained variance in the principal component analysis was quite low, reflecting the potential unmeasured factors we did not have to better describe these phenotypes. Second, we were unable to provide the exact timing of CCE following admission, which could alter our results, especially for the cluster 5 "still hypovolemic". However, we reported that these patients did not receive less fluid than the others before CCE performance, and even more, which may suggest a particular profile of capillary linkage. Third, we did not repeat the hemodynamic evaluation by CCE, as we only evaluated our cohort in the first 12 h after initial resuscitation of septic shock patients. It is of interest to assess in future studies whether the transition from one cardiovascular phenotype to another during the first 2–3 days could alter outcome, and whether it is related to spontaneous progression or induced by therapy. Fourth, variabilities of echo parameters were not calculated in the present cohort. However, the intraobserver and interobserver reproducibility of SVC collapsibility index were calculated as good to excellent in the Hemopred study [13]. The same authors, using the same route (TEE), in the same population (septic shock) previously reported interobserver variability of AoVTI, RV/LV EDA, LV volumes, and areas [10] which were below 10–11%. Fifth, we decided not to include in the analysis the 50 patients with a history of chronic heart failure. Indeed, we did not have any characterization of this failure; in particular, we did not know whether it was systolic, diastolic, valvular, injuring the right ventricle, and even the treatment. Finally, all CCE evaluations were performed by highly trained intensivists, which limits the external validity of our results, even though patients came from 12 different

ICUs and we reported in the past that the learning curve of TEE hemodynamic evaluation was steep and skills quickly achieved [36].

In conclusion, using a clustering approach in a large cohort of patients with septic shock evaluated early by CCE, we identified five distinct cardiovascular phenotypes which could help physicians to individualize the hemodynamic support. How this better characterization could change management and prognosis should be evaluated in the future.

Electronic supplementary material
The online version of this article (https://doi.org/10.1007/s00134-019-05596-z) contains supplementary material, which is available to authorized users.

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Author's contribution
GG, PV, AA and AVB designed the study and drafted the manuscript. PV, AA, ALF, CC, SS, XR and AVB collected the data. GG conducted the statistical analysis. AA, ALF, CC, SS and XR carefully revised the manuscript.

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Compliance with ethical standards

Conflicts of interest
GG, PV, AU, ALF, CC, SS, XR declared no conflict of interest. AVB has received grant from GSK for conducting clinical research and is a member of the scientific advisory board.

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Both cohorts (Hemosepsis and Hemopred) received Limoges ethics committee approval.

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ORIGINAL

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

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Abstract

Purpose: One potential way to protect patients from the physiological demands that are a consequence of fever is to aim to prevent fever and to treat it assiduously when it occurs. Our primary hypothesis was that more active fever management would increase survival among patient subgroups with limited physiological reserves such as older patients, patients with higher illness acuity, and those requiring organ support.

Methods: We conducted an individual-level patient data meta-analysis of randomised controlled trials to compare the outcomes of ICU patients who received more active fever management with the outcomes of patients who received less active fever management. The primary outcome variable of interest was the unadjusted time to death after randomisation.

Results: Of 1413 trial participants, 707 were assigned to more active fever management and 706 were assigned to less active fever management. There was no statistically significant heterogeneity in the effect of more active compared with less active fever management on survival in any of the pre-specified subgroups that were chosen to identify patients with limited physiological reserves. Overall, more active fever management did not result in a statistically significant difference in survival time compared with less active fever management [hazard ratio 0.91; (95% CI 0.75–1.10), $P = 0.32$].

Conclusions: Our findings do not support the hypothesis that more active fever management increases survival compared with less active fever management overall or in patients with limited physiological reserves.

Keywords: Sepsis, Fever, Septic shock, Paracetamol, Non-steroidal anti-inflammatory drugs, Physical cooling

Introduction

Fever occurs commonly in intensive care (ICU) patients and increases metabolic demand [1]. Increasing metabolic demand has important physiological consequences on oxygen consumption and cardiac output [1]. One potential way to protect patients from the physiological demands that are a consequence of fever is to aim to prevent fever and to treat it assiduously when it develops [2]. This strategy is an attractive candidate intervention to improve outcomes in the ICU setting because patients with a range of critical illnesses including major trauma, infection, acute myocardial infarction, and pancreatitis develop fever [3–5], and many such patients have limited physiological reserves.

Body temperature can be manipulated in ICU patients with medicines [6, 7] and physical cooling devices [8] allowing for more or less active approaches to fever

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management. In a recent systematic review and aggregate data meta-analysis evaluating the effect of fever management on all-cause mortality in ICU patients, we found that more active fever management neither increased nor decreased mortality in critically ill adults compared with less active fever management [9]. However, despite these findings, it is plausible that the balance of risks and benefits of active fever management in ICU patients varies based on the physiological reserves of the patients being treated and the nature of their illness [10]. In this context, we submit that physiological reserves are reasonably defined as the capacity of a patient to cope with the physiological demands associated with fever and depend on patients' physiology, illness severity, and the organ support they require.

Our primary hypothesis was that more active fever management would increase survival among patient subgroups with limited physiological reserves such as older patients, patients with higher illness acuity, and those with very high body temperature (≥ 39.5 °C). Because fever is part of the adaptive host response to infection [11], we further hypothesised that more active fever management would improve survival in the absence of infection but not in the presence of infection.

Take-home message

We conducted an individual level patient data meta-analysis of randomised controlled trials to compare the outcomes of ICU patients who received more active fever management with the outcomes of patients who received less active fever management. Our findings do not support the hypothesis that more active fever management increases survival compared with less active fever management overall, or in patients with limited physiological reserves.

Methods

Study design

To address our hypotheses, we conducted an individual-level patient data meta-analysis (IPDMA) using available data from randomised controlled trials identified in our recent systematic review and aggregate data meta-analysis [9]. We contacted lead investigators for all randomised controlled trials identified in the recent systematic review and requested access to individual patient-level data (see ESM for details). The search strategy used in our systematic review has been published previously [9]; however, in brief, we searched major databases for randomised controlled trials evaluating fever management in adult ICU patients excluding trials where the intervention involved therapeutic hypothermia. We included trials that evaluated any treatment administered commonly to febrile patients to reduce body temperature. The protocol for this IPDMA was posted online on 29 June 2018 at http://wellingtonicu.com/PubResPres/Protocols/ in advance of analyses being undertaken.

Data extraction and cleaning for analysis

Pre-randomisation (baseline) data points were extracted from individual study databases. These were age, gender, invasively ventilated (yes or no), receiving inotropes and/or vasopressors at baseline (yes or no), suspected infection at baseline (yes or no), Acute Physiology And Chronic Health Evaluation (APACHE) II score [12], mean arterial pressure (mmHg), heart rate (beats per min), serum creatinine (μmol/l), and body temperature (°C).

We sought to compare the outcomes of patients who received more active fever management with the outcomes of patients who received less active fever management. Accordingly, where studies compared an antipyretic drug with placebo, the patients allocated to the antipyretic drug were considered to have received more active fever management. Where studies compared different thresholds for temperature treatment, patients allocated to the group with the lowest body temperature target were considered to have received more active fever management.

Time to death after randomisation was defined as the difference between time zero (T0) and the date and time of death. T0 was generally defined as the date and time of randomisation. In one study, where the date and time of randomisation were not recorded, the date and time of administration of the first dose of study medication defined T0. Where no time, only a date, was available to define either T0 or the time of death, the time(s) were be assumed to be 12:00 p.m. The time for censored participants (those who did not die) was defined as the last time of observation in relation to the time of randomisation as described above. All patients who died on or before the date of ICU discharge were defined as dead for the purposes of evaluating the end point 'mortality at ICU discharge'. ICU and hospital length of stay were defined as the difference between T0 as described above and ICU and hospital discharge, respectively. Where no time, only a date, was available to define either T0 or the time of discharge, the time(s) were assumed to be 12:00 p.m. Body temperature at 6, 12, 24, 48, and 72 h after randomisation were included in the IPDMA database. One study reported temperature data at 4 h and 8 h after randomisation [7]. For this study, the 6-h temperature data point was calculated by averaging the values from the 4–8 h time points.

Outcomes

The primary outcome variable of interest was the time to death after randomisation. This outcome was chosen

Table 1 Baseline characteristics

Characteristic	More active fever control (*n* = 707)	Less active fever control (*n* = 706)
Age (years)	56.7 ± 17.2 (*n* = 705)	56.5 ± 17.2 (*n* = 705)
Male sex, *n/N* (%)	415/707 (58.7)	397/706 (56.2)
Intensive care support, *n/N* (%)		
Invasive mechanical ventilation	498/707 (70.4)	500/706 (70.8)
Receiving inotropes and/or vasopressors	358/707 (50.6)	377/706 (53.4)
APACHE-II score[a]	17.1 ± 7.1 (*n* = 605)	17.3 ± 7.4 (*n* = 605)
SAPS score[b]	76.9 ± 13.9 (*n* = 101)	78.3 ± 14.4 (*n* = 99)
Biochemistry and physiology		
Heart rate (beats per min)	106 ± 22 (*n* = 703)	107 ± 23 (*n* = 705)
Mean arterial pressure (mmHg)	78 ± 15 (*n* = 704)	77 ± 14 (*n* = 705)
Temperature (°C)	38.5 ± 1.0 (*n* = 703)	38.5 ± 0.9 (*n* = 706)
Serum creatinine (μmol/l)	134 ± 128 (*n* = 536)	129 ± 123 (*n* = 535)
Study, *n/N* (%)		
Bernard et al.	224/707 (31.7)	231/706 (32.7)
Niven et al.	14/707 (2.0)	12/706 (1.7)
Saxena et al.	21/707 (3.0)	20/706 (2.8)
Schortgen et al.	101/707 (14.3)	99/706 (14.0)
Young et al.	347/707 (49.1)	344/706 (48.7)
Suspected infection at baseline	672/707 (95.1)	673/706 (95.3)

Plus/minus values are mean ± SD

APACHE Acute Physiology and Chronic Health Evaluation, *ICU* intensive care unit, *SAPS* Simplified Acute Physiology Score

[a] Scores on the APACHE II range from 0 to 71, with higher scores indicating more severe disease and a higher risk of death. APACHE II scores were not collected for participants in the Schortgen et al. trial

[b] Scores on the SAPS III range from 0 to 263, with higher scores indicating more severe disease and a higher risk of death. SAPS III was only collected for participants in the Schortgen et al. trial

as the primary outcome because it allowed multiple trials with different durations of follow-up to be combined without loss of data. Secondary outcomes were mortality at ICU discharge, ICU and hospital length of stay, and body temperature at 6, 12, 24, 48, and 72 h following randomisation.

Statistical analyses

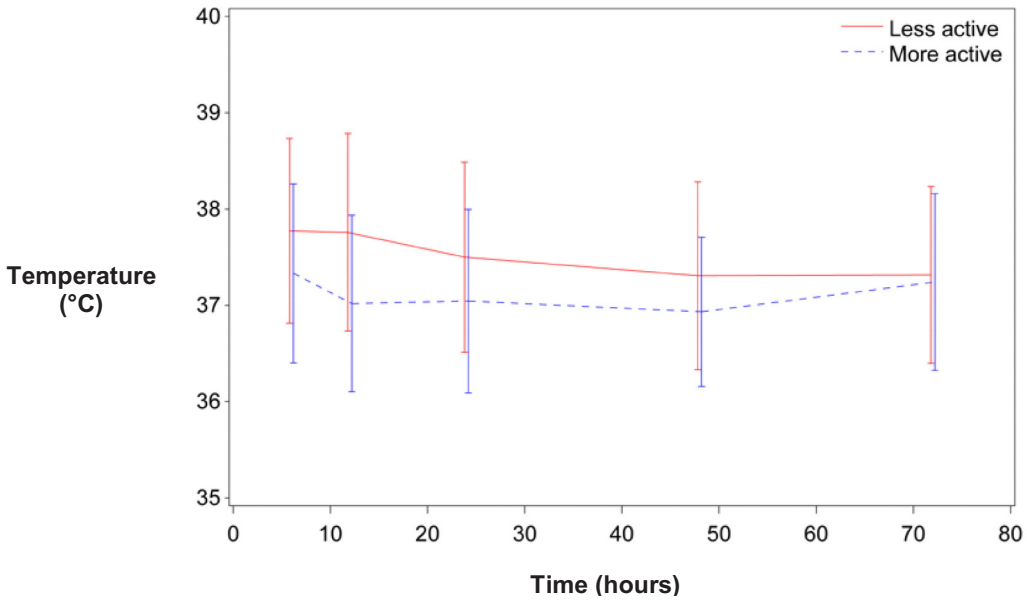
Data summaries by treatment group are frequency and proportions expressed as percentages for categorical data and mean ± standard deviation for continuous data.

Survival times use log-rank tests and are shown as Kaplan-Meier curves and relative survival estimated with a Cox proportional-hazards model. The primary analysis model was adjusted for study as fixed effect; however, a sensitivity analysis adjusted for baseline covariates of age, sex, and APACHE-II score was also performed incorporating individual study as a fixed effect. Because the APACHE-II score was not available for one study [8] two post hoc sensitivity analyses were used: one in which the APACHE-II score was not included in the adjusted model and another in which illness severity scores based on Simplified Acute Physiology Score (SAPS3) [13] were used in place of APACHE-II scores for the study that did not include APACHE-II data (see ESM for details).

ICU mortality was compared by treatment group using logistic regression with analyses performed in a similar fashion to those performed for survival. Mortality data by treatment group are reported as frequencies with proportions expressed as percentages with treatment effects reported as odds ratios.

ICU and hospital length of stay were highly skewed and were analysed on the logarithm transformed scale. These variables are summarised as geometric mean with treatment effects expressed as a ratio of geometric means. A post hoc analysis evaluating whether ICU length of stay differed in relation to randomised treatment for patients who did or did not die in the ICU used an ANCOVA-based interaction model.

Interaction analyses were also used to explore whether survival, ICU mortality, ICU length of stay, or hospital length of stay varied in pre-specified subgroups based on pre-randomisation characteristics. The subgroups of interest were: invasively ventilated or not; receiving inotropes and/or vasopressors or not; both invasively ventilated and receiving inotropes and/or vasopressors



Time point	Mean difference, °C (95% CI)	P value*
6 hours	-0.49 (-0.60 to -0.38)	<0.001
12 hours	-0.73 (-0.83 to -0.63)	<0.001
24 hours	-0.33 (-0.46 to -0.2)	<0.001
48 hours	0.33 (-0.46 to -0.2)	<0.001
72 hours	-0.04 (-0.15 to 0.08)	0.52

Fig. 1 Body temperature over time with more active fever management vs. less active fever management. **P* value for the interaction term evaluating temperature difference by time from randomisation was < 0.001 indicating a statistically significant variation in the temperature difference by treatment over time

or not; infection present or not; high fever (≥ 39.5 °C or < 39.5 °C); age (≥ 75 years or < 75 years); APACHE-II score ≥ 25 or < 25; and physical cooling included in the study intervention or physical cooling not included in the study intervention.

Temperature was analysed using a mixed linear model with a power exponential structure for the correlation between repeated measurements and the time by randomisation interaction term used to estimate temperature differences between randomised treatments at each time point.

A two-sided *P* value < 0.05 was considered to indicate statistical significance and estimates are shown with 95% confidence intervals (CI). No adjustment was made for multiple comparisons.

SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for analyses.

Results

Data sources

Individual-level patient data were obtained for 5 [6–8, 14, 15] of 13 randomised controlled trials identified in the systematic review conducted for our aggregate data meta-analysis [9]. This included the three largest trials [6–8] and resulted in data from 1413 of 1780 (79.4%) of the participants in the original trials being included in this analysis. Details of included trials and those trials from which data could not be obtained are shown in the ESM.

Patient characteristics

Of the 1413 participants included in this analysis, 707 were assigned to more active fever management and 706 were assigned to less active fever management. The study groups had similar characteristics at baseline (Table 1). More than 95% of participants were suspected to have an infection at baseline.

Effects of fever management on body temperature

Patients assigned to more active fever management had statistically significantly lower body temperature than patients assigned to less active fever management (Fig. 1). The effect of study treatment on body temperature varied with time with a maximum temperature difference

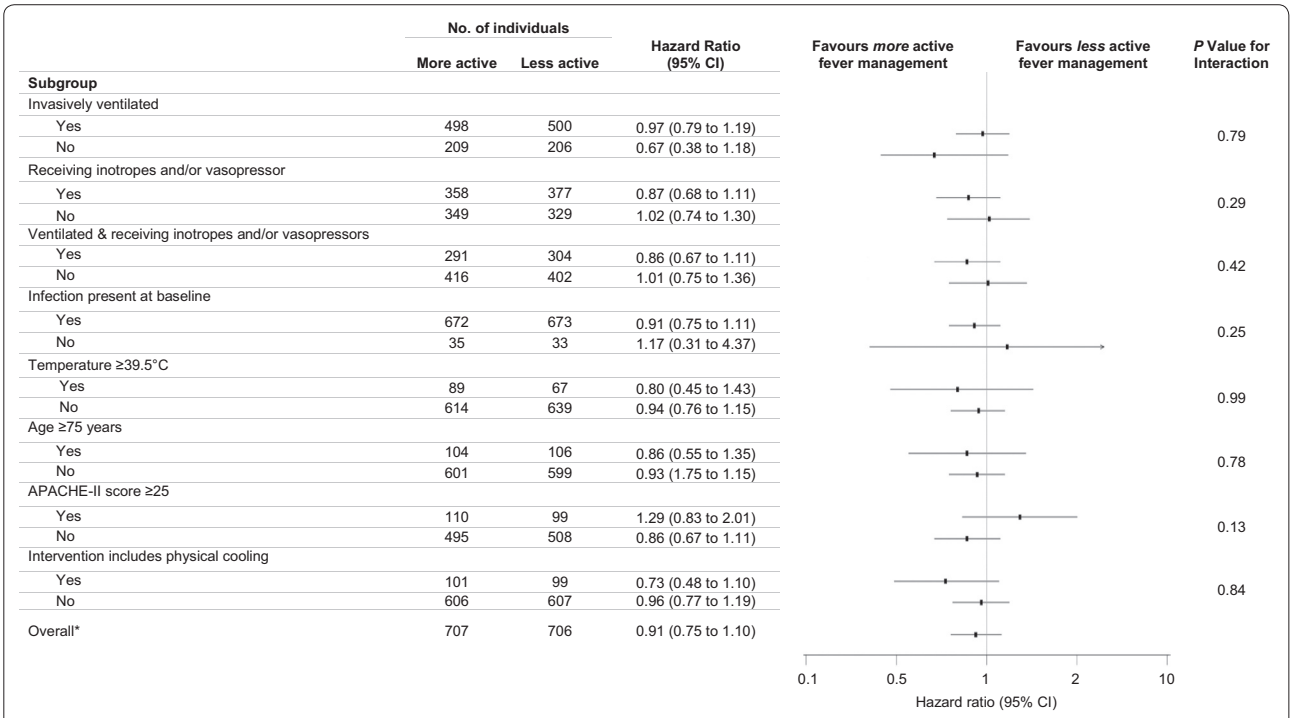


Fig. 2 Survival with more active fever management vs. less active fever management by subgroup. *Includes adjustment for study as a fixed effect

Table 2 Outcomes

	More active fever control (*n* = 707)	Less active fever control (*n* = 706)	Treatment effect estimate[a] (95% CI)	*P* value
Outcomes				
			Hazard ratio	
Survival (days), median (95% CI)	202 (146–N/A)	212 (143–390)	0.91 (0.75–1.10)	0.32
			Adjusted hazard ratio[b]	
			0.96 (0.77–1.19)	0.69
			Odds ratio	
ICU mortality—*n*/*N* (%)	98/707 (13.9)	121/706 (17.1)	0.78 (0.58–1.04)	0.09
			Adjusted odds ratio[a]	
			0.76 (0.52–1.09)	0.13
			Ratio of geometric means (95% CI)	
ICU length of stay (days), geometric mean (95% CI)	5.7 (5.2–6.2)	5.6 (5.2–6.2)	1.01 (0.89–1.14)	0.92
			Ratio of geometric means (95% CI)	
Hospital length of stay (days), geometric mean (95% CI)	14.6 (13.5–15.9)	13.5 (12.3–14.8)	1.08 (0.96–1.23)	0.19

IQR interquartile range, *CI* confidence interval

[a] The hazard ratio from the primary survival analysis includes an adjustment for study as a fixed effect. The widths of the confidence intervals for secondary analyses have not been adjusted for multiplicity and the intervals should not be used to infer definitive differences between the groups

[b] Adjusted for age, sex, study, and APACHE-II score; APACHE-II data were not available for the Schortgen et al. trial. Additional adjusted analyses are presented in the ESM

of 0.73 °C (95% CI 0.63–0.83 °C) between temperature groups evident at 12 h post randomisation.

Survival and mortality

In the analysis addressing the primary hypothesis that more active fever management would increase survival among patient subgroups with limited physiological

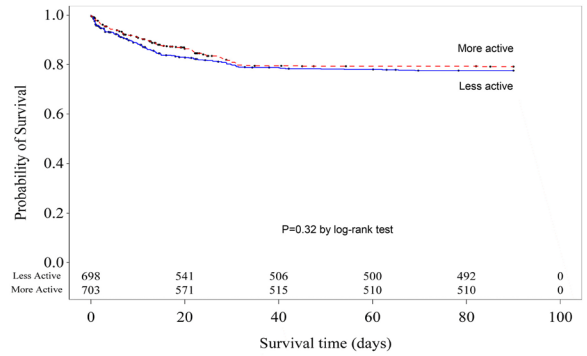


Fig. 3 Kaplan-Meier survival estimates of the probability of survival. Because the number of observations beyond day 90 is small, this figure is truncated at day 90 with data censored at day 90 if death had not occurred by then. An expanded Kaplan-Meier survival plot including all available data points is provided with the electronic supplementary material

reserves such as older patients, patients with higher illness acuity, and those requiring organ support, we found no statistically significant heterogeneity of treatment effect in any of the pre-specified subgroups (Fig. 2). Similarly, there was no heterogeneity of survival response by treatment allocation in patients with and without infections (Fig. 2). Overall, more active fever management did not result in a statistically significant difference in survival time compared with less active fever management [hazard ratio; 0.91; (95% CI 0.75–1.10), $P=0.32$] (Table 2, Fig. 3, Fig. S1 ESM). Findings were similar in analyses adjusting for pre-specified baseline covariates (Table 2 and Fig. 3) and in sensitivity analyses (ESM). Moreover, the estimates of the hazard ratios related to survival from the sensitivity analyses were more-or-less identical treating the studies as fixed effects (as was pre-specified) or as random effects.

There was no statistically significant heterogeneity in the effect of more active compared with less active fever management on ICU mortality in any of the pre-specified subgroups (Fig. 4). A total of 98 of 707 patients (13.9%) assigned to more active fever management and 121 of 706 patients (17.1%) assigned to less active fever management died in the ICU [absolute mortality difference, −3.3% points (95% CI −7.1 to 0.5% points); odds ratio, 0.78 (95% CI 0.58–1.04), $P=0.09$] (Table 2). Findings were similar in analyses adjusting for pre-specified baseline covariates (Table 2) and in sensitivity analyses (ESM).

Length of stay

Overall, ICU length of stay and hospital length of stay were similar between treatment groups (Table 2).

	No. of individuals		No. of Events (%)		Odds Ratio (95% CI)	Favours *more* active fever management / Favours *less* active fever management	*P* Value for Interaction
	More active	Less active	More active	Less active			
Subgroup							
Invasively ventilated							
Yes	498	500	91	111	0.78 (0.58 to 1.04)		0.79
No	209	206	7	10	0.68 (0.25 to 1.82		
Receiving inotropes and/or vasopressors							
Yes	358	377	68	93	0.72 (0.50 to 1.02)		0.29
No	349	329	30	28	1.01 (0.59 to 1.73)		
Ventilated & receiving inotropes &/or vasopressors							
Yes	291	304	66	88	0.72 (0.50 to 1.04)		0.42
No	416	402	32	33	0.83 (0.56 to 1.55)		
Infection present at baseline							
Yes	672	673	95	120	0.76 (0.57 to 1.02)		0.25
No	35	33	3	1	3.0 (0.30 to 30.4)		
Temperature ≥39.5°C							
Yes	89	67	16	15	0.76 (0.35 to 1.67)		0.99
No	614	639	81	106	0.76 (0.56 to 1.05)		
Age ≥75 years							
Yes	104	106	19	22	0.85 (0.43 to 1.69)		0.78
No	614	599	79	99	0.76 (0.56 to 1.05)		
APACHE-II score ≥25							
Yes	110	99	24	19	1.18 (0.60 to 2.31)		0.13
No	495	508	38	59	0.63 (0.41 to 0.97)		
Intervention includes physical cooling							
Yes	101	99	36	43	0.72 (0.41 to 1.27)		0.84
No	606	607	62	79	0.77 (0.54 to 1.10)		
Overall	707	706	98 (13.9)	121 (17.1)	0.78 (0.58 to 1.04)		0.09

Fig. 4 ICU mortality for more active fever management vs. less active fever management by subgroup

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	1.09 (0.95 to 1.26)	0.05
No	209	206	0.84 (0.68 to 1.04)	
Receiving inotropes and/or vasopressor				
Yes	358	377	1.12 (0.94 to 1.32)	0.10
No	349	329	0.90 (0.76 to 1.05)	
Ventilated & receiving inotropes and/or vasopressors				
Yes	291	304	1.21 (1.00 to 1.45)	0.019
No	416	402	0.90 (0.76 to 1.05)	
Infection present at baseline				
Yes	672	673	1.00 (0.88 to 1.14)	0.63
No	35	33	1.15 (0.65 to 2.01)	
Temperature ≥39.5°C				
Yes	89	67	1.26 (0.86 to 1.84)	0.22
No	614	639	0.98 (0.86 to 1.12)	
Age ≥75 years				
Yes	104	106	1.07 (0.77 to 1.48)	0.69
No	614	599	1.00 (0.87 to 1.14)	
APACHE-II score ≥25				
Yes	110	99	1.02 (0.74 to 1.40)	0.67
No	495	508	0.95 (0.82 to 1.09)	
Intervention includes physical cooling				
Yes	101	99	1.32 (0.96 to 1.82)	0.07
No	606	607	0.96 (0.84 to 1.09)	
Overall	707	706	1.01 (0.89 to 1.14)*	

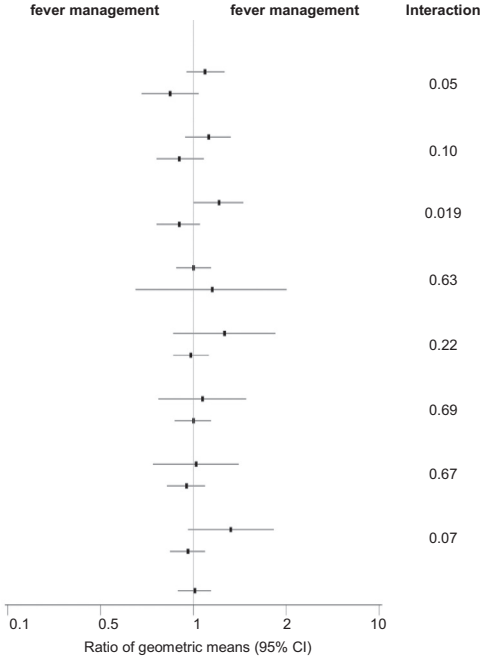


Fig. 5 ICU length of stay with more active fever management vs. less active fever management by subgroup. *Unadjusted *P* value for overall comparison, 0.92; *P* value adjusted for age, sex, study, and APACHE-II score (excluding Schortgen et al. trial), 0.52; *P* value adjusted for age, sex, study, and 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), 0.29; *P* value adjusted for age, sex, and study, 0.94

However, there was statistically significant heterogeneity of response to treatment in relation to length of stay variables in some subgroups (Fig. 5 and Fig. S2, ESM). In each case where significant heterogeneity in response to treatment was observed, the length of stay was statistically significantly shorter in a subgroup where the point estimate for ICU mortality risk favoured more active fever management. In a post hoc interaction analysis of ICU and hospital length of stay by treatment allocation, there was statistically significant heterogeneity in response in survivors compared with non-survivors (ESM). Compared with less active fever management, more active fever management was associated with longer ICU and hospital length of stay in patients who died in ICU and with shorter ICU and hospital length of stay in patients who survived ICU.

Discussion

In this individual-level patient data meta-analysis of randomised controlled trials, more active fever management did not increase survival compared with less active fever management in critically ill adults either overall or in those with limited physiological reserves. Survival by treatment group was similar in a range of subgroup pairs that divided the study population into groups based on age, illness severity, on receipt of specific organ supports, and in the presence or absence of high fever at baseline.

Overall, effect size estimates in relation to ICU mortality based on the 95% CI were consistent with an absolute effect on ICU mortality with active fever management ranging from a decrease of 7.1% points to an increase of 0.5% points. Although we observed statistically significant heterogeneity of treatment effect in relation to ICU and hospital length of stay effects, the interpretation of these findings is complicated because length of stay can be reduced by more rapid recovery or by early death. Moreover, a reduction in mortality, even a non-statistically significant one, can be associated with a statistically significant increase in length of stay when survivors have longer average lengths of stay than non-survivors. In patients who were receiving invasive mechanical ventilation, those receiving inotropes and/or vasopressors, or those receiving both of these, a relative increase in hospital length of stay was associated with lower ICU mortality based on point estimates. We also found that, compared with less active temperature management, more active temperature was associated with reduced ICU and hospital length of stay in patients who survived ICU and with increased length of stay in patients who died in ICU.

Our study is consistent with two recent aggregate data metaanalyses [9, 16] evaluating fever control in adult ICU patients; however, it extends their findings because the use of individual-level patient data allowed us to conduct analyses adjusting for important baseline covariates and to accurately evaluate subgroups of interest defined based on pre-randomisation characteristics.

Our study has a number of limitations. Because our analyses were not adjusted for multiple comparisons, they should be considered exploratory and should not be used to infer definitive treatment effects. Although we did not demonstrate statistically significant heterogeneity of treatment effect on survival for subgroups of interest, confidence intervals around hazard ratios were generally wide and the possibility of clinically important differences in survival responses by subgroup cannot be excluded. In particular, as nearly all patients were suspected of having an infection at baseline, our findings effectively neither confirm nor refute the hypothesis that the presence of infection is an important factor in determining the efficacy of active fever management [17]. We were only able to obtain data from 5 out of 13 trials identified in our recent systematic review. However, the three largest trials [6–8] conducted were included in our analysis and 79.4% of all potential data from prior randomised controlled trials were analysed. Most of the studies where data were not available were small single-centre studies. The studies included in our analysis used a variety of different therapies and it is not known whether these therapies have equivalent effects on patient outcomes. Nevertheless, as all therapies evaluated are given to patients to treat fever, we submit that combining trials in an IPDMA has both face validity and clinical relevance.

In conclusion, our findings do not support the hypothesis that more active fever management increases survival compared with less active fever management in patients with limited physiological reserves. However, as point estimates for the effect of active fever management on ICU mortality encompass potentially clinically important effects, further clinical trials are justified. The significant heterogeneity in treatment effects on length of stay in subgroups based on the receipt of organ support, combined with the finding that more active fever management increases ICU and hospital length in patients who die in ICU, and reduces length of stay in patient who survive ICU, suggests that further research in patients receiving organ support may be of interest.

Electronic supplementary material
The online version of this article (https://doi.org/10.1007/s00134-019-05553-w) contains supplementary material, which is available to authorized users.

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Compliance with ethical standards

Conflicts of interest
Dr. Paul Young and Dr. Manoj Saxena report receiving speaker's fees from Bard Medical Pty. Dr. Gordon Bernard reports having an equity stake and is on the Board of Directors for Cumberland Pharmaceuticals, Nashville, TN, makers of intravenous ibuprofen (Caldolor).

Ethical approval
An approval by an ethics committee was not applicable.

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ORIGINAL

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

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Abstract

Purpose: Reliable biomarkers for predicting subsequent sepsis among patients with suspected acute infection are lacking. In patients presenting to emergency departments (EDs) with suspected acute infection, we aimed to evaluate the reliability and discriminant ability of 47 leukocyte biomarkers as predictors of sepsis (Sequential Organ Failure Assessment score ≥ 2 at 24 h and/or 72 h following ED presentation).

Methods: In a multi-centre cohort study in four EDs and intensive care units (ICUs), we standardised flow-cytometric leukocyte biomarker measurement and compared patients with suspected acute infection (cohort-1) with two comparator cohorts: ICU patients with established sepsis (cohort-2), and ED patients without infection or systemic inflammation but requiring hospitalization (cohort-3).

Results: Between January 2014 and February 2016, we recruited 272, 59 and 75 patients to cohorts 1, 2, and 3, respectively. Of 47 leukocyte biomarkers, 14 were non-reliable, and 17 did not discriminate between the three cohorts. Discriminant analyses for predicting sepsis within cohort-1 were undertaken for eight neutrophil (cluster of differentiation antigens (CD) CD15; CD24; CD35; CD64; CD312; CD11b; CD274; CD279), seven monocyte (CD35; CD64; CD312; CD11b; HLA-DR; CD274; CD279) and a CD8 T-lymphocyte biomarker (CD279). Individually, only higher neutrophil CD279 [OR 1.78 (95% CI 1.23–2.57); $P = 0.002$], higher monocyte CD279 [1.32 (1.03–1.70); $P = 0.03$], and lower monocyte HLA-DR [0.73 (0.55–0.97); $P = 0.03$] expression were associated with subsequent sepsis. With logistic regression the optimum biomarker combination was increased neutrophil CD24 and neutrophil CD279, and reduced monocyte HLA-DR expression, but no combination had clinically relevant predictive validity.

Conclusions: From a large panel of leukocyte biomarkers, immunosuppression biomarkers were associated with subsequent sepsis in ED patients with suspected acute infection.

Clinical trial registration: NCT02188992.

Keywords: Sepsis, Infection, Mortality, Cohort study, Biomarker, risk prediction

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Introduction

Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. Host immune responses result from leukocytes sensing pathogen- and tissue damage-associated danger signals [2, 3]. Sepsis-related immune responses involve both humoral and leukocyte components of the innate and adaptive immune systems, with excessive inflammation and immunosuppression occurring simultaneously in most patients [2, 3]. These are thought to influence the resulting clinical phenotypes and outcomes [3, 4].

Leukocyte responses in sepsis measured using flow cytometry detect leukocyte biomarkers, including surface markers and/or leukocyte subsets [5]. Previous flow cytometry-based leukocyte biomarker studies in sepsis were mostly small, single-centre studies in patients with sepsis, typically focusing on a limited panel of biomarkers. These studies rarely evaluated biomarker reliability and reproducibility, which is methodologically and clinically relevant as it influences diagnostic validity [6]. In addition, few studies used robust unbiased designs to assess predictive ability for clinically relevant outcomes in unselected populations with suspected infections *prior* to developing organ dysfunction and established sepsis.

We hypothesized that among patients with clinically suspected acute infection, but without established sepsis, leukocyte biomarkers would identify patients who subsequently deteriorate clinically and develop sepsis, when measured within a few hours of presentation to the emergency department (ED). Our study objectives were: (1) to identify reliable leukocyte biomarkers; (2) to ascertain which of the reliable biomarkers could discriminate [6] acutely unwell patients with suspected infection from patients with community acquired sepsis-related critical illness in the intensive care unit (ICU) and/or ED patients with non-infective acute illness requiring hospitalisation; and (3) to ascertain whether any of the reliable biomarkers with cross-cohort discrimination could predict which patients with suspected infection in the ED subsequently develop sepsis. We also undertook a post hoc extreme phenotype analysis [7], to compare the biomarker profiles between acutely unwell patients with suspected infection who subsequently developed most severe illness with those who recovered rapidly.

Take-home message

In this first study of standardised multi-site flow cytometry in acutely unwell patients with suspected infections attending emergency departments, we explored which of 47 leukocyte biomarkers reliably discriminates which patients develop sepsis over the next 3 days, defined according to the Sepsis-3 sepsis criteria.

After highlighting the importance of test reliability (14 biomarkers lacked measurement reliability) and comparator cohorts (a further 17 biomarkers did not discriminate acutely unwell patients with suspected infection from patients with established sepsis-related critical illness and/or non-infective acute illness), we found that none of the remaining 16 biomarkers had clinically relevant predictive ability for subsequent sepsis or other important clinical outcomes. However, markers of early immune suppression (neutrophil and monocyte CD274 and CD279; monocyte HLA-DR) had the strongest associations with clinical outcomes. The optimum biomarker combination associated with clinical deterioration to sepsis was increased neutrophil CD24 and CD279 and reduced monocyte HLA-DR expression.

Methods

Study sites and ethics

We performed a prospective, multi-centre, observational cohort study at four sites in the United Kingdom. Ethical approval was granted by the Scotland A/Oxford C Research Ethics Committees (13/SS/0023;13/SC/0266). Consent was provided by patients or surrogate decision-makers according to capacity. We registered the study (NCT02188992) and published the protocol including the analysis plan [8].

Cohort definitions and eligibility criteria

We recruited three distinct patient cohorts using an a priori sampling method to achieve similar age and sex profiles across the ED cohorts. Detailed inclusion/exclusion criteria are listed in the electronic supplement and published protocol (emethods-1) [8]. Cohort-1 comprised acutely unwell patients with suspected infection and systemic inflammation presenting to ED and formed the "discovery cohort". Patients considered by clinical teams to already have established severe sepsis and/or require ICU admission when screened were excluded. Cohort-2 comprised ICU patients with established community acquired sepsis-related critical illness and formed the "true positive" cohort. Cohort-3 comprised acutely ill patients presenting to ED without infection or systemic inflammation, but requiring hospitalization and formed the "true negative" cohort. Inclusion criteria used throughout the study were based on the sepsis definitions by Levy et al. [9], as our study was designed prior to the Sepsis-3 definitions [1, 10]. All ED patients were enrolled within 12 h of hospital presentation. For all cohorts, we excluded patients with acute pancreatitis, haematological malignancy, chemotherapy in the past 2 weeks, myelodysplastic syndromes, known neutropenia, HIV infection, viral hepatitis infection, pregnancy, blood transfusion > 4 units in the past week, oral corticosteroids for > 24 h prior to enrolment, or a decision not to have active therapy/for palliative care [8].

Leukocyte surface biomarkers and cross-site standardization of flow cytometry

We devised five separate flow cytometry panels to assess 47 leukocyte biomarkers with biological plausibility for having predictive validity for subsequent sepsis (eMethods-1; eTable-1; eFigure-1). We developed, standardized and harmonized flow cytometry procedures across all four study sites [8]. We performed flow cytometry within 4 h of sample acquisition. All anti-human antibodies conjugated to fluorochromes for flow cytometry were from the same batch and clones [all Becton–Dickinson Biosciences (BDB)], standardized on the same platform (FACSCanto II; BDB, San Jose, CA, USA), using a common batch of Cytometer Setup and Tracking beads with the same beads for daily internal quality controls, at all clinical sites. All flow cytometry standard (FCS) files were read by expert technicians using standardized gating procedures developed for each biomarker prior to analysis. The gating strategy for estimating median fluorescence intensity (MFI) or proportions is reported in eMethods-1. All FCS analysis technicians were blinded from clinical data.

Sample size

We based sample size estimates on the confidence interval (CI) widths for positive and negative predictive values (PPV and NPV). The initial design had a primary outcome of septic shock, with an estimated event rate of 5–10% in cohort-1 [11, 12]. For a range in test performance for PPV/NPV of 50–90% we planned a sample size of: cohort-1, $n = 300$; cohort-2, $n = 100$; and cohort-3, $n = 100$, to give a CI width between $\pm 4.6\%$ to $\pm 9.8\%$ for PPV and $\pm 3.4\%$ to $\pm 6.3\%$ for NPV. At an interim analysis of clinical event rates, the incidence of septic shock was substantially lower than anticipated. We decided by consensus to change the primary outcome to severe sepsis (and subsequently adopted the sepsis-3 sepsis criteria [1] of Sequential Organ Failure Assessment (SOFA) score ≥ 2), with critical care admission a key secondary outcome, to ensure adequate clinically relevant events in the discriminant analyses. These changes occurred *prior to* study completion and were reported in the published protocol [8].

Statistical analysis

The primary study cohort was cohort-1. The primary exposure was suspected infection. The cohorts-2 and 3 were comparator populations for cross-cohort discrimination and biomarker selection.

Outcomes

The primary outcome was sepsis, defined as SOFA score ≥ 2 at 24 h and/or 72 h following presentation to hospital in patients with suspected infection in the ED (cohort-1) [1]. Secondary outcomes were: critical care admission or death within 72 h of presentation; SOFA ≥ 4 at 24 h and/or 72 h following presentation to hospital; development of septic shock; discharge home within 72 h; discharge to home or in hospital with no organ failure within 72 h; death from sepsis; confirmed infection and length of hospital stay [8]. All cohort-1 data are based on blood samples taken in the ED after recruitment.

Biomarkers selection strategy

Our analytic approach to discover biomarkers with potential diagnostic discrimination for risk of subsequent sepsis occurred in three a priori planned stages and one post hoc analysis.

Stage one: reliability

Inter- and intra-reader reliability for 47 different biomarkers was established according to the protocol [8]. To be included in subsequent evaluation stages, biomarkers needed to demonstrate both inter- and intra-reader reliability at the pre-defined intra-class correlation coefficient (ICC) between readers ≥ 0.9; see Fig. 1 and eMethods-2; eTable-2). For intra-reader reliability the ICC for each reader was calculated as the ratio of within-reader variability to the total variance (within-reader plus residual variance) from the normal linear mixed model. For inter-reader reliability the ICC was calculated as the ratio of between-reader variability to the total variance (between-reader plus residual variance) from the normal linear mixed model. Reliability analyses were done prior to linking leukocyte biomarkers data and clinical outcome data.

Stage two: cross-cohort discrimination

For reliable biomarkers, statistically significant inter-group differences between the three cohorts were explored using Kruskal–Wallis analysis of variance (ANOVA) tests (eTable-3) and visual inspection of data. Biomarkers that discriminated between cohort-1 and either cohort-2 (true-positive) and/or cohort-3 (true negative) and had variability within cohort-1 consistent with potential to discriminate clinical outcomes were selected for Stage-3 analysis. Other factors considered were cell counts, the magnitude of MFI, and potential linkage and co-linearity between groups of biomarkers. This was done in consensus meetings by researchers blinded from clinical outcomes within cohort-1.

Stage three: prediction of clinical outcomes in cohort-1

Within cohort-1 patients, the ability of the selected biomarkers to predict the primary and secondary outcomes

was calculated using univariate logistic regression. For the secondary outcomes of death from sepsis, septic shock and length of stay, we provided a descriptive summary as per the analysis plan [8]. The odds ratio (OR) for the outcome per standard deviation increase in biomarker, receiver operating characteristic (ROC) curves, and area under ROC curve (AUROC) were used to assess predictive ability. Youden's index identified the optimal cut-off point for each marker [13]. Candidate biomarkers that showed consistent inclusion were then taken forward for multivariable modelling.

We used best subsets regression [14] to identify optimal combinations of predictive markers. Specifically, models containing a given number of biomarkers were fitted for all potential biomarker combinations. The five best-fitting models of a given size, according to the Chi squared score statistic, were identified. Biomarkers that consistently appeared in the best-fitting models were selected for the final model. The change in Chi squared score statistic between the best fitting models containing different numbers of biomarkers was used to determine the number of biomarkers to be included in the final model. Linearity of biomarker associations on the logistic scale was checked using plots of deviance residuals. Based on consistency and model fit we identified optimal combinations of predictive markers.

Post hoc extreme phenotype comparison

On the recommendation of a pre-planned independent expert group (see eTable-4), we compared biomarker profiles between sub-populations within cohort-1 with extreme clinical phenotypes of organ dysfunction and outcome to further explore associations for the biomarkers evaluated. We defined *well* and *sick* extreme phenotypes [7] by consensus among clinical investigators using clinical data without knowledge of group differences in biomarkers (eFigure-2). The *well* phenotype had no positive microbiology, a SOFA score ≤ 2 at 24 *and* 72 h post-enrolment and were either discharged home by 72 h or were in hospital but no longer receiving antibiotics. The *sick* phenotype had a confirmed infection, SOFA score ≥ 2 at both 24 *and* 72 h post-enrolment and were still in hospital and receiving antibiotics at 72 h. We compared biomarker expression between the two phenotypes using two-sample t-tests or Mann–Whitney tests as appropriate, applying Bonferroni correction for multiple testing.

For additional comparison, we also measured C-reactive protein (CRP) and procalcitonin (PCT) concentrations at the same time point for cohort-1 patients, given the widespread clinical use of these biomarkers in assessing infection. We constructed ROC curves for CRP and PCT and estimated similar univariate predictive performance characteristics of these for outcomes reported, to enable direct comparison of predictive validity with the more novel biomarkers.

Results

Patient characteristics

Between January 2014 and February 2016, we recruited 272, 59 and 75 patients ($N=406$) to cohorts 1, 2, and 3, respectively. The clinical characteristics for the three cohorts and the cohort-1 outcomes are shown in Table 1. Cohorts-1 and 3 had a similar age and sex distribution. Cohort-2 patients tended to be older. The primary outcome in cohort-1, clinical deterioration to sepsis, occurred in 139 patients (51.1%).

Stage one: reliability

The step-wise assessment of intra-reader and then inter-reader reliability resulted in rejection of 14 biomarkers as non-reliable, leaving 33 reliable biomarkers for cross-cohort comparison (Fig. 1; eTable-2).

Stage two: cross-cohort discrimination

Statistical comparison, expert review, and cohort-1 data distribution resulted in rejection of a further 17 biomarkers (Fig. 1; eTable-2; eTable-3). The cross-cohort comparisons plots for the 16 selected biomarkers are shown in eFigure-3. Based on the stage-1 and -2 selections, eight neutrophil biomarkers [cluster of differentiation antigens (CD) CD15; CD24; CD35; CD64; CD312; CD11b; CD274; CD279], seven monocyte biomarkers (CD35; CD64; CD312; CD11b; HLA-DR; CD274; CD279) and one CD8 T-lymphocyte biomarker (CD279) were selected for evaluation of discrimination for clinical outcomes. Biological relevance of these markers in sepsis are summarized in Table 2.

Stage three: prediction of clinical outcomes in cohort-1

Most biomarkers lacked any clinically or statistically significant discrimination for predicting primary and secondary outcomes within cohort-1 patients. Amongst the individual biomarkers, clinical deterioration to sepsis was associated with higher neutrophil CD279 expression, higher monocyte CD279 expression and lower monocyte HLA-DR expression. The optimal MFI cutoff for neutrophil CD279 was 239 [sensitivity 0.88 (95% confidence interval 0.82–0.93); specificity 0.35(0.26–0.43)]; for monocyte CD279 was 141 [sensitivity 0.83(0.77–0.90); specificity 0.39(0.30–0.47)]; and for monocyte HLA-DR was 3572 [sensitivity 0.43(0.34–0.51); specificity 0.69(0.60–0.77)]. Although these associations were statistically significant, discriminant ability was poor and unlikely to be clinically useful in isolation.

Table 1 Cohort characteristics and cohort-1 outcomes

	Cohort-1 (infected ED cohort) *N* = 272	Cohort-2 (ICU-septic) *N* = 59	Cohort-3 (non-infected ED controls) *N* = 75
Cohort characteristics			
Age in years mean (SD)	62.1 (19.1)	67.9 (12.8)	61.6 (20.0)
Female *N* (%)	133 (48.9%)	23 (39.0%)	33 (44.0%)
FCI Score median (IQR)	2 (1,3)	2 (1,4)	1 (0,2)
White cell count median (IQR)			
Total	13.5 (10.7, 16.2)	16.9 (10.1, 19.6)	7.7 (6.4, 9.1)
Neutrophils	11.2 (8.5, 14.1)	14.1 (8.2, 17.5)	4.9 (4.1, 6.4)
Lymphocytes	0.9 (0.6, 1.4)	0.9 (0.6, 1.3)	1.7 (1.3, 2.1)
C-reactive protein median (IQR)	64 (21,168)	212 (86,309)	13 (2,27)
Procalcitonin Median (IQR)	29.4 (0.0, 337.3)	No data	No data
Confirmed infection	238 (87.5%)	59 (100%)	0
qSOFA score >=2			
At ED presentation	44 (16.2%)	No data	No data
At 24 h	6 (2.2%)		
At 72 h	5 (1.8%)		
APACHE II score median (IQR)	9 (6, 13)	16 (12, 21)	6 (3, 9)
SOFA score median (IQR)	2 (1, 3)	7 (5, 9)	1 (1, 2)
Site of infection *N* (%)			
Respiratory	124 (45.6%)		
Urinary	44 (16.2%)		
Unknown	40 (14.7%)		
Musculoskeletal, skin and soft tissue	32 (11.7%)		
Abdominal (including biliary)	28 (11.0%)		
Neurological	4 (1.5%)		
Outcomes for Cohort-1			
Primary outcome[1]			
SOFA ≥ 2 at 24 or 72 h	139 (51.1%)		
Secondary outcomes			
ICU admission or death within 72 h of hospitalization	22 (8.1%)		
SOFA >=4 at 24 or 72 h	36 (13.2%)		
Discharged home within 72 h of hospitalization	86 (31.6%)		
Discharged home or in hospital with no organ failure	148 (54.4%)		
Hospital mortality *N* (%)	1 (0.4%)		
Development of septic shock	1 (0.4%)		
Organ support			
On antibiotics at 72 h	144 (52.9%)		
Vasopressors	2 (0.7%)		
Ventilation invasive	2 (0.7%)		
Ventilation non-invasive	5 (1.8%)		
Hospital length of stay (days) median (IQR)	5 (2, 9)		

FCI functional co-morbidity index, *qSOFA* quick sepsis organ failure assessment, *SOFA* sepsis organ failure assessment, *APACHE II* Acute Physiology And Chronic Health Evaluation II, *ICU* intensive care unit, *ED* emergency department

[1] *N* = 3 missing data for primary outcome

With best subsets logistic regression, the optimum combination for predicting clinical deterioration to sepsis included increased neutrophil CD24; increased neutrophil CD279; and reduced monocyte HLA-DR expression [sensitivity 0.72(0.64–0.79); specificity 0.56(0.48–0.65)]. With best subsets logistic regression, the optimum combination for predicting the secondary outcome of discharge to home within 72 h, included increased neutrophil CD15,

Table 2 Biological relevance in sepsis patients of the reliable cell surface markers with discriminant value identified in cohort-1

Cell surface markers	Marker positive leukocytes in our study	Biological relevance in sepsis [2, 3, 22, 25, 32, 39–42]	Our key inferences
CD15	Neutrophil	Expressed on all myeloid cells and from the promyelocyte stage onwards on neutrophils. Although monocytes express CD15 at low levels, we were gating $CD15^{hi}$ granulocytes	Alongside CD14, CD16, CD11b, CD15, is a marker for myeloid derived suppressor cells [43, 44], which is implicated in suppressing T cell function
CD24	Neutrophil	Expressed on mature granulocytes and B cells; down-regulated on neutrophils in sepsis, induces neutrophil apoptosis which is delayed in sepsis	CD16 low/CD14 negative/CD24 positive myeloid-derived suppressor cells are cytotoxic to T cells [20]. Immature granulocytes in peripheral circulation in sepsis is associated with greater risk of death [45]
CD35	Neutrophil Monocytes	Receptors of complement activation (RCA) family expressed on leukocytes; potentially discriminates sepsis from inflammation	Understanding of major roles of CD35 alterations in sepsis is unclear
CD64	Neutrophil Monocytes	Fc gamma receptor expressed on leukocytes; Patients with sepsis have increased expression of CD64 has been consistently reported	Despite this association, CD64 as a single marker has limited diagnostic performance in sepsis [23, 40].
CD11b	Neutrophil Monocytes	Role in adhesive interactions of monocytes, macrophages and granulocytes; mediating the uptake of complement-coated particles; increased in sepsis following neutrophil activation	Neutrophil and monocyte increase in CD11b is inconsistent in the literature [46, 47]. Tissue resident CD11b positive T cells, secrete interferon gamma and may influence local host defence mechanisms in bacterial infections [48]
CD312	Neutrophil Monocytes	human myeloid-restricted class B seven-span transmembrane (TM7) subfamily of G-protein coupled receptors; acutely altered in sepsis secondary to leukocyte activation	Understanding of major roles of CD312 alterations in sepsis is unclear
CD274 CD279	Neutrophil Monocytes Neutrophil Monocytes CD8 T cells	PD-1 and PDL-1 form a checkpoint inhibitor complex and are considered markers of sepsis related immunosuppression. In sepsis, neutrophils, monocytes and lymphocytes express elevated levels of with CD274 and CD279 [2, 30, 31]. In sepsis, neutrophils are thought to impair T cell function through PD-L1 mechanism [33, 49]	Recently, it has been shown that the increasing functional deficit in multiple innate and adaptive immune responses in sepsis-related critical illness could be restored ex vivo in cells treatment with monoclonal antibodies targeting either arm of the PD-1:PD-L1 axis [30, 49]. Thus, measuring cellular levels of PD-1 and PD-L1 could inform trial design
HLA-DR	Monocyte	Consistently reported as a marker of immunosuppression in sepsis and in critically ill patients	Reduced HLA-DR expression on monocytes is associated with increased risk of nosocomial infection due to impaired monocyte competence. Monocyte HLA-DR expression less than 8000 monoclonal antibodies/cell for 2 or more days can be reversed with GM-CSF therapy, with potentially beneficial effects [25]. This is a useful biomarker for enrichment in future clinical trials

CD cluster of differentiation antigens; *HLA-DR* Human Leukocyte Antigen–antigen D Related

reduced neutrophil CD274 and increased total monocyte HLA-DR expression. No biomarkers had significant discriminant value for the outcome of critical care admission or death within 72 h. The performance of individual and optimized combinations of biomarkers for predicting the primary and secondary outcomes are shown in Table 3. No marked non-linearities in biomarker effects were identified. Overall, although statistically significant associations were demonstrated, discrimination of clinical outcomes was unlikely to be clinically useful (Fig. 1).

Extreme phenotype analysis

From 272 patients in cohort-1, we identified 40 "well' and 52 "sick" phenotypes (eFigure-2). "Sick" phenotype patients were characterized by being older, more often male, with a higher frequency of co-morbidities, more frequently lymphopenic, with higher APACHE II and SOFA scores at baseline. After Bonferroni correction for multiple comparisons, "sick" phenotypes had significantly higher monocyte CD279 and neutrophil CD279 in the ED, but no other biomarkers were different (Table 4; eFigure-4).

For both CRP and PCT, there was also no statistically or clinically significant discrimination for subsequent sepsis with univariate analysis (Table 3).

Discussion

In this multi-site cohort study, we reduced a candidate panel of 47 leukocyte biomarkers to 16 reliable biomarkers with potential for discriminating the risk of developing sepsis in patients with suspected infection presenting to the ED. The combination of higher neutrophil CD24, higher neutrophil CD279, and a lower monocyte HLA-DR expression best predicted the clinical deterioration to sepsis. Consistent with this association, a lower neutrophil CD279 expression and higher monocyte HLA-DR expression were associated with discharge home within 72 h (implying rapid recovery). Although our pre-defined biomarker discovery strategy identified these biomarkers as associated with development of sepsis and more severe illness, their discriminant value was insufficient to suggest utility for decision-making in routine clinical care.

Our findings have potential clinical relevance. The key pathophysiological insight is that leukocyte biomarkers of immunosuppression such as check-point inhibitors (CD279; CD274) and antigen processing ability (HLA-DR) were altered even in patients with *suspected infection* presenting to ED. We also demonstrate the importance of assessing reliability when standardising flow cytometry for large-scale time critical use. The development of clinically useable tests is likely to require a form of cross-platform calibration (such as multiparametric version of the Quantibrite system, BD Bioscience). Our study shows it is feasible to implement flow cytometry as a means of undertaking precision medicine in sepsis, for example to guide novel therapeutic interventions such as those tested recently in immunotherapy trials [15] and highlighted in recent expert reviews [16, 17]. However, our data suggest that for patients with suspected infection the predictive validity of panels of leukocyte biomarkers are unlikely to be useful as general clinical decision-making tools. Of note, both CRP and PCT also performed poorly.

Strengths of our study were well-defined hypothesis, pre-published protocol [8], internationally accepted primary outcome [1], clinically relevant secondary outcomes and hierarchical analytic approach to reduce biomarker selection bias. Reliability of multi-site flow cytometry is potentially problematic due to measurement error bias [18], which we addressed rigorously with fluorochrome-conjugated antibody titrated for optimal signal and kept constant throughout the study. Using hospitalized non-infected patients and ICU-sepsis patients as comparators during biomarker selection increased the chance of detecting infection related host responses and is superior to using healthy volunteer controls. Our blood sampling time point in the ED was prior to severe illness, before major clinical interventions, and much earlier than in previous studies of sepsis biomarkers, and we excluded patients who clinicians considered to have already established sepsis and/or critical illness. As such, our population was different from other recent studies, which evaluated leukocyte biomarkers for prediction of sepsis trajectory (by including patients with sepsis-2 defined sepsis, severe sepsis and septic shock) [19, 20] and stratified nosocomial infection risk in ICU patients [21] (see eTable-5, which highlights important differences). The post hoc extreme phenotype analysis enhanced face validity by considering multiple clinical variables simultaneously for phenotype definition.

Our study has potential weaknesses. Although we could not include all potential leukocyte biomarkers, we studied a range of leukocyte biomarkers (such as complement pathway receptors (CD35, CD11b), G protein-coupled receptors (CD312), Fc-gamma-receptors (CD64 [22, 23]), factors delaying neutrophil apoptosis (CD24 [22]), check-point molecules (CD274, CD279) [24]; HLA-DR expression [25–27]), that previous studies highlight association with adverse outcomes in established sepsis. We enrolled a smaller sample size than planned due to time and funding constraints. However, this had a limited impact since substantial differences in biomarker levels across cohorts still enabled selection of candidate biomarkers for further discriminant analysis. Supervised classification methods such as classification and regression trees (CART) is a valid alternative analytic approach for this research question. However, CART requires approximately 50 events

Table 3 Candidate biomarkers and combinations for predicting outcomes in cohort-1

Biomarker	Marker expression in cohort-1 as Median MFI (IQR)	Primary outcome [OR (95% CI) per SD increase in MFI; *p* value]		Secondary outcomes[1] [OR (95% CI) per SD increase in MFI; *p* value]				
		SOFA score ≥ 2 at 24 h and/or 72 h following presentation to hospital[2]	AUROC (95% CI)	ICU admission or death within 72 h of presentation	SOFA >=4 at 24 or 72 h after presentation	Discharge home within 72 h of presentation	Discharge home within 72 h of presentation or in-hospital with no organ failure	Confirmed infection
Neutrophils								
CD15	31,148 (22,261, 39,622)	0.94 (0.69–1.28); 0.70	0.50 (0.41–0.59)	1.36 (0.82–2.22); 0.23	1.01 (0.65–1.58); 0.97	1.38 (0.99–1.91); 0.06	1.13 (0.83–1.56); 0.42	0.89 (0.57, 1.41); 0.63
CD24	22,261 (16,398, 28,565)	1.20 (0.94–1.54); 0.15	0.56 (0.49–0.63)	1.26 (0.84–1.90); 0.17	1.48 (1.08–2.05); 0.01	1.00 (0.77–1.30); 1.00	0.79 (0.62–1.02); 0.07	1.31 (0.85, 2.04); 0.22
CD35	17,363 (10,021, 26,452)	0.98 (0.77–1.25); 0.87	0.51 (0.44–0.58)	1.18 (0.76–1.83); 0.45	0.90 (0.62–1.31); 0.59	1.18 (0.91–1.53); 0.21	1.15 (0.90–1.47); 0.28	1.34 (0.88, 2.06); 0.17
CD64	2384 (1353, 5522)	0.95 (0.71–1.29); 0.75	0.49 (0.41–0.58)	0.98 (0.55–1.75); 0.94	0.88 (0.53–1.45); 0.61	0.97 (0.70–1.33); 0.83	0.95 (0.71–1.28); 0.74	1.62 (0.84, 3.12); 0.15
CD312	685 (451, 845)	1.29 (0.99–1.67); 0.06	0.57 (0.50–0.64)	0.74 (0.43–1.29); 0.29	0.82 (0.55–1.23); 0.34	0.79 (0.59–1.06); 0.12	0.85 (0.67–1.09); 0.21	1.10 (0.73, 1.67); 0.64
CD11b	20,583 (13,210, 28,737)	1.25 (0.97–1.62); 0.08	0.56 (0.49–0.63)	1.45 (0.97–2.16); 0.07	1.36 (0.98–1.60); 0.57	1.12 (0.86–1.45); 0.39	0.84 (0.66–1.08); 0.18	1.27 (0.83, 1.96); 0.27
CD274	269 (207, 320)	1.25 (0.96–1.61); 0.10	0.57 (0.50–0.64)	0.91 (0.55–1.49); 0.70	1.16 (0.83–1.61); 0.39	0.70 (0.51–0.95); 0.02	0.77 (0.59–0.99); 0.045	1.56 (0.97, 2.52); 0.07
CD279	569 (300, 640)	1.78 (1.23–2.57); 0.002	0.59 (0.52–0.66)	0.96 (0.57–1.61); 0.86	1.06 (0.77–1.46); 0.72	0.57 (0.39–0.83); 0.003	0.60 (0.41–0.87); 0.007	1.06 (0.68, 1.66); 0.78
Monocyte								
CD35	21,018 (13,818, 28,565)	1.15 (0.89–1.48); 0.28	0.55 (0.48–0.62)	0.99 (0.62–1.57); 0.95	1.33 (0.97–1.83); 0.07	0.91 (0.70–1.20); 0.52	0.94 (0.73–1.20); 0.60	1.19 (0.77, 1.84); 0.44
CD64	30,848 (24,499, 39,622)	1.04 (0.77–1.39); 0.80	0.57 (0.49–0.66)	1.25 (0.77–2.03); 0.36	1.12 (0.74–1.71); 0.59	0.95 (0.69–1.30); 0.73	0.92 (0.69–1.24); 0.58	2.24 (1.11, 4.52); 0.02
CD312	1087 (649, 1617)	0.91 (0.71–1.16); 0.43	0.54 (0.47–0.61)	0.73 (0.41–1.29); 0.29	0.79 (0.52–1.21); 0.28	1.24 (0.96–1.61); 0.09	1.06 (0.83–1.36); 0.64	0.94 (0.65, 1.36); 0.73
CD11b	22,705 (14,413, 28,651)	1.21 (0.94–1.57); 0.14	0.58 (0.51–0.65)	1.25 (0.83–1.88); 0.28	1.27 (0.91–1.76); 0.16	1.15 (0.89–1.49); 0.30	0.87 (0.68–1.12); 0.27	1.24 (0.80, 1.93); 0.33
HLA-DR	4435 (2379, 8001)	0.73 (0.55–0.97); 0.03	0.56 (0.49–0.63)	0.69 (0.34–1.40); 0.30	0.76 (0.46–1.24); 0.27	1.35 (1.04–1.75); 0.02	1.34 (1.00–1.80); 0.052	0.96 (0.66, 1.38); 0.82
CD274	60 (0, 166)	0.90 (0.70–1.16); 0.41	0.50 (0.43–0.56)	1.06 (0.69–1.61); 0.80	1.03 (0.73–1.46); 0.85	0.84 (0.62–1.15); 0.28	0.99 (0.78–1.27); 0.95	0.89 (0.64, 1.23); 0.48
CD279	240 (129, 280)	1.32 (1.03–1.70); 0.03	0.58 (0.51–0.65)	0.89 (0.56–1.43); 0.31	1.21 (0.84–1.75); 0.27	0.68 (0.52–0.90); 0.006	0.80 (0.62–1.02); 0.07	0.98 (0.67, 1.44); 0.92
CD8 T cells CD279	117 (72, 169)	1.16 (0.81–1.66); 0.43	0.48 (0.41–0.55)	0.23 (0.02–2.29); 0.21	0.94 (0.58–1.93); 0.80	0.79 (0.43–1.45); 0.45	0.82 (0.55–1.23); 0.34	2.00 (0.44, 9.06); 0.37
Neutrophil CD24 +		1.48 (1.10 –1.98); 0.009	0.64 (0.58–0.71)	*	*	1.32 (0.94 –1.85); 0.10	0.65 (0.49–0.87); 0.004	*
Neutrophil CD279		2.23 (1.47 –3.38); < 0.001	0.67 (0.60–0.74)			0.59 (0.41 –0.86); 0.006	0.47 (0.31–0.71); 0.0004	
Neutrophil CD24 +		1.49 (1.10 –2.00); 0.009				1.48 (1.03–2.13); 0.04		
Neutrophil CD279 +		2.37 (1.54 –3.64); < 0.001						

Table 3 continued

Biomarker	Marker expression in cohort-1 as Median MFI (IQR)	Primary outcome [OR (95% CI) per SD increase in MFI; *p* value]		Secondary outcomes[1] [OR (95% CI) per SD increase in MFI; *p* value]				
		SOFA score ≥ 2 at 24 h and/or 72 h following presentation to hospital[2]	AUROC (95% CI)	ICU admission or death within 72 h of presentation	SOFA >=4 at 24 or 72 h after presentation	Discharge home within 72 h of presentation	Discharge home within 72 h of presentation or in-hospital with no organ failure	Confirmed infection
Monocyte HLA-DR		0.72 (0.53–0.97); 0.03						
Neutrophil CD15 +								
Neutrophil CD274 +								
Monocyte HLA-DR								
Other markers#								
CRP		1.20 (0.94–1.54): $p = 0.15$	0.56 (0.49, 0.63)	0.88 (0.55–1.42); 0.60	0.99 (0.69–1.42); 0.94	0.74 (0.55–0.99); 0.04	0.85 (0.66–1.08); 0.19	1.16 (0.99–2.65); 0.06
PCT		0.94 (0.72–1.21); $p = 0.61$	0.53 (0.46, 0.60)	0.93 (0.54–1.61); 0.57	0.81 (0.48–1.36); 0.42	0.83 (0.60–1.15); 0.27	1.02 (0.79–1.32); 0.89	4.00 (0.78–20.5); 0.10

2SOFA score sequential organ failure assessment score; *MFI* median fluorescence intensity; *CD* Cluster of differentiation; *ICU* intensive care unit

* Best subsets regression did not identify any combination models which provided better fit than individual marker models

[1] As pre-specified, secondary outcomes of hospital mortality, occurrence of septic shock and length of stayare not reported

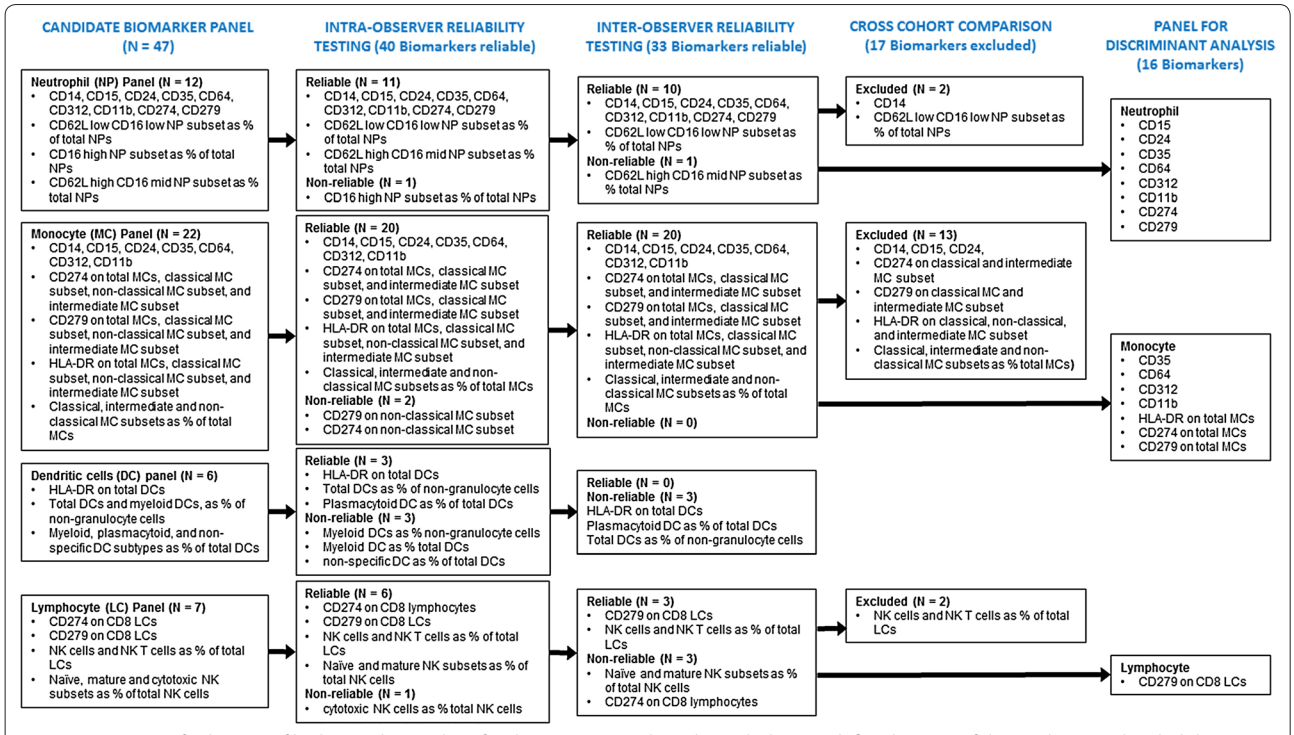

Fig. 1 Overview of selection of leukocyte biomarkers for discriminant analysis through the pre-defined stages of the study. For a detailed description of the rationale for biomarker selection see eMethods-1 and eMethods-2. Non-reliable refers to the analysis of cell populations that are not sufficiently distinct in bimodal FACS plots, are difficult to reliably standardize for a uniform gating approach and need further development. We are proposing that these biomarkers are necessarily of limited value

Table 4 Extreme phenotype description

	Well phenotype (*N* = 40)	Sick phenotype (*N* = 52)	*p* value
Age, median (IQR)	37.5 (27.3–56.8)	70.0 (56.0–81.0)	< 0.001
Female, *n* (%)	26 (65%)	19 (37%)	0.009
FCI Score Median (IQR)	1 (0–2)	2 (1–3)	0.03
White cell count Median (IQR)			
Total	13.2 (10.3–14.4)	13.1 (9.1–16.5)	0.78
Neutrophils	10.3 (8.1–12.0)	11.2 (7.5–15.1)	0.29
Lymphocytes	1.2 (0.7–1.8)	0.8 (0.5–1.2)	0.01
C-reactive protein Median (IQR)	58.5 (24.0–107.3)	56.0 (16.5–191.0)	0.85
qSOFA score ≥ 2			
At ED presentation	3 (7.5%)	10 (19.2%)	0.11
At 24 h	0	4 (7.7%)	0.07
At 72 h	0	2 (3.8%)	0.22
Source of infection*, *n* (%)			
Respiratory	13 (40.6%)	30 (57.7%)	0.13
Neurological	1 (3.1%)	2 (3.8%)	0.87
Urinary	2 (6.3%)	7 (13.4%)	0.31
Abdominal	5 (15.6%)	5 (9.6%)	0.41
Skin	9 (28.1%)	3 (5.8%)	0.005
Biliary	0 (0%)	5 (9.6%)	0.005
Sepsis of unknown origin	2 (6.3%)	0 (0%)	0.07
Baseline APACHE 2 score, median (IQR)	4.5 (2–7)	11.5 (9–16)	< 0.001
Baseline SOFA, median (IQR)	1 (1–1)	3 (2–4)	< 0.001
Discharged home within 72 h, *n* (%)	32 (80%)	0	< 0.001
Admitted to HDU/ICU within 72 h, *n* (%)	0	14 (26.9%)	< 0.001
Neutrophil biomarkers (MFI) median (IQR)			
Neutrophil CD15	30,848 (24,499–45,352)	30,848 (19,116–41,992)	> 0.10
Neutrophil CD24	23,815 (18,299–29,261)	24,034 (18,741–30,710)	> 0.10
Neutrophil CD35	19,485 (7985–26,580)	15,636 (10,988–25,117)	> 0.10
Neutrophil CD64	3098 (1528–6272)	2150 (1693–5378)	> 0.10
Neutrophil CD312	565.8 (382.7–712.9)	670.9 (493.6–853.9)	> 0.10
Neutrophil CD11b	16,089 (13,664–25,552)	22,154 (13,510–30,737)	> 0.10
Neutrophil CD27 s	279.0 (101.4–322.8)	284.3 (233.8–327.7)	> 0.10
Neutrophil CD279	326.4 (152.7–584.2)	584.2 (383.7–648.8)	**0.005**
Monocyte biomarkers (MFI) median (IQR)			
Monocyte CD35	16,556 (9974–27,488)	22,476 (15,067–27,681)	> 0.10
Monocyte CD64	29,685 (21,843–45,021)	33,323 (29,405–45,352)	> 0.10
Monocyte CD312	1243 (694–2001)	817.0 (470.5–1560.0)	> 0.10
Monocyte CD11b	20,205 (12,102–26,644)	26,660 (16,984–32,741)	> 0.10
Monocyte CD274	50.7 (0–167.2)	78.6 (0–199.7)	> 0.10
Monocyte CD279	151.2 (94.8–262.1)	245.4 (161.1–287.0)	**0.05**
Monocyte HLA-DR	6172 (3516–11,544)	4016 (2692–7170)	0.12
CD-8 T cell biomarker (MFI) median (IQR)			
CD8 T-Lymphocyte CD279	112.2 (78.7–153.3)	115.6 (58.5–167.9)	> 0.10

Categorical variables are given as numbers with percentages. Continuous variables are given as mean with standard deviation where data are parametric, and median with interquartile range otherwise. Comparisons between phenotypes were performed with Fisher exact test between percentages for categorical variables, unpaired *t*-test for continuous normally-distributed variables, and Mann–Whitney test for other continuous variables

Significant differences are shown in bold (*p*-value of <0.05 taken as significant). For biomarker comparisons, Bonferroni method was used to correct for multiple comparisons and the corrected *p*-values are reported

* For the well phenotype, the denominator for the 'source of infection' variable is 32, as only 32 patients had a final diagnosis of infection. Biomarker comparisons are also reported as dot plots in eFigure-4

per variable when predicting a dichotomised outcome, before predictions become stable and over-optimism is minimised [28]. As our observed number of sepsis events did not reach this threshold we opted to use the best subsets logistic regression approach as pre-specified in our statistical analysis plan [8]. As our cohort-1 inclusion criteria mandated SIRS, we have excluded SIRS negative patients with infection, who could have progressed to develop sepsis. However, this is unlikely to bias the results, as the prevalence of SIRS negative sepsis-3 sepsis in ICUs in England is only 3% [29]. As our objective was to study leukocyte biomarkers at an earlier time point than previously achieved and to identify biomarkers that predict deterioration within 72 h of hospitalisation, we excluded patients planned for direct admission to ICU from the ED at enrolment, which explains the lower than expected event rate for death and septic shock. Findings might be different for more severely ill patients studied later in sepsis, as observed in other recent flowcyometric studies (eTable-5) [19–21].

Our findings have biological plausibility, as the leukocyte biomarkers that best predicted the risk of developing sepsis in our study were on the key innate immune cells, namely neutrophils and monocytes, which are first responders to infection. The strongest biomarker predicting subsequent sepsis and extreme phenotypes was higher levels of CD279 (programmed death receptor 1, PD-1) on monocytes and neutrophils. CD279 expression is associated with neutrophil and monocyte suppressor subsets [30], memory lymphocyte subsets [31], is thought to regulate T cell responses and induce an inhibitory signal characterized by cell cycle arrest and reduced cytokine synthesis [2, 32]. This early role for CD279/PD-1 is consistent with animal models of sepsis [33] and sepsis cohorts [30]. CD279/PD-1 acts in conjunction with its ligand CD274 (PD-L1). In our study, lower CD274, together with lower CD279, higher monocyte HLA-DR, and lower neutrophil CD24, emerged as a predictor for rapid recovery sepsis phenotype. These novel findings require further confirmatory studies.

Although none of the biomarkers we studied had discriminant ability that could be used to guide clinical decision-making, our data imply that immunosuppression in infected patients precedes established sepsis and that higher CD279/PD-1 and lower HLA-DR are potential theragnostic and enrichment markers [34–37] for anti-PD-1/PDL-1 agents and granulocyte-monocyte colony stimulating factor [25], respectively, for carefully designed immunotherapy trials [3, 38].

Conclusions

We conclude that in a population of patients presenting with suspected infection prior to established sepsis, a sequential approach to identifying reliable potential leukocyte biomarkers from a large candidate panel that may predict the subsequent development of sepsis identified only a small number with discriminant properties. These were markers of immune suppression, namely CD279 and HLA-DR, suggesting this may be an early event, prior to development of sepsis.

Electronic supplementary material

The online version of this article (https://doi.org/10.1007/s00134-018-5389-0) contains supplementary material, which is available to authorized users.

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Author contributions

Drs. Shankar-Hari, Weir and Walsh had full access to all the data in the study and take responsibility for integrity of data and the accuracy of the data analyses. Concept and design: Walsh, Simpson, Conway Morris, Datta, Weir, Warner. Statistical analysis: Assi, Stephen, Weir, Datta, Wilson, Shankar-Hari. Drafting of manuscript: Shankar-Hari, Weir, Walsh. Acquisition, analysis and interpretation of data: All authors. Critical revision of the manuscript for important intellectual content: All authors. Obtained funding: Walsh, Conway Morris, Brown, Simpson, Warner, Keenan. Administrative, technical, or material support: Walsh, Weir, Warner, Judge. Keenan. Supervision: Walsh, Weir. All authors confirm to the accuracy or integrity of the work.

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Compliance with ethical standards

Conflicts of interests

Noel Warner, Kevin Judge, Jim Keenen and Alice Wang were all employees of BD biosciences whilst this work was being undertaken, and all four authors hold stock in BD Biosciences. Prof Simpson collaborated with BDB on a Wellcome Trust/Department of Health-funded Healthcare Innovation Challenge Fund (HICF) grant in suspected ventilator-associated pneumonia. He is Director of the NIHR Newcastle In Vitro Diagnostic Evidence Co-operative (formerly the NIHR Newcastle Diagnostic Evidence Co-operative)—these entities exist to evaluate in vitro diagnostics and have worked with (and continue to work with) BDB and other companies in this capacity. All other authors declare that they do have any personal conflict of interest directly related to this manuscript.

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UNDERSTANDING THE DISEASE

Non-antiarrhythmic interventions in new onset and paroxysmal sepsis-related atrial fibrillation

Antoine Vieillard-Baron[1,2,3*] and John Boyd[4]

Introduction

Although rarely the primary diagnosis, atrial fibrillation (AF) occurs frequently in critically-ill patients. For instance, in 1341 patients without pre-existing cardiac disease admitted to an intensive care unit, 8.4% exhibited supraventricular arrhythmia, representing AF in 77% of cases [1]. Patients with sepsis appear to be at highest risk; cumulative risk for de novo AF has been reported as 10, 22 and 40% in patients with sepsis, severe sepsis and septic shock, respectively [2]. AF mainly occurs in the first 3 days following admission and is associated with increased ICU-, 30 days-, 90 days-, and 1 year-mortality, even after adjusting for such confounding factors as age, gender, scores of severity, malignancy, and cardiovascular diseases [2]. In 8356 consecutive critically-ill adult patients admitted in medical and surgical ICU, Moss et al. recently reported that de novo AF is associated with increased hospital mortality and length of stay [3].

While it is possible that new onset AF is simply a marker of the overall severity of disease, AF may drive the poor outcome by worsening acute or pre-existing heart failure and by favoring development of thrombo-embolic events such as stroke or myocardial infarction, even though myocardial infarction in this situation is more the consequence of a rate-related oxygen demand–supply imbalance. Side effects of antiarrhythmic drugs (especially beta-blockers and amiodarone) and anticoagulation may also be directly related to morbidity and mortality in medically fragile patients [4], particularly those with acute or pre-existing heart failure. Due to this unknown benefit/risk balance, there is no consensus on the best treatment for patients who develop new AF but who are not so unstable that they require immediate cardioversion. This is particularly true in patients with sepsis, in which the combination of cardiac failure, microcirculation alterations and changes in vasomotor tone elevates the risks associated with anti-arrhythmic drugs, even compared to other critically-ill patients. Electrical cardioversion is associated with a poor success rate and a very high rate of immediate/early recurrence of AF. Prevention of thrombo-embolic complications of AF with anticoagulation is challenging in the critically ill, as these patients have a high prevalence of disseminated intravascular coagulation and are difficult to maintain in the therapeutic range with unfractioned heparin [5]. In less complex patients with postoperative AF, it is only recommended in hemodynamically unstable patients related to AF to perform a cardioversion, and that rate control is preferred over rhythm control in patients with "acceptable" symptoms [6]. Thus AF is both highly prevalent and associated with death and disability in complex critically ill patients, especially with sepsis. While there is no proven best strategy to treat new onset AF in these patients, we propose in this short piece a simple and systematic approach with a check-list, non-anti-arrhythmic-based, to prevent AF or to reduce AF heart rate. This approach represents the current clinical practice of the authors combined with an analysis of the literature, however it is not based upon high-grade randomized controlled trial evidence.

Pathophysiology of AF

The pathophysiology of AF is complex but may be separated into 2 parts, the cardiac substrate and the trigger.

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The substrate is for the most part unmodifiable in the ICU during what is typically a very short period of time and is related to left atrium remodeling (dilatation, fatty infiltration, fibrosis, and inflammation), which may favor atrial reentry. Pre-existing risk factors are chronic heart failure, male gender, coronary artery diseases, history of hypertension, left ventricular hypertrophy, diabetes mellitus, aging, obesity (BMI per unit) and excessive alcohol use [7]. Frailty, increased pulse pressure (as a measure of aortic stiffness), chronic kidney disease, valvular heart disease, and sleep apnea have also been reported as risk factors. All these factors are commonly observed in critically-ill patients. The trigger which initiates AF is atrial ectopic discharges, mainly derived from the pulmonary veins. It is this trigger that offers intensivists a therapeutic approach to reduce the incidence and duration of AF. Briefly, atrial ectopic discharges are secondary to diastolic leak of Ca^{2+} from the sarcoplasmic reticulum, leading to early and delayed afterdepolarization. Factors which shorten the refractory period of cardiac myocytes favor AF initiation. It has been reported that metabolic disturbances, as hypokalemia and hyponatremia, may favor de novo AF [8]. Indeed, a low K^+(< 3.5 mmol/L) or a low Na^+(< 135 mmol/L) induces delayed afterdepolarization by acting on the Na^+/Ca^{2+} exchanger [9]. Another important electrolyte is magnesium (Mg). In more than half of the patients with hypokalemia, hypomagnesemia is associated. Magnesium is known to modulate potassium and calcium channels in the atria and the ventricles and then to have membrane-stabilizing properties. In the Framingham heart study, a low serum Mg level ($\leq$ 1.77 mg/dL) was moderately associated with the development of AF [10] and the infusion of a K-Mg electrolyte solution facilitated electric cardioversion of AF without inducing side effects. Sympathetic activation is another risk factor for atrial ectopic discharges. This may be inherent to the disease, but also due to withdrawal of chronic anti-sympathetic therapies such as beta-blockers and the choice of the vasoactive drug therapy. In the classic report describing stress-induced cardiomyopathy, catecholamine levels were 2–5 fold those of patients with an acute myocardial infarction, equivalent to 3.5 ng/mL norepinephrine, while plasma levels of catecholamines during norepinephrine infusions range from 3 ng/mL up to 170 ng/mL. Clearly a patient with the appropriate cardiac substrate may be triggered into AF with this vasopressor induced chronotropy. Dopamine and epinephrine have the greatest chronotropic effects, both increasing the heart rate by 15% when compared to norepinephrine [11]. Vasopressin in its currently recommended usage, for refractory shock despite norepinephrine, is largely a hormone used as a beta-agonist sparing agent and reduces heart rate by 10%, likely mediated by a 50% reduction in norepinephrine dose [11]. The synthetic analogues Terlipressin and Selepressin both exert similar 10% reductions in heart rate; Selepressin is currently being studied for use in septic shock (clinical trial identifier NCT02508649). Levosimendan, a calcium sensitizing agent was recently found to exert its main hemodynamic effect via increases in heart rate (more than 10% compared to norepinephrine), with either no change or a decline in stroke volume [12]. Acute hypovolemia has been reported to favor AF [13], probably by inducing sympathetic stimulus. Given that left atrial (LA) diameter > 5 cm measured by echocardiography is the strongest predictor of chronic AF [14], it is likely (though unproven to date) that overprescription of fluid and fluid overload lead to LA dilation and increases the probability of new AF. Systemic inflammation, through inflammatory mediators (IL-6, IL-1, CRP, TNF-α) is another risk factor for AF. One may assume that fever control could limit AF. However, no data currently support such a hypothesis and in the randomized controlled trial by Schortgen et al. on the effect of fever control in septic patients, no information is given about the incidence of AF [15]. More, such an approach is probably not a benign intervention in septic patients and requires more investigation. Finally, it is suspected that pericardial effusion, as well as mal-positioned central venous catheter into the right atrium, might favor de novo AF. In cases of de novo AF, treating these factors, especially fever and sympathetic activation, may help control heart rate. Gillinov et al. have reported no difference for length of stay, 60 days-mortality and sinus rhythm between rate control targeting 90–110 beats per minute versus rhythm control groups in patients after cardiac surgery [16], although in a different population than medically complex critically ill patients.

Non-antiarrhythmic interventions

Based on the description above of AF pathophysiology and of the main risk factors for atrial ectopic discharges, we can propose for stable patients, i.e. those not requiring urgent electrical cardioversion, a list that has to be checked before discussing any anti-arrhythmic drugs (Table 1). Briefly, electrolytes have to be rigorously normalized, focusing on potassium, sodium and magnesium. Fluid status must be optimized, avoiding hypovolemia but also fluid overload, with a particularly conservative fluid administration if hemodynamic monitoring reveals a LA size enlargement. When selecting vasoactive drugs, avoid highly active chronotropes such as epinephrine and dopamine, and reduce the overall dose of adrenergic agent by choosing the lowest acceptable blood pressure target according to organ perfusion and history of chronic kidney disease. Strategies such as early vasopressin infusion to reduce norepinephrine dose could

Table 1 A standardized approach to prevent new atrial fibrillation in critically ill patients

Modifiable Risk Factor	Diagnosis	Strategy to avoid AF
Hypovolemia[a]	Tachycardia, hypotension, with low central venous pressure or positive dynamic parameters of fluid-responsiveness	Fluid resuscitation targeting parameters of fluid-responsiveness if the patient is still hypotensive
Volume overload[a]	Peripheral and/or pulmonary edema. Echocardiography with LA diameter > 5 cm[b]	Discontinue fluids in favor of non-chronotropic vasopressors. Try limiting central venous pressure below 8 mmHg
Potassium < 3.5 mmol Magnesium < 1.77 mg/dL Sodium < 135 mmol	Electrolyte screen	Concurrent replacement of potassium and magnesium. Avoid hypotonic solutions in initial resuscitation and consider correcting hyponatremia, if any
Excess chronotropy due to vasopressors	Recognition of high chronotropic potency of Epinephrine, dopamine and levosimendan	Avoid Epinephrine, dopamine and levosimendan in high risk patients. Vasopressin or synthetic analogue co-administration with noradrenaline in patients at high risk of AF could be discussed
Pericardial effusion	Echocardiography	Drain if hemodynamically significant
Mal-positioned central venous Catheter	Chest X-ray or ultrasonography	Withdraw catheter to caval-atrial junction
Persistent fever (> 38°1) despite adequate antibiotics	Routine vital sign monitoring	Check for any unexpected cause or complication (superinfection). External cooling and anti-pyretics could be discussed

LA left atrium

[a] As all the recommendations regarding fluid management, some of these aspects may be subject to controversy

[b] LA size reflects the cumulative effects of increased LV filling pressures over time. In other words, acute increase in LV filling pressures may exist without any LA dilatation

be discussed in patients at highest risk of AF. Echocardiography may be performed to look for LA diameter, a pericardial effusion that could be drained if significant and a chest X-ray (or an ultrasonography) to control the position of the central venous catheter, if any (a catheter too far into the right atrium must be slightly removed). Finally, reasons for persistent fever have to be investigated and external cooling or antipyretic medication could favor AF reduction, even though no data currently support this assumption.

Conclusion

Atrial fibrillation is frequent in critically-ill patients and its incidence increases in sepsis and septic shock. Despite a well-demonstrated association with length of stay and mortality, the need for a specific treatment, as well as the type of this treatment, cannot be recommended. Based on the pathophysiology of AF and its risk factors, mainly present in septic patients, we propose a systematic and simple non-antiarrhythmic-based approach to reduce AF, focusing on optimization of electrolytes and fluid status, limitation of sympathetic activation, whatever its cause (inflammation, fever, vasoactive drugs), and control of the central venous catheter position.

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Compliance with ethical standards

Conflicts of interest

The authors declare that they have no conflict of interest.

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EDITORIAL

Using multiple 'omics strategies for novel therapies in sepsis

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Sepsis is life-threatening organ dysfunction caused by dysregulated host response to infection [1]. Treatment is complicated because sepsis is heterogeneous, explaining the lack of effective drugs. Sepsis treatment includes broad-spectrum antibiotics, vasopressors, ventilation and dialysis. A limitation of antibiotics is that they do not directly remove the bacterial endotoxins and exotoxins, which may cause organ failure.

Exotoxins are potent immunologic stimulators—very low concentrations stimulate deleterious immunologic responses. Most exotoxins function as if they are superantigens [that bind to T cell receptors, activate T cells (especially T helper cells) and stimulate cytokine release].

Blocking endotoxin effects by blocking the TLR4 receptor with eritoran (a TLR4 blocker) was unsuccessful in severe sepsis [2]. This pivotal trial may have been negative because the timing was inadequate, the patients had severe sepsis rather than just septic shock, patients were not sick enough (placebo mortality 56% in the prior phase II trial but only 27% in the pivotal trial), eritoran may have worsened outcomes in gram-positive sepsis (46% of patients) (mortality rates: eritoran 34% vs. placebo 25%) or other causes.

There are no novel drugs available to treat sepsis. We propose a new drug discovery strategy that focuses on (1) the early infectious stage, (2) multiple 'omics and (3) an inverted drug discovery sequence to increase the chances of success.

Why focus on early sepsis?

Prior drug discoveries in sepsis that focused on the host inflammatory responses failed. Early antibiotics remain the only effective treatment [3], so we focus on the early infectious phase. "Early" is difficult to define for sepsis because determining 'time-zero' in human sepsis is impossible. Herein, we define early as inclusion within the first 24 h after emergency department arrival.

Antibiotics are recommended within 1 h of presentation [4] because each 1-h delay is associated with 4–6% decreased survival [5]. However, antibiotics do not directly remove bacterial endotoxins that stimulate immune, inflammatory, apoptotic and coagulation pathways causing organ failure and death [6].

Why multi-'omics?

Most sepsis drugs were developed by understanding the disease mechanism and targeting a relevant pathway. An 'omics association is typically an unbiased discovery that points to a possible mechanistic pathway. We define multi-'omics as measurement and examination of associations of at least two types of 'omics variables, from genomics, lipidomics, proteomics to metabolomics. Multi-'omics confirmation refines mechanistic understanding so that high probability drug targets can be identified.

Death due to infection is more heritable than death due to cancer or heart disease [7]. More recently, it has been proposed that environmental influences in early life may override genetic influences [8]. However, there is great value in evaluating the associations of genetic variations with impaired endotoxin clearance, organ dysfunction and death to facilitate drug discovery.

As an example, the endotoxin clearance cascade is a strong candidate pathway for study. Variation of endotoxin clearance cascade genes could alter endotoxin clearance, inflammation, bacterial load and survival. Key aspects of endotoxin cascade neutralization include binding to HDL, modulation by proprotein convertase subtilisin/kexin type 9 (PCSK9), transfer to LDL, LDL/

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endotoxin clearance via the hepatic LDL receptor, VLDL binding of endotoxin and the role of VLDL receptors in adipose tissue and transfer proteins (e.g., cholesterylester transfer protein).

Using a candidate gene approach, we discovered that PCSK9 inhibition acts as a broad-spectrum adjunct to all antibiotics in severe infection. We evaluated *PCSK9* because PCSK9 inhibitors were developed to lower cholesterol [9–12] and because endotoxins are lipid rich. LPS bound to LDL is cleared via hepatic LDL receptors and then excretion in bile. PCSK9 impedes LPS clearance by decreasing LDL receptor density [9]. Septic patients with *PCSK9* loss-of-function (LOF) genotypes have higher survival and lower plasma cytokine concentrations than wild type and patients carrying gain-of-function polymorphisms (GOF) [9].

The most common single-nucleotide polymorphisms of PCSK9 [13] are missense LOF variants rs11591147 (R46L), rs11583680 (A53V) and rs562556 (V474I); the most common missense GOF variant is rs505151 (G670E). The minor allele frequencies in sepsis patients are: rs11591147: 0.6–1.2%, rs11583680: 11–13%, rs562556: 16–17% and rs505151: 4–5% [9] similar to the general population. These PCSK9 mutations are pleiotropic [14]; the degree of cardiovascular protection is greater than expected by the LDL reduction perhaps because of other aspects of lipoprotein metabolism, inflammation, thrombosis, immune function (anti-viral and -malarial properties) and PCSK9 function in non-hepatic tissues.

Why an inverted drug discovery sequence?

Previous sepsis drugs arose from classic drug discovery: researchers identified mechanism(s) of sepsis in animal models and then did trials in humans. This strategy does not account for genetic heterogeneity of microorganisms and the host. We propose inverting (as in our PCSK9 discovery) the standard drug discovery sequence by starting with human 'omics, confirm mechanisms in models and then make go/no-go decisions for potential targets for clinical development.

One could extend our PCSK9 genomics-based approach, by adding multi-'omics to discover other novel targets. First, sequence genes of a relevant pathway (e.g., 32 endotoxin clearance cascade genes) and determine associations with 28-day survival. Then, measure multi-'omics in the same sepsis cohorts to determine associations of variants with multi-'omics in those cohorts. Next, examine associations of gene variants with multi-'omics in human volunteers administered low-dose lipopolysaccharide to select candidate targets meeting three criteria: variants with (1) significantly decreased survival, (2) significantly different level(s) of multi-'omics and (3) significantly different multi-'omics in the human lipopolysaccharide infusion cohort. Selected candidate targets would be evaluated for mechanisms in (1) human hepatocytes (because the liver clears endotoxins) and (2) murine gene knock-out models (e.g., peritonitis). Targets with mechanisms of action are taken to drug synthesis (antibody and small molecules). We did such a feasibility study of multi-'omics in 24 septic shock patients and 99 heathy controls and found significantly lower levels of

Table 1 Associations of PCSK9 genotype (wild type vs. loss of function) with protein, lipid and metabolite concentrations in patients with septic shock ($n = 24$)

Metabolites and lipids	PCSK9 genotype wild type ($n = 13$)	PCSK9 genotype LOF ($n = 11$)	p
Citrulline	18.5 (15.9–21.3)	21.9 (20.0–24.1)	0.026
Glutamic acid	37.3 (24.5–44.7)	38.9 (35.4–54.7)	0.043
Lysophosphatidylcholine C18:2	2.2 (1.5–7.3)	1.1 (0.9–3.2)	0.046
Ornithine	59.1 (44.8–72.7)	104.3 (68.3–116.6)	0.010
Phenyalainine	73.7 (64.9–81.2)	86.9 (77.0–96.8)	0.009
Phosphatidylcholine acyl-alkyl C30:1	0.03 (0.02–0.07)	0.06 (0.04–0.12)	0.042
Phosphatidylcholine diacyl C42:5	0.20 (0.17–0.25)	0.16 (0.12–0.18)	0.042
Trans-OH-proline	6.5 (5.3–9.1)	10.8 (8.4–18.3)	0.016
Proteins			
Apolipoprotein A-IV	41.1 (31.0–54.5)	60.3 (46.1–109.7)	0.046
Apolipoprotein B-100	694 (445–830)	354 (231–523)	0.003
Coagulation factor V	7.8 (6.0–8.5)	4.4 (3.7–7.0)	0.002
Complement component C7	38.0 (27.8–52.9)	62.1 (52.0–79.3)	0.004
IgGFc-binding protein	10.0 (8.3–12.4)	27.7 (11.0–42.7)	0.019
Serotransferrin	784 (618–1196)	1293 (878–1611)	0.025
Thyroxine-binding globulin	3.1 (2.5–4.0)	2.5 (2.0–2.8)	0.026

proteins, lipids and metabolites compared with controls (Genga KR 2018). We evaluated PCSK9 gene variants and found significant differences in proteins, lipids and metabolites between PCSK9 loss-of-function and wild-type patients (Table 1; supplement text).

In summary, focus on early sepsis, harnessing the power of multi-'omics and inverting the drug discovery sequence could enhance drug discovery in sepsis.

Electronic supplementary material
The online version of this article (https://doi.org/10.1007/s00134-018-5122-z) contains supplementary material, which is available to authorized users.

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