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Bolus therapy with 3% hypertonic saline or 0.9% saline in emergency department patients with suspected sepsis: A pilot randomised controlled trial☆

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ABSTRACT

Objective and design: Hypertonic saline administered during fluid resuscitation may mitigate endothelial glycocalyx (EG) shedding and inflammation. The objective of this pilot randomised controlled trial was to measure the effect of hypertonic saline, compared to isotonic saline, on biomarkers of EG shedding and inflammation in emergency department patients with suspected sepsis.

Methods: Patients received either 5 mL/kg of 3% saline (hypertonic group, n = 34) or 10 mL/kg of 0.9% saline (isotonic group, n = 31). Change in serum biomarker concentrations of syndecan-1, hyaluronan, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, interleukin-6, -8, -10, interferon-γ, neutrophil gelatinase-associated lipocalin and resistin were compared from baseline (T0) to after fluid (T1), 3 h (T3) and 12–24 h (T24) later, as was serum osmolality, using linear mixed effects models.

Results: The hypertonic group had significantly increased mean serum osmolality compared to the isotonic group at T1 ($P < .001$) and T3 ($P = .004$). Minor differences were found in some biomarker outcomes, including a decreased fold-change in syndecan-1 at T1 ($P = .012$) and in interleukin-10 at T24 ($P = .006$) in the isotonic group, compared to the hypertonic group.

Conclusions: Although a single bolus of hypertonic saline increased serum osmolality, it did not reduce biomarkers of EG shedding or inflammation, compared to patients that received isotonic saline.

Trial registration: ANZCTR.org.au, ACTRN12611001021965, Registered on 23rd September 2011.

1. Introduction

Sepsis is characterised by a dysregulated host response to an infection [1]. This can lead to microcirculatory alteration, reduced tissue perfusion and organ dysfunction. Current recommendations for improving perfusion in the early stages of treatment include bolus fluid administration, up to 30 mL/kg for patients with hypoperfusion [2]. However, patient fluid requirements and fluid responsiveness can be difficult to assess in regards to volume titration, and positive fluid balance has been associated with increased mortality in intensive care unit (ICU) patients with sepsis [3,4].

An association between large volumes of crystalloid and poorer outcomes in septic patients is currently an active area of investigation [3,5-7]. One proposed mechanism for this association is shedding of the endothelial glycocalyx (EG), causing fluid extravasation and interstitial oedema [8]. Shedding of the EG precipitates endothelial and leucocyte activation, and increased vascular permeability [9-11]. Our recent work in patients with pneumonia has shown positive associations between circulating EG and neutrophil activation biomarker concentrations [12]. Increased EG shedding in patients with sepsis has also been associated with cumulative fluid volumes, degree of organ failure

Abbreviations: ED, Emergency department; EG, Endothelial glycocalyx; ICU, Intensive care unit; IL, Interleukin; SIRS, Systemic Inflammatory Response Syndrome; SOFA, Sequential Organ Failure Assessment.

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and mortality [13-20]. Therefore, strategies aimed at mitigating shedding of the EG, such as modification of current resuscitation practices, may improve outcomes for patients with sepsis.

Hypertonic saline increases serum osmolality and draws water from the interstitium, therefore it may re-establish blood flow while avoiding administration of large volumes of fluid. In turn, this may reduce EG shedding and endothelial activation. Hypertonic saline has been shown in *in vitro* and rodent models to reduce inflammatory cytokine release, cell activation and adhesion [21-30]. Studies in trauma patients have shown evidence that hypertonic saline reduced endothelial activation and inflammation, compared to isotonic crystalloid [31-34]. However, there is currently little information on the endothelial and immunomodulatory effects of hypertonic saline, compared to isotonic crystalloid, when used in adult patients with sepsis.

In this randomised controlled pilot trial we sought to compare the effect of two initial fluid bolus strategies, 5 mL/kg of hypertonic saline or 10 mL/kg of isotonic saline, in adult patients with suspected infection meeting Systemic Inflammatory Response Syndrome (SIRS) criteria. We hypothesised that patients who received hypertonic saline would have an increased serum osmolality and reductions in biomarkers of endothelial activation and inflammation over time, compared to patients who received isotonic saline. We also hypothesised that hypertonic saline would reduce the volume of fluids subsequently given over the following 24 h, compared to isotonic saline.

2. Methods

2.1. Study design

This study was approved by the Royal Perth Hospital Human Research Ethics Committee (HREC 2011-091) and was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12611001021965) before commencement. The Sepsis-Saline Trial (SST) was a pragmatic, investigator-initiated, single-center, randomised controlled open-label pilot trial of adult patients presenting to the emergency department (ED) with suspected infection, who met SIRS criteria and required a fluid bolus as judged by the treating clinician. The HREC restricted enrolment to patients that could provide written informed consent. Patients were randomised *via* password-protected web-based interface to receive either intravenous 3% saline (hypertonic group) or 0.9% saline (isotonic group). Patients were recruited between November, 2011 and April, 2015. The primary outcome of this study was fold-change in endothelial activation and inflammatory biomarker concentrations. Secondary outcomes included serum osmolality, volume of fluids administered and development of organ failure.

2.2. Patients

Patients admitted to the ED were included if they met 2 of 4 SIRS criteria (temperature > 38 °C or < 36 °C, heart rate > 90 bpm, respiratory rate > 20 bpm or white cell count > 12×10^9/L or $<4 \times 10^9$/L) as well as clinical suspicion of infection and a clinical requirement for fluid administration. Patients were excluded if there was a contraindication to fluid volume loading, a plasma sodium concentration < 135 or >145 mmol/L, acidosis (venous pH < 7.25), established renal failure (eGFR < 45 mL/min/1.73 m^2), pregnancy, age < 18 years, patient deemed to receive palliative care only or they had received >500 mL total of pre-hospital crystalloid fluid. Patients that required immediate resuscitation were also excluded, as research processes including the ethics committee requirement for informed patient consent may have delayed treatment.

2.3. Intervention

Patients were randomised to receive either 5 mL/kg of 3% saline or 10 mL/kg of 0.9% saline, to be given over <1 h intravenously. Blinding of the study intervention was not possible due to the different volumes required. The dose of 0.9% saline was chosen to be consistent with the usual practice in the authors' ED, with a maximum dose of 1000 mL total allowed. The dose of 3% saline was equated to deliver twice the sodium load equivalent of 1 l of 0.9% saline at a maximum dose of 600 mL. Any treatment given after the study fluid, including additional crystalloid fluid, was open-label and not restricted. All fluids given after enrolment were recorded, including volume and type of fluid, up until the last blood sampling time point.

2.4. Data collection

Heart rate, mean arterial blood pressure and haemoglobin concentration were collected from the medical records, aligned with the research blood sampling time points below. The Sequential Organ Failure Assessment (SOFA) score [35] was slightly modified for the ED setting (Table 1) and calculated from data collected during three time periods; during ED stay, on day 1 (first 24 h in wards or ICU) and day 2 (second 24 h in wards or ICU). A single clinician blinded to treatment allocation reviewed medical records and assigned a discharge diagnosis category of either 'not infection', 'suspected infection' or 'confirmed infection', informed by review of diagnostic test results and subsequent clinical course. Charlson Comorbidity Score [36] was calculated from data at time of admission.

2.5. Laboratory parameters

Blood samples were taken prior to administration of the study fluid (T0), and 1 h (T1), 3 h (T3) and 12–14 h after fluid administration (T24), and were chosen to be consistent with the sampling protocol of a parallel study [37]. Samples were collected into a serum clot tube then centrifuged at 3000 rpm at 4 °C for 10 min. Serum was then aliquoted and stored at -80 °C. Inflammatory biomarkers, neutrophil gelatinase-associated lipocalin and resistin, as well as glycocalyx biomarkers, syndecan-1 and hyaluronan, were measured using commercial enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA). Inflammatory cytokines interleukin (IL)-6, IL-8, IL-10, interferon-γ and endothelial biomarkers, soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1, were measured using a commercial multiplex cytometric bead array kit (BD Biosciences, San Jose, CA, USA). Serum osmolality was measured by freezing point depression (6 M Osmometer, Löser Messetechnik, Berlin, Germany).

2.6. Statistical methods

Due to the exploratory nature of this study and lack of previous data to inform potential differences, a power calculation to estimate sample size was not performed. Normality of data was assessed by visual inspection of histograms and Q-Q plots. Patient characteristics were summarised using number (percentage) for categorical variables, and median [Q1–Q3] or mean (95% confidence interval) for continuous variables depending on normality. Between-group differences for baseline characteristics were tested using linear regression for normally distributed continuous variables, Fisher's exact test for dichotomous variables, negative binomial regression for count variables and Wilcoxon rank sum test for continuous variables not normally distributed.

Changes in clinical variables from baseline were compared using linear random effects regression models. Volumes of fluid delivered between each time point were compared using Wilcoxon rank sum test. Biomarker concentrations were log-transformed to produce normal or approximately normal distributions and summarised using geometric mean (95% confidence interval). Comparisons of change in biomarker concentration over time were made using either linear random effects regression or Tobit random effects regression models. For each biomarker, change from T0 was expressed as fold-change (95% confidence interval). Patients that had completed research blood sampling at T24

Table 1
Modified Sequential Organ Failure Assessment score scheme for Emergency Department patients with suspected sepsis.

Organ system	Criteria	0	1	2	3	4
Respiratory	$Pa0_2/Fi0_2$	>400	<400	<300	<200	<100
	$Sp0_2/Fi0_2$	>400	<400	<315	<235	<150
	$Sp0_2$	>94% on 0.21	>94% on 6 LPM	<90% on >6 LPM	+ resp support	+ resp support
Cardiovascular	Systolic blood pressure (mmHg)	SBP > 90 at all times	SBP > 90 after fluid bolus >20 mL/kg	SBP < 90 despite fluid bolus		
	Vasopressor			Dopamine ≤5	Dopamine > 5 Noradrenaline ≤ 0.1	Dopamine > 15 Noradrenaline > 0.1
Haematologic	Platelet count ($x10^9$/L)	>150	100–150	50–99	20–49	<20
GIT	Bilirubin (μmol/L)	<20	20–32	33–101	102–204	>204
CNS	GCS score	15	13–14	10–12	6–9	3–5
Renal	Creatinine (μmol/L)	<110	110–170	171–300	301–440	>440
	Urine output (mL/kg/h)		<0.5 for 2 h			

Abbreviations: CNS, central nervous system; GCS, Glasgow Coma Scale; GIT, gastrointestinal; $Fi0_2$, inspired oxygen concentration; LPM, litres per minute of oxygen; $Pa0_2$, arterial partial pressure oxygen concentration; SBP, systolic blood pressure; $Sp0_2$, pulsatile blood oxygen saturation.

were further followed for clinical progression by comparing components of their SOFA score. Proportion of patients with organ failure (SOFA score > 0) (Table 1) was compared between groups using Fisher's exact test for each time period. Hospital length of stay was compared between groups using Wilcoxon rank sum test.

All analyses were performed using Stata 14 (College Station, TX, USA) with significance set at two-sided $P < .05$.

3. Results

3.1. Baseline characteristics

This study included 31 patients randomised to the isotonic group and 34 patients randomised to the hypertonic group (Fig. 1). There were no significant differences between baseline characteristics

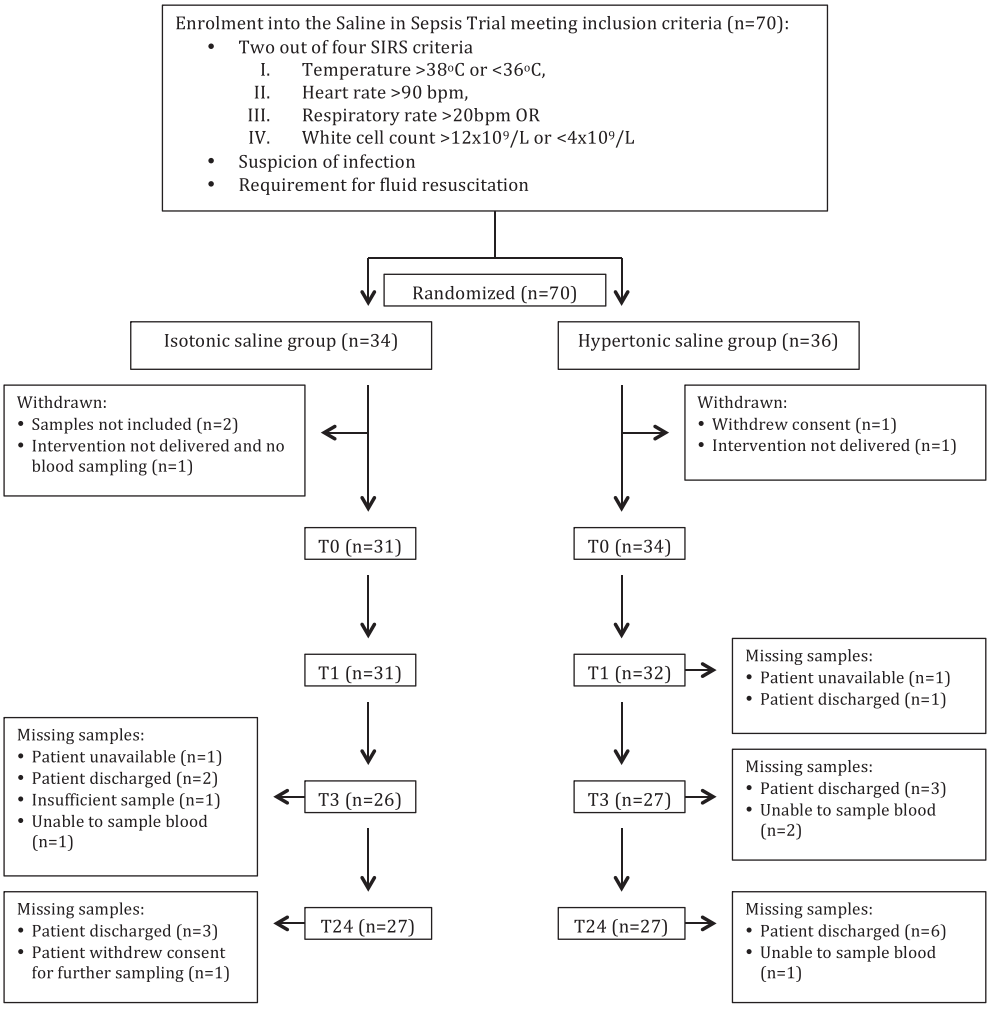

Fig. 1. Study flow chart.

Table 2
Characteristics of Emergency Department (ED) patients with suspected sepsis that received either 10 mL/kg of 0.9% NaCl (Isotonic) or 5 mL/kg of 3% NaCl (Hypertonic).

Characteristic	Isotonic group (n = 31)	Hypertonic group (n = 34)	*P* value
Age (years)	45 (39–52)	41 (35–47)	0.83
Male, n (%)	18 (58)	21 (62)	0.76
Body weight (kg)[a]	89 [75–95]	85 [70–95]	0.81
CCS	0 [0–1]	0 [0–1]	0.45
Discharge diagnosis, n (%)			
Not infection	3 (10)	3 (9)	–
Suspected infection	9 (29)	11 (32)	–
Confirmed infection	19 (61)	20 (59)	0.96[b]
Admission parameters			
Heart rate (bpm)	104 (97–111)	102 (97–108)	0.58
Respiratory rate (bpm)	21 (19–23)	22 (20−23)	0.50
Temperature (°C)	38.3 (37.9–38.7)	38.2 (37.8–38.6)	0.65
GCS	15 (15–15)	15 (15–15)	–
MAP (mmHg)	92 (86–98)	90 (86–95)	0.67
WBCC ($\times 10^9$/L)	10.6 [8.9–14.0]	12.7 [10–17]	0.16
SOFA score in ED	0 [0–1]	0 [0–1]	0.33
C-reactive protein (mg/mL)	97 [13–180]	99 [38–150]	0.91
Lactate (mmol/L)	1.5 [1.0–2.1]	1.4 [0.9–2.2]	0.96
Creatinine (μmol/L)	75 (69–81)	77 (70–84)	0.66
Time admit-to-fluid (mins)[c]	116 (90–133)	102 (88–117)	0.46

Data is presented as either median [Q1-Q3], mean (95% confidence interval) or number (percentage).
Abbreviations: CCS, Charlson Comorbidity Score; GCS, Glasgow Coma Score; MAP, mean arterial blood pressure; SOFA, sequential organ failure assessment; WBCC, white blood cell count.
[a] Body weight was available for 26 in the isotonic group and 33 in the hypertonic group.
[b] Fisher's exact test across groups.
[c] Time from ED admission to delivery of intervention fluid.

(Table 2). Of patients with confirmed infection, 21 had positive bacterial culture (13 in the isotonic group, 8 in the hypertonic group), 9 had infection confirmed on imaging results (3 in the isotonic group, 6 in the hypertonic group), and 4 patients had positive viral serology results (2 in each group). The most frequent source of infection was lung (n = 15), followed by urinary tract (n = 15) and soft tissue (n = 9).

3.2. Change in biomarker concentrations over time

Summarised biomarker concentrations and fold-change from T0 for each subsequent time point are presented in Table 3. There was a significantly decreased fold-change in syndecan-1 at T1 in the isotonic group, compared to the hypertonic group ($P = .012$). There were no significant differences between the two groups in fold-change for hyaluronan, soluble intercellular adhesion molecule-1 or soluble vascular cell adhesion molecule-1 at any time point.

There was a significantly decreased fold-change in IL-10 at T24 in the isotonic group, compared to the hypertonic group ($P = .006$). Otherwise, there were no significant differences between the two groups in fold-change for inflammatory cytokines IL-6, IL-8, neutrophil gelatinase-associated lipocalin or resistin. Interferon-γ was below the detectable limit for 78% of samples. There was no significant difference in number of samples with measurable interferon-γ, *versus* unmeasurable, at any time point between the two groups (data not shown).

3.3. Clinical variables

Serum osmolality in participants in the hypertonic group was significantly increased from baseline at T1 ($P < .001$) and T3 ($P = .004$), compared to the isotonic group, but there was no significant difference between groups by T24 ($P = .59$) (Fig. 2). There were no significant changes from baseline between the two groups in heart rate, mean arterial blood pressure or haemoglobin concentration over time (Table 4).

3.4. Fluids administered

Only patients in the hypertonic group received 3% saline during the study period (Fig. 3). The isotonic group received significantly more 0.9% saline and total volume of fluids between T0 and T1 (both $P <$

Table 3
Concentrations (geometric mean, 95% CI) of endothelial activation, glycocalyx shedding and inflammation biomarkers in Emergency Department patients with suspected sepsis that received either 10 mL/kg of 0.9% NaCl (Isotonic) or 5 mL/kg of 3% NaCl (Hypertonic) measured at baseline (T0), 1 h after start of fluid intervention (T1), and then 3 h (T3) and 12–24 h (T24) later. Comparison between groups in change from T0 (fold change (95% CI) is also provided).

	Biomarker concentration (geometric mean, 95% CI)				Fold change (95% CI) from T0					
	T0	T1	T3	T24	T1	*P*	T3	*P*	T24	*P*
Syndecan-1 (ng/mL)										
Isotonic	1.5 (1.2–1.9)	1.2 (1.0–1.6)	1.4 (1.0–2.0)	2.0 (1.6–2.5)	0.8 (0.7–0.9)		1.0 (0.9–1.2)		1.3 (1.2–1.5)	
Hypertonic	1.4 (1.1–1.8)	1.4 (1.1–1.8)	1.5 (1.1–2.0)	2.0 (1.5–2.7)	1.0 (0.9–1.1)	0.012	1.1 (1.0–1.3)	0.41	1.5 (1.3–1.7)	0.42
Hyaluronan (ng/mL)										
Isotonic	5.9 (3.6–9.6)	14.7 (10.3–21.1)	15.3 (10.4–22.4)	26.0 (16.4–41.3)	2.5 (1.7–3.7)		2.6 (1.7–3.9)		4.1 (2.8–6.1)	
Hypertonic	4.8 (2.5–9.1)	20.2 (13.8–29.7)	11.8 (6.7–20.5)	15.2 (8.5–27.4)	3.9 (2.7–5.7)	0.11	2.7 (1.8–4.0)	0.92	3.4 (2.3–5.0)	0.49
Interleukin-6 (pg/mL)										
Isotonic	77 (39–153)	62 (32−120)	65 (28–151)	38 (18–78)	0.8 (0.5–1.2)		0.8 (0.5–1.3)		0.5 (0.3–0.8)	
Hypertonic	69 (36–133)	62 (31–125)	48 (24–96)	36 (16–83)	0.9 (0.6–1.3)	0.73	0.7 (0.4–1.0)	0.43	0.4 (0.3–0.6)	0.44
Interleukin-8 (pg/mL)										
Isotonic	41 (25–66)	41 (28–58)	47 (27–83)	50 (32–80)	1.0 (0.7–1.4)		1.2 (0.9–1.6)		1.2 (0.9–1.6)	
Hypertonic	20 (12–34)	23 (13–39)	23 (14–40)	28 (18–46)	1.1 (0.8–1.5)	0.79	1.2 (0.8–1.6)	0.91	1.3 (0.9–1.8)	0.65
Interleukin-10 (pg/mL)										
Isotonic	1.9 (0.6–6.3)	1.3 (0.4–4.2)	0.7 (0.2–2.2)	0.4 (0.2–0.9)	0.7 (0.3–1.9)		0.3 (0.1–1.0)		0.1 (0.0–0.3)	
Hypertonic	0.5 (0.2–1.3)	0.5 (0.2–1.3)	0.8 (0.3–2.1)	0.5 (0.2–1.4)	0.9 (0.3–2.8)	0.72	1.2 (0.4–3.5)	0.10	0.8 (0.3–2.6)	0.006
NGAL (ng/mL)										
Isotonic	114 (91–142)	103 (84–126)	119 (93–151)	144 (91–230)	0.9 (0.7–1.3)		1.1 (0.7–1.5)		1.3 (0.9–1.9)	
Hypertonic	109 (80–149)	100 (67–150)	95 (63–142)	167 (121−231)	0.9 (0.7–1.3)	0.94	0.9 (0.7–1.3)	0.65	1.5 (1.1–2.1)	0.57
Resistin (ng/mL)										
Isotonic	31 (25–39)	28 (22–35)	33 (27–40)	42 (30–57)	0.9 (0.8–1.0)		1.1 (0.9–1.2)		1.4 (1.2–1.6)	
Hypertonic	36 (29–46)	34 (27–44)	31 (24–40)	42 (31–57)	1.0 (0.9–1.1)	0.45	1.0 (0.8–1.1)	0.32	1.2 (1.1–1.4)	0.15
sICAM-1 (μg/mL)										
Isotonic	2.2 (0.8–6.4)	1.9 (0.7–5.2)	0.9 (0.3–2.6)	2.1 (0.7–6.7)	0.9 (0.6–1.2)		0.8 (0.5–1.1)		1.0 (0.7–1.5)	
Hypertonic	0.3 (0.2–0.6)	0.4 (0.2–0.7)	0.4 (0.2–1.0)	0.3 (0.2–0.7)	1.1 (0.8–1.5)	0.32	1.2 (0.9–1.7)	0.06	1.0 (0.7–1.4)	0.89
sVCAM-1 (μg/mL)										
Isotonic	6.5 (2.2–19.5)	5.6 (2.0–16.2)	2.9 (1.0–8.3)	6.0 (1.8–19.3)	0.9 (0.6–1.2)		0.8 (0.6–1.1)		1.0 (0.7–1.4)	
Hypertonic	1.2 (0.6–2.7)	1.4 (0.6–3.1)	1.5 (0.6–3.4)	1.3 (0.6–2.6)	1.1 (0.8–1.4)	0.38	1.0 (0.7–1.3)	0.50	1.0 (0.7–1.4)	0.87

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular adhesion molecule-1.

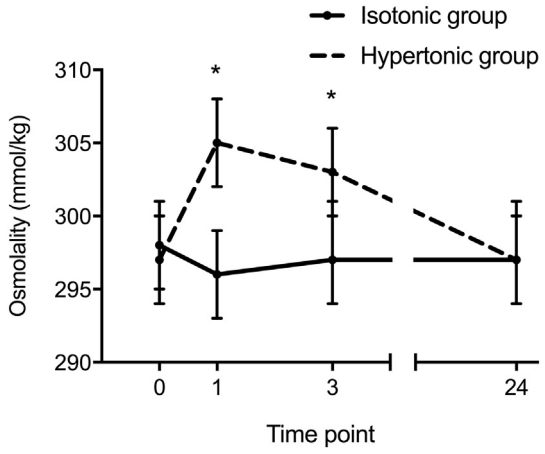

Fig. 2. Serum osmolality (mean, 95% confidence interval) in Emergency Department patients with suspected sepsis that received either 10 mL/kg of 0.9% NaCl (Isotonic) or 5 mL/kg of 3% NaCl (Hypertonic) measured at baseline (T0), 1 h after start of fluid intervention (T1), and then 3 h (T3) and 12–24 h (T24) later. Asterisks denote significant ($P < .05$) differences between groups in change from T0.

.001), compared to the hypertonic group, but there were no significant differences between groups in volume of 0.9% saline or total fluid volume administered between T1 to T3 ($P = .37$, $P = .68$, respectively) or T3 to T24 ($P = .94$, $P = .14$, respectively) (Fig. 3). One patient in the hypertonic group reported pain above the cannula insertion site therefore the study fluid rate was halved.

The most frequent fluid type administered after the study fluid was 0.9% saline. Eight patients in the isotonic group received an additional fluid type, compared to 3 patients in the hypertonic group ($P = .068$). Fluid types other than 0.9% saline included compound sodium lactate, 4% succinylated gelatine and albumin solution.

3.5. Clinical outcomes

There were significantly more patients with cardiovascular failure on day 1 in the isotonic group, compared to the hypertonic group ($P = .014$) (Table 5). On day 1, 4 patients in the isotonic group required at least 20 mL/kg of intravenous fluids to maintain blood pressure (n = 1) and/or required vasopressor support (n = 3) whereas none of the patients in the hypertonic group required either types of blood pressure support. Also, there were significantly more patients with haematologic failure in the isotonic group on day 1, compared to the hypertonic group ($P = .026$), confluent with decreases in platelet count (Table 5). Event rate was too low for central nervous system and renal failure to be compared (data not shown).

There was no significant difference in length of hospitalisation between the isotonic group (median 2.4 days, Q1–Q3 1.2–4.2) and hypertonic group (3.1 days, Q1–Q3 1.9–5.2) ($P = .26$). Only one patient was admitted to the ICU (isotonic group) and only one patient died in each of the groups.

Table 5
Number of participants that received either 10 mL/kg of 0.9% NaCl (Isotonic) or 5 mL/kg of 3% NaCl (Hypertonic) with organ failure detected in the Emergency Department (ED) or on day 1 or 2 of hospitalisation. Organ failure for each category was defined as a Sequential Organ Failure Assessment score > 0.

	In ED	Day 1	Day 2
Cardiovascular failure			
Isotonic group			
Yes	0	4	2
No	27	22	17
Hypertonic group			
Yes	2	0	1
No	25	27	21
P value	0.091	0.014	0.46
Hematologic failure			
Isotonic group			
Yes	4	8	6
No	23	18	13
Hypertonic group			
Yes	3	2	4
No	24	25	18
P value	0.69	0.026	0.32
Respiratory failure			
Isotonic group			
Yes	5	7	2
No	22	19	17
Hypertonic group			
Yes	5	7	3
No	22	20	19
P value	–	0.93	0.76
Gastrointestinal failure			
Isotonic group			
Yes	2	2	1
No	25	24	18
Hypertonic group			
Yes	5	6	3
No	22	21	19
P value	0.22	0.13	0.36

4. Discussion

This pilot study demonstrated that a single bolus of hypertonic saline given to ED patients with suspected sepsis resulted in a significant increase in serum osmolality, compared to a single bolus of isotonic saline. However, administration of hypertonic saline did not reduce biomarker concentrations of EG shedding, endothelial activation or inflammatory biomarker concentrations, though small differences were found in fold-change of syndecan-1 and IL-10 at isolated time points. These minor differences were considered of low relevance given the distribution of the raw biomarker data at each time point. Additionally,

Table 4
Clinical variables (mean (95% confidence interval)) of Emergency Department patients with suspected sepsis that received either 10 mL/kg of 0.9% NaCl (Isotonic) or 5 mL/kg of 3% NaCl (Hypertonic), measured at baseline (T0), 1 h after start of fluid intervention (T1), and then 3 h (T3) and 12–24 h (T24) later.

Variable	n	T0	n	T1	n	T3	n	T24
Heart rate (bpm)								
Isotonic	30	104 (97–111)	30	98 (92–104)	24	93 (87–100)	24	85 (79–91)
Hypertonic	34	102 (96–108)	31	95 (89–102)	27	87 (87–99)	26	82 (76–88)
P value				0.80		0.85		0.61
Mean arterial pressure (mmHg)								
Isotonic	30	92 (86–98)	30	87 (82–93)	24	86 (80–92)	24	85 (80–90)
Hypertonic	34	90 (86–95)	31	88 (82–94)	27	86 (80–91)	25	91 (85–96)
P value				0.43		0.88		0.075
Haemoglobin (g/L)								
Isotonic	30	143 (137–150)	25	133 (125–141)	17	139 (131–146)	23	131 (123–139)
Hypertonic	33	142 (137–147)	24	132 (127–137)	22	132 (127–137)	21	131 (125–136)
P value				0.95		0.86		0.26

P values are for differences between groups in change from baseline.

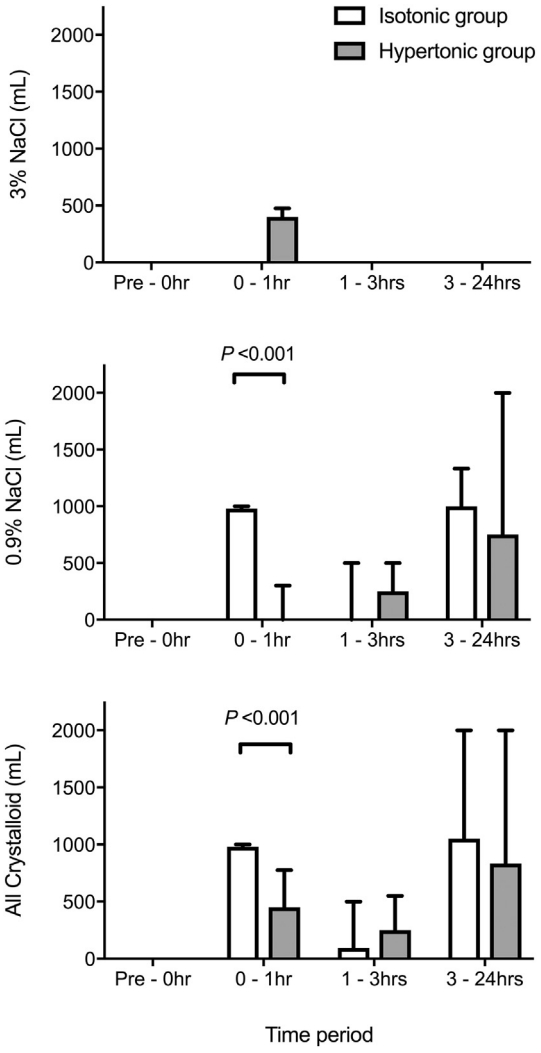

Fig. 3. Fluid volumes (median, interquartile range) of 3% NaCl, 0.9% NaCl and all crystalloid fluid delivered to Emergency Department patients with suspected sepsis that were randomised to receive either 10 mL/kg of 0.9% NaCl (Isotonic) or 5 mL/kg of 3% NaCl (Hypertonic) between 0 and 1 h.

hypertonic saline did not significantly reduce the volume of subsequent crystalloid administered.

Studies in trauma patients that have received hypertonic saline have found less leucocyte and endothelial activation, lower inflammatory cytokine concentrations and fewer post-operative infections, compared to isotonic crystalloid [31-34]. This effect has not been replicated thus far in patients with sepsis. Although one pilot study found that hypertonic saline reduced the volume of subsequent fluid administration [38], there were no significant differences in gene expression for inflammatory cytokines compared to an isotonic fluid [39]. These results are difficult to interpret as the fluids used in this study were combined with hydroxyethyl starch, known to suppress cytokine production [40-42].

Lack of treatment effect observed in this study may have been due to only achieving a modest increase of ~10 mmol/kg in serum osmolality (Fig. 3). A greater change in osmolality may have been required in order to mitigate inflammation. For the purpose of simplicity, studies that have measured osmolarity are described here as osmolality, with recognition that these measurements have slight differences. Previous studies demonstrating immunomodulatory effects of hypertonic saline *in vitro* have used concentrations of saline at an osmolality of ~340 mmol/kg or higher [21,22,26,29,43-45]. In animal models demonstrating anti-inflammatory effects of hypertonic saline, studies have achieved a minimum of ~325 to 350 mmol/kg within 1 h of infusion [22,46]. Lack of treatment effect in this study may also be due to the timing of hypertonic saline delivery in relation to injury. Two different studies support that hypertonic saline has little effect if given after leucocyte activation [23] or is delayed beyond the onset of inflammation [47]. This may negate the immunomodulatory effects of hypertonic saline in the setting of sepsis, whereby there is little opportunity to deliver a fluid intervention close to the time of illness onset.

In this study, there was no difference in volumes of subsequent crystalloid administered, due to additional open-label administration of 0.9% saline to patients in the hypertonic group. We were unable to determine from clinical data in medical records what prompted further fluid administration but this may have been driven by lack of blinding and clinician bias against withholding 0.9% saline. This additional fluid administration may have blunted any treatment effect on biomarker outcomes. The other reason for lack of treatment effect may have been due to the relatively mild severity of illness of this cohort; a consequence of the HREC requirement to restrict the trial only to those who could provide written consent. It is possible that inclusion of sicker patients or stricter protocolisation of subsequent fluid administration may have yielded different results.

Strengths of this study include delivery of the intervention fluid early in the treatment of suspected sepsis, as well as frequent blood sampling during initial patient stabilisation. This study demonstrates the feasibility of achieving hyperosmolality with a single dose of 3% saline, which has not been previously reported in this type of patient cohort. Patients in the hypertonic group also achieved similar blood volume expansion, based on no difference in haemoglobin concentration at T1, supporting the effect of hypertonic saline drawing fluid from the extravascular space. Limitations include the lack of blinding and mild severity of illness in this patient cohort. Although the finding of more patients in the isotonic group having cardiovascular failure on day 1 of hospitalisation is provocative, the result may be coincidental in such a small sample size. This study would have also been strengthened by assessment of serum sodium, chloride and base excess concentrations, however lack of these measurements at T24 for most patients precluded this analysis. The biomarkers used in this study were chosen on the basis of previous work demonstrating their association with severity of sepsis [13,48], however, broadening the spectrum of biomarker assessment may have been more informative. Given the small sample size, variations in baseline biomarker concentrations may have influenced the results, despite only statistically comparing fold-change in biomarker concentrations. Recommendations for future research in this area include selection of patients with a higher severity of illness, blinding of the intervention fluid and protocolisation of subsequent fluid administration aimed at achieving clinical endpoints.

In conclusion, delivery of a single bolus of 3% hypertonic saline in ED patients with suspected sepsis increased serum osmolality but did not reduce biomarkers of endothelial glycocalyx shedding, endothelial activation or inflammation, compared to patients that received 0.9% saline.

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Declarations of interest

None.

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