

# Fluid resuscitation associated with elevated angiotensin-2 and length of mechanical ventilation after cardiac surgery

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Intravenous fluid therapy is a ubiquitous component of post-operative intensive care unit (ICU) care for patients who had cardiac surgery.<sup>1</sup> Recent evidence suggests that fluid restriction results in more ventilator-free days, has shown better outcomes in general surgical populations,<sup>2,3</sup> and facilitates faster gastrointestinal functional recovery, including a reduction in tissue oedema and improved wound healing.<sup>4</sup> While a positive fluid balance is associated with poor lung,<sup>5-7</sup> renal<sup>8</sup> and gastrointestinal function<sup>2,9</sup> and an increased risk of morbidity and mortality,<sup>10-14</sup> the mechanism behind the increase in adverse effects with fluid therapy remains speculative.

Angiotensin-1 (Ang-1) is required for blood vessel stabilisation and angiotensin-2 (Ang-2) is a blood vessel destabiliser and promotes inflammation, which results in their ratio being important for vascular stability and integrity.<sup>15</sup> Ang-2 is also a marker of endothelial lung injury, which is increased in patients with acute respiratory distress syndrome,<sup>16-18</sup> and predicts development of lung injury in at-risk patients,<sup>19</sup> while the ratio of Ang-1/Ang-2 predicts mortality in patients with lung injury.<sup>20,21</sup> Recent evidence suggests Ang-2 plays a critical role in cytokine-induced vascular leak through inflammation,<sup>22</sup> endothelial glycocalyx breakdown<sup>23</sup> and increase in its permeability, especially in patients undergoing bypass surgery.<sup>24-26</sup> We recently demonstrated both an increase in Ang-2 and a corresponding decrease in Ang-1/Ang-2 ratio in healthy volunteers receiving bolus 0.9% saline who developed interstitial pulmonary oedema<sup>27</sup> and in an animal model of fluid induced direct lung injury.<sup>28</sup> These findings are consistent with conservative fluid therapy preferentially lowering plasma Ang-2 levels over time, decreasing endothelial inflammation in patients with lung injury.<sup>16</sup> Similarly, increases in both P-selectin<sup>29</sup> and phospholipase A<sub>2</sub> (PLA<sub>2</sub>)<sup>30</sup> have been associated with increased permeability of the alveolocapillary barrier, disruption to the alveolar septal barrier and with acute lung injury. Finally, previous evidence suggests that elevated hydrostatic pressures in the lung can induce pulmonary inflammation.<sup>29</sup> Consequently, we hypothesised that a conservative bolus fluid administration strategy may lead to decreased length of mechanical

## ABSTRACT

**Background:** Fluid restriction in patients with acute respiratory distress syndrome increases ventilator-free days while lowering plasma angiotensin-2 (Ang-2), a marker of pulmonary endothelial injury. We hypothesised that fluid resuscitation may lead to endothelial injury after cardiac surgery and analysed Ang-2, angiotensin-1 (Ang-1) and phospholipase A<sub>2</sub> (PLA<sub>2</sub>) levels and the impact of fluid management on ventilation time.

**Methods:** Patients enrolled in a single-centre, prospectively randomised interventional study of liberal or conservative fluid resuscitation strategy had plasma Ang-2, Ang-1 and PLA<sub>2</sub> levels measured at baseline (pre-operative), 6 and 24 hours after commencement of cardiopulmonary bypass, and analysed by linear mixed models as liberal v conservative (intention to treat) or high v low fluid group (actual treatment,  $\geq 3250$  mL of fluid administered), and further subclassified as EuroSCORE (European System for Cardiac Operative Risk Evaluation) II  $\geq 0.9$  or  $< 0.9$ .

**Results:** Over 9 months, 144 patients were randomly allocated to either liberal ( $n = 74$ ) or conservative ( $n = 70$ ) fluid. Patients in the liberal fluid arm tended to an increased Ang-2 ( $P = 0.12$ ) and had higher PLA<sub>2</sub> levels ( $P = 0.03$ ). Based on actual fluid administered, Ang-2 levels were higher, the Ang-1/Ang-2 ratio lower, and the length of mechanical ventilation and intensive care unit (ICU) stay was longer in the high fluid group ( $P < 0.001$ ). The highest levels of Ang-2 and corresponding lowest Ang-1/Ang-2 ratio, along with longest length of mechanical ventilation and ICU stay, were found with both the liberal and high fluid groups in patients with a EuroSCORE II  $\geq 0.9$  ( $P < 0.01$ ).

**Conclusion:** Liberal fluid resuscitation after cardiac surgery was associated with both pulmonary endothelial injury and prolonged length of mechanical ventilation.

**Clinical trial registration:** ACTRN12612000754842

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ventilation and length of stay after cardiac surgery when compared with a liberal fluid strategy, and that this may be associated with lessened endothelial injury.

To examine this hypothesis, we analysed blood samples from our previously published randomised controlled trial, which used stroke volume variation to reduce intravenous fluid administration after scheduled cardiac surgery.<sup>31</sup> The aim of this supplementary study was, therefore, to examine temporal changes to plasma markers of endothelial injury and activation, Ang-1, Ang-2, PLA<sub>2</sub> and soluble P-selectin in these patients managed with conservative (intervention) versus liberal (usual care) fluid bolus strategy.

## Methods

The fluid therapy after cardiac surgery study (Australia and New Zealand Clinical Trials Registry, ACTRN12612000754842) was a single-centre, prospectively randomised interventional study designed to assess the ability of a fluid administration protocol (a stroke volume variation-based algorithm) to provide a clinically significant reduction in intravenous fluid after cardiac surgery.<sup>30</sup> It included patients (aged > 16 years) expected to have cardiac surgery using cardiopulmonary bypass, and excluded patients undergoing an emergency procedure, patients with an intra-aortic balloon pump and patients with atrial fibrillation. The intervention was a protocolised strategy for administering bolus fluid after the operation, from admission to ICU until extubation or 24 hours, as compared with usual care. The study methods, fluid administration algorithm and results have been previously published.<sup>30</sup>

The study complied with the Declaration of Helsinki, the supplementary protocol was approved by the Southern Adelaide Clinical Human Research Ethics Committee, Flinders Medical Centre, and all participants provided written informed consent. Participants enrolled in the main study were informed of the supplementary study and given the opportunity to opt out of it. All patients' data were used for the study. Ethics approval for the study was obtained from the Northern A Health and Disability Ethics Committee, New Zealand (12/NTA/2).

## Preparation of blood samples

Between 31 January 2013 and 11 October 2013, venous blood samples were collected at baseline (before the operation); 6 hours after commencement of bypass and 24 hours after commencement of bypass, then centrifuged and 1.5 mL plasma frozen at -80°C on site at Auckland City Hospital. Batched plasma samples were transported by overnight courier on dry ice to the Lung Injury Research Laboratory at Flinders University in Adelaide, South Australia, and returned to -80°C until analysis (undertaken 18 January 2016 – 9 March 2016).

## Immunoassays

Plasma samples were assayed by EIA for PLA<sub>2</sub> (Cayman Chemical, Ann Arbor, MI, USA), and by ELISA for sP-selectin, Ang-1 (R and D Systems, Minneapolis, MN, USA) and Ang-2 (AbCam, Cambridge, MA, USA), according to the manufacturers' instructions.

## Statistical analysis

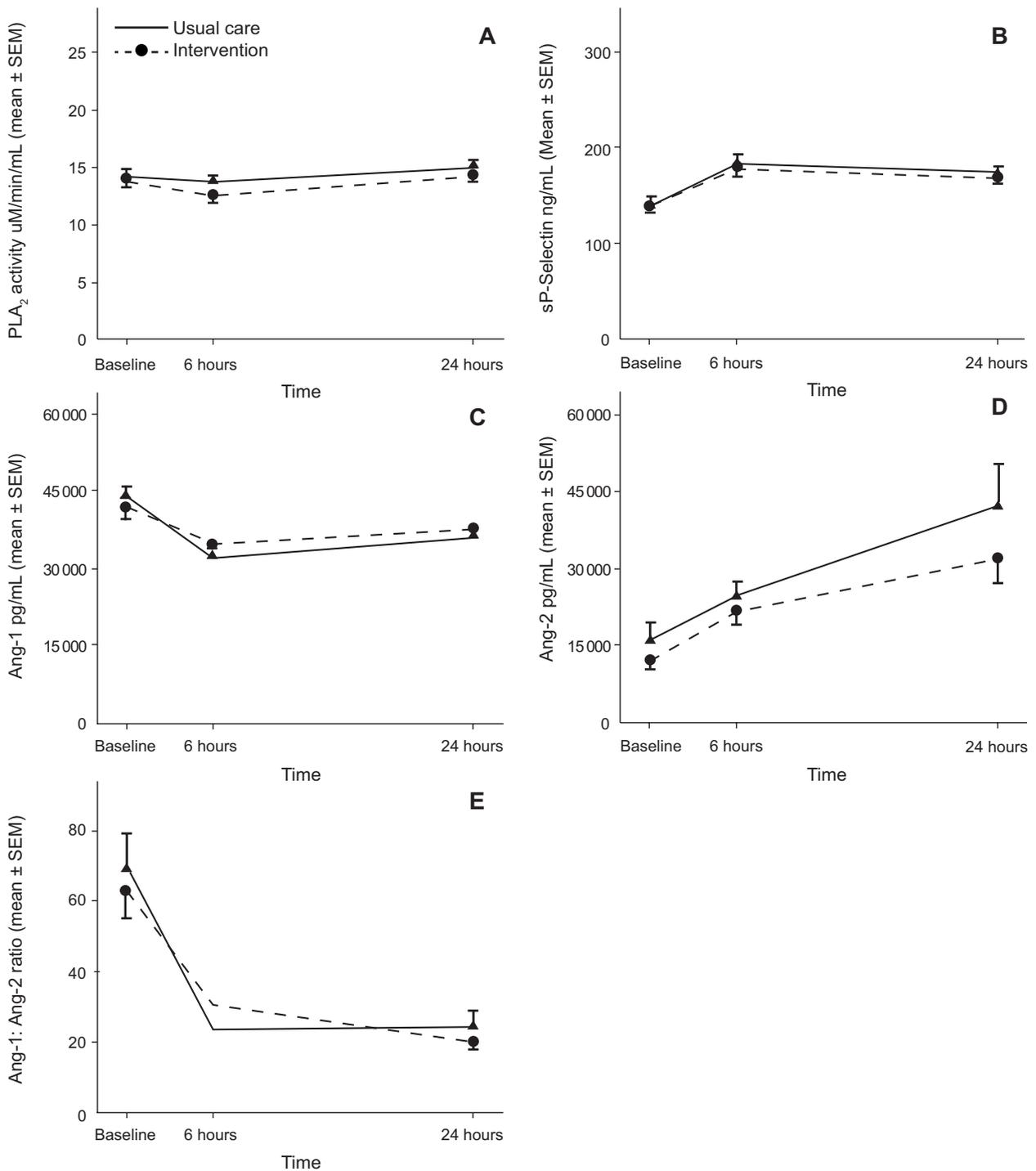
Statistical analyses were performed using SPSS Statistics 23.0 (IBM). Variables were assessed for normality by the Kolmogorov–Smirnov test and log-transformed when necessary for statistical analyses. Baseline and clinical continuous variables were compared using independent samples *t* test or two-way ANOVA, and categorical variables by Pearson's  $\chi^2$  test as appropriate. Temporal plasma mediator data were examined by linear mixed models as liberal versus conservative (intention to treat) and high versus low fluid group (dichotomised according to actual treatment, based on the median 3250 mL of fluid administered). Secondary analysis further subclassified each group according to EuroSCORE (European System for Cardiac Operative Risk Evaluation) II  $\geq 0.9$  or  $< 0.9$ . EuroSCORE is a validated method of calculating predicted operative mortality for patients undergoing cardiac surgery.<sup>31,32</sup> By using a cut-off of 0.9, we have dichotomised patients into two groups: those that have a EuroSCORE II  $< 0.9$  and have no comorbidities present at baseline, and those with a EuroSCORE II  $\geq 0.9$  and have one or more comorbidities at baseline. We theorised that patients with no comorbidity may behave differently to those with one or more comorbidities, and had found in our feasibility study a significant difference in both fluid administration reduction and ICU length of stay in the group with a EuroSCORE II  $\geq 0.9$ . Further,  $P \leq 0.05$  was considered significant.

**Table 1. Baseline levels of Ang-1, Ang-2, PLA<sub>2</sub> and P-selectin between the groups (usual care and interventional arm)\***

Baseline plasma markers	Intervention	Usual care	<i>P</i>
Ang-1 (ng/mL)	42.0 ± 19.7	44.0 ± 18.1	0.33
Ang-2 (ng/mL)	1.2 ± 1.0	1.6 ± 2.8	0.72
Ang-1/Ang-2	62.9 ± 64.6	69.2 ± 83.4	0.87
PLA <sub>2</sub> (μM/min/mL)	13.9 ± 4.9	14.3 ± 3.8	0.53
P-selectin (ng/mL)	137 ± 43	143 ± 44	0.58

Ang-1 = angiotensin-1. Ang-2 = angiotensin-2. PLA<sub>2</sub> = phospholipase A<sub>2</sub>. \* Values are shown in mean (standard deviation), by independent samples *t* test. Variables were tested for normality by Kolmogorov–Smirnov and log-transformed where necessary.

**Figure 1. Plasma markers of endothelial injury and activation in patients managed with conservative (intervention) versus liberal (usual care) fluid bolus strategy, by linear mixed model analysis**



Ang-1 = angiotensin-1. Ang-2 = angiotensin-2. PLA<sub>2</sub> = phospholipase A<sub>2</sub>. SEM = standard error of the mean. **A.** PLA<sub>2</sub>: group (*Gp*), *P* = 0.03; time, *P* = 0.007; *Gp*\*time, *P* = 0.69. **B.** Soluble P-selectin: group, *P* = 0.29; time *P* ≤ 0.001; *Gp*\*time, *P* = 0.99. **C.** Ang-1: group, *P* = 0.72; time, *P* ≤ 0.001; *Gp*\*time, *P* = 0.55. **D.** Ang-2: group, *P* = 0.12; time, *P* ≤ 0.001; *Gp*\*time, *P* = 0.78. **E.** Ang-1/Ang-2: group *P* = 0.81; time, *P* ≤ 0.001; *Gp*\*time, *P* = 0.19.

**Table 2. Patients in usual care and interventional arm further examined based on pre-operative EuroSCORE II\***

	Intervention		Usual care		<i>P</i> ≤
	EuroSCORE < 0.9 <i>n</i> = 29	EuroSCORE ≥ 0.9 <i>n</i> = 41	EuroSCORE < 0.9 <i>n</i> = 31	EuroSCORE ≥ 0.9 <i>n</i> = 43	
Length of mechanical ventilation (h) <sup>†</sup>	13.0 ± 6.3	17.3 ± 18.2	14.2 ± 8.1	42.8 ± 110.6	0.008
Ventilator free hours (24 h) <sup>†</sup>	11.5 ± 4.7	10.7 ± 5.5	11.0 ± 5.3	7.5 ± 6.7	0.002
ICU length of stay (h) <sup>†</sup>	30.3 ± 29.4	43.4 ± 45.6	23.5 ± 12.8	69.7 ± 115.3	0.001
Total fluid bolus admission to 24 h <sup>†</sup>	3114 ± 1758	3208 ± 2489	3559 ± 2206	4447 ± 2649	0.052
Total fluid admission to 24 h <sup>†</sup>	4703 ± 1999	4698 ± 2629	5033 ± 2217	6051 ± 2562	0.037
Fluid balance admission to 24 h	1026 ± 1253	1729 ± 2013	1566 ± 1310	2108 ± 1834	0.068
<b>Baseline plasma markers</b>					
Ang-1 (ng/mL) <sup>†</sup>	45.9 ± 20.7	39.2 ± 18.8	44.9 ± 17.2	43.4 ± 18.9	0.229
Ang-2 (ng/mL) <sup>†</sup>	0.8 ± 0.5	1.4 ± 1.2	1.2 ± 1.1	1.9 ± 3.6	0.359
Ang-1/Ang-2 <sup>†</sup>	88.9 ± 87.2	45.3 ± 84.6	64.7 ± 76.9	72.4 ± 88.5	0.154
PLA <sub>2</sub> (μM/min/mL) <sup>†</sup>	13.5 ± 2.9	14.2 ± 6.0	14.7 ± 3.7	14.1 ± 3.9	0.756
P-selectin (ng/mL) <sup>†</sup>	143 ± 40	133 ± 46	140 ± 47	144 ± 41	0.668

Ang-1 = angiotensin-1. Ang-2 = angiotensin-2. EuroSCORE = European System for Cardiac Operative Risk Evaluation. ICU = intensive care unit. PLA<sub>2</sub> = phospholipase A<sub>2</sub>. \* Values are shown in mean (standard deviation), by one-way ANOVA. † Variables were tested for normality by Kolmogorov-Smirnov and log transformed where necessary.

## Results

Over a 9-month period, 144 patients were randomly assigned to a group: 74 to the control arm and 70 to the intervention arm. There were 117 (81%) males in the study, with the participants undergoing coronary artery bypass graft (CABG) surgery alone (*n* = 83), valve surgery (*n* = 44) and combination of CABG and valve surgery (*n* = 16). There were no significant differences in baseline characteristics between the randomised groups.<sup>30</sup> Overall, less bolus fluid and less total overall fluid volume was administered in the intervention group, which also had greater ventilator-free hours when compared with the usual care group, as previously published.<sup>30</sup>

### Intention to treat analysis (usual care and intervention arm)

Baseline levels of Ang-1, Ang-2, PLA<sub>2</sub> and P-selectin were not different between the groups (Table 1). PLA<sub>2</sub> activity was reduced by 24 hours after surgery in the intervention group (*P* = 0.03), with a trend toward increased Ang-2 levels in the control group (*P* = 0.12) (Figure 1). There was no difference in Ang-1 or P-selectin levels between the groups at 6 or 24 hours.

When these patients were further classified as EuroSCORE II ≥ 0.9 and EuroSCORE II < 0.9, the length of mechanical

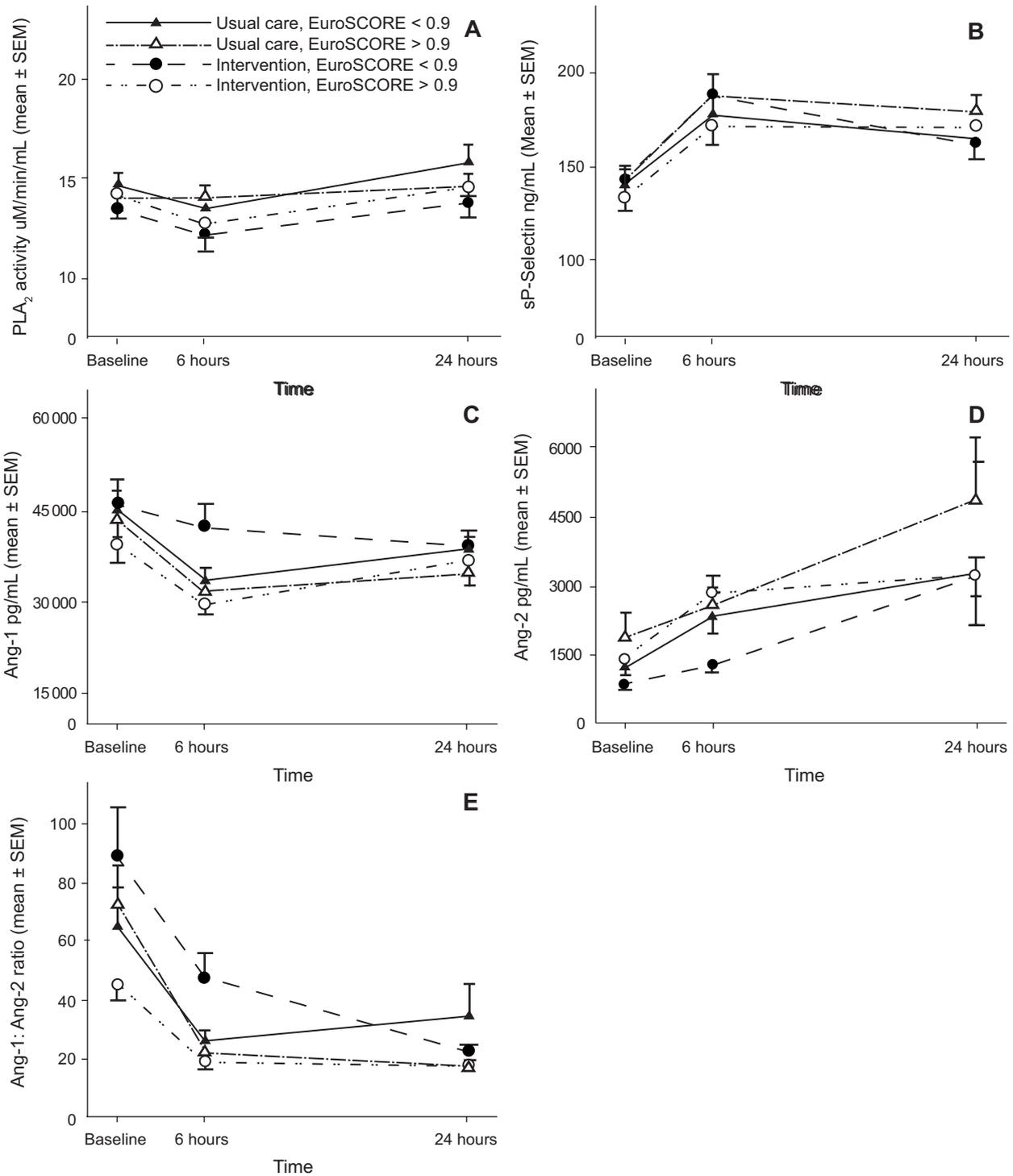
ventilation was longer in patients with EuroSCORE II ≥ 0.9 receiving high fluid intake; however, there was no difference in patients with EuroSCORE II < 0.9 (Table 2). Correspondingly, the greatest concentration of Ang-2 and the lowest Ang-1/Ang-2 ratio were found in the EuroSCORE II ≥ 0.9 subgroup of usual care patients (*P* < 0.01) (Figure 2).

### Analysis of high and low fluid groups (*n* = 72 in each group) dichotomised based on the median of 3250 mL fluid bolus

The low fluid group had lower positive fluid balance and both decreased length of mechanical ventilation and ICU length of stay (Table 3). Baseline plasma Ang-1, Ang-2, PLA<sub>2</sub> and P-selectin levels were not different between the groups (Table 3). However, Ang-2 was increased and, correspondingly, the Ang-1/Ang-2 ratio decreased in the high fluid group up to 24 hours after surgery (Figure 3). There was an increase in P-selectin with time, but there was no difference between PLA<sub>2</sub> and P-selectin between the groups.

When these patients were further classified ≥ 0.9 and < 0.9, based on EuroSCORE II, the length of mechanical ventilation and ICU stay were longer in patients with EuroSCORE II ≥ 0.9 receiving high fluid intake (Table 4), and the greatest concentration of Ang-2 and lowest Ang-1/Ang-2 ratio were found in patients with EuroSCORE II ≥ 0.9 receiving high fluid intake (*P* < 0.01) (Figure 4).

**Figure 2. Plasma markers of endothelial injury and activation in patients managed with conservative (intervention) versus liberal (usual care) fluid bolus strategy categorised by EuroSCORE II (low, < 0.9; or high, ≥ 0.9), by linear mixed model analysis**



Ang-1 = angiotensin-1. Ang-2 = angiotensin-2. EuroSCORE = European System for Cardiac Operative Risk Evaluation. PLA<sub>2</sub> = phospholipase A<sub>2</sub>. SEM = standard error of the mean. **A.** PLA<sub>2</sub>: group (Gp),  $P = 0.08$ ; time,  $P = 0.003$  (baseline [BL] v 6 h,  $P = 0.03$ ; 6 h v 24 h,  $P = 0.001$ ); Gp\*time,  $P = 0.81$ . **B.** Soluble P-selectin: group,  $P = 0.26$ ; time,  $P \leq 0.001$  (BL v 6 h and 24 h,  $P \leq 0.001$ ); Gp\*time,  $P = 0.83$ . **C.** Ang-1: group,  $P = 0.006$  (intervention\*EuroScore [euro] < 0.9 v usual care\*euro > 0.9 and intervention\*euro > 0.9;  $P \leq 0.007$ ); time,  $P \leq 0.001$  (BL v 6 h and 24 h,  $P \leq 0.002$ ); Gp\*time,  $P = 0.41$ . **D.** Ang-2: group,  $P = 0.005$  (intervention\*euro < 0.9 v all other groups;  $P \leq 0.04$ ); time,  $P \leq 0.001$  (all times v all groups;  $P \leq 0.001$ ); Gp\*time,  $P = 0.88$ . **E.** Ang-1/Ang-2: group,  $P = 0.003$  (intervention\*euro < 0.9 v usual care\*euro > 0.9 and intervention\*euro > 0.9;  $P \leq 0.02$ ; intervention\*euro > 0.9 v usual care\*euro < 0.9;  $P = 0.03$ ); time,  $P \leq 0.001$  (BL v 6 h and 24 h,  $P \leq 0.001$ ); Gp\*time,  $P = 0.02$ .

**Table 3. Baseline characteristics, levels of Ang-1, Ang-2, PLA<sub>2</sub> and P-selectin and outcomes between the high and low fluid groups\***

	Fluid bolus < 3250	Fluid bolus ≥ 3250	P ≤
Group (intervention) <sup>†</sup>	38 (56%)	32 (42%)	0.10
Age	62 ± 14	64 ± 12	0.38
Sex (male) <sup>†</sup>	55 (81%)	62 (82%)	0.92
BMI	29 ± 6	29 ± 5	0.42
Pre-surgical creatinine (μmol/L)	89 ± 24	88 ± 27	0.72
EuroSCORE	1.87 ± 2.22	2.77 ± 3.50	0.07
Length of mechanical ventilation (h)	12.1 ± 6.0	33.5 ± 84.4	0.03
Ventilator-free hours (24 h)	12.5 ± 4.2	7.7 ± 6.3	0.001
ICU length of stay (h)	25.8 ± 29.9	60.8 ± 90.4	0.002
Total fluid bolus admission to 24 h	1705 ± 959	5361 ± 1924	0.001
Total fluid admission to 24 h	3261 ± 1197	6888 ± 1975	0.001
Fluid balance admission to 24 h	705 ± 1194	2524 ± 1655	0.001
<b>Baseline plasma markers</b>			
Ang-1 (pg/mL)	42.3 ± 18.1	43.7 ± 19.7	0.89
Ang-2 (pg/mL)	1.4 ± 2.7	1.4 ± 1.7	0.69
Ang-1/Ang-2	65.3 ± 65.1	66.9 ± 82.7	0.71
PLA <sub>2</sub> (μM/min/mL)	14.5 ± 5.1	13.8 ± 3.5	0.38
P-selectin (ng/mL)	144 ± 41	136 ± 46	0.24

Ang-1 = angiotensin-1. Ang-2 = angiotensin-2. BMI = body mass index. EuroSCORE = European System for Cardiac Operative Risk Evaluation. ICU = intensive care unit. PLA<sub>2</sub> = phospholipase A<sub>2</sub>. \* Values are shown in mean (standard deviation), by independent samples t test. Variables were tested for normality by Kolmogorov–Smirnov and log-transformed where necessary. †  $\chi^2$ .

## Discussion

We analysed plasma samples previously collected as part of a single-centre study of fluid administration after cardiac surgery, which found that patients had a shorter length of mechanical ventilation and ICU stay if their fluid administration was restricted through either randomisation (intervention group) or clinical treatment (low fluid administration cohort). This was accompanied by maintenance of low plasma Ang-2 levels and a corresponding higher ratio of Ang-1/Ang-2. Patients in the intervention group also had a smaller rise in PLA<sub>2</sub> levels.

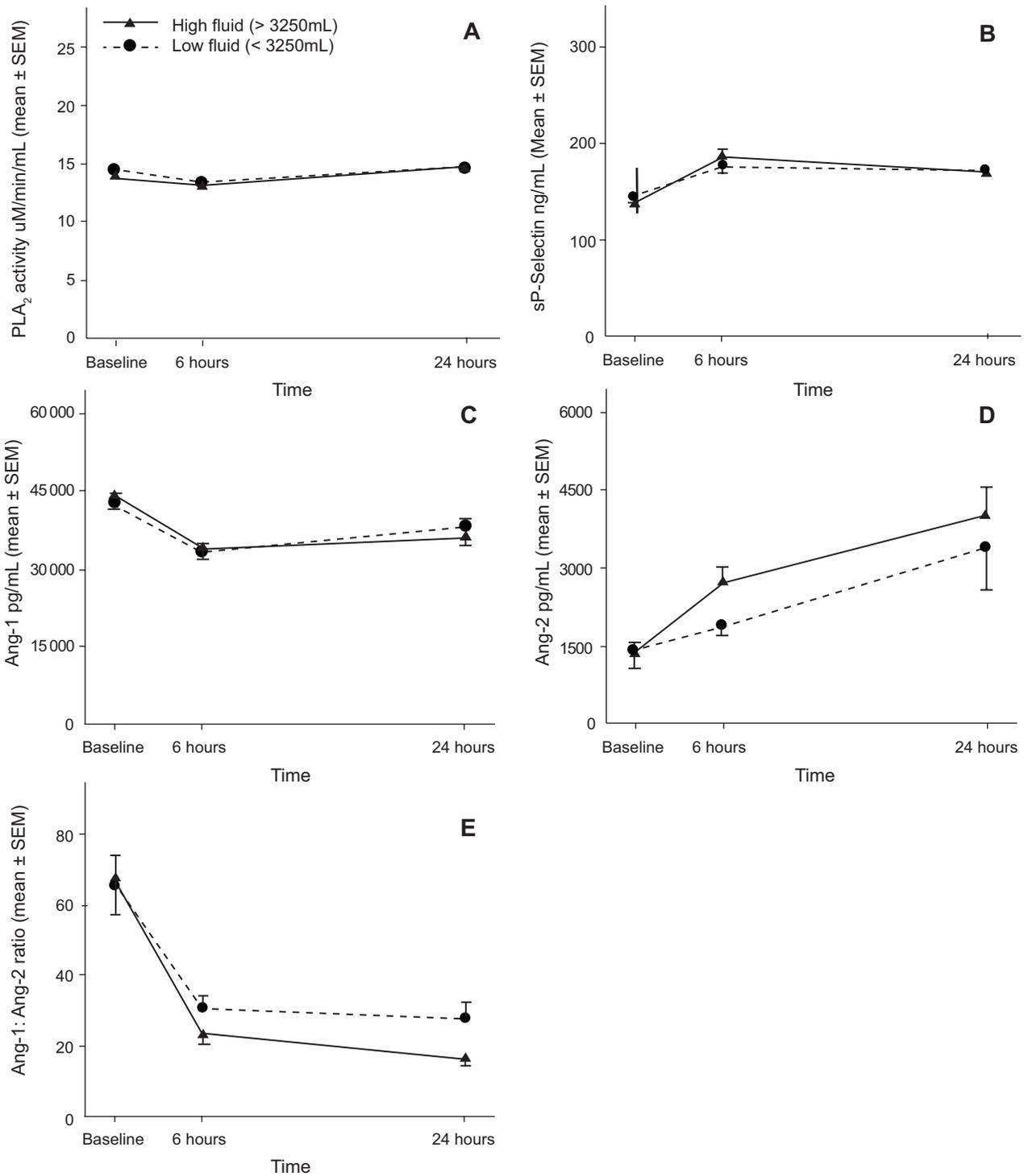
Increased plasma Ang-2 levels is a marker of patients with or at risk of lung injury and sepsis,<sup>19,33,34</sup> and follows the disease course.<sup>16</sup> Fluid-conservative therapy results in a greater decline in Ang-2 levels from Day 0 to Day 3 in patients with infection-related acute lung injury.<sup>18</sup> Although demonstrated in healthy volunteers,<sup>27</sup> the direct relationship of increased plasma Ang-2 levels with fluid boluses in patients who previously had normal lungs has not been reported. We now report a rise in Ang-2 levels after liberal fluids administration, which was also associated with both an increased length of mechanical ventilation and ICU stay.

Finally, this difference was more apparent in patients with both a higher pre-operative risk score and those managed with liberal fluid boluses.

Ang-2 is not only a marker of lung injury but it also sensitises endothelial cells to the action of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ )<sup>34</sup> and has a crucial role in induction and perpetuation of inflammation leading to endothelial barrier dysfunction.<sup>24</sup> With its inhibitory action on Tie-2 receptors, it blocks the regulation and stabilisation function of Ang-1, leading to a greater permeative action of TNF- $\alpha$  on the blood vessel and further perpetuation of inflammation through the release of adhesion molecules such as ICAM (intercellular adhesion molecule) and VCAM (vascular cell adhesion molecule).<sup>15</sup> Ang-2 appears to be an essential component of cytokine-induced vascular leakage.<sup>22</sup> Liberal fluids alter the ratio of Ang-1 and Ang-2 levels, particularly in high risk surgical patients. This is important because a prolonged bypass time exposes these patients to a higher risk of inflammation, which is further perpetuated with liberal fluid administration.

Ang-2, along with P-selectin and other vascular modulators, is stored in endothelial Weibel–Palade bodies from where it is released upon stimulation.<sup>35,36</sup> Rapid

**Figure 3. Plasma markers of endothelial injury and activation in patients receiving high fluid (> 3250 mL) versus low fluid (< 3250 mL) total fluid bolus, by linear mixed model analysis**



Ang-1 = angiotensin-1. Ang-2 = angiotensin-2. PLA<sub>2</sub> = phospholipase A<sub>2</sub>. SEM = standard error of the mean. **A.** PLA<sub>2</sub>: group (Gp),  $P = 0.29$ ; time,  $P = 0.004$ ; Gp\*time,  $P = 0.81$ . **B.** Soluble P-selectin: group,  $P = 0.63$ ; time,  $P \leq 0.001$ ; Gp\*time,  $P = 0.43$ . **C.** Ang-1: group,  $P = 0.81$ ; time,  $P \leq 0.001$ ; Gp\*time,  $P = 0.54$ . **D.** Ang-2: group,  $P = 0.002$ ; time,  $P \leq 0.001$ ; Gp\*time,  $P = 0.26$ . **E.** Ang-1/Ang-2: group,  $P = 0.001$ ; time,  $P \leq 0.001$ ; Gp\*time,  $P = 0.15$ .

**Table 4. Baseline characteristics, levels of Ang-1, Ang-2, PLA<sub>2</sub> and P-selectin and outcomes between the high and low fluid groups further examined based on pre-operative EuroSCORE II\***

	Fluid bolus < 3250		Fluid bolus ≥ 3250		P ≤
	EuroSCORE < 0.9 n = 32	EuroSCORE ≥ 0.9 n = 36	EuroSCORE < 0.9 n = 28	EuroSCORE ≥ 0.9 n = 48	
Length of mechanical ventilation (h) <sup>†</sup>	12.3 ± 6.9	11.9 ± 5.0	15.2 ± 7.4	44.2 ± 105.0	0.001
Ventilator-free hours (24 h) <sup>†</sup>	12.5 ± 4.4	12.4 ± 4.1	9.7 ± 5.3	6.6 ± 6.6	0.001
ICU length of stay (h) <sup>†</sup>	20.4 ± 9.6	30.6 ± 39.7	34.1 ± 29.9	76.4 ± 108.9	0.001
Total fluid bolus admission to 24 h <sup>†</sup>	1832 ± 935	1592 ± 978	5071 ± 1383	5530 ± 2175	0.001
Total fluid admission to 24 h <sup>†</sup>	3363 ± 1106	3170 ± 1282	6600 ± 1570	7056 ± 2175	0.001
Fluid balance admission to 24 h <sup>†</sup>	666 ± 1054	740 ± 1319	2034 ± 1177	2810 ± 1829	0.001
Baseline plasma markers					
Ang-1 (ng/mL) <sup>†</sup>	47.7 ± 16.9	37.6 ± 17.9	42.9 ± 20.8	44.2 ± 19.2	0.130
Ang-2 (ng/mL) <sup>†</sup>	1.1 ± 1.1	1.7 ± 3.6	1.0 ± 0.6	1.6 ± 1.9	0.669
Ang-1/Ang-2 <sup>†</sup>	83.5 ± 85.1	49.7 ± 35.3	67.9 ± 79.4	66.4 ± 85.4	0.126
PLA <sub>2</sub> (μM/min/ml) <sup>†</sup>	14.0 ± 3.5	15.0 ± 6.2	14.3 ± 3.2	13.5 ± 3.7	0.509
P-selectin (ng/mL) <sup>†</sup>	147 ± 49	141 ± 33	135 ± ±36	137 ± 51	0.704

Ang-1 = angiotensin-1. Ang-2 = angiotensin-2. EuroSCORE = European System for Cardiac Operative Risk Evaluation. ICU = intensive care unit. PLA<sub>2</sub> = phospholipase A<sub>2</sub>. \* Values are shown in mean (standard deviation), by one-way ANOVA. † Variables were tested for normality by Kolmogorov-Smirnov and log transformed where necessary.

administration of intravenous fluid bolus leads to a transient rise in shear forces across the pulmonary endothelium and rapid influx of calcium ions through activation of the TRPV4 channels, leading to exocytosis of endothelial storage vesicles and an increase in Ang-2.<sup>28</sup> This not only provides rationale to “wet lung”, “shock lung” or “Da Nang lung”, where lung failure followed successful resuscitation from circulatory collapse,<sup>37</sup> but explains the increased length of stay on mechanical ventilation with these patients, and provides insight to the increased early mortality seen in children with severe infection managed with fluid resuscitation.<sup>12</sup>

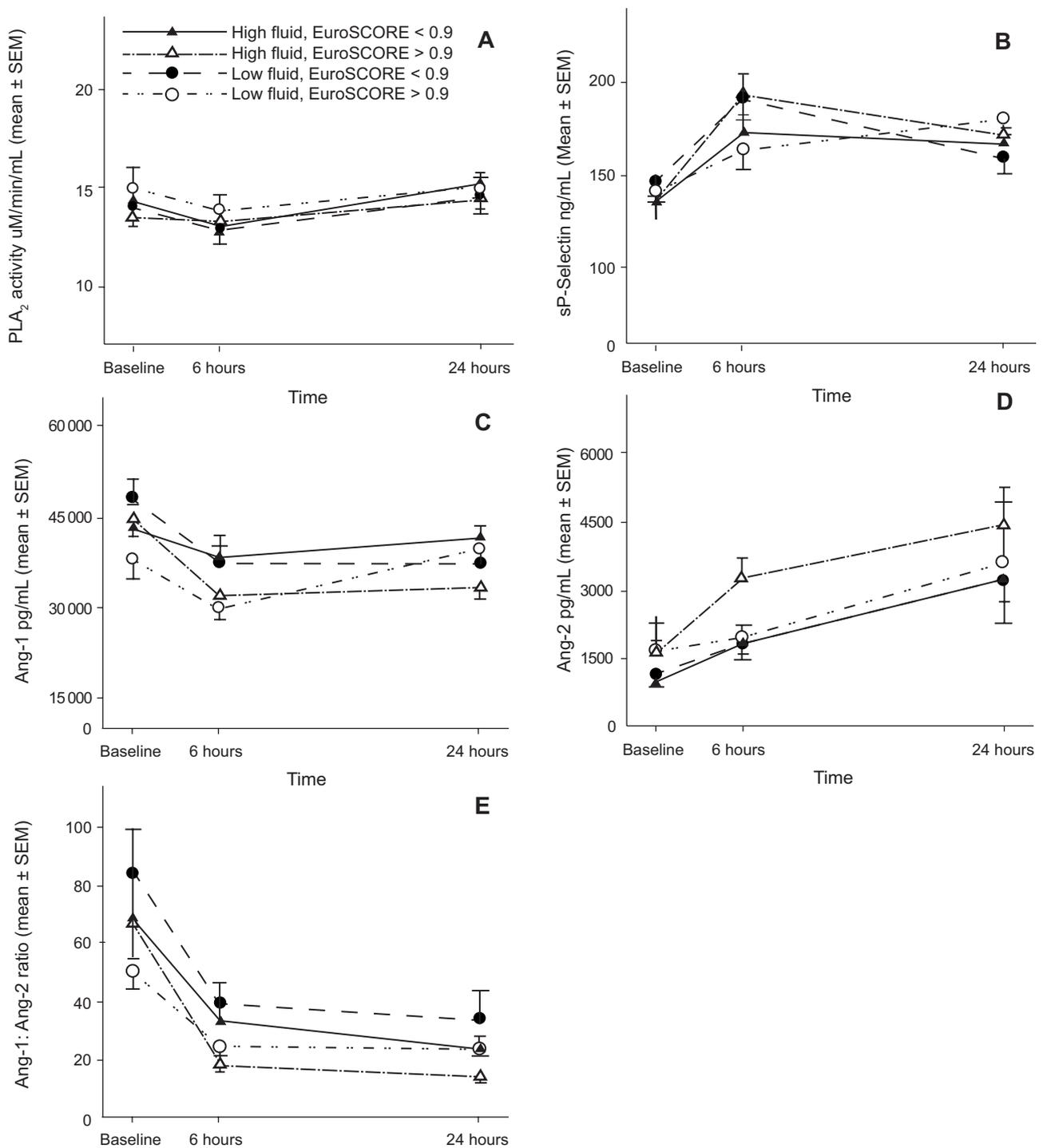
Rapid administration of 0.9% saline in human volunteers leads to interstitial oedema, associated with raised distal airway resistance, increased Ang-2 levels and corresponding decrease in Ang-1/Ang-2 ratio.<sup>27,38,39</sup> An increase in Ang-2 is not only an indication of damage to the endothelial glycocalyx layer<sup>40</sup> but also causes direct endothelial glycocalyx degradation and breakdown.<sup>23</sup> The resultant alteration in the revised Starling equation leads to interstitial lung oedema.<sup>41</sup> Rapid administration of a fluid bolus results in lung injury in an animal model<sup>28</sup> and a decrease in Pao<sub>2</sub>/Fio<sub>2</sub> (arterial partial pressure of oxygen/fraction of inspired oxygen) ratio in patients with sepsis.<sup>42</sup>

As a fluid bolus likely leads to an associated rise in shear forces, this may activate TRPV4 channels via PLA<sub>2</sub>-mediated

arachidonic acid release and subsequent formation of epoxyeicosatrienoic acids.<sup>43</sup> We found a rise in PLA<sub>2</sub> levels in patients managed with liberal fluids consistent with this, and increases in PLA<sub>2</sub><sup>44</sup> have been associated with acute lung injury.

We have examined consecutive blood samples in a cohort of patients undergoing cardiac surgery. A pragmatic study design was used, to ensure timely completion and enrolment, aiding in generalisability of study results, as all patients presenting for cardiac surgery were screened for enrolment and invited to participate. Only 15% of patients approached declined to participate and there was no loss to follow-up. Blood was collected and analysed from all the subjects. Although the sample size is small, to our knowledge, it is the largest study examining fluid resuscitation in patients who had cardiac surgery. There was a time delay between the conduct of the study and sample analysis; however, the samples were collected and stored according to a standard operating procedure. The separation between the intervention and the usual care group was small in terms of the amount of administered fluid, but we still found differences in PLA<sub>2</sub> and Ang-2 between the groups. We also examined the groups based on the actual amount of fluid received. We only had plasma samples, as no lung lavage was collected in the original study, owing to the pragmatic nature of the study design. In addition,

**Figure 4. Plasma markers of endothelial injury and activation in patients receiving high fluid (> 3250 mL) versus low fluid (< 3250 mL) total fluid bolus, categorised by EuroSCORE II (low, < 0.9) or (high, ≥ 0.9), by linear mixed model analysis**



Ang-1 = angiotensin-1. Ang-2 = angiotensin-2. EuroSCORE = European System for Cardiac Operative Risk Evaluation. PLA<sub>2</sub> = phospholipase A<sub>2</sub>. SEM = standard error of the mean. **A.** PLA<sub>2</sub>: group (Gp),  $P = 0.40$ ; time,  $P = 0.007$  (6 h v 24 h,  $P = 0.002$ ); Gp\*time,  $P = 0.97$ . **B.** Soluble P-selectin: group,  $P = 0.52$ ; time,  $P \leq 0.001$  (baseline [BL] v 6 h and 24 h;  $P \leq 0.001$ ); Gp\*time,  $P = 0.14$ . **C.** Ang-1: group,  $P = 0.02$  (EuroSCORE [euro] < 0.9\*low fluid v euro > 0.9\*high fluid and euro > 0.9\*low fluid;  $P \leq 0.03$ ; euro < 0.9\*high fluid v euro > 0.9\*low fluid and euro > 0.9\*high fluid;  $P \leq 0.04$ ); time,  $P \leq 0.001$  (all times v all groups;  $P \leq 0.03$ ); Gp\*time,  $P = 0.07$ . **D.** Ang-2: group,  $P = 0.001$  (euro > 0.9\*high fluid v all groups;  $P \leq 0.02$ ); time,  $P \leq 0.001$  (all times v all groups;  $P \leq 0.001$ ); Gp\*time,  $P = 0.79$ . **E.** Ang-1/Ang-2: group,  $P \leq 0.001$  (euro < 0.9\*low fluid bolus v euro > 0.9\*low fluid and euro > 0.9\*high fluid;  $P \leq 0.02$ ; euro > 0.9\*low fluid v euro > 0.9\*high fluid;  $P = 0.006$ ; euro < 0.9\*high fluid v euro > 0.9\*high fluid;  $P \leq 0.001$ ); time,  $P \leq 0.001$  (all times v all groups;  $P \leq 0.03$ ); Gp\*time,  $P = 0.21$ .

there was no control over the use of anti-inflammatories or steroids, which may affect some of the measured markers, such as PLA<sub>2</sub>, although this is likely to be insignificant as their use is not part of usual practice. Although the use of vasopressors was similar between the groups,<sup>30</sup> their effect cannot be delineated in a study design such as this. Finally, we did not find a difference in soluble P-selectin levels, which does not rule out changes at its expression within the lung tissue.

## Conclusion

Fluid resuscitation after cardiac surgery leads to an increase in endothelial markers of lung injury and prolonged length of mechanical ventilation.

## Competing interests

None declared.

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## Author contributions

All authors have been involved in the conception and study design, and in the drafting of the manuscript and final approval. Rachael Parke and Shailesh Bihari were responsible for the study design, funding and data analysis and for drafting the manuscript. Shay McGuinness was responsible for the study design, funding and manuscript approval. Eileen Gilder was involved in the design and conduct of the study, sample collection and processing and final approval of the manuscript. Dani-Louise Dixon, Elena Cavallaro and Andrew Bersten were responsible for the study design, sample processing and data analysis.

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