THE LANCET
Respiratory Medicine

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplementary Appendix

1·0 Study Group

1·1 Management Committee

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1·2 Statistician

John Moran FRACP, FCICM, MD

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1·3 Website & Randomisation

Gordon Doig PhD

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1·4 Participating Centres & Personnel

Bendigo Hospital, Bendigo VIC, Australia: Emma Broadfield (PI); Timothy Chimunda, John Edington, Jason Fletcher, Cameron Knott, Sanjay Porwal, Janice Yeung (AIs); Catherine Boschert, Julie Smith (RCs).

Frankston Hospital, Frankston VIC, Australia: Sachin Gupta (PI); Cameron Green (AI).

Footscray and Sunshine Hospitals, Western Health, Sunshine VIC, Australia: Craig French (PI); John Mulder, Sathyajith Koottayi, Forbes McGain, Gerard Fennessy, Irma Bilgrami, James Douglas, Yang Yang (AIs); Samantha Bates, Rebecca Morgan, Anna Tippett, Miriam Towns (RCs).

Monash Medical Centre, Clayton VIC, Australia: Timothy Crozier (PI); Jonathan Devine, David Ernest, Craig Walker, Hellen Karonga, Paul Ritchie, Stephen Thornhill (AIs); Dhiraj Bhatia, Lauren Bulfin, Suzanne Eliott, Michelle Fernando, Pauline Galt, Tammy Lamac, Klaudija Lavrans, Oakleigh Mullan, Angela Wang (RCs).

Northern Hospital, Epping VIC, Australia: Angajendra Ghosh (PI); Andrew Casamento, David Crosbie, Anthony Cross, John Green, Honey Ong (AIs); Mary Park, Simone Said (RCs).

The Canberra Hospital, Woden ACT, Australia: Frank van Haren (PI); Peter Velloza (AI); Helen Rodgers, Josie Russell-Brown, Rebecca Cartwright-Williams, Katie Jefferson, Rebecca Millar, Mary Nourse, Shakira Spiller (RCs).

The Royal Melbourne Hospital, Parkville VIC, Australia: Christopher MacIsaac, Thomas Rechnitzer (PIs); Roberto Citrioni, Rohit D'Costa, Kieron Gorman, Nerina Harley, Christian Karcher, Shyamala Sriram (AIs); Deborah Barge, Kathleen Byrne, Sarah Doherty, Andrea Jordan (RCs).

St Vincent's Hospital Melbourne, Fitzroy VIC, Australia: Barry Dixon (PI); Espedito Faraone, Jennifer Holmes, John Santamaria, Roger Smith, Antony Tobin (AIs); Leanne Barbazza (RC).

University Hospital Geelong, Geelong VIC, Australia: Nicholas Simpson (PI); Tania Elderkin, Neil Orford, Tania Salerno (AIs); Allison Bone (RC).

PI = principal investigator. AI = associate investigator. RC = research coordinator.

2·0 Supplementary Methods

2·1 Human Research Ethics Committee (HREC) Approval for Each New Participating Centre

Participating Centre	HREC Approval Date*
St Vincent's Hospital Melbourne, Fitzroy VIC, Australia	16 Mar 2012
The Royal Melbourne Hospital, Melbourne Health, Parkville VIC, Australia	16 Mar 2012
The Canberra Hospital, ACT Health, Woden ACT, Australia	11 Nov 2013
University Hospital Geelong, Barwon Health, Geelong VIC, Australia	20 Mar 2013
The Northern Hospital, Northern Health, Epping VIC, Australia	20 Mar 2013
Monash Medical Centre, Monash (Southern) Health, Clayton VIC, Australia	30 Apr 2013
Bendigo Hospital, Bendigo Health, Bendigo VIC, Australia	11 June 2014
Frankston Hospital, Peninsula Health, Frankston VIC, Australia	27 Aug 2014
Footscray and Sunshine Hospitals, Western Health, Sunshine VIC, Australia	19 Feb 2018

* St Vincent's Hospital Melbourne HREC, Fitzroy VIC, Australia was the responsible HREC for all participating centres except The Canberra Hospital, which was responsible to ACT Health HREC, Woden ACT, Australia. St Vincent's Hospital Melbourne HREC Reference HREC/12/SVHM/6; ACT Health HREC Reference ETH·8·13·206.

2·2 Protocol Amendments and Human Research Ethics Committee (HREC) Approval Dates

Version 1, 12 Dec 2011; HREC Approval 16 Mar 2012, Original Protocol

Version 2, 10 Apr 2012; HREC Approval 01 May 2012, prior to commencement of participant screening at any study centre. Summary of Changes: Increased sample size from 250 to 256; collect blood samples for cytokine and coagulation testing at baseline and on day 3 for participants at St Vincent's Hospital Melbourne and Royal Melbourne Hospital; conduct participant follow-up at six-months.

Version 3, 15 Dec 2017; HREC Approval 25 Jan 2018. Summary of changes: updated the details of study personnel and contact information, updated background and safety information, and updated recruitment rate and feasibility; clarified process-of-care and secondary endpoints; provided procedures for assessing co-enrolment in other clinical trials; provided advice about use of the study nebuliser for administering non-study nebulised medications; clarified unclear statements about the data collected e.g. for 'arterial blood gases' the specific arterial blood gas items collected were identified; updated safety reporting procedures and definitions in keeping with recently revised National Health and Medical Research Council (Australia) recommendations; aligned the protocol with the Data Safety Monitoring Board (DSMB) Charter in relation to DSMB responsibilities.

2·3 Pre-Start Participating Centre Investigator Meetings

A half-day meeting for site Investigators and research coordinators was conducted at each study centre prior to site activation. These were led by the Chief Investigator and Project Manager. Presentations covered the study rationale, eligibility criteria, consent and randomisation procedures, set-up of the nebuliser and ventilator circuit, administration of the study drug and drug accountability, methodology for assessing chest imaging, the data dictionary and case report form, and definitions and reporting of adverse events.

2·4 Eligibility Criteria

Inclusion criteria

To be eligible, patients must have met all of these inclusion criteria:

- Age 18 years or older

- Receiving ventilation via an endotracheal tube

- Started ventilation via an endotracheal tube yesterday or today

- Expected to require invasive ventilation all of today and all of tomorrow

- P_aO_2 to F_IO_2 ratio less than 300 or S_pO_2 to F_IO_2 ratio less than 315 at any time since commencing invasive ventilation. Note: Only S_pO_2 values $\leq 97\%$ can be used to calculate the S_pO_2 to F_IO_2 ratio

- Active ventilator circuit humidification or the treating Intensivist has decided to start active humidification.

Exclusion criteria

Patients were excluded if any of the following criteria were met:

- Previously enrolled in this study

- Enrolled in another randomised intervention study, unless approved*

- Allergy to heparin

- Any history of heparin induced thrombocytopaenia

- Platelet count less than 50 x 10^9/L

- APTT is prolonged to greater than 80 seconds and this is not due to anticoagulant therapy

- Uncontrolled bleeding

- Pulmonary bleeding during this hospital admission (Pulmonary bleeding is frank bleeding in the lungs, trachea or bronchi with repeated hemoptysis, or requiring repeated suctioning, and temporally associated with acute deterioration in respiratory status)

- Neurosurgical procedures during this hospital admission or such procedures are planned

- Epidural catheter in place

- Any history of intracranial, spinal or epidural hemorrhage

- Hepatic encephalopathy or any history of gastrointestinal bleeding due to portal hypertension or biopsy proven cirrhosis with documented portal hypertension

- Tracheostomy in place

- Usually receives home oxygen

- Usually receives any type of assisted ventilation at home e.g. continuous positive airway pressure for obstructive sleep apnoea

- Cervical spinal cord injury associated with reduced long-term ability to breathe independently

- Spinal or peripheral nerve disease with a likely prolonged reduction in the ability to breathe independently e.g. Guillain-Barré syndrome, motor neurone disease

- Receiving high frequency oscillation ventilation or the treating Intensivist has made a definite decision to commence high frequency oscillation ventilation

- Receiving extra-corporeal membrane oxygenation or the treating Intensivist has made a definite decision to commence extra-corporeal membrane oxygenation

- Treatment limits restrict the provision of renal replacement therapy, inotropes, vasopressors or prolonged invasive ventilation

- Usually treated with hemodialysis or peritoneal dialysis for end-stage renal failure

- Dementia

- Death is deemed imminent or inevitable or there is underlying disease with a life expectancy of less than 90 days

- Pregnant or might be pregnant. Women aged 18 to 49 are excluded unless there is documented menopause, hysterectomy or surgical sterilisation, or a pregnancy test is negative

- Objection from the treating clinician

- Consent refused by the patient or substitute decision maker.

* The Management Committee assessed requests for co-enrolment. In general, co-enrolment was viewed favourably, except for studies of interventions likely to affect coagulation or acute lung injury.

2·5 Nebuliser and Ventilator Circuit Set-Up
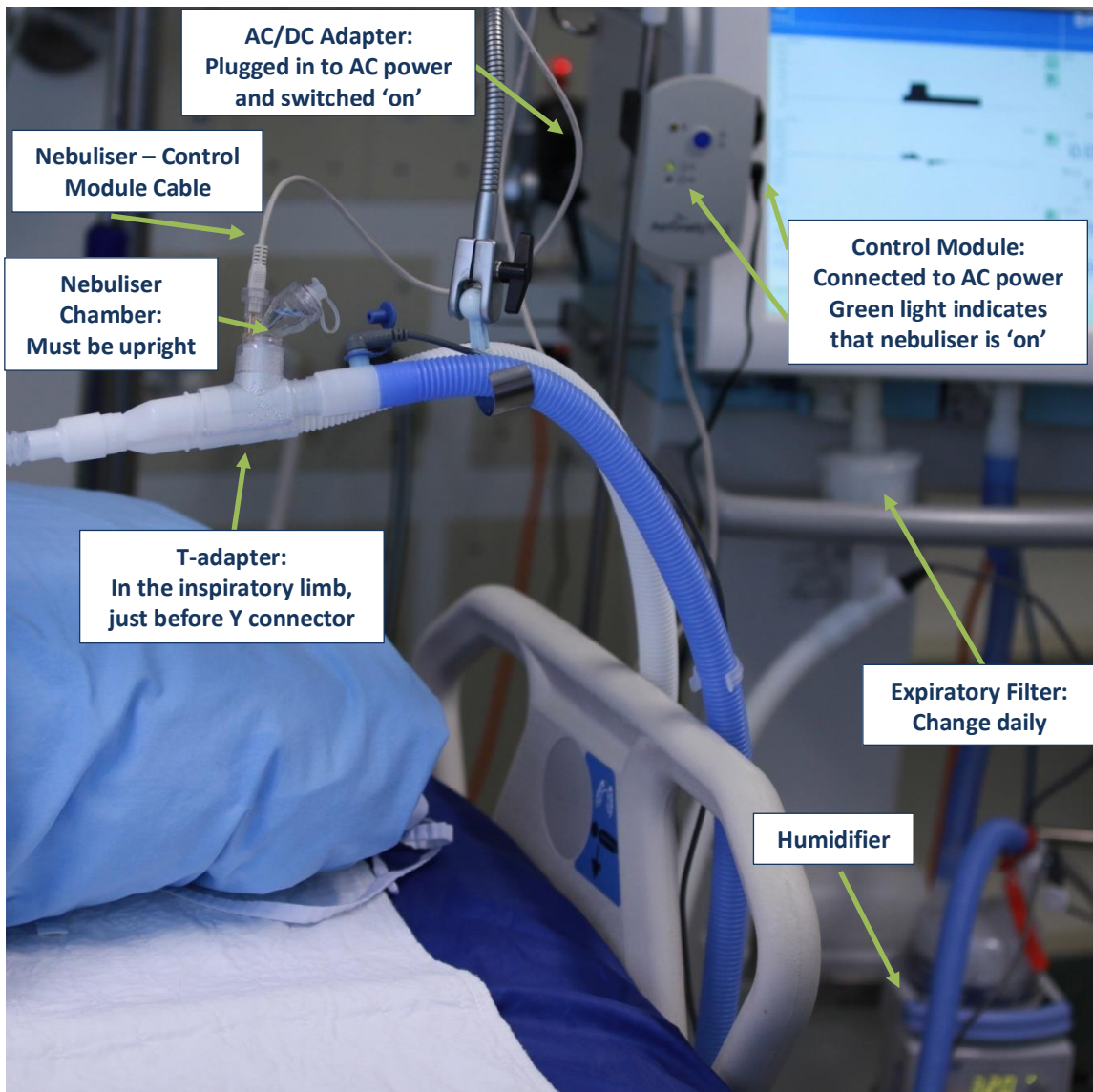
The study medication was administered using a vibrating mesh nebuliser (Aeroneb Solo, Aerogen, Galway, Ireland), positioned in the inspiratory limb of the ventilator circuit just before the Y-piece. An expiratory filter (Servo Duo Guard, Maquet Critical Care AB, Solna, Sweden) was employed to prevent the study drug from impairing ventilator function. Active circuit humidification was used throughout.

2·6 Chest Imaging Assessment Tool

Site Investigators assessed chest imaging using the following methodology:

1. How many lung quadrants have acute opacities that are not fully explained by effusions, lobar/lung collapse, or nodules? (If 'zero', skip to the next image)

2. Are these lung opacities present bilaterally?

3. Given all the medical information about the patient, are these lung opacities entirely attributable to cardiac failure or fluid overload? (If 'Yes', skip to the next image)

4. Was the patient exposed to any risk factor for lung injury at any time in the last 7 days? (If 'Yes', skip to the next image)

5. Is there objective evidence (e.g. echocardiography) that excludes the possibility of cardiac failure or fluid overload?

2·7 Data Safety Monitoring Board (DSMB) Responsibilities

The DSMB was charged with providing independent, expert advice to the Management Committee concerning the safety of study participants. Meetings occurred after the 50th, 100th and 200th enrolment. The DSMB was required to review all serious adverse reactions and serious adverse events with knowledge whether a patient received study drug A or study drug B but blinded to the identity of the study drug. For events that were study-related (possibly, probably or definitely), the DSMB, using the Haybittle-Peto approach, was required to determine if there emerged a difference in serious adverse events between study groups that exceeded three standard deviations in magnitude. If such a difference had occurred, the DSMB was empowered to conduct a blinded interim analysis on the primary outcome to ensure patient safety. If a difference in the primary outcome was found that exceeded three standard deviations, the DSMB may have recommended to the Management Committee that the trial be stopped. No recommendation to stop the trial was made.

2·8 Data Quality Monitoring

Source data were verified during on-site monitoring for all data points from screening to hospital discharge for at least the first participant at each study centre. Remote data monitoring was conducted according to pre-defined criteria.

2·9 Database Management

It emerged during blinded analysis that four participants, all in the same study group, had been transferred to a non-participating hospital intensive care unit prior to day 28; three on day 1 and the other on day 18. The Management Committee elected to have the health records of the participants at the receiving hospitals interrogated to ascertain major secondary outcomes: onset of the acute respiratory distress syndrome, use of extracorporeal membrane oxygenation, use of prone positioning, tracheotomy, ventilator separation, intensive care separation, and hospital separation. Other outcomes at days 5, 10 and 28 that were unknown at the time of transfer were censored in these patients. Outcomes at days 60 and 180 were unaffected.

Participants excluded from analysis: 256 patients were randomised, 131 to nebulised heparin and 125 to placebo. Three participants withdrew consent and were excluded from analysis and another, who was ineligible due to thrombocytopaenia, was excluded from analysis by the blinded Chief Investigator in consultation with the Site Investigator before any study treatment had been given and any outcome data collected. Data were therefore analysed for 128 participants assigned to heparin and 124 to placebo.

2·10 Statistical Analysis

The protocol listed the primary, secondary, safety and process-of-care outcomes and provided a simple statistical outline, with a strategy by the Management Committee to develop the detailed statistical plan prior to analysis. Analyses were agreed by the Management Committee and the statistician prior to the database lock. We did not publish the statistical analysis plan in the public domain.

Power calculation: To demonstrate a clinically important 10-point improvement in the primary outcome, the SF-36 Health Survey Physical Function Score, a total of 98·6 patients were required in each group assuming an improvement in the score from 45 to 55, standard deviation 25, power 80% and alpha 0·05.[1,2] To account for the loss of information due to death, a mortality rate by day 60 of up to 30% was assumed, giving a sample size of 256.

Analysis principles: Analyses followed the intention-to-treat principle, considering all participants in the treatment group to which they were assigned, except for cases lost to follow-up and withdrawn. There was no imputation of missing data. Hypothesis tests are 2-sided and are taken to signify statistical significance if < 0.05. P-values of secondary outcomes have not been adjusted for multiple comparisons, so should not be used to infer definite differences between groups. Analyses were performed using Stata statistical software, Version 15·1 (StataCorp LLC, College Station, Texas, USA).

Study day: Day 0 was defined as the period from randomisation to midnight on the day of enrolment, day 1 the first calendar day after the day of enrolment, and so forth. In this study the median duration of the period from randomisation to midnight on the day of enrolment (day 0) was 10 hours.

Independent analysis: Analysis of the primary and secondary endpoints was performed by a statistician (John L Moran). The statistician had knowledge of whether participants were assigned to drug A or drug B but was blinded to the identity of the study drug.

Baseline characteristics: Continuous variables are presented as mean and standard deviation and categorical variables as number/(total number per group) and percentage.

Continuous outcomes: Groups were compared on continuous outcomes, including the primary outcome, using the Student t-test assuming unequal variance and data are displayed as mean and standard deviation. In the case of small subgroups with skewed distributions, group medians were compared using quantile regression and data are displayed as median and interquartile range.

Time-to-event analyses: The groups were compared on each of onset of ARDS to day 5, days to ventilator separation to day 28 and days to ICU separation to day 28 by hazard ratio calculated using the Fine-Gray methodology to account for the competing risk of death.[3] Data for onset of ARDS were presented as the number/(total number) and percentage of the at-risk participants in each group and as cumulative incidence curves. Data for days to ventilator separation and days to ICU separation are presented as mean and standard deviation, and as Kaplan-Meier curves, with non-survivors at day 28 treated as though not separated from the ventilator or the ICU.

Ventilator separation was deemed to have occurred if the patient had not received either invasive or non-invasive ventilatory support at any time during the remainder of the day of stopping ventilatory support or the next day. If a participant achieved ventilator separation more than once, it was the final separation that was used to calculate the outcome. Similarly, if ICU separation occurred more than once, it was the final separation that was used to calculate the outcome. The date and the time of day of ventilator separation and of ICU separation were recorded on the case report form, which enabled precise measurement of these outcomes.

On the number of days to ventilator separation to day 28 in survivors and on the number of days to ICU separation to day 28 in survivors, the groups were compared by hazard ratio calculated using Cox regression and data were presented as the mean and standard deviation and as Kaplan-Meier curves.

Hazard ratio calculated by Cox regression was used for comparing the number of days from enrolment to discharge from the study hospital for non-survivors and for survivors, and for those discharged to home, to rehabilitation and to another hospital, with the data presented as median and interquartile range.

Hazard ratio calculated by Cox regression was used for comparing survival to day 180 and data presented as Kaplan-Meier survival curves. The number/(total number) and percentage deceased by day 180 in each group were also reported.

The proportional hazards assumption was verified for all Cox models by assessment of Schoenfeld residuals.

Categorical outcomes: Binary outcomes were compared using the odds ratio assessed by logistic regression but, when the event rate in a group was zero, exact logistic regression was used. The Fisher exact test was used to compare groups across categories of each domain of the EQ-5D-3L quality of life instrument and across categories of participant-reported health compared to one year ago. Categorical data are displayed as number/(total number per group) and percentage.

Subgroup analyses: Interactions between treatment effect and each of ten dichotomous baseline subgroups (median age, sex, enrolment period, enrolment centre, median hours of pre-randomisation invasive ventilation, presence of ARDS, median Murray Lung Injury Score, presence of pneumonia, treatment with unfractionated heparin, and treatment with unfractionated heparin or low-molecular-weight heparin) were separately assessed on the primary outcome and ventilator separation to day 28. Interaction was determined using linear regression or competing risk regression, as appropriate, with models including main effect and interaction terms.

2·11 Future Analyses

Analyses of secondary outcomes listed in the protocol that pertain to biomarkers of inflammation and to cost-effectiveness will be undertaken and reported in future publications.

3·0 Supplementary Results

The number randomised over time from the first randomisation on 15 Sep 2012 to the last on 23 August 2018.
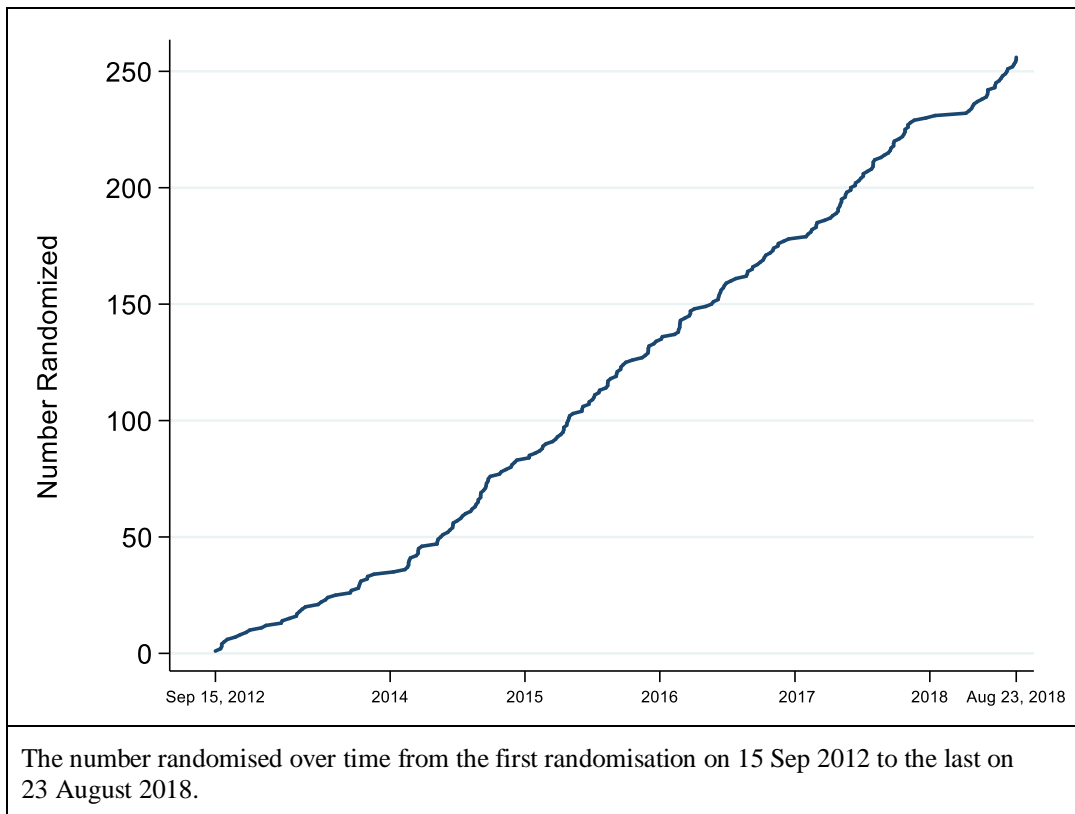
sFigure 1. **Recruitment**

	Nebulised Heparin Group (n=128)	Nebulised Saline Group (n=124)
Comorbid conditions		
Psychiatric illness[a]	47 (36·7%)	36 (29·0%)
Arthro-skeletal disease[b]	44 (34·4%)	32 (25·8%)
Diabetes mellitus	27 (21·1%)	37 (29·8%)
Alcohol or substance misuse	29 (22·7%)	21 (16·9%)
Immunocompromised[c]	14 (10·9%)	13 (10·5%)
Heart failure[d]	6 (4·7%)	9 (7·3%)
ICU Admission source		
Emergency department	56 (43·8%)	51 (41·1%)
Ward	23 (18·0%)	34 (27·4%)
Inter-hospital transfer – Not from ICU	29 (22·7%)	24 (19·4%)
Inter-hospital transfer – From ICU	7 (5·5%)	4 (3·2%)
Theatre – Not emergency surgery	6 (4·7%)	7 (5·6%)
Theatre – Emergency surgery	7 (5·5%)	4 (3·2%)
Medications[e]		
Sedative or opiate infusion	128 (100·0%)	123 (99·2%)
Antibacterial	126 (98·4%)	121 (97·6%)
Antiviral	35 (27·3%)	25 (20·2%)
Antifungal	6 (4·7%)	6 (4·8%)
Biochemistry and hematology		
Creatinine[f] (μmol/L)	149 (153)	141 (113)
Bilirubin[f] (μmol/L)	15 (14), n=125	15 (12), n=121
Haemoglobin[g] (g/L)	116 (23)	114 (24)
Platelet count[g] (x 10^9/L)	233 (121), n=127	229 (117), n =124
APTT[g] (seconds)	40 (23), n=122	37 (10), n=120
INR[g]	1·3 (0·4), n=122	1·4 (0·4), n=119
Ventilatory ratio[h]	1·78 (0·52), n=125	1·79 (0·77), n=121

Data are n (% of the total number) and mean (SD). The number of participants available for specific variables is stated in each cell if different from the total number of participants in the treatment group.

APTT = activated partial thromboplastin time. ICU = intensive care unit. SD = standard deviation.

[a] Documented history of depression, anxiety, schizophrenia, bipolar disorder, personality disorder, eating disorder or deliberate self-harm.

[b] Documented history of joint injury, deformity, infection or surgery, osteomyelitis, limb fracture or amputation, chronic joint pain or inflammation, or chronic neck or back pain.

[c] According to the APACHE II criteria.

[d] Documented history of congestive cardiac failure, cardiomyopathy or impaired left ventricular function.

[e] In the 24 hours before enrolment.

[f] Highest recorded value in the 24 hours prior to enrolment.

[g] Value recorded closest to and before enrolment and within the 24 hours prior to enrolment.

[h] Ventilatory ratio = (minute ventilation x P_aCO_2) $\div$ (predicted ventilation x ideal P_aCO_2); predicted ventilation = predicted body weight kg x 100; and ideal P_aCO_2 = 37·5 mmHg.

sTable 1. **Additional baseline characteristics of the participants**

	Nebulised Heparin Group (n=128)	Nebulised Saline Group (n=124)	Type of Difference Estimate, Difference Estimate (95% CI)	P
Logistical reasons				
Any logistical reason	27 (21·1%)	22 (17·7%)	OR, 1·24 (0·66 to 2·32)	0·50
Nebuliser fault	1 (0·8%)	1 (0·8%)	OR, 0·97 (0·06 to 15·66)	0·98
Drug unavailable or prescription error	7 (5·5%)	5 (4·0%)	OR, 1·38 (0·43 to 4·46)	0·59
Extra-corporeal membrane oxygenation	0 (0·0%)	1 (0·8%)	OR, 0·97 (0 to 37·78)	0·98
Temporarily outside ICU	6 (4·7%)	2 (1·6%)	OR, 3·00 (0·59 to 15·16)	0·18
Tracheotomy	3 (2·3%)	6 (4·8%)	OR, 0·47 (0·12 to 1·93)	0·30
Surgery	7 (5·5%)	5 (4·0%)	OR, 1·38 (0·43 to 4·46)	0·59
Consent for drug withdrawn	1 (0·8%)	0 (0·0%)	OR, 0·97 (0·02 to und.)	1·00
Treatment limitation	3 (2·3%)	1 (0·8%)	OR, 2·95 (0·30 to 28·77)	0·35
Other logistical reason	4 (3·1%)	3 (2·4%)	OR, 1·30 (0·29 to 5·94)	0·73
Safety precautions				
Any safety precaution	15 (11·7%)	4 (3·2%)	OR, 3·98 (1·28 to 12·36)	0·0168
Sputum too bloody	8 (6·3%)	2 (1·6%)	OR, 4·07 (0·85 to 19·54)	0·08
APTT too prolonged	6 (4·7%)	0 (0·0%)	OR, 8·20 (1·17 to und.)	0·0324
HIT Screen[a]	2 (1·6%)	2 (1·6%)	OR, 0·97 (0·13 to 6·98)	0·97
Safety concerns				
Any safety concern	7 (5·5%)	2 (1·6%)	OR, 3·53 (0·72 to 17·33)	0·12
Bleeding, non-pulmonary	3 (2·3%)	1 (0·8%)	OR, 2·95 (0·30 to 28·77)	0·35
Increase in airway pressure	3 (2·3%)	0 (0·0%)	OR, 3·77 (0·40 to und.)	0·26
Haemoptysis[b]	1 (0·8%)	0 (0·0%)	OR, 0·97 (0·02 to und.)	1·00
Hypoxaemia	0 (0·0%)	1 (0·8%)	OR, 0·97 (0 to 37·78)	0·98
HIT Confirmed[a]	0 (0·0%)	0 (0·0%)		
Ventilator circuit malfunction	0 (0·0%)	0 (0·0%)		
Any reason	38 (29·7%)	26 (21·0%)	OR, 1·59 (0·90 to 2·83)	0·11

Data are n (% of the total number).

Odds ratios were assessed using logistic regression or, if there was a count of zero, exact logistic regression.

APTT = activated partial thromboplastin time. CI = confidence interval. HIT = heparin-induced thrombocytopaenia. OR = odds ratio. Und = undefined.

At least one dose must have been withheld. Participants could have the study medication withheld for more than one reason. Three participants, each in the heparin group, were transferred to a non-participating hospital ICU on day 1 precluding further administration of the drug; study medication usage is reported up to the time of transfer.

[a] Two participants in each group had the study medication temporarily withheld to permit heparin antibody testing and clinical evaluation of possible heparin-induced thrombocytopaenia. There were no confirmed cases.

[b] Described by the site Investigator as medically important haemoptysis. The patient did not deteriorate clinically.

sTable 2. **Reasons for withholding the study medication**

	Nebulised Heparin Group (n=128)	Nebulised Saline Group (n=124)	Type of Difference Estimate, Difference Estimate (95% CI)	*P*
APTT Average (seconds)				
APTT average	39 (16), n=125	36 (13), n=122	MD, 3 (-1 to 6)	0·15
APTT average, if *any* IV or SC UFH	47 (20), n=56	40 (17), n=62	MD, 8 (1 to 15)	0·0270
APTT average, if *no* IV or SC UFH	32 (5), n=69	33 (6), n=60	MD, -1 (-3 to 1)	0·53
APTT average, if *any* IV or SC LMWH	37 (12), n=91	34 (7), n=86	MD, 2 (-1 to 5)	0·16
APTT average, if *no* IV or SC LMWH	46 (21), n=34	41 (21), n=36	MD, 5 (-5 to 15)	0·36
APTT Highest (seconds)				
APTT highest	52 (31), n=125	44 (20), n=122	MD, 7 (1 to 14)	0·0260
APTT highest, if *any* IV or SC UFH	69 (38), n=56	51 (25), n=62	MD, 18 (6 to 30)	0·0037
APTT highest, if *no* IV or SC UFH	37 (9), n =69	37 (9), n=60	MD, 0 (-3 to 3)	0·81
APTT highest, if *any* IV or SC LMWH	46 (24), n=91	42 (14), n=86	MD, 5 (-1 to 11)	0·10
APTT highest, if *no* IV or SC LMWH	65 (42), n =34	50 (30), n=36	MD, 15 (-2 to 32)	0·09
APTT > 120 seconds				
APTT > 120	7 (5·6%), n=125	2 (1·6%), n=122	OR, 3·56 (0·72 to 17·49)	0·12
APTT > 120, if *any* IV or SC UFH	7 (12·5%), n=56	2 (3·2%), n=62	OR, 4·29 (0·85 to 21·57)	0·08
APTT > 120, if *no* IV or SC UFH	0 (0·0%), n=69	0 (0·0%), n=60		
APTT > 120, if *any* IV or SC LMWH	2 (2·2%), n=91	0 (0·0%), n=86	OR, 2·30 (0·18 to und.)	0·53
APTT > 120, if *no* IV or SC LMWH	5 (14·7%), n=34	2 (5·6%), n=36	OR, 2·93 (0·53 to 16·25)	0·22

Data are n (% of the total number) and mean (SD). The number of participants available for specific variables is stated in each cell if different from the total number of participants in the treatment group.

Mean differences were assessed using Student t-test. Odds ratios were assessed using logistic regression or, if there was a count of zero, exact logistic regression.

APTT = activated partial thromboplastin time. CI = confidence interval. LMWH = Low molecular weight heparin. MD = mean difference. OR = odds ratio. SD = standard deviation. UFH = unfractionated heparin. Und = undefined.

***sTable 3*. APTT in the Intensive Care Unit to day 10**

	Nebulised Heparin Group (n=128)	Nebulised Saline Group (n=124)	Type of Effect Estimate, Effect Estimate (95% CI)	P
Haemoglobin (g/L)				
Average	99 (20), n=125	99 (20)	MD, 0 (-5 to 5)	0·95
Lowest	88 (20), n=125	89 (22)	MD, -1 (-6 to 5)	0·84
Fall[a]	27 (17), n=125	24 (17)	MD, 3 (-2 to 7)	0·21
< 70	24 (19·2%), n=125	21 (16·9%)	OR, 1·17 (0·61 to 2·23)	0·64
Platelet count (x 10^9/L)				
Average	248 (120), n=124	248 (135)	MD, -1 (-33 to 31)	0·96
Lowest	176 (93), n=124	180 (110)	MD, -4 (-30 to 21)	0·73
Percentage fall[b]	22 (26), n=124	21 (25)	MD, 1 (-5 to 7)	0·74
< 20	0 (0·0%), n=124	2 (1·6%)	OR, 0·41 (0 to 5·32)	0·50

Data are n (% of the total number) and mean (SD). The number of participants available for specific variables is stated in each cell if different from the total number of participants in the treatment group.

Mean differences were assessed using Student t-test. Odds ratios were assessed using logistic regression.

CI = confidence interval. MD = mean difference. OR = odds ratio. SD = standard deviation.

[a] Difference of the baseline haemoglobin measurement and the nadir haemoglobin measurement to day 10.

[b] Difference of the baseline platelet count and the nadir platelet count to day 10 as a percentage of the baseline count.

sTable 4. **Haemoglobin and platelet count in the Intensive Care Unit to day 10.**

	Nebulised Heparin Group (n=128)	Nebulised Saline Group (n=124)	Type of Difference Estimate, Difference Estimate (95% CI)	P
Fluid balance (mL)				
Cumulative to day 1	1405 (1954), n=125	1255 (2300)	MD, 151 (-382 to 684)	0·58
Cumulative to day 3	829 (3233), n=114	874 (3837), n=104	MD, -44 (-997 to 908)	0·93
Cumulative to day 5	193 (4363), n=90	1033 (4687), n=87	MD, -840 (-2185 to 504)	0·22
Cumulative to day 10	1553 (5978), n=35	1155 (5648), n=42	MD, 397 (-2263 to 3058)	0·77
Medications (treatment days to day 28 for treated patients[a])				
Sedative or opiate infusion	6·5 (4·1), n=124	7·3 (6·0)	MD, -0·8 (-2·1 to 0·5)	0·21
Antibacterial	8·0 (4·9), n=122	8·8 (6·4), n=123	MD, -0·8 (-2·3 to 0·6)	0·25
Antiviral	4·0 [1·0 to 6·0], n=34	4·5 [2·0 to 6·0], n=28	MedD, -1·0 (-3·4 to 1·4)	0·40
Antifungal	4·0 [2·0 to 9·0], n=19	4·0 [2·0 to 8·0], n=23	MedD, 0·0 (-3·6 to 3·6)	1·00
Corticosteroid	6·1 (4·6), n=69	6·5 (5·5), n=67	MD -0·4 (-2·1 to 1·3)	0·63

Data are n (% of the total number), mean (SD) and median [IQR]. The number of participants available for specific variables is stated in each cell if different from the total number of participants in the treatment group.

Mean differences were assessed using Student t-test. Median differences were assessed using quantile regression.

CI = confidence interval. IQR = inter-quartile range. MedD = median difference. MD = mean difference. SD = standard deviation.

[a] If the therapy was administered at any time on a calendar day, this constituted a day of therapy.

sTable 5. **Fluid Balance, sedatives, corticosteroids and antimicrobials in the Intensive Care Unit**

	Nebulised Heparin Group (n=128)	Nebulised Saline Group (n=124)	Type of Effect Estimate, Effect Estimate (95% CI)	P
Deterioration in Murray Lung Injury Score[a]				
ARDS – At risk	-0·00 (0·49), n=59	0·10 (0·50), n=71	MD, -0·10 (-0·28 to 0·07)	0·23
ARDS – Present	-0·09 (0·48), n= 64	0·08 (0·45), n=52	MD, -0·16 (-0·33 to 0·01)	0·07

Data are mean (SD). The number of participants available for specific variables is stated in each cell if different from the total number of participants in the treatment group.

Mean differences were assessed using Student t-test.

ARDS = acute respiratory distress syndrome. CI = confidence interval. MD = mean difference. SD = standard deviation.

[a] Calculated by subtracting the baseline score from the highest of the scores measured daily on days 1 to 5. The score was calculated for all participants while receiving invasive or non-invasive ventilatory support; respiratory compliance was calculated by tidal volume divided by the difference of peak inspiratory and expiratory pressures.

The outcome was not calculated for 1 participant in the heparin group whose baseline ARDS status was not known, for 1 participant in each group who had separation from ventilatory support by day 1, and for 3 participants in the heparin group who were transferred to another intensive care unit on day 1.

***sTable 6.** Deterioration in Murray Lung Injury Score to day 5, according to baseline ARDS status (post hoc)*

A. **Murray Chest X-Ray Score – Change from Baseline**

Study Day	1	2	3	4	5
Neb Hep, n/N	118/127	103/110	84/90	70/76	54/58
Neb Sal, n/N	113/123	92/100	81/89	69/75	57/61

B. **Murray P_aO_2:F_IO_2 Score – Change from Baseline**

Study Day	1	2	3	4	5
Neb Hep, n/N	123/127	103/110	80/90	66/76	52/58
Neb Sal, n/N	119/123	94/100	82/89	69/75	57/61

C. Murray PEEP Score – Change from Baseline

Study Day	1	2	3	4	5
Neb Hep, n/N	127/127	110/110	90/90	76/76	54/58
Neb Sal, n/N	123/123	100/100	89/89	75/75	57/61

D. **Murray Compliance Score – Change from Baseline**

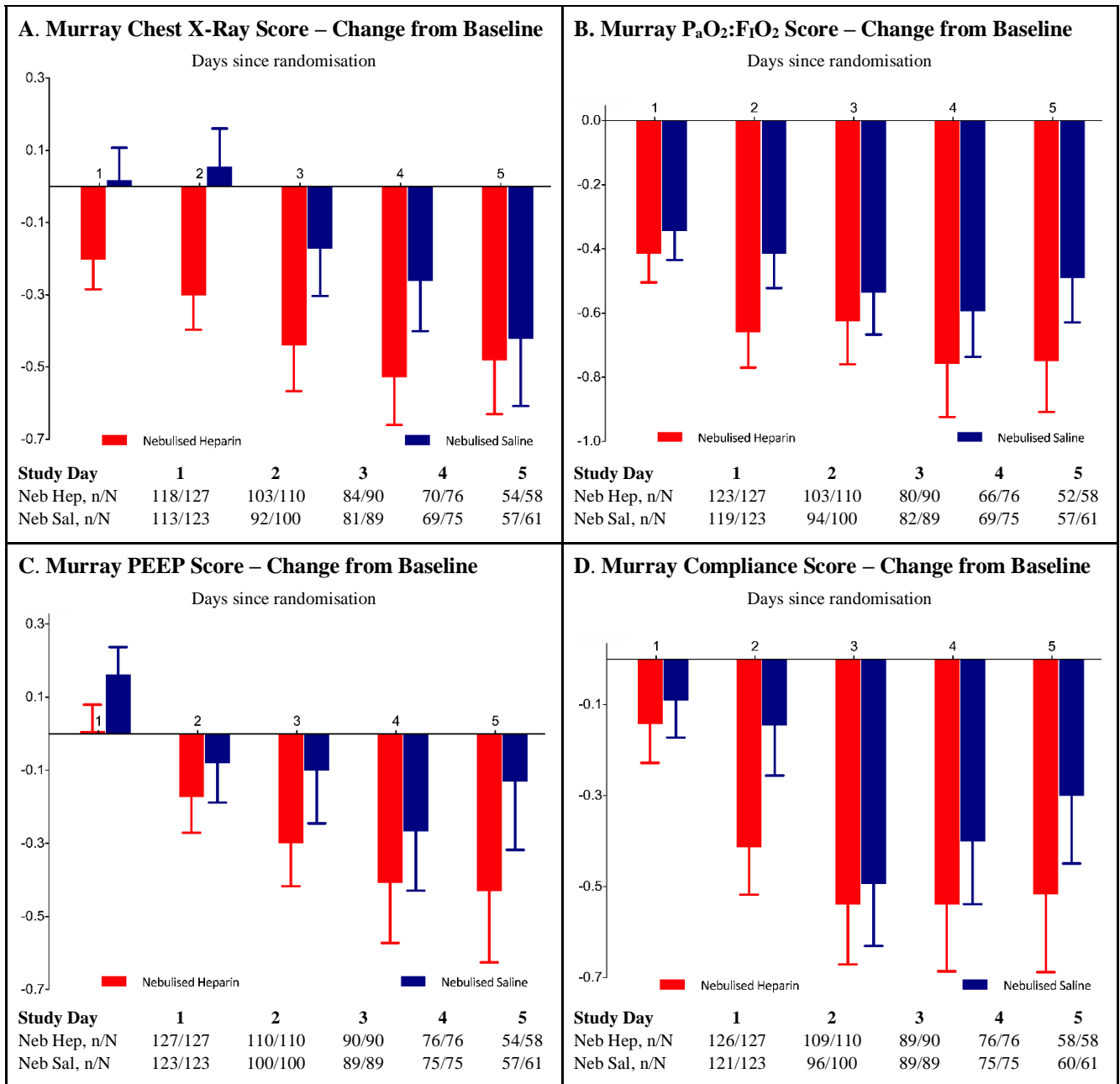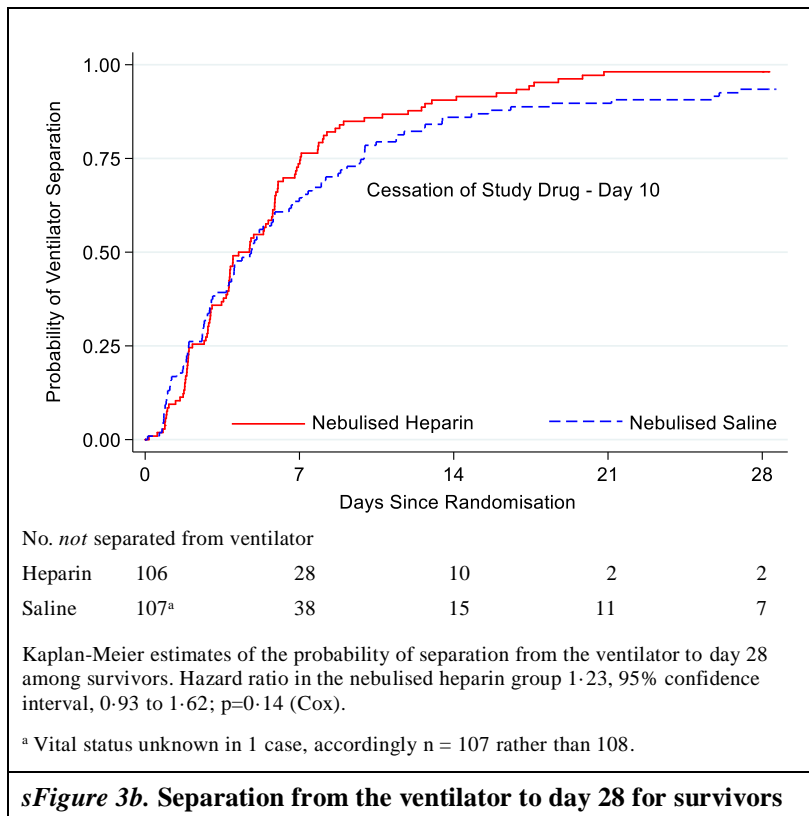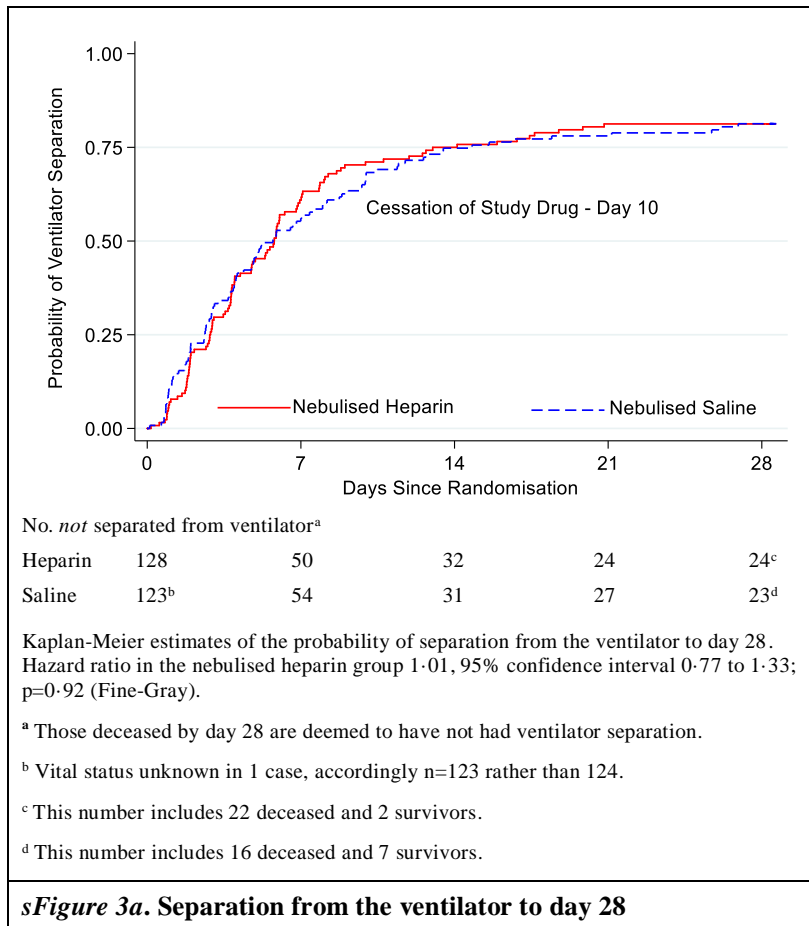| Study Day	1	2	3	4	5
Neb Hep, n/N	126/127	109/110	89/90	76/76	58/58
Neb Sal, n/N	121/123	96/100	89/89	75/75	60/61

sFigure 2. **Change from baseline for each component of the Murray Lung Injury Score among mechanically ventilated patients by treatment group**

Bar height (or depth) indicates mean change from baseline, and whisker indicates upper (or lower) boundary of the standard error of the mean.

Neb Hep = Nebulised Heparin. Neb Sal = Nebulised Saline.

n = number for whom the component was available. N = number for whom the Murray Lung Injury Score was calculated.

At baseline there were 128 in the heparin group and 124 in the saline group. Loss of data for the Murray Lung Injury Score is due to death (6 in each group by day 5), transfer to another intensive care unit (3 in the heparin group on day 1) and separation from mechanical ventilation.

No. *not* separated from ventilator[a]

Heparin	128	50	32	24	24[c]
Saline	123[b]	54	31	27	23[d]

Kaplan-Meier estimates of the probability of separation from the ventilator to day 28. Hazard ratio in the nebulised heparin group 1·01, 95% confidence interval 0·77 to 1·33; p=0·92 (Fine-Gray).

[a] Those deceased by day 28 are deemed to have not had ventilator separation.

[b] Vital status unknown in 1 case, accordingly n=123 rather than 124.

[c] This number includes 22 deceased and 2 survivors.

[d] This number includes 16 deceased and 7 survivors.

sFigure 3a. **Separation from the ventilator to day 28**

No. *not* separated from ventilator

Heparin	106	28	10	2	2
Saline	107[a]	38	15	11	7

Kaplan-Meier estimates of the probability of separation from the ventilator to day 28 among survivors. Hazard ratio in the nebulised heparin group 1·23, 95% confidence interval, 0·93 to 1·62; p=0·14 (Cox).

[a] Vital status unknown in 1 case, accordingly n = 107 rather than 108.

sFigure 3b. **Separation from the ventilator to day 28 for survivors**

No. *not* separated from ICU[a]

Heparin	128	72	38	28	25[c]
Saline	123[b]	73	39	31	26[d]

Kaplan-Meier estimates of the probability of separation from the Intensive Care Unit (ICU) to day 28.[a] Hazard ratio in the nebulised heparin group, 1·08; 95% confidence interval, 0·82 to 1·42; p=0·59 (Fine-Gray).

[a] Those deceased by day 28 are deemed to have not had ICU separation.

[b] Vital status unknown in 1 case, accordingly n = 123 rather than 124.

[c] This number includes 22 deceased and 3 survivors.

[d] This number includes 16 deceased and 10 survivors.

sFigure 4a. **Separation from ICU to day 28**

No. *not* separated from ICU

Heparin	106	50	16	6	3
Saline	107 [a]	57	23	15	10

Kaplan-Meier estimates of the probability of separation from the Intensive Care Unit to day 28 among survivors. Hazard ratio in the nebulised heparin group 1·31, 95% confidence interval 0·99 to 1·74; p=0·06 (Cox).

[a] Vital status unknown in 1 case, accordingly n = 107 rather than 108.
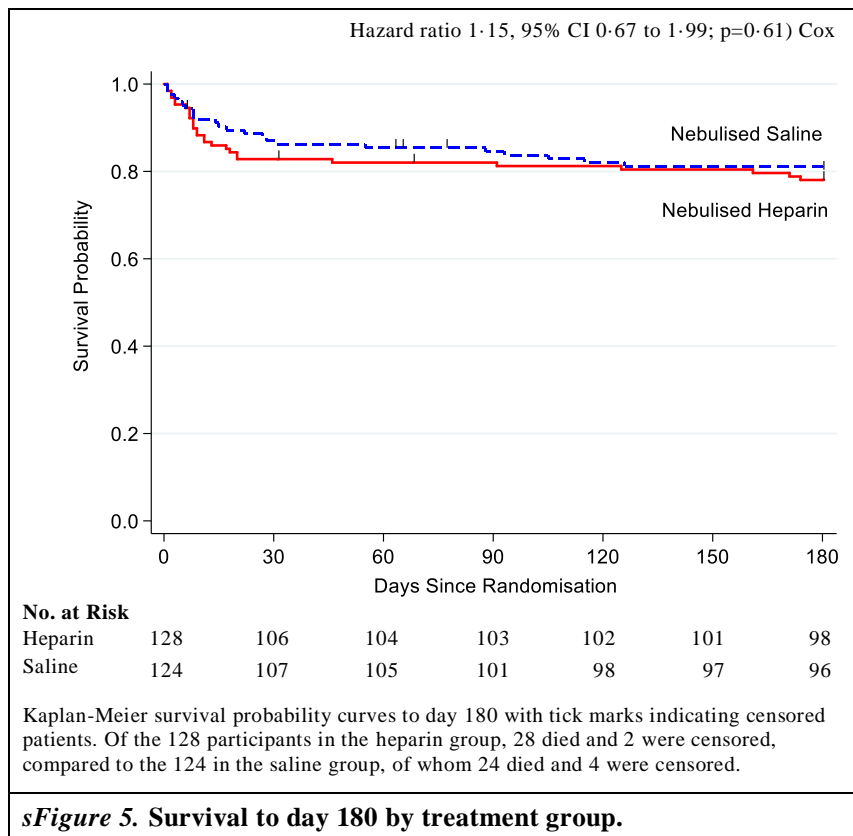
sFigure 4b. **Separation from ICU to Day 28 for survivors**

	Nebulised Heparin Group (n=128)	Nebulised Saline Group (n=124)	Type of Effect Estimate, Effect Estimate (95% CI)	P
Vasopressor-free days[a]	20·6 (10·2), n=124	21·5 (9·5), n=123	MD, -0·9 (-3·4 to 1·6)	0·48
Vasopressor-free days[a] of day 28 survivors	25·1 (3·7), n=102	24·7 (4·9), n=107	MD, 0·3 (-0·8 to 1·5)	0·57
RRT-free days[b]	22·7 (11·1), n=124	23·8 (10·1), n=123	MD, -1·1 (-3·8 to 1·6)	0·41
RRT-free days[b] of day 28 survivors	27·6 (3·8), n=102	27·3 (4·4), n=107	MD, 0·2 (-0·9 to 1·3)	0·69

Data are mean (SD). The number of participants available for specific variables is stated in each cell if different from the total number of participants in the treatment group.

Mean differences were assessed using Student t-test.

CI = confidence interval. MD = mean difference. RRT = renal replacement therapy. SD = standard deviation.

[a] Vasopressor-free days are the number of days to day 28 where the participant did not receive an inotrope or vasopressor infusion in the ICU; deceased participants at day 28 were assigned zero vasopressor-free days. There were 22 deaths in the heparin group and 16 in the saline group·

[b] RRT-free days are the number of days to day 28 where the participant did not receive renal replacement therapy in the ICU; deceased participants at day 28 were assigned zero RRT-free days. There were 22 deaths in the heparin group and 16 in the saline group·

sTable 7. **Vasopressor therapy and renal replacement therapy to day 28**

	Nebulised Heparin Group (n=128)	Nebulised Saline Group (n=124)	Type of Effect Estimate, Effect Estimate (95% CI)	P
Discharge destination				
Home	60 (46·9%)	67 (54·0%)	OR, 0·75 (0·46 to 1·23)	0·26
Residential low-level care	1 (0·8%)	0 (0·0%)	OR, 0·97 (0·02 to und.)	1·00
Rehabilitation	26 (20·3%)	22 (17·7%)	OR, 1·18 (0·63 to 2·22)	0·60
Transfer to another hospital	18 (14·1%)	16 (12·9%)	OR, 1·10 (0·54 to 2·28)	0·79
Hospice	0 (0·0%)	1 (0·8%)	OR, 0·97 (0 to 37·78)	0·98
Deceased	23 (18·0%)	18 (14·5%)	OR, 1·29 (0·66 to 2·53)	0·46
Time to discharge				
If alive	14·0 (10·0 to 20·0), n=105	15·5 (9·0 to 29·0), n=106	HR, 1·14 (0·87 to 1·50)	0·34
If discharged home	12·0 (8·0 to 15·0), n=60	12·0 (8·0 to 20·0), n=67	HR, 1·30 (0·91 to 1·89)	0·16
If discharged to rehabilitation	18·5 (15·0 to 38·0), n=26	25·5 (17·0 to 43·0), n=22	HR, 0·95 (0·52 to 1·72)	0·86
If transferred to another hospital	18·0 (9·0 to 32·0), n=18	21·0 (11·5 to 42·0), n=16	HR, 1·25 (0·63 to 2·49)	0·52
If deceased	8·0 (3·0 to 13·0), n=23	8·0 (4·0 to 22·0), n=18	HR, 1·56 (0·80 to 3·05)	0·19

Data are n (% of the total number) and median (IQR). The number of participants available for specific variables is stated in each cell if different from the total number of participants in the treatment group.

Odds ratios were assessed using logistic regression or, if there was a count of zero, exact logistic regression. Hazard ratios were assessed using Cox regression.

CI = confidence interval. HR = hazard ratio. IQR = interquartile range. OR = odds ratio. Und = undefined.

sTable 8. **Discharge from the study hospital**

	Nebulised Heparin Group (n=97)	Nebulised Saline Group (n=94)	Type of Effect Estimate Effect Estimate (95% CI)	*P*
Study day of interview	64 (62 to 68)	63 (62 to 66)	HR, 0·81 (0·60 to 1·07)	0·14
Interview candidate				
Participant	74 (76·3%)	74 (78·7%)	OR, 0·87 (0·44 to 1·72)	0·69
Proxy	23 (23·7%)	20 (21·3%)	OR, 1·15 (0·58 to 2·27)	0·69

Data are n (% of the total number) and median (IQR).

Hazard ratio was assessed using Cox regression. Odds ratios were assessed using logistic regression.

CI = confidence interval. HR = hazard ratio. IQR = inter-quartile range. OR = odds ratio.

sTable 9. **Additional information about the primary outcome interview at day 60**

	Nebulised Heparin Group (n=128)	Nebulised Saline Group (n=124)	Type of Effect Estimate, Effect Estimate (95% CI)	P
SF-36 Health Survey Physical Function Score of survivors and non-survivors[a]	43·3 (35·4), n=120	40·9 (37·3), n=112	MD, 2·4 (-7·0 to 11·9)	0·61
EQ-5D-3L Domains[b]				
Mobility				0·24
No problems	48 (49·5%), n=97	46 (47·9%), n=96		
Some problems	47 (48·5%), n=97	43 (44·8%), n=96		
Confined to bed	2 (2·1%), n=97	7 (7·3%), n=96		
Personal care				0·06
No problems	69 (71·1%), n=97	61 (63·5%), n=96		
Some problems	26 (26·8%), n=97	25 (26·0%), n=96		
Unable to wash or dress	2 (2·1%), n=97	10 (10·4%), n=96		
Usual activities				0·20
No problems	40 (41·2%), n=97	37 (38·5%), n=96		
Some problems	46 (47·4%), n=97	39 (40·6%), n=96		
Unable to perform	11 (11·3%), n=97	20 (20·8%), n=96		
Pain or discomfort				0·17
None	53 (54·6%), n=97	48 (50·0%), n=96		
Moderate	36 (37·1%), n=97	45 (46·9%), n=96		
Extreme	8 (8·3%), n=97	3 (3·1%), n=96		
Anxiety or depression				0·32
None	54 (55·7%), n=97	50 (52·1%), n=96		
Moderate	31 (32·0%), n=97	39 (40·6%), n=96		
Extreme	12 (12·4%), n=97	7 (7·3%), n=96		

Data are n (% of the total number) and mean (SD). The number of participants available for specific variables is stated in each cell if different from the total number of participants in the treatment group.

CI = confidence interval. MD = mean difference. SD = standard deviation.

Mean differences were assessed using Student t-test. The Fisher exact test compared the groups for each domain of the EQ-5D-3L.

[a] Score range 0 to 100; higher is preferable. Non-survivors were assigned a score of zero.

[b] EuroQol quality of life instrument.

sTable 10. **Physical function and quality of life at day 60**

	Nebulised Heparin Group (n=128)	Nebulised Saline Group (n=124)	Type of Effect Estimate, Effect Estimate (95% CI)	P
SF-36 Health Survey Physical Function Score[a] of survivors	60·5 (31·0), n=91	61·3 (33·7), n=93	MD, -0·7 (-10·1 to 8·7)	0·88
SF-36 Health Survey Physical Function Score[a] of survivors and non-survivors[b]	46·3 (37·4), n=119	48·7 (39·0), n=117	MD, -2·4 (-12·2 to 7·4)	0·63
EQ-5D-3L Domains[c]				
Mobility				0·61
No problems	55 (60·4%), n=91	52 (55·9%), n=93		
Some problems	35 (38·5%), n=91	38 (40·9%), n=93		
Confined to bed	1 (1·10%), n=91	3 (3·2%), n=93		
Personal care				0·53
No problems	73 (80·2%), n=91	73 (78·5%), n=93		
Some problems	17 (18·7%), n=91	16 (17·2%), n=93		
Unable to wash or dress	1 (1·1%), n=91	4 (4·3%), n=93		
Usual activities				0·85
No problems	47 (51·7%), n=91	51 (54·8%), n=93		
Some problems	37 (40·7%), n=91	34 (36·6%), n=93		
Unable to perform	7 (7·7%), n=91	8 (8·6%), n=93		
Pain or discomfort				0·97
None	49 (53·9%), n=91	51 (54·8%), n=93		
Moderate	36 (39·6%), n=91	37 (39·8%), n=93		
Extreme	6 (6·6%), n=91	5 (5·4%), n=93		
Anxiety or depression				0·0046
None	52 (57·1%), n= 91	59 (63·4%), n=93		
Moderate	25 (27·5%), n= 91	32 (34·4%), n=93		
Extreme	14 (15·4%), n= 91	2 (2·2%), n=93		

Data are n (% of the total number) and mean (SD). The number of participants available for specific variables is stated in each cell if different from the total number of participants in the treatment group.

CI = confidence interval. MD = mean difference. SD = standard deviation.

Mean differences were assessed using Student t-test. The Fisher exact test compared the groups for each domain of the EQ-5D-3L.

[a] Score range 0 to 100; higher is preferable.

[b] Non-survivors were assigned a score of zero.

[c] EuroQol quality of life instrument.

sTable 11. **Physical function and quality of life at day 180**

Hazard ratio 1·15, 95% CI 0·67 to 1·99; p=0·61) Cox

No. at Risk

Heparin	128	106	104	103	102	101	98
Saline	124	107	105	101	98	97	96

Kaplan-Meier survival probability curves to day 180 with tick marks indicating censored patients. Of the 128 participants in the heparin group, 28 died and 2 were censored, compared to the 124 in the saline group, of whom 24 died and 4 were censored.

sFigure 5. **Survival to day 180 by treatment group.**

	Nebulised Heparin Group (n=28)	Nebulised Saline Group (n=24)	Odds Ratio (95% CI)	*P*
Distributive shock	12 (46·2%), n=26	4 (20·0%), n=20	3·43 (0·90 to 13·09)	0·07
Hypoxic respiratory failure	8 (30·8%), n=26	8 (40·0%), n=20	0·67 (0·20 to 2·26)	0·52
Arrhythmia or cardiogenic shock	0 (0·0%), n=26	4 (20·0%), n=20	0·13 (0 to 1·08)	0·06
Brain injury[a]	2 (7·7%), n=26	2 (10·0%), n=20	0·75 (0·10 to 5·84)	0·78
Metabolic disturbance	1 (3·9%), n=26	0 (0·0%), n=20	0·77 (0·02 to und.)	1·00
Hypovolemic shock	0 (0·0%), n=26	0 (0·0%), n=20		
Other (Unspecified)	3 (11·5%), n=26	2 (10·0%), n=20	1·17 (0·18 to 7·79)	0·87
Unknown	2 (7·1%)	4 (16·7%)	0·38 (0·06 to 2·31)	0·30

Data are n (% of the total number). The number of participants available for specific variables is stated in each cell if different from the total number of deceased participants in the treatment group.

CI = confidence interval. Und. = undefined.

Odds ratios were assessed using logistic regression or, if there was a count of zero, exact logistic regression.

[a] Traumatic or non-traumatic, with or without brain death.

sTable 12. **Proximate cause of deaths to day 180**

Subgroup	Nebulised Heparin Group (n=97)[a]	Nebulised Saline Group (n=94)	Mean Difference[b] (95% CI)	P [c]
ARDS				
No ARDS	50·9 (31·6), n=52	50·9 (36·5), n=55	0·0 (-13·1 to 13·0)	
ARDS	56·7 (31·9), n=44	45·6 (34·8), n=39	11·1 (-3·6 to 25·7)	0·26
Murray Lung Injury Score				
< 2·25	47·1 (32·7), n=38	48·2 (34·8), n=45	-1·1 (-15·9 to 13·7)	
≥ 2·25	57·8 (30·4), n=59	49·2 (36·9), n=49	8·6 (-4·5 to 21·7)	0·33
Invasive ventilation hours				
< 18	59·6 (29·6), n=52	53·0 (36·6), n=45	6·6 (-7·0 to 20·2)	
≥ 18	46·7 (32·7), n=45	44·8 (34·8), n=49	1·9 (-12·0 to 15·7)	0·63
Pneumonia				
No pneumonia	42·5 (33·1), n=30	46·8 (37·0), n=31	-4·3 (-22·3 to 13·7)	
Pneumonia	58·6 (29·8), n=67	49·7 (35·3), n=63	8·9 (-2·5 to 20·3)	0·21
UFH or LMWH				
No heparin	51·8 (30·4), n=20	40·0 (37·1), n=23	11·8 (-9·1 to 32·6)	
UFH or LMWH	54·1 (32·0), n=77	51·5 (35·1), n=71	2·5 (-8·4 to 13·5)	0·43
UFH				
No UFH	59·0 (30·3), n=69	49·3 (36·3), n=64	9·7 (-1·8 to 21·2)	
UFH	40·4 (31·3), n=28	47·5 (35·0), n=30	-7·1 (-24·6 to 10·3)	0·11
Age (years)				
< 60	59·8 (30·0), n=53	53·8 (36·6), n=53	6·0 (-6·8 to 18·9)	
≥ 60	46·1 (32·2), n=44	42·2 (33·9), n=41	3·9 (-10·3 to 18·2)	0·83
Sex				
Female	47·1 (28·3), n=35	47·4 (35·2), n=35	-0·3 (-15·5 to 15·0)	
Male	57·3 (32·9), n=62	49·5 (36·3), n=59	7·8 (-4·7 to 20·3)	0·43
Enrolment period				
Prior to 1 September 2015	52·1 (30·6), n=43	51·9 (35·1), n=47	0·2 (-13·6 to 14·0)	
From 1 September 2015	54·8 (32·6), n=54	45·5 (36·4), n=47	9·3 (-4·5 to 23·0)	0·35
Enrolment site				
Not lead recruiter	49·7 (33·5), n=51	45·2 (33·9), n=42	4·5 (-9·5 to 18·4)	
Lead recruiter	57·9 (29·1), n=46	51·5 (37·2), n=52	6·4 (-6·9 to 19·7)	0·84

Values are mean (SD) of the SF-36 Health Survey Physical Function Score of survivors at day 60.

ARDS = acute respiratory distress syndrome. CI = confidence interval. LMWH = low molecular weight heparin. SD = standard deviation. UFH = unfractionated heparin.

[a] ARDS at baseline is unknown in 1 case, accordingly n = 96 rather than 97 for the ARDS subgroup.

[b] Student t-test.

[c] For interaction assessed using linear regression; unadjusted for multiple tests.

***sTable 13a*. SF-36 Health Survey Physical Function Score of survivors at day 60 according to baseline subgroup**

Subgroup	Nebulised Heparin Group (n=128)[a]	Nebulised Saline Group (n= 123)[b]	Hazard Ratio[c] (95% CI)	P [d]
ARDS				
No ARDS	8·1 (8·9), n=62	8·6 (9·4), n=71	1·03 (0·72 to 1·49)	
ARDS	11·6 (10·4), n=65	12·4 (10·8), n=52	1·07 (0·70 to 1·64)	0·85
Murray Lung Injury Score				
< 2·25	8·4 (9·5), n=49	6·2 (7·7), n=55	0·73 (0·49 to 1·10)	
≥ 2·25	10·8 (9·9), n=79	13·4 (10·8), n=68	1·32 (0·91 to 1·91)	0·0439
Invasive ventilation hours				
< 18	9·1 (9·6), n=69	9·3 (10·1), n=60	1·02 (0·69 to 1·50)	
≥ 18	10·8 (10·1), n=59	11·1 (10·2), n=63	0·99 (0·67 to 1·47)	0·91
Pneumonia				
No pneumonia	10·3 (10·7), n=41	8·4 (8·3), n=33	0·82 (0·50 to 1·34)	
Pneumonia	9·7 (9·4), n=87	10·9 (10·7), n=90	1·09 (0·79 to 1·52)	0·35
UFH or LMWH				
No heparin	10·9 (10·5), n=29	8·1 (8·0), n=30	0·75 (0·43 to 1·31)	
UFH or LMWH	9·6 (9·6), n=99	10·9 (10·7), n=93	1·12 (0·82 to 1·53)	0·19
UFH				
No UFH	9·4 (9·9), n=89	10·4 (10·2), n=83	1·08 (0·78 to 1·51)	
UFH	10·9 (9·7), n=39	9·8 (10·1), n=40	0·87 (0·53 to 1·41)	0·42
Age (years)				
< 60	8·5 (8·6), n=64	8·0 (8·7), n=62	0·93 (0·64 to 1·36)	
≥ 60	11·2 (10·8), n=64	12·5 (11·0), n=61	1·08 (0·72 to 1·61)	0·57
Sex				
Female	10·0 (9·8), n=45	8·4 (9·1), n=49	0·83 (0·54 to 1·29)	
Male	9·8 (9·9), n=83	11·4 (10·7), n=74	1·14 (0·80 to 1·62)	0·30
Enrolment period				
Prior to 1 September 2015	8·8 (9·3), n=57	9·9 (9·8), n=58	1·14 (0·76 to 1·70)	
From 1 September 2015	10·7 (10·2), n=71	10·5 (10·5), n=65	0·94 (0·64 to 1·36)	0·51
Enrolment site				
Not lead recruiter	9·9 (9·5), n=68	11·3 (10·2), n=63	1·14 (0·78 to 1·67)	
Lead recruiter	9·8 (10·3), n=60	9·1 (10·1), n=60	0·90 (0·61 to 1·34)	0·43

Values are mean (SD) of time to ventilator separation (days). Non-survivors at day 28 were treated as though not separated from the ventilator. There were 22 deaths in the heparin group and 16 in the saline group.

ARDS = acute respiratory distress syndrome. CI = confidence interval. LMWH = low molecular weight heparin. SD = standard deviation. UFH = unfractionated heparin.

[a] ARDS at baseline is unknown in 1 case, accordingly n = 127 rather than 128 for the ARDS subgroup.

[b] Vital status at day 28 is unknown in 1 case, accordingly n = 123 rather than 124.

[c] Analysed to account for the competing risk of death; there were 22 deaths in the heparin group and 16 in the saline group.

[d] For interaction assessed using competing-risk regression; unadjusted for multiple tests.

sTable 13b. **Ventilator separation to day 28 according to baseline subgroup**

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Nebulised Heparin for Lung Injury
Protocol
Version 3, Dated 15 December 2017

1. General information

1.1 Title

Nebulised heparin for lung injury: A multi-centre, randomised, double-blind, placebo-controlled trial

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1.4 Management Committee

The committee members are:

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- Dr Duncan Campbell FRACP, FCSANZ, PhD, GradDipEpidBiostat
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- Mr Roger Smith (Project Manager) RN, BN, PostgradDipAdvNurs (Critical Care), MPH
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2. Background and trial rationale

2.1 Diffuse alveolar damage and lung injury

The clinical syndrome of acute respiratory failure due to acute inflammatory changes in the lungs was first described in a 12-patient case series by Ashbaugh et al in 1967.[1] These patients developed an acute onset of rapid breathing, low oxygen levels in the blood and stiff lungs after a variety of stimuli including trauma, pancreatitis and pneumonia. Chest radiograph changes included bilateral lung infiltrates. At autopsy the lungs had the macroscopic appearance of liver. Microscopic examination demonstrated engorged lung capillaries, areas of air sac collapse, interstitial and air sac oedema and haemorrhage. White cells and hyaline membranes in the air sacs were numerous.

The histological changes found by Ashbaugh have been termed diffuse alveolar damage.[2] These changes represent the acute inflammatory response to a tissue insult. The hallmark histological characteristic of diffuse alveolar damage is hyaline membrane formation in the air sacs as a result of fibrin deposition.[2] Blockage of small blood vessels with fibrin is also an important feature.[3-7] A host of conditions can precipitate diffuse alveolar damage including pneumonia, sepsis, aspiration, transfusion, cardiac surgery and trauma.[8] Mechanical ventilation may itself trigger diffuse alveolar damage through ventilator-induced lung injury and ventilator-associated pneumonia.[9]

A number of approaches have been developed to quantify the severity of lung injury based on the clinical features associated with diffuse alveolar damage, including the extent of infiltrates in the chest radiograph and oxygen levels in the blood. These approaches include the Murray Lung Injury Score and the Berlin Criteria, which are used to define Acute Lung Injury (ALI) and the Acute Respiratory Distress Syndrome (ARDS).[10,11]

2.2 Hyaline membrane formation

Hyaline membrane formation is a consistent and early manifestation of diffuse alveolar damage.[1,3,20,21] Hyaline membrane formation occurs when inflammatory fluid carrying plasma borne coagulation factors enters the air sacs and tissue factor, expressed by alveolar epithelial cells and white cells, triggers the conversion of these coagulation factors to fibrin.[22]

Hyaline membrane formation contributes to lung injury through a number of mechanisms. Firstly, it causes shunting by creating a physical barrier that limits the diffusion of gases between the air spaces and the blood. Secondly, it contributes to air sac collapse by directly reducing compliance of the sacs and by impairing the action of surfactant. Finally, the laying down of a fibrin membrane may promote infiltration by white cells and subsequent lung scarring.[22,23]

2.3 Microvascular thrombosis

The acute inflammatory response in lung capillary beds triggers the expression of tissue factor on endothelial cells, which in turn triggers the conversion of coagulation factors in the plasma into fibrin, resulting in the formation of microvascular thrombi.[24] Extensive microvascular thrombosis has been demonstrated in a number of histological studies of ALI and ARDS.[3-7] Angiographic and other studies demonstrated the extent of microvascular obstruction correlated with both the severity of respiratory failure and mortality.[5,25-28] Microvascular thrombosis may contribute to lung injury through a number of mechanisms. Firstly, it reduces air sac blood flow and this reduces the quantity of oxygen taken up by the blood. Secondly, microvascular thrombosis may cause ischaemic air sac injury. And thirdly, it may contribute to high pressures in the pulmonary circulation and right ventricular failure.[29]

2.4 Heparin reduces fibrin formation

Heparin is a chemical normally produced by the body. Heparin has been in clinical use for 60 years primarily to prevent fibrin formation. Heparin reduces fibrin formation through a range of mechanisms including catalysing the action of antithrombin, promoting tissue factor pathway inhibitor expression, reducing tissue factor expression and increasing endothelial expression of heparan sulfate. In addition to its anticoagulant effects heparin has anti-inflammatory, fibrinolytic, antibacterial and antifibrotic properties. These properties suggest nebulised heparin would be beneficial in promoting recovery from diffuse alveolar damage.

2.5 Systemic heparin for lung injury and sepsis

Clinical and experimental models of lung injury demonstrated that intravenous heparin and other anticoagulants reduced fibrin deposition in the lungs and improved clinical outcomes.[30-34] We were the first to demonstrate that preoperative intravenous heparin reduced histological evidence of lung capillary fibrin deposition during cardiac surgery.[35]

Previous work in septic states has found heparin may limit injury in other acutely inflamed organs of the body.[30] Post-hoc analysis of three large trials of patients with life-threatening infections found subcutaneous heparin was associated with improved clinical outcomes and reduced mortality (32 vs. 42%; $P = 0.0001$).[30,36-38] A large randomised prospective study of subcutaneous heparin in patients with life-threatening infections all treated with activated protein C also found a trend to reduced mortality (28 vs. 32%; $P = 0.08$).[39]

2.6 Nebulised heparin for asthma, hay fever, cystic fibrosis and smoke inhalation

Nebulisation is the optimal method of administering heparin to limit fibrin deposition in the lungs. Nebulised heparin has been used for over 30 years in adults and children to treat a range of respiratory conditions including asthma, hay fever, cystic fibrosis and smoke inhalation. Studies found heparin had a number of beneficial actions including reduced bronchospasm in asthma[40] reduced rhinorrhoea in hay fever[41], reduced sputum elasticity in cystic fibrosis[42] and reduced lung damage and death, compared with historical controls, in patients with smoke inhalation.[43] In all of these trials administration of nebulised heparin was safe.

2.7 Phase I trial of nebulised heparin for lung injury

In 2008 we performed a phase I trial that demonstrated, for the first time, that nebulised heparin reduced the extent of pulmonary coagulation in patients with ALI and ARDS.[44,45] We showed that nebulised heparin at doses up to 400,000 Units per day was safe and reduced levels of pulmonary coagulation, as indicated by reduced levels of thrombin-anti-thrombin complexes and fibrin degradation products in fluid samples taken from the lungs before and after two days of treatment.

2.8 Phase II trial of nebulised heparin for lung injury

Based on these findings we undertook a phase II double-blind randomised trial of nebulised heparin in patients expected to require mechanical ventilation for the more than 48 hours.[46] This inclusion criterion was chosen to allow for enrolment of patients with ARDS and also patients with less severe forms of lung injury. Patients with less severe forms of lung injury are at high risk of progressing to ARDS. It has been shown that for patients requiring mechanical ventilation for the more than 48 hours, 26% have ALI at commencement of mechanical ventilation, while a further 18% will develop ALI, predominantly over the first three days of mechanical ventilation.[47]

Fifty (50) patients were randomised to receive either nebulised heparin (25,000 Units) or placebo (0.9% sodium chloride) 4-hourly or 6-hourly, depending on patient height. The study drug was continued while the patient remained ventilated for a maximum of 14 days. All had a Murray Lung Injury Score above 0.5 (indicating at least mild lung injury). The mean Murray Lung Injury Score at enrolment was 2.4, suggesting on average moderate to severe lung injury. Nebulised heparin was associated with an improvement in ventilator-free days amongst survivors at day 28 (22.6 ± 4.0 versus 18.0 ± 7.1, treatment difference 4.6 days, 95% CI 0.9 to 8.3, $P = 0.02$)

In comparison with placebo, nebulised heparin was also associated with reduced use of nitric oxide, which is used as a lung rescue therapy when oxygenation of the blood is inadequate despite maximal ventilator settings [0/25 (0%) vs. 5/25 (19%), $P = 0.05$]. Positive trends were present in other important clinical endpoints, including a lower tracheostomy rate (28 vs. 48%, $P = 0.1$), a lower rate of development of ARDS (0% vs. 19%, $P = 0.1$) and a shorter stay in the intensive care unit (9.4 vs. 14.0 days, $P = 0.2$). Furthermore, a post hoc analysis found heparin accelerated the rate of recovery in the Murray Lung Injury Score over the first five days for patients with a baseline score ≥ 2 ($P < 0.05$). Nebulised heparin was not associated with adverse events.

2.9 Low-dose nebulised heparin for prevention of ventilator-associated pneumonia

In a recent Australian study (IPHIVAP study), 214 adults expected to require more than 48 hours of mechanical ventilation were randomised to nebulised heparin, nebulised sodium chloride 0.9% or usual care.[48] The study had several important methodological limitations including the low dose of heparin (5000 Units, 4 times daily) and the use of non-standardised methods of nebulisation and humidification. No difference was found for the primary outcome of Klompas-defined ventilator associated pneumonia, though the study was underpowered to detect a difference. The study did not raise safety concerns.

2.10 Nebulised heparin for cardiac surgery

Our group investigated whether prophylactic nebulised heparin could limit the inflammatory response to cardiac surgery with cardiopulmonary bypass. We undertook a single-centre double-blind randomised trial. Forty (40) patients undergoing elective cardiac surgery with cardiopulmonary bypass were randomised to prophylactic nebulised heparin (50,000 Units) or placebo.[49] The primary endpoint was the change in arterial oxygen levels over the operative period. Secondary endpoints included end-tidal CO_2, the alveolar dead space fraction and bleeding complications. Nebulised heparin was not found to improve arterial oxygen levels. However, nebulised heparin was associated with a lower alveolar dead space fraction ($P < 0.05$) and lower tidal volumes at the end of surgery ($P < 0.01$) suggesting better alveolar perfusion. Nebulised heparin was not associated with bleeding complications.

2.11 Pharmacodynamics of nebulised heparin

Drug delivery during mechanical ventilation depends on the type of nebuliser, its position in the ventilator circuit and the type of ventilator circuit.[50] In this study the nebuliser and its position in the ventilator circuit are the same as were used in our phase I and phase II studies of nebulised heparin.[44-46]

The airway distribution and kinetics of nebulised heparin were quantified in a study of healthy subjects who were administered 90,000 Units of [99m]technetium-labelled sodium nebulised heparin. Approximately 8% of the nebulised dose was deposited in the lower respiratory tract, 40% of which remained present in the lungs 24 hours after inhalation; less than 1% of the nebulised dose was found in the systemic circulation.[51]

The effect of nebulised heparin on systemic coagulation was assessed in a study of healthy subjects who received a dose of nebulised heparin of 100,000 Units, 200,000 Units, 300,000 Units or 400,000 Units.[52] There were no significant changes in activated partial thromboplastin time (APTT), except at the dose of 400,000 Units, where peak values were found at 1 and 3 hours after the start of nebulisation. There was a small and dose-dependent increase in anti-Xa activity, which peaked 6 hours after nebulisation and returned to baseline after 24 hours. None of the participants reported spontaneous, prolonged or excessive bleeding, or breathing difficulties after heparin inhalation.

2.12 Specific safety aspects of nebulised heparin for lung injury

- *Systemic bleeding:* None of the 41 patients administered nebulised heparin in our phase I and II studies developed systemic bleeding as a complication.[44-46] The average APTT in the phase II study was higher in the heparin group by 4 seconds.[46] Such a difference is not clinically significant. There was one case where a clinically relevant rise in APTT necessitated temporarily withholding the study drug. Coagulation parameters and platelet count are monitored daily as part of routine ICU care. The possibility of systemic bleeding cannot be excluded, but the risk is extremely low.

- *Pulmonary bleeding:* In the phase II study, blood staining of the sputum occurred on 31% of study treatment days in the placebo group and on 41% in the heparin group ($P = 0.3$).[46] The increase in blood staining of the sputum was not associated with adverse clinical effects. The possibility of serious bleeding in the lungs or airways cannot be excluded, but the risk is very low.

- *Heparin-induced thrombocytopaenia and thrombosis (HITT)*: HITT is a recognised but rare complication of heparin therapy. Subcutaneous heparin for deep vein thrombosis prophylaxis is routine care for critically ill patients. It is therefore expected that most patients will already be exposed to heparin. There is no evidence that nebulised heparin carries additional risk of HITT compared to other routes of administration or that nebulised heparin increases the risk of HITT in patients receiving heparin via other routes. No patients administered nebulised heparin in our phase I and II studies developed HITT.[44-46]

- *Ventilator dysfunction*: To ensure that the exhaled nebulised medication does not impair function of the mechanical ventilator, a filter is placed at the end of the expiratory limb. In this study a *Servo Duo Guard* (Maquet Critical Care AB), which is designed for use with nebulised medications, is placed between the expiratory limb of the circuit and the expiratory valve of the ventilator. This filter is changed daily. Careful attention to study procedures should all but eliminate any risk to the patient from impaired function of the ventilator or ventilator circuit.

- Increased peak inspiratory pressure (PIP): We have reports for two patients in this study where administration of the study drug was associated with a transient increase in the peak inspiratory pressure. Before the study drug was commenced the patients had severe bronchospasm necessitating the use of low minute ventilation with end-tidal CO_2 of 80-100 mmHg, deep sedation, neuromuscular blockers, corticosteroids and regular nebulised bronchodilators. The increase in PIP occurred within a few breaths of administering the drug and resolved within minutes of stopping the study drug. In neither case was the study drug unblinded. Neither patient experienced adverse sequelae. Subsequent doses were withheld until the patients' bronchospasm showed some improvement. In both cases the study drug was reintroduced without event. This problem is probably confined to patients with very severe bronchospasm and may be caused by a layer of study drug coating the bronchioles and temporarily impeding air flow.

2.13 Incidence and burden of lung injury and the potential impact of nebulised heparin

In Australia the incidence of ALI and ARDS is 33/100 000 or around 8000 cases each year.[12] Mortality remains high at around 25–45% and, for surviving patients, significant and persistent poor health is common five years after hospital discharge.[12,13] The healthcare costs are enormous as patients require prolonged periods in the intensive care unit and hospital and also require rehabilitation and community-based health services.[14]

We showed nebulised heparin promoted faster recovery of lung injury and earlier freedom from mechanical ventilation. Previous studies suggest that faster recovery from lung injury and earlier freedom from mechanical ventilation leads to reductions in hospital and intensive care stays, improved long-term health, better quality of life and reduced healthcare costs.[14-19] Treating lung injury with nebulised heparin could potentially save as much as $100 million dollars annually in intensive care costs alone.

3. Trial design and feasibility

This is a multi-centre, randomised, double-blind trial of nebulised heparin (25,000 Units in 5 mL) versus nebulised placebo (sodium chloride 0.9%, 5 mL) in 256 intensive care patients with mild to severe lung injury who are receiving invasive ventilation and are expected to require invasive ventilation in intensive care for more than two days. Patients are administered the study medication every six hours for 10 days while receiving assisted ventilation via an endotracheal or tracheostomy tube in intensive care; the maximum number of doses of study drug is 40.

Our earlier, single-centre study of nebulised heparin recruited 50 patients over 18 months. It is reasonable to expect participating centres to randomise an average of 6 patients per year. On this basis the recruitment target of 256 patients can be achieved at eight sites in approximately 5½ years.

The Investigators, having participated in many multi-centre trials and having conducted phase I and phase II trials of nebulised heparin in mechanically ventilated patients and having established links within the intensive care community, are in an excellent position to preemptively identify and promptly resolve potential threats to the successful conduct of the trial.

4. Trial aims and hypotheses

4.1 Primary aim

The primary aim is to determine if treatment with nebulised heparin improves long-term physical function in patients with mild to severe lung injury who are expected to require at least two days of invasive mechanical ventilation in intensive care.

The null hypothesis assumes no difference in physical function, assessed 60 days after randomisation using the SF-36 physical function score, for patients treated with nebulised heparin compared to patients treated with placebo (nebulised saline). The physical function component of the SF-36 health survey determines the extent to which participants are limited in performing activities that might be undertaken during a typical day such as bathing and dressing, lifting and carrying groceries, bending, kneeling and stooping, walking and climbing stairs.

Previous studies have shown that faster recovery in the Murray Lung Injury Score and earlier freedom from mechanical ventilation are associated with better long-term health assessed by the SF-36 physical function score.[14,16,18]

4.2 Secondary aims

The major secondary aims are:

- To determine if there is any difference in the change in plasma thrombin time, thrombin-antithrombin levels and D-dimer levels from baseline to Day 3 between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in the change in serum tumour necrosis factor, interleukin-1 beta, interleukin-6, interleukin-8, and interleukin-12 from baseline to Day 3 between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in the change in Murray Lung Injury Score from baseline during Days 1 to 5 between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in the onset and progression of ARDS from baseline during Days 1 to 5 between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in fluid balance in the 10 days after randomisation between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in blood transfusion requirements in the 10 days after randomisation between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in haemoglobin, platelet count and APTT in the 10 days after randomisation between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in the dose of study drug administered and in the reasons for omitting the study drug between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in the incidence of major bleeding, pulmonary bleeding, heparin-induced thrombocytopenia, respiratory deterioration due to ventilator dysfunction and other adverse events between patients treated with nebulised heparin and those treated with placebo

- To determine if there is any difference in the use of neuromuscular blockers, inhaled nitric oxide, nebulised prostacyclin, prone positioning, high frequency oscillation ventilation (HFOV), extra-corporeal membrane oxygenation (ECMO) and recruitment manoeuvres in the 28 days after randomisation between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in the rate of separation from mechanical ventilation, censored at Day 28, between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in mechanical ventilation-free days, vasopressor-free days, renal replacement therapy-free days and ICU-free days in the 28 days after randomisation between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in the incidence of tracheostomy insertion in the 28 days after randomisation between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in hospital length of stay, hospital mortality, cause of death and hospital discharge destination between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in mortality, cause of death, accommodation, modified SF-36 physical function score (where those who died on or before Day 60 are allocated a physical function score of 0) and quality of life at Day 60 between patients assigned to nebulised heparin and those assigned to placebo

- To determine if there is any difference in mortality, cause of death, hospital re-admission, accommodation, physical function and quality of life at Six-Months between patients assigned to nebulised heparin and those assigned to placebo

- To determine the cost-effectiveness of nebulised heparin.

5. Assessment of process of care, safety and efficacy

5.1 Primary outcome

The primary outcome is the level of physical health assessed 60 days after randomisation using the physical function component of the SF-36 health survey.

5.2 Secondary outcomes

The major secondary outcomes are:

- Hours from commencing invasive ventilation to randomisation

- Hours from randomisation to first dose of the study drug

- Change in plasma thrombin time, thrombin-antithrombin levels and D-dimer levels between baseline and Day 3

- Change in serum tumour necrosis factor, interleukin-1 beta, interleukin-6, interleukin-8, and interleukin-12 between baseline and Day 3

- Change in Murray Lung Injury Score from baseline during Days 1 to 5

- Onset and progression of ARDS from baseline during Days 1 to 5

- Cumulative fluid balance at Day 1, 3, 5 and 10

- Treatment with open-label unfractionated heparin in the 10 days after randomisation; average daily dose of open-label unfractionated heparin in the 10 days after randomisation; average daily dose of open-label unfractionated heparin > 15,000 Units per day in the 10 days after randomisation

- Treatment with open-label low molecular weight heparin in the 10 days after randomisation; average daily dose of open-label low molecular weight heparin in the 10 days after randomisation; average daily dose of open-label low molecular weight heparin > 40 mg per day in the 10 days after randomisation

- Treatment with non-heparin anticoagulants in the 10 days after randomisation; days of treatment with non-heparin anticoagulants in the 10 days after randomisation

- Transfusion of packed red cells or whole blood in the 10 days after randomisation; volume of packed red cells or whole blood transfused in the 10 days after randomisation

- Average of lowest daily haemoglobin measures in the 10 days after randomisation; lowest haemoglobin in the 10 days after randomisation; haemoglobin < 70 g/L in the 10 days after randomisation

- Average of lowest daily platelet count in the 10 days after randomisation; lowest platelet count in the 10 days after randomisation; platelet count < 20 x 10^9/L in the in the 10 days after randomisation

- Average of highest daily APTT in the 10 days after randomisation; highest APTT in the 10 days after randomisation; APTT > 120 seconds in the 10 days after randomisation

- Dose of study drug administered per 24 hours of invasive ventilation in the 10 days after randomisation

- Reasons for omitting a scheduled dose of study drug in the 10 days after randomisation

- Instances of major bleeding, pulmonary bleeding, heparin-induced thrombocytopenia, respiratory deterioration due to ventilator dysfunction and other adverse events

- Days of treatment with sedative infusions, opiate infusions, corticosteroids, antibacterials, antivirals and antifungals in the 28 days after randomisation

- Days of treatment with neuromuscular blockers, inhaled nitric oxide, nebulised prostacyclin, prone positioning, HFOV, ECMO and recruitment manoeuvres in the 28 days after randomisation

- Rate of separation from mechanical ventilation, censored at Day 28

- Mechanical ventilation-free days, vasopressor-free days, renal replacement therapy-free days and ICU-free days in the 28 days after randomisation

- Tracheostomy in the 28 days after randomisation

- Hospital length of stay, hospital mortality, cause of death and hospital discharge destination

- Mortality, cause of death, accommodation, modified physical function score (where those who died before on or before Day 60 are allocated a physical function score of 0) and quality of life at Day 60

- Mortality, cause of death, hospital re-admission, accommodation, physical function and quality of life at Six-Months

- Cost-effectiveness of nebulised heparin.

Day 0 is the period from randomisation to midnight on the day of enrolment. Day 1 is the first calendar day after the day of enrolment, Day 2 the second calendar day after the day of enrolment, and so on. 'In the 10 days after randomisation' means from Day 0 to Day 10 inclusive. 'In the 28 days after randomisation' means from Day 0 to Day 28 inclusive.

Plasma thrombin time, thrombin-antithrombin levels, D-dimer and serum cytokines are measured in a subset of patients. Other outcomes will be assessed for the entire cohort.

A component of both the Murray Lung Injury Score and the Berlin Definition of ARDS requires that the number of lung quadrants with an infiltrate be determined. For this purpose, when assessing the chest radiograph: the quadrants are defined by dividing the chest vertically at the spine and horizontally at the level of the carina and; an infiltrate is defined according to the Berlin criteria of 'acute opacities not fully explained by effusions, lobar/lung collapse or nodules'.[11]

Separation from mechanical ventilation is deemed to have occurred if the patient did not receive either invasive or non-invasive ventilation in intensive care at any time during the remainder of the day of stopping mechanical ventilation or during the next day. If a patient achieves separation from mechanical ventilation more than once, it is his/her final separation that is used to calculate the outcome. Patients who die in the in the 28 days after randomisation are deemed never to have had separation from mechanical ventilation.

Mechanical ventilation-free days are the days that follow the day of final separation from mechanical ventilation. Patients who die at any time in the 28 days after randomisation are assigned zero (0) mechanical ventilation-free days.[53]

A vasopressor-free day is a day where the patient did not receive an inotrope or vasopressor infusion in intensive care. A renal replacement therapy-free day is a day where the patient did not receive any type of renal replacement therapy in intensive care. An ICU-free day is a day where the patient did not spend any time in intensive care. For vasopressor-free days, renal replacement therapy-free days and ICU-free days, patients who die in the 28 days after randomisation are assigned zero (0) -free days.

Major bleeding is defined according to the International Society of Thrombosis and Hemostasis criteria.[54,55]

Quality of life will be assessed at Day 60 and at Six-Months using the EQ-5D instrument, a questionnaire that consists of the EQ-5D descriptive system, which measures health-related quality of life on five dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression), and the EQ VAS, which generates a self-rating of health related quality of life. EQ-5D has previously been shown to be a valid and reliable measure of health-related quality of life following intensive care admission.[56,57]

Health economic modelling will be used to estimate cost-effectiveness. Decision analysis will be used to compare the downstream consequences of nebulised heparin versus placebo. The incorporation of Markov and life-tabling techniques will allow for the modelling of outcomes beyond the duration of the trial. Incremental cost-effectiveness ratios, in terms of net costs per unit of health gained, will be calculated. Health gains, in addition to clinical outcomes, will include estimating years of life gained and quality-adjusted life-years gained. To account for uncertainty in the model inputs, sensitivity and uncertainty analyses will be undertaken via Monte Carlo simulation.

6. Selection of participants

6.1 Inclusion criteria

To be eligible, patients must meet all of these inclusion criteria:

- Age 18 years or older

- Receiving ventilation via an endotracheal tube

- Started ventilation via an endotracheal tube yesterday or today

- Expected to require invasive ventilation all of today and all of tomorrow

- P_aO_2 to F_IO_2 ratio less than 300 or S_pO_2 to F_IO_2 ratio less than 315[58] at any time since commencing invasive ventilation. Note: Only S_pO_2 values ≤ 97% can be used to calculate the S_pO_2 to F_IO_2 ratio

- An active ventilator circuit humidification system is in use or the treating Intensivist has decided to start active circuit humidification.

6.2 Exclusion criteria

Patients are excluded if any of the following criteria are met:

- Previously enrolled in this study

- Enrolled in another randomised intervention study, unless approved*

- Allergy to heparin

- Any history of heparin induced thrombocytopenia

- Platelet count less than 50×10^9/L

- APTT is prolonged to greater than 80 seconds and this is not due to anticoagulant therapy

- Uncontrolled bleeding

- Pulmonary bleeding during this hospital admission. Pulmonary bleeding is frank bleeding in the lungs, trachea or bronchi with repeated haemoptysis, or requiring repeated suctioning, and temporally associated with acute deterioration in respiratory status.

- Neurosurgical procedures during this hospital admission or such procedures are planned

- Epidural catheter in place

- Any history of intracranial, spinal or epidural haemorrhage

- Hepatic encephalopathy or any history of gastrointestinal bleeding due to portal hypertension or biopsy proven cirrhosis with documented portal hypertension

- Tracheostomy in place

- Usually receives home oxygen

- Usually receives any type of assisted ventilation at home e.g. continuous positive airway pressure for obstructive sleep apnoea

- Cervical spinal cord injury associated with reduced long-term ability to breathe independently

- Spinal or peripheral nerve disease with a likely prolonged reduction in the ability to breathe independently e.g. Guillain-Barré syndrome, motor neurone disease

- Receiving HFOV or the treating Intensivist has made a definite decision to commence HFOV

- Receiving ECMO or the treating Intensivist has made a definite decision to commence ECMO

- Treatment limits restrict the provision of renal replacement therapy, inotropes, vasopressors or prolonged invasive ventilation

- Usually treated with haemodialysis or peritoneal dialysis for end-stage renal failure

- Dementia

- Death is deemed imminent or inevitable or there is underlying disease with a life expectancy of less than 90 days

- Pregnant or might be pregnant. Women aged 18 to 49 are excluded unless there is documented menopause, hysterectomy or surgical sterilisation, or a pregnancy test is negative

- Objection from the treating clinician

- Consent refused by the patient or substitute decision maker.

*The Management Committee assesses requests for co-enrolment. In general, co-enrolment is viewed favourably, except for studies of interventions likely to affect coagulation or acute lung injury.

7. Screening procedures

Research Coordinators and Investigators at each site will review patients admitted to the intensive care unit to identify potential candidates for enrolment. All patients meeting the inclusion criteria will be entered on the screening log. For patients that meet the inclusion criteria, but who are not enrolled, the reason for exclusion will be recorded on the screening log.

8. Randomisation and allocation concealment

Allocation concealment will be maintained throughout by the use of a central, secure web randomisation process hosted at the University of Sydney's Northern Clinical School Intensive Care Research Unit (NCS ICRU) under the supervision of Associate Professor Gordon Doig. Blocks of variable size and a random seed will be used to ensure allocation concealment cannot be violated by deciphering the sequence near the end of each block. To further protect from deciphering, block size will not be revealed to site Investigators. Randomisation is stratified by site.

At randomisation each participant is assigned a box of study medication. Each box has a unique 4-letter code and contains 48 nebules, each labeled with the matching unique 4-letter code. Each box contains the maximum number of nebules that can be administered (40 doses) and a further 8 nebules to allow for potential wastage/loss of study drug as might occur in the course of clinical activities.

9. Blinding, storage and dispensing of the study drug

The active study drug is heparin sodium 25,000 Units in 5 mL ampoules (Pfizer Australia Pty Ltd). The placebo is sodium chloride 0.9% 5 mL (Pfizer Australia Pty Ltd), presented in ampoules identical to the active treatment. The manufacturer guarantees that the product has been manufactured and released in accordance with Australian standards. The product has a three-year window for use. The blinding and labelling of ampoules (nebules) is in compliance with TGA requirements and follows a Standard Operating Procedure.

Study medications will be distributed by the study Coordinating Centre and delivered by the Project Manager to the intensive care units of the participating centres. The study medications will be stored and dispensed within the intensive care units of these centres under the supervision of the site Principal Investigators.

10. Treatment of participants

10.1 Active ventilator circuit humidification

An active humidification system will be used (Figure 1). Use of an active ventilator circuit humidification system is an inclusion criterion for this study.

10.2 Nebuliser type and its position in the ventilator circuit

The *Aeroneb Solo* (Aerogen Ltd) single patient use, vibrating mesh nebuliser is placed in the inspiratory limb of the ventilator circuit prior to the Y and attached to the *Aeroneb Pro Controller* (Aerogen Ltd) (Figure 1).

10.3 Expiratory filter type, position and maintenance

A *Servo Duo Guard* (Maquet Critical Care AB) filter is placed between the expiratory limb of the circuit and the expiratory valve of the ventilator (Figure 1). This filter is changed daily.

10.4 Prescription of the study medication

The study medication must be prescribed on the patient's medication administration record by a treating physician. The prescription must specify the drug name (nebulised heparin trial medication), the dose (5 mL), the frequency (6 hourly), the route (nebulised inhalation), the duration (10 days) and the indication (while receiving invasive ventilation in intensive care). The maximum number of doses is 40. It is preferable to include the unique drug code in the prescription.

10.5 Administration of the study medication

Study medications are administered by nursing and medical staff under the supervision of site Principal Investigators and Research Coordinators.

Each dose of study drug is instilled into the nebuliser chamber using a sterile, single-use 5 mL syringe (B Braun).

The nebuliser chamber must be upright during nebulisation (Figure 1). The ventilator circuit should be inspected during nebulisation to verify that the study medication is being delivered: the medication should be seen 'misting'. Nebulisation of each dose of the study drug should be complete within 10 minutes.

Administration of the drug is recorded on the patient's hospital medication administration record. All nebules of study drug that are administered and discarded/wasted are entered on the study Bedside Drug Accountability Log.
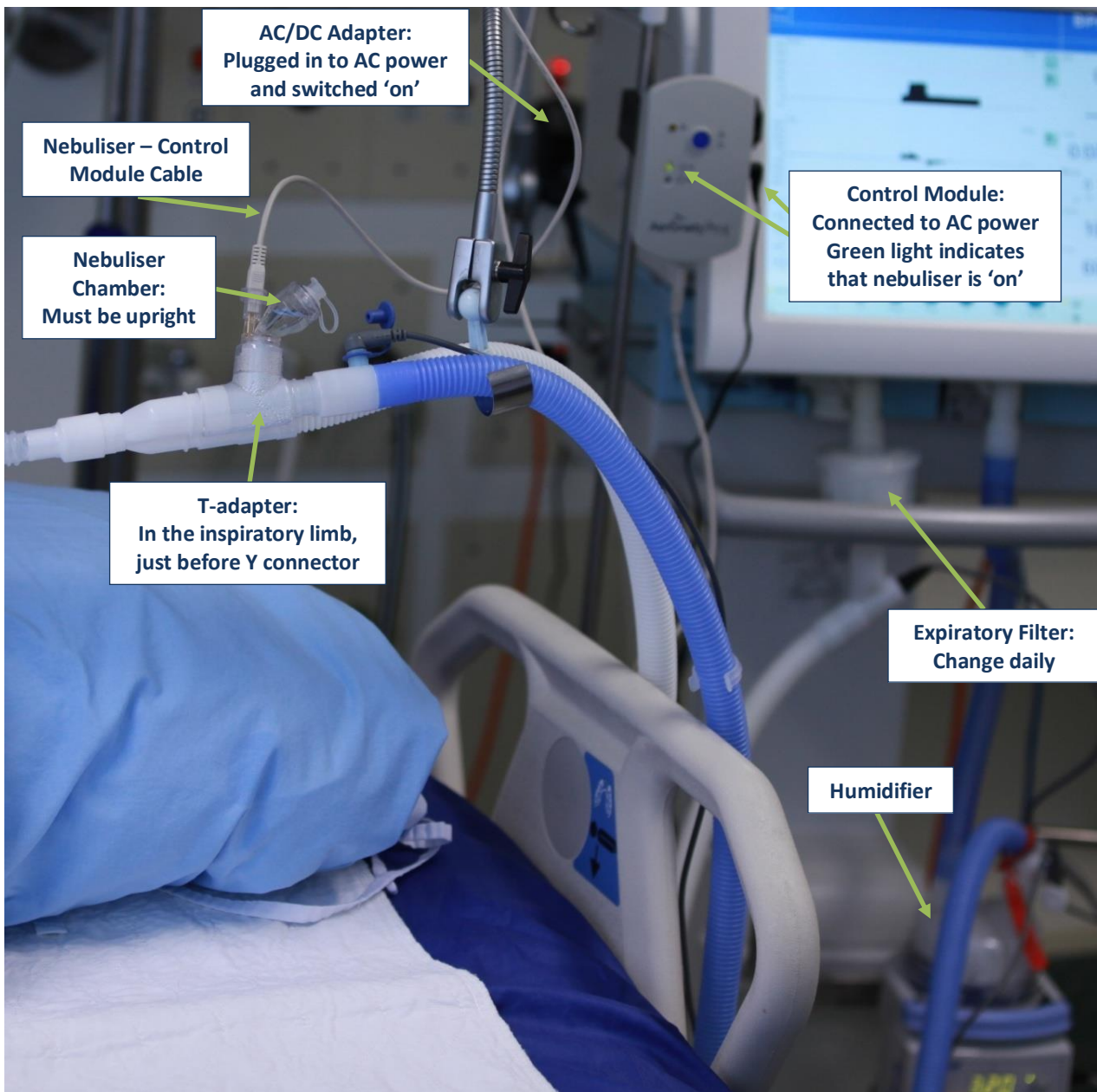
Figure 1. Ventilator circuit and nebuliser set-up

10.6 Withholding the study medication

The study drug should be withheld if any of the following occurs:

- Greater than 10 days have elapsed since randomisation

- The patient is outside of intensive care

- The patient is no longer receiving invasive ventilation

- There is an unexplained increase in APTT to greater than 80 seconds

- In the opinion of the treating Intensivist the APTT is hazardously elevated

- In the opinion of the treating Intensivist there is excessive blood staining of respiratory secretions

- There is pulmonary bleeding (refer to definition in Section 12 of this document)

- There is major bleeding (refer to definition in Section 12 of this document)

- There is suspected or confirmed heparin-induced thrombocytopenia

- The patient is receiving ECMO

- The patient is receiving HFOV

- Tracheostomy or major surgery is scheduled in the next 4 hours or has occurred in the last 4 hours.

The study drug should be recommenced if:

- Having been withheld because the patient was outside intensive care, the patient returns to intensive care

- Having been withheld because the patient was not receiving invasive ventilation, invasion ventilation is reinstituted

- Having been withheld because of an unexplained increase in the APTT to more than 80 seconds, the APTT returns to below 80 seconds

- Having been withheld because in the opinion of the treating Intensivist the APTT was hazardously elevated, the APTT returns to an acceptable level in the opinion of the treating Intensivist

- Having been withheld because in the opinion of the treating Intensivist there was excessive blood staining of respiratory secretions, the blood staining of the respiratory secretions has resolved in the opinion of the treating Intensivist

- Having been withheld for pulmonary bleeding or major bleeding, the bleeding has been definitively controlled

- Having been withheld for suspected heparin-induced thrombocytopenia, the patient is found on antibody testing not to have this condition

- Having been withheld for ECMO, the treatment with ECMO is stopped

- Having been withheld for HFOV, the treatment with HFOV is stopped.

Treatment with any or all of the following therapies is <u>not</u> reason to withhold the study medication:
- Deep vein thrombosis prophylaxis with heparin, including low molecular weight heparin

- 'Full' therapeutic dose heparin, including low molecular weight heparin

- Non-heparin anticoagulants

- Anti-thrombotic medications

- Protamine

- Prone ventilation

- Inhaled nitric oxide

10.7 Unblinding

The site Investigator is able to unblind if they consider there is a safety-related need for unblinding. The precise reason for unblinding and any documentation that supports the reason for unblinding will be submitted to the Coordinating Centre. Unblinding will only be performed when knowledge of the treatment allocation will influence the participant's management in a significant fashion. In any case of unblinding, the follow-up schedule of data collection will be maintained and full analysis of patient data will be performed on an intention-to-treat basis.

10.8 Other treatments, including other nebulised medications

The study medication is given in addition to the standard care that the patient requires, as determined by the treating team. The study nebuliser may be used to administer preparations of salbutamol, ipratropium and budesonide that are approved for use by nebulisation. Non-study nebulised drugs should be scheduled so that the administration times of these drugs do not coincide with those of the study drug. The study drug should never be mixed with another drug or given concurrently. Prior to instilling the study drug in the nebuliser chamber, the chamber must be inspected to ensure that the chamber is empty: drugs should never be mixed in the nebuliser chamber. The study nebuliser is very efficient and may deliver larger doses of non-study drugs to the lungs compared to other nebulisers that the site investigators and clinicians may have used. The patient's response to non-study nebulised medications should be carefully monitored, especially if the recommended dose is exceeded.

11. Data collection and management

11.1 Storage and disposal of personal and health information

Paper records will be stored in locked rooms accessible only to authorised study personnel. Electronic information will be kept on password protected computers accessible only to authorised study personnel.

All study material, including case report forms and the study database, will be stored for a minimum period of 15 years after the conclusion of the study.

Any paper study material that requires disposal will be shredded using a commercial grade shredder. Any electronic data requiring disposal will be thoroughly erased from its electronic media (hard drive, backup tapes or copies) such that it cannot be retrieved with commercially available software.

11.2 Site master patient log

Each participating centre will maintain a log of enrolled patients that includes patient identifiers. Patient identifiers are not transferred to the study Coordinating Centre but it must be possible to reidentify patients to allow future audit against source documents.

11.3 Case report form

Data will be collected by trained staff at each site under the supervision of the site Principal Investigator using a paper case report form and data dictionary developed by the Coordinating Centre. This information will be re-identifiable by the participating site to facilitate audits against source documents. This information will not be re-identifiable by the study Coordinating Centre.

11.4 Baseline data

Baseline data will be gathered from health records at the recruiting institution. It may be necessary in a small number of cases to obtain information from other healthcare providers. The following baseline data is gathered:

- Eligibility criteria
- Invasive ventilation commencement date and time
- Randomisation date and time (generated electronically by the study website)
- Birth date, gender, height and weight
- Medical history, cigarette smoking status
- Hospital admission date
- Intensive care admission date and time, admission source and admission diagnosis
- Risk factors for lung injury in the 7 days before randomisation: pneumonia, aspiration, inhalation, contusion, submersion, non-pulmonary sepsis, pancreatitis, cardiopulmonary bypass, massive transfusion, skin burns, trauma, other(s) (specified), and none
- APACHE II Severe chronic health states: liver, cardiovascular, respiratory, immunocompromised; renal is not assessed because chronic dialysis is a study exclusion criterion
- APACHE II Physiology in the 24 hours before randomisation: temperature (lowest and highest), mean arterial pressure (lowest and highest), heart rate (lowest and highest), respiratory rate (lowest and highest), alveolar-arterial oxygen gradient on $F_IO_2 \geq 0.5$, lowest P_aO_2 on $F_IO2 < 0.5$, arterial pH (lowest and highest), sodium (lowest and highest), potassium (lowest and highest), creatinine (lowest and highest), presence of acute renal failure, white cell count (lowest and highest), haematocrit (lowest and highest) and lowest GCS
- Highest bilirubin in the 24 hours before randomisation
- Sites of infection in the 24 hours before randomisation: blood stream, cardiovascular, chest, abdomen, neurologic, urinary, reproductive, skeletal, skin or soft tissue, other, unknown and none
- Dose of unfractionated open-label heparin and dose of low molecular weight heparin in the 24 hours before randomisation; treatment with non-heparin anticoagulants in the 24 hours before randomisation
- Treatment with renal replacement therapy and inotrope or vasopressor infusion in the 24 hours before randomisation; hours of non-invasive ventilation in the 24 hours before randomisation
- Treatment with neuromuscular blockers, inhaled nitric oxide, nebulised prostacyclin, recruitment manoeuvres and prone positioning in the 24 hours before randomisation

- Treatment with sedative infusions, opiate infusions, corticosteroids, antibacterials, antivirals and antifungals in the 24 hours before randomisation

- Haemoglobin, platelet count, APTT and INR closest to and before randomisation

- The chest radiograph (or chest CT) performed closest to and before randomisation is interpreted by a site Investigator who is a medical doctor. The following information is ascertained: the number of lung quadrants with acute opacities that are not fully explained by effusions, lobar/lung collapse or nodules; whether the opacities are present bilaterally; whether, given all the medical information about the patient, the opacities are entirely attributable to cardiac failure or fluid overload; whether the patient was exposed to any risk factor for acute lung injury in the previous 7 days and; whether there is objective evidence to exclude the possibility of cardiac failure or fluid overload

- Arterial blood gas (pH, P_aCO_2, P_aO_2, bicarbonate) and corresponding ventilation parameters (respiratory rate, F_IO_2, S_pO_2, peak airway pressure, PEEP or CPAP and tidal volume) closest to and before randomisation

- Blood samples are collected for plasma thrombin clotting time, D-dimer and thrombin-antithrombin levels and serum tumour necrosis factor, interleukin-1 beta, interleukin-6, interleukin-8, and interleukin-12

- Date and time of the first dose of study drug.

11.5 Coagulation and cytokine tests Day 3 while in intensive care

Blood samples are collected for plasma thrombin clotting time, D-dimer and thrombin-antithrombin levels and serum tumour necrosis factor, interleukin-1 beta, interleukin-6, interleukin-8, and interleukin-12.

11.6 Chest imaging data Days 1 to 5 while in intensive care

The chest radiograph (or chest CT) performed earliest on each study day is interpreted by a site Investigator who is a medical doctor. The following information is ascertained: the number of lung quadrants with acute opacities that are not fully explained by effusions, lobar/lung collapse or nodules; whether the opacities are present bilaterally; whether, given all the medical information about the patient, the opacities are entirely attributable to cardiac failure or fluid overload; whether the patient was exposed to any risk factor for acute lung injury in the previous 7 days and; whether there is objective evidence to exclude the possibility of cardiac failure or fluid overload

11.7 Daily data Days 0 to 10 while in intensive care

The following data is gathered from health records at the recruiting institution for each day the patient is in intensive care up to and including the 10th day after the day of randomisation:

- AM: Arterial blood gas (pH, P_aCO_2, P_aO_2, bicarbonate) and corresponding ventilation parameters (respiratory rate, F_IO_2, S_pO_2, peak airway pressure, PEEP or CPAP, tidal volume)

- PM: Arterial blood gas (pH, P_aCO_2, P_aO_2, bicarbonate) and corresponding ventilation parameters (respiratory rate, F_IO_2, S_pO_2, peak airway pressure, PEEP or CPAP, tidal volume)

- Dose of unfractionated open-label heparin

- Dose of low molecular weight heparin

- Treatment with non-heparin anticoagulants

- Volume of packed red cells and whole blood transfused

- Fluid balance

- Pulmonary bleeding, major bleeding, suspected heparin-induced thrombocytopenia and respiratory deterioration due to ventilator dysfunction

- Dose (volume) of study drug administered today and, if a complete daily dose of study drug (20 mL) was not given, the reason(s) for not administering the study drug

11.8 Daily data Days 0 to 28 while in intensive care

The following data is gathered from health records at the recruiting institution for each day the patient is in intensive care up to and including the 28th day after the day randomisation:

- Whether readmitted to intensive care and, if so, time of readmission

- Hours of invasive ventilation, hours of non-invasive ventilation and time last ventilated

- Extubation or decannulation; airway at the end of the day

- Treatment with renal replacement therapy and inotrope or vasopressor infusion

- Treatment with neuromuscular blockers, inhaled nitric oxide, nebulised prostacyclin, recruitment manoeuvres, prone positioning, high frequency oscillation ventilation and extra-corporeal membrane oxygenation

- Treatment with sedative infusions, opiate infusions, corticosteroids, antibacterials, antivirals and antifungals

- Highest creatinine, highest bilirubin, lowest haemoglobin, highest white cell count, lowest platelet count, highest APTT, highest INR

- Whether discharged from ICU and, if so, the time of discharge and vital status at discharge

11.9 Drug accountability data

Data are gathered from the health records at the recruiting institution, in particular the patient's hospital medication administration record, and the Bedside Drug Accountability Log.

The following information is ascertained: drug code; number of nebules administered; number of nebules discarded or wasted during clinical activities; the number of nebules unused; reconciliation of all assigned nebules and, if not reconciled, the reason for any discrepancy.

11.10 Hospital discharge data

Hospital discharge data are gathered from health records at the recruiting institution and, where necessary, other healthcare providers. The discharge date and discharge destination (including deceased) is ascertained.

11.11 Day 60 Follow-Up data

Day 60 data are gathered from health records at the recruiting institution and, where necessary, other healthcare providers as well as from the patient or proxy.

The following information is ascertained: date of follow-up; accommodation (including deceased); interview subject (patient, proxy); physical function; health score; health compared to one year ago and; quality of life.

11.12 Six-Month Follow-Up data

Six-Month data are gathered from health records at the recruiting institution and, where necessary, other healthcare providers as well as from the patient or proxy.

The following information is ascertained: date of follow-up; re-admission to hospital; accommodation (including deceased); interview subject (patient, proxy); physical function; health score; health compared to one year ago and; quality of life.

11.13 Cause of death data

Cause of death data are gathered from health records at the recruiting institution and, where necessary, other healthcare providers. Date of death, proximate cause of death and underlying causes of death are ascertained.

11.14 Adverse event or adverse reaction data

Adverse event or adverse reaction data are gathered from health records at the recruiting institution and, where necessary, other healthcare providers and the patient or proxy.

The following information is ascertained: the type of event or reaction; date and time of onset; date and time of most recent dose of study drug; the extent of any causal link to the study; whether life-threatening or fatal; whether medically significant; whether hospitalisation was prolonged; whether persistent or significant disability resulted; action taken regarding study drug (none, withheld temporarily, permanently discontinued); whether the study drug was unblinded; concomitant medications and; a freeform summary.

12. Assessment of safety

Assuring patient safety is essential. Each site Principal Investigator has primary responsibility for the safety of the individual participants under his or her care.

12.1 Definitions of adverse events

An Adverse Event (AE) is as any untoward medical occurrence in a patient. The event does not necessarily have to have a causal relationship with the intervention.[59]

A Serious Adverse Event (SAE) is an event that is medically important, or fatal, or life-threatening, or persistently disabling or incapacitating, or prolongs hospitalisation, or is a congenital abnormality or birth defect.[59] Life-threatening means that the patient was, in the view of the site Principal Investigator, at immediate risk of death from the reaction as it occurred. It does not include a reaction that, had it occurred in a more serious form, might have caused death.

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is an adverse reaction, the nature or severity of which is not consistent with the reference safety information.[59]

A Significant Safety Issue (SSI) is an issue that could adversely affect the safety of participants or materially impact on the continued ethical acceptability of the trial.[59]

12.2 Non-reportable adverse events

Abnormalities of blood tests and physiology, organ failures and even death are relatively common in the study population and should not be reported as adverse events or reactions unless: in the site Principal Investigator's judgement, the event is not part of the expected clinical course and could (at least possibly) be related to the study or; the event is a reportable event as set out in section 12.3.

Blood transfusion is common the study population and is recorded daily on the case report form. Blood transfusion should not be reported as an AE unless the reason for transfusion is bleeding (see section 12.3).

Abnormalities of platelet count and haemoglobin are common in the study population and should not be reported as AEs unless due to bleeding or to suspected heparin-induced thrombocytopaenia (see section 12.3). Lowest haemoglobin and lowest platelet count are recorded daily on the case report form.

Simple blood-staining of the respiratory secretions is common in the study population and should not be reported as an AE. Blood-staining of the sputum that is considered to be heavier than usual should not need to be reported as an AE unless it is associated with deterioration in the patient's condition or requires treatment (see section 12.3). The reasons for withholding the study drug, including excessive blood in the sputum, are recorded daily on the case report form.

Changes in APTT should not be reported as AEs. Highest APTT is recorded daily on the case form. The reasons for withholding the study drug, including unexplained elevation of the APTT, are recorded daily on the case report form.

12.3 Reportable adverse events

The following adverse events must be reported:

- *Major bleeding*. Bleeding that results in: death and/or; is symptomatic and occurs in a critical area or organ (intra-cranial, intra-spinal, intra-ocular, retroperitoneal, intra-articular, or intramuscular with compartment syndrome) and /or; results in a fall in haemoglobin of 20g/L or more, or results in transfusion of two or more units of whole blood or red cells.[54,55]

- *Pulmonary bleeding.* Frank bleeding in the lungs, trachea or bronchi with repeated haemoptysis, or requiring repeated suctioning, and temporally associated with acute deterioration in respiratory status.

- *Suspected heparin-induced thrombocytopenia*. Suspected heparin induced thrombocytopenia is an unexplained fall in platelet count and a decision by the treating Intensivist to withhold all forms of heparin and a decision by the treating Intensivist to request a heparin antibody test.

- *Respiratory deterioration due to ventilator dysfunction*. Hypoventilation, hypoxaemia or other signs of respiratory deterioration temporally associated with ventilator or ventilator circuit dysfunction and which could reasonably be considered to be due to the study medication, nebuliser equipment or the expiratory filter.

- SAEs that, in the site Principal Investigator's judgement, are not part of the expected clinical course and could be related (at least possibly) to the study.

- SUSARs.

12.4 Screening period for adverse reactions and events

The occurrence (or not) of major bleeding, pulmonary bleeding, suspected heparin-induced thrombocytopenia and respiratory deterioration due to ventilator dysfunction is collected systematically as part of the daily data in intensive care from Day 0 to 10. However, taking into account the pharmacodynamic profile of nebulised heparin and allowing a margin of safety, Investigators should be alert to possible adverse events or reactions during the period from enrolment until 4 days (96 hours) after the last dose of study drug.

12.5 Safety reporting process and timeline

SAEs and SUSARs should be reported by the site Investigators to the Coordinating Centre by telephone within 24 hours of becoming aware of the event. The completed written report should be sent promptly to the Coordinating Centre; in general this would be no later than 3 days after the event. Other adverse events and reactions should be reported to the Coordinating Centre within 5 days of Investigators becoming aware the event.

The Management Committee will assess all safety reports received from Investigators. The Chief Investigator will notify the Therapeutic Goods Administration (TGA) of SUSARs within 7 days of being made aware of the case. The Chief Investigator will notify the TGA, the Human Research Ethics Committee (HREC) and Investigators of all significant safety issues; this will occur within 72 hours for issues that require an urgent safety measure and within 15 days in other cases.

The Management Committee will report SAEs and SUSARs to the Data Safety and Monitoring Board (DSMB) as set out in Section 13 (below). The Management Committee, after considering all the available safety information and the advice of the DSMB, will provide the HRECs and Investigators with an annual safety report.

13. Independent Data Safety and Monitoring Board

13.1 Responsibilities

The Data Safety and Monitoring Board (DSMB) provides independent, expert advice to the Management Committee about the safety of study participants. The DSMB does not make decisions about the trial, but rather makes recommendations to the Management Committee.

The DSMB is required to review all serious adverse reactions and serious adverse events with knowledge whether a patient received study drug A or study drug B but blinded to the identity of the study drug. For events that are study-related (possibly, probably or definitely), the DSMB shall, using the Haybittle-Peto approach determine if there emerges a difference in the number of serious adverse events between study groups that exceeds three standard deviations in magnitude. If such a difference occurs, the DSMB is empowered to conduct a blinded interim analysis on the primary outcome to ensure patient safety. If a difference in the primary outcome exceeds three standard deviations, the DSMB may recommend to the Management Committee that the trial be stopped.

13.2 Timing of meetings

Meetings will occur within three months of the Day 60 follow-up milestone of the 50th, 100th and 200th enrolment. An ad hoc meeting of the DSMB may be called by the DSMB Chair if a significant and unexpected safety concern is raised by a DSMB member or by the Management Committee.

13.3 Membership

The DSMB consists of a biostatistician, a respiratory physician and an intensive care physician.

The members are:

- Dr Matthew Anstey (Chair), FCICM, FACEM, MPH
  Intensive Care Physician, Sir Charles Gairdner Hospital

- Dr Daniel Steinfort FRACP, PhD
  Respiratory Physician, Royal Melbourne Hospital

- Dr James Anstey FRACP, FCICM, GradDipBiostat (candidate)
  Intensive Care Physician, Royal Melbourne Hospital

14. Statistical analysis

14.1 Statistical methods

Independent statisticians blinded to treatment allocation will perform data analysis. Crude differences in outcomes will be compared using Student's t-test for normally distributed data and Wilcoxon rank-sum tests otherwise. Crude categorical outcomes will be assessed using Fisher's exact test. Secondary, adjusted analyses will be performed if there are imbalances in baseline characteristics. All analyses will be performed on an intention-to-treat basis.

14.2 Sample size calculation

Previous studies have shown that faster recovery in the Murray Lung Injury Score and earlier freedom from mechanical ventilation improves long-term health assessed by the physical function component of the SF-36 health survey.[14,16,18] To demonstrate a clinically significant 10-point improvement in the SF-36 physical function score at Day 60 a total of 98.6 patients are required in each group (198 patients in total). This calculation assumes an improvement in the score from 45 to 55, SD of 25, with power of 80% and alpha of 0.05.[18,60,61] To account for the loss of physical function information due to death, assuming a mortality rate prior to Day 60 of up to 30%, 256 patients will be enrolled.

15. Quality control and quality assurance monitoring

Conduct and progress of this trial will be monitored on an ongoing basis by the study Management Committee. Case report forms from the researchers will be monitored for protocol compliance and appropriateness of conduct.

Members of the Management Committee will conduct study monitoring visits to all sites, which may be random or planned, and will review source data, consent documentation and other study related activities.

Prior to study commencement a meeting will be held for all site Investigators and Research Coordinators to ensure they thoroughly understand the study protocol and procedures.

A data manager with a background in the critical care setting and experience managing trial data will be employed. Under the supervision of the Chief Investigator this person will coordinate data management including establishment of the study database, establishment of a data quality assurance program, resolution of data queries and preparation of the data for analysis.

16. Ethical considerations

16.1 Ethical principles

The study will be performed in accordance with the ethical principles of the Australian Code for the Responsible Conduct of Research (2007),[62] The Declaration of Helsinki (June 1964 and amended 1975, 1983, 1989, 1996, 2000 and 2008, and Notes of Clarification 2002 and 2004),[63] ICH GCP Notes for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with Therapeutic Goods Administration comments (2000)[64] and the National Statement on Ethical Conduct in Research Involving Humans (2007).[65]

16.2 Human research ethics committee approval

The protocol will be submitted to a HREC. Approval of the protocol and related documents will be obtained prior to the start of the study at each site. The Investigators will ensure that all conditions for approval of the study are met prior to commencing screening and that amendments to the protocol, SAEs and other requisite information is submitted to the responsible HREC.

16.3 Risks and benefits of nebulised heparin

Currently there is no specific treatment for lung injury: patients usually receive supportive care such as supplemental oxygen and assistance to breathe.

Mortality for patients with the most severe forms of lung injury is around 25-40%. For surviving patients long-term physical weakness is a common complication. This contributes to reduced quality of life and increased need for rehabilitation services, hospital readmissions and residential care.

In phase I and phase II trials, nebulised heparin was found to promote faster recovery from lung injury and earlier freedom from mechanical ventilation. Previous studies suggest faster recovery from lung injury leads to shorter hospital and intensive care lengths of stay, improved long-term health and quality of life and marked cost savings. We estimate that treating lung injury with nebulised heparin could reduce intensive care costs by as much as $100 million dollars annually in Australia.

Heparin has been widely used in clinical practice for over 50 years. Its risks and benefits are therefore well established. The majority of patients admitted to intensive care routinely receive subcutaneous heparin to prevent clots forming in large leg veins.

Nebulised heparin has been used for over 30 years in adults and children to treat a range of respiratory conditions including asthma, hay fever, cystic fibrosis and smoke inhalation. Studies found heparin had beneficial actions in these conditions and was safe. In the phase I and phase II trials of nebulised heparin lung injury, nebulised heparin was safe.

16.4 Justification of the placebo

The study medication is given in addition to standard care. Therefore, no patient will be denied standard treatment by the use of a placebo. The placebo in the proposed study is nebulised 0.9% sodium chloride. Sodium chloride is extremely unlikely to cause harm and may even have a mild beneficial effect on lung function, as demonstrated in a recent study that compared nebulised sodium chloride with sham nebulisation.[66]

16.5 Informed consent from the participant

When possible, prior informed consent will be sought in writing from the participant. A member of the research team will determine capacity to provide consent. Competence involves the capacity to comprehend and retain relevant information, believe this information, understand the implications of the decision and weigh information in arriving at a decision. Members of the research team who approach the patient for consent will have a thorough understanding of the study and the requirements for informed consent.

Potential participants are intensive care patients with lung injury who require the assistance of a mechanical ventilator to breathe and are expected to require assistance to breathe for at least two days. These patients are likely to have impaired judgment due to serious acute illness and/or the effects of sedative medications. Patients with cognitive impairment for some other reason or who have an intellectual disability or who have a mental illness may also be potentially eligible for the study. When the patient does not have capacity to provide consent, consent will be sought from their person responsible.

16.6. Informed consent from the person responsible

Where appropriate, the person responsible will be identified by asking the potential participant. Existing hospital records will also be consulted to identify the person responsible. When speaking to a potential person responsible, a member of the research team will confirm the relationship the person has to the potential participant. Members of the research team who approach a person responsible for consent will have a thorough understanding of the study and the requirements for informed consent.

When consent is sought from the person responsible, this will be written informed consent whenever possible. However, circumstances may arise where a person responsible can provide verbal informed consent but cannot give written informed consent because, for example, they are unable to attend the hospital. In this situation verbal consent will be sought, and then written consent will be obtained from the person responsible or the participant, as appropriate, at the earliest appropriate opportunity.

16.7 Procedural authorisation

It is likely that for some patients who are potentially eligible for this study, but who are lacking the capacity to provide consent, it will not be possible to identify the person responsible or it will not be possible to contact the person responsible. Patients are sometimes admitted to intensive care unexpectedly, some are socially isolated and some are from remote locations. This can make it difficult to identify and locate the person responsible within a reasonable timeframe despite the best efforts of the research team. In these circumstances it may be appropriate, in the judgement of the site principal Investigator, to enrol the patient using the process known as procedural authorisation. When a patient is enrolled using procedural authorisation, consent to continue participation will be obtained from the person responsible, should they become known and/or available, or the participant, as appropriate, at the earliest appropriate opportunity.

16.8 Consent to continue participation

When a member of the research team determines that the participant has regained the capacity to provide consent, a member of the research team will meet with the participant to explain the research project and obtain consent. The research team and the treating team will monitor each participant closely after enrolment and review the participant's capacity to consent on a regular basis.

16.9 People in dependent relationships

Members of the research team may sometimes also be responsible for aspects of a potential participant's routine health care. In these situations, great care will be taken to ensure the potential participant does not feel coerced to provide consent or continue participation. Whenever possible a member of the research team who is not directly responsible for the patient's health care will approach the patient or the person responsible for consent.

All potential participants or their person responsible will be reassured that any decision to accept or decline participation will not influence the quality of the care that will be provided. All potential participants or their person responsible will be provided with sufficient time to make their decisions and be given the opportunity to ask questions and be given the opportunity to consult with other people. No patient will be enrolled in this study if the researcher believes the patient or their person responsible has not given consent freely. No patient will be enrolled in this study without the prior approval of the patient's treating physician.

16.10 People highly dependent on medical care

Potential participants are intensive care patients with lung injury who require the assistance of a mechanical ventilator to breathe and are expected to require assistance to breathe for at least two days. These patients are highly dependent on medical care. The researchers will be sensitive to the vulnerable situation of these patients when approaching them or their persons responsible about participation in the study.

All potential participants or their person responsible will be reassured that any decision to accept or decline participation will not influence the quality of the care that will be provided. All potential participants or their person responsible will be provided with sufficient time to make their decisions and be given the opportunity to ask questions and be given the opportunity to consult with other people. No patient will be enrolled in this study if the researcher or delegate believes the patient or their person responsible has not given consent freely. No patient will be enrolled in this study without the prior approval of the patient's treating physician.

16.11 People whose primary language is other than English

Information and consent forms will be written in English. Participation, rights, risks and benefits will be clearly explained to either the patient or their person responsible by a member of the research team. When a person is approached whose primary language isn't English, and if they appear to have any difficulty understanding English, the researcher will ensure that the study is explained to them, and that the study information and consent form is read to them, in a language that they understand. The participating hospitals' Interpreter Services may be utilised, when necessary, to ensure this occurs. No patient will be enrolled in this study if the researcher believes the patient or their person responsible does not understand the study, including the rights of participants, the study procedures and the potential risks and benefits.

16.12 Aboriginal and Torres Strait Islander peoples

It is possible that Aboriginal and Torres Strait Islander peoples may be cared for in hospitals participating in this study and may qualify for enrolment into this trial. We are unaware of any aspects of this trial that conflict with Aboriginal and Torres Strait Islander culture.

16.13 People who may be involved in illegal activity

People involved in illegal activity may, by coincidence, be potentially eligible to participate in this study. This research does not seek to study or uncover illegal activity, and inadvertent discovery of illegal activity is unlikely. Information is gathered from existing medical records, as documented by healthcare providers. Questionnaires administered by the researchers to participants or their proxies do not seek information about illegal activities.

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