Supplemental Material

Detailed Methods

Pre-operative Care

ACE inhibitors, diuretics and alpha blockers were withheld on the day of surgery except in cases of severe diastolic hypertension or poorly controlled left ventricular failure. All other cardiac medications, including beta blockers, calcium channel blockers and statins, were given as usual. Half the usual morning beta blocker dose was administered if heart rate (HR) was below 55 and for those patients taking warfarin, treatment was ceased 5 days prior to surgery and a baseline international normalised ratio measure of coagulation was checked pre-operatively. Clopidogrel and, in patients without recent symptoms of angina, aspirin were similarly withheld for 5 days before surgery wherever possible. Low molecular weight heparin was stopped a minimum of 24 hours pre-operatively and, in patients with ongoing unstable angina, was substituted with unfractioned heparin to maintain an activated partial thromboplastin time (ACT) between 60 - 80 seconds. Metformin and other oral hypoglycaemic agents were stopped the night before and on the day of surgery, respectively. Patients with diabetes mellitus were treated with glucose-insulin-potassium therapy as necessary to maintain a target blood sugar level of 4 - 10 mmol/L. Sildenafil (50 mg) was administered pre-operatively to patients with chronic pulmonary hypertension, defined as pulmonary artery systolic pressure > 60mmHg.

Anaesthesia

Pre-medications consisted of zopiclone (7.5 - 15 mg) the night before surgery with or without midazolam (3.75 - 15 mg) and oxygen (6 L/min via face mask) on the morning of the operation, depending on the patient's age and physical state. Anaesthesia was induced with midazolam (0.05 - 0.15 mg/kg) and fentanyl (5 - 15 mcg/kg), with vecuronium (0.1 - 0.2 mg/kg) or rocuronium (0.5 - 1.5 mg/kg). Before cardiopulmonary bypass (CPB), anaesthesia was maintained with isoflurane (0.3 - 2.0%) to keep the bispectral index $\leq$ 60. Propofol infusion (2 mg/kg/h) was given with additional boluses of fentanyl (100 - 250 mcg) as required, up to a total dose of 2000 mcg. At commencement of CPB, prior to aortic cross-clamping, morphine (20 mg) was administered and further doses given (10 - 20 mg) if necessary. Anaesthesia while on CPB consisted of isoflurane (0.3 - 3.0%) and propofol infusion (2 mg/kg/h). In addition to routine monitoring, a Swan-Ganz catheter (Edwards Lifesciences, CA, USA) was inserted into the internal jugular vein to continuously monitor cardiac output and haemodynamic pressures. Cefazolin (2 g) was administered on induction, with further doses in the perfusion pump prime (1 g), and the earlier of 4 h post induction or separation from CPB. Subsequent doses were 4 hourly while the sternum remained open, then 8 hourly for 2 final doses. In patients with penicillin allergy, antibiotic treatment consisted of vancomycin (1 g over 30 min) and gentamicin (4 mg/kg over 30 min). A bolus of tranexamic acid (20 mg/kg), was given on induction, followed by smaller doses at the start of CPB and for each hour of CPB (10 mg/kg). Heparin (350 U/kg) was given and supplemented as needed to maintain an ACT $\geq$ 480 s until reversal with protamine (1 mg/100 U of estimated residual heparin). Actrapid insulin was infused as necessary to keep blood sugar level $\leq$ 10 mmol/L. Glucocorticoids were not administered unless there was evidence of adrenal suppression. Before CPB, PlasmaLyte 148 was used to replace pre-operative fluid deficits and ongoing insensible losses with additional boluses in cases of hypovolemia. Post CPB, PlasmaLyte was administered as necessary to assist with optimal ventricular filling.

Perfusion

Non-pulsatile perfusion was performed using a VHK Venous Hardshell Reservoir, Quadrox oxygenator system, Jostra Rotaflow centrifugal pump head (Macquet, Rastatt, Germany), Affinity 38 micron in-line arterial filter (Carmeda, AB, Sweden) and Softline coated biocompatible tubing (Macquet). The pump was primed with PlasmaLyte 148 (2 L) and heparin (10,000 U) without mannitol, HCO_3 , or colloid. Tepid bypass (32 - 34ºC nasopharyngeal) was performed, with a pump flow of 2.4 L/min/m^2. ACT was kept $\geq$ 480 s, and checked at 30 min intervals. Alpha-stat management was used to evaluate pH levels. Cardiotomy suction was reserved for fresh bleeding directly from the heart or aorta, and a cell saver was used for pooled blood collected from the

mediastinum and pleura which, together with the remaining pump blood, was washed and reinfused following CPB. A "hotshot" of normothermic blood was given immediately before removal of the aortic cross-clamp, and rewarming to 36.5ºC was induced with the application of a sterile forced air blanket to the patient's lower body prior to separation from CPB. Metabolic acidosis was treated by HCO_3^-, or haemofiltration. Haemoglobin was kept at ≥ 70 g/L while on CPB, and anaemia was treated with packed red blood cells or haemoconcentration, as appropriate. The cardioplegia solution contained potassium chloride (KCl; 50 mmol/L), $MgSO_4$ (20 mmol/L), 8.4% $NaHCO_3$ (20 mmol/L) with 5% dextrose and was infused at 400 ml/h into tepid blood flowing at 300 ml/min. Induction cardioplegia consisted of 4 min antegrade, then retrograde, perfusion with an additional 1 - 2 min if required to achieve electrical silence. Maintenance cardioplegia was administered every 15 - 25 min for 2 min to reach a coronary sinus pressure of 20 - 60 mmHg.

Haemodynamic Management

The haemodynamic aims during surgery of maintaining a mean arterial pressure (MAP) ≥ 65 mmHg, CI ≥ 2 L/min/m^2, SVO_2 ≥ 65% and Hb ≥ 70 g/L were considered violated in the event of failure to achieve acceptable values despite 5 min of maximal appropriate therapy. The nature and duration of any violations were documented in the patient data. Briefly, MAP was increased in cases of hypotension prior to CPB using phenylephrine (0.01 - 1 mcg/kg/min). During and following CPB, phenylephrine (250 mcg boluses up to every 2 min), noradrenaline infusion (0.05 - 0.4 mcg/kg/min), vasopressin (2 - 3 U/h), or methylene blue (2mg/kg over 10 min) were used as necessary to raise MAP. In situations of hypertension (MAP > 80), isoflurane was increased (≤ 3.0%) and glycerol trinitrate (0.1 - 2.5 mcg/kg/min), administered. Milrinone was infused at 0.375 - 0.75 mcg/kg/min to treat reduced cardiac index, ventricular dysfunction or pulmonary hypertension (PHT). In cases of persistently low CI despite milrinone and noradrenaline dosing, adrenaline (0.05 - 0.5 mcg/kg/min) or inhaled nitric oxide (40 ppm) was administered, with the latter also used for severe PHT with right ventricular dysfunction. Sub-optimal SVO_2 (< 65%) in the presence of target pump flow and normal arterial carbon dioxide pressure was treated by setting the sweep gas at 100% and increasing the pump speed. In the first 12 hours following surgery, the haemodynamic goals were CI ≥ 1.7 L/min/m^2, mixed venous ≥ 55%, and MAP 60 - 75 mmHg. These goals were altered from CI ≥ 2 L/min/m^2, mixed venous ≥ 60% and MAP 65 - 75 mmHg after the first 40 patients after data comparing these targets with historical non-PA catheter driven in the study unit. However, equal numbers of RIPC and control group patients received each fluid protocol due to block randomization in groups of 8. If any of the goals were not met within the first 12 hours, the patients were managed according to the protocol shown in Online Figure 2.

Cytokine Analysis

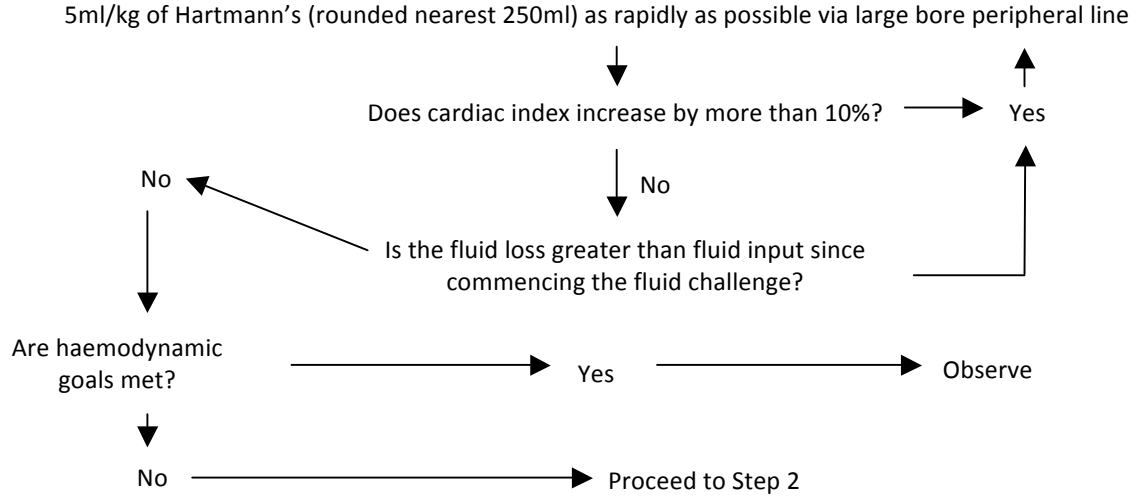
Blood samples were collected from the radial artery pre-operatively, and at 1, 2, 3, 6 and 12 hours following cross-clamp removal. The samples were incubated at room temperature for 30 minutes, then centrifuged, and the serum collected for storage at -80ºC until analysis. The cytokines were quantified using a human inflammatory cytokine cytometric bead array (CBA) kit (BD Biosciences, San Jose, CA, USA). The CBA kit consists of six bead populations with distinct fluorescence intensities that can be resolved in the red FL-3 channel. Each population is coated with capture antibodies specific for IL-1β, IL-6, IL-8, IL-10, IL-12p70 or TNF-α. After these beads are incubated with test samples or standards, phycoerythrin (PE)-conjugated cytokine-specific detection antibodies are added to enable the cytokine-bound beads to be distinguished from unbound beads. The PE fluorescence intensity of the resulting sandwich complexes can then be detected in the yellow FL-2 channel during flow cytometry and compared to a range of standards to ascertain the cytokine concentrations. The cytometric assay beads were mixed, washed, and incubated in serum enhancement buffer. Following bead preparation, 25 uL of undiluted sample and duplicate standards ranging from 20 pg/mL to 5000 pg/mL were incubated with 25 uL of the mixed beads for 1.5 hours. At the end of the incubation the samples were washed twice with wash buffer and all but 50 uL of supernatant was aspirated. Twenty-five uL of the PE-conjugated anti-cytokine antibodies were then added to each sample. After a 1.5 hour incubation, the samples were washed twice, resuspended in 200 uL wash buffer, and acquired on a FACSCanto™ II flow cytometer. The data was analysed with BD FACSDiva and FCAP Array software (BD Bioscience).

Clinical End points

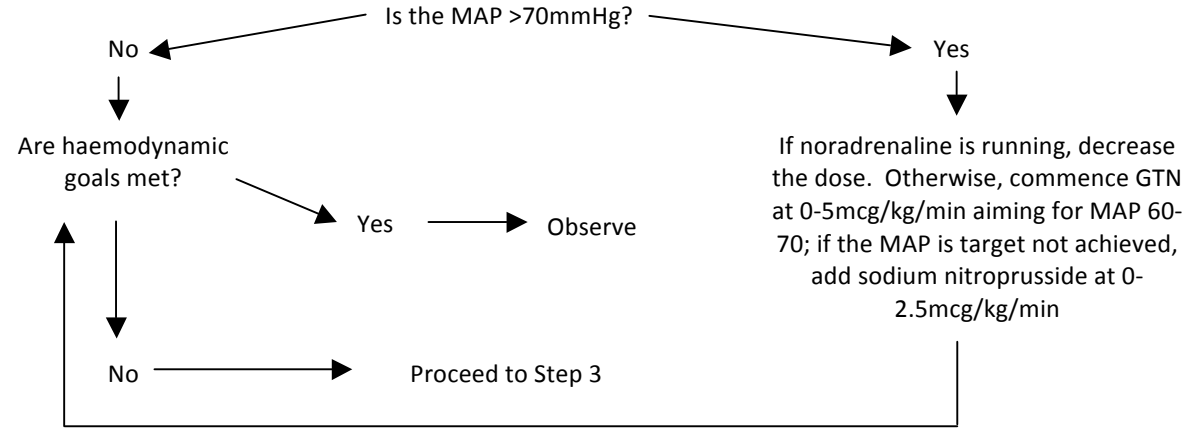
There were four main clinical end points in the study: post-operative plasma high sensitivity troponin T levels measured 6 and 12 hours after cross-clamp removal, duration of noradrenaline use among ICU survivors, and worst post-operative renal injury determined by RIFLE criteria[1]. Additional outcome measures included haemodynamic parameters of cardiac index, mixed venous oxygen saturation and mean arterial pressure recorded at 3, 6 and 12 hours from the time of ICU admission. Duration of noradrenaline use was defined as the number of hours from ICU admission until noradrenaline was permanently ceased during the index ICU admission. If a noradrenaline infusion was stopped, and then re-started within the index ICU admission, the time when noradrenaline was ceased permanently was recorded. The duration of mechanical ventilation was also measured, determined using the extubation time based on the final time a patient was extubated if more than one period of mechanical ventilation was required.

Supplemental Figures

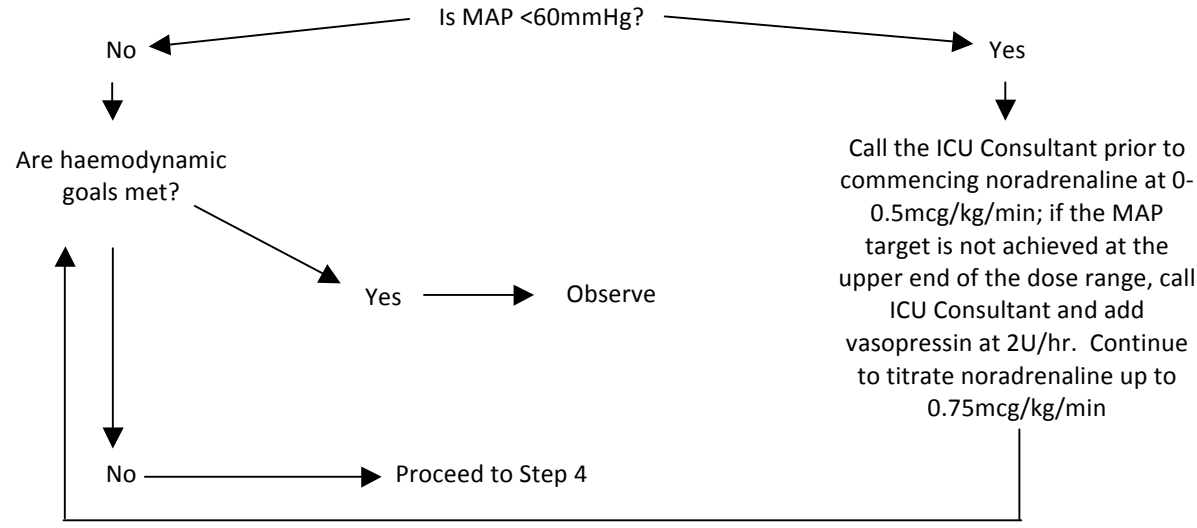
Step 1: Assessment of volume responsiveness (Omit step 1 if MAP>70)[1]



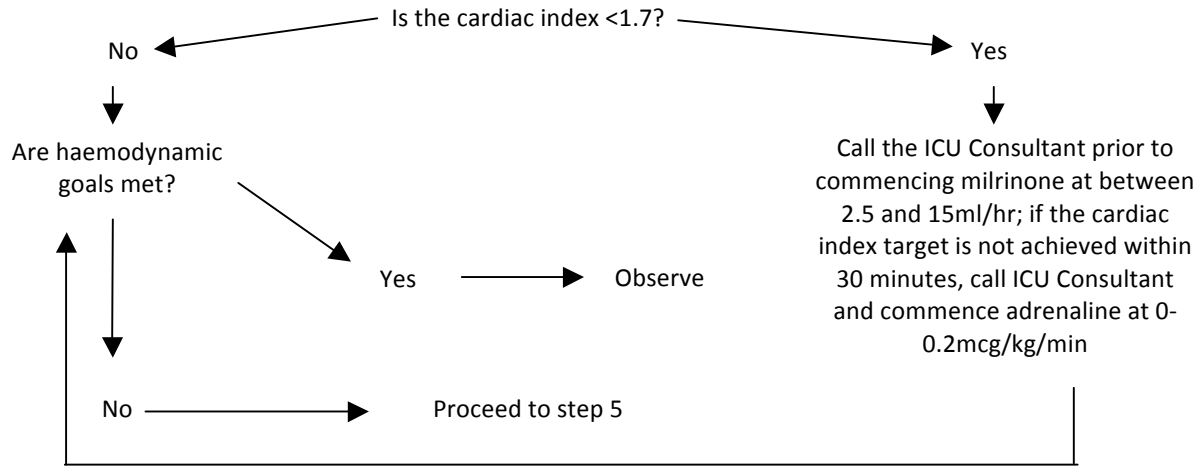
Step 2: Afterload reduction



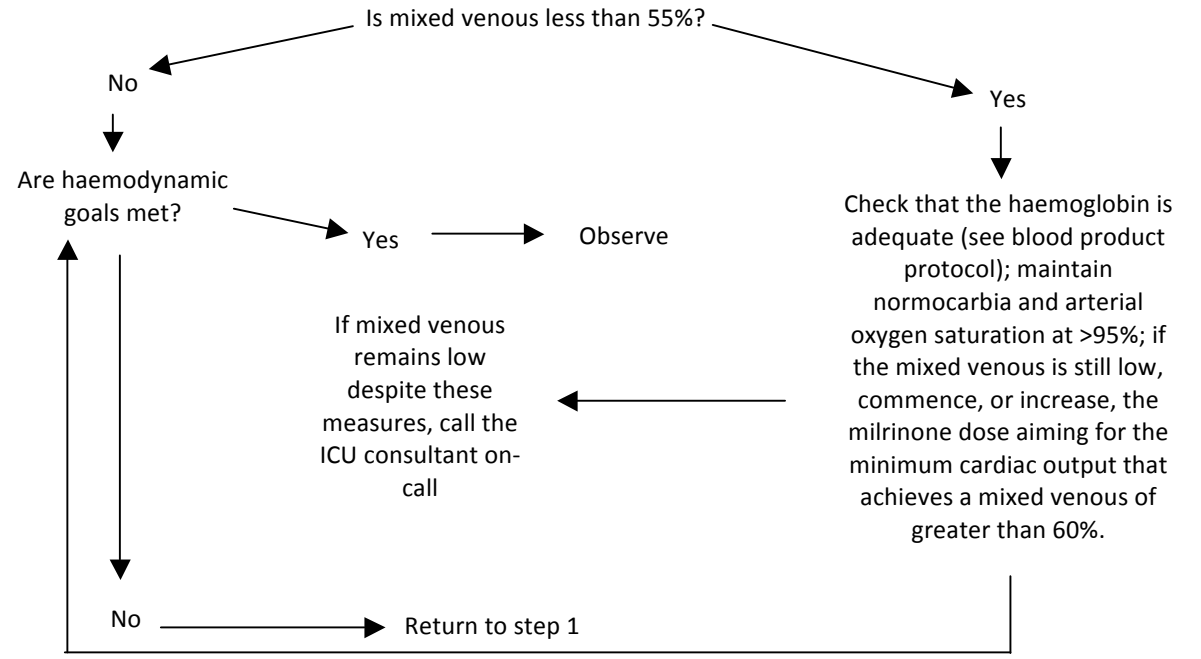
Step 3: Maintain adequate blood pressure (should occur simultaneously with step 4)



Step 4: Maintain adequate cardiac output[2] (should occur simultaneously with step 3)



Step 5: Maintain adequate mixed venous



Online Figure 2: Protocol for haemodynamic management of high-risk patients in the first 12 hours after cardiac surgery

Patients should be maintained in sinus rhythm at 80 - 110 bpm. If the rate is < 80 then DDD pacing is the preferred mode. If HR < 80, sinus rhythm may still be preferred to VVI if there are no atrial wires.

Maintenance fluid is dextrose 5% in water at 0.8 mL/kg/hr (rounded to the nearest 10 mLs).

All dosing on the protocol is based on the patient's actual pre-operative body weight.

[1] A sustained rise in CVP or PA diastolic in response to fluid boluses usually indicates adequate volume resuscitation. If the patient develops new 'instability' and filling pressures have not dropped, it may be appropriate to omit step 1 of the algorithm. A straight leg raise may give you an idea of the patient's volume responsiveness and may be performed at step 1 if the patient is felt to be 'well filled'. If the BLOOD PRESSURE does not rise in response to a straight leg raise the patient is unlikely to be volume responsive.

[2] If the cardiac index is > 3.5 and the mixed venous is adequate, positive inotropes (milrinone, adrenaline, dobutamine, dopamine) should be weaned. Milrinone can generally be ceased without weaning due to its long half life. An intra-aortic balloon pump should be considered if adequate cardiac index is not achieved with adequate fluid resuscitation and milrinone at 0.75 mcg/kg/min. Unexpected low cardiac output should be evaluated by echocardiography.

Supplemental Tables

Online Table 1: Peri-operative cytokine concentrations

	Mean Conc, pg/mL (SD)		Mean Log Conc (SD)	
	RIPC (n = 47)	Control (n = 48)	RIPC (n = 47)	Control (n = 48)
IL-6				
Pre-op	7.17 (8.14)	5.75 (6.36)	1.62 (0.79)	1.41 (0.74)
1 h	865.7 (1573)	391.3 (620.0)	5.90 (1.23)	5.52 (0.81)
2 h	825.3 (1386)	514.2 (747.6)	5.99 (1.13)	5.76 (0.86)
3 h	797.7 (1280)	540.6 (816.3)	5.99 (1.11)	5.80 (0.85)
6 h	572.2 (787.4)	372.1 (558.7)	5.71 (1.17)	5.51 (0.77)
12 h	508.3 (865.5)	273.2 (273.0)	5.57 (1.11)	5.28 (0.78)
IL-8				
Pre-op	18.2 (14.5)	13.6 (8.25)	2.66 (0.69)	2.47 (0.54)
1 h	239.8 (423.1)	130.8 (113.4)	4.89 (1.01)	4.56 (0.76)
2 h	216.9 (303.1)	147.1 (137.5)	4.89 (0.93)	4.66 (0.79)
3 h	190.6 (252.6)	149.6 (144.6)	4.84 (0.84)	4.72 (0.72)
6 h	170.8 (208.8)	122.7 (141.9)	4.71 (0.90)	4.53 (0.67)
12 h	164.0 (233.6)	96.5 (99.3)	4.60 (0.91)	4.29 (0.70)
IL-10				
Pre-op	1.92 (1.71)	2.58 (2.88)	0.02 (1.44)	0.44 (1.25)
1 h	228.8 (218.1)	231.2 (242.6)	4.89 (1.21)	4.88 (1.20)
2 h	67.6 (85.4)	65.1 (89.8)	3.62 (1.15)	3.54 (1.16)
3 h	36.9 (54.0)	44.4 (78.9)	3.06 (1.05)	2.89 (1.26)
6 h	19.1 (33.8)	21.9 (56.3)	2.32 (1.03)	2.25 (1.08)
12 h	13.7 (12.5)	20.7 (47.7)	2.25 (0.86)	2.26 (1.03)

Supplemental References

1. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care.* 2004;8(4):R204-212.