

**THE ROLE OF EARLY MANAGEMENT AND HORMONE REPLACEMENT
THERAPY IN POTENTIAL HEART AND LUNG DONORS**

By

R. V. Venkateswaran

A thesis submitted to the Faculty of Medicine

Of The University of Birmingham

For the degree of

Doctor of Medicine

Department of Heart and Lung Transplantation

University Hospital Birmingham NHS Foundation Trust

Birmingham

and

Department of Physiology

Medical School

The University of Birmingham

2010

UNIVERSITY OF
BIRMINGHAM

University of Birmingham Research Archive

e-theses repository

This unpublished thesis/dissertation is copyright of the author and/or third parties. The intellectual property rights of the author or third parties in respect of this work are as defined by The Copyright Designs and Patents Act 1988 or as modified by any successor legislation.

Any use made of information contained in this thesis/dissertation must be in accordance with that legislation and must be properly acknowledged. Further distribution or reproduction in any format is prohibited without the permission of the copyright holder.

Abstract

Heart and lung transplantation activity is frustrated by the lack of availability of donor organs. The haemodynamic, hormonal and inflammatory changes that follow brain stem death leads to donor organ injury. Appropriate donor management is essential to avoid further damage to already injured donor heart and lungs. Hormone replacement therapy with tri-iodothyronine and methylprednisolone in organ donors has been reported to increase the retrieval rate of heart and lungs with improved function. In a prospective, randomised, double blind, controlled trial the role of hormone replacement therapy and early donor management on the donor heart and lung function and their retrieval rate was studied.

Heart donor Outcome

Early donor management was associated with significant improvement in donor heart function. It may also increase the retrieval rate of hearts for transplantation. However, administration of hormones neither influenced the donor heart function nor the heart retrieval rate. Serial echocardiography guides in identifying suitable donor hearts for transplantation during intensive management.

Lung donor outcomes

Early donor management was associated with significant increase in lung retrieval rate for transplantation. Despite management donor lung function deteriorated following brain stem death. Hormone replacement therapy did not increase the lung retrieval rate or affect the donor lung function. However, methylprednisolone administration leads to significant reduction in progressive donor lung water

accumulation. Measurement of thermodilution lung water index predicted ultimate lung suitability for transplantation and recipient outcome.

Conclusion

Early donor management is the cornerstone to improve the donor heart function and to increase the lung retrieval rate. It may also increase the heart retrieval rate. Serial transthoracic echocardiography may guide in identifying suitable hearts that respond to donor management.

Dedication

To Apurna, Prabha and Vaikhundh for their love, support and forbearance

I would like to thank my supervisors Prof. R. S. Bonser and Prof. J. H. Coote for their help, guidance and enthusiasm without which I could not have completed this research work. I would also like to thank Mr. I. C. Wilson, Mr. J. Mascaro and Dr. R. Thompson for their help in completing and editing each of the manuscripts. I would like to convey my sincere thanks to Dr. R. Steeds for training me to perform transthoracic echocardiography, analysing the echo pictures and help me complete the echocardiography part of the manuscript.

I am also thankful to Dr. Peter Lambert, Aston University, Birmingham who helped me in analysing the cytokines from serum samples. Finally I would like to thank Dr. Val Patchell for her help in gravimetric lung water measurement. I would like to thank the British Heart Foundation for their generous financial grant to help me complete the analysis of serum samples.

Finally I would like to convey my gratitude towards the donor coordinators and donor families for providing me the opportunity to undertake such a study in difficult circumstances.

List of abbreviations

ADH	Anti-diuretic hormone
BSD	Brain stem death
CI	Cardiac index
CO	Cardiac output
CRP	C-Reactive protein
CVP	Central venous pressure
CPO	Cardiac power output
CTnI	Cardiac troponin-I
EF	Ejection fraction
EVLWI	Extravascular lung water index
EVLW-G	Gravimetric lung water
EVLW-T	Thermodilution derived lung water
FS	Fractional shortening
IL	Interleukins
ICP	Intracranial pressure
LV	Left ventricle
LAP	Left atrial pressure
MAP	Mean arterial pressure
MPAP	Mean pulmonary arterial pressure
MP	Methylprednisolone
PAWP	Pulmonary artery wedge pressure
PCT	Procalcitonin

PGD	Primary graft dysfunction
PVR	Pulmonary vascular resistance
RV	Right ventricle
SVR	Systemic vascular resistance
SWI	Stroke work index
TNF	Tumour necrosis factor
T3	Tri-iodothyronine
T4	Thyroxine
VP	Vasopressin
TOE	Transoesophageal echocardiography
TTE	Transoesophageal echocardiography

Published manuscripts generated from this research work

1. Venkateswaran R. V., Townend J, Wilson I.C, Mascaro J. G, Bonser R. S, Steeds R. P. Echocardiography in the potential heart donor. *Transplantation*, Jan-2010 (Epub-ahead of print).
2. Dronavalli V, Ranasinghe A, Venkateswaran R. V, James S, McCabe C, Wilson I C, Mascaro J G, Bonser. N-terminal pro-brain natriuretic peptide: A biochemical surrogate of cardiac function in the potential heart donor. *Eur J Cardio-thorac Surgery*, Feb-2010 (Epub-ahead of print).
3. Venkateswaran RV, Dronavalli V, Lambert PA, Wilson I, Mascaro J, Thompson R and Bonser R. The pro-inflammatory environment in potential heart and lung donors: Prevalence and impact of donor management and hormonal therapy. *Transplantation*, 2009; 88(4):582-588.
4. Venkateswaran RV, Ganesh JS, Thekkudan J, Steeds RP, Wilson IC, Mascaro JG, Thompson RD and Bonser RS. Donor cardiac troponin-I: A bio-chemical surrogate of heart function. *Eur J Cardio-thorac Surgery*, 2009; 36 (2): 286-92.
5. Venkateswaran RV, Steeds RP, Quinn DW, Wilson IC, Mascaro JG, Thompson RD, Townend JN and Bonser RS. The haemodynamic effects of adjunctive hormone therapy in potential heart donors: A prospective randomized double blind factorially designed controlled trial. *Eur Heart J*, 2009; 30 (14):1771-80.
6. Venkateswaran RV, Patchell VB, Wilson IC, Mascaro JG, Thompson RD, Quinn DW, Stockley RA, Coote JH, Bonser RS. Early donor management increases the retrieval rate of lungs for transplantation. *Annals Thorac Surg*. 2008; 85: 278-286.

7. Venkateswaran RV, Bonser RS, Steeds RP. Echocardiographic detection of dysfunctions in donor hearts. Review- *Future Cardiol.* 2007;3(1):99-104
8. Venkateswaran RV, Bonser RS, Steeds RP. The echocardiographic assessment of donor heart function prior to cardiac transplantation. *Eur J Echocardiogr.* 2005; 6: 260-63.

Table of Contents

SECTION-1: INTRODUCTION	1
1. HEART AND LUNG TRANSPLANTATION	2
1.1. History and development	2
1.1.1. Heart transplantation	2
1.1.2. Lung transplantation.....	3
1.2. Indications.....	4
1.2.1. Heart transplantation	4
1.2.2. Lung transplantation.....	5
1.3. Outcome following heart and lung transplantation	6
1.4. Current status of heart and lung transplantation	7
2. BRAIN STEM DEATH	9
2.1. Anatomy.....	9
2.2. Coning	10
2.3. Pathophysiological changes during and after brain stem death.....	11
2.3.1. Haemodynamic changes.....	11
2.3.1. 1. Pathophysiology of neurogenic pulmonary oedema.....	12
2.3.2. Hormonal changes following BSD	15
2.3.3. Inflammatory changes following BSD.....	18
2.3.3.1. Interleukins.....	18
2.3.3.2. Tumour necrosis factor.....	20
2.3.4. Bio-chemical changes following BSD	22

2.4. Summary	22
3. DONOR MANAGEMENT AND HORMONE REPLACEMENT THERAPY	24
3.1. Rationale behind donor management.....	24
3.2. Hormone replacement therapy-Thyroid hormone	26
3.2.1. Synthesis and release	26
3.2.2. Regulation of thyroid hormone	27
3.2.3. Cardiovascular effects of thyroid hormone	29
3.2.4. Euthyroid sick syndrome	30
3.2.5. Thyroid hormone replacement in multi-organ donors	30
3.2.5.1. Animal studies	31
3.2.5.2. Retrospective clinical studies	31
3.2.5.3. Prospective studies	33
3.3. Hormone replacement therapy-Steroids	35
3.3.1. Synthesis and release	35
3.3.2. Regulation	36
3.3.3. Anti-inflammatory effects of steroids	37
3.3.4. Steroids replacement in multi-organ donors.....	38
3.4. Hormone replacement therapy- Vasopressin.....	39
3.4.1. Synthesis and release	39
3.4.2. Actions of vasopressin	40
3.4.3. Role of vasopressin therapy in multi-organ donors	40
4. ECHOCARDIOGRAPHY ON POTENTIAL HEART DONORS	42
4.1. Transthoracic and transoesophageal echocardiography	42
4.2. Resting abnormalities of donor heart function.....	45

4.3. Limitations to resting echocardiography.....	46
5. EXTRAVASCULAR LUNG WATER INDEX MEASUREMENT IN POTENTIAL LUNG DONORS.....	49
5.1. Thermodilution lung water index measurement	49
5.2. Validation of thermodilution lung water measurement by gravimetry	53
5.3. Clinical uses of lung water index measurement.....	54
6. SUMMARY AND OBJECTIVES	56
SECTION 2- METHODS	58
7. CORE METHODOLOGY.....	59
7.1. Introduction	59
7.2. Recruitment	60
7.2.1. Patient selection	60
7.2.2. Ethical approval.....	61
7.3. Randomisation.....	61
7.4. Treatment groups	62
7.4.1. Group-1 T3 therapy	62
7.4.2. Group-2 Placebo therapy	62
7.4.3. Group-3 MP therapy.....	62
7.4.4. Group-4 T3 and MP therapy.....	63
7.4.5. Blinding of trial solutions.....	63
7.5. Preparation to leave to the donor intensive care units	63
7.6. Donor Management	64
7.6.1. Insertion of invasive monitoring lines.....	64
7.6.2. Bronchoscopic examination of airways.	65

7.6.3. Transthoracic echocardiography	65
7.6.4. Administration of randomised drugs	66
7.6.5. Haemodynamic management	66
7.6.6. Lung management	69
7.6.7. Bio-chemical management.....	69
7.7. Intra-Operative assessment of heart and lung function.....	69
7.8. Myocardial biopsy technique and retrieval of lungs for gravimetry	70
7.9. Statistical analysis	71
7.9.1. Study design	71
7.9.2. Hypothesis	71
7.9.3. Power calculations	71
7.9.4. Primary outcome measures	72
7.9.5. Secondary outcome measures.....	72
7.9.6. Data collection and storage.....	73
7.9.7. Data analysis.....	73
SECTION 3- RESULTS	75
8. A STUDY OF THE HAEMODYNAMIC EFFECTS OF ADJUNCTIVE HORMONE THERAPY IN POTENTIAL HEART DONORS	76
8.1- Introduction.....	76
8.2-Methods.....	77
8.2.1-Study design	77
8.2.2-Initial assessment and management.....	77
8.2.3-Endpoints and statistics	79
8.3-Results.....	80

8.3.1-Donor heart function within the 4 treatment groups of the Trial.....	85
8.3.2-Donor heart function according to receipt of T3 or MP	89
8.3.3-Donor heart function according to pre-treatment thyroid function	96
8.3.4-Donor heart function and noradrenaline administration.....	99
8.3.5-Marginal and non-marginal donor hearts, transplant suitability and outcomes.....	99
8.4-Discussion	102
8.5-Conclusion.....	106
9. A STUDY OF PRO-INFLAMMATORY ENVIRONMENT IN POTENTIAL HEART AND LUNG DONORS AND IMPACT OF DONOR MANAGEMENT AND HORMONAL THERAPY.....	107
9.1-Introduction.....	107
9.2-Methods.....	108
9.2.1-Study design	108
9.2.2-Serum immunoassay	110
9.2.3-Statistical analysis.....	110
9.3-Results.....	111
9.3.1-The prevalence of a pro-inflammatory environment.....	114
9.3.2-The effect of donor management and hormonal therapy	114
9.3.3-Initial biomarker levels and donor organ function.....	119
9.3.4-Biomarker levels and heart and lung suitability for transplantation	122
9.4-Discussion	124
9.5-Conclusion.....	126

10. A STUDY INTO THE MEASUREMENT OF CARDIAC TROPONIN-I IN POTENTIAL HEART DONORS AS A BIOCHEMICAL SURROGATE OF FUNCTION	128
10.1-Introduction.....	128
10.2-Materials and methods	129
10.2.1-Donor management and data collection.....	129
10.2.2-Recipient data collection	130
10.2.3-Statistical analysis.....	130
10.3-Results.....	131
10.3.1-Donor demographics.....	131
10.3.2-Donor haemodynamic parameters.....	134
10.3.3-The relationship of cTnI and function with the time between coning and assessment.....	139
10.3.4-Suitability for transplantation	139
10.3.5-Recipient parameters	140
10.4-Discussion	143
10.5-Conclusion.....	145
11. A STUDY OF SERIAL ECHOCARDIOGRAPHY IN THE POTENTIAL HEART DONORS AND THE IMPACT OF DONOR MANAGEMENT AND HORMONAL THERAPY	146
11.1. Introduction	146
11.2-Materials and methods	148
11.2.1-Initial assessment and management.....	148
11.2.2-Echocardiography	149

11.2.3-Endpoints and statistics	149
11.3-Results.....	151
11.3.1-Echocardiographic correlation with haemodynamic measurements	153
11.3.2-Initial echocardiographic function and thyroid functional status	157
11.3.3-Hormonal treatment and echocardiographic changes.....	157
11.3.4-Repeat echocardiographic assessment	159
11.3.5-Predictors of end-assessment functional suitability.....	163
11.3.6-Findings at operating room inspection, transplantation outcome	165
11.4-Discussion	166
11.5-Conclusion.....	170
12. A STUDY INTO THE IMPACT OF EARLY DONOR MANAGEMENT AND HORMONE REPLACEMENT THERAPY IN INCREASING THE RETRIEVAL RATE OF LUNGS FOR TRANSPLANTATION.....	171
12.1-Introduction.....	171
12.2-Materials and methods	172
12.2.1-Study design	172
12.2.2-Assessment and management of non-Trial donors.....	173
12.2.3- Assessment and management of trial donors	173
12.2.4-Endpoints and statistics	174
12.3-Results.....	175
12.3.1-Donor lung function within the treatment groups of the Trial	179
12.3.2-Donor lung function according to receipt of hormonal therapy	181
12.3.3-Observations in the whole <i>Trial</i> population	183
12.3.4-Suitability of lungs for transplantation and transplant outcomes	186

12.3.5. Effect of Trial management on other solid organ retrieval	188
12.4-Discussion	188
12.5-Conclusion.....	191
13. A STUDY OF MEASUREMENT OF EXTRAVASCULAR LUNG WATER IN POTENTIAL LUNG DONORS.....	192
13.2. Methods	193
13.2.1. Study design	193
13.2.2. Donor management.....	194
13.2.3. Retrieval of donor lungs	194
13.2.4. Gravimetric measurement of lung water.....	195
13.2.5. Endpoints and Statistics	196
13.3. Results.....	197
13.3.1. Donor demographics.....	197
13.3.2. Baseline parameters	198
13.3.3. Pre-retrieval parameters.....	198
13.3.4. Noradrenaline use and extravascular lung water index.....	199
13.3.5. Extravascular lung water index and suitability of lungs for transplantation	200
13.3.6. Recipient outcome after transplantation	202
13.3.7. Lung water index validation.....	204
13.4. Discussion	207
13.5. Conclusion	209
SECTION 4- SUMMARY	210
14. SUMMARY AND POTENTIAL AREAS FOR FUTURE RESEARCH	211

14.1. Summary of findings	211
14.2. Early donor management-Future	213
14.3. Hormone replacement therapy in organ donors-Future	215
14.4. Transthoracic echocardiography assessment-Future	216
14.5. Inflammatory and bio-chemical marker assessment-Future	217
14.6. Conclusion	218
14.7. Study impact	218
SECTION-5-APPENDICES	219
APPENDIX-A - Equations for calculation of derived variables	220
SECTION-6-REFERENCES	221

LIST OF FIGURES

Figure 1-1- Number of heart transplant procedures reported to the ISHLT registry by year, stratified by continent	8
Figure 2-1- Demonstrating various changes that follow brain stem death impacting on donor heart and lung function.....	10
Figure 2-2- Graph demonstrating changes to SVR following coning	12
Figure 2-3- Graph demonstrating the relationship between systemic and pulmonary blood flow following BSD leading to blood pooling in the lungs	13
Figure 2-4- Graph demonstrating the increased trans-pulmonary gradient following BSD	14
Figure 2-5- Bar chart demonstrating increased levels of serum IL-6 in hearts that were unused for transplantation compared to the normal donor hearts	19
Figure 2-6- Bar chart showing the significantly elevated Serum TNF- α level in donor hearts that were unused for transplantation compared with normal donor hearts	21
Figure 3-1- Regulation of TSH secretion and negative feedback effects of T3 and T4	28
Figure 3-2- Regulation of ACTH and the negative feedback effect of cortisol on CRH and ACTH.....	37
Figure 5-1- Estimation of cardiac output using Stewart-Hamilton principle	50
Figure 5-2- Estimation of ITTV and PTV using thermodilution curve	51
Figure 5-3- Estimation of GEDV from ITTV	51
Figure 5-4- Estimation of various blood volumes and EVLW using PiCCO plus method	52
Figure 5-5- Validation of EVLW-T with EVLW-G	53

Figure 5-6- Demonstrates significantly less duration of ventilation and ICU stay for patients managed using EVLW measurement	55
Figure 7-1-Donor pathway through the study	59
Figure 7-2-Managing an organ donor with MAP > 70 mm Hg	67
Figure 7-3- Flow diagram demonstrating management of a hypotensive donor.....	68
Figure 8-1-CONSORT diagram demonstrating enrolment, exclusion, randomisation and number of hearts transplanted.....	81
Figure 8-2- Cardiac index (mean \pm 95% confidence interval) between initial (I) assessment and end-assessment (E) in the four treatment groups.	88
Figure 8-3-Cardiac index (mean \pm 95% confidence interval) in donors receiving or not receiving T3 (n=40 in each group).....	92
Figure 8-4–Cardiac index (mean \pm 95% confidence interval) in donors receiving (n=39) or not receiving MP (n=41) in each group.	95
Figure 8-5-Cardiac index (mean \pm 95% confidence interval) between initial (I) assessment and end-assessment (E) in biochemically euthyroid or low T3 or T4 donors receiving or not receiving T3 during donor management.....	98
Figure 8-6-Organisation chart of donor heart outcomes within the study	101
Figure 9-1–Consort diagram showing the donor inclusion, exclusion and allocation to randomised treatment groups.....	113
Figure 9-2-The relationship of CI, LVEF and RVEF with procalcitonin levels	120
Figure 9-3-Receiver operating characteristic curve demonstrating the relationship between sensitivity and 1-specificity in determining the predictive value of initial PCT level in identifying subsequent heart usability for transplantation.....	123
Figure 10-1-Distribution of CTnI levels in the entire donor cohort	132

Figure 10-2-a-Correlation between left ventricular ejection fraction and CTnI levels in 79 heart donors. 10-2-b- ROC curve demonstrating the relationship between sensitivity and 1-specificity in determining the predictive value of cTnI>1 μ g.L ⁻¹ for identifying decreased donor LV ejection fraction (LVEF <50%)	134
Figure 10-3-a-Correlation between baseline cardiac index and cTnI levels 10-3-b- ROC curve demonstrating the relationship between sensitivity and 1-specificity in determining the predictive value of CTnI>1 μ g.L ⁻¹ for identifying decreased donor cardiac index	135
Figure 10-4-Correlation between LV-Tei index and cTnI levels in 79 donor hearts .	136
Figure 10-5--Kaplan-Meier survival curve demonstrating early recipient outcome following transplantation	142
Figure 11-1-Consort diagram of donor inclusion, exclusion and recruitment into the trial and withdrawal from the echocardiographic study with allocation to the four randomized treatment groups.....	152
Figure 11-2-Scattergram and correlation of left ventricular ejection fraction with cardiac index	154
Figure 11-3-Scattergram and correlation of left ventricular Tei index with cardiac index.....	155
Figure 11-4-Flow diagram in donors in which repeat assessment of LV echocardiographic function was possible (n=52).....	160
Figure 11-5-Echocardiographic and haemodynamic change in donors with subnormal LVEF according to degree of LV impairment, where repeat measurement was available (n=14).....	161

Figure 11-6-Echocardiographic and hemodynamic change in donors with abnormal LV-Tei index where repeat measurement was available (n=18).....	162
Figure 11-7-Flow diagram in the 66 donors with initial echocardiographic functional assessment showing outcome towards hemodynamic functional suitability for transplantation.....	164
Figure 12-1-CONSORT diagram demonstrating study enrolment, exclusion, allocation to treatment groups and number of lungs transplanted from Trial and non-Trial cohorts.....	177
Figure 12-2-Changes in PaO ₂ /FiO ₂ ratio ± 95% confidence intervals in donors receiving (MP n=29) or not receiving (non-MP n=31) methylprednisolone	184
Figure 13-1- ROC curve demonstrating the relationship between sensitivity and 1-specificity in determining the predictive value of baseline PaO ₂ in identifying lungs suitable for transplantation	201
Figure 13-2- ROC curve demonstrating the relationship between sensitivity and 1-specificity in determining the predictive value of baseline EVLWI for identifying lungs suitable for transplantation	202
Figure 13-3-Kaplan-Meier survival curve demonstrating early recipient outcome following transplantation.....	203
Figure 13-4-Correlation between EVLWI measurement by thermodilution and gravimetry (r=0.7; Spearman's correlation p=0.002)	205
Figure 13-5-Bland-Altman plot demonstrating limits of agreement between EVLWI measurement by thermodilution and gravimetry.....	206

LIST OF TABLES

Table 2-1- showing hormonal changes following BSD and their effect on the donor.	17
Table 3-1- Principles behind donor management.....	25
Table 3-2- Haemodynamic Targets used by Wheeldon et al.....	26
Table 4-1- Established Aims of Echocardiography in the Assessment of Donor Hearts	44
Table 7-1-Study inclusion and exclusion criteria	60
Table 8-1- Characteristics of Trial and non-Trial donors	82
Table 8-2-Hemodynamic parameters for the entire Trial cohort	84
Table 8-3-Donor characteristics in the 4 Trial treatment groups.....	86
Table 8-4-Characteristics of Trial donors according to T3 receipt	90
Table 8-5-Characteristics of Trial donors according to MP receipt	93
Table 8-6-Thyroid function changes according to T3 receipt.....	97
Table 9-1- Donor demographic parameters.....	112
Table 9-2-Initial and pre-retrieval inflammatory marker levels in 79 donors according to randomised group	115
Table 9-3-Baseline and pre-retrieval cytokine levels in donors receiving methylprednisolone compared to non-MP donors	117
Table 9-4-Baseline and pre-retrieval cytokine levels in donors receiving T3 compared to non-T3 donors	118
Table 9-5-Initial and change in heart function in donors with low and high baseline PCT level.....	121
Table 10-1-Donor demographic parameters.....	133
Table 10-2-Donor parameters between the CTnl groups	138

Table 10-3-Comparison of donor variables between suitable and unsuitable hearts for transplantation.....	140
Table 10-4-Recipient parameters between the CTnI groups.....	141
Table 11-1-Echocardiographic parameters LVEF, LVFS and LV Tei index versus cardiac index at initial assessment.....	156
Table 11-2-Echocardiographic parameters of initial and post-management ventricular function by treatment group.....	158
Table 11-3-Demonstrating normal E-function predicting suitable hearts for transplantation.....	165
Table 12-1-Characteristics of Trial and non-Trial donors	178
Table 12-2-Donor parameters between the four Trial treatment groups.....	180
Table 12-3-Parameters in Trial donors receiving or not receiving methylprednisolone	182
Table 12-4-Overall parameters of lung function within Trial donors (n=60).....	185
Table 12-5-Comparison of (Trial) donor parameters between suitable and non-suitable lungs for transplantation (Univariate analysis)	187
Table 13-1- Donor demographic data.....	197
Table 13-2- Univariate analysis of parameters in donors with end-assessment EVLWI<10ml.kg ⁻¹ and 10ml.kg ⁻¹	199

SECTION-1: INTRODUCTION

1. HEART AND LUNG TRANSPLANTATION

1.1. History and development

1.1.1. Heart transplantation

Heart and lung transplantation are established treatment options for patients suffering from end stage heart and lung diseases. This is relatively a young speciality with a first successful heart transplantation performed just over 40 years ago. Although the first successful transplantation was performed in December, 1967 by Dr. Christiaan Barnard a South African surgeon, heart transplantation evolved through much earlier experimental development. Alexis Carrel a French surgeon first performed experimental transplantation of puppy's heart into the neck of an adult dog in 1905. In the mid-1940s Demikhov a Russian surgeon performed series of animal experiments on intrathoracic heterotopic heart transplantation. Most of his work was not reported in the West until 1960. Demikhov successfully performed first heart-lung transplantation in the dog. He had performed a total of 67 procedures with two of the recipients surviving 5 and 6 days. Most of the initial experimental procedures were carried out as heterotopic or parallel heart transplantation. The development of cardiopulmonary bypass in 1950s paved way for further refinement of surgical technique of heart transplantation (1;2).

Norman Shumway and colleagues from Stanford University in California developed the surgical technique of performing heart transplantation by suturing the atrial cuffs, aorta and pulmonary artery separately using cardiopulmonary bypass on dogs. Following successful operation the animal survived for 3 weeks followed by death due to haemorrhagic rejection. This led to further understanding of the role of

immunological mechanisms involved in rejection of the graft and Immunosuppressant was used to prevent rejection leading to long term survival of experimental animals.

Following this initial success a series of heart transplantations was performed worldwide. However, disappointing results, multiple complications and rejections lead to dampening of initial enthusiasm. Following the development of endomyocardial biopsy techniques which was pioneered and developed by Dr. Philip Caves a British surgeon, to monitor rejection and the discovery of cyclosporine in 1980s lead to explosion in number of heart transplantation performed worldwide (1).

1.1.2. Lung transplantation

Experimental lung transplantation developed simultaneously with heart transplantation as most of the researchers performed combined heart-lung transplantations. Demikhov performed the first lung transplantation by implanting canine lobes. The concept of suturing left atrial cuff for pulmonary venous anastomosis was first introduced by a French surgeon Dr. Henry Metras. The first human lung transplantation was performed by Dr. James Hardy at the University of Mississippi. The recipient survived for 18 days. Michael Woodruff in Edinburgh (1968) and Donald Ross in London (1969) attempted the initial lung transplantation in the UK without success. First successful lung transplantation in the Europe was performed by Dr. Fritz Derom, a Belgian surgeon. Following this initial success many attempts on lung transplantation continued with disappointing results. Rejection, infection and airway complication were major contributors for failure. Following the advent of cyclosporine the lung transplantation activity started to expand. Joel Cooper in Toronto performed single lung transplantation for idiopathic pulmonary

fibrosis with the recipient surviving for 6 years. Followed by Alec Patterson in St. Louis introduced the concept of double-lung transplantation with both lungs implanted with single tracheal anastomosis. However, the technical difficulties and tracheal anastomotic complication of this procedure, lead to the development bilateral sequential single lung transplantations, where the lungs were implanted individually. There had been a gradual increase in lung transplant activity in late 1990s and currently the lung transplant activity has taken over by larger number than heart transplantations performed in the UK (1;3).

1.2. Indications

1.2.1. Heart transplantation

Patients with end stage heart failure of any aetiology with severe ongoing symptoms despite optimal medical therapy are suitable candidates.

1. Peak oxygen uptake on exercise less than 14.ml/kg or less than 12 ml/kg for patients receiving beta-blockers
2. History of recurrent admission to hospital with worsening heart failure
3. Refractory ischaemia not amenable to revascularisation with severe left ventricular impairment
4. Recurrent symptomatic ventricular arrhythmia associated with severe impairment of ventricular function

1.2.2. Lung transplantation

Patients suffering with severe chronic lung disorder with an expected 2 year survival of less than 50%

1. Patients with COPD-emphysema (alpha-1 antitrypsin deficiency)

- a. $FEV_1 < 25\%$ predicted
- b. Resting $PCO_2 > 6$ KPa
- c. Resting $PO_2 < 8$ KPa
- d. Poor quality of life
- e. Oxygen dependency and repeated hospitalisation
- f. Rapid decline in FEV_1 with life threatening exacerbations

2. Patients with cystic fibrosis

- a. $FEV_1 < 30\%$ predicted
- b. Rapid decline in lung function
- c. $PO_2 < 7.5$ KPa
- d. $PCO_2 > 6.5$ KPa

3. Idiopathic pulmonary fibrosis

- a. Resting hypoxaemia with TLC and VC $< 60\%$ predicted
- b. DLCO $< 50\%$ predicted
- c. Symptomatic disease on non-responsive to medical therapy
- d. Secondary pulmonary hypertension

4. Primary pulmonary hypertension

- a. NYHA class 3 or 4
- b. Poor exercise tolerance- less than 350 metres on 6 minute walk test
- c. Mean RA pressure >15 mm Hg
- d. Mean pulmonary artery pressure > 50 mm Hg
- e. Uncontrolled syncope, haemoptysis or right heart failure
- f. Cardiac index <2.5 L.min⁻¹.m⁻²

1.3. Outcome following heart and lung transplantation

The operative mortality for the heart and lung transplantation procedure remains at 10%. The median survival for the entire cohort of heart transplantation recipients on the ISHLT database (the time at which 50% of those transplanted remain alive) is currently 10 years. However, the median survival increases to 13 years for those who survive the operation and the first year following transplantation (4). Despite accepting marginal donor hearts and operating on sicker older recipients the outcome following heart transplantation in the recent era is better due to the improved survival in the first year of transplantation.

The outcome following lung transplantation is 79% at 1 year, 65% at 3 years, 54% at 5 years and 29% at 10 years. The median survival for the entire cohort of patients in ISHLT database is 5.4 years and for those who survive the operation and the first year post-transplantation the median survival increases to 7.4 years (5). Similar to heart transplantation the outcome for lung transplantation recipients since 2000 is better than the previous years due to the improved operative mortality and one year survival.

1.4. Current status of heart and lung transplantation

The number of heart transplantation performed worldwide peaked to 4,460 in mid 1990s followed by a steady decline in the activity. The activity rate has achieved a steady plateau down to 3,300 in the last few years (Figure-1.1). The number of adult heart transplantation performed in the UK has been declining from 350 per year in early-1990s to less than 100 in the last year. This reduction in activity is due to an improvement in neurosurgical intensive care, reduction in road traffic accidents following strict legislation on the use of seat belts, change in donor demographics and finally donors with pre-existing co-morbidities that preclude them from donating their heart.

In contrast the lung transplantation activity has been increasing over the last decade. The number of single lung transplants performed worldwide is stable with consistent growth in the activity of bilateral lung transplantation. In the UK the lung transplantation activity is higher than the number of heart transplantation performed per year. This is due to liberalisation of donor acceptance criteria, increase in non-heart beating lung donation and resuscitation of unsuitable lungs in ex-vivo lung perfusion rigs (EVLP).

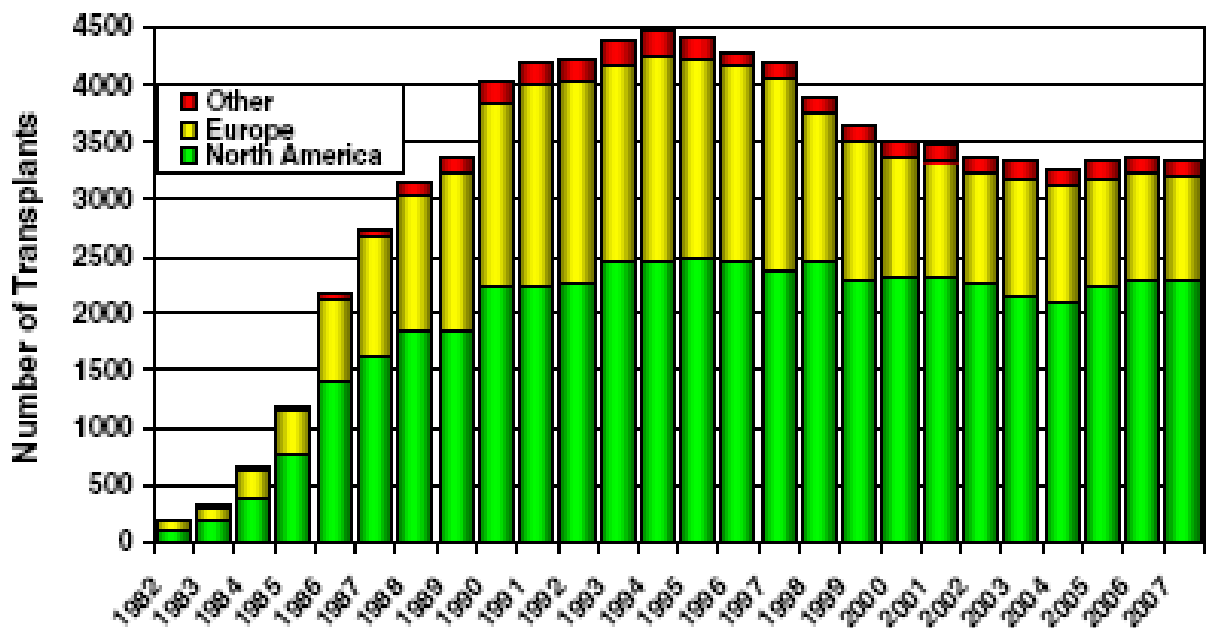


Figure 1-1- Number of heart transplant procedures reported to the ISHLT registry by year, stratified by continent

Despite initiatives to increase the number of available donor pool, there is an imbalance between supply and demand of donor heart and lungs. This leads to increase in waiting times and higher waiting list mortality (6). One of the major factors is the poor retrieval rate of hearts and lungs from the existing donor pool. The current heart retrieval rate remains between 30% and the lung retrieval rate is around 20 and 25%. Donor organs with marginal functions are considered for transplantation that could lead to increased incidence of primary graft dysfunction (PGD) and worse recipient outcome.

Therefore efforts need to be directed towards increasing the retrieval rate of donor organs from the existing donor pool and also to improve the quality of the organs to reduce the incidence of PGD. This could be achieved only by better understanding of the pathophysiology of brain stem death, its impact on heart and lung function and the role of various interventions that could improve the donor heart and lung function.

2. BRAIN STEM DEATH

2.1. Anatomy

The normal functioning of the heart and lungs is regulated by groups of neurons located in the brain stem. From here parasympathetic (vagal) neurones and pre-sympathetic neurones play a vital role in regulating heart rate, blood pressure, cardiac output and vascular tone as well as smooth muscle tone of the airways. In addition normal rhythmic respiration is regulated by groups of neurones in the brain stem, controlling the spinal phrenic motor neurones to the diaphragm. Importantly, the activity of the brain stem cell groups is regulated by afferent input from receptors situated in the cardiovascular system and the airways (7). Therefore any neurological insult involving the brain stem can lead to loss of neural control of both heart and lungs.

The brain, spinal cord and spinal fluid along with cerebral vessels are encased in a rigid bony enclosure. Because brain tissue and spinal fluid are essentially incompressible, the volume of brain tissue, blood and spinal fluid in the cranium must remain constant at any one time (Monro-Kelly doctrine). When the intracranial pressure (ICP) increases the cerebral vessels are compressed leading to ischaemia. The cerebral blood flow is maintained relatively at constant levels between 50 to 150 mm Hg (6.7 to 20 KPa) by a process of autoregulation keeping the normal steady state of flow despite variations in perfusion pressure (8)

2.2. Coning

Any CNS pathology (head injury, sub-arachnoid haemorrhage, sub-dural haemorrhage, intracranial haemorrhage, infections and tumours) that increases the pressure within the skull leads to increase in ICP. As the brain lies within a closed non-expandable bony cavity any increase in ICP leads to decrease in brain blood flow. The resultant ischaemia stimulates the vasomotor area in the brain stem leading to increase in blood pressure and fall in heart rate (bradycardia). This response is called Cushing's reflex. However, as the ICP increases beyond certain limit the brain blood flow completely ceases and the brain stem is pushed through the foramen magnum (8). This process is called coning. The process of coning leads to haemodynamic, hormonal, inflammatory and bio-chemical changes, each one of these changes inflict cardio-pulmonary damage (Figure-2.1).

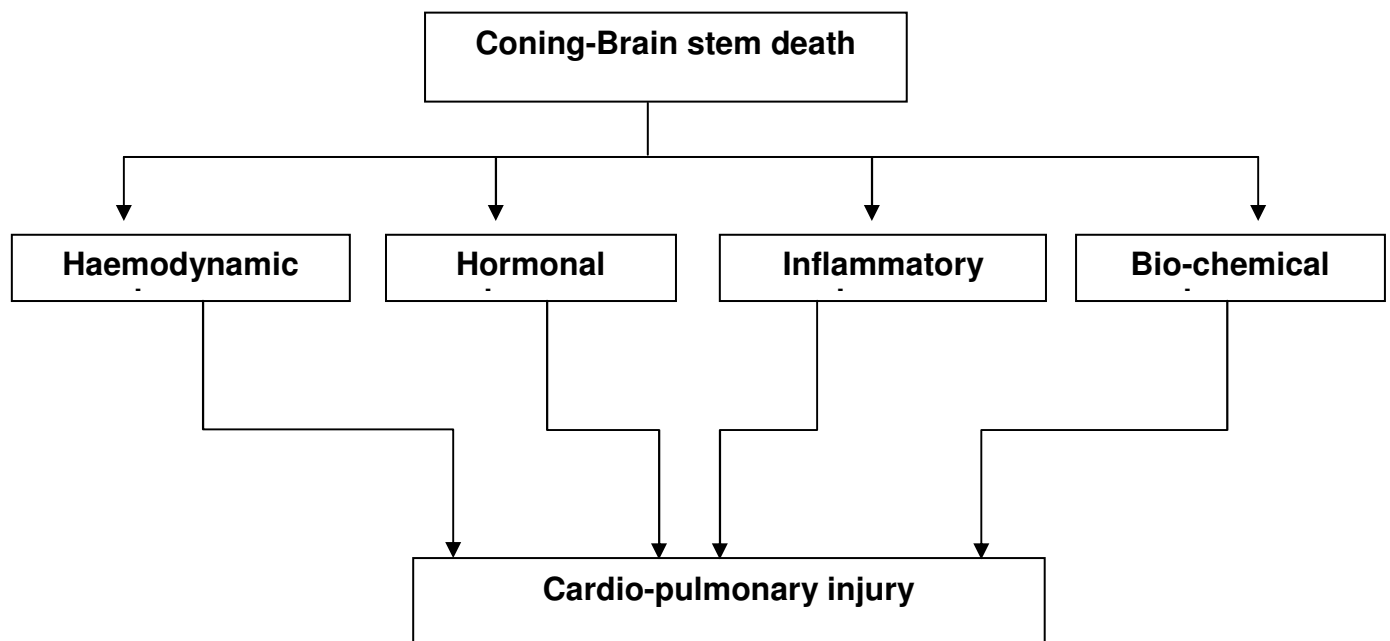


Figure 2-1-Demonstrating various changes that follow brain stem death impacting on donor heart and lung function

2.3. Pathophysiological changes during and after brain stem death

2.3.1. Haemodynamic changes

Haemodynamic effects following BSD demonstrate biphasic changes. Immediately after coning an initial massive sympathetic neural outflow is associated with a catecholamine surge (catecholamine storm) with release of both adrenaline and nor-adrenaline from the sympathetic nerve terminals (9;10). This results in increased blood pressure, systemic vascular resistance (SVR), and cardiac work-load which in turn lead to increased myocardial oxygen consumption (11). Due to the high SVR the peripheral tissue perfusion is reduced leading to tissue hypoxia and metabolic acidosis. Experimental studies have shown that the catecholamine release follows a biphasic pattern with an initial release followed by a return to normal levels and another release into the systemic circulation after 2 hours associated with myocardial injury (12).

This catecholamine storm is followed by vasoparetic phase which leads to reduction in sympathetic neural output and complete loss of vascular tone. As a consequence blood pressure falls together with cardiac output and vascular tone (13;14). During this phase vasopressin (VP) (a vasoconstrictor) infusion can help in maintaining SVR (15) (Figure-2.2).

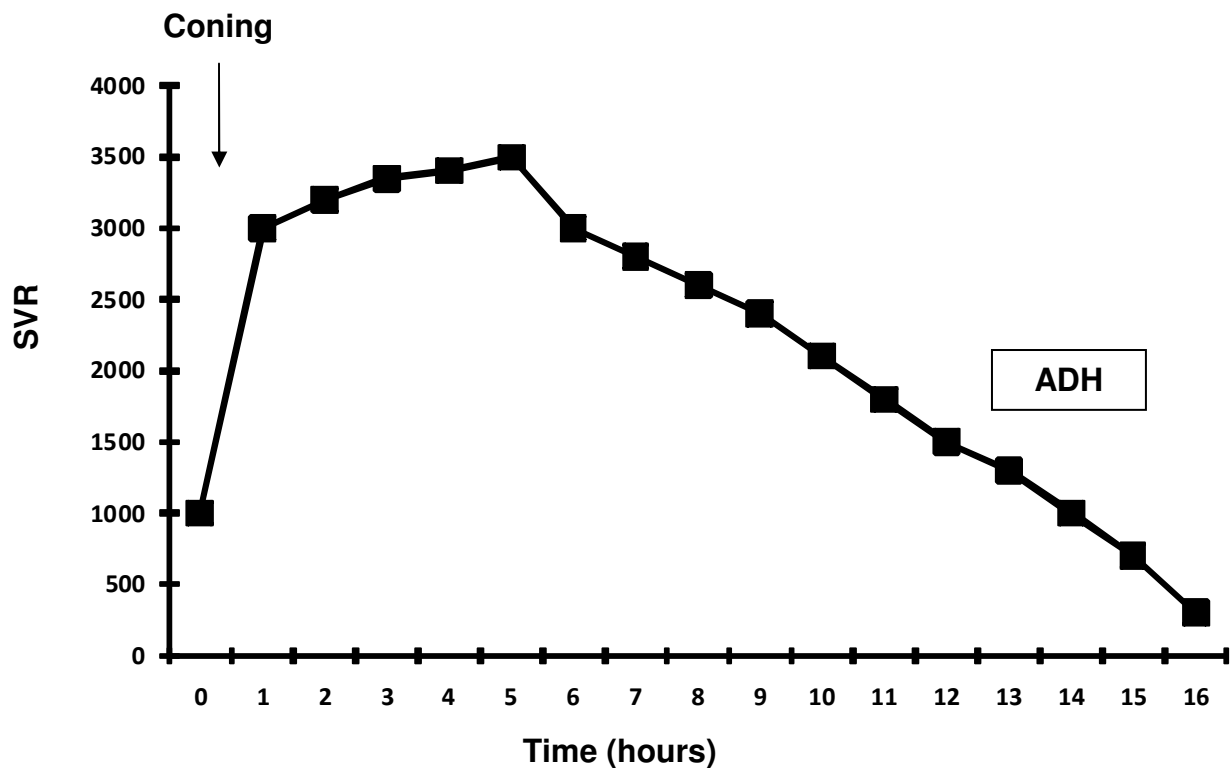


Figure 2-2- Graph demonstrating changes to SVR following coning

2.3.1. 1. Pathophysiology of neurogenic pulmonary oedema

Novitzky and colleagues using an experimental brain death animal model demonstrated the mechanism of development of neurogenic pulmonary oedema (16). During the catecholamine storm phase there is an increase in SVR and mean arterial blood pressure (MAP) along with a reduction in systemic blood flow (Qs). During the same time the pulmonary vascular resistance (PVR) (resistance against which the right ventricle pumps blood to the pulmonary arteries) is also elevated along with mean pulmonary artery pressure (MPAP) with a reduction in pulmonary blood flow (Qp). However, the PVR then falls below normal levels, this occurring much earlier

than a drop in SVR. This leads to reduction in MPAP and increase in pulmonary blood flow with still reduced systemic blood flow due to high SVR. This imbalance between the pulmonary and systemic blood flow ($Q_p > Q_s$) leads to blood pooling in the lungs (Figure-2.3).

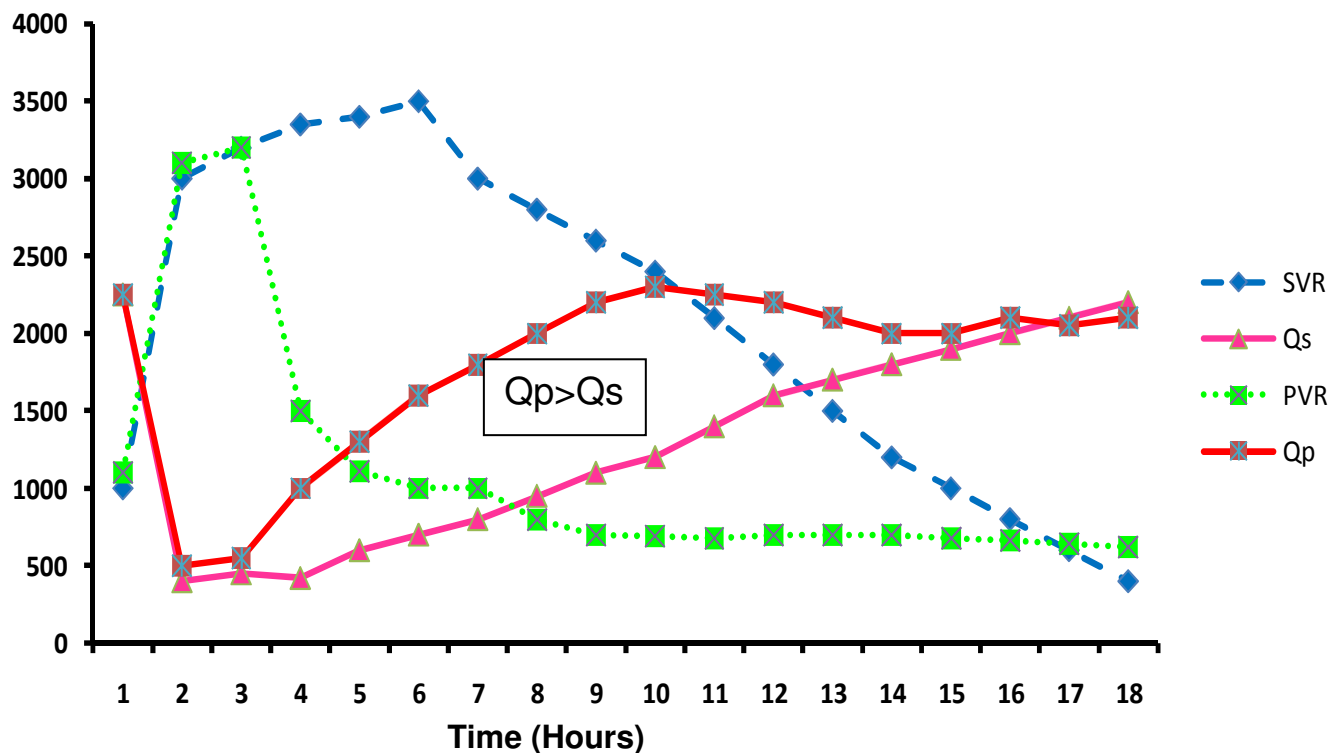


Figure 2-3-Graph demonstrating the relationship between systemic and pulmonary blood flow following BSD leading to blood pooling in the lungs
Y-axis represents SVR, PVR and systemic and pulmonary blood flow (ml)

Due to this imbalance in pulmonary and systemic blood flow, the left atrial pressure increases with associated increase in MPAP. This imbalance leads to an increase in gradient across the pulmonary vascular tree (trans-pulmonary gradient (TPG)) which in turn causes pulmonary oedema. The increase in LAP and TPG also induces sub-endocardial ischaemic damage of the myocardium (Figure-2.4).

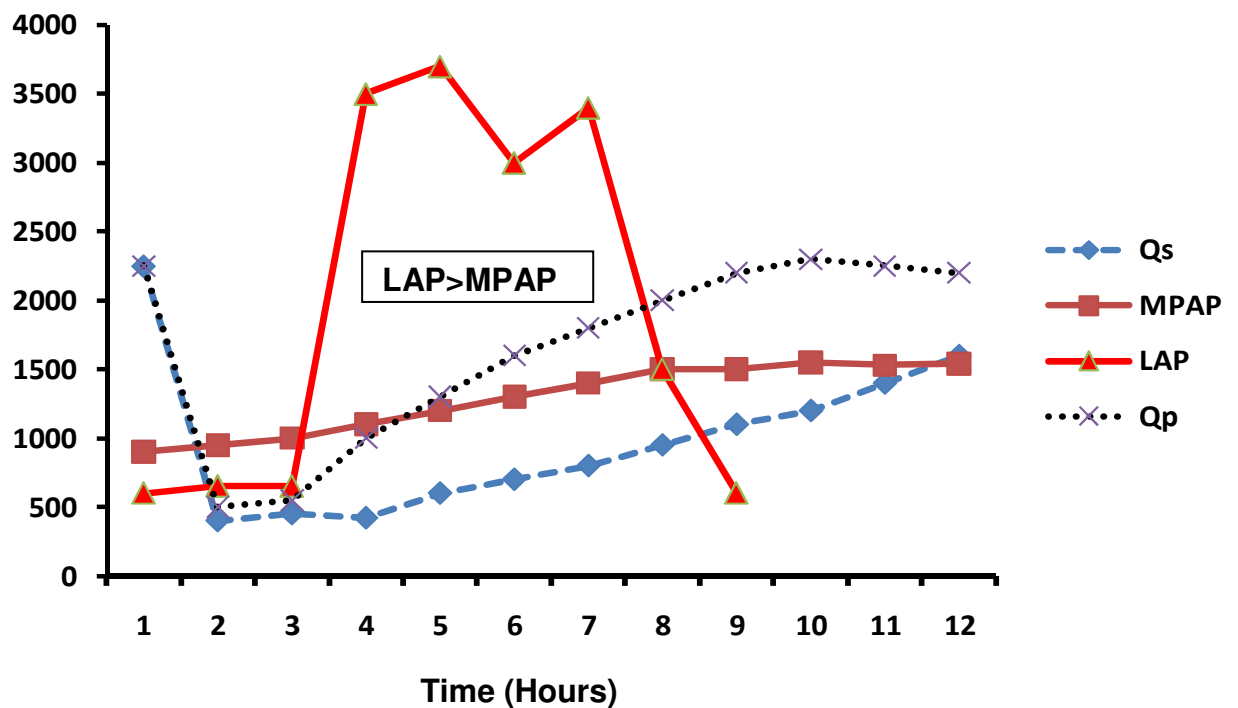


Figure 2-4-Graph demonstrating the increased trans-pulmonary gradient following BSD

2.3.2. Hormonal changes following BSD

Immediately following the coning induced catecholamine storm, increased plasma levels of both adrenaline and nor-adrenaline by many folds was noted (12). Shivalker et al showed that the catecholamine release was increased with an acute increase in ICP, as compared to gradual increase (17;18). In experimental studies bilateral sympathectomised animals did not show any histological evidence of myocyte injury in comparison with animals with no or incomplete cardiac denervation (19) or β -blockers (20). This demonstrates that most of the catecholamines are released from the sympathetic nerve terminals. High concentration of catecholamines is known to cause cardiac damage and myocardial dysfunction. Areas of myocytolysis, contraction band necrosis, focal coagulative necrosis, sub-endocardial haemorrhage and oedema with infiltration of mononuclear cells have all been described (21;22).

There are many theories of the mechanism of catecholamine induced cardiac damage. These include hypoxia, direct catecholamine toxicity (21), alteration in coronary microcirculation (23;24), alteration in membrane permeability and rapid desensitisation of myocardial β -adrenoreceptors (25). Therefore catecholamine release following coning causes severe myocardial damage by its haemodynamic changes and direct effects which manifests as cardiac dysfunction (26) affecting both left and right ventricle. In experimental brain death canine model Bittner and colleagues demonstrated significant right ventricular dysfunction which further deteriorated following graft (27;28) preservation and transplantation.

Following coning the neural connections between the midbrain and the brain stem are severed (Hypothalamo-pituitary axis). This leads to loss of hypothalamic control over the pituitary gland and rapid significant decrease of thyroid, adrenal hormones

and vasopressin (VP) (29). This is followed by a rapid reduction in serum catecholamines (30) and rapid fall in posterior pituitary hormone (31). Lack of VP impairs water retention in the kidneys causing diabetes insipidus. During this time the patients produce large volumes of urine leading to rapid fluid shift in the vascular compartment which further worsens the hypotension during the vasoparetic phase. Serum levels of tri-iodothyronine (T3), cortisol and insulin levels gradually fall after BSD (31). T3 levels are found to be reduced in serum in 85% of donors following BSD and the T4 (thyroxine) levels are found to normal with a low to normal levels of TSH (thyroid stimulating hormone) and elevated levels of reverse T3 (rT3) (32;33). This condition is called the euthyroid sick syndrome. Administration of thyroxine T4 will lead to preferential conversion of T4 to rT3. This hormone depleted state has been shown to be associated with deterioration in cardiac function and depletion of myocardial energy stores and lactic acidosis (34;35). Administration of T3 in animal BSD model was associated with reduction in plasma lactate and free fatty acid levels suggesting a reversal from anaerobic to aerobic metabolism (36). Fall in cortisol and insulin levels will lead to abnormal anti-inflammatory mechanism and abnormal blood sugar control respectively (14). Table-2.1 describes the hormonal changes that occur following BSD and their effect on the donor physiology.

Table 2-1- showing hormonal changes following BSD and their effect on the donor

Hormone	Effects
↑ Catecholamines	<p>Increase in heart rate, MAP, cardiac output, SVR</p> <p>Increase in myocardial work-load and oxygen consumption</p> <p>Catecholamine induced direct injury</p>
↓ Vasopressin	Diabetes insipidus
↓ T3/T4	Essential for aerobic myocardial metabolism
↓ Insulin	Altered glucose metabolism-high blood sugars
↓ Adrenal steroids	Poor response to stress, Loss of normal anti-inflammatory mechanisms

2.3.3. Inflammatory changes following BSD

Brain stem death generates a pro-inflammatory environment after catecholamine storm. This promotes the release of large amounts of pro-inflammatory cytokines that worsens the already injured cardio-pulmonary function (37). Studies have shown that cytokines, like interleukin-1, 6 and 8 (IL-1, IL-6 and IL-8), tumour necrosis factor- α (TNF- α) and procalcitonin (PCT), levels were elevated in both serum and the myocardium following BSD especially in dysfunctional hearts (38).

2.3.3.1. Interleukins

Interleukins are signalling molecules from the super family of cytokines and are mainly involved in communicating signals between the white cells. The term interleukin refers to inter (communicating between) and leukins (white blood cells) was given after their function. IL-1 is the first to be described. Both IL-1 and IL-6 are secreted by the T-cells and also from the activated macrophages. Their main function is to further stimulate more T cells and is involved in elaboration of inflammation and acute phase proteins. Elevated levels of IL-1 and IL-6 have been associated with worse donor heart function. Serum and myocardial IL-6 levels were found to be significantly elevated in hearts that were not used for transplantation compared to the transplanted hearts (39) (Figure-2.5). Elevation of such cytokines are associated not only with donor heart dysfunction (40) but also implicated in increased risk of post-transplant rejection (41-43) and increased post-operative inotrope requirement.

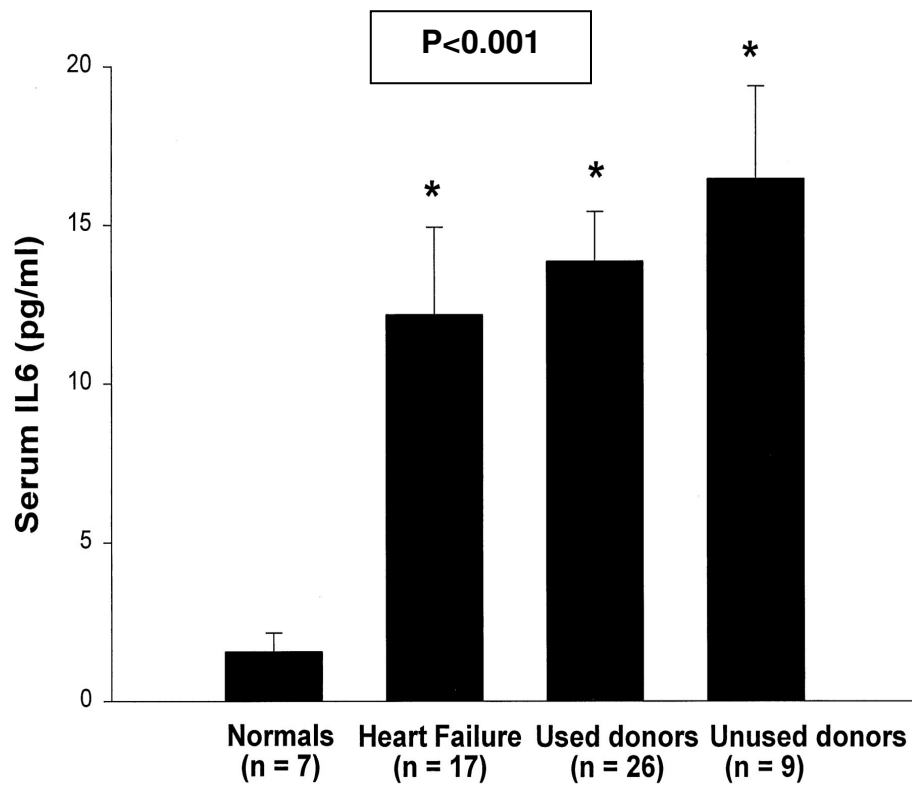


Figure 2-5-Bar chart demonstrating increased levels of serum IL-6 in hearts that were unused for transplantation compared to the normal donor hearts

Interleukin-8 is another cytokine secreted by macrophages and endothelial cells. It is associated with immune response to bacterial infections. IL-8 has been implicated in acute inflammatory lung diseases. It is a potent chemotactic factor promotes neutrophilic adhesion, aggregation and degranulation. It has been implicated in ischaemia-reperfusion injury of lungs following transplantation. The level of IL-8 from lung tissue was found to be directly related to poor lung function ($\text{PaO}_2/\text{FiO}_2$ ratio), high airway pressure, and prolonged ICU stay thereby predicting early graft function post-transplantation (44). In another study the level of IL-8 in donor broncho-alveolar lavage (BAL) predicted the early graft function and also the recipient outcome (45).

2.3.3.2. Tumour necrosis factor

TNF- α is another cytokine produced by the activated lymphocytes that can induce inflammation and apoptosis (programmed cell death). TNF- α mRNA is expressed in the failing or stressed nucleated myocyte generating and releasing TNF- α (46). In patients with heart failure, serum TNF- α levels correlate with disease severity and are predictive of mortality (11;37;42;47). In heart donors, myocardial upregulation of TNF- α mRNA and elevated serum TNF- α levels have been reported previously and the greatest increase has been found in donor hearts rejected for transplantation due to poor function (38;39;48). In these donors, TNF- α levels exceed those seen in patients with advanced heart failure (39) (Figure-2.6).

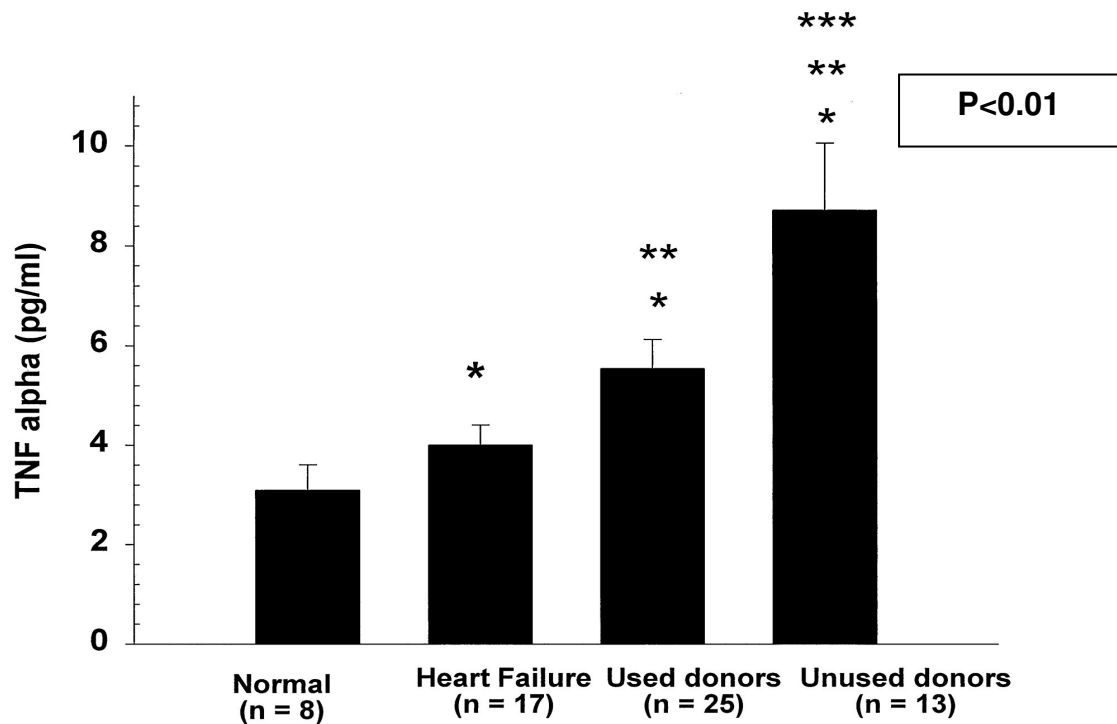


Figure 2-6-Bar chart showing the significantly elevated Serum TNF- α level in donor hearts that were unused for transplantation compared with normal donor hearts

Similarly other inflammatory markers like procalcitonin (precursor of hormone calcitonin) and C-reactive protein, which is prognostically valuable in heart failure, have also been predictive of primary graft failure and graft dysfunction following heart transplantation (49-51). Wagner et al. retrospectively analyzed the serum levels of PCT and CRP in 79 donors. They grouped the donor hearts into two, based on the post-transplant mortality related to graft dysfunction. Assessment of donor heart function was limited and ischemic times were significantly greater in the high PCT donors. They reported that higher PCT levels were associated with significantly elevated graft failure-related mortality and that a PCT $>2\text{ng.ml}^{-1}$ had 96% specificity and 50% sensitivity for predicting post-transplant mortality (50).

2.3.4. Bio-chemical changes following BSD

Troponin-I and T are myocardial proteins released into the blood following injury and cell death (52). Measurement of troponins is an invaluable, non-invasive tool for the assessment of myocardial injury (53;54). Haemodynamic changes that follow BSD leads to sub-endocardial ischaemia and muscle death. Cardiac troponin I (CTnI) elevation has also been observed in patients with subarachnoid haemorrhage and in this setting is associated with increased LV dysfunction, pulmonary oedema, hypotension requiring inotropic support and is predictive of worse outcome (55).

Serum cardiac troponin-I and T levels are found to be elevated in most donors. Elevation of CTnI has been associated with worse donor heart function and increased inotropic requirement following transplantation and increased risk of acute graft failure (56;57). Elevated troponins may predict adverse recipient outcome, being associated in some studies with increased risk of early graft failure and increased rates of inotrope requirement post-transplantation and post-transplant rejection (49;56-59) However, this association remains controversial as equivalent outcomes have been achieved in other studies with no increased risk of rejection (60-62). Elevated CTnI levels represent a marker of myocardial injury but whether they predict the ultimate heart usability following donor management is unknown.

2.4. Summary

Each of the above described changes that happen following BSD injures the heart and lungs either directly or indirectly. The catecholamine storm induced haemodynamic changes leads to direct ischaemic injury to the myocardium. This also

leads to direct changes in the pulmonary and systemic vascular resistance causing haemodynamic shear force in pulmonary endothelium causing pulmonary oedema. Following the catecholamine storm the inflammatory response sets in along with hormonal deficiency state, indirectly affecting donor heart and lungs.

The inflammatory environment leads to release of large amounts of cytokines which further worsens already injured heart and lungs. These changes may add insult to the injuries sustained by the heart and lungs prior to BSD, like trauma to chest, aspiration, infection, positive pressure ventilation. In addition the donor heart may be suffering from pre-existing coronary artery disease and hypertension. Therefore heart and lungs even in an otherwise healthy donor gets severely injured during BSD and the management of the donor following BSD is an important aspect of organ retrieval. This also will influence the outcome of the recipient following successful transplantation.

Therefore understanding the pathophysiology of the BSD and further management of the organ donor are the key to successful organ retrieval and transplantation.

3. DONOR MANAGEMENT AND HORMONE REPLACEMENT THERAPY

3.1. Rationale behind donor management

Donor management has unfortunately been most neglected part of thoracic transplantation. This is due to the fact that most of the donor work is done away from the recipient centres in a new hospital intensive care unit environment and in most cases it happens at night time. There is always a shortage of trained practitioners to manage an organ donor. However, donor management is the most essential part of successful thoracic organ transplantation.

The principles behind donor management involve attention to details on the basic physiological parameters (Table-1). Prior to BSD the mean arterial pressure on the patient is maintained at higher level in order to achieve adequate cerebral perfusion. Once the BSD testing is done and consent for organ donation is available the MAP does not need to be very high. Therefore accepting a MAP of 70 mm Hg is adequate to maintain end organ perfusion. Achieving adequate pre and after-load along with maintaining normal electrolyte, temperature homeostasis and trachea-bronchial clearance are the main objectives of donor management. In order to achieve these parameters insertion of invasive monitoring lines including pulmonary artery flotation catheter (PAFC) is essential. This will help in optimising pre-loads on both right and left ventricle and their after load. Performing bronchoscopy will help in airway clearance and check the position of the endotracheal tube.

Table 3-1- Principles behind donor management

-
- Attention to detail
 - Adequate Mean arterial pressure
 - Optimise pre-load and after-load
 - Meticulous fluid balance
 - Tracheobronchial clearance
 - Maintain normal blood sugar, serum potassium and temperature homeostasis
 - Monitoring haemodynamic performance
-

The role of invasive monitoring and protocol driven donor management could lead to increase in number of transplantable donor organs. Wheeldon et al. over 15 years ago reported the role of PAFC guided optimisation in transforming unacceptable donors to usable ones. They studied 150 donors over 3 year period and found over 35% (52/150) of donors were outside the criteria for heart transplant suitability. Forty four of the 52 donor hearts were suitable at the end of protocol driven donor management (Table-2) (63).

Table 3-2- Haemodynamic targets used by Wheeldon et al

Mean arterial pressure	>65-70 mmHg
Central venous pressure	< 12mmHg
Wedge pressure	<12mmHg
Cardiac Index	2.5 L.min ⁻¹ .m ⁻²
With dopamine use	<5ug/Kg/min

The same group demonstrated using a simple nomogram of protocol driven optimisation they could increase their donor organ pool by over 30% (64). Following this many authors reported the role of protocol driven donor management in increasing potential donor organ pool (65-67). The crystal city consensus conference report published in 2002 also recommends the utilisation of PAFC guided donor management pathway in maximising the use of organs from the cadaveric donor (68).

3.2. Hormone replacement therapy-Thyroid hormone

3.2.1. Synthesis and release

The thyroid gland is endodermal in origin and is located in the anterior portion of the neck where it lies on either side of the trachea. The functional unit of the thyroid gland is the follicle surrounded by a rich capillary plexus. The follicular epithelium consists of a single layer of cuboidal cells. The lumen of the follicle is filled with hormone containing colloid; this is the major constituent of thyroid mass. This is the site of iodination. The major products of the thyroid gland are the iodothyronines.

Iodine enters the thyroid follicular cells as inorganic iodide and is transformed in a series of steps into the thyroid hormones. T₄ and T₃ are the two iodothyronines with biological activity. The thyroid gland contains 5-8mg of iodide on average. Nearly all of this is in the form of iodothyronines. Approximately 1% of this is released from the gland per day, 75% as thyroid hormones (TH) and the remainder as free iodide (69).

Active iodide uptake occurs at the basal membrane of the follicular cell. Iodide then diffuses along an electric gradient into the lumen. Once within the lumen iodide is oxidised by a peroxidase and is incorporated into tyrosine molecules. Monoiodotyrosine (MIT) and diiodotyrosine (DIT) are formed as a result of iodination. Two DIT molecules are coupled to form T₄ or one DIT and one MIT to form T₃ or rT₃. Once thyroglobulin has been iodinated it is stored as colloid in the follicular lumen. Release of T₄ and T₃ into the circulation requires proteolysis of the thyroglobulin. Once T₄ and T₃ are released into the plasma the vast majority is bound to plasma proteins. Thyroxine-binding globulin binds approximately 75% of the plasma T₄ and almost all (99.5%) of T₃. Very small quantities of T₄ (0.05%) and T₃ (0.5%) are unbound (free) within the plasma (69).

3.2.2. Regulation of thyroid hormone

The most important regulator of the thyroid gland is thyroid-stimulating hormone (TSH). TSH secreting cells are located within the anterior portion of the pituitary gland. Its synthesis is stimulated by hypothalamic thyrotropin-releasing hormone (TRH). The secretion of TSH is regulated by two factors. Firstly, TRH increases the rate of secretion and secondly, circulating levels of T₄ and T₃ decrease it as part of a

negative feedback loop (Figure 3.1). The regulation of TSH is under a negative feedback loop and circulating levels of free T4 and T3 produce feedback to the pituitary to decrease TSH production. As levels drop then TSH secretion increases.

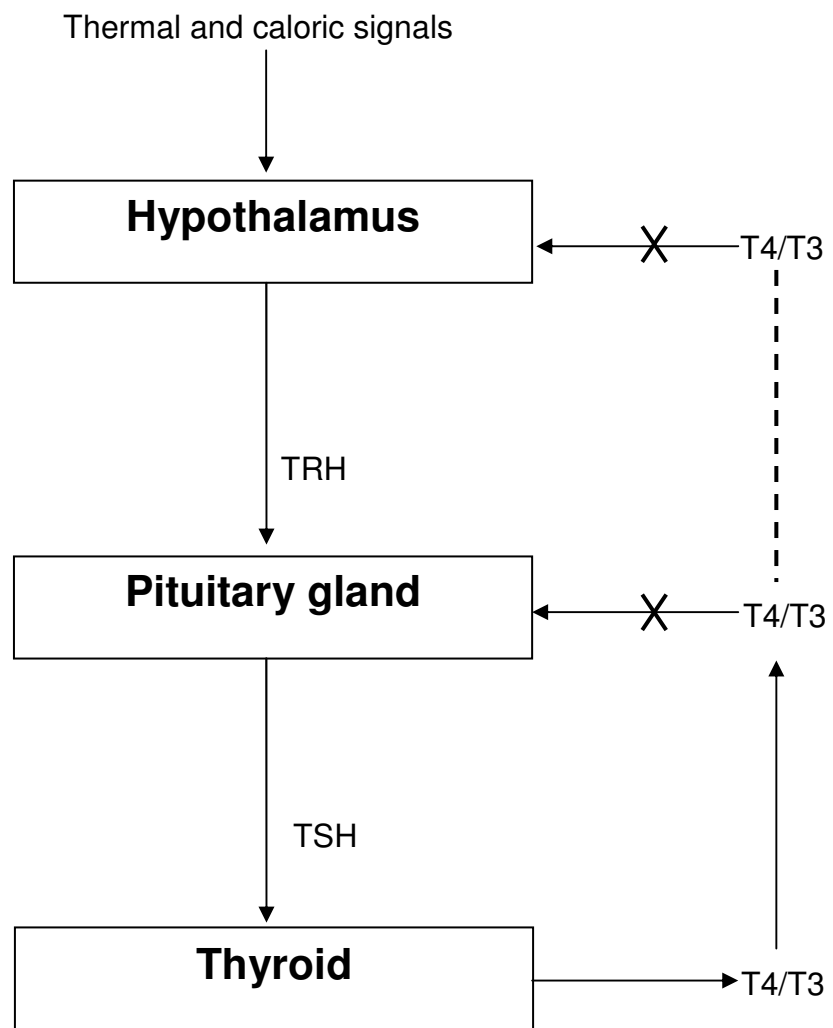


Figure 3-1-Regulation of TSH secretion and negative feedback effects of T3 and T4

3.2.3. Cardiovascular effects of thyroid hormone

Thyroid hormone increases the basal metabolic rate, oxygen consumption and heat production within the body. It increases the cardiac output and has positive inotropic and chronotropic effect on the heart. T₃ is the biologically relevant component of thyroid hormone in cardiac myocytes. As in other tissues T₃ exerts its effects by both genomic and non-genomic mechanisms. The predominant mechanism by which T₃ affects myocardial action is by exerting a direct effect in cardiac myocytes by binding to nuclear receptor isoforms (70). Secondly, T₃ affects cardiac function by its actions on the vascular system, in particular reduction of left ventricular afterload (70). The immediate effects are due to the extra-nuclear action of thyroid hormone. It increases the oxidative metabolism and increased sensitivity to the circulating catecholamines (71). The late effects are due to the genomic action after 6 to 8 hours of T₃ infusion (72). This increases the protein synthesis and m-RNA expression. It also increases the β -adrenergic receptor density (73).

Reduction in systemic vascular resistance is one of the earliest changes that occur to patients on administration of TH. This may in part be due to the release of local vasodilators that are liberated as a result of increasing metabolic activity and oxygen consumption. This reduction in systemic vascular resistance decreases afterload and leads to an increase in cardiac output (70). The subsequent increase in cardiac output leads to an increased oxygen delivery to the periphery supporting the increased basal metabolic rate and increased oxygen consumption. TH administration also leads to an increase in circulating blood volume. This leads to an increase in venous return and preload, which further contribute to increased cardiac output.

3.2.4. Euthyroid sick syndrome

Serum levels of TH drop in response to physical stress such as starvation or illness. This can occur rapidly. Most commonly circulating T4 and TSH levels return to normal and T3 levels then remain low. This phenomenon is often referred to as the “euthyroid sick syndrome” (ESS) on the basis that these patients are not actually clinically hypothyroid despite their biochemical profile. It is characterised by low levels of total and free T3, low or normal levels of thyroxine, high levels of reverse T3 and normal thyroid stimulating hormone (TSH). The reduction in T3 is thought to be secondary to a reduction in the peripheral conversion of T4 to rT3. After recovery from the precipitating cause thyroid function invariably returns to within normal limits.

3.2.5. Thyroid hormone replacement in multi-organ donors

Brain stem death leads to a rapid decline in serum levels of thyroxine, cortisol and insulin. These observations have been confirmed in animal experiments (74). However, the changes in thyroid hormone levels following BSD in human organ donors are controversial. T3 levels are found to be reduced in serum in 85% of donors following BSD and the T4 levels are found to normal with a low to normal levels of TSH and elevated levels of reverse T3 (rT3) (32;33). There is abnormal peripheral conversion of T4 to inactive rT3, all these findings pointing towards euthyroid sick syndrome (35;75). The association between low levels of serum T3 and myocardial dysfunction and the use of T3 in potential organ donors following BSD is not confirmed (76).

3.2.5.1. Animal studies

Studies on pigs and baboons following experimental brain death have shown rapid decline in hormone levels and an increase in anaerobic metabolism. This leads to deterioration in cardiac function and depletion of myocardial energy stores and lactic acidosis (77). These abnormalities were reversed on administration of T4, cortisol and insulin (36;78). However, a load-independent analysis of cardiac function in pigs following experimental brain death and T3 administration failed to show any advantage over the control group in terms of preserving left ventricular function or prolonging survival.

Furthermore, marked decreases in myocardial blood flow (MBF) in the T3 group versus control group without a significant change in cardiac function was also identified. Analysis of endocardial to epicardial flow ratios disclosed no significant differences between groups at any time. In summary, animals treated with T3 had a greater decline in MBF than the control group at 4 hours, without any benefit to cardiac function (79).

3.2.5.2. Retrospective clinical studies

Following BSD same events as described in animal experiments have been observed in human organ donors. 85% of BSD patients show low levels of T3. But, the presence of T3 deficiency and its association between myocardial dysfunction and the improvement in haemodynamics following its administration is controversial.

There are few prospective studies published in the literature on the use of T3 in multi-organ donors.

A retrospective analysis of all brain dead donors in the United States, from January 2000 to September 2001, was conducted. The donors were divided to two groups depending on whether they received hormone replacement therapy (HRT Group) (T3, Methylprednisolone and Vasopressin) or not. Out of 10,292 donors, 701 (6.8%) of donors received HRT. On univariate analysis the mean number of organs retrieved from HRT group was 22.5% greater than non-HRT group donors (3.1 versus 3.8). On multivariate analysis 4.7% increased probability of retrieving heart from the HRT group was observed compared to the non-HRT group (80;81). Unfortunately, in this study only 47 out of the 701 HRT group donors received T3 infusion (0.45%). The rest of the 654 donors received T4. It is well known from the above discussion that there is a euthyroid sick syndrome status following BSD and the serum levels of T4 in the donors were found to be normal and abnormal peripheral conversion of T4 to rT3 has been observed following BSD. Administration of T4 will be of no use in these donors. The dose, mode, timing (before retrieval) and the duration of administration of thyroid hormone in this group of patients was not available. The duration of donor management and other management strategy used to stabilize the donor until retrieval has not been mentioned.

3.2.5.3. Prospective studies

Novitzky et al observed the effects of routine hormone administration on twenty one consecutive donors (82) by administering IV T3 (0.2µg), cortisol (100mg) and insulin (10-30 IU), and repeating the dose hourly. Donors were monitored between 3 to 8 hours with a mean duration of 5.5 hours. The results were compared with 26 donors managed routinely without hormone supplement. They observed a significant improvement in mean arterial pressure (MAP), fall in central venous pressure (CVP) by 35%, significant reduction in lactate levels and reduction in bicarbonate requirement to treat metabolic acidosis compared to the control group. The serum T3 levels were initially low in all donors and rose to normal levels following T3 replacement. All 21 hearts were used for transplantation with a good post-operative outcome. The study observation confirms the presence of T3 deficiency following BSD and return to normal levels following replacement therapy. However, the study is a non-randomised controlled trial, the donor demographic details are unknown and haemodynamics were observed only from the right side of the heart. They continued the same observational study to include 116 consecutive donors and found the same haemodynamic improvement and decrease in the inotropic support (83).

In an observational study Jeevanandam and colleagues used T3 replacement therapy in dysfunctional donor hearts that normally might have been considered unsuitable for transplantation (High CVP, decreased LVEF <45%, inotrope requirement and episodes of cardiac arrest). The donors received 0.2µg bolus of T3 repeated hourly to a maximum dose of 0.6µg. The donors were monitored between 2-3 hours, from the time of administration of drugs to procurement of heart. A

significant improvement in the MAP and fall in CVP and left atrial pressure was observed following T3 administration. All 6 dysfunctional hearts were used for transplantation with good post-operative outcome. All the donors in this study were young with a mean age of 16.5 years (range of 8-30years). They are unlikely to have any coronary artery disease. All donors received 80 mg of frusemide intravenously after T3 administration. This could have lead to massive diuresis and resulted in fall in CVP and LAP. All donors received dopamine infusion between 5 to 10µg/Kg/min which might have improved the cardiac contractility and MAP. The study was not a randomised controlled trial and apart from filling pressures no other haemodynamic and echocardiographic data were available. Serum T3 levels were not measured in the study group (84;85).

The role of routine use of T3 in resuscitating all organ donors was studied in an open label non-blinded randomised study by Jeevanandam et al. All donors received bolus of 0.6µg of IV T3. All donors were on less than 10µg/kg/min of dopamine. They found no difference between the parameters tested in the T3 and the control groups (73).

In a prospective, randomised, controlled study on 25 consecutive organ donors, Randell and colleagues used T3 and placebo as an infusion to stabilize the organ donors. In contrary to the findings observed by Novitzky and Jeevanandam et al, they noted no difference in the haemodynamic parameters, inotrope requirement between the two groups of donors. They also noticed a worsening metabolic acidosis and increase in requirement of bicarbonate infusion in T3 group, quite opposite to the findings reported by Novitzky and colleagues. The sample size of this study was

small and they compared MAP and temperature maintenance between the groups. Serum T3 levels were not measured before and after treatment (86).

Goarin et al conducted a prospective, randomised, and blinded controlled trial to determine the haemodynamic and myocardial effects of T3 administration on 37 brain dead organ donors. The donors either received 0.2µg bolus of T3 or saline placebo. All donors were monitored using pulmonary artery catheter and transoesophageal echocardiography (TOE). They found very low levels of T3 in 85% of donors and normal T4 levels in all donors. Following administration of T3 the serum levels returned to normal in all treatment group donors. There was no difference in the haemodynamic parameters between the groups. Even in the group with decreased fractional axis change (FAC) on TOE T3 administration did not improve the function and FAC. The study concluded that T3 has no important effect on myocardial function and haemodynamics of brain dead organ donors (87).

3.3. Hormone replacement therapy-Steroids

3.3.1. Synthesis and release

Glucocorticoides are produced from the adrenal glands which lie above both kidneys in the retroperitoneum. Adrenal glands are divided into an outer cortex and an inner medullary region. The adrenal cortex has 3 further layers called zona glomerulosa which produces aldosterone a mineralocorticoid, zona fasciculata which produces cortisol and corticosterone or glucocorticoides and zona reticularis which produces sex hormones. Adrenal medulla produces the catecholamines both adrenaline and nor-adrenaline. Cortisol is synthesised from cholesterol. The synthesis is stimulated by

the adrenocorticotrophic hormone (ACTH) which is released from the anterior lobe of pituitary gland. ACTH increases the concentration of cholesterol within the mitochondria of adrenal cortex. Cholesterol is converted into pregnenolone within the adrenal cortex which is the rate limiting step in steroid synthesis. Pregnenolone undergoes further hydroxylation process to progesterone, deoxycorticosterone and finally to cortisol. Following release from the adrenals cortisol is bound to proteins called corticosteroid binding globulin. Most of the cortisol is protein bound and only 4% of cortisol is available for the receptors. The amount of cortisol available in the serum undergoes diurnal variation with the highest levels in the early morning and lowest levels present during late evening and at night time. Cortisol is metabolised by oxidation in liver by the cytochrome P450 enzyme (88).

3.3.2. Regulation

Cortisol synthesis is stimulated by ACTH release from anterior lobe of pituitary gland. ACTH release is further controlled by a peptide hormone called corticotropin-releasing hormone (CRH) secreted from the hypothalamus. ACTH increases the availability of cholesterol within the adrenal cortex. The released cortisol in turn acts as a negative feedback to inhibit release of ACTH and CRH. In the presence of active inflammation activated macrophages secrete IL-1 which synergistically increases the CRH and ACTH release thereby increasing cortisol and suppression of immune response (88).

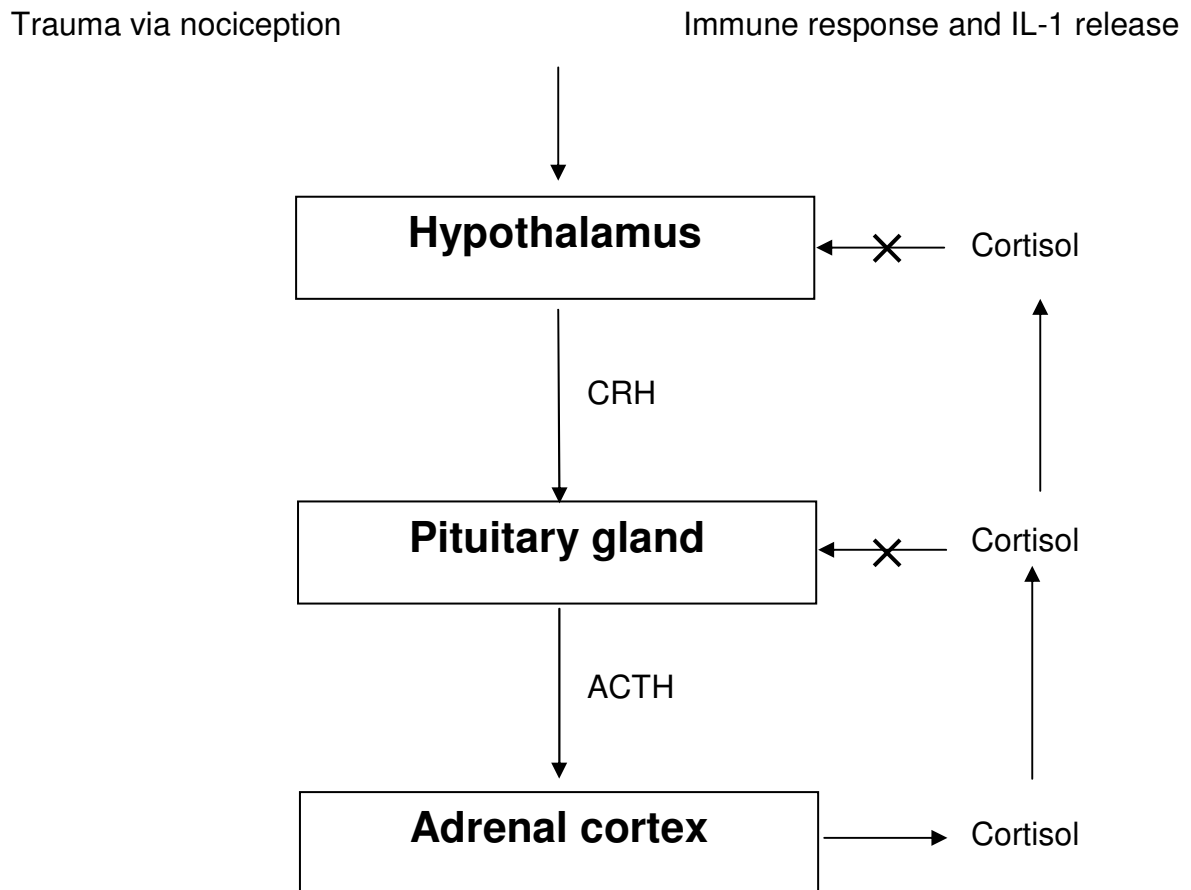


Figure 3-2-Regulation of ACTH and the negative feedback effect of cortisol on CRH and ACTH

3.3.3. Anti-inflammatory effects of steroids

Glucocorticoides are the hormones released in response to any stress in our body. They have various actions including effects on glucose metabolism, collagen loss, epithelial fluid transport, and water and sodium re-absorption. However, its effects on immune system are of considerable importance. Steroids have been used extensively in inflammatory mediated diseases like, multiple sclerosis, rheumatoid

arthritis, bronchial asthma and systemic lupus erythematosus. It acts at various levels starting from cellular level acting on lymphocytes and neutrophils. At vascular endothelium it prevents leucocyte adhesion and aggregation and locally at tissue level it stabilises the mast cells and avoids degranulation. It has a potent anti-inflammatory action by blocking the proliferation of T-lymphocytes. The activated T-cells secrete IL-2 and produce the T-cell growth factor. Cortisol binds to the IL-2 receptors thereby prevents activated T-cells responsive to IL-1 stimulation and T-cell proliferation (88). Cortisol also has a negative feedback effect on IL-1. In addition they exert their anti-inflammatory effect by stabilisation of cellular membranes, reduction of HLA antigen up-regulation and inhibition of cytokine elaboration (89). Glucocorticoides stimulate the epithelial Na⁺/K⁺-channels in alveolar epithelium and have an important role in absorption of excessive fluid from the alveolar space (90). This particular action is beneficial in early clearance of lung oedema (91;92).

3.3.4. Steroids replacement in multi-organ donors

The role of steroid administration in multi-organ donors is studied in most trials with combination of T3 and vasopressin (80;81). The anti-inflammatory and immunosuppressive actions of steroids in post-transplant recipients are well established. The role of steroids in organ donor is still in debate. In an experimental brain death model both systolic and diastolic heart function was better preserved in animals receiving methylprednisolone than in controls and this was associated with a significant reduction in inflammatory markers like IL-6, TNF- α and IL-10, 6 hours after BSD (93;94). In a sheep model of brain death and heart transplantation the measures of left ventricular functions were significantly higher up until 6 hours of

transplantation in steroid pre-treated animals. This was also associated with stable recovery of transplanted animals than compared with non-steroid group (95). In all these animal trials steroids were administered immediately prior to or after BSD. However, in clinical studies steroids are usually administered only after confirming BSD and consent for organ donation is available. This is usually many hours after coning and established BSD. Therefore by the time the steroids are administered the inflammatory cascade had well established with elaboration of pro-inflammatory cytokines. To achieve the benefits the steroids should be administered as soon as possible after clinical signs of coning. But, it is currently unethical in the UK to treat a patient as an organ donor prior to the confirmation of BSD and consent for organ donation.

In lung transplantation, steroid administration was reported to be associated with improved oxygenation and increased lung retrieval rate in a retrospective study (96). This improved oxygenation could have been due to its up regulation of alveolar epithelial fluid transport (97). However, there are no prospective randomised trials evaluating the role of early steroids administration in potential lung donors.

3.4. Hormone replacement therapy- Vasopressin

3.4.1. Synthesis and release

Arginine vasopressin (VP) also called as anti diuretic hormone (ADH) is found in most mammals. It is a peptide hormone synthesised as a pre-hormone in the hypothalamus and is stored in vesicles in posterior pituitary gland. It is released into

the blood stream and also has some direct effects on brain. Vasopressin is released into the blood stream in response to the changes in plasma volume and osmolality. Whenever there is a reduction in plasma volume activated by the stretch receptors in carotid and aortic bodies the posterior pituitary gland releases the hormone. There are 3 different types of receptors for VP action. Changes in plasma osmolality and plasma volume lead to regulation of its secretion.

3.4.2. Actions of vasopressin

One of the major functions of VP is to maintain body water content and maintaining normal osmolality. Its major action is at the level of the distal convoluting tubules and collecting tubules of the kidneys. It concentrates urine by reabsorbing water and reducing urine volume. Because of this action it is called anti-diuretic hormone. Vasopressin in high concentrations increases peripheral vascular resistance and thus increases arterial blood pressure. This effect appears small in healthy individuals; however it becomes an important compensatory mechanism for restoring blood pressure in hypovolaemic shock. This particular effect is useful in post BSD vasoparetic state.

3.4.3. Role of vasopressin therapy in multi-organ donors

The use of vasopressin (VP) in treatment of post-BSD vasoparesis is well established than the other two hormones. Following BSD the serum levels of VP is decreased due to the transection of hypothalamo-pituitary axis. This leads to diabetes insipidus

and intravascular fluid depletion. Due to this massive fluid shift the donor becomes hypotensive and also loses the vascular tone. Blaine et al. reported the effect of lack of vasopressin in a BSD animal model and demonstrated that the animal becomes hypernatremic and haemoconcentrated. Administration of small dose of VP leads to normalisation of plasma osmolality and serum sodium levels (98). Chen and colleagues reported the improvement in MAP in organ donors who did not manifest diabetes insipidus. This increase in MAP allowed weaning of catecholamines (99). The same observation was demonstrated by other authors and has also been shown to be safe and not to affect the transplanted kidney function (15;100).

The large fluid shift due to post-BSD diabetes insipidus is treated with crystalloids solutions. This could lead to interstitial and intra-cellular oedema and deterioration of organ function. Pennefather and colleagues demonstrated the detrimental effect of crystalloids transfusion on the arterial oxygen concentration in organ donors (101). Use of VP infusion would avoid the need for large volumes of crystalloids infusion. Moreover, Post-BSD diabetes insipidus could lead to increased plasma osmolality and hypernatraemia, which had been shown to be associated with worse graft function following liver transplantation (102). Finally VP use in the management of post-BSD vasoplegia has been reported to be associated with reduced pulmonary capillary leak, lung oedema and inflammation thereby reduced lung injury (103).

4. ECHOCARDIOGRAPHY ON POTENTIAL HEART DONORS

4.1. Transthoracic and transoesophageal echocardiography

Brain stem death leads to hormonal, inflammatory and haemodynamic changes which either directly or indirectly affects the heart function. The average heart utilisation after consent for organ donation remains at 30 to 40% (104). This is due to the concerns regarding the function of hearts post-transplantation and the risk of primary graft dysfunction. Although the failure to use donor hearts is multifactorial, left ventricular dysfunction is the commonest single cause and is responsible for approximately 26% of the unused organs (105). Heart utilisation rates might be improved if techniques were able to select those borderline donor hearts that were previously felt to be unsuitable yet could be transplanted with a good outcome. Echocardiography is a non-invasive, widely available investigation that is ideally suited to the accurate assessment of donor heart function and could guide donor optimization. However, usage varies widely, partly because of the limited published evidence to support routine echocardiographic assessment of donor hearts.

The potential of transthoracic echocardiography (TTE) as a screening tool for cardiac donors was first identified in 1988 (106). Despite the inherent difficulties of imaging ventilated subjects, TTE was successfully performed in all but one of 74 potential donors. Nine potential donor hearts with grossly abnormal echocardiograms were excluded from transplantation (8 hearts with severe left ventricular dysfunction and 1 heart with severe mitral and tricuspid regurgitation). Of the remaining 64 studied, TTE was normal in 46, 9 had pericardial effusions, 5 had mild septal hypokinesis, and 4

had possible mitral valve prolapse without significant regurgitation. In the absence of TTE, 21 (29%) of these donor hearts would have been excluded on clinical criteria (chest trauma, prolonged hypotension, cardiac arrest and prolonged catecholamine use). However, transplantation was successful in each case, including those with mild abnormalities on TTE. Therefore, TTE screening of donor hearts is feasible. It may exclude donor hearts with severe cardiac dysfunction and avoid direct surgical inspection. Perhaps more importantly, this study raised the possibility that TTE could identify donor hearts that could be used successfully in transplantation despite the presence of other clinical factors that would previously have led to exclusion. The objectives of echocardiographic assessment of donor heart function are presented in Table 4.1. Subsequent studies have helped to define limitations to this early promise of standard TTE to distinguish between those donor hearts with ventricular dysfunction that could proceed successfully to transplantation and those that could not.

There remain however, technical difficulties in performing standard trans-thoracic echocardiography (TTE) in brain stem dead (BSD) patients who are intubated and mechanically ventilated in the critical care environment, including lack of acoustic window, patient positioning, multiple instrumentation, and increased acoustic impedance during ventilation. A comparative study of TTE with transoesophageal echocardiography (TOE) suggested that TTE assessment may be incomplete in up to 29%. The yield of abnormal studies was higher with TOE (9/24) compared with TTE (3/24) but the study numbers were inadequate to show a difference in outcome based on imaging modality (107). TOE may be preferable but a more critical factor

may be the availability of adequate experience in the assessment of potential donor hearts, particularly given the considerable changes in loading pressures to which the hearts are subject (108).

Table 4-1- Established aims of echocardiography in the assessment of donor hearts

-
- a. Exclude pre-existing congenital abnormalities
 - b. Exclude significant valvular dysfunction
 - c. Assessment of the left ventricle:
 - a. Size and global function
 - b. Regional wall motion abnormalities:
 - i. Catecholamine storm
 - ii. Regional ischaemia
 - iii. Contusion
 - c. Response to inotropic and vasodilator therapy
 - d. Assessment of the right ventricle:
 - a. Size and global function
 - b. Effects of pulmonary pressure and volume overload
 - c. Response to volume therapy
 - e. Detect pericardial effusion and assess haemodynamic significance
-

4.2. Resting abnormalities of donor heart function

The main aim in imaging the donor heart is to select those hearts likely to succeed when transplanted and to exclude those that are likely to fail. Unfortunately, current standard echocardiographic techniques are not adequate to make this distinction without reference to other clinical and invasive haemodynamic markers of outcome. Left ventricular (LV) dysfunction is a common finding in patients with intracranial pathologies and BSD. In 147 patients with subarachnoid haemorrhage, global or regional LV dysfunction was found in 30 (20%) patients on echocardiography. Regional wall motion abnormalities tended to cover multiple arterial territories, often spares LV apex and occur in the absence of coronary artery disease (109).

The incidence of LV dysfunction in potential pediatric donors is even higher, with abnormalities demonstrable on TTE in over half of the donors. This increase may be due to heightened sensitivity to catecholamine storm of young compared to adult myocardium (110). These echocardiographic abnormalities do not however correspond to any demonstrable pathological abnormality at post-mortem (111). More importantly, such abnormalities do not preclude successful transplantation. In one study, 9/40 hearts with severe regional wall motion abnormalities (defined as diffuse hypokinesis of all segments in a 6 segment model) were successfully transplanted with resolution of left ventricular dysfunction 15 months post transplantation (112). In a further study of urgent transplantation in sick paediatric recipients, the use of donor hearts despite the presence of LV dysfunction (defined as LV fractional shortening <28%, mean LV shortening fraction $24.5 \pm 3\%$) and mitral regurgitation did not result in an increase in 30 day mortality or inotrope requirement compared with normal donor hearts. In each case, LV function was normal on

echocardiography at 30 days post-transplantation (113). LV abnormalities on TTE may be caused by the catecholamine storm that occurs in BSD, a potentially transient phenomenon that spares the ventricular apex, (109).

4.3. Limitations to resting echocardiography

A major disadvantage of standard TTE/TEE assessment of donor ventricular function is that the common methods used are load dependant. The ideal index of contractility would be sensitive to contractile change, independent of loading conditions, non-invasive, simple to apply and repeatable (114). The current gold standard method of assessment of contractile performance is end-systolic elastance (E_{es}), as derived from conductance catheter techniques (114;115). However, this method is invasive, requires special equipment and technical expertise to perform, and is therefore unlikely to be used in the routine assessment of donor heart function. A number of echocardiographic parameters have been proposed which may give load-independent information on LV performance, including myocardial performance index of Tei (116), isovolumic acceleration on tissue Doppler imaging (117;118). As yet, these have not been tested in donor heart assessment and their role is not understood.

The role of the myocardial performance (Tei) index in donor assessment has not been reported. The Tei index is relatively load-independent, simple to calculate and reproducible (116;119). Secondly, tissue doppler examination using isovolumetric acceleration (myocardial acceleration during isovolumetric period) (IVA) may provide

load-independent assessment of biventricular function (120). IVA is a sensitive index of left and right ventricular contractility, which is relatively resistant to physiological changes in loading conditions (118;121). Until recently, tissue Doppler has not been available on machines with sufficient portability to be used for donor heart assessment but this situation has now changed. Although tissue Doppler is easy to perform, assessment of IVA needs high frame rates to ensure adequate accuracy and also requires trained echocardiographer to acquire and interpret the findings

It is clear that a single snap shot TTE/TEE examination alone is unlikely to provide a stand-alone, 'gold standard' decision regarding transplantability of the donor heart. There are a number of potential alternatives that may provide improvements. Firstly, response to stimulation with low dose dobutamine during stress echocardiography (DSE) has been studied in a small prospective study of 30 consecutive BSD patients. Seven donor hearts were identified with impaired LV function (fractional shortening (FS) <30%) and proceeded to DSE. In 3, LV function improved but there was no response in the remaining 4. Troponin-t levels were markedly higher in the non-responsive group compared to the responsive patients (122). Although none of these hearts were used for transplantation, the inference is that DSE may identify those donor hearts with less myocardial necrosis that could proceed to successful transplantation. The disadvantage of DSE is that this is a demanding technique and the skills required may not be readily available at the hours when many donor heart evaluations occur. Secondly, monitoring changes in function using serial echocardiography may be more useful than single assessment, since these are more likely to detect positive responses to intensive donor management. Serial

echocardiography is feasible in these circumstances and detects progressive change in donor heart function. In a prospective study of 49 donor hearts with reduced ejection fraction ($EF < 50\%$) or RWMA sufficient for the organs to be initially rejected, prolonged donor management resulted in an improvement in EF or RWMA in 38 (78%) donors. Serial TTE was successful in identifying this improvement, resulting in the successful transplantation of 34 donor hearts (104;123).

In summary echocardiography is an ideally suited non-invasive investigation which can identify the presence of major cardiac abnormality and in addition may guide during donor management. However, the role of echocardiogram during protocol driven donor management with invasive monitoring has not been investigated in a prospective study.

5. EXTRAVASCULAR LUNG WATER INDEX MEASUREMENT IN POTENTIAL LUNG DONORS

5.1. Thermodilution lung water index measurement

Extravascular lung water (EVLW) refers to the amount of water in the pulmonary interstitium and alveolar spaces. This is an accurate measure of pulmonary oedema and it could be measured in number of ways by using double, single dye dilution techniques (124;125), radio-isotope labelled dye dilution techniques (126) or nuclear magnetic resonance imaging (127). However, these are technically difficult and cumbersome methods of estimating EVLW. A well validated method to derive a measure of EVLW is pulse-induced contour cardiac output (PiCCO), a single thermodilution technique based on the Stewart-Hamilton principle (128).

PiCCO plus (Pulsion UK Ltd) derives its haemodynamic variables based on injecting a known volume of cold saline into SVC through a central venous line and the thermistor sensor placed in distal aorta via femoral artery detecting changes in temperature. The cold saline distributes within the thorax volumetrically and thermally before being detected by the thermistor in distal aorta. Using Stewart-Hamilton principle the cardiac output is calculated. From the thermodilution curve the mean transit time (MTT-half of the indicator passed detection) and downward slope time (DST-exponential down slope time) are calculated (Figure-5.1).

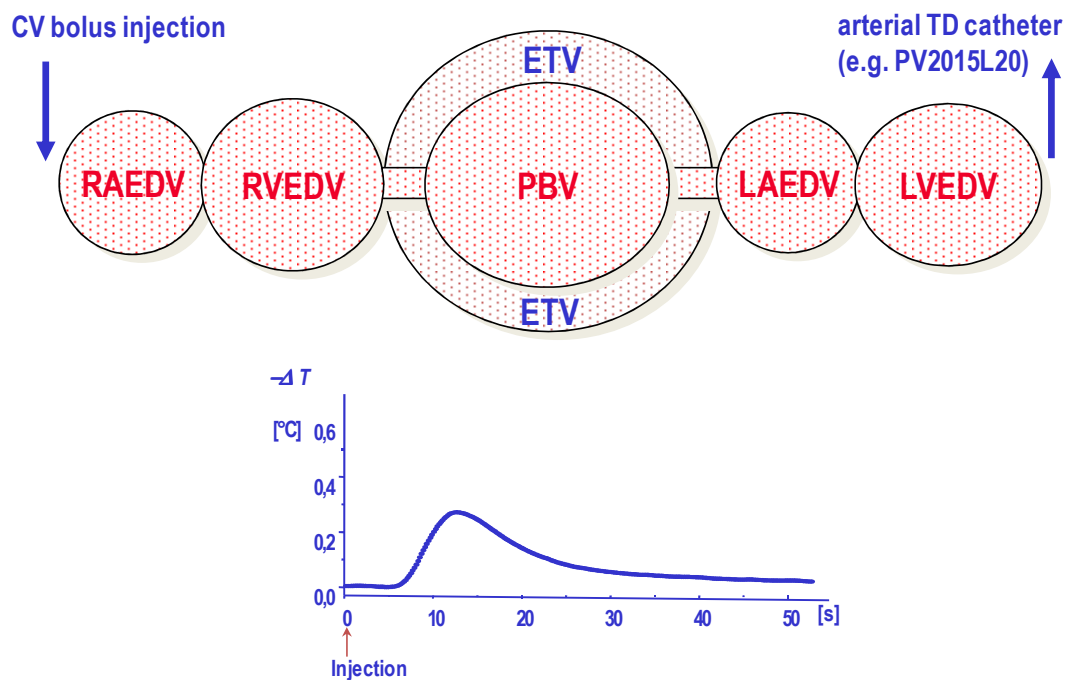
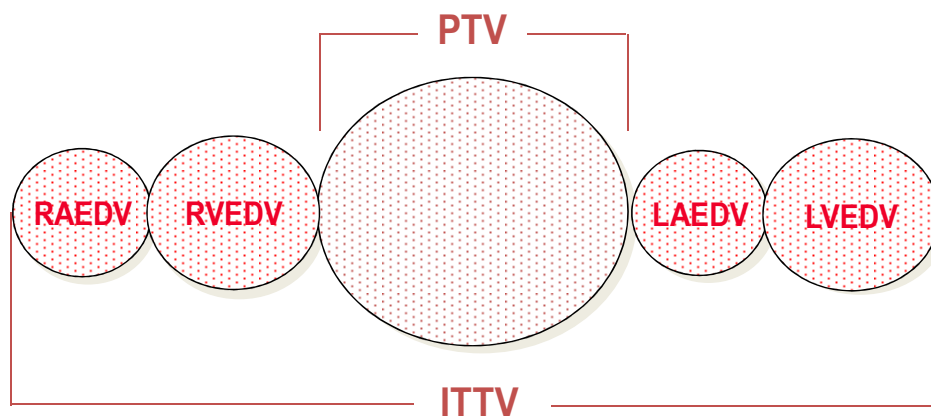


Figure 5-1- Estimation of cardiac output using Stewart-Hamilton principle

By multiplying the mean transit time with the cardiac output the entire intra-thoracic thermal volume (ITTV) is calculated and downward slope time with cardiac output the pulmonary thermal volume (PTV) is measured (Figure-5.2). By subtracting PTV from ITTV the volume of blood in all 4 chambers of the heart is calculated. This is called the global end-diastolic volume (GEDV) (Figure-5.3)



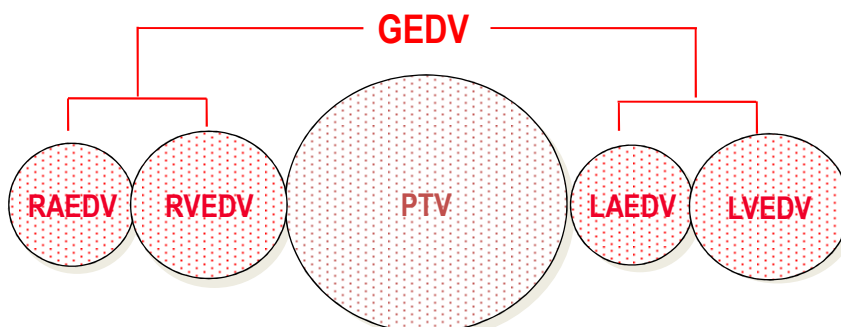
PTV = Pulmonary Thermal Volume

largest thermal volume in the series of mixing chambers (DSt – Volume)

ITTV = Intrathoracic Thermal Volume

volume from the point of injection to the point of detection (MTt – Volume)

Figure 5-2- Estimation of ITTV and PTV using thermodilution curve



GEDV = Global end-diastolic volume

End-diastolic volume of the 4 heart chambers

GEDV = ITTV - PTV

Figure 5-3- Estimation of GEDV from ITTV

By multiplying GEDV with a constant value of 1.25, intrathoracic blood volume is calculated (ITBV). This consists of blood volume in all 4 chambers of the heart and blood volume in the pulmonary circulation excluding the water content in pulmonary interstitium. Sakka et al reported the ITBV calculated by single thermodilution correlated well with GEDV calculated using double dye dilution technique in 57 critically ill patients (129). Extravascular lung water is calculated by subtracting ITBV from ITTV (Figure-5.4).

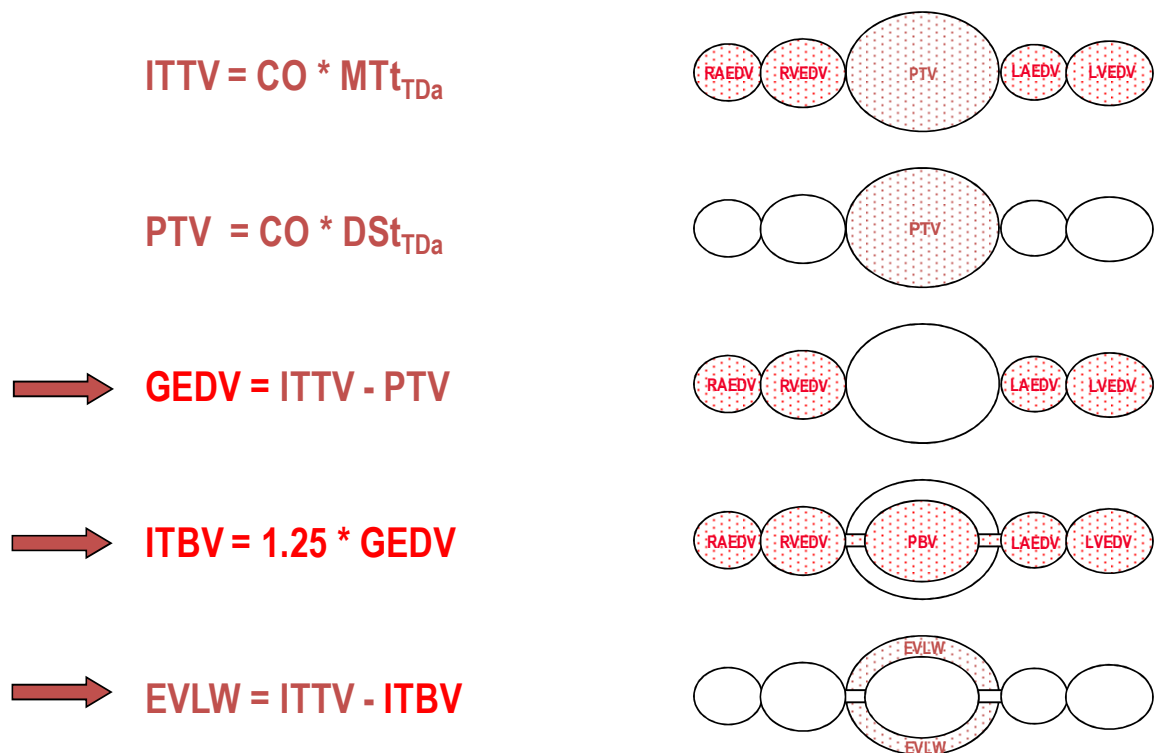


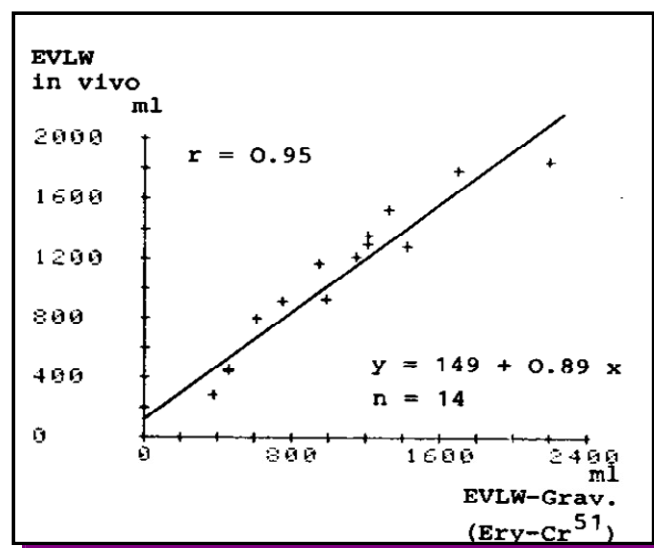
Figure 5-4- Estimation of various blood volumes and EVLW using PiCCO plus method

Extravascular lung water measured by single thermodilution technique has been shown to correlate well with double dye dilution technique in critically ill patients (129).

5.2. Validation of thermodilution lung water measurement by gravimetry

The thermodilution EVLW (EVLW-T) has been validated against the gold standard gravimetric lung water measurement (EVLW-G). The EVLW-T correlated well with EVLW-G in both animal and human studies (130-133) (Figure-5.5).

EVLW by indicator dilution compared to gravimetric EVLW measurement in brain-dead humans



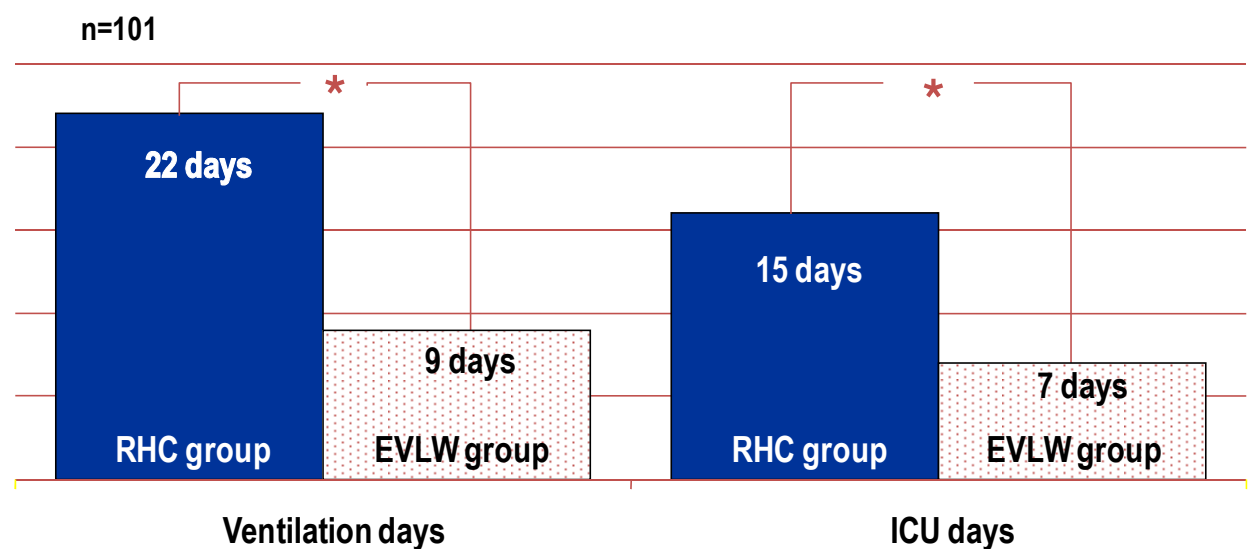
Sturm, In: Practical Applications of Fiberoptics in Critical Care Monitoring, Springer Verlag Berlin - Heidelberg - New York 1990, pp 129-139

Figure 5-5-Validation of EVLW-T with EVLW-G

Early data, using a thermal dye double indicator dilution technique in humans following BSD suggested that although such techniques over-estimated EVLWI-G, they provided a reliable index of lung water content and could be of clinical utility (132).

5.3. Clinical uses of lung water index measurement

Thermodilution measurement is well validated, simple, reliable and easily available at the bedside which gives direct quantification of EVLW. Higher EVLW has been shown to negatively correlate with arterial oxygenation (124) and increased mortality. In clinical studies higher EVLW was associated with worse outcome, prolonged intensive care unit stay and is an independent predictor of outcome (134;135). Mitchell et al managed 101 patients with pulmonary oedema randomised either to receive pulmonary artery catheter or indicator dilution measured EVLW. The patients fluid management was guided either by wedge pressure measured by PA catheter or EVLW. Patients managed with EVLW guided fluid management required significantly less number of days of ventilation and their intensive care unit stay was also significantly low (136) (Figure-5.6).



Mitchell et al, Am Rev Resp Dis 145: 990-998, 1992

Figure 5-6- Demonstrates significantly less duration of ventilation and ICU stay for patients managed using EVLW measurement

The role of EVLW measurement in a potential lung donor has not been evaluated in a prospective study. A properly conducted prospective trial evaluating EVLW with protocol driven donor management and its validation by gravimetry will clarify its role in managing a potential lung donor.

6. SUMMARY AND OBJECTIVES

Protocol guided donor management using invasive monitoring is an essential part of successful transplantation. The basic principles are simple but require trained personnel and equipments to carry out the management. From the available evidences we can summarise that there is acute T3 deficiency after BSD in most of the donors and administration of T3 lead to a normal levels in the serum. However there is no evidence to show the association between T3 deficiency and myocardial dysfunction that follows brain death and also its reversal on T3 administration.

The anti-inflammatory properties of steroids are well established, however, its role in multi-organ donors needs to be investigated. The role of vasopressin use in treating post-BSD vasoplegia and diabetes insipidus is well established. A properly designed prospective randomised controlled (blinded) trial using T3 and methylprednisolone will clarify the role of routine use of these hormones in resuscitating multi-organ donors.

Echocardiography has a significant role to play in donor heart assessment. However, with current practice over two thirds of donor hearts are still rejected for transplantation. Organ retrieval is costly and labour intensive, and donor heart malfunction is the commonest cause for aborted retrieval. Such abortive attempts may be reduced by a detailed echocardiographic examination. Studies combining echocardiography along with haemodynamic monitoring and protocol driven donor management would be required to evaluate the potential role of echocardiography in donor assessment.

OBJECTIVES

The objectives of this study were following

1. To evaluate the role of early donor management on donor heart and lung function and their retrieval rate for transplantation
2. To evaluate the role of early hormone replacement therapy with T3 and methylprednisolone in a prospective randomised double blind placebo controlled trial
3. To study the effect of hormone replacement therapy on donor heart and lung function
4. To study the effect of hormone replacement therapy on heart and lung retrieval rate
5. To study the role of serial transthoracic echocardiography to assess donor heart function during protocol guided donor management in the background of hormone replacement therapy
6. To study the prevalence of post-BSD inflammatory environment, their effect on donor heart and lung function and the impact of donor management and hormone replacement therapy
7. To study the prevalence of donor cardiac troponin-I release following BSD and its impact on donor heart function
8. To study the role of extravascular lung water index measurement using single thermodilution technique in evaluating donor lung function
9. To validate the thermodilution lung water index measurement using gravimetry

SECTION 2- METHODS

7. CORE METHODOLOGY

7.1. Introduction

This study was a prospective, randomized, double blind, factorially designed, placebo controlled trial investigating the effect of tri-iodothyronine (T3), methylprednisolone (MP) and combination of both T3 and MP in brain stem dead (BSD) potential heart and lung donors between January 2004 and April 2006. A diagram of study flow is shown in figure 7.1

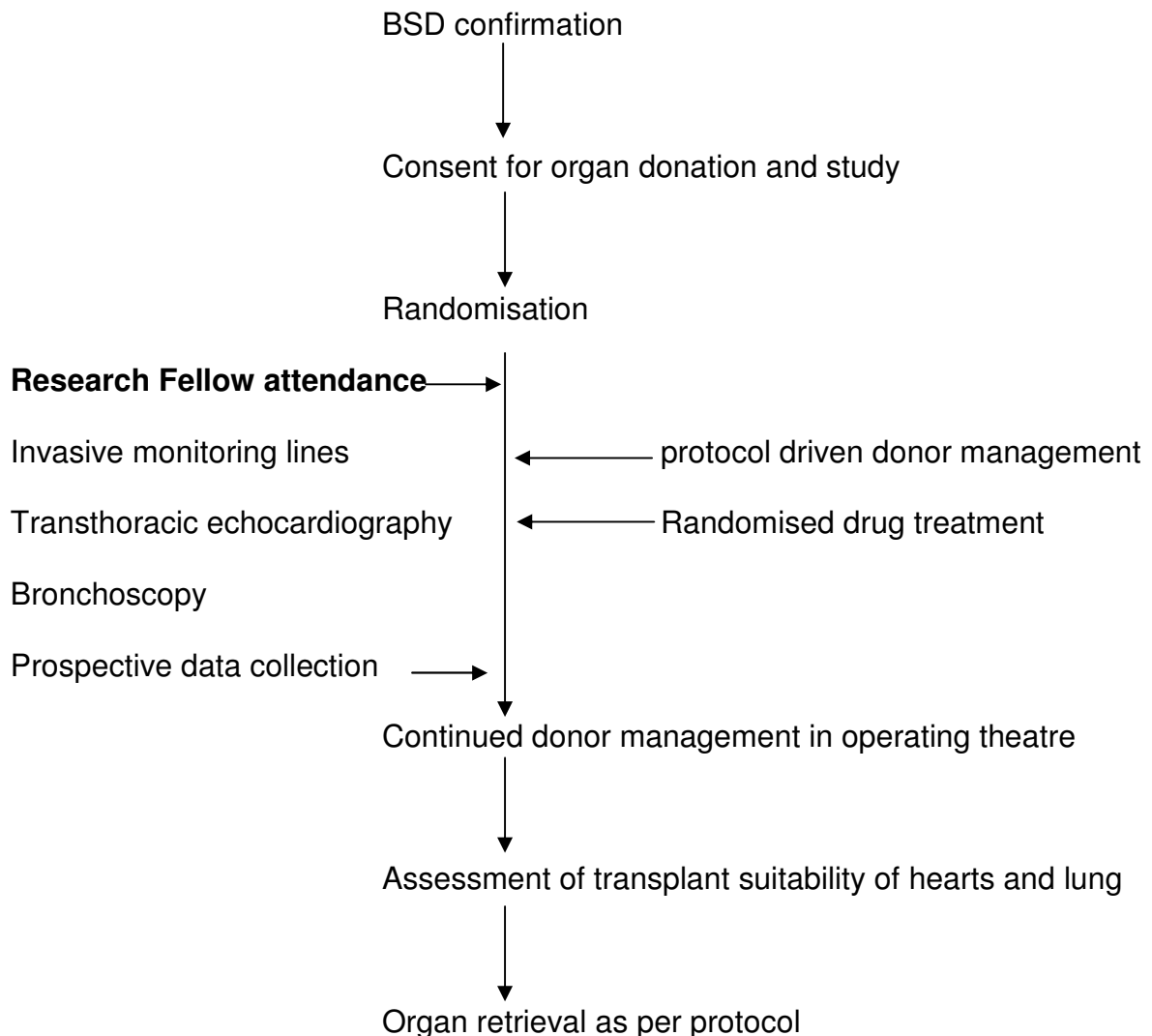


Figure 7-1-Donor pathway through the study

7.2. Recruitment

7.2.1. Patient selection

All brain stem death confirmed potential multi-organ donors were considered recruitment into this study. Following confirmation of brain stem death and consent for organ donation the next-of-kin of all suitable donors were approached for lack of objection for the study. The donor procurement co-ordinators informed the donors' family regarding the study and a patient information sheet was made available to them. Having counselled them and given time to read and discuss about the study, a lack of objection was obtained from the donors' next-of-kin. A separate consent was obtained for myocardial biopsy and retrieval of lungs not used for transplantation for laboratory research. The study inclusion and exclusion criteria are detailed in Table 7.1.

Table 7-1-Study inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age >16, <65 years	<16 and >65 years
Arterial PaO ₂ >230 mm of Hg	Blunt/penetrating chest trauma
No evidence of pneumonia	Radiologically confirmed pneumonia
	History of ischaemic heart disease

7.2.2. Ethical approval

Initially the study was commenced with local research ethics committee approval from Birmingham Black Country ethical committee, however to increase the study recruitment a multicenter research ethics committee (MREC) approval with no local investigator category was obtained. Following this separate approval and permission was obtained from the Research & Development department of each hospital included in the trial. Some of the hospitals required me to obtain an honorary contract to implement the donor management in their intensive care units. We recruited 33 intensive care units within 100 mile radius of Birmingham heart and lung transplant unit within the retrieval zone.

We also obtained permission from the zonal liver, renal transplant teams and the study was supported by the intensive care units within the zone.

7.3. Randomisation

The donors were randomised according to a computer generated sequence allocating to one of 4 treatment groups, T3, placebo, MP and T3 & MP combination. Randomisation took place in blocks of 4 (1:1:1:1) without any stratification. All staffs involved in organ retrieval and implantation were blinded to the treatment allocation by a central randomisation process in which a pre-coded opaque sealed envelope containing the notes indicating assignment of a donor to a particular treatment group was placed in the theatre office. The donor procurement co-ordinator contacted me once a donor was identified and consent for organ donation and study was available. The donor's body weight was informed to the on call operating department practitioner (ODP) who was not involved in the trial, was able to pick up the coded

envelope from the office. The trial solutions were then prepared as per the nomogram by the sister in-charge of the critical care unit and the treatment allocation document was signed and counter signed and was placed back in the envelope which was returned back to the office.

7.4. Treatment groups

7.4.1. Group-1 T3 therapy

Group-1 received 5% dextrose 20mls as an intravenous bolus (syringe labelled as trial drug-B) and T3 (Goldshield pharmaceuticals, UK) $0.8\mu\text{g.kg}^{-1}$ IV bolus (made up to a volume of 20mls with 5% dextrose, syringe labelled as trial drug-A bolus) followed by $0.113\mu\text{g.kg}^{-1}.\text{hr}^{-1}$ IV infusion (made up to a volume of 60mls using 5% dextrose, syringe labelled as trial drug-A infusion, run at 10mls.hr^{-1}).

7.4.2. Group-2 Placebo therapy

Group-2 received intravenous bolus of 20mls of 5% dextrose (syringe labelled as trial drug-B) and further bolus of 20mls of 5% dextrose (syringe labelled as trial drug-A bolus) followed by an intravenous infusion of 5% dextrose at 10mls.hr^{-1} (syringe labelled as trial drug-A infusion).

7.4.3. Group-3 MP therapy

Group-3 received 1000mg of methylprednisolone made up to a volume of 20mls using 5% dextrose as an intravenous bolus (syringe labelled as trial drug-B) and further bolus of 20mls of 5% dextrose (syringe labelled as trial drug-A bolus) followed by an intravenous infusion of 5% dextrose at 10mls.hr^{-1} (syringe labelled as trial drug-A infusion).

7.4.4. Group-4 T3 and MP therapy

Group-4 received 1000mg of methylprednisolone made up to a volume of 20mls using 5% dextrose as an intravenous bolus (syringe labelled as trial drug-B) and T3 (Goldshield pharmaceuticals, UK) $0.8\mu\text{g.kg}^{-1}$ IV bolus (made up to a volume of 20mls with 5% dextrose, syringe labelled as trial drug-A bolus) followed by $0.113\mu\text{g.kg}^{-1}.\text{hr}^{-1}$ IV infusion (made up to a volume of 60mls using 5% dextrose, syringe labelled as trial drug-A infusion, run at 10mls.hr^{-1}).

7.4.5. Blinding of trial solutions

To ensure blinding of all personnel involved in organ retrieval the syringes containing trial drugs were labelled as 'Trial Drug-A bolus' (for T3 iv bolus), 'Trial drug-A infusion' (for T3 iv infusion) and 'Trial drug-B' (for MP iv bolus). The drugs were prepared by the sister in-charge of cardiothoracic critical care unit as per the weight based nomogram and labelled accordingly.

7.5. Preparation to leave to the donor intensive care units

The donor co-ordinators contacted me regarding the availability of the donor once the consent for organ donation and research study was available. I then prepared a sterile central line insertion kit along with the pulmonary artery flotation catheter (PAFC) sheath and a PAFC - VoLEF catheter (Pulsion Medical UK Ltd) and femoral arterial thermodilution catheter (PiCCO, Pulsion Medical UK Ltd). The invasive monitoring insertion kit along with the monitors and cables were packed. A transthoracic echocardiography (Acuson Cypress, Siemens, USA) portable machine and a sterile fiberoptic bronchoscope with light source were also packed. An ice box

with all the trial drugs and empty vials to collect the serum sample was prepared and finally a cryoflask with liquid nitrogen to snap freeze the left ventricular tru-cut biopsy was also packed. All the equipments were carried by me and I drove to the hospital to attend the donor as soon as possible following the consent for the study.

7.6. Donor Management

7.6.1. Insertion of invasive monitoring lines

Upon arrival to the intensive care unit the donor co-ordinator introduced me to the nursing staff looking after the donor. I checked the donor's case notes for relevant history and confirmation of BSD testing and checked the identity of the donor. After checking the consent for organ donation and for study the invasive lines were inserted. Under aseptic precautions PiCCO thermodilution catheter was inserted in the femoral artery. Right femoral artery was preferred if it was unavailable then left femoral artery was used. Following that, a PAFC sheath was inserted through the right internal jugular vein. If the donor did not have a jugular central line, a quadruple lumen central line was also inserted at the same time. A VoLEF pulmonary artery catheter was then floated. The monitor was set up and demographic parameters were entered. Then baseline haemodynamic parameters were obtained (central venous (CVP), mean arterial pressure(MAP), pulmonary capillary wedge pressures (PCWP), cardiac index (CI), right and left ventricular stroke work index (RVSWI, LVSWI), right ventricular ejection fraction (RVEF), cardiac power output, systemic vascular resistance(SVR), extravascular lung water index (EVLWI) (normal 3-8ml.kg⁻¹), pulmonary vascular permeability index (PVPI) and pulmonary vascular resistance(PVR)).

7.6.2. Bronchoscopic examination of airways.

Bronchoscopy was performed to assess anatomy and endotracheal tube placement, to aspirate secretions, to detect evidence of active bronchitis or aspiration and to obtain a broncho-alveolar lavage (BAL) specimen for culture. The donor FiO_2 was increased to 100% oxygen with positive end expiratory pressure (PEEP) of 5 cm of H_2O . After examination of airways and clearing all secretions the bronchoscope was wedged into middle lobe bronchus and 20mls of normal saline was used to obtain a BAL specimen. The same procedure was repeated on the left side after end management prior to retrieval to obtain further BAL specimen. After completion of bronchoscopic examination the FiO_2 was reduced to maintain arterial oxygen concentration of 80mm of Hg and CO_2 concentration of 35-55 mm of Hg.

7.6.3. Transthoracic echocardiography

After bronchoscopic examination using Acuson Cypress (Siemens, USA) portable machine with second harmonic imaging (3.6 MHz probe) a transthoracic echocardiography was performed. The aim was to obtain adequate echo windows to interpret left ventricular function (LV). Using M-Mode measurement LV wall thickness was calculated. Using standard transthoracic views the LV ejection fraction was calculated using fractional shortening formula and LV biplane Simpson's formula. Using 2D-colour doppler any significant valve pathology was excluded. All parameters were measured in triplicate and averaged. The left and right myocardial performance indices were calculated using standard formulae as described by Tei et al. The index was calculated by measuring the mitral closure-to-opening interval at

the mitral inflow (a) and ejection time at the LV outflow (b). The sum of isovolumetric contraction time (IVCT) and isovolumetric relaxation time (IVRT) was calculated by subtracting 'b' from 'a'. The Tei index is calculated by using the formula $IVCT+IVRT/ejection\ time\ (a-b/b)$. The echocardiographic findings were unavailable to recipient centres. Echocardiography was performed by me and I was trained to obtain the images only. The accrued images were stored on optical disc and analyzed off-line, after removal of patient identifiers and random coding of examination timing, by two independent observers blinded to group allocation and time order using Cypress viewer software.

7.6.4. Administration of randomised drugs

After obtaining baseline haemodynamic parameters, bronchoscopy and echocardiography the trial drugs were administered. The trial drug-A & B bolus was given followed by the infusion of Trial drug-A intravenously at 10mls.hr^{-1} . Following which the donors were managed according to a strict protocol.

7.6.5. Haemodynamic management

Haemodynamic management directed by the PAFC finding was initiated after administration of trial drugs. The main aim is to achieve where possible a $CI > 2.5\text{L.min}^{-1}\text{m}^{-2}$ with CVP and PCWP $\leq 12\text{mmHg}$, MAP 70mmHg and SVR $800\text{-}1200\text{ dynes.cm.sec}^{-5}$. In donors with low pre-load volume replacement was commenced using preferably colloids solutions provided the serum sodium level was < 160

mmol.L⁻¹. Crystalloids were not used for volume replacement unless in donors with high serum sodium levels. Vasopressin was commenced in all donors (pitressin 20 units in 40mls of 5% dextrose) with 1 unit bolus and was started on infusion of 2 units per hour. This allowed active weaning of noradrenaline (NA) in all donors (Fig-7.2).

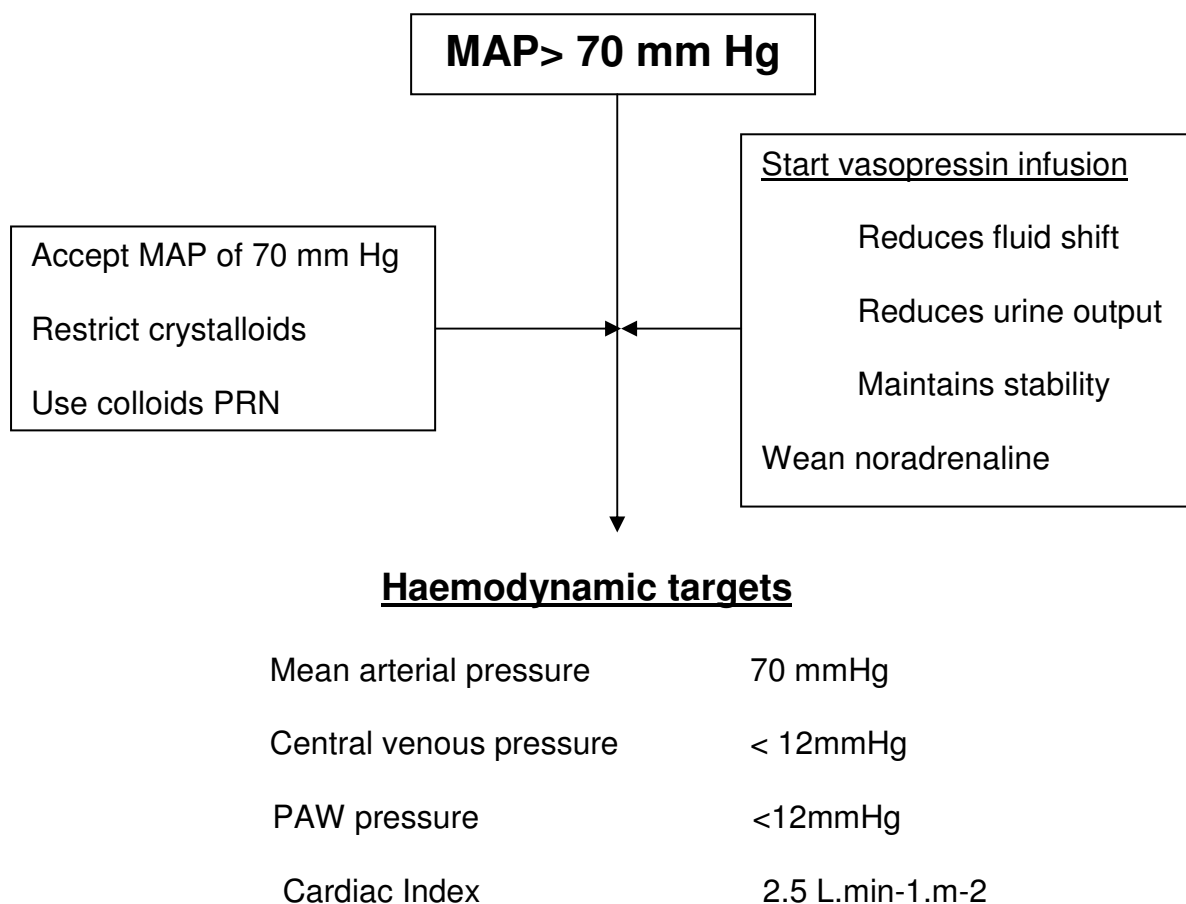


Figure 7-2-Managing an organ donor with MAP > 70 mm Hg

In donors with high pre-load venesection was commenced using the side arm port of PAFC sheath. The blood was collected in a citrated blood bag and was used to make up the lung preservative solution (Papworth solution). Donors with adequate CVP with low blood pressure due to low cardiac index were treated with inotropes. Dopamine was the first line of choice started at $5 \mu\text{g}.\text{kg}^{-1}.\text{min}^{-2}$ (Fig-7.3).

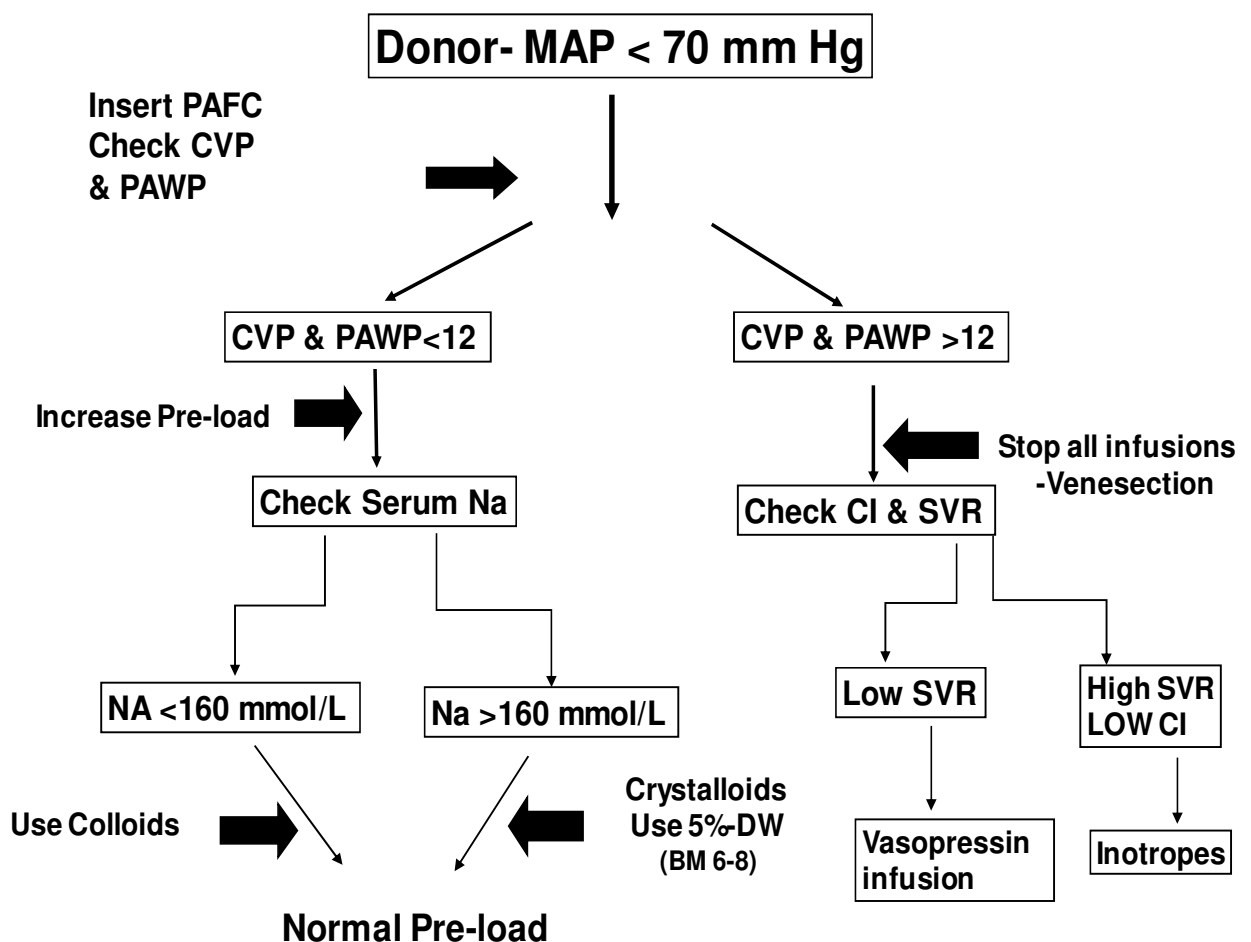


Figure 7-3- Flow diagram demonstrating management of a hypotensive donor

7.6.6. Lung management

At initial assessment all donors underwent arterial blood gas ABG (mmHg) measurement (Fractional inspired oxygen (FiO_2) 1.0), positive end-expiratory pressure (PEEP) 5cmH₂O). Following baseline ABG and bronchoscopy the FiO_2 was reduced to maintain PaO_2 of >80 mm of Hg. The donor was turned in the bed every 2 hours and active oral and endotracheal tube suctioning was performed every 2 hours. Hourly ABG was performed.

7.6.7. Bio-chemical management

During donor management the homeostasis was maintained by keeping normal body temperature with the use of warming blankets. Serum potassium (4.5-5.5 mmol.L⁻¹) and blood glucose (4-6 mmol.L⁻¹) levels were maintained within normal limits. Donors with high blood glucose were started on actrapid insulin on a sliding scale to normalise the levels. Haemoglobin was maintained at 10 g.dl⁻¹.

7.7. Intra-Operative assessment of heart and lung function

Donor management was continued in the operating theatre as per the above protocol. Following dissection of abdominal organs a median sternotomy was performed using handheld saw. After achieving haemostasis the pericardium was opened and stay sutures were placed on the pericardial edges. Both pleurae were opened widely to assess the lungs for any gross abnormalities. Any collapsed lung segments were actively recruited by bagging with 100%oxygen. The heart was

assessed for contractility, RV function, hypo/ akinetic segments and any palpable coronary artery disease. The donor was put on 100% FiO₂ with PEEP of 5 cm of H₂O. Arterial blood gas and selective pulmonary venous blood gas sampling were performed after 15 minutes of recruitment of collapsed segments. The operative findings and blood gas results were conveyed to the recipient centres if the organs were accepted for transplantation. If the heart and lungs were suitable for transplantation they were retrieved as per the standard procedure.

7.8. Myocardial biopsy technique and retrieval of lungs for gravimetry

Myocardial biopsy was performed immediately prior to the application of cross clamp only in donors where the consent was available. Biopsies were full thickness and taken from the left ventricular (LV) free wall. LV biopsies were obtained using a Trucut biopsy needle (Allegiance Healthcare, McGaw Park, IL). Biopsies were immediately frozen in liquid nitrogen and stored at -80°C. The recipient centers were informed about the biopsy and the site was used for de-airing the heart after implantation.

Donor lungs accepted for transplantation were flush perfused with single dose of antergrade pneumoplegia (Papworth solution- ringer lactate 700mls, human albumin (20%) 200mls, 20% mannitol 100mls and heparin 10,000 units along with donor blood (300ml), citrate/phosphate/dextrose chelating agent (56mL)) and a single dose of epoprostenol (prostacycline) infusion (0.5 mg in 20mls (Flolan, (GlaxoSmithKline, UK Ltd))). Donor lungs were retrieved for gravimetric lung water assessment without pneumoplegia or prostacycline infusion. Donor lungs were retrieved as a whole block

after explanting the donor heart. The trachea was stapled after expansion of both lungs. The retrieved lung blocks were transported in cold ringer lactate solution in 3 plastic bags in 4°C. Donor lungs accepted for transplantation were used in a number of UK centres.

7.9. Statistical analysis

7.9.1. Study design

This is a randomised double blind factorially designed controlled study of the effects of T3 and methylprednisolone (alone or in combination) and an observational study to assess the impact of early donor management.

7.9.2. Hypothesis

- a. The use of T3 in potential heart donors will increase the cardiac index by $0.7 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$.
- b. Early donor management will increase the retrieval rate of both hearts and lungs for transplantation.

7.9.3. Power calculations

The hypothesis was derived from the previously conducted trial in our institute using T3 and glucose insulin potassium solution in coronary artery bypass surgery patients. The power calculation was also estimated after analysing data from a pilot study on 10 donors. The data was analysed without breaking the randomisation code. We noted an increase in cardiac index in all donors from baseline to retrieval. Therefore we hypothesised that T3 will increase in cardiac index by $0.7 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$.

To detect an increase in cardiac index of $0.7 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$ between groups at end-assessment, using a 2×2 factorial design, would require 19 donors in each cell with an α of 0.05 and $1-\beta$ 0.85. This would lead to 38 donors receiving early T3 and we assumed no interaction between T3 and MP. The study was also powered to detect an increase in retrieval rate of both hearts and lungs for transplantation by 20% from the non-research contemporary cohort of donors.

7.9.4. Primary outcome measures

The primary endpoint of the study was the difference in CI between groups at end-assessment. For a standardized difference of 0.7, using a 2×2 factorial design, 19 donors in each cell were required (α 5%; $1-\beta$ 0.85) (i.e. 38 would receive early T3) and assumed no interaction between treatments.

7.9.5. Secondary outcome measures

Planned statistical analyses were performed for the following secondary outcomes:
Heart donors: Right and left ventricular stroke work index, cardiac power output, systemic vascular resistance, the effect of donor thyroid status on donor heart function, impact of donor management on the heart retrieval rate, the effect of donor management and noradrenaline (NA) withdrawal on marginal hearts, bio-chemical markers in evaluating suitable donor hearts for transplantation and the role of cytokines in evaluating donor heart function.

On lung donors: The effect of T3 and MP administration on lung donors, the impact of NA administration on donor lung function, impact of donor management on lung retrieval rate, the role of extra-vascular lung water index (EVLWI) measurement in potential lung donors, the validation of EVLWI measured by thermodilution with

gravimetric lung water measurement and the role of cytokine measurement in evaluating donor lung function.

7.9.6. Data collection and storage

All data were prospectively collected and stored on a pre designed data sheet. Once data acquisition was complete for a patient the data were entered onto a Microsoft Access database. Following interrogation of the database, data for analysis were electronically transferred to a Microsoft Excel spreadsheet and automatically transferred into a statistical package, SPSS version 15.0 software (Chicago, IL) prior to performing statistical analysis.

7.9.7. Data analysis

Continuous data were assessed for normality and are presented as mean \pm SD or median [25, 75 centiles]. Normally distributed variables were tested using two-way ANOVA that included factors for the two treatments given and also their interaction. The post-treatment variables were tested using ANCOVA using the pre-treatment value as a covariate along with the two treatments given and their interaction. The impact of donor management was analysed on the entire cohort of patients using the paired sample t-test. Skewed data were tested using non-parametric tests (Mann–Whitney and Kruskal–Wallis test). Categorical data were analysed using χ^2 and Fisher's exact tests. Serial measurements were compared with repeated measures ANOVA. Univariate and multivariate analysis (stepwise logistic regression) was used to identify factors that predicted suitability for transplantation at end-assessment. Receiver operating curves (ROC), sensitivity, specificity and predictive values of parameters for heart usability were generated using standard formulae. Kaplan-Meier

survival analysis was performed to calculate the recipient outcome and log rank test was used to compare the outcome between groups. Statistical significance was assigned when $P < 0.05$ and all tests were two-sided. Further grouping and statistical presentation has been described in individual chapters.

SECTION 3- RESULTS

8. A STUDY OF THE HAEMODYNAMIC EFFECTS OF ADJUNCTIVE HORMONE THERAPY IN POTENTIAL HEART DONORS

8.1- Introduction

Following brain stem death (BSD), there is commonly a decline in circulating cortisol, insulin and thyroxine (T4) (74). Tri-iodothyronine (T3) may also be reduced, sometimes with normal T4, low thyroid stimulating hormone (TSH) and elevated reverse T3 constituting a “sick euthyroid” syndrome (32;33;35;75). These phenomena led to the use of hormonal replacement therapy (HRT) in potential heart donors. Retrospective studies suggest that HRT comprising steroid, thyroid hormone and vasopressin, enhances retrieval rate with improved post-transplant function (80;81). However, while the control of post-BSD vasoparesis using vasopressin (VP) is well established (137), the role of T3 and steroids is less clear. Some studies report a hemodynamic improvement following T3 administration, while others, suggest T3 to be either of no benefit or detrimental (83;85-87). Steroids which have a membrane stabilising effect and inhibit the elaboration of cytokines (89) may be beneficial in the pro-inflammatory post-BSD environment, particularly in lung transplantation (96) but an effect on haemodynamic function has not been examined. I therefore investigated the individual or combined effects of MP and T3 on donor heart function in potential donors in a randomised double blind factorially designed trial.

8.2-Methods

8.2.1-Study design

In the UK, transplant centres are responsible for heart retrieval within a specified zone. Between, January-2004 and April-2006, the next-of-kin of eligible potential heart donors in an intensive care unit (ICU) within 2 hours road distance of our recipient centre were approached for consent to enter the donor into a prospective, double-blind, placebo-controlled, factorially designed randomised trial. The research was supported by 33 accessible ICUs and approved by a multi-centre research ethics committee, all UK cardiothoracic transplant centres and all zonal liver and kidney retrieval teams. The trial was conducted independently of whether donor hearts were provisionally accepted for transplantation by any centre. Hearts that were not provisionally accepted were not re-offered following management. Consent was obtained from the next-of-kin for all participating donors according to the ethical approval.

8.2.2-Initial assessment and management

As soon as possible following consent to both donation and the study, I attended donors, drew blood for thyroid function assessment and inserted a pulmonary artery flotation catheter (PAFC - VoLEF catheter-Pulsion Medical UK Ltd) and femoral arterial thermodilution catheter (PiCCO, Pulsion Medical UK Ltd). Following measurement of central venous (CVP), mean arterial pressure(MAP), pulmonary capillary wedge pressures (PCWP), cardiac index (CI), right and left ventricular stroke work index (RVSWI, LVSWI), right ventricular ejection fraction (RVEF), cardiac power output, systemic vascular resistance(SVR), the study medication was

administered. Donors were randomly assigned to receive T3 ($0.8\mu\text{g.kg}^{-1}$ IV bolus followed by $0.113\mu\text{g.kg}^{-1} \cdot \text{hr}^{-1}$ IV infusion); MP (1000mg as a single IV bolus), both drugs or placebo (dextrose 5%) on 1:1:1:1 basis using a computerised model with permuted blocks and a sealed envelope system. The T3 dose was based on reports of positive hemodynamic effect following cardiac surgery (138;139). The T3 or placebo infusion continued until retrieval. The MP dose was an empirical immunosuppressive dose, approximating 15mg.kg^{-1} for a 70kg donor. The drugs were pre-prepared in identical volumes in code-labelled syringes by uninvolved recipient centre staff according to a weight-based nomogram. All medical, nursing and technical staff involved in the research or donor care remained blinded to treatment allocation until analysis. PAFC-directed haemodynamic management then commenced according to specific algorithms to achieve where possible a $\text{CI} > 2.5\text{L.min}^{-1}\text{m}^{-2}$ with CVP and $\text{PCWP} \leq 12\text{mmHg}$, MAP 65-85mmHg and SVR 800-1200 dynes.cm.sec^{-5} (64). Volume adjustment was restricted to limited blood or colloid transfusion to achieve a haemoglobin $\geq 10\text{g.dL}^{-1}$ and to maintain filling pressures. Any pre-study inotrope and noradrenaline (NA) were weaned and VP administered to modulate SVR. Blood sugars were maintained between 4.4-6.1 $\mu\text{mol.L}^{-1}$ using IV insulin and hypothermia prevented by standard measures. Management, continued in the ICU and operating room until retrieval or end-assessment. The duration of treatment and time of coning (detection of fixed dilated pupils and blood pressure surge) were noted. Haemodynamic studies were repeated hourly for 3 hours and immediately pre-retrieval. Prior to retrieval repeat blood samples were drawn for thyroid function. No donors underwent angiography. Trans-thoracic echocardiograms were performed but were analysed off-line and were not

available at initial assessment. Thus, assessment of left ventricular hypertrophy (LVH) and palpable coronary artery disease (CAD) did not occur until after the final functional measurements. Recipient 30-day and 1 year survival (100% follow-up) were provided by transplant centres.

8.2.3-Endpoints and statistics

The primary endpoint of the study was the difference in cardiac index between groups at end-assessment. For a standardised difference of 0.7, using a 2x2 factorial design, 19 donors in each cell were required (α 5%; $1-\beta$ 0.85) (i.e. 38 would receive early T3) and assumed no interaction between treatments. Secondary endpoints included comparisons of RVSWI and LVSWI and CPO. Post-hoc analyses allowed assessment of the response of marginal donor hearts to management, the effects of NE withdrawal on donor circulatory function and the impact of initial thyroid functional status. Hearts were classified as marginal if the MAP was <70 mm Hg with CVP or PCWP >14 mm Hg and CI < 2.4 L.min⁻¹.m⁻² at initial assessment or on inotropes (dopamine > 5 µg.kg⁻¹.min⁻² or adrenaline >0.03 µg.kg⁻¹.min⁻²) or NA >0.06 µg.Kg⁻¹.min⁻²) (63). When establishing the number of hearts functionally suitable for transplantation we set the following criteria; CVP ≤ 12 mm Hg, PCWP ≤ 14 mm Hg, CI ≥ 2.5 l.min⁻¹.m⁻² on minimal support without gross LVH or palpable CAD on direct inspection.

Data were analyzed using SPSS v15.0 (Chicago, IL). Continuous data were assessed for normality and are presented as mean ± SD or median (inter-quartile range (IQR)). Normally distributed variables were tested using two-way ANOVA that included factors for the two treatments given and also their interaction. The post-

treatment variables were tested using ANCOVA using the pre-treatment value as a covariate along with the two treatments given and their interaction. The interaction between treatments was not significant and so the p values for the main effects are reported for the model without interaction. The impact of donor management was analysed on the entire cohort of patients using the paired sample t test. Skewed data were tested using non-parametric tests (Mann-Whitney and Kruskal-Wallis test). Categorical data were analyzed using χ^2 and Fisher's Exact tests. Serial measurements of cardiac indices were compared with repeated measures ANOVA.

On the basis of statistical advice received, I included a within subjects factor with 5 levels for the five repeated measurements of cardiac index (primary outcome measure of the study) and the two treatments were entered as between subjects factors. I used a full factorial model with type III partitioning of the sums of squares and the p values quoted are those obtained after applying the Huynh-Feldt correction. The interaction of the within subjects factor and each treatment was used to assess whether the treatment had any effect on change in cardiac index. No interaction between treatments was identified and the p values for the main effects are reported for the model without interaction. Statistical significance was assigned when $p \leq 0.05$ and all tests were two sided.

8.3-Results

During the period of recruitment, of 250 zonal donors, 116 were eligible for study. Consent was withheld in 36 giving a sample size of 80 (Figure-8.1).

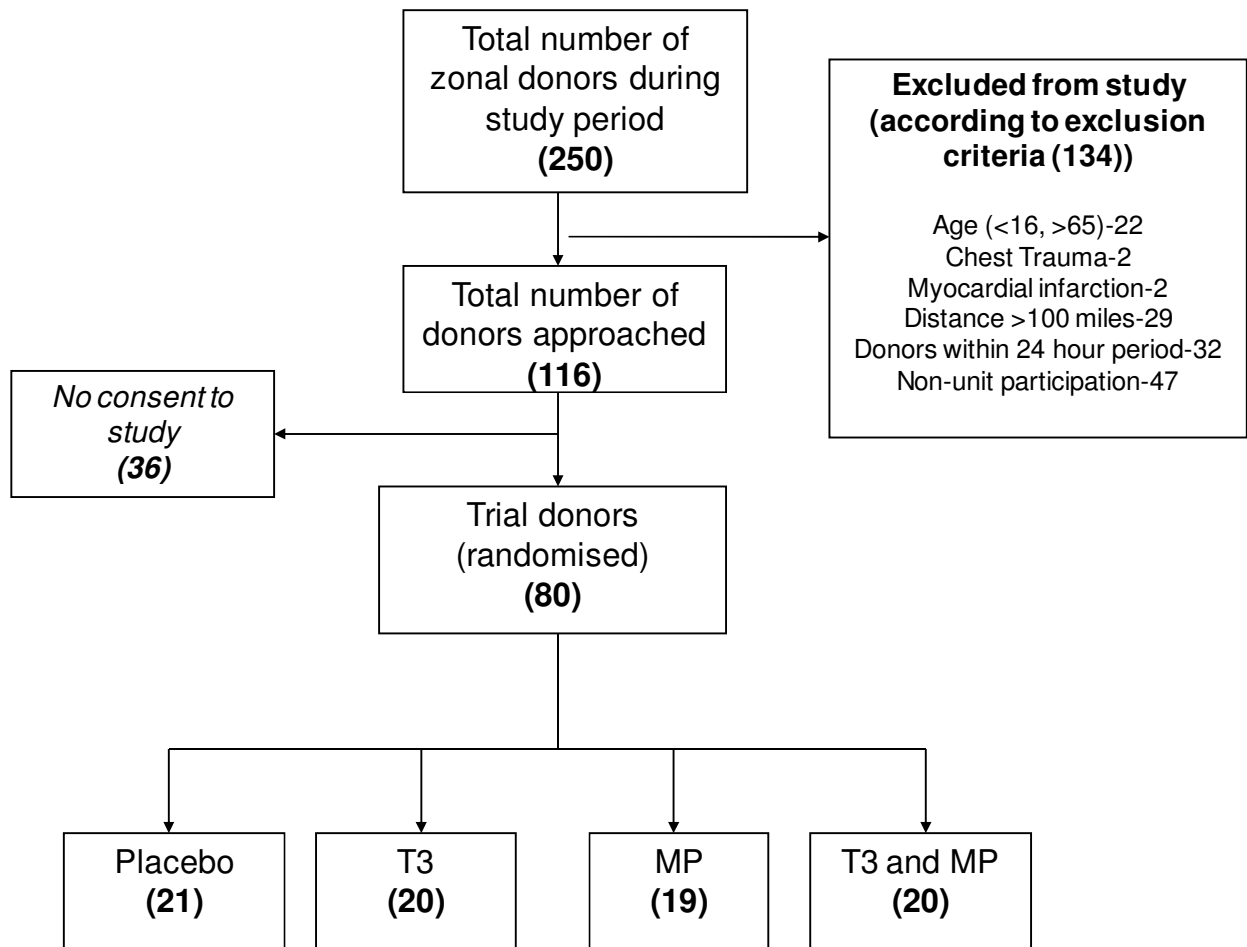


Figure 8-1-CONSORT diagram demonstrating enrolment, exclusion, randomisation and number of hearts transplanted

The donor characteristics of both trial (80) and the non-trial donors (134) are presented in Table-8.1.

Table 8-1- Characteristics of Trial and non-Trial donors

Parameter	Trial donor (n=80)	Non-Trial donor (n=134)	p-value
Age	43(36, 55)	44(37, 54)	=0.7
Donor Cause of death			
Vascular & Tumour	51/80 (64%)	86/134 (64%)	=1
Traumatic	19/80 (24%)	31/134 (23%)	=1
Hypoxic	5/80 (6%)	12/134 (9%)	=0.61
CNS infection	5/80 (6%)	5/134 (4%)	=0.51
Provisional acceptance	35/80 (44%)	73/134 (54%)	=0.16
Heart-yield	25/80 (31%)	41/134(31%)	=1

Age is presented as median (25th, 75th centiles)

CNS- central nervous system

Trial donor demographics (n=80)

Gender	38 female (48%)
BMI	25.55±4.67
Ethnicity	74-Caucasian, 4-Asian and 2-Black-British
Hypertension	11 donors (14%)
Diabetes	1 (1.25%)
Smoking (current)	32 donors (40%)

The median age was 43 (IQR 36, 55), 52% were male and 11(14%), 1(1.25%) and 32(40%) respectively had a history of hypertension, diabetes or smoking. BSD was caused by trauma in 19/80(24%), vascular event or tumour in 51/80(64%) and hypoxia or infection in 10/80(13%).

Assessment commenced within a median of 2 (IQR 0.5-3.5) hours of consent, within 12.7 ± 8.3 hours of coning and 6.9 ± 1.3 hours prior to retrieval or end-assessment. Study medication was administered for 5.9 ± 1.3 hours prior to retrieval or end-assessment; the T3 or placebo infusion being continued until OR inspection. Between initial and final measurements, donors received 346 ± 344 ml of colloid raising CVP and PCWP significantly (Table-8.2). In the study population overall, CI, CPO, RVSWI and LVSWI all increased ($p < 0.001$) while SVR fell (Table-8.2).

Table 8-2-Hemodynamic parameters for the entire Trial cohort

Parameters	Baseline value	Pre-retrieval value	<i>p</i>-value
CVP	8.6 ±4 (7.8-9.6)	11±3.7 (10-11.9)	<0.001
PCWP	9.4±4.4 (8.4-10.4)	12.6 ±4.1 (11.7-13.6)	<0.001
CI	3.2±1 (2.9-3.5)	3.9 ±1.2 (3.6-4.1)	<0.001
RVEF	25.6±7.5 (23.8-27.3)	26.5±7.4 (24.8-28.3)	=0.09
LVSWI	40.6±14.5 (37.3-43.9)	46 ±17.8 (41.6-49.7)	=0.007
RVSWI	4.9±3 (4.3-5.6)	7.5 ±4.1 (6.6-8.4)	<0.001
CPO	1.2±0.5 (1.1-1.3)	1.5 ±0.6 (1.4-1.6)	<0.001
SVR	1213±506 (1100-1325)	1082±650 (937-1226)	=0.001

CVP- Central venous pressure, PCWP- Pulmonary capillary wedge pressure, CI-cardiac index, RVEF- Right ventricular ejection fraction, LV/RVSWI- Left and right ventricular stroke work index, CPO- Cardiac power output, SVR Systemic vascular resistance.

Units: CVP and PCWP- mmHg, CI- L.min⁻¹m⁻², RVEF- %, LVSWI and RVSWI- g-m.m⁻².beat⁻¹, CPO- Watts, SVR- dyne.cm.sec⁻⁵.

8.3.1-Donor heart function within the 4 treatment groups of the Trial

Demographic details, donor heart function and suitability for transplantation for the 4 treatment groups are summarized in Table 8.3. The administration of T3 and MP alone or in combination did not affect CI (Figure-8.2) or any other parameter when compared to placebo.

Table 8-3-Donor characteristics in the 4 Trial treatment groups

Parameters	T3	MP	T3+MP	Placebo	p-value		
	(20)	(19)	(20)	(21)	T3	MP	MP+T3
Age	54 (39, 56)	39 (26, 51)	40 (36, 45)	51 (37, 59)			
BSD to treatment	12±6.3	10±6.7	15±9.3	12±10.2			
Donor management	7.5±1.3	6.5±1.2	6.7±1.6	6.7±1.2			
Drug treatment	6.4±1.3	5.6±1.2	5.8±1.6	5.7±1.2	0.14	0.29	0.43
Hearts suitable	8	10	12	10			

Donor heart function (Baseline and post-donor management parameters)

CVP	8.6±4.5	8.7±5	9±3.5	8.2±2.5			
PCWP	9.3±4.8	9.4±3.9	9.9±5.1	9.9±5.4			
CI	3.2±0.9	3±1.1	3.5±1.2	3.2±0.9			
RVEF	26.8±6.8	23.8±7	25.7±10.3	25.4±5.7			
LVSWI	39.4±11.3	35.4±15.2	41.1±15.9	43.7±16			
RVSWI	4.8±2.6	4.7±2.5	5.1±2.9	5.6±3.4			
CPO	1.2±0.4	1.1±0.4	1.4±0.6	1.2±0.5			
SVR	1189±529	1225±405	1175±605	1283±478			
P- CVP	12.1±2.2	10.3±5.1	10.4±2.7	10.9±2.8	0.42	0.02	0.93
P- PCWP	12.8±3.2	12.6±5.7	11.4±3.9	13.4±3.8	0.40	0.07	0.79
P- CI	3.7±1.1	3.7±0.9	4.8±1.1	3.8±1	0.25	0.14	0.15
P-RVEF	26.1±7.5	23.4±6.6	29.4±8.6	27.5±5.5	0.96	0.52	0.05
P-LVSWI	40±16.6	47.1±19.2	50±16.6	47.8±18.7	0.34	0.14	0.65
P- RVSWI	6±3.8	7.6±2.8	9.6±3.4	8.6±3.5	0.96	0.09	0.07

P-CPO	1.3±0.47	1.6±0.53	1.8±0.68	1.5±0.6	0.88	0.02	0.41
P-SVR	960±556	1181±423.7	1029±811	1156.6±716	0.38	0.71	0.82

Units as per Table-8.2

Data presented as median (25th, 75th centiles) and mean ± standard deviation.

Post treatment values were presented with a prefix-P. P-values for T3 and MP were generated from ANCOVA analysis for the two main treatment effects after removal of the T3 + MP interaction from the model.

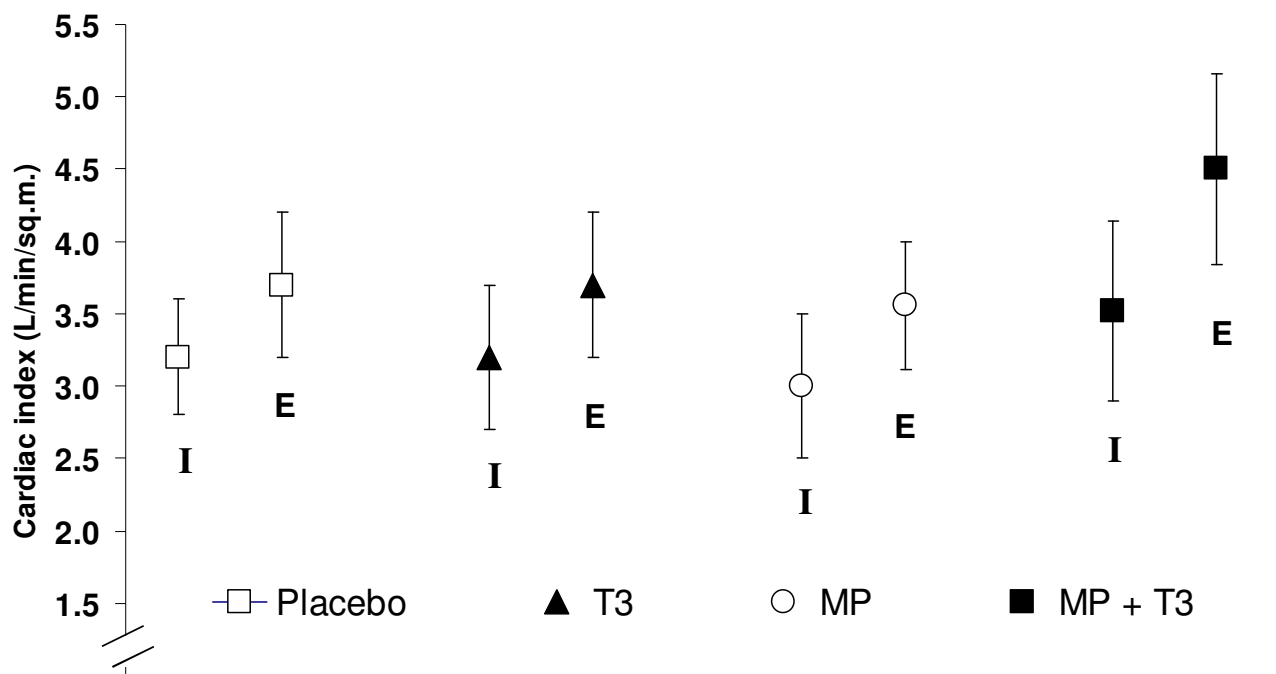


Figure 8-2- Cardiac index (mean \pm 95% confidence interval) between initial (I) assessment and end-assessment (E) in the four treatment groups.

CI improved significantly in the entire cohort ($p < 0.001$) but there was no difference between groups (T3 $p = 0.22$; MP $p = 0.14$; MP+T3 $p = 0.15$).

8.3.2-Donor heart function according to receipt of T3 or MP

In a secondary analysis, outcomes in donors according to receipt of T3 (i.e. T3 group plus T3 + MP group) or MP (MP group plus T3 +MP group) were compared (Tables- 8.4 and 8.5). T3 administration was not associated with any identifiable beneficial haemodynamic effect (Figure-8.3). The receipt of MP did not influence circulatory functional changes or retrieval rate (Figure-8.4).

Table 8-4-Characteristics of Trial donors according to T3 receipt

	Non-T3 (n=40)	T3 donors (n=40)	Mean Treatment difference (95% CI)
Age	44 (37, 54)	45 (30, 56)	
BSD to management (h)	11±8.6	13.5±7.9	
Donor management (h)	6.6±1.2	7.1±1.5	
Randomized treatment (h)	5.6±1.5	6±1.5	
Hearts suitable for transplantation			
	20	20	
Donor heart function (Baseline and post-donor management parameters)			
CVP	8.4±3.8	8.8±3.9	
PCWP	9.9±5.2	9.3±4.3	
CI	3.2±1	3.4±1.1	
RVEF	24±6.4	26±8.6	
LVSWI	40±16	40±14	
RVSWI	5.2±3	5±2.7	
CPO	1.2±0.4	1.3±0.5	
SVR	1254±439	1182±562	
P-CVP	10.6±3.9	11.3±2.6	-0.55 (-1.9, 0.8)
P-PCWP	13.1±4.7	12.1±3.6	0.56 (-0.8, 1.8)
P-CI	3.8±0.9	4.2±1.3	-0.23 (-0.63, 0.16)
P-RVEF	25.7±6.3	27.7±8.1	-0.06 (-2.3, 2.2)
P-LVSWI	47.4±18.7	44.8±17.2	3.3 (-3.7, 10.3)

P-RVSWI	8.2±3.2	7.7±4	-0.04 (-1.7, 1.6)
P-CPO	1.5±0.54	1.6±0.6	-0.02, (-0.2, 0.23)
P-SVR	1168±585	996±691	115 (-143, 372)

Data presented as median (25th, 75th centiles) and mean ± standard deviation (95% confidence limits). Post treatment values were presented with a prefix-P. Units as per Table 8.2

The p values are as for Table-8.3 and were generated from ANCOVA analysis for two main treatment effects after the non-significant T3+MP interaction was removed from the model.

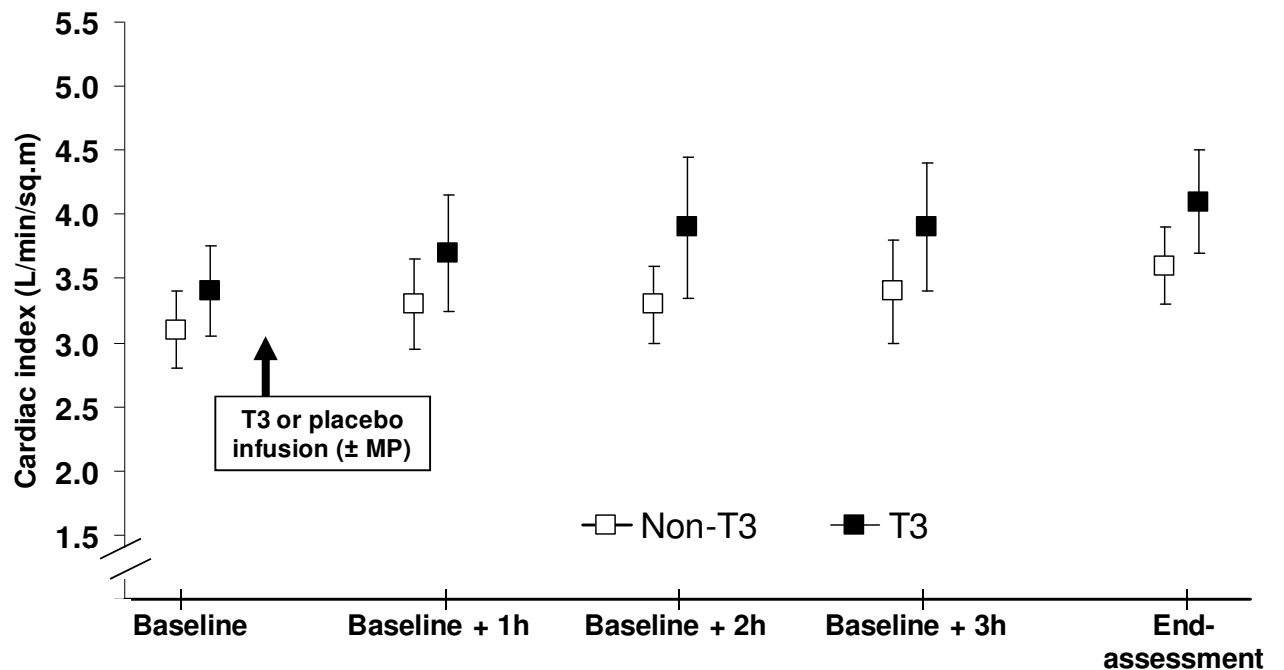


Figure 8-3-Cardiac index (mean \pm 95% confidence interval) in donors receiving or not receiving T3 (n=40 in each group).

The x axis denotes time in hours following initial assessment after which T3 or placebo was administered. Note the interval between the 3rd hour and end-assessment was 1 - 2 hours. Both groups improved cardiac index significantly ($p < 0.001$) between baseline and end-assessment. On repeated measure ANOVA analysis no significant difference was noted between the groups (T3 $p = 0.17$; MP $p = 0.9$; T3+MP $p = 0.252$; T3 and time $p = 0.56$; MP and time $p = 0.47$; T3+MP and time $p = 0.86$)

Table 8-5-Characteristics of Trial donors according to MP receipt

	Non-MP (n=41)	MP donors (n=39)	Mean treatment difference (95% CI)
Age	53 (38, 56)	39 (29, 47)	
BSD to management (h)	12.3±8.5	13.2±8.2	
Donor management (h)	7.1±1.2	6.6±1.4	
Randomized treatment (h)	5.9±1.2	5.7±1.4	
Hearts suitable for transplantation			
	18	22	
Donor heart function (Baseline and post-donor management parameters)			
CVP	8.4±3.6	8.9±4.2	
PCWP	9.6±5.1	9.6±4.4	
CI	3.2±0.9	3.3±1.2	
RVEF	26±6.2	24±8.9	
LVSWI	42±13.8	39±15.6	
RVSWI	5.2±3	5±2.7	
CPO	1.2±0.4	1.2±0.5	
SVR	1237±505	1187±512	
P-CVP	11.5±2.6	10.3±3.9	1.7 (0.30, 3)
P-PCWP	13.1±3.5	12±4.8	1.2 (-0.1, 2.5)
P-CI	3.8±1.1	4.3±1.2	-0.29 (-0.69, 0.1)
P-RVEF	26.8±6.6	26.6±8.2	0.72 (-2.9, 1.5)

P-LVSWI	43.8±17.9	48.6±17.7	-5.2 (-12.2, 1.8)
P-RVSWI	7.3±3.9	8.7±3.2	-1.4 (-3.1, 0.24)
P-CPO	1.4±0.5	1.7±0.6	-0.3 (-0.5, -0.05)
P-SVR	1060±643	1103±648	-49 (-306, 208)

Data presented as median (25th, 75th centiles) and mean ± standard deviation (95% confidence limits). Post treatment values were presented with a prefix-P. Analysis by ANCOVA analysis for the main treatment effect after removal of T3 and MP interaction from the model

Note the ‘p’ values are the same as in Tables 8.3 and 8.4. Units as per Table 8.3

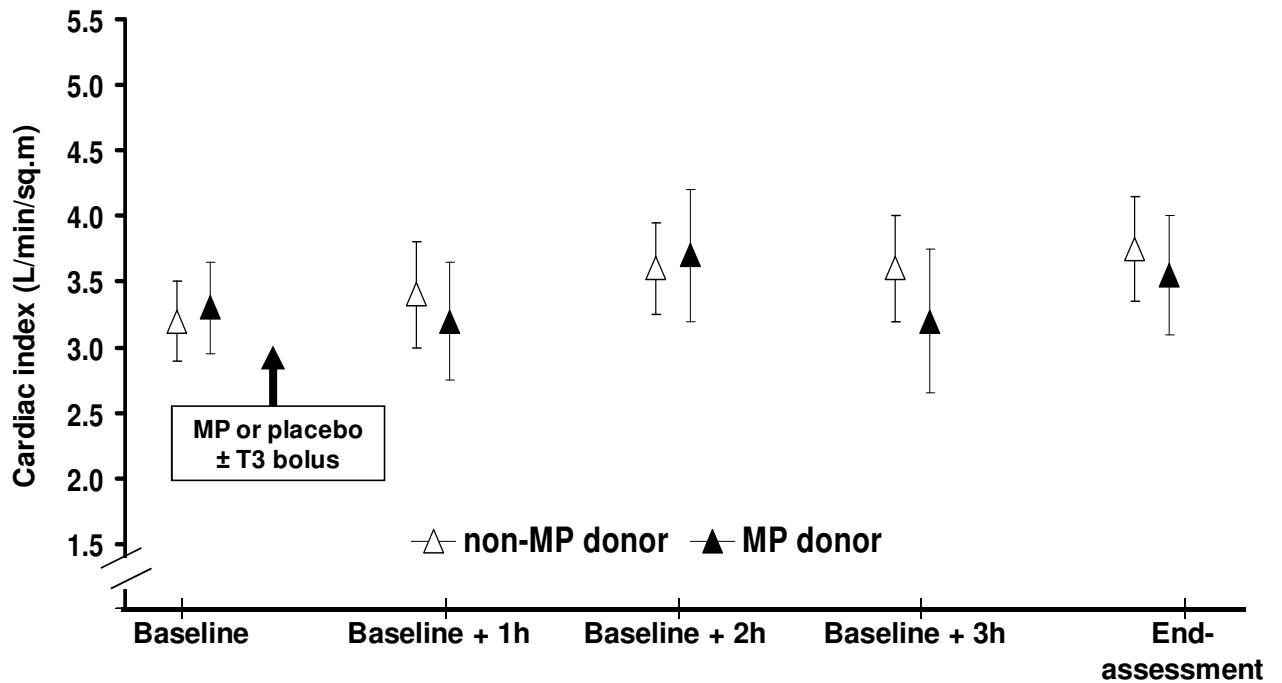


Figure 8-4—Cardiac index (mean \pm 95% confidence interval) in donors receiving (n=39) or not receiving MP (n=41) in each group.

The x axis denotes time in hours following initial assessment after which MP or placebo was administered. Note the interval between the 3rd hour and end-assessment was 1 - 2 hours. Both groups improved cardiac index significantly ($p=0.002$) between baseline and end-assessment and treatment and time interactions were not significant (p values as per figure 8.3).

8.3.3-Donor heart function according to pre-treatment thyroid function

Further examination of the effect of T3 therapy according to the pre-treatment thyroid hormone levels was performed (Table-8.6). Over half the donors (46/80; 58%) had low free T3 or free T4 levels initially while only 18 had coexistent low thyroid stimulating hormone levels. Of the 46 donors with low T3 or T4 levels, 24 received T3, thereby achieving supra-physiological T3 levels. In donors not receiving T3, free T3 levels fell significantly ($p<0.001$). The change in cardiac index according to initial T3/T4 levels and T3 receipt was then compared. Initial cardiac index was not different in donors with low T3 or T4 levels. The management protocol improved CI regardless of initial T3/T4 levels or T3 administration (Figure-8.5).

Table 8-6-Thyroid function changes according to T3 receipt

	Non-T3 Group (n=40)	T3-Group (n=40)	Mean treatment difference (95% CI)	p-value (T3)
Baseline value				
Free T3 (pmol.L ⁻¹)	3.2±1	5.9±6.6		
Free T4 (pmol.L ⁻¹)	11.5±3.7	11.4±4.8		
TSH (mIU.L ⁻¹)	1.1±1.4	1±1.2		
Pre-retrieval value				
Free T3 (pmol.L ⁻¹)	3.1±0.9	15.2±3.9	-11.5 (-13.1, -9.9)	<0.001
Free T4 (pmol.L ⁻¹)	12.4±4.7	12.3±5.2	-0.23 (-1.1, 0.64)	0.59
TSH (mIU.L ⁻¹)	1.4±1.4	1.3±1.5	-0.1 (-0.37, 0.17)	0.45

Data presented as mean ± standard deviation. The reference laboratory ranges are free T₃ (3.3-7.5 pmol.L⁻¹), free T₄(11-26 pmol.L⁻¹) and TSH (0.4-6.3 mIU.L⁻¹).

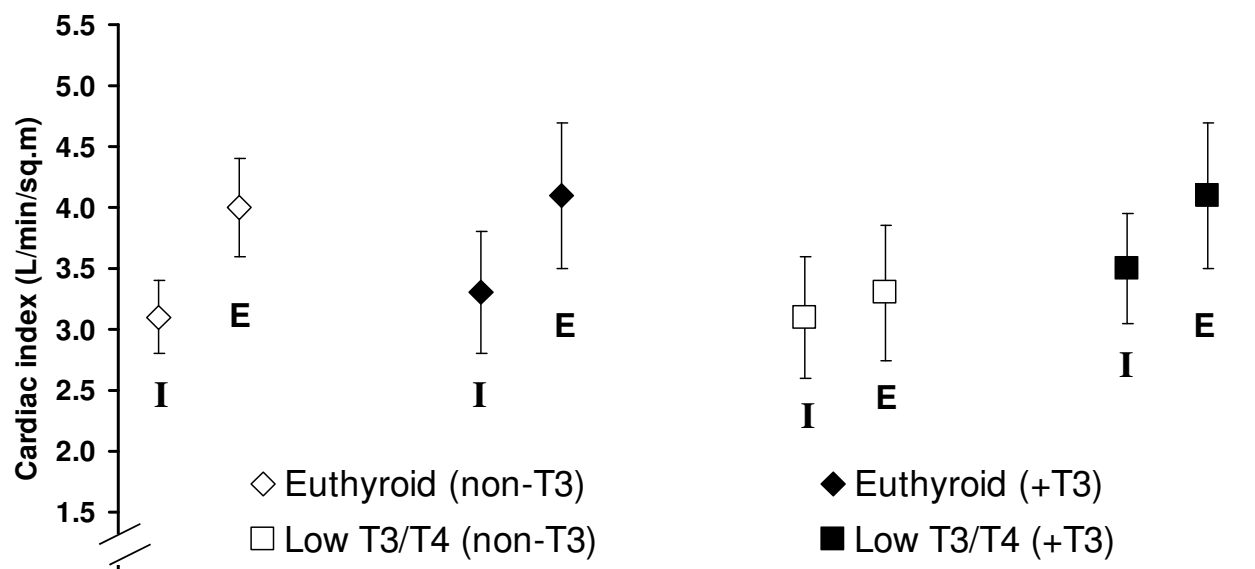


Figure 8-5-Cardiac index (mean \pm 95% confidence interval) between initial (I) assessment and end-assessment (E) in biochemically euthyroid or low T3 or T4 donors receiving or not receiving T3 during donor management.

Cardiac index improved in the entire cohort ($p<0.001$). Administration of T3 ($p=0.13$), the pre-treatment thyroid status ($p=0.27$) or the interaction between the thyroid status and T3 administration ($p=0.158$) did not affect the cardiac index

8.3.4-Donor heart function and noradrenaline administration

Within the data, an apparent association between NA withdrawal and cardiac functional improvement was noted and further post-hoc analysis performed. At assessment, 7 donors were on inotropes and 48/80 (60%) were receiving NA ($0.2 \pm 0.3 \mu\text{g.kg}^{-1}.\text{min}^{-1}$). None were receiving VP. Thirty six out of 48 donors were receiving $>0.06 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ of NA support at the time of baseline measurement of parameters. Their baseline LVSWI (43.5 ± 14.9 vs 36.6 ± 13.5 ($p=0.03$)) and CPO (1.3 ± 0.52 vs 1.0 ± 0.42 ($p=0.03$)) were significantly lower when compared to those not receiving NA support. VP was introduced in 60/80, allowing NA to be withdrawn (26/48) or reduced to $0.06 \pm 0.16 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ ($p<0.001$). NA reduction was accompanied by a rise in CI from 3.18 ± 1.1 to $3.72 \pm 1.5 \text{ L.min}^{-1}\text{m}^{-2}$ ($p<0.001$) and a fall in SVR from 1190 ± 572 to 964 ± 381 ($p=0.001$).

8.3.5-Marginal and non-marginal donor hearts, transplant suitability and outcomes

At initial assessment, half of the donor hearts met the functional criteria of suitability for transplantation. Of these, 26/40 remained suitable at the end-assessment and 15/26 hearts were transplanted. The reasons for rejection or non-use are detailed in Figure 8.6. Of 40/80 of the donor hearts with initial marginal status, 14/40 (35%) attained the functional suitability criteria at end-assessment and 10 (25%) were transplanted with no recipient 30 day mortality. Administration of T3 and MP alone or in combination did not influence the hemodynamic parameters or transplant rate.

Twenty donors (25%; median age 55 (range 48-57)) had palpable coronary artery disease on direct inspection, despite a negative medical history and 7/20 donors with CAD also had LVH. At end-assessment 20 further hearts were deemed unsuitable for transplant on the basis of inadequate function or macroscopic right (n=11) or left (n=9) heart dysfunction. Thus, from 80 potential donors assessed, 40 hearts (50%) were ultimately suitable for transplant and 25 (31%) were actually transplanted. As 15 additional hearts, not provisionally accepted for transplantation, fulfilled the suitability criteria, the actual percentage of potentially usable hearts was 50%. Of these 15, 5 had been rejected for reasons of donor age ≥ 60 (n=3), history of hypotension (n=1) or inotrope use (n=1) and 10 were rejected due to an inability to identify suitable recipients. They were not re-offered at end-management. Following transplantation, 24/25 recipients survived beyond 30-days (96%) and 1-year survival was 88%. The early death was unrelated to graft function.

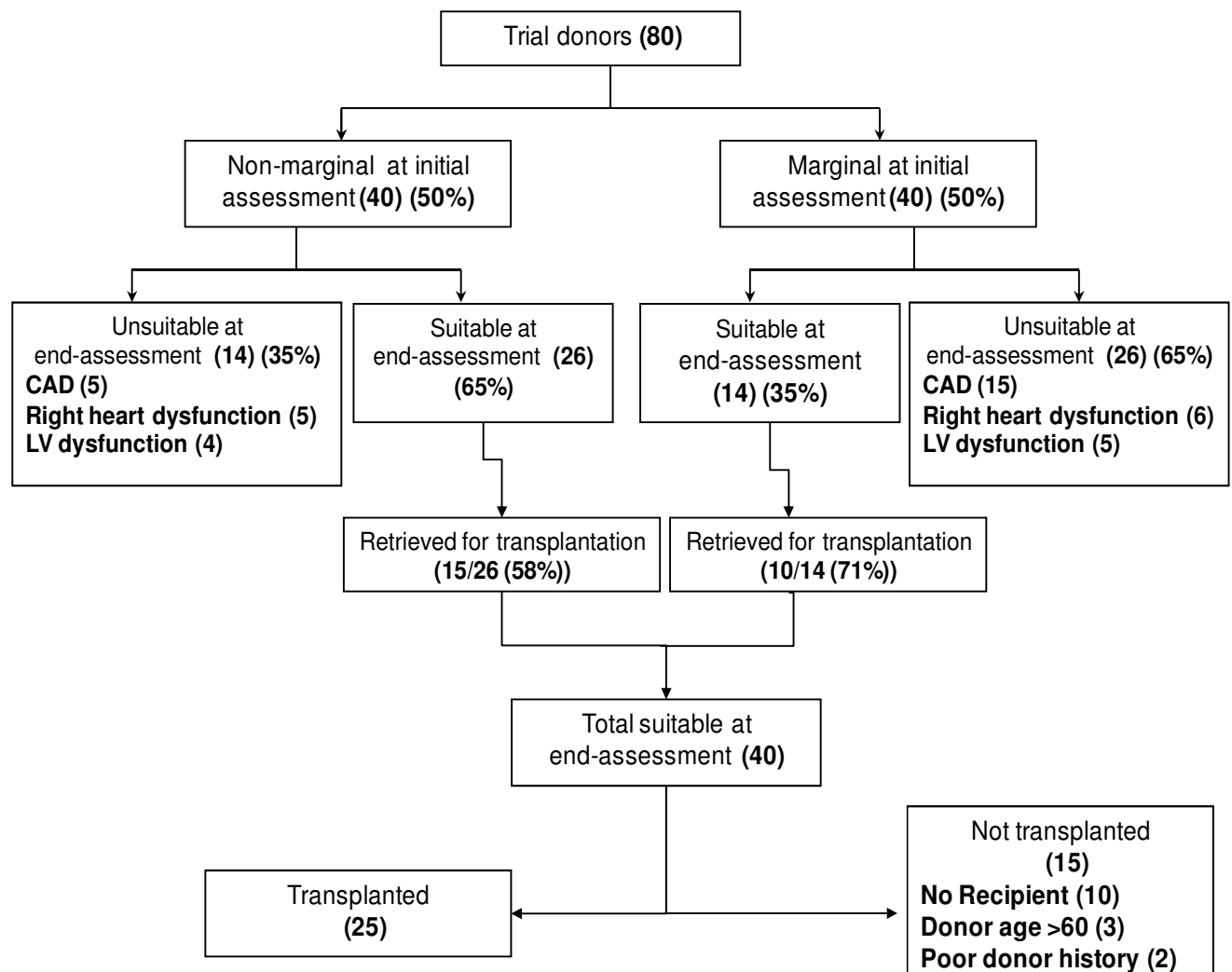


Figure 8-6-Organisation chart of donor heart outcomes within the study

8.4-Discussion

Active donor management improves circulatory function and has the capacity to increase the yield of suitable hearts from the existing pool of potential donors. Neither T3 nor MP, alone or in combination appear fundamental to this improvement.

The clinical relevance and management of post-BSD thyroid dysfunction remains an area of controversy (32;67;76;140-142). In experimental studies of BSD, rapid falls of T3 have been documented. This led to supplementation of T3 in the human donor with salutary results compared to historical controls. Observational studies with limited hemodynamic assessment then showed a general beneficial effect of T3 (83;143). In a report of 6 dysfunctional donor hearts (mean age 16.5 years) with high CVP, LVEF <45%, inotrope requirement and episodes of cardiac arrest, T3 administration was followed by an increase in mean arterial pressure, a fall in filling pressures and successful transplantation (85). However, the donors also received furosemide and dopamine which may have been responsible for the fall in pre-load and increased MAP (84;85). Thereafter T3 was used in management regimes that incorporated PAFC-guided manipulation of pre-load, afterload and inotropy (63). These measures led to improved cardiovascular performance and increased donation rates but as HRT was administered to all, a specific beneficial effect of T3 could not be evaluated (144). High dose T4 therapy has also been reported to reduce catecholamine requirements but does not change cardiac output and actually increases oxygen consumption and base deficit (84). In this study and previous randomised studies, low T3 levels were not associated with worse haemodynamics and T3 administration did not affect retrieval rate or haemodynamics beyond that achieved by the other components of management (73;86;87). This suggests that T3

reduction is not the mechanism of donor circulatory dysfunction. Low T3 levels were found in >50% of donors in the current study and all T3 recipients achieved T3 levels above the upper reference limit. The change in CI was not different regardless of initial T3/T4 levels or T3 administration. Additionally, T3 donors received the drug for a longer period than reported previously and our weight-based nomogram provided slightly higher dosages which have previously been shown to improve post- coronary artery bypass surgery haemodynamics without increasing oxygen consumption (139). The reasons for these discrepant findings are unclear. T3 has been found to ameliorate post-ischaemic cardiac dysfunction (138;139), to improve contractile performance after excessive catecholamine stimulation (145) and to stabilise pressor requirements following brain death (146;147). I suspect that the reasons relate to vascular resistance modulation with vasopressin. Vasopressin levels fall profoundly following BSD (99;146) in parallel with the progressive vasoparesis. Administration of vasopressin, reverses this phenomenon, stabilising blood pressure and reducing catecholamine requirements as in this study (99;148;149). Vasopressin administration, which was not used in the early observational studies that suggested improvement with T3, therefore appears to have a more fundamental role in donor management than T3.

In a large retrospective survey, HRT was associated with an increased retrieval rate of the heart and other organs. However, only 701/10,292 (6.8%) donors received HRT and only 47/701 (<0.5%) received T3. The remainder received T4 which may be preferentially converted to the inactive r-T3 in the 'sick euthyroid' state and be thereby clinically ineffective. Donors receiving HRT were significantly younger and less likely to have died from cerebrovascular accidents or have diabetes,

hypertension or renal dysfunction; factors predictive of a lower yield of hearts for transplantation. Moreover, although donors that received HRT had a statistically higher thoracic organ donation rate, this is intuitive as only donors under active consideration for heart donation may have been prescribed T3 or T4 (80). A second retrospective report from the same database suggested that triple HRT (i.e. T3 or T4, steroids and vasopressin) reduced recipient mortality and the incidence of early graft dysfunction. Duration of therapy was not reported but non-HRT hearts had longer ischemic times and non-HRT recipients had a higher incidence of congenital heart disease, ventilator dependency and female donor-to-male recipient matching; factors predictive of worse post-transplant outcome. Only a triple HRT combination was associated with a beneficial effect, the administration of T3 or T4 alone was not beneficial. Thus, the investigation of the individual roles of the hormonal agents remains relevant.

The current study suggests that neither T3 nor a T3/MP combination lead to an additional improvement in donor circulatory function beyond that achieved by active management. Their routine use for this indication remains, therefore, an area of debate.

Noradrenaline (NA) is known to be cardiotoxic and a coronary vasoconstrictor (150). NA accelerates cardiovascular decline in the vasoparetic phase that follows the catecholamine storm (15).and has been associated with reduced right ventricular contractility and worse heart recipient survival (151;152). This association between NA and reduced function was confirmed in the post hoc analyses in the current study and NA withdrawal and substitution with vasopressin was associated with improved function, attributable at least in part to normalisation of systemic vascular resistance

and vasopressin's lack of cardiotoxicity; a finding consistent with previous reports (15;31;153). Despite these adverse effects, NA remains the most commonly used pressor agent in hypotensive organ donors. The study data supports the view that the use of NA infusions for post-BSD pressor support should be abandoned and vasopressin utilised in preference. In conjunction with invasive cardiac functional assessment, this allows manipulation of pre-load and afterload and discrimination between those donor hearts that can be resuscitated from those that cannot.

The sample size, although larger than previously reported, reflects the logistical difficulties of undertaking such prospective, randomised, blinded and controlled studies in this population. It remains relatively numerically small and thereby under-powered to confidently exclude some positive haemodynamic effects of multi-hormone therapy particularly in donors with initially low T3 or T4 levels or an advantageous post-ischemic effect following recipient implantation. The randomisation process permitted age differences to occur in the trial treatment groups but this does not, we believe, significantly alter the interpretation of the data. Although we have not reported recipient outcomes in detail, overall graft survival appears satisfactory regardless of HRT use. The donor age reported in this study reflects a typical change in the donor population observed world-wide. The incidence of coronary artery disease (CAD) is of concern, particularly as coronary angiographic screening is not available in many countries and alternative methods of screening to rule out CAD in donors need to be identified.

8.5-Conclusion

The study demonstrates that not only may donor circulatory status be improved by active management but that also there is the potential to increase the yield of transplantable hearts if decisions on organ acceptance are deferred until a period of resuscitation and assessment is complete. Active donor management with PAFC monitoring is the cornerstone of this objective but this has implications for planning donor retrieval services. The simple introduction of hormone therapy is not a substitute for the detailed hemodynamic assessment and management of the potential heart donor.

9. A STUDY OF PRO-INFLAMMATORY ENVIRONMENT IN POTENTIAL HEART AND LUNG DONORS AND IMPACT OF DONOR MANAGEMENT AND HORMONAL THERAPY

9.1-Introduction

Brain stem death (BSD) generates a pro-inflammatory environment, promoting pro-inflammatory cytokine m-RNA gene expression (38;154;155), leading to elevated plasma levels of interleukins-1 and 6 (IL-1 & IL-6) and tumour necrosis factor-alpha (TNF- α). In heart transplantation, high tissue and plasma levels of these cytokines have been associated with worse donor heart function (39-41;48) and post-transplantation graft outcome (11;43;154). C-reactive protein (CRP) and procalcitonin (PCT), a precursor of the hormone calcitonin, are also markers of inflammation and abnormal levels of both have been observed following BSD and high levels may be predictive of adverse heart transplantation recipient outcome (49;50). Thus, the pro-inflammatory environment which exists in the prospective organ donor may be a target for therapeutic intervention which could potentially improve organ function and yield.

In light of the known propensity of the organ donor to have diminished circulating thyroid hormone levels, tri-iodothyronine (T3) is commonly administered to heart donors as a putative optimising agent. Its effect on the pro-inflammatory environment is unknown.

The administration of steroids may be beneficial in the post-BSD environment by virtue of their anti-inflammatory effects involving stabilization of cellular membranes,

reduction of HLA antigen up-regulation and inhibition of cytokine elaboration (89). In experimental animal studies, post-BSD elevations of IL-6 and TNF- α levels have been diminished by steroid administration and this reduction is paralleled by better maintenance of heart function (93;94). This effect has not been confirmed in humans. In clinical lung transplantation, the early administration of steroids has been reported to improve oxygenation and organ yield and to attenuate lung water accumulation (97;156). It is not known if these observations reflect an anti-inflammatory effect, modification of alveolar fluid clearance or other phenomena. We hypothesised that donor hormonal treatment would reduce inflammatory marker levels. Thus, the objectives of this study were to (i) prospectively evaluate the prevalence of elevated inflammatory markers in potential heart and lung donors, (ii) to evaluate the impact of T3 and steroids administered during donor management, (iii) to correlate the presence of biomarker levels with donor heart and lung function and (iv) to explore the value of these biomarkers in predicting donor organ outcome.

9.2-Methods

9.2.1-Study design

This study reports the cytokine analyses, a secondary outcome measure, of the main trial. The clinical outcomes have been previously reported (156;157). In brief, donors were randomized to receive T3 ($0.8\mu\text{g.kg}^{-1}$ IV bolus followed by $0.113\mu\text{g.kg}^{-1} \cdot \text{hr}^{-1}$ IV infusion); MP (1000mg as a single IV bolus), both T3 and MP or placebo (dextrose 5%) following an initial comprehensive assessment of cardiopulmonary function. The T3 or placebo infusion continued until retrieval. The details of recruitment have been explained previously. Following donor identification and consent, I conducted the

study. Blood samples were collected prior to the initiation of donor management. The samples were initially stored on ice and then transferred to a core laboratory where they were centrifuged and stored at -70°C until batch analysis. Following initial sampling, invasive monitoring lines were inserted as described previously. Following hemodynamic and blood gas measurements and an echocardiogram (for off-line assessment of left ventricular ejection fraction (LVEF)), donors were randomly assigned to hormonal treatment groups. Hemodynamic management of the donor, directed by the PAFC monitoring, then commenced according to specific algorithm. Following volume adjustment, any pre-study inotropes were weaned if possible. In donors receiving noradrenaline (NA), this was actively weaned and substituted with vasopressin (VP). Lung management comprised initial bronchoscopic sputum clearance, alveolar recruitment manoeuvres and fluid limitation. The duration of treatment and time of coning (detection of fixed dilated pupils and blood pressure surge) and white cell count on the day of retrieval were noted. Hemodynamic studies were repeated hourly for 3 hours and immediately pre-retrieval. Prior to retrieval blood gases were repeated. Blood samples were drawn for repeat cytokine measurement after 4 hours of initial sampling.

For the purpose of this study, marginal donor hearts were pre-defined as those with initial higher filling pressures CVP and/or PAWP >14 mm Hg), and $CI \leq 2.4 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$) \pm inotropes or NA >0.06 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Marginal donor lungs were pre-defined as those with an initial $\text{PaO}_2/\text{FiO}_2$ ratio >230 but ≤ 320 at initial assessment (156). Heart suitability for transplantation at end-assessment was defined as $CI > 2.4 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ with CVP and PCWP $\leq 12 \text{ mmHg}$ in the absence of palpable coronary artery disease during direct inspection. Lung suitability was defined as a PaO_2/FO_2 of ≥ 300

(marginal = <300) in the absence of lung contusion, aspiration, infection, or non-recrutable atelectasis during the direct assessment at end-management. The number of organs transplanted was noted together with their 30-day survival. Information on primary organ function was not available.

9.2.2-Serum immunoassay

Serum measurement of IL-1, IL-6 and TNF- α were performed from the serum with commercially available immunoassay kits (Human Quantikine HS, R & D systems, Oxford, UK). PCT was measured using flash chemiluminescence (Liaison® Flash chemiluminescence), repeat measurements were not performed in light of PCT's known longer plasma half-life. C-reactive protein (CRP) measurement was performed using standard bio-assay kits. Coefficients of variation for each assay were <10%. All the measurements were performed in Aston University, Birmingham.

9.2.3-Statistical analysis

The study was powered to detect a standardised difference of 1.0 ($\alpha=0.05$; $1-\beta=0.85$) in biomarker levels post-therapy. Data were analyzed using SPSS v15.0 (Chicago, IL). Continuous data were assessed for normality and are presented as mean \pm SD or median (inter-quartile range (IQR)). Normally distributed variables were tested using two-way ANOVA that included factors for the two treatments given and also their interaction. The post-treatment variables were tested using ANCOVA using the pre-treatment value as a covariate along with the two treatments given and their interaction. However, throughout the analysis we found the T3+MP interaction did not affect any of the measured biomarkers, therefore the interaction was removed from the analysis and the main effects were tested. Categorical data were analyzed

using X^2 and Fisher's Exact tests. Receiver operating curves, sensitivity, specificity and predictive values of procalcitonin as a predictor of heart usability were generated using standard formulae. Statistical significance was assigned when $p \leq 0.05$ and all tests were two sided.

9.3-Results

Eighty potential heart donors (60 were eligible for lung donation) were recruited into the study. No donors were pyrexial and none had received steroids prior to the study. Study blood samples were drawn in all but one (positive hepatitis C) donor. A Consort diagram demonstrating the study exclusion and randomisation has been presented in Figure-9.1. Demographic details for the 79 study donors are presented in Table-9.1. The median donor age was 45 (range 36-56) years and 38 were female (48%). The mean time to initiation of donor management from the time of clinical coning was 12.2 ± 8.3 hours. The donors were managed for a mean period of 6.9 ± 1.4 and the randomized hormonal treatment administered 5.9 ± 1.4 hours prior to final functional assessment.

Table 9-1- Donor demographic parameters

Age (years)	45 (36, 56)
Sex (Female)	38/79 (48%)
Donor cause of death (no)	
Vascular	51/79 (65%)
Trauma	19/79 (24%)
Hypoxic brain damage	4/79 (5%)
CNS infection	5/79 (6%)
Time to donor management after coning (hours)	12.2±8.3
Duration of donor management (h)	6.9±1.4
Duration of drug treatment (h)	5.9±1.4
White cell count ($\times 10^9 \cdot L^{-1}$)	13.1±5.9

Values presented as median (25, 75 centiles) or mean±SD

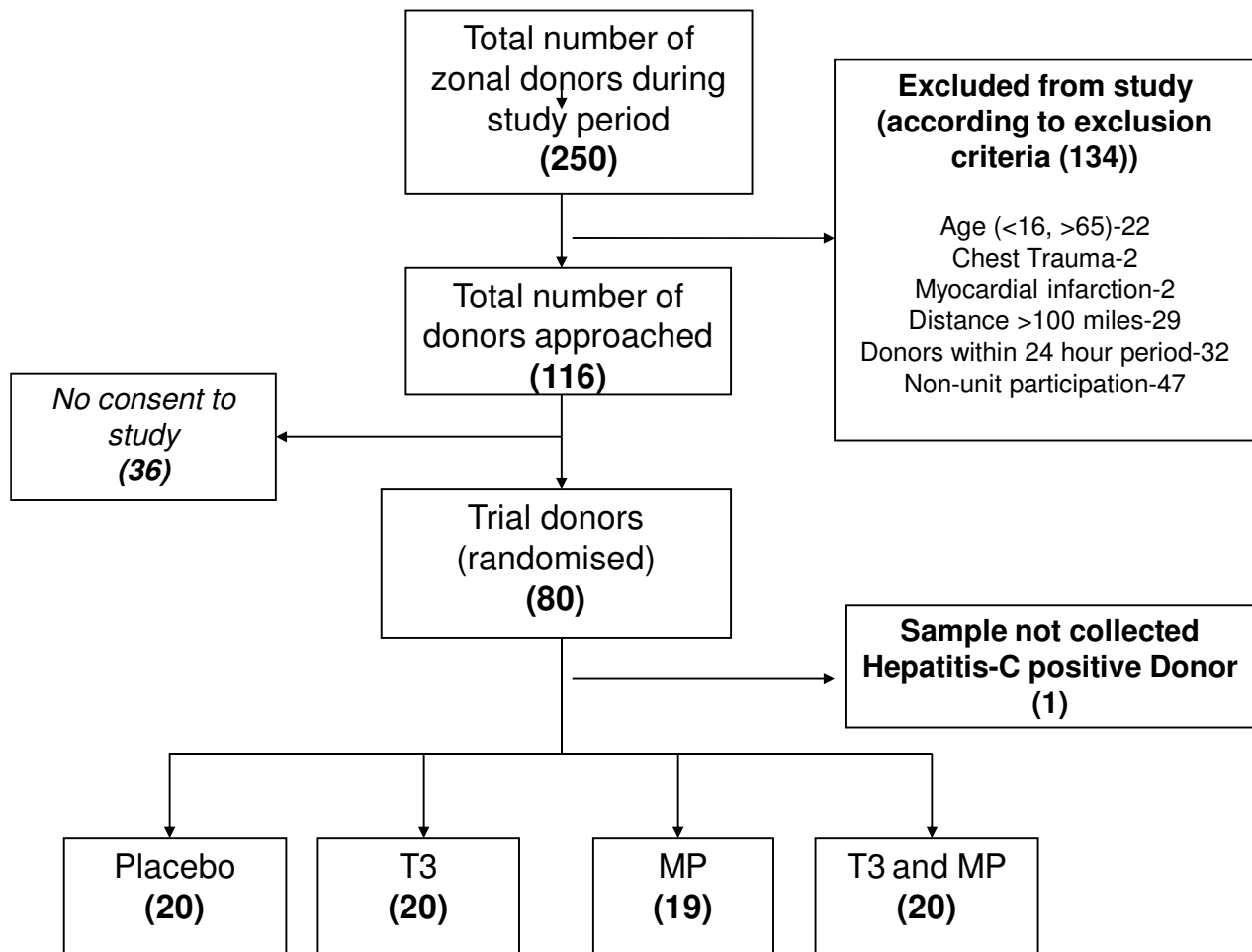


Figure 9-1–Consort diagram showing the donor inclusion, exclusion and allocation to randomised treatment groups

9.3.1-The prevalence of a pro-inflammatory environment

Interleukin-1 (mean $2.3 \pm 4.1 \text{ pg.mL}^{-1}$; upper reference limit (URL) 9 pg.mL^{-1}) was above URL in 13/79(16%) donors while IL-6(mean 91.9 ± 28.2 ; URL 4.5 pg.mL^{-1}) was elevated in all and was $>10 \times$ URL in 70/79 donors. Tumour necrosis factor- α (mean 8 ± 2.3 ; URL 20 pg.mL^{-1}) levels were above URL in 22/79(28%). C-reactive protein (mean 113 ± 63) levels were $>5 \text{ mg.L}^{-1}$ (URL) in 78/79(98%) donors (mean 113 ± 63) and procalcitonin levels (mean 5.2 ± 15.3) were $>0.1 \text{ ng.mL}^{-1}$ (URL) in 69/79(87%). Thus, all donors had evidence of a pro-inflammatory environment manifest predominantly as elevation of IL-6, CRP and PCT levels.

9.3.2-The effect of donor management and hormonal therapy

Table-9.2 reports the initial and final biomarker levels according to treatment groups and their interaction. There was no difference in final biomarker levels (IL-1, IL-6, TNF- α and CRP) according to treatment allocation and any change detected was not dependent on the time-interval of treatment.

Table 9-2-Baseline and pre-retrieval inflammatory marker levels in 79 donors according to 4 treatment groups

Parameter	T3	MP	T3+MP	Placebo	p-value		
	(20)	(19)	(20)	(20)	T3	MP	T3+MP
CRP	127±4	91±54	90±70	101±55			
CRP-P	124±7	119.6±42	105±86	111±51	0.62	0.56	0.55
IL-1	2.2±3.8	1.8±2.3	2.1±3.4	2.8±5.7			
IL-1-P	1.5±2.3	3.9±7.6	2.2±3.6	2.2±6.1	0.53	0.19	0.46
IL-6	96±29	102±20	88±30	87±26			
IL-6-P	98±24	103±20	90±32	100±24	0.12	0.35	0.79
TNF- α	7.9±1.8	8.2±2.3	7.4±2.5	8.4±2.9			
TNF- α-P	7.8±2.2	7.7±2.4	7.8±2.1	7.7±2.9	0.23	0.75	0.79
PCT	3.3±7.2	1.6±2.9	2.4±4.7	8.4±19.3			
PCT-P	3.6±7.9	1.8±2.3	2.9±4.5	22.9±63.5	0.32	0.8	0.69

Comparison of treatment effects post-management using ANCOVA analysis with initial biomarker value as co-variate, *P*-values for T3 and MP were generated from ANCOVA analysis for the two main treatment effects after removal of the T3 + MP interaction from the model. Results presented as mean±SD. CRP =C-reactive protein, IL =Interleukin, TNF =-tumour necrosis factor and PCT-procalcitonin. IL-1, IL-6 and TNF- α expressed in pg.ml⁻¹.PCT expressed in ng.ml⁻¹ and CRP in mg.L⁻¹. The suffix -P identifies the post-management, pre-retrieval value.

The factorial design of the study allowed comparison of biomarker change according to the receipt of T3 or steroids. Thirty-nine donors received MP (\pm T3) and 40 no-MP (\pm T3) and similarly 40 received T3 (\pm MP) and 39 no-T3 (\pm MP) following initial assessment. Within these groupings we compared the post-treatment biomarker levels using ANCOVA with baseline value as a co-factor. Administration of neither T3 nor MP affected the marker levels when compared to no-T3 (\pm MP) or no-MP (\pm T3) groups (Tables-9.3 and 9.4).

Table 9-3-Baseline and pre-retrieval cytokine levels in donors receiving methylprednisolone compared to non-MP donors

Parameters	non-MP donors	MP donors	Mean difference	
	(n=40)	(n=39)	(95%confidence	intervals)
CRP	114±66	91±62		
IL-1	2.5±4.8	2.0±2.9		
IL-6	91±27	95±26		
TNF- α	8.1±2.4	7.8±2.4		
PCT	5.8±14.6	2.1±3.9		
CRP-P	119±60	112±69	-5.6 (-22.8, 11.7)	
IL-1-P	1.8±4.2	2.9±5.7	-1.7 (-4.1, 0.71)	
IL-6-P	98±23	96±28	+4.1 (-4.5, 12.7)	
TNF-α-P	7.8±2.5	7.8±2.2	-0.21 (-1.4, 0.97)	
PCT-P	11.8±41.8	2.4±3.3	+0.7 (-5.1, 6.5)	

Value presented in mean±SD. CRP-C-reactive protein, IL-Interleukin, TNF-tumour necrosis factor and PCT-procalcitonin. The suffix -P identifies the post-management, pre-retrieval value. The *p* values are as for Table-9.2 and were generated from ANCOVA analysis for two main treatment effects after the non-significant T3+MP interaction was removed from the model.

Table 9-4-Baseline and pre-retrieval cytokine levels in donors receiving T3 compared to non-T3 donors

Parameters	non-T3 donors	T3 donors	Mean difference	
	(n=39)	(n=40)	(95%confidence	intervals)
CRP	96±54	109±74		
IL-1	2.3±4.4	2.2±3.6		
IL-6	94±24	92±29		
TNF- α	8.3±2.6	7.6±2.1		
PCT	5.2±14.4	2.8±6.0		
CRP-P	115±46	116±75	+4.8 (-12.4, 21.9)	
IL-1-P	3±6.8	1.8±2.9	+0.48 (-1.5, 3.2)	
IL-6-P	101±22	94±27	+6.6 (-2, 15.1)	
TNF- α -P	7.7±2.7	7.8±2.1	-0.7 (-1.8, 0.49)	
PCT-P	14.4±49.2	3.4±7	+2.4 (-3.3, 8.1)	

Value presented in mean±SD. IL-1, IL-6 and TNF- α expressed in pg.ml^{-1} . PCT expressed in ng.ml^{-1} and CRP in mg.L^{-1} . The suffix -P identifies the post-management, pre-retrieval value. The *p* values are as for Table-9.2 and were generated from ANCOVA analysis for two main treatment effects after the non-significant T3+MP interaction was removed from the model.

9.3.3-Initial biomarker levels and donor organ function

In post-hoc analyses relationship between initial marker levels, donor diagnostic category, time from coning, white cell count and donor organ function was explored. There was no difference in biomarker levels according to donor cause of death and no correlation with time from coning or white cell count. There was no correlation between IL-1, IL-6, TNF- α and CRP levels and CI, L or RVEF or left ventricular stroke work index (LVSWI). However, baseline PCT level correlated inversely with LVEF ($p=0.045$ Spearman rank coefficient $r=-0.297$) and RVEF ($p<0.001$ Spearman rank coefficient $r=-0.494$). To illustrate this effect, we arranged the data into tertiles grouping of donor PCT levels (Figure-9.2).

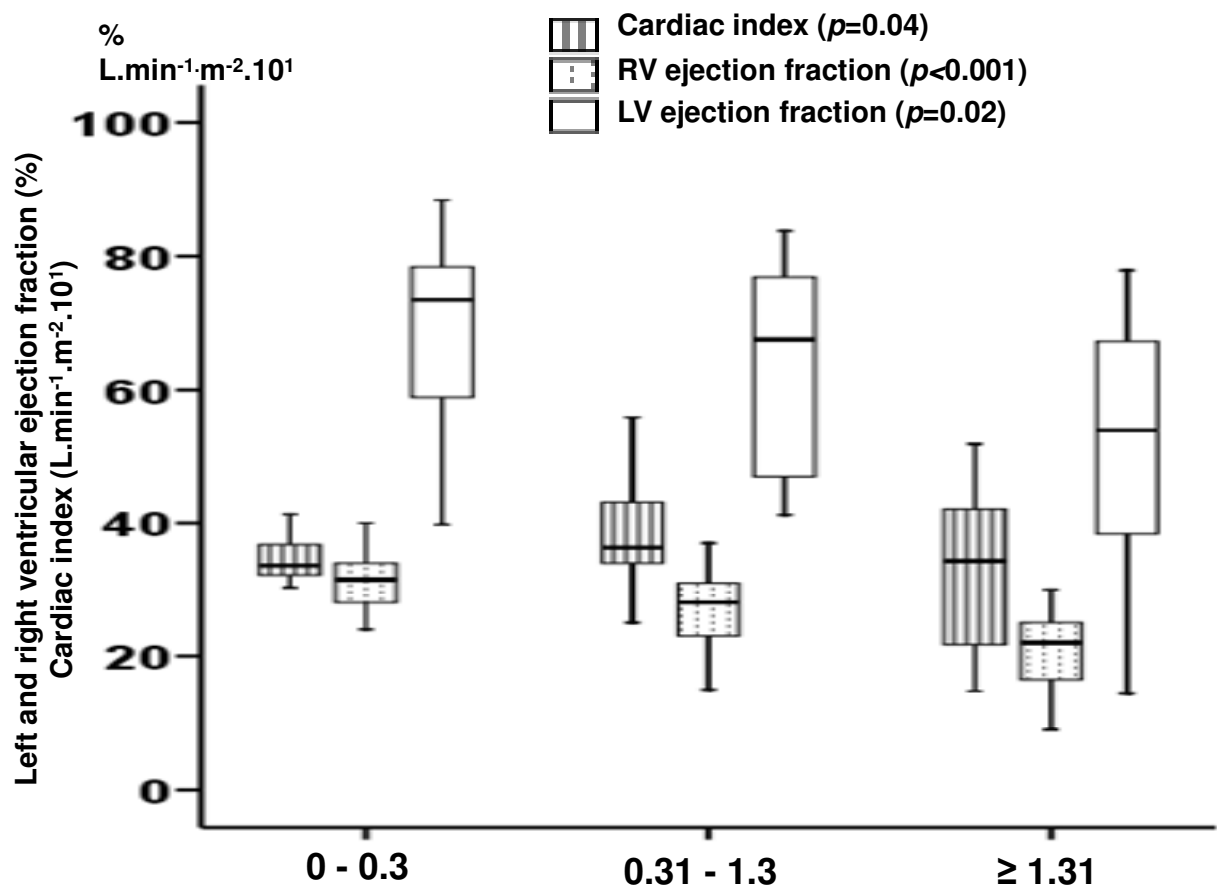


Figure 9-2-The relationship of CI, LVEF and RVEF with procalcitonin levels

On the x-axis donor procalcitonin levels ($\text{ng} \cdot \text{ml}^{-1}$) are presented as tertiles (0 – 0.3 $n=26$, 0.31 - 1.3 $n=26$, ≥ 1.3 $n=27$). Higher PCT levels were associated with significantly, incrementally worse CI ($p=0.04$), LVEF ($p=0.02$ and RVEF ($p<0.001$). N.B. for presentation the cardiac index is multiplied by 10^1 .

Note RVEF was calculated by pulmonary catheter while LVEF using echocardiography.

Previous studies have suggested a PCT cut-off of 2ng.ml^{-1} , above which donor hearts have a higher risk of dysfunction (50). On univariate analysis, a PCT level $>2\text{ng.ml}^{-1}$ was noted to be associated with significantly worse donor cardiac index, stroke work index and bi-ventricular ejection fractions and appeared less able to increase cardiac index with donor management (Table-9.5).

Table 9-5-Initial and change in heart function in donors with low and high baseline PCT level

Parameter	PCT $<2\text{ ng.ml}^{-1}$ (n=62)	PCT $>2\text{ ng.ml}^{-1}$ (n=17)	p value
Initial CI ($\text{L.min}^{-1}\text{m}^{-2}$)	3.5 ± 0.98	2.9 ± 1.1	0.037
LVEF (%)	66.1 ± 17.1	53.8 ± 19.3	0.026
RVEF (%)	28.6 ± 6.6	20.8 ± 7.9	<0.001
LVSWI ($\text{g-m.m}^{-2}.\text{beat}^{-1}$)	44.8 ± 12.9	35.1 ± 14.9	0.003
RVSWI ($\text{g-m.m}^{-2}.\text{beat}^{-1}$)	5.1 ± 2.7	4.6 ± 3.9	0.55
Δ -CI ($\text{L.min}^{-1}\text{m}^{-2}$)	0.75 ± 0.9	0.11 ± 0.6	0.013

Value presented in mean \pm SD. PCT- procalcitonin, CI – cardiac index, LVEF- left ventricular ejection fraction, RVEF- right ventricular ejection fraction, LVSWI- left ventricular stroke work index, RVSWI- right ventricular stroke work index. Δ -CI represents the change in cardiac index following donor management (final CI – initial CI)

Using the pre-determined definition of heart marginality (*vide supra*) 27/79 hearts were marginal at initial assessment. In marginal heart donors, TNF- α (8.7 ± 2.9 versus $7.5 \pm 1.9 \text{ pg.mL}^{-1}$; $p=0.044$) and PCT (6.1 ± 16.2 versus $2.9 \pm 6.6 \text{ ng.mL}^{-1}$; $p=0.02$) levels were higher than in non-marginal donors but IL-1 (2.6 ± 5.1 versus $2.0 \pm 3.2 \text{ pg.mL}^{-1}$; $p=0.5$), IL-6 (93.2 ± 28 versus $92 \pm 27 \text{ pg.mL}^{-1}$; $p=0.8$) and CRP (95 ± 67 versus $105 \pm 63 \text{ mg.mL}^{-1}$; $p=0.55$) were not different. In the 60 potential lung donors, biomarker levels showed no correlation with initial pO_2/FiO_2 ratio or extravascular lung water index (EVLWI) and were none were higher in those with marginal initial pO_2/FiO_2 ratio.

9.3.4-Biomarker levels and heart and lung suitability for transplantation

As I had detected no effect of treatment on biomarker levels, further assessment was performed whether initial marker levels predicted the end-assessment and management suitability of organs for transplantation. As the initial PCT level significantly correlated with the donor heart function we performed ROC analysis of baseline PCT level predicting the subsequent transplant suitability of the donor heart. It demonstrated that baseline PCT was a potentially useful tool in predicting the end-management heart usability for transplantation (Area under the curve=0.668; SE=0.06; $p=0.01$) (Figure-9.3). Baseline PCT level $\leq 2 \text{ ng.mL}^{-1}$ had a sensitivity of 88%, specificity of 31%, positive predictive value of 57% and a negative predictive value of 71% in detecting subsequent heart suitability for transplantation. Despite, this 5/17 hearts with initial PCT $>2 \text{ ng.mL}^{-1}$ were suitable. Overall, at end-management 40/79 hearts and 60/120 possible lungs met the pre-defined functional and patho-anatomical suitability criteria. Apart from PCT marker levels were not

different between those suitable or unsuitable for either organ (data not shown). Twenty-five donor hearts and 48 donor lungs (21 bilateral and 6 single lung transplantations) were transplanted and the 30-day survival for both hearts and lungs transplanted was 96%.

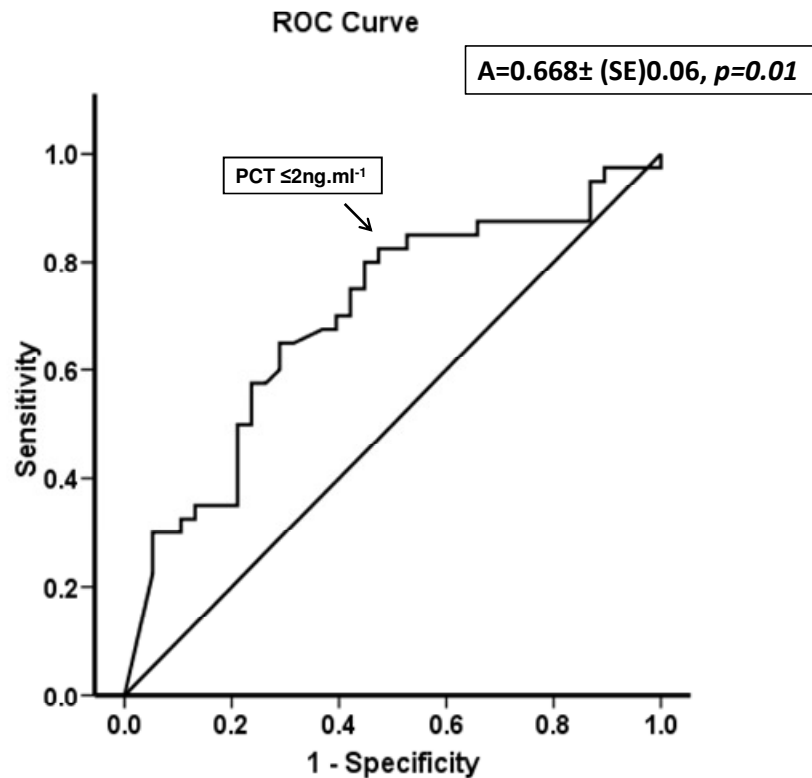


Figure 9-3-Receiver operating characteristic curve demonstrating the relationship between sensitivity and 1-specificity in determining the predictive value of initial PCT level in identifying subsequent heart usability for transplantation

The area under ROC curve (A) ± SE is reported

9.4-Discussion

There is a very high prevalence of a pro-inflammatory response in potential heart and lung donors predominantly manifest as elevated IL-6, CRP and procalcitonin. The response is unrelated to factors such as donor cause of death or time from coning. The pro-inflammatory response is potentially detrimental to the heart and other organs and provides a target for intervention during optimisation. However, the donor management strategy and hormonal treatment did not affect biomarker concentrations.

Over 25% of donors display elevated levels of the cardiotoxic cytokine, TNF- α . TNF- α mRNA is expressed in the failing or stressed nucleated myocyte generating and releasing TNF- α (46). In patients with heart failure, serum TNF- α levels correlate with disease severity and are predictive of mortality (11;37;42;47). In heart donors, myocardial upregulation of TNF- α mRNA and elevated serum TNF- α levels have been reported previously and the greatest increase has been found in donor hearts rejected for transplantation due to of poor function (38;39;48). In these donors, TNF- α levels exceed those seen in patients with advanced heart failure (39). These findings are also replicated for interleukin-6 (IL-6), a promoter of myocyte hypertrophy and a negative inotrope (39). In this study IL-6 levels were >URL in all donors but levels were not associated with marginal function. Although higher TNF- α levels were noted in the initially marginal category, neither initial IL-6 nor TNF- α level predicted whether a heart was functionally suitable for transplantation at end-assessment.

Similarly other inflammatory markers, procalcitonin and C-reactive protein, which is prognostically valuable in heart failure, have also been predictive of primary graft failure and graft dysfunction following heart transplantation (49-51). Wagner et al.

retrospectively analyzed the serum levels of PCT and CRP in 79 donors. They grouped the donor hearts into two, based on the post-transplant mortality related to graft dysfunction. Assessment of donor heart function was limited and ischemic times were significantly greater in the high PCT donors. They reported that higher PCT levels were associated with significantly elevated graft failure-related mortality and that a PCT $>2\text{ng.ml}^{-1}$ had 96% specificity and 50% sensitivity for predicting post-transplant mortality (50). In the current study, I found an important inverse relationship between PCT levels and donor heart function which potentially explains these previous observations. I also found that donor hearts with PCT levels $>2\text{ng.ml}^{-1}$ may not augment cardiac index with management. However, as the change in cardiac index is less than the measurement error for the thermodilution method, this finding should be viewed with caution. With the exception of PCT, no marker was predictive of ultimate suitability for transplantation of either hearts or lungs. As some hearts with elevated PCT and TNF- α were successfully transplanted with satisfactory recipient survival, this suggests that while PCT and TNF- α may be surrogate indices of donor heart function; their presence may not preclude successful optimisation. This observation should be viewed with caution as numbers transplanted were small and the finding requires corroboration by larger studies.

One of the study hypothesis was, that hormone replacement therapy, particularly methylprednisolone because of its anti-inflammatory properties, might favourably influence biomarker levels. In an experimental brain death model both systolic and diastolic heart function was better preserved in animals receiving methylprednisolone than in controls and this was associated with a significant reduction in IL-6, TNF- α and IL-10, 6 hours after BSD (93;94). This finding was not corroborated in the current

clinical study in which MP failed to influence any biomarker concentration at 4 hours and this, therefore could represent a sampling time effect. The discrepancy could also be related to a varying time interval of initial sampling following coning (1.2 to 37.5 hours) but this is unlikely as no time-dependent effect was observed. Future appropriately powered studies, with earlier administration of steroids post-BSD and a longer observation period might yield an effect.

Steroid administration has been reported to increase donor lung yield and improve function but the mechanism of this effect is unclear (96). The failure of MP to attenuate the cytokine response, in this study, suggests that this is not the mechanism of action. An alternative explanation may be the effect of steroids on enhancing alveolar fluid clearance; a finding demonstrated experimentally (97) and in human lung donors (156). It is also possible that other cytokines e.g. IL-8 may be both more relevant in lung transplantation and more steroid responsive.

9.5-Conclusion

This study has implications for clinical cardiac donor management. Elevated levels of TNF- α and PCT may identify donors with cardiac dysfunction which, if left unrecognised and unmanaged may worsen recipient outcome (48;50). Their measurement may therefore alert transplant teams to adopt an aggressive optimization management to restore donor hearts to a transplant-suitable status. Biomarker assessment might thus become a prompt for more intensive management and evaluation rather than organ rejection. It is also possible that additional biomarkers of cardiac ischemia, injury or stress may add discrimination in assessing the suitability of the donor heart. Thus, larger studies assessing an array of

biomarkers, including inflammatory probes, analyzed singly or in combination may facilitate donor heart assessment and better predict recipient outcomes.

10. A STUDY INTO THE MEASUREMENT OF CARDIAC TROPONIN-I IN POTENTIAL HEART DONORS AS A BIOCHEMICAL SURROGATE OF FUNCTION

10.1-Introduction

Although donor heart assessment remains a critical factor in determining post-transplant outcome, the criteria for donor heart selection are ill-defined and some hearts that appear unacceptable on initial data may in fact become satisfactory for transplantation. Brain-stem death (BSD) may cause myocardial injury if undetected may lead to primary graft dysfunction (PGD) and death of the recipient (67). Despite best attempts to match the donor to the recipient, PGD remains the major cause for 30 day mortality (158). In an attempt to stratify, the suitability of donor hearts for transplantation a number of biomarkers have been studied (39;48). Among these are cardiac troponins, markers of myocardial injury or infarction and an important prognostic index in ischaemic heart disease and advanced heart failure (159). Elevated troponins have been correlated with donor heart dysfunction and may predict adverse recipient outcome, being associated in some studies with increased risk of early graft failure and increased rates of inotrope requirement post-transplantation and post-transplant rejection (49;56-59) However, this association remains controversial as equivalent outcomes have been achieved in other studies with no increased risk of rejection (60-62). The objectives of this study were to evaluate the prevalence of cTnI elevation in heart donors, its relationship to donor

heart function and time from coning, its predictive value of donor heart suitability and post-transplant recipient outcome in a prospective study.

10.2-Materials and methods

10.2.1-Donor management and data collection

This report represents serum troponin-I measurement, one of the secondary outcome measures, of the main trial. The study population was previously discussed. As soon as feasible following identification, I attended the donors. Blood samples were collected prior to the initiation of donor management, initially stored on ice and within 8 hours were centrifuged, serum collected and stored at -70°C for later core laboratory batch analysis. Following initial sampling, invasive monitoring lines were inserted as previously discussed. Haemodynamic measurements an echocardiogram for off-line assessment of left ventricular ejection fraction (LVEF), fractional shortening, wall motion score and the LV Tei index were obtained. Donors were randomly assigned to hormonal treatment groups and haemodynamic management of the donor, directed by the PAFC monitoring was then commenced according to specific algorithm. Following volume adjustment, any pre-study inotropes were weaned if possible. In donors receiving noradrenaline (NA), this was actively weaned and substituted with vasopressin (VP). This management, continued in the intensive care unit and operating room until retrieval or end-assessment. Changes in donor organ function were conveyed to the recipient centers, which had provisionally accepted organs for transplantation. The duration of treatment and time of coning (detection of fixed dilated pupils and blood pressure surge) were noted. Hemodynamic studies were repeated hourly for 3 hours and immediately pre-retrieval

or direct inspection. Dysfunctional donor hearts were defined as those hearts with initial higher filling pressures CVP and/or PAWP >14 mm Hg), and $CI < 2.4 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$) \pm inotropes or NA $> 0.06 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$.

10.2.2-Recipient data collection

The recipient demographic data, ischaemic time, length of hospitalization, use of post-operative mechanical support, 30 day and 1 year outcome were obtained from the UK Cardiothoracic Transplantation Audit (UKCTA) database.

10.2.3-Statistical analysis

Data was analyzed using SPSS v15.0 (Chicago, IL). Continuous data were assessed for normality and are presented as mean \pm SD or median (inter-quartile range (IQR)). Normally distributed variables were tested using independent sample t test. Skewed data were tested using non-parametric tests (Mann-Whitney and Kruskal-Wallis test). Categorical data were analyzed using χ^2 and Fisher's Exact test. Spearman rank correlation, regression and receiver operating curve (ROC) analyses of cTnI levels with parameters of cardiac function were performed. As previous studies (61) have found a cut-off value of cTnI of $1 \mu\text{g} \cdot \text{L}^{-1}$, to be discriminant we also compared functional and outcome parameters for donor hearts with serum cTnI $\leq 1 \mu\text{g} \cdot \text{L}^{-1}$ and cTnI $> 1 \mu\text{g} \cdot \text{L}^{-1}$. Recipient survival was reported using Kaplan-Meier analysis and the inter-group comparison was analysed using the log rank test. Statistical significance was assigned to p value ≤ 0.05 . Univariate and multivariate analysis (stepwise logistic regression) was used to identify factors that predicted suitability for transplant at the end assessment.

10.3-Results

10.3.1-Donor demographics

Eighty potential heart donors were recruited into the study. Study blood samples were drawn in all but one (positive hepatitis C) donor. The median donor age was 45 (range 36 to 56) years and 38 (48%) were female. The median time to initiation of donor management from the time of clinical coning was 9.8 (25, 75 centiles 6.5-16.1) hours. The donors were managed for a mean period of 6.9 ± 1.4 hours and the randomized hormonal treatment administered 5.9 ± 1.4 hours prior to final functional assessment. The laboratory upper reference limit (URL) for cTnI was $0.01 \mu\text{g.L}^{-1}$ and all 79 donors exceeded this URL with a median value of $0.25 \mu\text{g.L}^{-1}$ (0.03 to 3.1). The distribution of cTnI levels is shown in Figure-10.1.

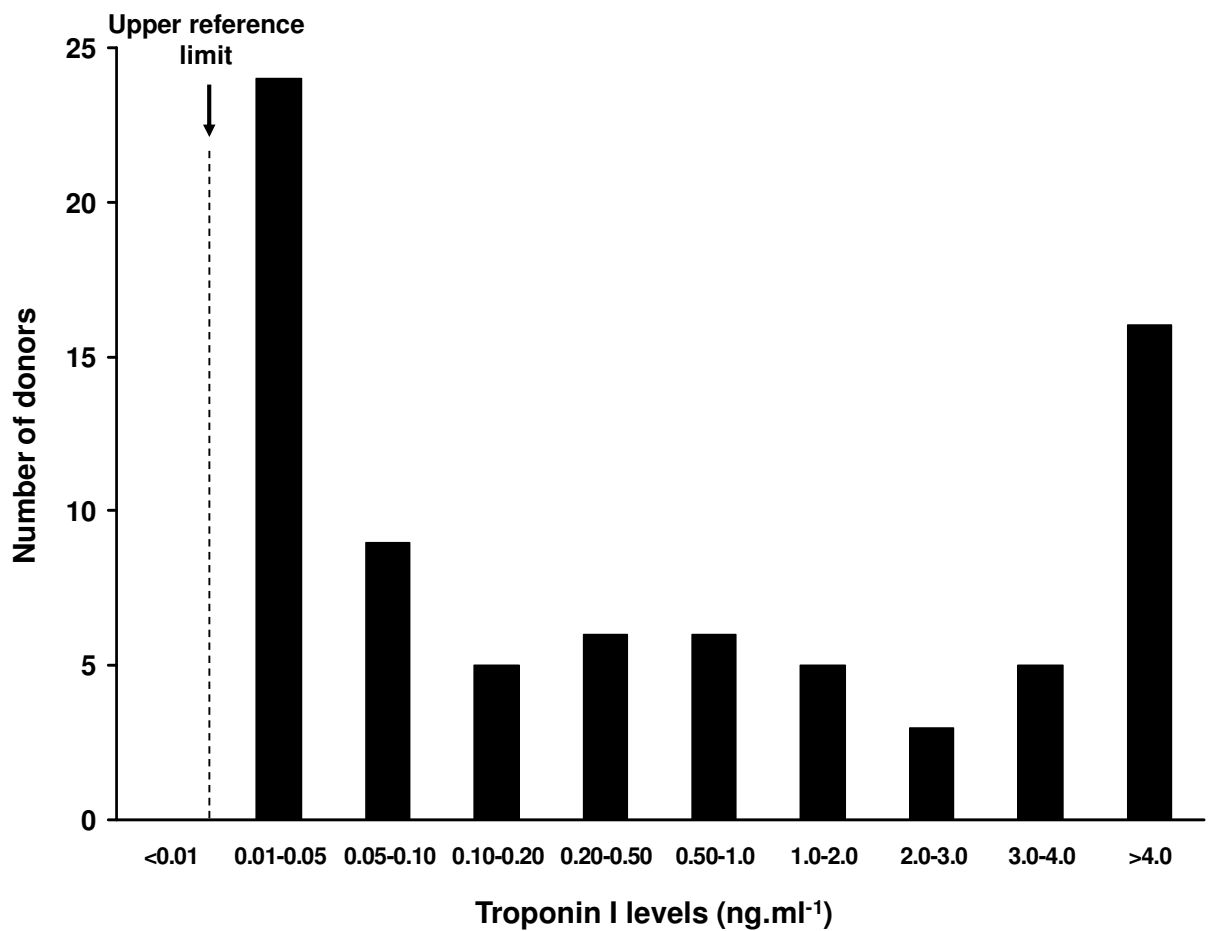


Figure 10-1-Distribution of CTnI levels in the entire donor cohort

Serum cTnI was $>1 \mu\text{g.L}^{-1}$ in 29/79 (37%) donors and was greater than $10\mu\text{g.L}^{-1}$ in 5/29(17%). There was no difference in any of the donor demographic parameters between donors with $\text{cTnI} \leq 1 \mu\text{g.L}^{-1}$ or $\text{cTnI} > 1 \mu\text{g.L}^{-1}$ groups (Table-10.1) with the exception of the time from clinical coning to blood sampling. This time duration was significantly shorter in the $\text{cTnI} > 1 \mu\text{g.L}^{-1}$ group (8.5 ± 5.4 versus 14.5 ± 9.1 ($p=0.006$) hours).

Table 10-1-Donor demographic parameters

Parameter	CTnI $\leq 1 \mu\text{g.L}^{-1}$	CTnI $> 1 \mu\text{g.L}^{-1}$	p-value
	(n=50)	(n=29)	
Age	44.1 (37-55)	42.5 (37-55)	=0.59
Sex (Female)	21(42%)	17(59%)	=0.17
Donor cause of death			
Vascular (n)	31 (62%)	20 (69%)	=0.62
Trauma (n)	13 (26%)	6 (21%)	=0.78
Hypoxic brain damage (n)	3 (6%)	1 (3%)	=0.99
CNS infection (n)	3 (6%)	2 (7%)	=0.99
Time to donor management (hours (h))	14.7 \pm 9.1	8.5 \pm 5.4	=0.006
Received tri-iodothyronine	27 (54%)	13 (45%)	=0.49
Received methylprednisolone	24 (48%)	15 (52%)	=0.82
Duration of donor management (h)	6.9 \pm 1.5	6.6 \pm 1.2	=0.32
Duration of drug treatment (h)	5.9 \pm 1.5	5.7 \pm 1.1	=0.26
Noradrenaline therapy	32(64 %)	17(59%)	=0.06

Values presented as median (25 and 75 centiles), mean \pm SD or number (percentage)

CNS-central nervous system

10.3.2-Donor haemodynamic parameters

LVEF measurement by transthoracic echocardiography was available in 47/79 (59%), LV Tei index in 64/79 (81%), wall motion score 48/79 (61%), CI in 79/79 and thermodilution RVEF in 79/79. A strong negative correlation was found between baseline donor cardiac functional parameters and cTnI levels; LVEF (Spearman's correlation $r=-0.487$, $p=0.001$) (Figure-10.2a), CI (Spearman's correlation $r=-0.335$, $p=0.003$) (Figure-10.3a) and RVEF (Spearman's correlation $r=-0.414$, $p<0.001$).

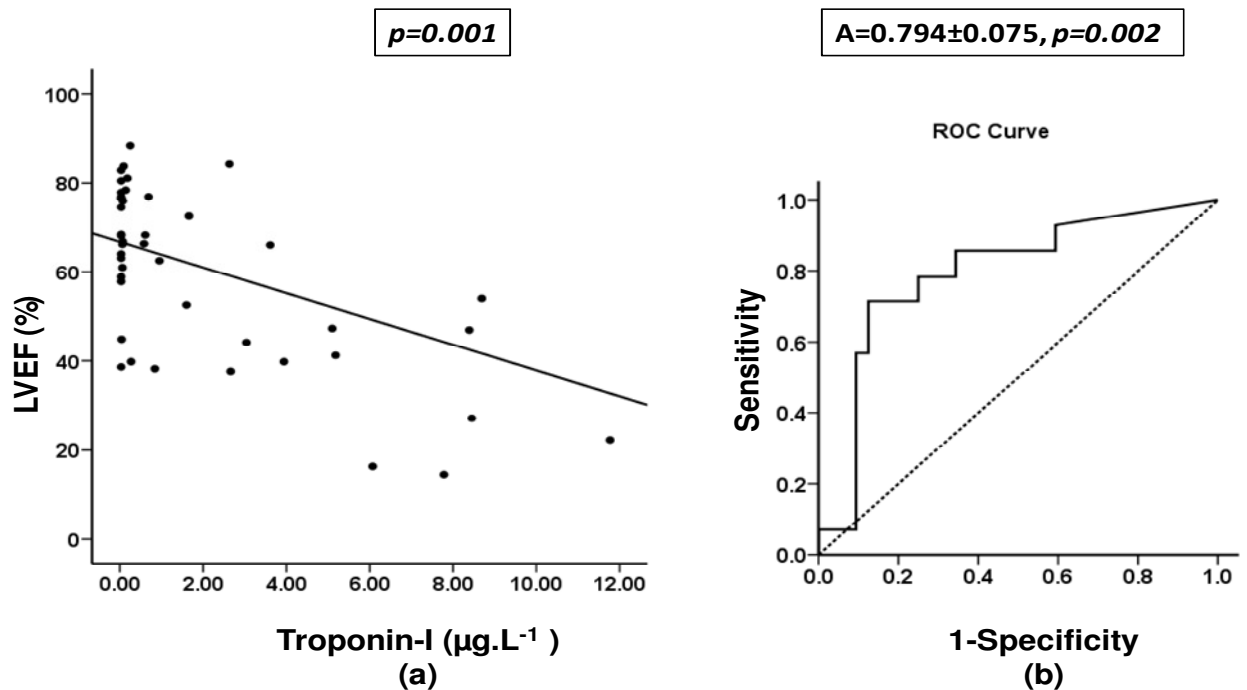
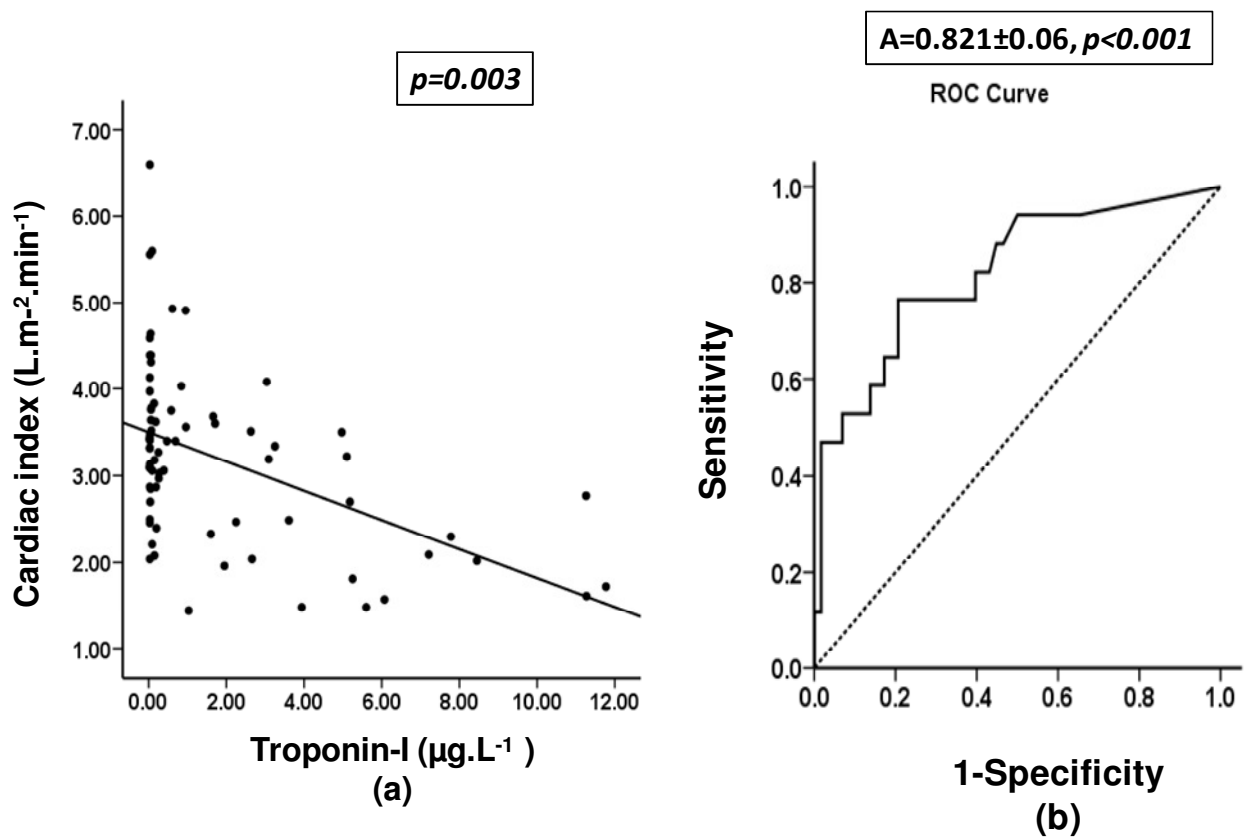


Figure 10-2-a-Correlation between left ventricular ejection fraction and cTnI levels in 79 heart donors. 10-2-b- ROC curve demonstrating the relationship between sensitivity and 1-specificity in determining the predictive value of $\text{cTnI}>1\mu\text{g.L}^{-1}$ for identifying decreased donor LV ejection fraction (LVEF $<50\%$)



**Figure 10-3-a-Correlation between baseline cardiac index and cTnI levels 10-3-
b-ROC curve demonstrating the relationship between sensitivity and 1-
specificity in determining the predictive value of CTnI>1μg.L⁻¹ for identifying
decreased donor cardiac index**

The area under ROC±SD (or SE) (A) is reported.

The donor LV-Tei index (Spearman's correlation $r=0.336$, $p=0.008$ (Figure-10.4) positively correlated with cTnI level indicative of worse myocardial performance with increased cTnI.

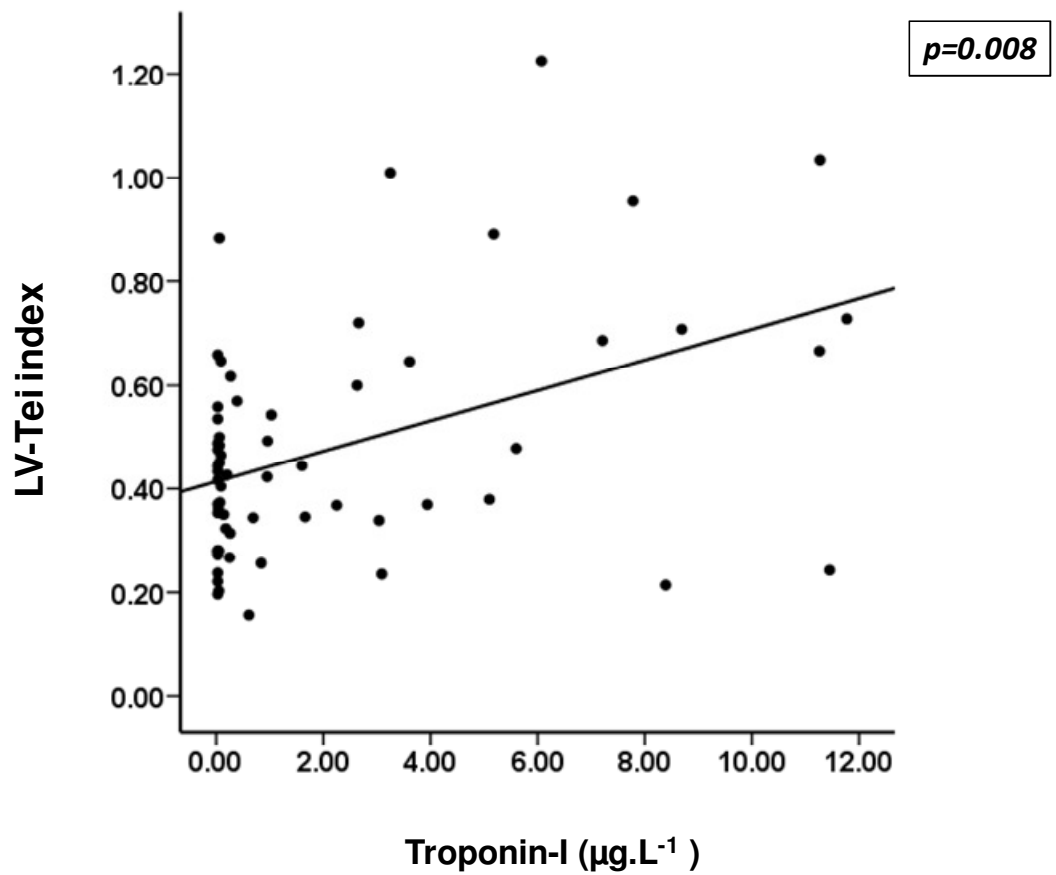


Figure 10-4-Correlation between LV-Tei index and cTnI levels in 79 donor hearts

(Spearman's correlation $r=0.336$, $p=0.008$). Normal LV-Tei index is 0.39 ± 0.05 .

There was a strong positive correlation between the cTnI level and LV-Tei index

Of the 47 donors with measurable LVEF, this was <50% in 15/47 (32%) donors. In these 15 donors, cTnI was significantly higher than in those donors with normal LVEF (LVEF >50%) ($4.5 \pm 3.7 \mu\text{g.L}^{-1}$, $1.4 \pm 0.54 \mu\text{g.L}^{-1}$, $p=0.005$). A ROC curve analysis was performed to evaluate the role of a pre-assessment cTnI level $>1 \mu\text{g.L}^{-1}$ in predicting donor hearts with decreased LVEF (<50%) and baseline CI $<2.4 \text{ L.m}^{-2}.\text{min}^{-1}$. It demonstrated that cTnI was an accurate tool in predicting decreased donor LVEF ($A=0.794 \pm 0.075$, $p=0.002$) (Figure-10.2b) and decreased baseline CI ($2.4 \text{ L.min}^{-2}.\text{min}^{-1}$) ($A=0.821 \pm 0.06$, $p<0.001$) (Figure-10.3b).

The baseline haemodynamic and echocardiographic parameters were significantly worse in 29 donors with cTnI $> 1 \mu\text{g.L}^{-1}$ than the donors with cTnI $< 1 \mu\text{g.L}^{-1}$ (Table-10.2). In addition donors with cTnI $> 1 \mu\text{g.L}^{-1}$ were receiving higher doses of inotropes and noradrenaline. Following the protocol-driven donor management, haemodynamic parameters improved significantly (160) and the change in donor parameters did not differ between the cTnI groups (Table-10.2). Administration of hormone replacement alone or in combination did not affect any of the parameters.

Table 10-2-Donor parameters between the CTnl groups

Parameter	CTnl $\leq 1 \mu\text{g.L}^{-1}$ (n=50)	CTnl $> 1 \mu\text{g.L}^{-1}$ (n=29)	p-value
Central venous pressure (CVP)	7.9 \pm 2.9	10 \pm 5.1	=0.026
Pulmonary artery wedge pressure (PAWP)	8.1 \pm 3.1	12 \pm 5.4	=0.002
Cardiac Index (CI)	3.6 \pm 0.9	2.7 \pm 1.1	=0.001
LV-stroke work index (LVSWI)	45.6 \pm 12.3	31 \pm 14.1	<0.001
Fractional shortening (FS) (%)	32.6 \pm 9.4	20.4 \pm 11.2	=0.001
Ejection fraction (EF) (%)	67.7 \pm 13.7	46.7 \pm 20.1	=0.001
LV-Tei index	0.41 \pm 0.15	0.62 \pm 0.29	=0.002
Wall motion score	1.4 \pm 0.51	2.3 \pm 0.58	<0.001
Δ -CVP	2.8 \pm 3.1	1.7 \pm 4.4	=0.17
Δ -PAWP	3.4 \pm 3.1	2.8 \pm 3.5	=0.46
Δ -CI	0.74 \pm 0.9	0.5 \pm 0.9	=0.28
Δ -LVSWI	4.9 \pm 16.5	5.1 \pm 16.1	=0.95
Δ -FS	3.3 \pm 8.9	-1 \pm 6.5	=0.17
Δ -EF	4.8 \pm 11.5	-2.8 \pm 12.9	=0.08
Δ -LV-Tei index	0.04 \pm 0.2	-0.02 \pm 0.2	=0.29
Δ -Wall motion score	1.9 \pm 8.2	3.5 \pm 11.1	=0.58

Value presented in mean \pm SD-(Δ -value is derived by subtracting the pre-retrieval value from the baseline value)-Units: CVP and PCWP- mmHg, CI- $\text{L.min}^{-1}\text{m}^{-2}$, RVEF- %, LVSWI - $\text{g.m.m}^{-2}.\text{beat}^{-1}$ Tei index is calculated by pulse wave Doppler across the right and left ventricular inflow and outflow tracts. The following formula is used to calculate the index: $\text{Tei}=\text{IVCT}+\text{IVRT}/\text{ET}$ (IVCT-isovolumetric contraction time, IVRT-isovolumetric relaxation time and ET-ejection time)

10.3.3-The relationship of cTnI and function with the time between coning and assessment

As the time from clinical coning to blood sampling was significantly lower in the cTnI group $>1 \mu\text{g.L}^{-1}$ the relationship between coning time and cTnI was analysed. There was a significant inverse correlation between the two, demonstrating falling cTnI level with increase in coning time (Spearman's correlation $r=-0.26$ $p=0.025$). The donor parameters CI (Spearman correlation $r= -0.261$ $p=0.02$) and wall motion score (Spearman's correlation $r= -0.25$ $p=0.04$) were higher after a longer period since coning.

10.3.4-Suitability for transplantation

Twenty five out of 79 (32%) donor hearts were ultimately retrieved for transplantation. An additional 15 of the remaining 54 (28%) hearts were found to meet the suitability criteria at the end of donor management but were not transplanted. Of these 15, 5 had been rejected for reasons of donor age ≥ 60 ($n=3$), history of hypotension ($n=1$) or inotrope use ($n=1$) and 10 were rejected due to an inability to identify suitable recipients. They were not re-offered at end-management. The remaining 39 donor hearts were not retrieved for transplantation due to the presence of coronary artery disease ($n=20$), left heart dysfunction ($n=9$) and right heart dilatation and failure ($n=10$). I used baseline CI, fractional shortening, $\text{CTnI} \leq 1 \mu\text{g.L}^{-1}$, $\text{cTnI} > 1 \mu\text{g.L}^{-1}$, T3 and MP group to identify factors that predicted suitability for transplant at the end assessment. On univariate analysis baseline cardiac index ($p=0.009$), $\text{CTnI} \leq 1 \mu\text{g.L}^{-1}$ ($p=0.01$) predicted suitability of hearts for transplantation (Table-10.3). On stepwise logistic regression analysis none of the factors significantly predicted the ultimate heart suitability. (Baseline CI (odds ratio (OR), 1.9; 95% CL, 0.98, 3.7 $p=0.058$).

Table 10-3-Comparison of donor variables between suitable and unsuitable hearts for transplantation

Variable	Unsuitable hearts (n=40)	Suitable hearts (n=40)	p-value
Baseline CI	2.9±1.2	3.5±0.85	=0.009
Baseline FS	23.9_13.7	30.8±8.9	=0.06
CTnI≤ 1 µg.L ⁻¹	19/40 (48%)	31/40 (78%)	=0.01
CTnI > 1 µg.L ⁻¹	20/40 (50%)	9/40 (23%)	=0.01
T3-positive	19/40 (48%)	21/40 (52%)	=0.82
MP-positive	17/40 (43%)	22/40 (56%)	=0.37

Value (age) presented in median (25 and 75 centiles) or mean±SD

10.3.5-Recipient parameters

Nineteen/50 cTnI≤1µg.L⁻¹ and 6/29 cTnI>1µg.L⁻¹ hearts were transplanted (p=0.137). Recipient demographic and outcome parameters were not different (Table-10.4) and 30 day (95%, 100%) and the 1 year survival (89%, 83%) were similar (p=0.252) (Figure-10.5).

Table 10-4-Recipient parameters between the CTnI groups

Parameter	CTnI $\leq 1 \mu\text{g.L}^{-1}$ (n=19)	CTnI $> 1 \mu\text{g.L}^{-1}$ (n=6)	p-value
Age	46.4 (40-57)	40.3 (41.5-56)	=0.35
Sex (Female)	3/19 (16%)	3/6 (50%)	=0.125
Donor triiodothyronine	9 (47%)	2 (33%)	=0.66
Donor methylprednisolone	14 (73%)	3 (50%)	=0.34
Ischaemic time (min)	205 \pm 79	188 \pm 75	=0.66
Mechanical support	2/19 (11%)	2/6 (33%)	=0.23
ITU stay (days)	6.4 \pm 9.1	8.2 \pm 5.2	=0.67
Hospital stay (days)	23 \pm 10.7	21.8 \pm 4.7	=0.65
30 day survival	18/19 (95%)	6/6 (100%)	=1
1 year survival	17/19 (89%)	5/6 (83%)	=1

Value (age) presented in median (25 and 75 centiles) or mean \pm SD

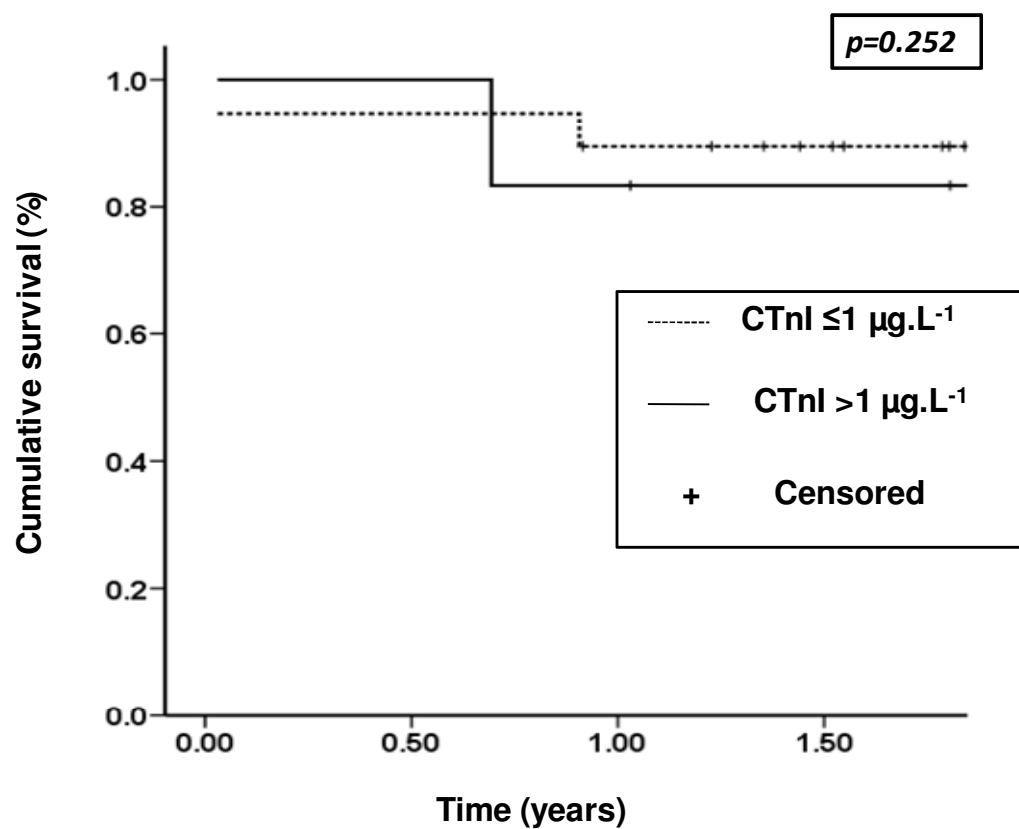


Figure 10-5--Kaplan-Meier survival curve demonstrating early recipient outcome following transplantation

Log rank test showed no difference in outcome between the CTnI groups in early recipient outcome (p=0.252)

10.4-Discussion

In this prospective study, serum cTnI was found to be above the upper reference limit in all donors and correlated strongly and negatively with haemodynamic and echocardiographic parameters of cardiac function. Thus, cTnI appears to be a useful surrogate index of cardiac function and may indicate which donors require intensive evaluation and management to assure their transplant suitability.

Troponin-I and troponin-T of the tropomyosin complex, are proteins present in the thin filament of the cardiac myofibril. They are released into the blood during episodes of myocyte injury and cell death (53;54). Circulating serum cTnI is a highly sensitive and specific marker of myocardial injury and is potentially useful tool in determining the quality of donor hearts. Troponin release into the recipient's blood post-transplantation has been previously evaluated as a marker of rejection (161). Cardiac troponin I elevation has also been observed in patients with subarachnoid haemorrhage and in this setting is associated with increased LV dysfunction, pulmonary oedema, hypotension requiring inotropic support and is predictive of worse outcome (55).

Riou et al. measured cardiac troponin-T and LV ejection fraction using transoesophageal echocardiography in 100 donors. They observed an LV ejection fraction <50% in 39% of donors and 14% had a LVEF <30%. Troponin-T level was significantly elevated in donors with LVEF <30% versus donors with normal LVEF and cTnT was found to be a significant predictor of severe LV dysfunction (56). However, they did not report the actual number of hearts retrieved for transplantation from these groups or their post-transplant outcome. Khush et al., in a retrospective

study measured cTnI in 263 heart donors, of which 139 hearts were utilised for transplantation (61). They found the cTnI level was $>1\mu\text{g.L}^{-1}$ in 43/139 donors but in contrast to the present study, they did not find any difference in non-invasive haemodynamic parameters between groups. They noted that the post-transplant 30 day and one year outcome were similar in the recipients. In a further study of 159 donor hearts, cTnI levels were found to be significantly higher in donor hearts with ejection fraction $<50\%$ and worse wall motion abnormalities but again recipient outcome appeared unaffected by the donor cTnI levels (62). In the current study, although a cTnI $>1\mu\text{g.L}^{-1}$ was associated with significantly worse baseline donor heart function, this did not preclude improvement with optimisation or successful transplantation. However, the numbers transplanted were small and this finding needs to be corroborated by larger studies.

An important and novel finding of the current study is the relationship of cTnI levels and functional parameters with the duration of the time-period between coning and initial assessment. The higher cTnI and worse function observed in donors with shorter post-coning period offers an attractive pathophysiological hypothesis of a time-dependent myocardial stunning phenomenon, the severity of which diminishes with time. This raises the possibility of extending periods of donor management until the donor heart is in a recovery phase and has obvious implications for the logistical coordination of heart transplantation retrieval services. This finding corroborates the observation that wall motion abnormalities observed in the cardiac donor or subarachnoid haemorrhage victim may improve over time (104;109). In addition, in the current study, the functional improvement in donor hearts subjected to active management did not differ by cTnI level; an indication of the recovery potential of this

stunning phenomenon and further, recipient outcome was similar although there was a suggestion of a higher need for mechanical support in the cTnl $>1\mu\text{g.L}^{-1}$ group.. This observation should be viewed with caution as the number of transplants in the cTnl $>1\mu\text{g.L}^{-1}$ group was small and our finding needs corroboration by larger studies. There is also a possibility that greater myocyte injury in the donor may increase the risk of rejection and graft vasculopathy in the recipient; again larger studies are required to investigate this. The impact of time on post-BSD cardiac function requires further studies to investigate whether this phenomenon of an apparent time-dependent recovery is a real effect which could ultimately be utilised to determine best donor care and the optimal timing of retrieval. One caveat to this approach, however, is the contrasting observation in lung donors where longer time from coning is predictive of worse donor lung function (156).

10.5-Conclusion

The use of cTnl as a surrogate index of donor heart function represents an attractive, non-invasive assessment tool for donor evaluation. It can aid in identifying donor hearts with dysfunction where the targeted donor management could lead to successful optimisation and increased heart utilisation without compromising recipient outcome. The combination of cTnl measurement with other biomarkers that have been demonstrated to have predictive value of either function or outcome may ultimately become a useful point-of-care assessment. Further, large prospective studies are required test this hypothesis.

11. A STUDY OF SERIAL ECHOCARDIOGRAPHY IN THE POTENTIAL HEART DONORS AND THE IMPACT OF DONOR MANAGEMENT AND HORMONAL THERAPY

11.1. Introduction

Many patients listed for heart transplantation die awaiting a satisfactory donor heart. The shortfall in hearts obliges centres to consider 'marginal' organs with less assured outcomes (158) but the proportion of hearts ultimately retrieved for heart transplantation remains low (105).

The best way to assess the donor heart is debated. Consensus guidelines recommend an initial echocardiogram with progression to more invasive, pulmonary artery catheter (PAFC) investigations if subnormal LV echocardiographic systolic function (E-function) is identified (68). However, in some organ procurement networks, including the UK, that have a low availability of echocardiography, there is reliance on PAFC-guided assessment and management of hearts provisionally accepted for heart transplantation (63). No studies have systematically examined the relationship between these two different modes of assessment.

If echocardiography is used, subnormal E-function even in the absence of a prior history of heart disease is the most common cause for rejecting a heart for transplantation (67). However, although a normal LV ejection fraction (LVEF) may predict heart usage and satisfactory recipient outcome, subnormal E-function does not necessarily preclude successful heart transplantation as the dysfunction may be reversible either within the donor or in the recipient following implantation (162). Thus, the utility of echocardiography in donor heart assessment has been questioned

as adherence to strict limits of E-function may inappropriately exclude hearts that could be used successfully. Conversely, failure to perform echocardiography as an entry investigation may lead to arbitrary rejection of hearts on “soft” clinical criteria and non-progression to formal PAFC-guided assessment e.g. a heart from an older potential donor with a history of hypertension and some inotrope requirement may be rejected for further assessment on the basis of presumed left ventricular hypertrophy (LVH) and dysfunction when in fact the heart has no or minimal LVH and is receiving inotropic support inappropriately for hypovolaemia and vasodilatation. Echocardiographic studies to-date have been limited to single snap shot assessment of E-function (106;111), whereas the phenomenon of reversibility suggests that serial studies may have better discriminative value (123). The best measure of E-function to predict heart transplant usability is not known.

Hormonal manipulation of the organ donor using tri-iodothyronine (T3), steroids and vasopressin is believed to augment cardiac function and improve yield (80) but its effects on haemodynamic function (H-function) and E-function are debated. In this prospective study, I had the opportunity to assess both and therefore sought to evaluate (a) the association between haemodynamic parameters and echocardiography (b) whether measurement of echocardiographic parameters performed at the initiation of donor management predicted functional suitability for heart transplantation; (c) whether repeat TTE might track changes that improved prediction of outcome and (d) the impact of donor management and hormonal manipulation on E-function.

11.2-Materials and methods

This report represents the echocardiographic assessment, a secondary outcome measure, of the main trial. At the time of the study, the provision of echocardiography at the donor hospital was rarely available and heart assessment was primarily based upon the results of PAFC-acquired pressure and cardiac output studies followed by direct inspection of the organ. Between, January-2004 and April-2006, the next-of-kin of eligible potential heart donors were approached for consent to enter the donor into the study independently of whether the donor heart was provisionally accepted for transplantation by any centre. The presumed time of coning was identified as the time of the first recorded observation of fixed dilated pupils accompanied by a blood pressure surge. Consent was obtained from the next-of-kin for all participating donors according to the ethical approval.

11.2.1-Initial assessment and management

As soon as possible following consent, I attended the donor and drew blood for thyroid function and inserted invasive monitoring lines as discussed previously. I then undertook TTE imaging. Coronary angiography was not performed as this is an exceptional investigation of donor hearts in the UK. Active algorithm-based, donor management was then instituted as per algorithm guided by the invasive monitoring. Pre-load was adjusted using either colloid administration or venesection, and after-load modulated by pressor therapy. The SVR was manipulated to a target range of 800-1200 dynes.cm.sec⁻⁵. Pre-management noradrenaline (NA) was actively substituted with vasopressin. This management continued in the ICU and OR until final heart inspection, assessment and retrieval. Each heart was finally inspected in

the operating room to assess macroscopic right and left ventricular function, LVH and the presence of palpable coronary artery disease.

11.2.2-Echocardiography

The trans-thoracic echocardiography (TTE) was for image acquisition not interpretative reporting and was performed using an Acuson Cypress (Siemens, USA) portable machine with second harmonic imaging (3.6 MHz probe). Images were repeated after 4 hours of management and stored on optical disc. Repeat echocardiography was performed specifically to look at the changes in myocardial function during intensive management and weaning of vasoconstrictors. After removal of patient identifiers and random coding of examination timing, studies were analysed by two observers (Dr Rick Steeds and I) who remained blinded to group allocation and time order using Cypress viewer software. E-function parameters were measured in triplicate and averaged, with LVEF (%) measured by Simpson's biplane. LV fractional shortening (LVFS), LV and RV ventricular myocardial performance (Tei) indices were calculated as previously described (116). As I accrued but did not interpret the images, echocardiographic findings were not conveyed to centres considering hearts for transplantation. Thus, echocardiographic findings did not influence the decision-making process of organ acceptance, which was still based on PAC measurements and direct inspection.

11.2.3-Endpoints and statistics

In this observational study I assessed the frequency of satisfactory initial and repeat TTE image accrual and the inter-class correlation between the reporting observers. Donor LVH, assessed using M-mode measurements of interventricular and/or

posterior wall thickness, was categorized as mild (1.2-1.3cm), moderate (1.4-1.7 cm) or severe (>1.7 cm) (163). Severe LVH is considered a contra-indication to heart donation and if detected prospectively would have precluded further management towards heart donation. In view of this, donors with severe LVH were withdrawn from subsequent analyses. Thus, the study cohort submitted to further analysis comprised those donors with satisfactory acoustic windows for at least initial image accrual who did not have severe LVH.

The incidence of echocardiographic abnormalities was noted and the correlation between initial E-function and H-function and the effect of hormonal treatment allocation on E-function assessed. Further analyses allowed assessment of echocardiographic response to management in donors with initially subnormal LV systolic E-function and the impact of initial thyroid functional status on echocardiographic function. For the purpose of this study, the echocardiographic criteria of normality were LVEF $\geq 50\%$, LVFS $\geq 30\%$ and LV Tei index ≤ 0.45 . Subnormal E-function was defined as either an LVEF $< 50\%$, an LVFS $< 30\%$ or LV Tei index > 0.45 . To be categorized as having normal E-function, donor hearts had to have no subnormal values in the measured parameters. Thus, a heart with a LV Tei < 0.45 was designated normal E-function even if images allowing LVEF and LVFS assessment were unobtainable. Echocardiographic improvement in function was pre-defined as an increase in LVEF or LVFS of $\geq 5\%$ or a fall in LV-Tei index of 0.2 (1 standard deviation). Data were analyzed using SPSS v12.0 (Chicago, IL). Continuous data were assessed for normality and are presented as mean \pm standard deviation or median (inter-quartile range). Normally distributed variables were tested using two-way ANOVA that included factors for the two treatments given and also

their interaction. The post-treatment variables were tested using ANCOVA using the pre-treatment value as a covariate along with the two treatments given and their interaction. As no interaction between T3 and MP was identified, the interaction was excluded from the model when reporting significance levels for the separate effects of T3 and MP. Skewed data were tested using non-parametric tests (Mann-Whitney and Kruskal-Wallis test). Categorical data were analyzed using χ^2 and Fisher's Exact tests and correlations using Spearman rank analysis. Observer variability was tested using interclass correlation co-efficient. Sensitivity, specificity, positive and negative predictive values of E-function variables in detecting hearts functionally suitable for transplantation at end-assessment was calculated using standard formulae. Statistical significance was assigned when $p \leq 0.05$ and all tests were two sided.

11.3-Results

Trial recruitment, TTE image acquisition and repeatability are demonstrated in Figure 11.1.

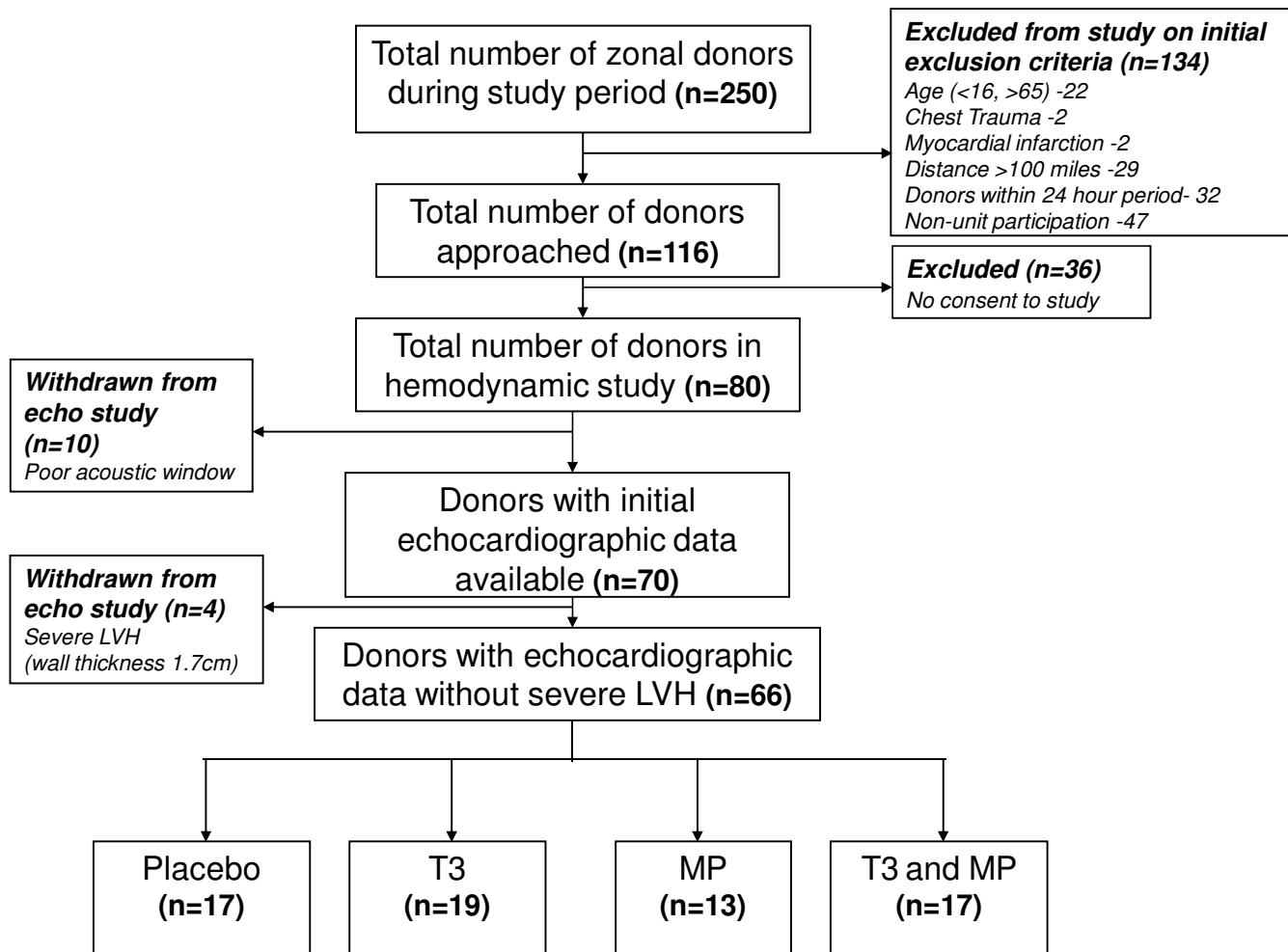


Figure 11-1-Consort diagram of donor inclusion, exclusion and recruitment into the trial and withdrawal from the echocardiographic study with allocation to the four randomized treatment groups

Of 80 donors within the trial, 70(87.5%) had acoustic windows sufficient to assess at least one initial measure of E-function, thus overall, LVEF, LVFS, LV Tei and RV Tei could be assessed in 59%, 59%, 80% and 50% respectively. The interclass correlation co-efficient between observers was 0.99 (95% (CI) confidence intervals

0.99-0.991); $p < 0.001$. Seven donors were found to have LVH; mild $n=1$, moderate $n=2$ or severe $n=4$ (6%). Hearts with severe LVH were withdrawn from further analyses leaving a study cohort of 66 donors. In this cohort, LVEF (mean $59 \pm 19\%$) was assessable in 44/66 and was $< 50\%$ in 15/44 (34%; mean $36 \pm 11\%$). LVFS (mean $28 \pm 12\%$) could be assessed in 44/66 and was $< 30\%$ in 23/44 (52%; mean $18 \pm 7\%$). The LV Tei index (mean 0.48 ± 0.23) could be assessed in 60/66 donors and was abnormal in 26/60 (43%; mean 0.68 ± 0.21). The RV Tei index (mean 0.48 ± 0.21) could be assessed in 36/66 and was abnormal in 18/36 (50%; mean 0.63 ± 0.18). No donor hearts were found to have significant valvular heart disease (more than mild stenosis or regurgitation of any valve). Overall, the incidence of sub-normal E-function by any measure was 44%.

The study cohort's median age was 43 (IQR 35, 56), 54% were male and 11 (16%), 1 (1.4%) and 27 (39%) respectively had a history of hypertension, diabetes or smoking. BSD was caused by trauma in 18/70 (26%), vascular event or tumour in 44/70 (63%) and hypoxia or infection in 8/70 (11%).

Assessment commenced within a median of 2 (IQR 1.5-2.0) hours of consent, within 12.4 ± 8.1 hours of coning and 6.8 ± 1.7 hours prior to retrieval or end-assessment. Study medication was administered for 5.8 ± 1.6 hours prior to retrieval or end-assessment; the T3 or placebo infusion being continued until OR inspection.

11.3.1-Echocardiographic correlation with haemodynamic measurements

In the 66 donor study cohort, baseline LVEF (Figure-11-2) , LVFS and LV Tei index (Figure-11.3) correlated as expected with initially measured cardiac index but neither initially normal E-function nor conversely, satisfactory initial H-function, clearly

defined hearts that were haemodynamically or echocardiographically suboptimal
(Table 11.1)

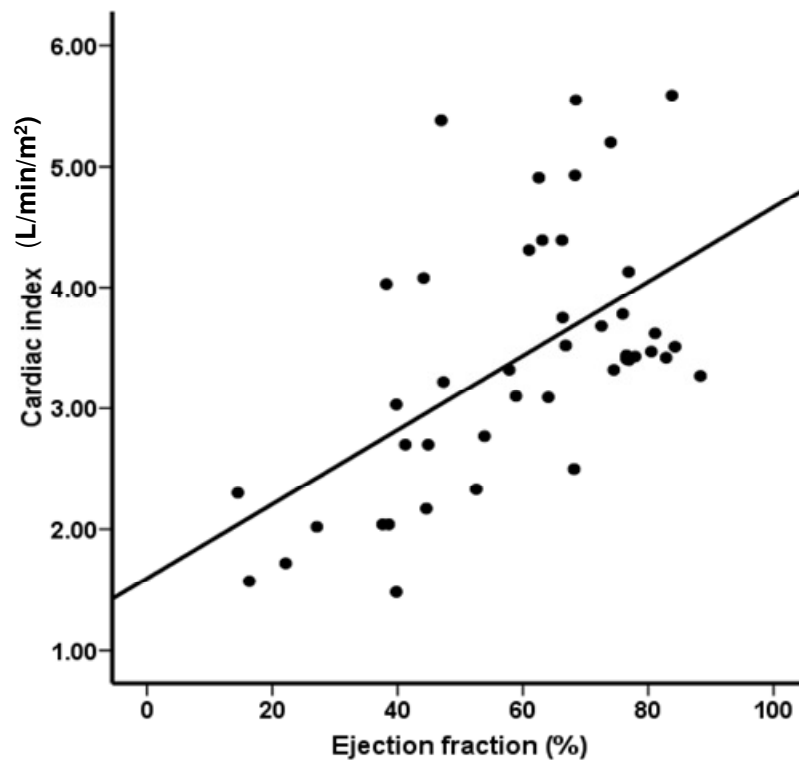


Figure 11-2-Scattergram and correlation of left ventricular ejection fraction with cardiac index
($\text{L}\cdot\text{min}^{-1}\text{m}^{-2}$) ($r=0.56$, $p<0.001$)

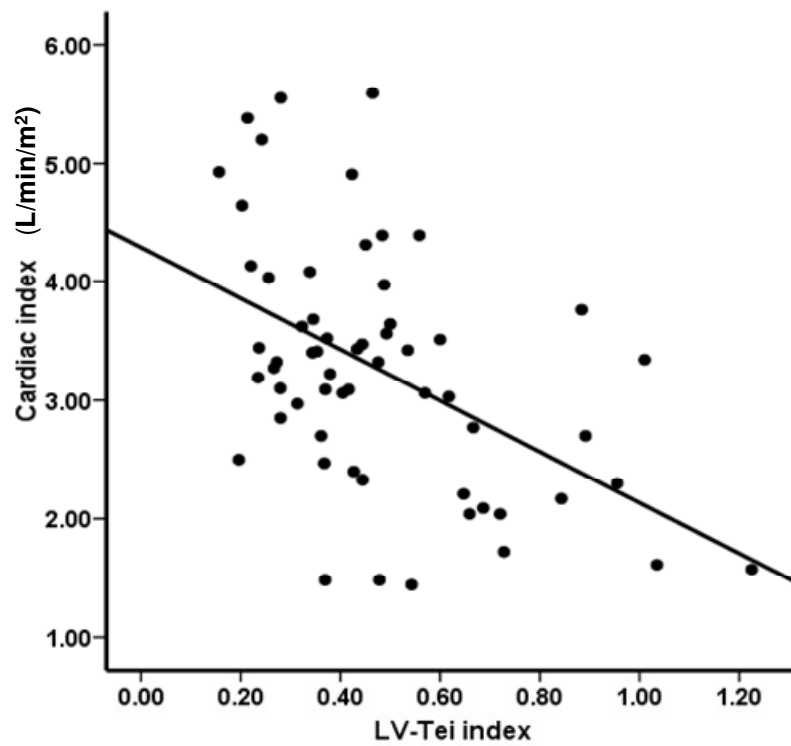


Figure 11-3-Scattergram and correlation of left ventricular Tei index with cardiac index
($\text{L} \cdot \text{min}^{-1} \text{m}^{-2}$) ($r = -0.49$, $p < 0.001$)

Table 11-1-Echocardiographic parameters LVEF, LVFS and LV Tei index versus cardiac index at initial assessment

Echo parameter	CI >2.4L.min⁻¹m⁻² (n)	CI<2.4L.min⁻¹m⁻² (n)	Total
LVEF≥50%	28	1	29
LVEF<50%	7	8	15
Total	35	9	44
LVFS≥30%	21	0	21
LVFS<30%	14	9	23
Total	35	9	44
LV-Tei≤0.45	30	3	33
LV-Tei>0.45	15	11	26
Total	45	14	59

On the pre-defined criteria, 29/66 (44%) hearts had initially subnormal LV systolic E-function. Donor hearts with times from coning above the median value of 11 hours were observed to have significantly better LV and RV systolic E-function (LVEF $65\pm13.3\%$ vs $53\pm23\%$ $p=0.03$; RV-Tei index 0.39 ± 0.14 vs 0.52 ± 0.22 $p=0.03$).

11.3.2-Initial echocardiographic function and thyroid functional status

Thirty-six of 66 study donors had subnormal T3 or thyroxine levels (157). Initial LVEF ($57\pm21\%$ vs. $62\pm18\%$; $p=0.41$), LVFS ($26.4\pm12\%$ vs. $29\pm11.3\%$; $p=0.45$), LV Tei index (0.43 ± 0.22 vs. 0.53 ± 0.24 ; $p=0.09$) and RV Tei index (0.43 ± 0.16 vs. 0.53 ± 0.24 ; $p=0.12$) were not statistically significantly different between those donors with subnormal or normal thyroid hormone levels.

11.3.3-Hormonal treatment and echocardiographic changes

There was no difference in any initial LV E-function parameter between those randomised to T3 and MP, alone or in combination (Table-11.2). Fifty-two donors underwent repeat echocardiographic assessment but echocardiographic parameters did not change in any treatment group with the exception of the RV Tei index which improved in T3 but not T3+MP donors ($p=0.04$). No interaction between T3 and MP was identified.

Table 11-2-Echocardiographic parameters of initial and post-management ventricular function by treatment group

Parameter	Placebo	T3	MP	T3+MP	p-value		
	(17)	(9)	(13)	(17)	T3	MP	T3+MP
LVFS	29±10(10)	32±12(14)	20±9(11)	30±11(12)			
LVEF	62±16(10)	65±18(14)	46±19(11)	63±18(12)			
LV-Tei	0.44±0.2(16)	0.51±0.2(18)	0.59±0.3(13)	0.45±0.2(17)			
RV-Tei	0.38±0.1(8)	0.53±0.1(14)	0.54±0.2(8)	0.44±0.3(10)			
WMS	1.8±0.7(17)	1.7±0.7(19)	2.0±0.6(14)	1.7±0.8(17)			
P-LVFS (%)	36±11	33±12	24±8	31±11	0.52	0.63	0.39
P-LVEF (%)	71±18	67±20	55±15	65±17	0.57	0.77	0.65
P-LV-Tei	0.45±0.2	0.45±0.2	0.58±0.2	0.46±0.2	0.66	0.12	0.91
P-RV-Tei	0.49±0.2	0.36±0.1	0.61±0.2	0.34±0.2	0.04	0.08	0.99

Data are presented as mean±SD (number of donors with available repeat measurements in each group). The repeat value after 4 hours of management is represented by a prefix-P. P-values for T3 and MP were generated from ANCOVA analysis for the two main treatment effects after removal of the T3 + MP interaction from the model. LVFS-left ventricular fractional shortening (%), LVEF-left ventricular ejection fraction (%)

11.3.4-Repeat echocardiographic assessment

At least one repeated measure of E-function could be obtained in 52/66(79%) of hearts. Repeat measurements could be more frequently obtained using the Doppler-based LV-Tei index (50/66) than using 2D Simpson biplane estimation of LVEF or LVFS (36/70; $p = 0.01$). The reasons for being unable to repeat echocardiography were donor haemodynamic deterioration requiring urgent transfer to theatre for retrieval of abdominal organs ($n= 5$) and other technical and logistic difficulties precluding repeat assessment ($n=9$).

Of the 52 hearts with repeat assessment, 33 had normal initial E-function. At end-assessment, this was retained in 30. Cardiac index changed from 3.8 ± 0.8 to $4.3 \pm 1.2 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$; $p=0.002$ and 24/33 met the H-function criteria for transplant. Following donor management 10/19 (53%) hearts with initial subnormal LV systolic E-function improved echocardiographically on the pre-defined criteria (Figure-11.4). Improvement was accompanied by a change in CI from 3.4 ± 0.9 to $4.1 \pm 0.82 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$, $p=0.03$. The remaining 9/19 hearts failed to improve echocardiographically but nevertheless CI increased from 2.5 ± 0.8 to $3.6 \pm 1.32 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$, $p=0.004$. Eight of 10 echocardiographic improvers and 8/9 non-improvers achieved functional suitability at end-assessment. Thus, neither initial subnormal LV E-function nor a failure to improve echocardiographically precluded hemodynamic augmentation or attainment of the H-function suitability criteria for transplant.

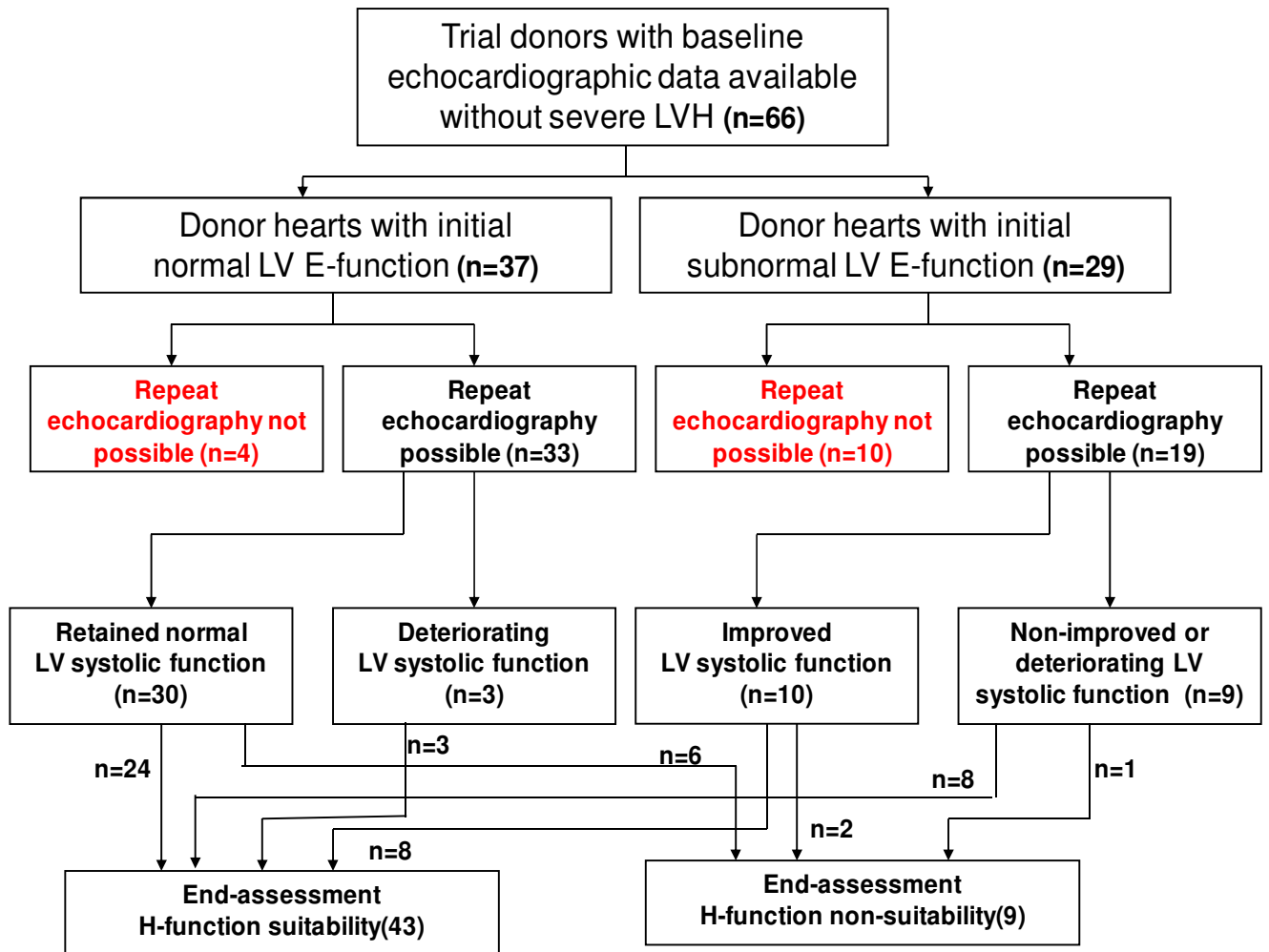


Figure 11-4-Flow diagram in donors in which repeat assessment of LV echocardiographic function was possible (n=52).

Twenty-seven of 33 donors with initially normal LV echocardiographic (E) function attained hemodynamic functional suitability criteria. Sixteen donors with subnormal LV E-function improved to suitability which was not dependent on E-function change.

The echocardiographic and hemodynamic change in hearts with subnormal function, according to the degree of LV impairment is shown for LVEF and LV-Tei index in Figure-11.5 & 11.6. LVFS changes paralleled those of LVEF. No hearts with an initial LVEF<30% improved to H-function suitability but above this threshold and for LV-Tei indices, no degree of LV dysfunction precluded hemodynamic \pm echocardiographic improvement.

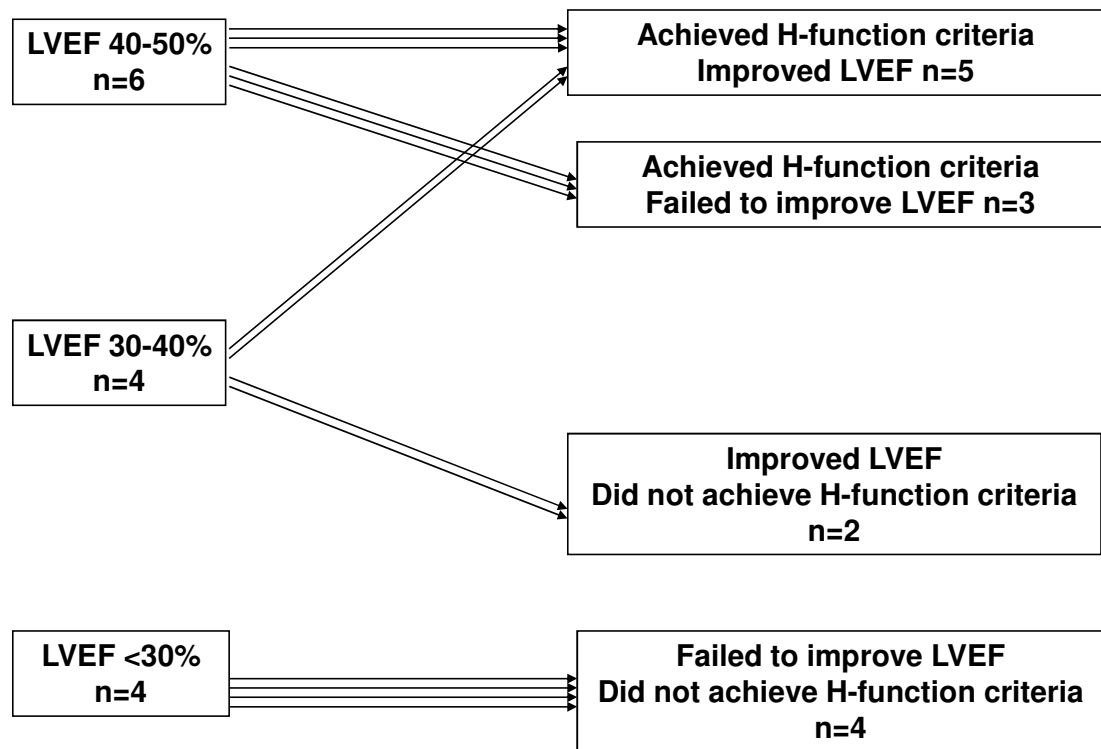


Figure 11-5-Echocardiographic and haemodynamic change in donors with subnormal LVEF according to degree of LV impairment, where repeat measurement was available (n=14).

Each arrow denotes a single donor. Eight of 14 donors attained hemodynamic suitability.

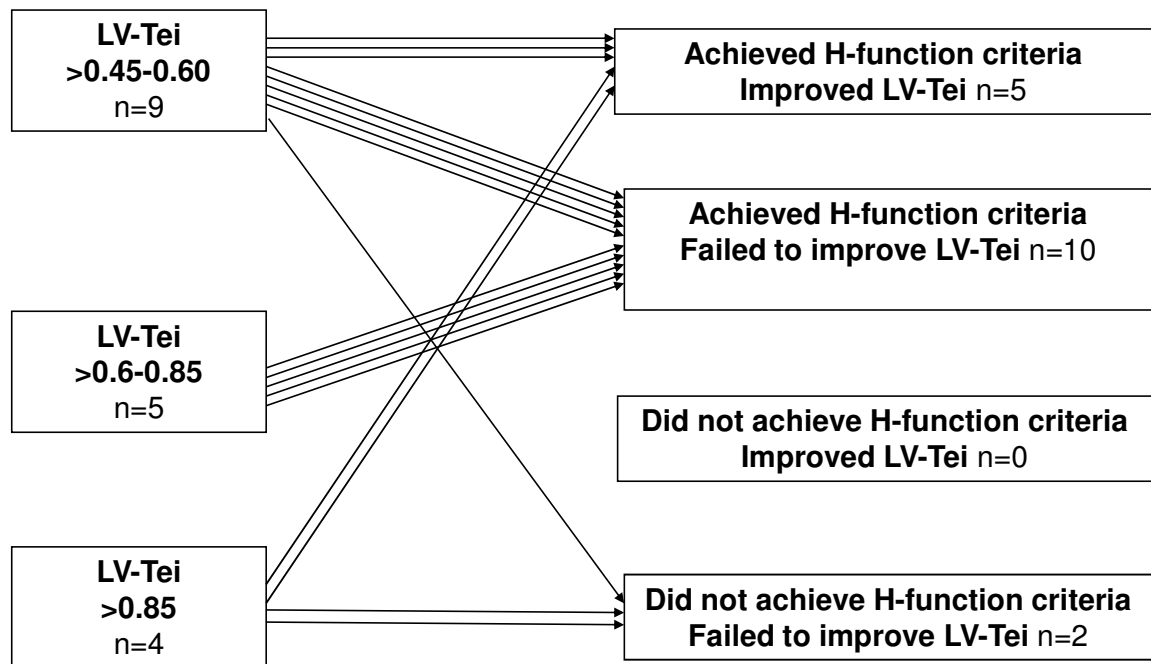


Figure 11-6-Echocardiographic and hemodynamic change in donors with abnormal LV-Tei index where repeat measurement was available (n=18).

Each arrow denotes a single donor. Fifteen of 18 donors attained hemodynamic suitability. Numbers are slightly different to Figure-11.3 which included all modes of LV E-function assessment and some donors had both LVEF and LV-Tei indices assessed.

11.3.5-Predictors of end-assessment functional suitability

Within the 66 donor study cohort, 37 had initially normal LV E-function and 28/37 of these had H-function suitable for transplant at end-assessment. In the 29(44%) hearts with initial subnormal LV systolic E-function, 17/29 achieved the H-function suitability criteria at end-assessment; a total of 45/66(68%) of investigated hearts achieving the H-function criteria (Figure11-7).

To assess whether the initially measured E-function parameters, LVEF, LVFS and LV Tei index predicted end-assessment H-function suitability, initial LVEF, LVFS, LV index were compared as continuous variables by univariate and multivariate analysis, along with donor age, time from coning, receipt of T3 and MP including all cases with missing data. On univariate analysis baseline higher initial LVFS ($p=0.02$) and LVEF ($p=0.006$, lower LV-Tei index ($p=0.03$) and a longer time after coning ($p=0.01$) predicted subsequent heart usability. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for normal E-function are shown in Table-11.3.

On stepwise logistic regression analysis, baseline LVEF (Odds ratio=1.05; 95%CL 1.007, 1.088; $p=0.021$) independently predicted ultimate functional suitability of hearts for transplantation. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for normal E-function (by any or all parameters) in predicting end-assessment functional suitability were 62%, 66%, 70% and 58% respectively.

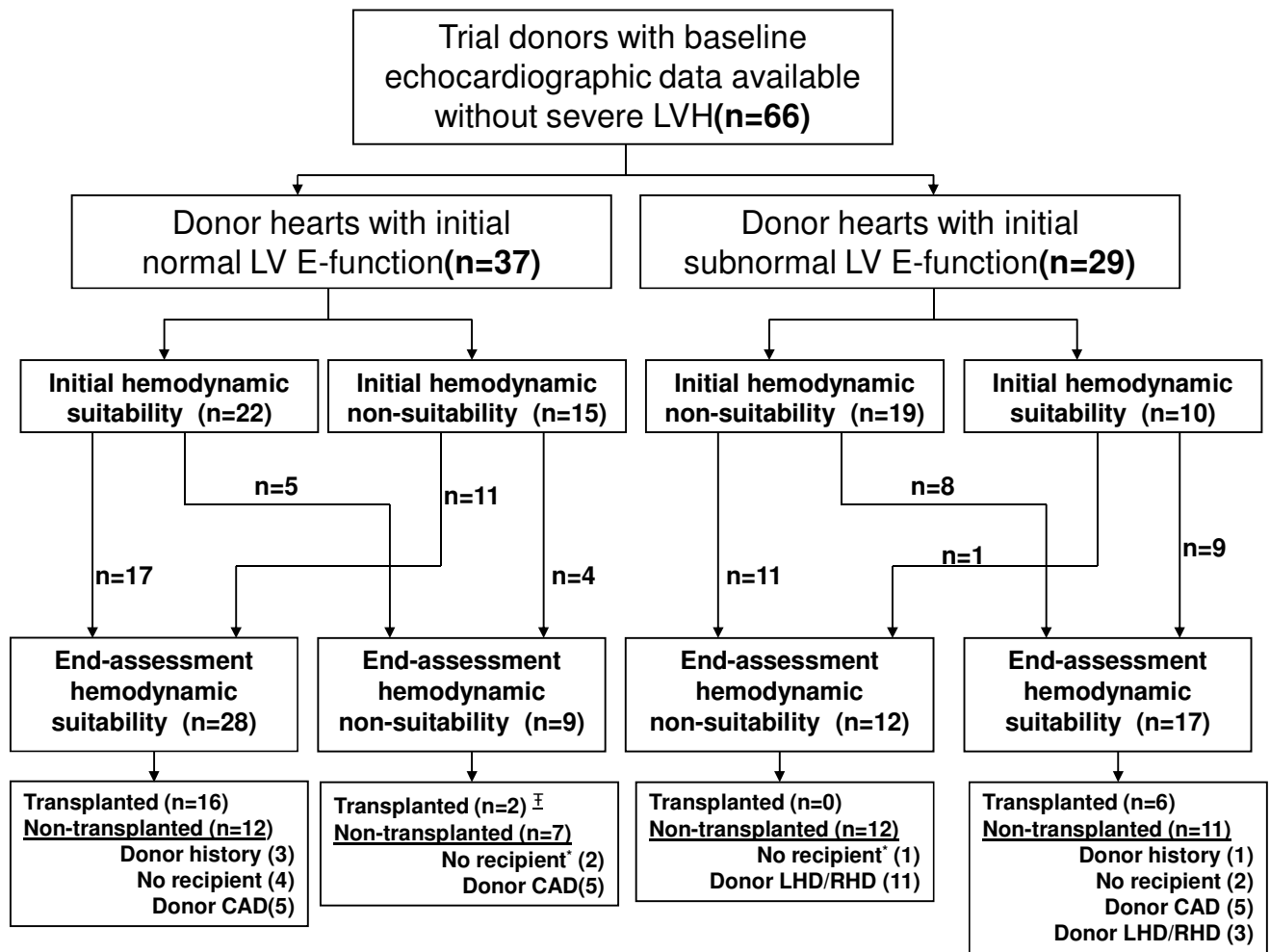


Figure 11-7-Flow diagram in the 66 donors with initial echocardiographic functional assessment showing outcome towards hemodynamic functional suitability for transplantation

Two hearts[‡] below H-function criteria were transplanted, one had a lower mean arterial pressure and one a high pulmonary capillary wedge pressure while all other criteria were attained. Three hearts^{*} not achieving hemodynamic function suitability had been declined for transplant prior to full assessment due to no suitable recipients identified. Ten hearts, functionally suitable were rejected due to palpable coronary artery disease.

CAD -coronary artery disease, L-HD and R-RHD – left- or right- heart dysfunction

Table 11-3-Demonstrating normal E-function predicting suitable hearts for transplantation

Parameter	Sensitivity	Specificity	PPV	NPV
LVEF>50%	85%	65%	79%	73%
LVFS>30%	56%	65%	71%	48%
Normal LV systolic function	62%	66%	70%	58%
LV Tei index ≤ 0.45	62%	50%	62%	50%

11.3.6-Findings at operating room inspection, transplantation outcome

Regardless of provisional acceptance for transplantation, each echocardiographically assessed heart was inspected in the operating room. LVH was notable in the 4 (wall thickness>1.7 cm) hearts subsequently confirmed to have severe echocardiographic LVH and in each of these there was concomitant palpable coronary artery disease (CAD). Palpable coronary artery disease (CAD) was also found in a further 15 donor hearts and in the absence of coronary angiography these hearts were rejected for transplantation. Neither demographic descriptors nor echocardiographic parameters were different in these 15 donors.

The outcomes for the 66 hearts are described in Figure 5. Twenty-four hearts were retrieved and transplanted with 100% graft and patient survival at 30 days and no

hearts required mechanical assistance. Thirteen functionally suitable hearts were not retrieved due to inability to identify suitable recipients (n=9) or non-acceptance due to adverse donor history (n=4). Each of the remaining 14 donor hearts that did not achieve functional suitability had macroscopic right or left heart dysfunction on direct inspection.

11.4-Discussion

The accrual of echocardiographic images without immediate interpretation in this study provides a unique insight into the inter-relationship of echocardiography and haemodynamic assessment in potential heart donors. The two methods of investigation appear complimentary. Echocardiography can identify structural valve abnormalities and ventricular hypertrophy (10% in this study) as well as providing an initial ventricular function assessment which, if normal, is predictive that functional suitability will be attained. PAFC-guided donor assessment can confirm the functional suitability of echocardiographically normal hearts and guide the optimisation of those hearts with either subnormal echocardiographic function or sub-optimal haemodynamic findings to suitable status. This dual investigational approach should be considered in all potential heart donors.

Although normal E-function at initial assessment is predictive of post-management functional suitability, specificity is poor and with optimisation, a significant number of hearts with initially abnormal echocardiographic function could improve their H-function and achieve transplantable status even if E-function does not change. This confirms that subnormal echocardiographic LV systolic function does not preclude successful transplantation (112;122;162), provided that other risk factors for early

graft failure are not disregarded (164-166). Only hearts with an initial LVEF <30% failed to improve with management and baseline LVEF independently predicted the subsequent heart usability for transplantation.

From a practical perspective, LVEF, LVFS and LV Tei are relatively easily-acquired objective assessments of LV systolic function. Of these, LV Tei index was obtained most frequently during donor assessment. The LV Tei index is a Doppler-derived index which, although requiring correct alignment, does not demand the same high image quality as biplane LVEF. It correlates closely with invasive measures of both systolic (+dP/dt) and diastolic function (-dP/dt and Tau), and is independent of heart rate and of LV geometry (116). Thus, although the Tei index is not load-independent, it is less dependent than volumetric LVEF, which is particularly sensitive to the large variations in loading, common in the cardiac donor (167). Previous data have documented that the Tei index has incremental prognostic significance beyond volumetric LVEF in a number of other clinical situations (168-170).

Despite the inherent difficulties of TTE in BSD patients who are intubated, mechanically ventilated and have relatively fixed supine positioning, image acquisition was possible in 87.5% of the trial cohort and repeat examination was feasible in 79% of these. Serial echocardiography in donor heart assessment has only previously been reported in smaller studies (104;123) but has demonstrated the capacity for E-function to improve. Our study corroborates this finding, demonstrating the potential for both echocardiographic and hemodynamic improvement. H-function improvement, to a level permitting heart transplantation may occur independently of E-function change and thus repeat echocardiography seems unnecessary in

determining the response to management provided that haemodynamic optimisation is achieved.

The use of hormonal therapy, particularly T3 in potential heart donors remains controversial. Several observational studies have suggested that T3 therapy may improve donor heart function but a number of randomized studies have found T3 to be of no benefit (86;157). In this study, neither initial thyroid functional status, nor T3 or MP therapy affected any parameter of LV function or predicted end-assessment functional suitability. In patients receiving T3, there was a significant improvement in the RV Tei index. This effect should be viewed with caution as numbers are small and we have previously reported that thermodilution RVEF does not change with management (157). However, as the right is potentially the more compromised ventricle following BSD a possible beneficial effect of T3 on RV dysfunction in both donor and recipient should be investigated further.

The association of better E-function with longer time from coning is concordant with the findings of haemodynamic and cardiac troponin studies and is consistent with the hypothesis that BSD generates a time-dependent reversible stunning phenomenon within some donor hearts (168). If corroborated, this finding has implications for donor management inferring that a longer period of donor observation and management following BSD may lead to more donor hearts becoming utilizable for transplantation and allowing exclusion of those hearts destined to fail to respond to therapy.

The study has several limitations. The pragmatic study design assessing all possible donors meant that there were a large number of changing variables that could influence detectable changes on echocardiography between the first and second

study e.g. loading conditions, inotrope and noradrenaline usage and time from coning. Echocardiographic contrast to optimise border detection and accuracy was not used and might improve prediction (169). Also, Doppler data on cardiac output from velocity-time integral data were not collected, which would have been useful to attempt to correlate changes in echocardiographic parameters with invasive data. Importantly, we were not able to assess E-function in recipients as these were dispersed nationwide. However, the 100% 30-day survival indicates that initial donor heart function remained satisfactory without early graft failure; indicative of the appropriateness of the H-function criteria used. Additionally, the study remains numerically small and this reflects the logistical difficulties of undertaking such investigations in multiple intensive care units in the setting of BSD. Further studies are required to determine if there is a cut-off in LV systolic function below which attempted donor resuscitation is futile.

The third component of donor heart assessment is inspection in the operating room. This allows further assessment of LVH and allows visual inspection of, particularly the right ventricle, potentially the most vulnerable part of the donor heart. In this study only those hearts not attaining the haemodynamic functional criteria displayed macroscopic LV or RV dysfunction on inspection.

If donor coronary arteriography is unavailable, there is a reliance on direct inspection and palpation to detect CAD which, if present, usually leads to rejection of the heart for transplantation. Any relationship between subjective CAD assessment, based on inspection and palpation, and haemodynamically significant CAD assessed objectively using coronary angiography remains undefined. Palpable CAD was the cause for heart rejection in 15 functionally suitable donors in this study.

Unfortunately, explantation and bench angiography to assess the relevance of this finding was not available. It is quite possible that some donor hearts with palpable CAD will not have flow-limiting lesions and could be safely utilised for transplantation. Conversely, in view of the ubiquitous nature of coronary atheroma, it is also likely that significant CAD could go undetected. At a time when donor numbers are diminishing, there is a need to increase the availability of coronary arteriography or other objective investigations both to further explore the possible yield from the existing donor pool and to exclude hearts with covert but extensive disease (68).

Finally, TTE has limitation in image acquisition due to lack of acoustic windows in supine, intubated, ventilated organ donor. Smaller TOE probes, with portable echocardiography consoles incorporated with tissue doppler facilities are more preferable in an organ donor. This will increase the image acquisition and repeatability during donor management. Studies using TOE analysis of donor heart function during management will help to address this issue in the future.

11.5-Conclusion

TTE is possible in the majority of potential heart donors. Normal LV systolic function is a strong predictor that hearts will be functionally suitable for transplantation but subnormal function does not preclude haemodynamic improvement. Serial echocardiography is feasible but does not improve prediction of post-optimisation functional suitability. Neither initial thyroid functional status, nor T3±MP therapy affect E-function. The study leads me to recommend that both echocardiography and PAC-guided assessment and management should be standard approaches to the potential heart donor.

12. A STUDY INTO THE IMPACT OF EARLY DONOR MANAGEMENT AND HORMONE REPLACEMENT THERAPY IN INCREASING THE RETRIEVAL RATE OF LUNGS FOR TRANSPLANTATION

12.1-Introduction

Lung transplantation is increasing (171) but demand outweighs supply with a significant waiting list mortality (172). Only approximately 20% of post-brainstem death (BSD) cadaveric donor lungs are ultimately used for transplantation and there is a need to investigate whether this utilisation rate can be increased without jeopardizing outcome. Lung donation may be compromised by lung injury, which may occur before and after BSD due to trauma, aspiration, infection, fluid overload and ventilatory barotrauma. Also, during BSD, hemodynamic shear forces and pro-oedematous hydrostatic pressures may cause direct injury. Each of these modes of injury may be exacerbated by a pro-inflammatory post-BSD environment (16;45;173;174). Steroids may stabilise cellular membranes, reduce up-regulation of HLA antigens (175), inhibit or prevent alterations in cytokines (89) and upregulate alveolar fluid clearance (AFC) (92;97). A retrospective study reported increased yield and improved donor lung function after early methylprednisolone (MP) administration (96). However, the effect of MP has not been investigated prospectively. Tri-iodothyronine (T3) is commonly used to putatively improve donor heart function. Improved cardiac function, with reduced hydrostatic pressures might limit lung water accumulation and in addition, T3 increases AFC (97). I therefore investigated the

effects of MP and T3 (administered early following consent for organ donation) on heart and lung function in potential heart and lung donors in a randomized trial.

Within this Trial, in addition to receiving hormone therapy, potential donors were actively managed to try and maintain organ function. As combination, MP, T3 and vasopressin (VP) therapy has been associated with higher organ retrieval rates (80) and as active management of marginal lung donors is reported to increase retrieval rate (176-179), I additionally compared lung yield between the Trial donors and a contemporary cohort of non-Trial donors.

12.2-Materials and methods

12.2.1-Study design

Between, January-2004 and April-2006, I accrued data on potential lung donors within our zone. Study inclusion and exclusion criteria are already discussed. Marginal organs were defined as a $\text{PaO}_2/\text{FiO}_2$ ratio <320 but >230 at referral. The lungs of all such donors were offered to transplant units via donor procurement coordinators (DPCs) and accepted or rejected on the basis of size, blood group, chest x-ray findings, arterial blood gases (ABG), the presence of endotracheal secretions and medical history. Within this cohort, we approached the next of kin for consent to enter into a prospective, double-blind, placebo-controlled, randomized trial. Consent was obtained from the next-of-kin for all participating donors according to the MREC approval. The Trial was conducted independent of whether donor lungs were provisionally accepted for lung transplantation. Lungs previously declined were not re-offered following management.

12.2.2-Assessment and management of non-Trial donors

Non-Trial donors were managed using a standard protocol by DPCs in collaboration with local ITU staff with the aims of maintaining haemodynamic stability, limiting fluid overload and substituting noradrenaline (NA) with VP in donors provisionally accepted for cardiac transplantation. In non-trial donors, PAFC insertion, bronchoscopy and hormone replacement therapy with MP and T3 only occurs in accepted heart or lung donors following transfer to the operating room (OR) for the retrieval procedure several hours following initial assessment. EVLWI measurement would not be routinely undertaken in this cohort.

12.2.3- Assessment and management of trial donors

Trial donors were attended on the ICU by me as soon as feasible, following donation and study consent. At initial assessment they underwent ABG (mmHg) measurement (Fractional inspired oxygen (FiO_2) 1.0), Positive end-expiratory pressure (PEEP) 5cmH₂O). This was followed by insertion of invasive monitoring lines as describe previously. In addition to all haemodynamic parameters, extravascular lung water index (EVLWI) (normal 3-7ml.kg⁻¹), pulmonary vascular permeability index (PVPI) and pulmonary vascular resistance (PVR) were measured. The underlying principles and analysis of lung water assessment have been previously reported (128). Following initial assessment, bronchoscopy was performed to assess anatomy and endotracheal tube placement, to aspirate secretions, to detect evidence of active bronchitis or aspiration and to obtain a broncho-alveolar lavage (BAL) specimen for culture. Thereafter, lung management protocol was followed as described previously. Trial medication was then administered; T3 (0.8µg.kg⁻¹ IV bolus followed by 0.113µg.kg⁻¹. hr⁻¹ IV infusion); MP (1000mg IV) as a single dose; both drugs or

placebo (dextrose 5%) and the T3/placebo infusion continued until retrieval. All medical, nursing and technical staff involved in the research or donor care remained blinded to treatment allocation until study completion.

Active hemodynamic management was simultaneously commenced according to specific algorithm. Systemic vascular resistance was maintained in the range 800-1200 dynes.cm.sec⁻⁵ by actively substituting VP for NE. Crystalloid infusions were not used to replace urine output except in hypernatremic donors (n=3).

Management, continued in the ICU and OR until retrieval. Changes in lung function and bronchoscopy findings were conveyed to the recipient centers which had provisionally accepted lungs for transplantation. The duration of treatment and time of coning (detection of fixed dilated pupils and BP surge) were noted.

Hemodynamic studies, PaO₂/FiO₂ ratio at FiO₂ 1.0, EVLWI, PVPI and PVR were performed at baseline and repeated after initial bronchoscopic examination, 1 hour thereafter and immediately pre-retrieval. Pre-retrieval ABG measurements were performed after direct inspection and manual inflation to recruit atelectatic lung segments where possible. After each measurement, FiO₂ was reduced, as after initial assessment.

12.2.4-Endpoints and statistics

The pre-planned primary Trial end-point was a difference in PaO₂/FiO₂ of one standard deviation (SD)) between groups at end-management. The Trial had 80% power ($\alpha = 0.05$) to detect this with recruitment of 4 groups of 24 allowing for multiple comparisons. Actual recruitment did not reach this target but the study remained powered to allow satisfactory comparison of this end-point in donors receiving MP or no MP or T3 or no T3. The Trial was also initially powered to detect a 20% absolute

increase in the number of lungs transplanted versus a historical or non-Trial cohort. Secondary Trial end-points included other functional data, the effect of NA administration in donors and the transplant suitability at the end-management. Suitability was defined as a $\text{PaO}_2/\text{FiO}_2 \geq 300$ without identifying lung trauma, aspiration, infection or non-recrutable atelectasis during assessment and at direct inspection. Data were analyzed using SPSS v12.0 (Chicago, IL). Continuous data were assessed for normality and are presented as mean \pm SD (95% confidence limits (CL)) or median (inter-quartile range (IQR)). Normally distributed variables were tested using independent or paired sample t tests. Skewed data were tested using non-parametric test (Mann-Whitney and Kruskal-Wallis test). Categorical data were analyzed using χ^2 and Fisher's Exact testing. Serial measurements were compared using repeated measures ANOVA. Statistical significance was assigned when $p \leq 0.05$. Univariate and multivariate analysis (stepwise logistic regression) was used to identify factors that predicted suitability for transplant at end-assessment.

12.3-Results

Study enrolment is summarized in Figure-12.1. During the period of Trial recruitment, there were a total of 254 donors, of which 182 fulfilled the study inclusion criteria with consent to lung donation. Of these 118 were within the specified distance for inclusion into the Trial. Thirty could not be considered for logistical reasons and study consent was withheld in 28 giving a Trial group (T) of $n=60$ and a consent rate of 68%. The non-Trial (non-T) group ($n=122$) comprised those donors outside the specified inclusion distance, those excluded for logistical reasons and those in whom

consent was obtained for donation but not the Trial. Twenty of the overall 254 donors were only eligible for the cardiac study and are excluded from the analyses.

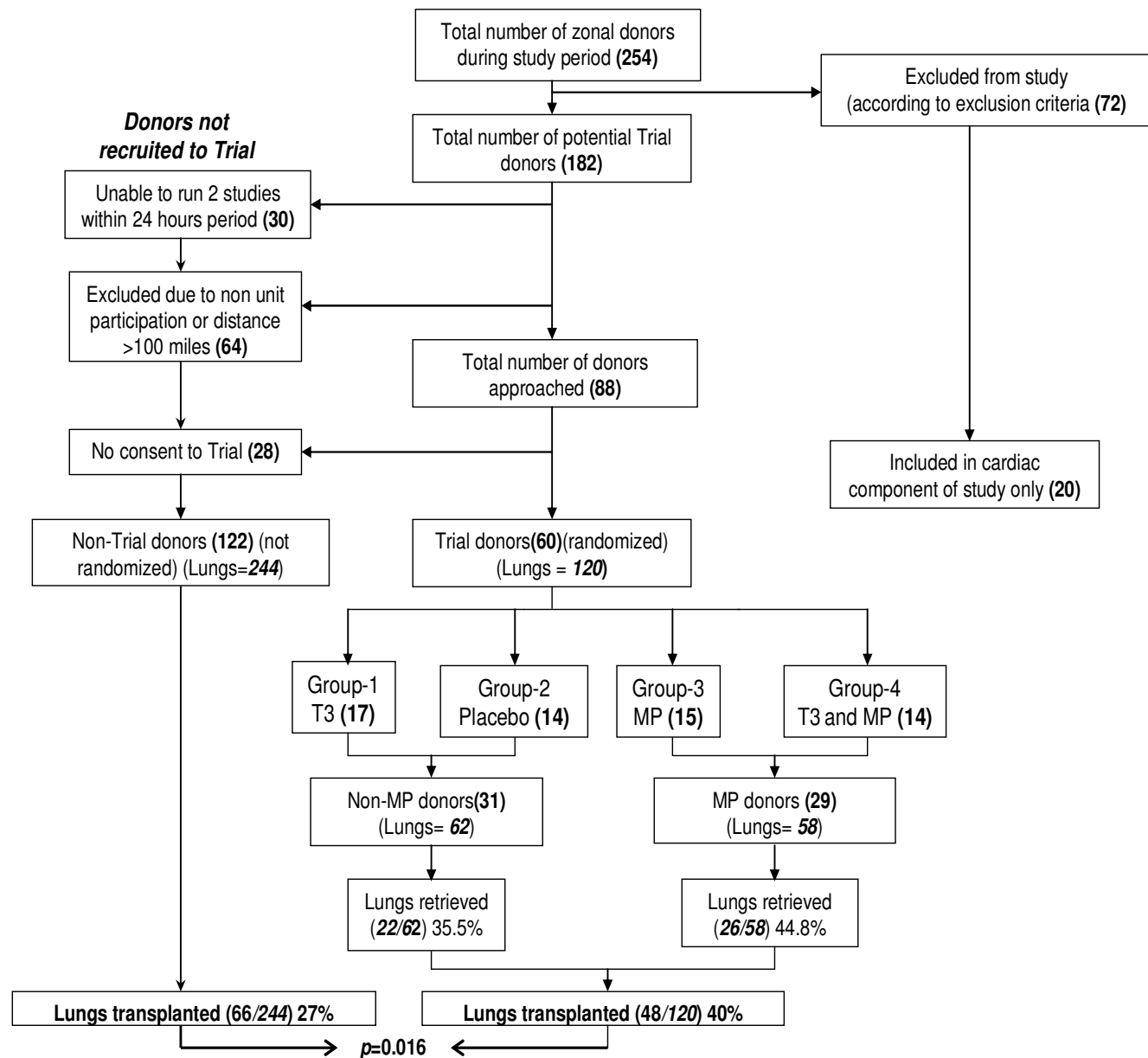


Figure 12-1-CONSORT diagram demonstrating study enrolment, exclusion, allocation to treatment groups and number of lungs transplanted from Trial and non-Trial cohorts.

Donor numbers are in bold and donor lungs in bold italics. The proportion of lungs transplanted was higher in the Trial cohort (p=0.0061)

The median age, mean PaO₂/FiO₂ ratio, donor cause of death, percentage provisional acceptance for transplantation and fraction of marginal donors between T and non-T were not different (Table-12.1)

Table 12-1-Characteristics of Trial and non-Trial donors

Parameter	Trial donor	Non-Trial donor	p-value
Age	47(36, 56)	45(37, 53)	=0.65
Baseline PaO ₂ /FIO ₂ ratio	396±79 (377-418)	394±102 (375-412)	=0.85
Donor Cause of death			
Vascular & Tumour	83/122 (68%)	40/60 (67%)	=0.98
Traumatic	28 (23%)	14/60 (23%)	=0.89
Hypoxic	8/122 (6.5%)	3/60 (5%)	=0.93
CNS infection	3/122 (2.5%)	3/60 (5%)	=0.64
Provisional acceptance and marginal fraction of donor lungs			
Provisional acceptance	64/120 (53%)	128/244 (52.5%)	=0.96
Marginal donors	15/60 (25%)	33/122 (27%)	=0.9

Characteristics of Trial and non-Trial donors

Values are presented as median (25, 75 centiles), mean ± SD (95%CL) and numerator/ denominator (percentage). CNS = central nervous system

Within the Trial, management commenced within a median of 2 (IQR 0.5-3.5) hours of consent, within a mean of 12.5 ± 8.1 (95%CL10.3-14.6) hours of coning and continued for 6.9 ± 1.2 (95%CL6.6-7.3) hours. Trial drugs were commenced 5.9 ± 1.2 (95%CL5.7-6.3) hours prior to retrieval or end-assessment. There was no difference in these variables between treatment groups. During the management period Trial donors received 376ml (95%CL289-463) of colloid to maintain CVP/PCWP and 27ml (95%CL4-50) of crystalloid. Bronchoscopy revealed abnormalities in 20 donors, including endotracheal tube malposition (7), evidence consistent with aspiration (4), edema (2) and excessive purulent secretions (7). Malposition was corrected and secretions cleared. Broncho-alveolar lavage yielded positive cultures in 31 donors (5/7 with excessive secretions). Organisms cultured included staphylococcal aureus (10), streptococcus pneumoniae (6), Gram positive anaerobes (1), Serratia spp. (3), Escherichia coli (3), Pseudomonas spp. (2), Klebsiella pneumoniae (4) and Candida albicans (2).

12.3.1-Donor lung function within the treatment groups of the Trial

Demographic details, suitability and donor lung function for the 4 treatment groups are summarized in Table-12.2. Baseline lung function did not differ and the administration of MP and T3, alone or in combination, did not affect the $\text{PaO}_2/\text{FiO}_2$ ratio or any other parameter compared to placebo.

Table 12-2-Donor parameters between the four Trial treatment groups

Parameters	T3 Group (n=17)	MP Group (n=15)	T3+MP Group (n=14)	Placebo (n=14)	p-value
Demographics					
Age	55(40, 56)	41(38, 47)	34(26, 53)	52(35, 56)	0.15
Hospital stay (days)	2(1, 3)	1(0, 2)	2(0, 4)	2(0, 4)	0.56
Duration of ventilation	1(0, 2)	1(0, 2)	2(0, 4)	2(1, 3)	0.2
BSD to treatment (h)	12.4±6.6	10.3±6.5)	13.4±7.6	14.1±11.2	0.81
Duration of treatment (h)	7.5±1.2	6.6±1.1	6.7±1.2	7±1.1	0.44
Drug treatment duration (h)	6.3±1.3	5.7±1.2	5.9±1.1	6±1.1	0.19
Lung suitable for transplantation					
	18	18	12	12	0.09
Donor lung function					
Baseline PaO ₂ /FIO ₂ ratio	398±79	392±70	383± 85	413± 87	0.78
Baseline EVLWI	9± 5.2	9.9± 4.3	9.5±4.2	9.9± 3.7	0.78
Baseline PVPI	2.2± 0.7	2.4± 0.9	2.6±1.4	2.4±0.8	0.67
Baseline PVR	151± 159	117± 73	106±65	111± 67	0.65
Baseline PCWP	9.2± 5.1	6.7±4.5	8.3±3.5	9.4±6.1	0.43
Δ- PaO ₂ /FIO ₂ ratio	50± 125	29± 113	35± 157	37± 132	0.98
Δ-EVLWI	2.3±2.8	-0.6±3.9	0.5±2.1	1.5±2.3	0.06
Δ-PVPI	0.6± 1.2	0.04± 0.4	0.08± 0.8	0.1± 0.9	0.21
Δ-PVR	8.5±123	2.2± 24	-35±114	-23.9± 87	0.58

Demographic and pulmonary function parameters in the 4 treatment groups of the Trial values are presented as median (25, 75 centiles) or mean ± SD. EVLWI as ml.kg⁻¹ and PVR as dyne.sec.cm⁻⁵. Changes in PaO₂/FiO₂ ratio, EVLWI, PVPI and PVR are presented as Δ-(Baseline value-pre-retrieval value)

12.3.2-Donor lung function according to receipt of hormonal therapy

In the post hoc analysis I compared outcomes in donors receiving or not receiving MP (\pm T3) or T3 (\pm MP). MP donors were younger ($p=0.036$) (Table-12.3). MP administration did not affect any absolute parameter (Figure 12-2) although it was associated with a reduced accumulation of EVLWI ($p=0.009$) and a lower pre-retrieval PCWP ($p=0.03$). T3 administration did not affect any parameter.

**Table 12-3-Parameters in Trial donors receiving or not receiving
methylprednisolone**

	Non-MP donors (n=31)	MP donors (n=29)	p-value
Donor demographics			
Age	53(38, 56)	39(30, 48)	0.027
Hospital stay (d)	2(0, 4)	1(0, 2)	0.36
Duration of ventilation (d)	2(1, 3)	1(0, 2)	0.4
BSD to treatment(h)	13.2±9 (9.8-16.6)	11.7±7 (8.9-14.5)	0.53
Duration of donor management (h)	7.2±1.2 (6.8-7.7)	6.7±1.2 (6.2-7.7)	0.15
Duration of drug administration (h)	6.2±1.2 (5.7-6.6)	5.8±1.1 (5.3-6.2)	0.07
Lungs suitable for transplantation			
	30	30	1
Donor lung function			
Baseline PaO ₂ /FIO ₂ ratio	407± 81 (377-437)	388±77 (359-417)	0.35
Baseline EVLWI	9.4±4.6 (7.7-11.1)	9.8±4.1 (8.2-11.4)	0.47
Baseline PVPI	2.3±0.7 (2.0-2.6)	2.5±1.1 (2.1-2.9)	0.17
Baseline PVR	133±126 (87-180)	114±68 (87-141)	0.38
Baseline PCWP	9.3± (7.3-11.3)	7.4± (5.8-9.1)	0.15
Δ- PaO ₂ /FIO ₂ ratio	44±126 (-4-,91)	32±133 (-19-83)	0.73
Δ-EVLWI	1.9±2.6 (1.0-2.9)	0.03±3.1 (-1.1-1.2)	0.009
Δ-PVPI	0.4± 1.1 (0.02-0.9)	0.1±0.6 (-0.1-0.3)	0.25
Δ-PVR	-6±107 (-45-33)	-15±80 (-47-17)	0.65
Δ-PCWP	-2.9± 6.3 (-5.2- -0.6)	-2.4±2.7 (-3.5- -1.3)	0.74
Pre-retrieval PaO ₂ /FIO ₂ ratio	361±124 (315-408)	356±115 (312-399)	0.85
Pre-retrieval EVLWI	11.4±5.9 (9.2-13.4)	10.2±4.4 (8.5-11.9)	0.37
Pre-retrieval PVPI	2.7±1.4 (2.2-3.2)	2.7±1.1 (2.2-3.1)	0.93

Pre-retrieval PVR	139±109 (99-179.4)	126±99 (86-166)	0.64
Pre-retrieval PCWP	12.2±4 (10.7-13.6)	9.8±4.2 (8.1-11.5)	0.03

Characteristics and pulmonary function parameters in Trial donors according to receipt of methylprednisolone (MP) Values are presented as mean± SD (95% CL) or median (25, 75centiles). EVLWI presented as ml.kg⁻¹ and PVR as dyne.sec.cm⁻⁵. Changes in PaO₂/FiO₂ ratio, EVLWI, PVPI and PVR are presented as Δ (Baseline value-pre-retrieval value).

12.3.3-Observations in the whole *Trial* population

In the 60 Trial donors, between baseline assessment and retrieval, lung function deteriorated significantly (Table-12.4). PaO₂/FiO₂ fell while EVLWI and PVPI both increased, PVR remained unchanged. PaO₂/FiO₂ rose following lung inspection and recruitment of atelectatic segments (Figure-12.2)

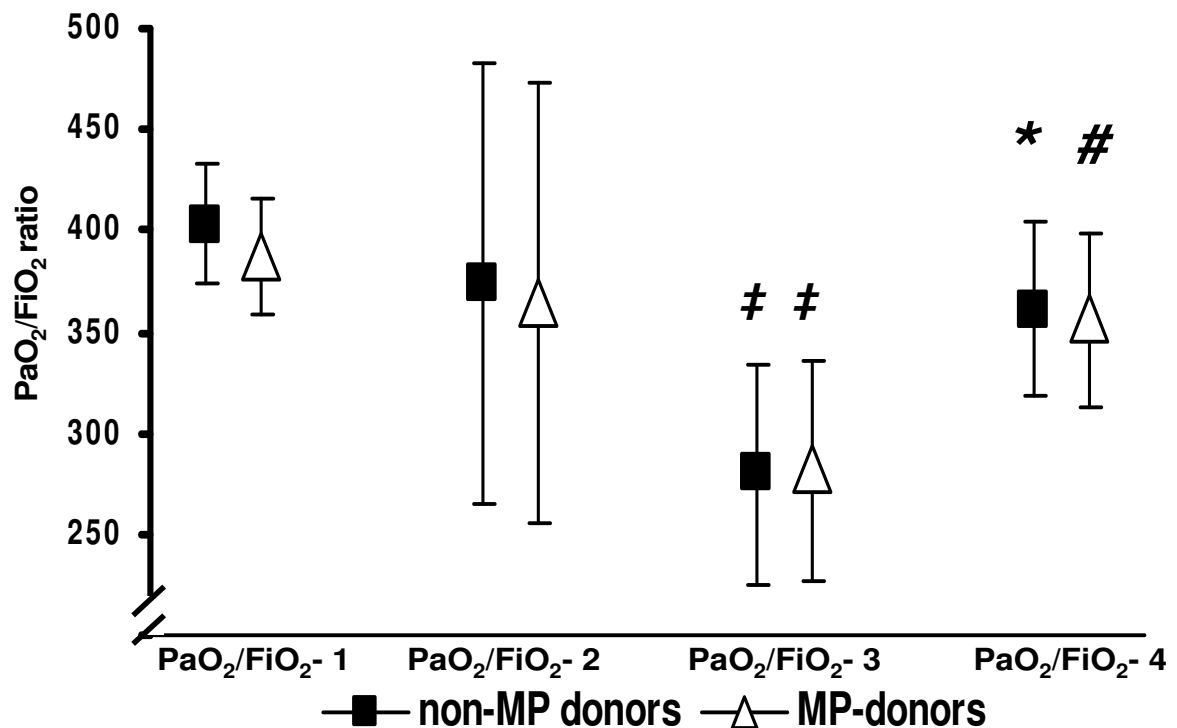


Figure 12-2-Changes in PaO₂/FiO₂ ratio \pm 95% confidence intervals in donors receiving (MP n=29) or not receiving (non-MP n=31) methylprednisolone

PaO₂/FiO₂-1 was measured before initial bronchoscopic examination and hemodynamic studies. PaO₂/FiO₂-2 was measured following bronchoscopy and PaO₂/FiO₂-3 was measured one hour after the 2nd measurement. PaO₂/FiO₂-4 was measured at end-assessment in the OR after inspection of the donor lungs. The mean time between PaO₂/FiO₂-1 and end-assessment PaO₂/FiO₂ was 6.9 hours. Note the y axis does not commence at zero. There was a significant deterioration between baseline and PaO₂/FiO₂-3 ($p < 0.001$) in both groups but improvement following inspection and recruitment of atelectatic segments (non-MP* $p = 0.005$; MP# $p = 0.0135$). There was no difference between groups ($p = 0.969$).

Table 12-4-Overall parameters of lung function within Trial donors (n=60)

Parameter	Baseline value	Pre-retrieval value	p-value
PaO ₂ /FIO ₂ ratio	397±78 (376- 417)	359±126 (328-390)	=0.028
EVLWI	9.7±4.5 (8.6-10.9)	10.8± 5.2 (9.4-12.2)	=0.009
PVPI	2.4±0.9 (2.2-2.7)	2.7±1.2 (2.4-3.0)	=0.025
PVR	123±103 (96-151)	133±104 (106-161)	=0.50

Changes in pulmonary function observed in all Trial donors (n=60).

Values are presented as mean± SD (95% CL). EVLWI presented as ml.kg⁻¹ and PVR as dyne.sec.cm⁻⁵.

At initial assessment 35/60 (58%) of Trial donors were receiving NA. By adding VP, it was possible to withdraw NA completely in 21/35(60%) and reduce NA from 0.25±0.3µg.kg⁻¹min⁻¹ (95%CL0.13-0.36) to 0.11±0.27µg.kg⁻¹min⁻¹(95%CL0.01-0.2) in the remainder (p<0.001). In donors receiving NA at initial assessment, PaO₂/FiO₂ ratio deteriorated (p=0.038) from 398± 84(95%CL368-427) to 350±131 (95%CL304-396) compared to 395±73 (95%CL364-425) and 370±100 (95%CL328-412) in non-NA donors (p=0.35). EVLWI also increased (p=0.04) in NA donors from 10.1±5.2 (95% CL8.3-11.9) to 11.4±6.2ml.kg⁻¹(95%CL 9.3-13.4). These changes were not influenced by the ability to withdraw NA.

12.3.4-Suitability of lungs for transplantation and transplant outcomes

More donors yielded lungs for transplantation in the Trial cohort (26/60 versus 35/122; $p=0.054$) and the number of lungs transplanted was significantly higher ($p=0.016$) in the Trial cohort (48/120; 43%) than the non-Trial cohort (66/244; 27%) (Fishers Exact test) (Figure-12.1). Although 48/120 Trial donor lungs were transplanted, a further 12 lungs (not provisionally accepted for transplantation) were also found to be suitable for transplantation at end-management.

Sixty lungs were deemed unsuitable due to deteriorating $\text{PaO}_2/\text{FiO}_2$ or hemodynamic features ($n=30$), abnormality on inspection (bullous disease, adhesions, intractable atelectasis ($n=16$), marginal $\text{PaO}_2/\text{FiO}_2$ ratio \pm excessive secretions in conjunction with adverse donor history (age >60 years, mild asthma and smoking) ($n=10$) and hepatitis C positivity ($n=4$). In marginal Trial donors ($n=15$), $\text{PaO}_2/\text{FiO}_2$ increased slightly (292 ± 34 (95%CL273-311) to 331 ± 152 (95%CL247-416) although this change was not significant ($p=0.34$). Administration of methylprednisolone in these marginal lung donors did not influence the change in EVLWI ($p=0.15$) during management. Four of 30 marginal lungs were transplanted and 8/30 lungs met suitability criteria.

On univariate analysis, lower baseline EVLWI, particularly $\text{EVLWI} < 10 \text{ml.kg}^{-1}$ and shorter time from coning, predicted suitability for transplant at end-management (Table-12.5).

Table 12-5-Comparison of (Trial) donor parameters between suitable and non-suitable lungs for transplantation (Univariate analysis)

Parameter	Unsuitable lungs (n=60)	Suitable lungs (n=60)	p-value
Donor age	48(30, 55)	45(37, 56)	=0.89
Time from coning (h)	12±8.6 (0.75-23.25)	8.5±6.3 (2-15)	=0.02
Baseline PaO ₂ /FIO ₂ ratio	377±90 (342- 412)	416±64 (393-440)	=0.09
Baseline EVLWI	11.2±5.6 (8.9-13.3)	8.4±2.9 (7.4-9.6)	=0.02
Baseline PVPI	2.6±0.8 (2.2-2.9)	2.3±0.9 (1.9-2.6)	=0.13
Δ- PaO ₂ /FIO ₂ ratio	66±146 (10-123)	12±107 (-28-+53)	=0.1
Δ-EVLWI	-1.1±3.8 (-2.5, +0.5)	-0.9±1.9 (-1.6, -0.2)	=0.73
Δ-PVPI	-0.4±0.7 (-0.6, -0.04)	-0.08±0.6 (-0.3, 0. 2)	=0.09
MP positive	30/60 (50%)	30/60 (50%)	=1
NA positive	30/60 (50%)	38/60 (63%)	=0.3
Baseline- EVLWI <10ml.kg ⁻¹	34/60 (56%)	54/60 (90%)	=0.019

Univariate comparison of (Trial) donor factors between suitable (n=60) and non-suitable (n=60) lungs for transplantation. Values presented as median (25, 75 centiles), mean±SD (95%CL) or numerator/ denominator (percentage).

EVLWI presented in ml.kg⁻¹. MP-positive=donor receiving methylprednisolone.

NA-positive=donor receiving noradrenaline at initial assessment.

On stepwise logistic regression analysis higher baseline $\text{PaO}_2/\text{FiO}_2$ ratio (Odds ratio (OR) =1.014(95%CL1.0-1.025); $p=0.01$ and lower $\Delta\text{-EVLWI}$ (OR=1.66(95%CL1.13-2.5); $p=0.01$, independently predicted suitability. These data were not available in non-Trial donors preventing further analysis. The 30-day and 1 year survivals for Trial and non-Trial lung transplant recipients were 96.3% versus 92.1% ($p=0.636$) and 77.8% versus 76.3% respectively ($p=1$).

12.3.5. Effect of Trial management on other solid organ retrieval

Within the Trial, 25 hearts, 57 livers and 118 kidneys were transplanted. Non-use of these organs was based upon intrinsic abnormality or failure to identify suitable recipients. In 4 donors, the study assessment identified poor heart function and hemodynamic decline despite maintained MAP. In these, cardiothoracic organ offering was abandoned allowing rapid procurement of liver and kidneys.

12.4-Discussion

Active and early donor management, as undertaken in the *Trial* cohort, increased the yield of transplantable lungs from the existing donor pool with equivalent recipient outcomes and without jeopardizing other organ retrieval.

A third of donors with normal chest X-ray have bronchoscopic abnormalities that may be correctable (180). Bronchoscopy, by clearance of secretions and blood clots and correction of endotracheal tube malposition may improve lung function. It may also identify factors precluding lung donation and may thereby avoid delay in retrieving

other organs. Limited crystalloid administration also appears important and may maintain better gas exchange (101).

Combination of both MP±T3 might improve donor lung function via their effects on the heart, endothelium and lung epithelium. T3 had no effect but MP attenuated the accumulation of EVLWI, supporting its use in lung donors. Despite such active management, PaO₂/FiO₂ deteriorated with rising EVLWI and PVPI suggesting increasing permeability. The deterioration of PaO₂/FiO₂ ratio was not affected by MP or T3. Early MP (15 mg.kg⁻¹) administration has been associated with improved lung yield in a retrospective report but in this report, MP-treated donors were actively managed for a longer period which may have contributed to the observed changes. The study may have been under-powered to corroborate these findings but suggests that MP use in isolation is not a substitute for active management. Neither T3 nor MP administration was associated with worsening lung function and a beneficial post-transplantation effect cannot be excluded. Moreover, MP administration took place 12.5hours after coning was recognized and earlier administration may be necessary to realise any beneficial effect.

Vasoparetic hypotension post-BSD is commonly treated by NA, a potent but cardiotoxic α -adrenergic agonist (150). In the Trial, PaO₂/FiO₂ ratio and EVLWI deteriorated in donors receiving NA and this was not prevented by NA withdrawal. The reason for the association between NA and lung dysfunction is unclear. NA requirement may reflect greater cardiac dysfunction, a greater pro-inflammatory response or a deleterious effect on pulmonary endothelium. Donor pressor support with NA has been associated but worse prognosis in heart and lung transplantation

(152) and inferior post-transplant gas exchange (181)., Although NA usage did not affect yield in this study its use in potential lung or heart donors may be inadvisable.

EVLWI is a validated index of pulmonary oedema and may be elevated before changes in gas exchange, clinical status or chest X-ray (182;183). On univariate analysis, a normal EVLWI ($<10\text{ml}/\text{kg}^{-1}$) and on multivariate analysis, a lower Δ -EVLWI and higher baseline $\text{PaO}_2/\text{FiO}_2$ predicted the eventual suitability of lungs for transplantation. AFC, the capacity to remove alveolar water by active ion transport, remains normal in most donor lungs, even those rejected for transplantation (184;185) and normal AFC hastens resolution of post-transplantation edema (91). As increased AFC, in response to steroids and β -adrenergic stimulation has been documented in experimental neurogenic pulmonary oedema and human acute lung injury this may be the mechanism for the reduced Δ -EVLWI observed with MP. Thus, measurement and manipulation of lung water may become important in the assessment and management of potential lung donors (186;187).

On univariate analysis, a shorter time from coning predicted transplant suitability. This may reflect less time for secondary lung injury to occur due to fluid overload, aspiration, ventilatory barotrauma and nosocomial colonisation and a shorter duration of lung exposure to a hostile hemodynamic and pro-inflammatory environment. Earlier truncation of this exposure may retain better lung function.

Although, prospective, randomized, blinded and controlled, this Trial remains numerically small and as recruitment did not reach the intended sample size, it is under-powered to detect differences between individual therapy groups. However, as

both Trial and non-Trial donor cohorts had similar ages, initial $\text{PaO}_2/\text{FiO}_2$ ratios, proportions with marginal $\text{PaO}_2/\text{FiO}_2$ ratios and provisional acceptance rates, the study demonstrates the importance of donor management in increasing yield especially as Trial lungs were not re-offered following initial rejection. Although non-Trial donor care was based on a similar management protocol albeit without bronchoscopy or invasive monitoring, management was overseen by DPCs who are simultaneously engaged in a logistical process including acquisition of consent, donor family support, offering of organs to recipient centers, arranging the multi-organ retrieval procedure and transportation of organs and tissue. In contrast, I was wholly dedicated to the donor management and I would suggest that this dedicated donor management role is fundamental to maximize yield and this could be increased further if repeat offering of lungs improving post-management occur.

12.5-Conclusion

Lung transplant availability can be increased by better management of lung donors. Early steroid administration is a component part of this management but is not a substitute for intensive donor care.

13. A STUDY OF MEASUREMENT OF EXTRAVASCULAR LUNG WATER IN POTENTIAL LUNG DONORS

13.1. Introduction

Extra-vascular lung water (EVLW) may increase in brain-stem dead potential lung donors for a number of reasons, particularly over-zealous crystalloid fluid replacement, direct lung injury associated with the cardiovascular events of coning (16) and secondary effects related to the pro-inflammatory response or cardiac dysfunction (45;174). In these circumstances, lung water measurement may have clinical utility. EVLW can be measured in different ways (124;125) and but a widely validated method to derive a measure of EVLW is pulse-induced contour cardiac output (PiCCO), a single thermodilution technique based on the Stewart-Hamilton principle (128). The EVLW may be indexed to body weight and has a normal range of 3-7ml.kg⁻¹. Experimentally, EVLWI measured using this thermodilution method (EVLWI-T) has been shown to correlate well with lung water measured by gravimetry (EVLW-G) (133;188). Early data, using a thermal dye double indicator dilution technique in humans following BSD suggested that although such techniques over-estimated EVLWI-G, they provided a reliable index of lung water content and could be of clinical utility (132).

EVLWI elevation precedes clinical, radiological and oxygenation manifestations of lung oedema (182;183) and is a predictor of outcome in a critically ill patients with acute pulmonary oedema and acute respiratory distress syndrome (134).

In vivo assessment of the donor lung is currently reliant on donor history, chest-X-ray evaluation, arterial and pulmonary venous blood gas measurement, bronchoscopy and direct inspection. Despite best efforts during assessment, retrieval and transplantation, there is a disturbing incidence of lung injury manifest as primary graft dysfunction with impaired gas exchange and pulmonary infiltrates. In view of this, estimation of lung water content may enhance the assessment process of the donor lung. The aims of this observational study were to present the EVLWI findings in potential lung donors and to validate this measurement in lungs not used for transplantation. We also evaluated the role of EVLWI measurement during protocol guided donor management, assessed the changes in EVLWI in response to administration of methylprednisolone, its role in predicting lungs suitable for transplantation and recipient outcome following transplantation.

13.2. Methods

13.2.1. Study design

Between, January-2004 and April-2006, contemporaneous PiCCO EVLWI and arterial blood gas measurement (ABG) was performed in 60 potential lung donors recruited into a prospective study on early donor management and hormonal therapy. Additional consent was obtained when possible to retain lungs rejected for transplantation for gravimetric lung water assessment.

13.2.2. Donor management

At initial assessment all donors underwent ABG (mmHg) measurement at a fractional inspired oxygen (FiO_2) 1.0 and 5cmH₂O positive end-expiratory pressure (PEEP). Invasive monitoring lines were inserted as previously described for measurement of hemodynamic parameters, EVLWI (normal range 3-7ml.kg⁻¹) and pulmonary vascular permeability index (PVPI). The underlying principles and analysis of lung water assessment have been previously reported (128;129). The PVPI is the EVLW/pulmonary blood volume ratio. Elevated EVLW with low PVPI implies hydrostatic edema whereas a high EVLW and PVPI imply permeability edema. Following initial assessment, bronchoscopy was performed to assess anatomy and endotracheal tube placement, to aspirate secretions, to detect evidence of active bronchitis or aspiration and to obtain a broncho-alveolar lavage (BAL) specimen for culture. Donors were then randomized to receive either T3 (0.8µg.kg⁻¹ IV bolus followed by 0.113µg.kg⁻¹. hr⁻¹ IV infusion); MP (1000mg IV) as a single dose; both T3 and MP or placebo (dextrose 5%) and the T3/placebo infusion continued until retrieval. The duration of treatment and time of coning (detection of fixed dilated pupils and BP surge) were noted. All medical, nursing and technical staff involved in the research or donor care remained blinded to treatment allocation until study completion. The donors were managed according to a strict protocol. Management, continued in the ICU and OR until retrieval.

13.2.3. Retrieval of donor lungs

Donor lungs accepted for transplantation were flush perfused with single dose of antergrade pneumoplegia (Papworth solution- ringer lactate 700mls, human albumin

(20%) 200mls, 20% mannitol 100mls and heparin 10,000 units along with donor blood (300ml), citrate/phosphate/dextrose chelating agent (56mL)) and a single dose of epoprostenol (prostacycline) infusion (0.5 mg in 20mls (Flolan, (GlaxoSmithKline, UK Ltd)). Donor lungs were retrieved for gravimetric lung water assessment without pneumoplegia or prostacycline infusion. Donor lungs were retrieved as a whole block after explanting the donor heart. The trachea was stapled after expansion of both lungs. The retrieved lung blocks were transported in cold ringer lactate solution in 3 plastic bags in 4 degree C.

13.2.4. Gravimetric measurement of lung water

Gravimetric analysis of lung water was calculated in the laboratory by the Holcroft and Trunkey modification of the Pearce method (189;190). This method is based on the comparison between the wet and dry weight of the lungs, which gives the total pulmonary water content. The lung blood volume is calculated by determining the haemoglobin in the lung tissue and systemic circulation. The lung blood weight is then subtracted from the total lung water to give gravimetric extravascular lung water.

10mls of terminal pulmonary venous effluent blood was collected prior to retrieval of lungs along with venous blood sample in EDTA bottle for haemoglobin estimation. Lungs were retrieved in a standard manner and transported at 4°C in Ringer lactate solution in an inflated state. Within 8 hours of retrieval, lungs were split on table and were weighed. Lung samples from upper and lower lobes were weighed and then homogenised with a known amount of distilled water and centrifuged at 12,000 rpm for 30 minutes at 4°C. The wet weights of supernatant, lung sediments and the

pulmonary venous blood were obtained. Haemoglobin levels from the pulmonary venous effluent blood and supernatant samples were measured. Supernatant, blood and the lung sediments were then freeze dried for 72 hours and were weighed again to obtain their dry weight. EVLW was calculated according to the formula described by Holcroft and Trunkey.

13.2.5. Endpoints and Statistics

All hemodynamic and lung function parameters including EVLWI and PVPI was measured at baseline and repeated after initial bronchoscopic examination, 1 hour thereafter and immediately pre-retrieval. Data were analyzed using SPSS v15.0 (Chicago, IL). Continuous data were assessed for normality and are presented as mean \pm SD (95% confidence limits (CL)) or median (inter-quartile range (IQR)). Normally distributed variables were tested using independent or paired sample t tests. Skewed data were tested using non-parametric test (Mann-Whitney and Kruskal-Wallis test). Comparison between gravimetry and thermodilution was performed using spearman's correlation and Bland-Altman analysis. Categorical data were analyzed using X^2 and Fisher's Exact testing. Recipient survival was compared using log-rank test according to EVLWI dichotomised at 10ml.kg⁻¹ and Kaplan-Meier survival. Statistical significance was assigned when $p \leq 0.05$.

13.3. Results

13.3.1. Donor demographics

The demographic details of all 60 donors are described in Table-13.1. The donor management commenced within a median of 2 (IQR 0.5-3.5) hours of consent, within a mean of 12.5 ± 8.1 (95%CL10.3-14.6) hours of clinical coning and continued for 6.9 ± 1.2 (95%CL6.6-7.3) hours. During management the donors received 376ml (95%CL289-463) of colloid to maintain CVP/PCWP and 27m l (95%CL4-50) of crystalloid.

Table 13-1- Donor demographic data

Parameter	n=60
Age	47(36, 56)
Sex (Female: Male)	31: 29
Baseline PaO ₂ /FIO ₂ ratio	396 \pm 79 (377-418)
Donor Cause of death	
Vascular & Tumour	40/60 (67%)
Traumatic	14/60 (23%)
Hypoxic	3/60 (5%)
CNS infection	3/60 (5%)
Duration of ventilation (hours)	24 (24, 48)
Hospital stay (d)	2 (1, 2)

Data are presented in mean \pm standard deviation or median (25, 75 centiles).

CNS- central nervous system

13.3.2. Baseline parameters

The mean overall initial EVLWI-T was 9.7 ± 4.5 . In 39/60 it was $>7 \text{ ml.Kg}^{-1}$ and $>10 \text{ ml.kg}^{-1}$ 16/60. Baseline EVLWI values of ≤ 7 , 7-10 and $>10 \text{ ml.kg}^{-1}$ corresponded to arterial pO_2/FiO_2 ratios to 359 ± 71.1 , 413 ± 71 and 428 ± 83 respectively. $\text{PaO}_2/\text{FiO}_2$ ratios in donors with EVLWI $>10 \text{ ml.kg}^{-1}$ were only slightly higher than lower than values $\leq 10 \text{ ml.kg}$ (387 ± 75 versus 428 ± 83 ; $p=0.08$). There was no correlation initial EVLWI and $\text{PaO}_2/\text{FiO}_2$ ratio measurements. Baseline PVPI was mean 2.4 ± 0.9 and it significantly correlated with initial EVLWI ($r=0.8$, $p<0.001$). Donors with EVLWI-T $>7 \text{ ml.kg}^{-1}$, had significantly higher PVPI values (2.8 ± 1 vs 1.9 ± 0.4 ; $p=0.003$) suggesting increased permeability as a contributor to lung water excess.

13.3.3. Pre-retrieval parameters

In the whole cohort EVLWI and PVPI increased significantly from 9.7 ± 4.5 to 10.8 ± 5.2 ; $p=0.009$ and 2.4 ± 0.9 to 2.7 ± 1.2 ($p=0.025$) respectively while the $\text{PaO}_2:\text{FiO}_2$ ratio fell (397 ± 78 to 359 ± 126 ($p=0.028$)) suggesting increasing oedema and pulmonary permeability. The final EVLWI was >10 in 22 donors. On univariate analysis initial EVLWI, initial PVPI, duration from coning were each higher in lungs with an end-EVLWI $>10 \text{ ml.kg}^{-1}$ and significantly lower number of lungs were ultimately suitable for transplantation (Table-13.2).

Table 13-2- Univariate analysis of parameters in donors with end-assessment EVLWI<10ml.kg⁻¹ and 10ml.kg⁻¹

	End-EVLWI<10ml.kg ⁻¹ (n=38)	End-EVLWI>10ml.kg ⁻¹ (n=22)	p-value
Age	45.5±12.1	41.8±14.5	=0.32
Duration from coning (h)	11.1±6.1	16.5±9.5	=0.03
Baseline PO ₂ (mm Hg)	386±82	418±71	=0.12
Duration of ventilation (h)	45±43	61±59	=0.09
Baseline EVLWI (ml.kg ⁻¹)	7.8±2.4	13.1±5.4	<0.001
Baseline PVPI	2.1±0.4	3±1.2	<0.001
Lungs suitability	24/38 (63%)	6/22 (27%)	<0.001

13.3.4. Noradrenaline use and extravascular lung water index

At initial assessment 35/60 (58%) of *Trial* donors were receiving noradrenaline (NA). By adding vasopressin (VP), it was possible to withdraw NA completely in 21/35(60%) and reduce NA from 0.25±0.3µg.kg⁻¹min⁻¹ to 0.11±0.27µg.kg⁻¹min⁻¹ in the remainder (p<0.001). In donors receiving NA at > 0.06 µg.kg⁻¹min⁻¹ dose at initial assessment, the EVLWI (8.5±3.2 vs 11.6±5.7; p=0.011) and PVPI (2.1±0.5 vs

2.8±1.2; $p=0.01$) were significantly higher. These changes were not influenced by the ability to withdraw NA.

13.3.5. Extravascular lung water index and suitability of lungs for transplantation

Lung suitability for transplantation in this study was defined as a $\text{PaO}_2/\text{FiO}_2 \geq 300$ without identifying lung trauma, aspiration, infection or non-recrutable atelectasis during assessment and at direct inspection. Initial EVLWI was lower in suitable lungs (8.4±2.9 versus; 11.2±5.6 $p=0.02$) and on univariate analysis an initial EVLWI $\leq 10 \text{ ml.Kg}^{-1}$ was predictive of suitability ($p=0.019$). We undertook receiver operating characteristic curve analysis for both initial pO_2 and EVLWI as a guide to ultimate lung transplant suitability. It demonstrated both baseline PO_2 ($A=0.66 \pm (\text{SE}) 0.07$ (95% CI 0.52, 0.8), $p=0.03$) (Figure-13.1) and baseline EVLWI ($A= 0.67 \pm (\text{SE}) 0.07$ (95% CI 0.53, 0.81); $p=0.025$) predicted end-assessment lung suitability for transplantation (Figure-13.2).

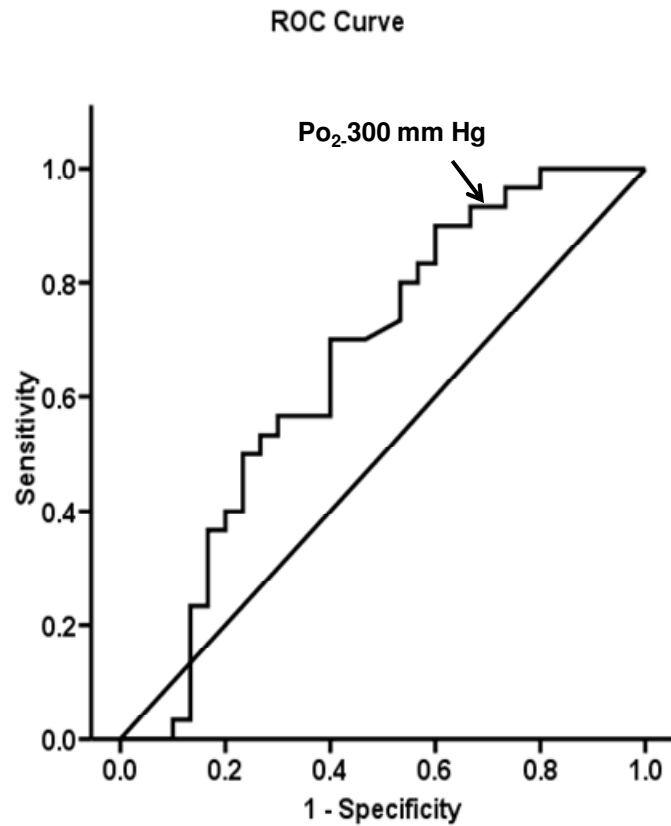


Figure 13-1- ROC curve demonstrating the relationship between sensitivity and 1-specificity in determining the predictive value of baseline PaO₂ in identifying lungs suitable for transplantation

The area under ROC curve (A) \pm SE is reported

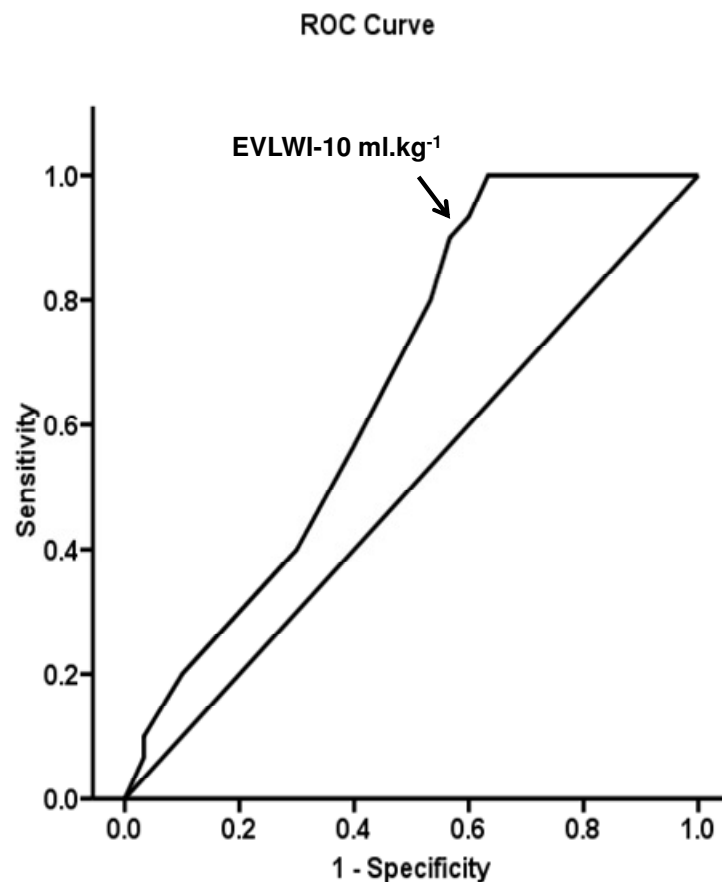


Figure 13-2- ROC curve demonstrating the relationship between sensitivity and 1-specificity in determining the predictive value of baseline EVLWI for identifying lungs suitable for transplantation

The area under ROC curve (A) \pm SE is reported.

13.3.6. Recipient outcome after transplantation

Twenty six out of 60 donor lung pairs were retrieved for transplantation. However, 6 single lung transplantation and 21 bilateral lung transplantation were performed. The

overall 30 day and 1 year mortality following transplantation was 96% and 74%. However, recipients who received lungs from donors with baseline EVLWI $>10 \text{ ml.kg}^{-1}$ performed significantly worse after transplantation. The peri-operative mortality was significantly higher ($p=0.03$) and their long term outcome was also inferior compared to recipients who had received lungs from donors with $\text{EVLWI} < 10 \text{ ml.kg}^{-1}$ ($p=0.03$) (Figure-13.3).

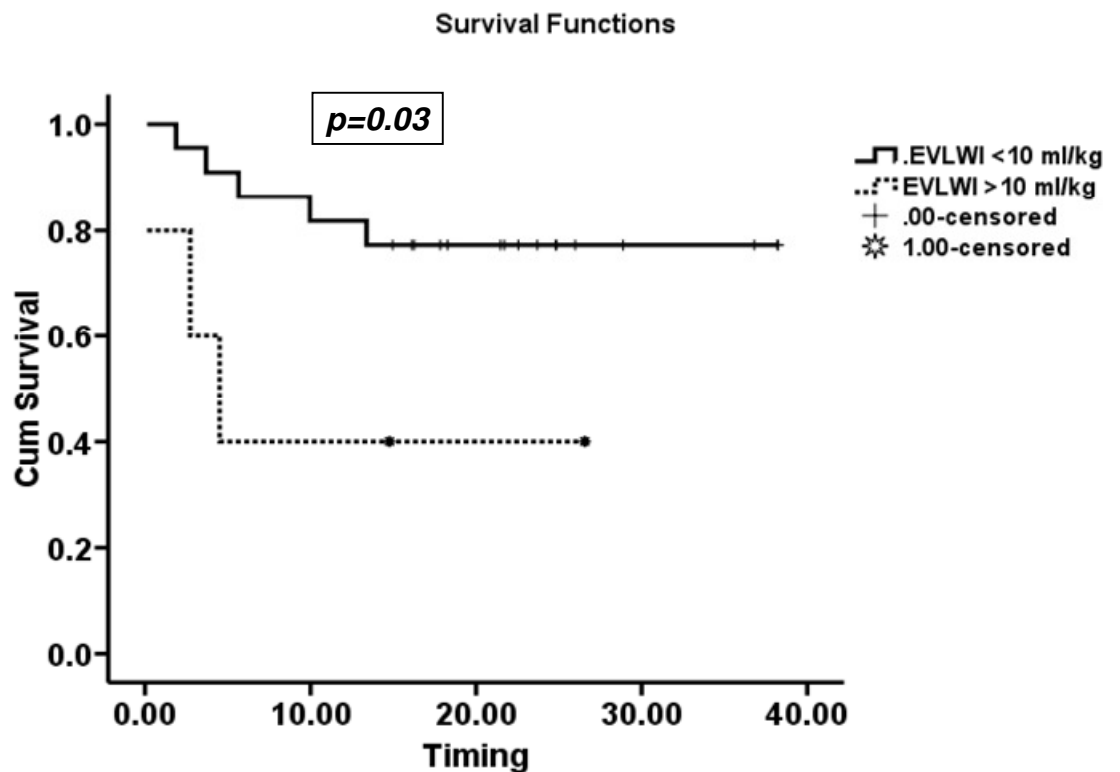


Figure 13-3-Kaplan-Meier survival curve demonstrating early recipient outcome following transplantation

Log rank test showed significantly worse outcome for recipients who had received lungs from donors with elevated baseline $\text{EVLWI} > 10 \text{ ml.kg}^{-1}$.

13.3.7. Lung water index validation

Twenty donor lungs not accepted for transplantation were removed for research analysis (9 bilateral lungs and 2 single lungs). EVLWI measured by thermodilution using single cold saline indicator correlated well with gravimetric lung water measurement ($r=0.7$; Spearman's correlation $p=0.002$) (Figure-13.4). On regression analysis a relationship between the two measurements was noted ($EVLW-T = 3.04 + 0.85 \times EVLW-G$). The thermodilution method over-predicted the EVLWI compared with gravimetry with a mean difference (positive bias) between EVLWI-T and EVLWI-G on Bland-Altman analysis of $1.6 \pm 2.14 \text{ ml.kg}^{-1}$. The upper and lower limits of agreement (± 2 standard deviations) were 5.8 and -2.6 ml.Kg^{-1} respectively (Figure-13.5). Positive bias increased with higher EVLWI.

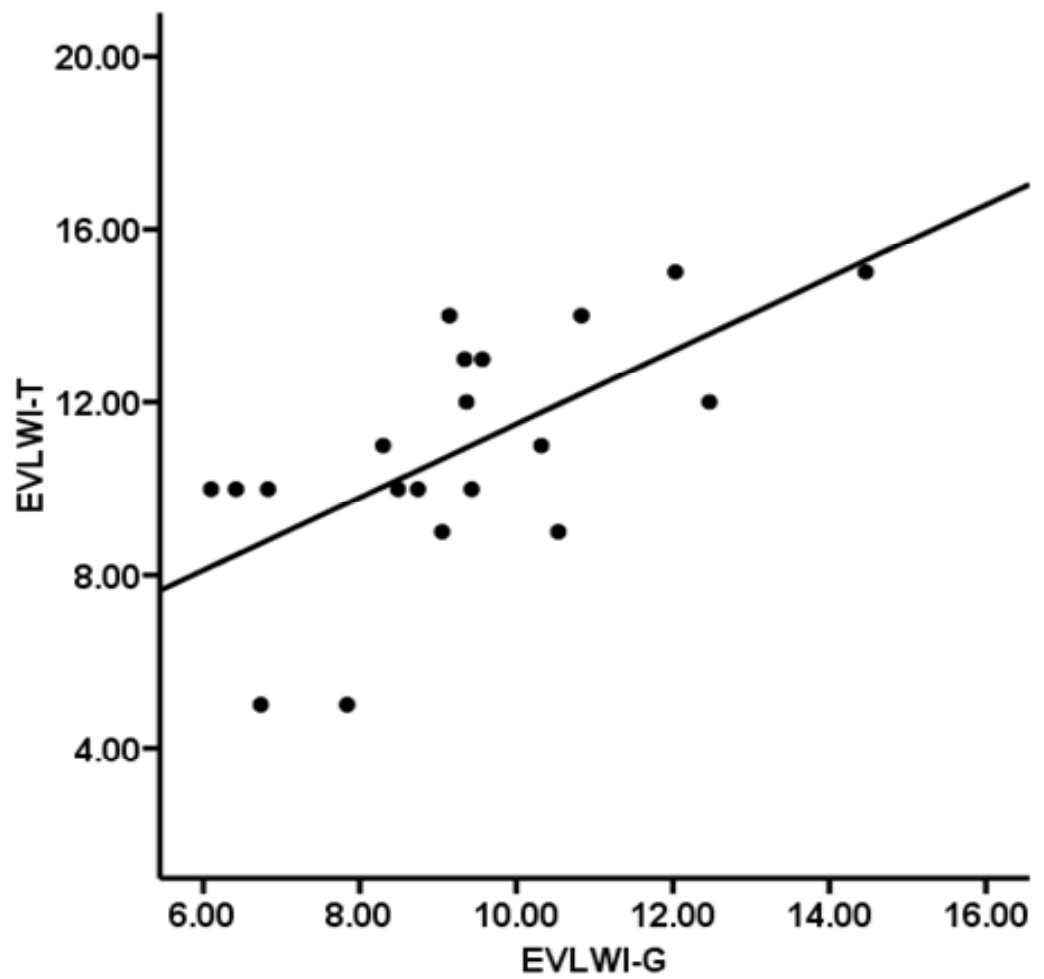


Figure 13-4-Correlation between EVLWI measurement by thermodilution and gravimetry ($r=0.7$; Spearman's correlation $p=0.002$)

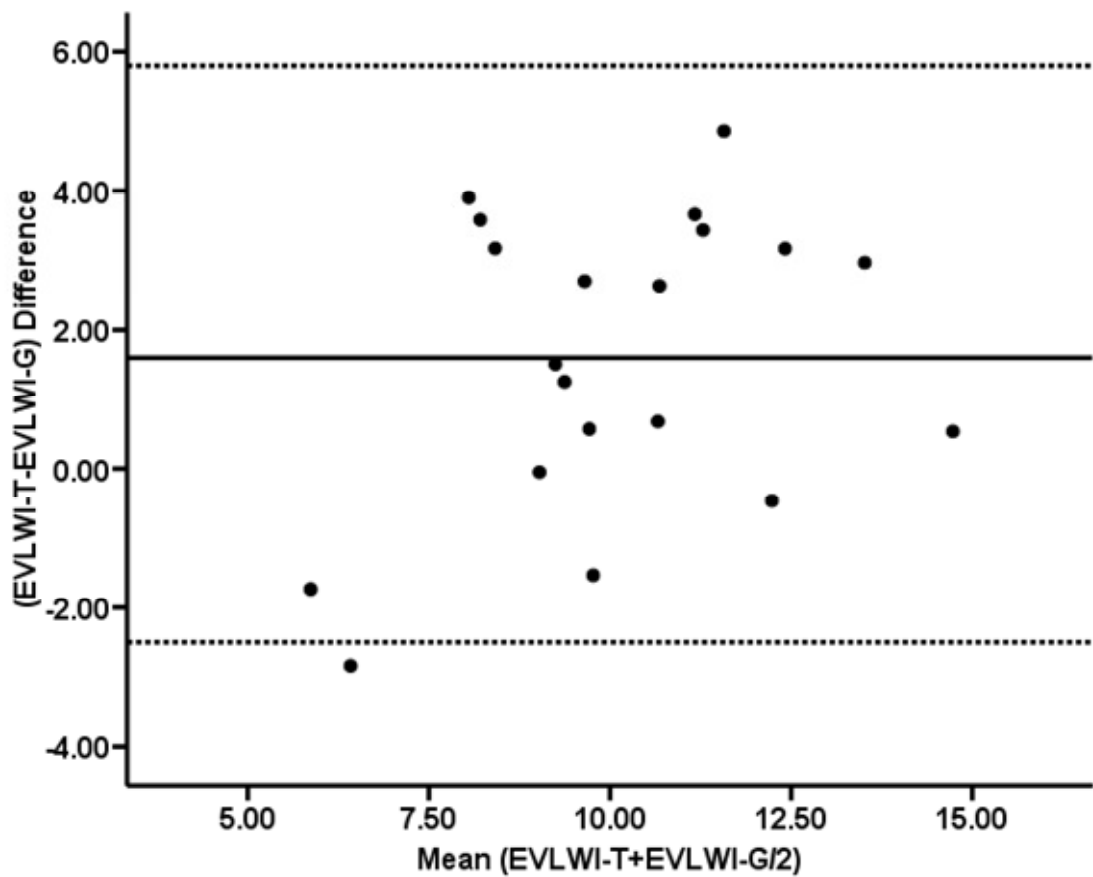


Figure 13-5-Bland-Altman plot demonstrating limits of agreement between EVLWI measurement by thermodilution and gravimetry

EVLWI-T over predicted the EVLWI-G and there was a positive bias which increased with higher EVLWI-T. However the limits of agreement vales lie between the 2 standard deviation

13.4. Discussion

This prospective observational study demonstrated that EVLWI is elevated in majority of the potential lung donors, its measurement predicted the suitability of lungs for transplantation and also recipient outcome. Lung water may increase in donor lungs by various mechanisms of injury like trauma, aspiration, infection, fluid overload and ventilatory barotrauma. These may occur before or after BSD. Also, during BSD, hemodynamic shear forces and pro-oedematous hydrostatic pressures may cause direct injury to the lungs. Each of these modes of injury may be further exacerbated by a pro-inflammatory post-BSD environment (16;173;174;191). All these mechanisms could lead to pulmonary oedema, worsening lung function and is a primary reason for rejection of lungs for transplantation (90).

Elevation of EVLWI precedes clinical, radiological and oxygenation manifestations of lung oedema (182;183). EVLWI measurement by thermodilution method is well validated, it guides treatment in critically ill patients (129;135). In a retrospective analysis of 373 critically ill patients EVLWI correlated significantly with poor outcome and on multivariate analysis found to be an independent predictor of prognosis (134). It is relatively non-invasive procedure requiring insertion of femoral arterial line and can guide fluid management in an organ donor.

The epithelial Na^+/K^+ -channels in alveolar epithelium have an important role in absorption of excessive fluid from the alveolar space. Steroids with their anti-inflammatory property and up regulation of alveolar fluid clearance (AFC) may be beneficial in manipulating the lung water in a post-BSD lung donor (92;97). In a prospective placebo controlled trial (BALTI trial) Perkins et al. had demonstrated that intravenous administration of beta-adrenergic agonist, salbutamol was associated

with significant reduction in EVLWI-T and a trend towards lower lung injury (187). Administration of nebulised beta-adrenergic agonists can achieve therapeutic levels in plasma and pulmonary oedema fluid and can augment the AFC (192). I had demonstrated that administration of methylprednisolone in the same cohort of donors was associated with significant reduction in progressive lung water accumulation (156).

Pulmonary oedema as a result of reperfusion injury occurs in 15-35% of recipients and is associated with poor outcome (90). Although there was no correlation between EVLWI and arterial oxygenation, our study demonstrated donor lungs with EVLWI $>10 \text{ ml.kg}^{-1}$ was associated with worse peri-operative mortality and long term outcome. Measurement of EVLWI-T helps in identifying donors with elevated lung water and can guide in donor fluid management and to use interventions that would increase AFC. Improved AFC has been shown to be associated with rapid resolution of hypoxia and improvement in radiological changes following transplantation (91).

Thermodilution EVLWI measurement is well validated and in animal studies it has been reported to correlate well with the gold standard gravimetric lung water measurement (130;131). In both these animal trials the EVLWI-T has over predicted the EVLWI-G. In our study we noted the same with thermodilution over predicting the gravimetric values. This discrepancy increased with progression of pulmonary oedema and in extreme levels the over prediction was higher. However, they both correlated well and this is a first study to demonstrate the use of ELWI measurement in potential lung donors.

Vasoparetic hypotension post-BSD is commonly treated by NA infusion, a potent but cardiotoxic α -adrenergic agonist (150). In our study EVLWI and PVPI were significantly elevated in donors receiving NA and this was not affected by NA withdrawal. The reason for the association between NA and elevation of lung water may reflect greater cardiac dysfunction, a greater pro-inflammatory response or a deleterious effect on pulmonary endothelium. Donor pressor support with NA has been associated with worse prognosis in heart and lung transplantation (152) and inferior post-transplant gas exchange (181). Therefore NA usage in potential organ donors is inadvisable.

13.5. Conclusion

In summary, measurement of thermodilution EVLWI is easy and reliable. It is found to be elevated in majority of potential lung donors. It predicted recipient outcome following transplantation. Elevated EVLWI-T is a target for modifying therapy during optimisation. Donor treatment with noradrenaline is associated with lung water excess. Even though EVLWI-T intrinsically over-estimates gravimetric lung water, its measurement may aid assessment of organ suitability.

SECTION 4- SUMMARY

14. SUMMARY AND POTENTIAL AREAS FOR FUTURE RESEARCH

14.1. Summary of findings

This research study has examined the impact of early protocol driven management and the effects of tri-iodothyronine, methylprednisolone, combination of both or placebo in a randomised, factorially designed, double blind , controlled clinical trial in potential heart and lung donors. The effects of these interventions were examined and discussed in relation to donor heart and lung functions, impact on heart and lung retrieval rate, impact on pro-inflammatory post-BSD environment and echocardiographic assessment. The study demonstrated that early donor management was associated with improved donor heart function and can potentially increase the retrieval rate of hearts for transplantation. All haemodynamic parameters were significantly better prior to retrieval when compared with baseline parameters. This was achieved simply by adjusting the pre and afterload of the donors guided by invasive monitoring. Commencing vasopressin infusion lead to stability on donor haemodynamics and enabled weaning of noradrenaline. This was associated with significant improvement in donor cardiac index. In contrast to heart function, the donor lung function continued to deteriorate despite donor management but with significant increase in retrieval rate of lungs for transplantation.

Hormone replacement therapy with T3 and methylprednisolone either alone or in combination was not associated with any improvement in donor heart or lung function

when compared with placebo. It also does not influence the retrieval rate of hearts and lungs for transplantation. However, MP administration was associated with significantly less progressive lung water accumulation.

Donor echocardiography is an effective non-invasive tool that correlates well with invasive monitoring data and helps in identifying hearts that are definitely likely to be successful and also helps in identifying hearts with moderate left ventricular function for targeted donor management. The hearts with poor LV ejection fraction were found to be unsuitable for transplantation and are unlikely to improve despite donor management.

The study evaluated the prevalence of pro-inflammatory cytokines and found profound increase in IL-6 in all donors. High levels of TNF- α and PCT was associated with poor donor heart function. The dysfunction correlated with levels of PCT elevation. The cytokine level was not influenced by steroid administration and did not preclude hearts from transplantation. The study also evaluated the role of donor cardiac troponin-I levels in heart donors. CTnI levels were elevated in all donors and it negatively correlated with left ventricular function. Haemodynamic and echocardiographic parameters of LV function were significantly worse in donors with CTnI $>1\mu\text{g.L}^{-1}$. However, the number of hearts transplanted from this group did not differ when compared with CTnI $<1\mu\text{g.L}^{-1}$. The operative and early outcome following transplantation of hearts from each group did not differ. It demonstrated that donor CTnI is a surrogate of donor heart function and guides in targeted donor management but does not affect outcome.

The study evaluated the role of EVLWI measurement in lung donors. It demonstrates that EVLWI deteriorated despite donor management. The deterioration was worse in donors requiring noradrenaline support. The EVLWI correlated with PVPI suggesting permeability oedema. Measurement EVLWI differentiated the lungs that would be ultimately suitable for transplantation and also predicted operative and early post-transplant outcome. The EVLWI measured by single injection thermodilution method correlated well with gravimetric lung water estimation. Early steroid administration reduced the progressive lung water accumulation but did not influence the oxygenation.

14.2. Early donor management-Future

Early donor management was associated with improved donor heart function however the study did not show a statistically significant increase in heart retrieval rate. The study demonstrated that 20% of more hearts could have been used for transplantation at the end of assessment. As it was a research trial the normal donor heart offering sequence took place simultaneous to the study conduct. Hearts that were rejected by transplant units earlier were not reoffered even if they were found to be suitable after a period of donor management. The study clearly demonstrated the need for trained donor management practitioners (DMP) to be involved in every retrieval operation. In future DMP should attend the donors early and institute the invasive monitoring and protocol driven management. The donor offering sequence should also be delayed by at least 2 to 3 hours enabling insertion of monitoring lines and institution of management and weaning of vasoconstrictors. Offering donor

organs with more detailed invasive data will be preferable and will increase the donor heart acceptance rate. This should hopefully lead to increased heart retrieval and transplantation in future.

Early donor management has another advantage, as seen during the study. A small proportion of donors with very poor heart function or gross aspiration/infection in airways on bronchoscopy, precluding lung donation, were identified early. Heart/lung offering was stopped in these donors thereby avoiding unnecessary delay and facilitating early mobilisation of retrieval teams for intra-abdominal organ retrieval. If the same process could be followed with the implementation DMP attending every donor there will be a reduction in the number of abandoned retrieval procedures. This will also avoid unnecessary delay in retrieval and even wastage of other organs due to sudden cardiovascular compromise of the donor.

Donor retrieval is an expensive, labour intensive procedure. Each abandoned donor retrieval procedure impacts on the recipient centres financially and also affects the conventional cardiac surgery work load the following day. Therefore early donor management should probably be instituted by every retrieval teams in the UK. Following this trial results we hope that this will be initiated by every transplant unit in the country and in future we should see an increase in both the quality and the quantity of the donor organs available for transplantation.

14.3. Hormone replacement therapy in organ donors-Future

Hormone replacement therapy using T3 and methylprednisolone did not affect any of the measured parameters of donor heart function or retrieval rate in this study. This could be due to number of reasons. The study although prospective was numerically small which demonstrates the difficulties in undertaking such trial in donor population. The donors were managed for 6 to 7 hours prior to retrieval and the time duration of T3 therapy was probably inadequate to produce any haemodynamic effect in the donors. Based on our study I could not recommend avoiding hormone replacement therapy for multi-organ donors. In future studies involving larger number of donors managed for a longer duration may be required to clearly define the role of hormone replacement therapy on organ donors.

I also did not assess the effect of hormone therapy on recipient haemodynamic function. The delayed genomic effects of T3 therapy may influence the recipient haemodynamics and outcome post-transplantation. Analysis of myocardial biopsy specimens from the donor left ventricle prior to heart retrieval to study the genomic expression of T3 administration would be required. The effect of early steroid therapy and noradrenaline withdrawal on recipient organ rejection episodes is also unknown. Studies comparing the outcome on recipients from donor managed cohorts with matched contemporary non-donor managed recipients might answer some of the questions raised.

Early steroid administration was associated with significantly reduced lung water accumulation during management in this study, but it was not associated with any

change in oxygenation. Steroids should probably be administered earlier, immediately after coming to get the benefit. However, managing a patient as an organ donor is ethically wrong at present before confirmation of BSD and consent for organ donation. A change in legislation to presumed consent for organ donation would be required in order to make changes in treatment. Laboratory analysis of alveolar epithelial fluid transport (alveolar fluid clearance) in donor lungs not used for transplantation would also help in evaluating the role of steroids in reducing lung oedema. Identifying such measures would be helpful in resuscitating marginal donor lungs in ex-vivo lung perfusion (EVLP) rigs.

14.4. Transthoracic echocardiography assessment-Future

Assessment of donor heart function correlated well with haemodynamic parameters in this study. It also guided hearts where targeted donor management is helpful. In this observational study we noted 4 hearts with baseline LVEF <30%. None achieved haemodynamic suitability for transplantation but their initial inotrope or vasoconstrictor requirements were not different. The size of the study does not allow us to make a general recommendation that hearts with an LVEF <30% should not enter a PA catheter guided optimisation protocol. Larger studies may be required to make a recommendation on rejecting hearts with poor LVEF.

Acquisition of echocardiographic pictures to assess at least one parameter was possible in 70/80 (88%) donors but LVEF assessment was available only in 47/80 (59%) donors. This demonstrates the difficulties in performing TTE in a supine, ventilated patient. Transoesophageal echocardiography would lead to much higher

data acquisition but it is invasive and requires expensive equipment and trained personnel to undertake the procedure. The use of echo contrast for LV opacification would increase the percentage of LVEF measurement by TTE. Most of the TTE parameters used in this trial are load dependant. The use of tissue dopplers for measurement of isovolumetric acceleration to evaluate biventricular function would be ideal. Studies using these parameters on donor population would be required in future to further establish the role of echocardiography in donor assessment.

14.5. Inflammatory and bio-chemical marker assessment-Future

This study demonstrated the prevalence of elevation of bio-chemical markers in organ donors and predicted poor donor organ function. Higher level of bio-marker was associated with worse outcome. However, this does not preclude successful resuscitation of organs for transplantation. Administration methylprednisolone did not affect the cytokine levels. This probably due to the administration steroids many hours after established BSD. Studies in future directed at earlier administration of methylprednisolone soon after coning and measurement of cytokines would be required to confirm the role of early steroids on post-BSD pro-inflammatory state. Larger studies using combination of bio-markers and other clinical investigations including electrocardiography and echocardiography will help in differentiating hearts that will be suitable for transplantation from donor hearts that would fail the recipient.

14.6. Conclusion

Early donor management is the cornerstone to improve the donor heart function and increase the lung retrieval rate. It may also increase the heart retrieval rate. Serial transthoracic echocardiography may guide in identifying suitable hearts that respond to donor management. Hormone replacement therapy with T3 and methylprednisolone did not affect the donor haemodynamics, oxygenation or retrieval rate of heart and lungs for transplantation. Steroid administration was associated with reduced progressive lung water accumulation.

14.2. Study impact

The data generated from the trial was presented to the cardiothoracic transplant advisory group (CTAG) and to the national specialist commissioning advisory group (NSCAG) for thoracic organ transplantation. The data was well received and the need for early donor management and Donor Management Practitioners (DMP) was recognised. The NSCAG has funded the Birmingham heart and lung transplantation unit along with University of Birmingham to train 5 donor management practitioners who will be fully trained to initiate early donor management. They will undergo 18 months of training and will receive a diploma in donor management. At present 3 DMPs are in training in Birmingham and they will most probably starting early management by the end of 2010. Two further trainees will be employed this year. If the DMPs are successful in increasing the quality and the quantity of donor heart and lungs for transplantation and reduce the number of abandoned retrieval then this program will most probably be rolled to the rest of the transplant units in the country.

SECTION-5-APPENDICES

APPENDIX-A - Equations for calculation of derived variables

Systemic vascular resistance indexed

$$SVRI = \frac{(MABP-CVP)}{CI} \times 80$$

Pulmonary vascular resistance indexed

$$PVRI = \frac{(MPAP-PAWP)}{CI} \times 80$$

Left ventricular stroke work indexed

$$LVSWI = \frac{(MABP-PAWP) \times CI}{HR} \times 0.0136 \times 1000$$

Right ventricular stroke work indexed

$$RVSWI = \frac{(MPAP-CVP) \times CI}{HR} \times 0.0136 \times 1000$$

Cardiac power output indexed

$$CPOi = CI \times MABP \times 0.00222$$

SECTION-6-REFERENCES

References

- (1) Reitz BA. History of heart and heart-lung transplantation. In: Baumgartner WA, Reitz BA, Kasper E, Theodore J, editors. Heart and Lung transplantation. W. B. Saunders company, 2002: 3-14.
- (2) Kirklin J, Young JB, McGiffin D. History of cardiac transplantation. Churchill Livingstone, 2002: 3-13.
- (3) Rutherford R, Lordan J, Fisher A, Corris. Historical overview of lung and heart-lung transplantation. In: Lynch III JA, Ross DJ, editors. Lung and heart-lung transplantation. 2006: 1-16.
- (4) Taylor DO, Stehlik J, Edwards LB, Aurora P, Christie JD, Dobbels F et al. Registry of the international society for heart and lung transplantation: twenty-sixth official adult heart transplant report-2009. J Heart Lung Transplant 2009; 28(10):1007-1022.
- (5) Christie JD, Edwards LB, Aurora P, Dobbels F, Kirk R, Rahmel AO et al. The registry of the international society for heart and lung transplantation: twenty-sixth official adult lung and heart-lung transplantation Report-2009. J Heart Lung Transplant 2009; 28(10):1031-1049.

- (6) Anyanwu AC, Rogers CA, Murday AJ. Review of the current state of thoracic transplantation: A national prospective cohort study. *Transplant Proc* 1999; 31(1-2):165.

- (7) De Burgh D. Peripheral arterial chemoreceptors and respiratory-cardiovascular integration. Oxford university press, 1997.

- (8) Ganong WF. Circulation through special regions. Review of medical physiology. Appleton and Lange, 1995: 555-574.

- (9) Cooper DK, Basker M. Physiologic changes following brain death. *Transplant Proc* 1999; 31(1-2):1001-1002.

- (10) Marshall VC. Pathophysiology of brain death: effects on allograft function. *Transplant Proc* 2001; 33(1-2):845-846.

- (11) Wilhelm MJ, Pratschke J, Laskowski IA, Paz DM, Tilney NL. Brain death and its impact on the donor heart - Lessons from animal models. *J Heart Lung Transplant* 2000; 19(5):414-418.

- (12) Chiari P, Hadour G, Michel P, Piriou V, Rodriguez C, Budat C et al. Biphasic response after brain death induction: prominent part of catecholamines release in this phenomenon. *J Heart Lung Transplant* 2000; 19(7):675-682.

- (13) Szabo G, Hackert T, Sebening C, Melnitchuk S, Vahl CF, Hagl S. Role of neural and humoral factors in hyperdynamic reaction and cardiac dysfunction following brain death. *J Heart Lung Transplant* 2000; 19(7):683-693.

- (14) Sebening C, Hagl C, Szabo G, Tochtermann U, Strobel G, Schnabel P et al. Cardiocirculatory effects of acutely increased intracranial pressure and subsequent brain death. *Eur J Cardiothorac Surg* 1995; 9(7):360-372.

- (15) Pennefather SH, Bullock RE, Mantle D, Dark JH. Use of low dose arginine vasopressin to support brain-dead organ donors. *Transplantation* 1995; 59(1):58-62.

- (16) Novitzky D, Wicomb WN, Rose AG. Pathophysiology of pulmonary edema following experimental brain death in the chacma baboon. *Ann Thorac Surg* 1987; 43(3):288-294.

- (17) Shivalkar B, van Loon J, Wieland W, Tjandra-Maga TB, Borgers M, Plets C et al. Variable effects of explosive or gradual increase of intracranial pressure on myocardial structure and function. *Circulation* 1993; 87(1):230-239.

- (18) van Loon J, Shivalkar B, Plets C, Goffin J, Tjandra-Maga TB, Flameng W. Catecholamine response to a gradual increase of intracranial pressure. *J Neurosurg* 1993; 79(5):705-709.

- (19) Novitzky D, Wicomb WN, Cooper DK, Rose AG, Reichart B. Prevention of myocardial injury during brain death by total cardiac sympathectomy in the Chacma baboon. *Ann Thorac Surg* 1986; 41(5):520-524.

- (20) Galinanes M, Hearse DJ. Brain-death-induced cardiac contractile dysfunction: studies of possible neurohormonal and blood-borne mediators. *J Mol Cell Cardiol* 1994; 26(4):481-498.

- (21) Rona G. Catecholamine cardiotoxicity. *J Mol Cell Cardiol* 1985; 17(4):291-306.

- (22) Rose AG, Novitzky D, Factor SM. Catecholamine-associated smooth muscle contraction bands in the media of coronary arteries of brain-dead baboons. *Am J Cardiovasc Pathol* 1988; 2(1):63-67.

- (23) Oishi Y, Nishimura Y, Imasaka K, Kajihara N, Morita S, Masuda M et al. Impairment of coronary flow reserve and left ventricular function in the brain-dead canine heart. *Eur J Cardiothorac Surg* 2003; 24(3):404-410.

- (24) Szabo G, Sebening C, Hackert T, Hoffmann L, Melnitchuk S, Vahl CF et al. The role of coronary perfusion changes in cardiac dysfunction associated with brain death. *Thorac CardiovasC Surg* 1998; 46(6):339-343.

- (25) D'Amico TA, Meyers CH, Koutlas TC, Peterseim DS, Sabiston DC, Jr., Van Trigt P et al. Desensitization of myocardial beta-adrenergic receptors and

deterioration of left ventricular function after brain death. J Thorac Cardiovasc Surg 1995; 110(3):746-751.

- (26) Arab D, Yahia AM, Qureshi AI. Cardiovascular manifestations of acute intracranial lesions: pathophysiology, manifestations, and treatment. J Intensive Care Med 2003; 18(3):119-129.
- (27) Bittner HB, Chen EP, Biswas SS, Van TP, III, Davis RD. Right ventricular dysfunction after cardiac transplantation: primarily related to status of donor heart. Ann Thorac Surg 1999; 68(5):1605-1611.
- (28) Bittner HB, Chen EP, Kendall SW, Van Trigt P. Brain death alters cardiopulmonary hemodynamics and impairs right ventricular power reserve against an elevation of pulmonary vascular resistance. Chest 1997; 111(3):706-711.
- (29) Bittner HB, Kendall SW, Chen EP, Van Trigt P. Endocrine changes and metabolic responses in a validated canine brain death model. J Crit Care 1995; 10(2):56-63.
- (30) Powner DJ, Hendrich A, Nyhuis A, Strate R. Changes in serum catecholamine levels in patients who are brain dead. J Heart Lung Transplant 1992; 11(6):1046-1053.

- (31) Chen EP, Bittner HB, Kendall SW, Van Trigt P. Hormonal and hemodynamic changes in a validated animal model of brain death. Crit Care Med 1996; 24(8):1352-1359.

- (32) Powner DJ, Hendrich A, Lagler RG, Ng RH, Madden RL. Hormonal changes in brain dead patients. Crit Care Med 1990; 18(7):702-708.

- (33) Mariot J, Sadoune LO, Jacob F, Dousset B, Perrier JF, Jacob C et al. Hormone levels, hemodynamics, and metabolism in brain dead organ donors. Transplant Proc 1995; 27(1):793-794.

- (34) Novitzky D. Heart transplantation, euthyroid sick syndrome, and triiodothyronine replacement. J Heart Lung Transplant 1992; 11(4 Pt 2):S196-S198.

- (35) Novitzky D. Triiodothyronine replacement, the euthyroid sick syndrome, and organ transplantation. Transplant Proc 1991; 23(5):2460-2462.

- (36) Novitzky D, Cooper DKC, Morrell D, Isaacs S. Change from aerobic to anaerobic metabolism after brain death, and reversal following triiodothyronine therapy. Transplantation 1988; 45(1):32-36.

- (37) Pratschke J, Wilhelm MJ, Kusaka M, Hancock WW, Tilney NL. Activation of proinflammatory genes in somatic organs as a consequence of brain death. Transplant Proc 1999; 31(1-2):1003-1005.

- (38) Birks EJ, Yacoub MH, Burton PS, Owen V, Pomerance A, O'Halloran A et al. Activation of apoptotic and inflammatory pathways in dysfunctional donor hearts. *Transplantation* 2000; 70(10):1498-1506.
- (39) Birks EJ, Burton PB, Owen V, Mullen AJ, Hunt D, Banner NR et al. Elevated tumor necrosis factor-alpha and interleukin-6 in myocardium and serum of malfunctioning donor hearts. *Circulation* 2000; 102(suppl-III):352-358.
- (40) Plenz G, Eschert H, Erren M, Wichter T, Bohm M, Flesch M et al. The interleukin-6/interleukin-6-receptor system is activated in donor hearts. *J Am Coll Cardiol* 2002; 39(9):1508-12.
- (41) Birks EJ, Burton PB, Owen VJ, Latif N, Nyawo B, Yacoub MH. Molecular and cellular mechanisms of donor heart dysfunction. *Transplant Proc* 2001; 33(5):2749-2751.
- (42) Wilhelm MJ, Pratschke J, Beato F, Taal M, Kusaka M, Hancock WW et al. Activation of the heart by donor brain death accelerates acute rejection after transplantation. *Circulation* 2000; 102(19):2426-2433.
- (43) Deng MC, Plenz G, Labarrere C, Marboe C, Baba HA, Erre M et al. The role of IL6 cytokines in acute cardiac allograft rejection. *Transplant Immunol* 2002; 9(2-4):115-120.

- (44) De Perrot M, Sekine Y, Fischer S, Waddell TK, McRae K, Liu M et al.
Interleukin-8 release during early reperfusion predicts graft function in human lung transplantation. *Am J Respir Crit Care Med* 2002; 165(2):211-215.
- (45) Fisher AJ, Donnelly SC, Hirani N, Haslett C, Strieter RM, Dark JH et al.
Elevated levels of interleukin-8 in donor lungs is associated with early graft failure after lung transplantation. *Am J Respir Crit Care Med* 2001; 163(1):259-265.
- (46) Torre-Amione G, Kapadia S, Lee J, Durand JB, Bies RD, Young JB et al.
Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. *Circulation* 1996; 93(4):704-711.
- (47) Deswal A, Petersen NJ, Feldman AM, Young JB, White BG, Mann DL.
Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone trial (VEST). *Circulation* 2001; 103(16):2055-2059.
- (48) Birks EJ, Owen VJ, Burton PB, Bishop AE, Banner NR, Khaghani A et al.
Tumor necrosis factor-alpha is expressed in donor heart and predicts right ventricular failure after human heart transplantation. *Circulation* 2000; 102(3):326-31.

- (49) Potapov EV, Wagner FD, Loebe M, Ivanitskaia EA, Muller C, Sodian R et al. Elevated donor cardiac troponin T and procalcitonin indicate two independent mechanisms of early graft failure after heart transplantation. *Int J Cardiol* 2003; 92(2-3):163-167.
- (50) Wagner FD, Jonitz B, Potapov EV, Qedra N, Wegscheider K, Abraham K et al. Procalcitonin, a donor-specific predictor of early graft failure-related mortality after heart transplantation. *Circulation* 2001; 104(12 Suppl 1):I192-I196.
- (51) Wagner FD, Jonitz B, Potapov E, Qedra N, Wegscheider K, Weinmann E et al. Procalcitonin: a donor-specific predictor of early graft failure and early graft failure mortality after heart transplantation. *J Heart Lung Transplant* 2001; 20(2):206.
- (52) Dhoot GK, Perry SV. Distribution of polymorphic forms of troponin components and tropomyosin in skeletal muscle. *Nature* 1979; 278(5706):714-718.
- (53) Adams JE, III, Bodor GS, Davila-Roman VG, Delmez JA, Apple FS, Ladenson JH et al. Cardiac troponin I. A marker with high specificity for cardiac injury. *Circulation* 1993; 88(1):101-106.

- (54) Katus HA, Remppis A, Looser S, Hallermeier K, Scheffold T, Kubler W.
Enzyme linked immuno assay of cardiac troponin T for the detection of acute myocardial infarction in patients. *J Mol Cell Cardiol* 1989; 21(12):1349-1353.
- (55) Naidech AM, Kreiter KT, Janjua N, Ostapkovich ND, Parra A, Commichau C et al. Cardiac troponin elevation, cardiovascular morbidity, and outcome after subarachnoid hemorrhage. *Circulation* 2005; 112(18):2851-2856.
- (56) Riou B, Dreux S, Roche S, Arthaud M, Goarin JP, Leger P et al. Circulating cardiac troponin T in potential heart transplant donors. *Circulation* 1995; 92(3):409-414.
- (57) Anderson JR, Hossein-Nia M, Brown P, Holt DW, Murday A. Donor cardiac troponin-T predicts subsequent inotrope requirements following cardiac transplantation. *Transplantation* 1994; 58(9):1056-1057.
- (58) Potapov EV, Ivanitskaia EA, Loebe M, Mockel M, Muller C, Sodian R et al. Value of cardiac troponin I and T for selection of heart donors and as predictors of early graft failure. *Transplantation* 2001; 71(10):1394-1400.
- (59) Vijay P, Scavo VA, Morelock RJ, Sharp TG, Brown JW. Donor cardiac troponin T: a marker to predict heart transplant rejection. *Ann Thorac Surg* 1998; 66(6):1934-1939.

- (60) Deibert E, Aiyagari V, Diring MN. Reversible left ventricular dysfunction associated with raised troponin I after subarachnoid haemorrhage does not preclude successful heart transplantation. *Heart* 2000; 84(2):205-207.
- (61) Khush KK, Menza RL, Babcock WD, Zaroff JG. Donor Cardiac Troponin I Levels Do Not Predict Recipient Survival After Cardiac Transplantation. *J Heart Lung Transplant* 2007; 26(10):1048-1053.
- (62) Boccheciampe N, Audibert G, Rangeard O, Charpentier C, Perrier JF, Lalot JM et al. Serum troponin Ic values in organ donors are related to donor myocardial dysfunction but not to graft dysfunction or rejection in the recipients. *Int J Cardiol* 2008.
- (63) Wheeldon DR, Potter CD, Oduro A, Wallwork J, Large SR. Transforming the "unacceptable" donor: outcomes from the adoption of a standardized donor management technique. *J Heart Lung Transplant* 1995; 14(4):734-742.
- (64) Potter CD, Wheeldon DR, Wallwork J. Functional assessment and management of heart donors: a rationale for characterization and a guide to therapy. *J Heart Lung Transplant* 1995; 14(1 Pt 1):59-65.
- (65) Delgado DH, Rao V, Ross HJ. Donor management in cardiac transplantation. *Can J Cardiol* 2002; 18(11):1217-1223.

- (66) Lindop MJ. Basic principles of donor management for multiorgan removal. *Transplant Proc* 1991; 23(5):2463-2464.
- (67) Wood KE, Becker BN, McCartney JG, D'Alessandro AM, Coursin DB. Care of the potential organ donor. *N Engl J Med* 2004; 351(26):2730-2739.
- (68) Zaroff JG, Rosengard BR, Armstrong WF, Babcock WD, D'Alessandro A, Dec GW et al. Consensus conference report: maximizing use of organs recovered from the cadaver donor: cardiac recommendations, March 28-29, 2001, Crystal City, Va. *Circulation* 2002; 106(7):836-841.
- (69) Ganong WF. The thyroid gland. In: Ganong WF, editor. *Review of medical physiology*. Appleton and Lange, 1995: 290-305.
- (70) Dillmann WH. Cellular action of thyroid hormone on the heart. *Thyroid* 2002; 12(6):447-452.
- (71) Danzi S, Klein I. Thyroid hormone-regulated cardiac gene expression and cardiovascular disease. *Thyroid* 2002; 12(6):467-472.
- (72) Danzi S, Ojamaa K, Klein I. Triiodothyronine-mediated myosin heavy chain gene transcription in the heart. *Am J Physiol Heart Circ Physiol* 2003; 284(6):H2255-H2262.

- (73) Jeevanandam V. Triiodothyronine: spectrum of use in heart transplantation. *Thyroid* 1997; 7(1):139-145.
- (74) Novitzky D, Horak A, Cooper DK, Rose AG. Electrocardiographic and histopathologic changes developing during experimental brain death in the baboon. *Transplant Proc* 1989; 21(1 III):2567-2569.
- (75) Masson F, Thicoipe M, Latapie MJ, Maurette P. Thyroid function in brain-dead donors. *Transpl Int* 1990; 3(4):226-233.
- (76) Gramm HJ, Meinhold H, Bickel U, Zimmermann J, von Hammerstein B, Keller F et al. Acute endocrine failure after brain death? *Transplantation* 1992; 54(5):851-7.
- (77) Cooper DK, Novitzky D, Wicomb WN. The pathophysiological effects of brain death on potential donor organs, with particular reference to the heart. *Ann R Coll Surg Engl* 1989; 71(4):261-266.
- (78) Cooper DK, Novitzky D, Wicomb WN. Hormonal therapy in the brain-dead experimental animal. *Transplant Proc* 1988;(5 (Suppl 7)):51-54.
- (79) Meyers CH, D'Amico TA, Peterseim DS, Jayawant AM, Steenbergen C, Sabiston DC, Jr. et al. Effects of triiodothyronine and vasopressin on cardiac function and myocardial blood flow after brain death. *J Heart Lung Transplant* 1993; 12(1 Pt 1):68-79.

- (80) Rosendale JD, Kauffman HM, McBride MA, Chabalewski FL, Zaroff JG, Garrity ER et al. Aggressive pharmacologic donor management results in more transplanted organs. *Transplantation* 2003; 75(4):482-487.
- (81) Rosendale JD, Kauffman HM, McBride MA, Chabalewski FL, Zaroff JG, Garrity ER et al. Hormonal resuscitation yields more transplanted hearts, with improved early function. *Transplantation* 2003; 75(8):1336-1341.
- (82) Novitzky D, Cooper DK, Reichart B. Hemodynamic and metabolic responses to hormonal therapy in brain-dead potential organ donors. *Transplantation* 1987; 43(6):852-854.
- (83) Novitzky D, Cooper DK, Chaffin JS, Greer AE, DeBault LE, Zuhdi N. Improved cardiac allograft function following triiodothyronine therapy to both donor and recipient. *Transplantation* 1990; 49(2):311-316.
- (84) Jeevanandam V, Todd B, Regillo T, Hellman S, Eldridge C, McClurken J. Reversal of donor myocardial dysfunction by triiodothyronine replacement therapy. *J Heart Lung Transplant* 1994; 13(4):681-7.
- (85) Jeevanandam V, Todd B, Hellman S, Eldridge C, McClurken J, Addonizio VP. Use of triiodothyronine replacement therapy to reverse donor myocardial dysfunction: creating a larger donor pool. *Transplant Proc* 1993; 25(6):3305-06.

- (86) Randell TT, Hockerstedt KA. Triiodothyronine treatment in brain-dead multiorgan donors--a controlled study. *Transplantation* 1992; 54(4):736-738.
- (87) Goarin JP, Cohen S, Riou B, Jacquens Y, Guesde R, Le Bret F et al. The effects of triiodothyronine on hemodynamic status and cardiac function in potential heart donors. *Anesth Analg* 1996; 83(1):41-7.
- (88) Ganong WF. Adrenal medulla and adrenal cortex. In: Ganong WF, editor. *Review of medical physiology*. Appleton and Lange, 1995: 327-351.
- (89) Wan S, DeSmet JM, Antoine M, Goldman M, Vincent JL, LeClerc JL. Steroid administration in heart and heart-lung transplantation: is the timing adequate? *Ann Thorac Surg* 1996; 61(2):674-678.
- (90) Ware LB, Fang X, Wang Y, Sakuma T, Hall TS, Matthay MA. Selected contribution: mechanisms that may stimulate the resolution of alveolar edema in the transplanted human lung. *J Appl Physiol* 2002; 93(5):1869-1874.
- (91) Ware L, Golden J, Finkbeiner W, Matthay MA. Alveolar Epithelial Fluid Transport Capacity in Reperfusion Lung Injury after Lung Transplantation. *Am J Respir Crit Care Med* 1999; 159(3):980-988.

- (92) Noda M, Suzuki S, Tsubochi H, Sugita M, Maeda S, Kobayashi S et al.
Single dexamethasone injection increases alveolar fluid clearance in adult rats. Crit Care Med 2003; 31(4):1183-1189.
- (93) Lyons JM, Pearl JM, McLean KM, Akhter SA, Wagner CJ, Pandalai PK et al.
Glucocorticoid administration reduces cardiac dysfunction after brain death in pigs. J Heart Lung Transplant 2005; 24(12):2249-2254.
- (94) McLean KM, Duffy JY, Pandalai PK, Lyons JM, Bulcao CF, Wagner CJ et al.
Glucocorticoids alter the balance between pro- and anti-inflammatory mediators in the myocardium in a porcine model of brain death. J Heart Lung Transplant 2007; 26(1):78-84.
- (95) Segel LD, Follette DM, Castellanos LM, Hayes R, Baker JM, Smolens IA.
Steroid pretreatment improves graft recovery in a sheep orthotopic heart transplantation model. J Heart Lung Transplant 1997; 16(4):371-380.
- (96) Follette DM, Rudich SM, Babcock WD. Improved oxygenation and increased lung donor recovery with high-dose steroid administration after brain death. J Heart Lung Transplant 1998; 17(4):423-429.
- (97) Folkesson HG, Norlin A, Wang Y, Abedinpour P, Matthay MA.
Dexamethasone and thyroid hormone pretreatment upregulate alveolar epithelial fluid clearance in adult rats. J Appl Physiol 2000; 88(2):416-424.

- (98) Blaine EM, Tallman RD, Jr., Frolicher D, Jordan MA, Bluth LL, Howie MB. Vasopressin supplementation in a porcine model of brain-dead potential organ donors. *Transplantation* 1984; 38(5):459-464.
- (99) Chen JM, Cullinane S, Spanier TB, Artrip JH, John R, Edwards NM et al. Vasopressin deficiency and pressor hypersensitivity in hemodynamically unstable organ donors. *Circulation* 1999; 100(19 Suppl):II244-II246.
- (100) Guesde R, Barrou B, Leblanc I, Ourahma S, Goarin JP, Coriat P et al. Administration of desmopressin in brain-dead donors and renal function in kidney recipients. *Lancet* 1998; 352(9135):1178-1181.
- (101) Pennefather SH, Bullock RE, Dark JH. The effect of fluid therapy on alveolar arterial oxygen gradient in brain- dead organ donors. *Transplantation* 1993; 56(6):1418-1422.
- (102) Totsuka E, Fung JJ, Ishii T, Urakami A, Moras NP, Hakamada K et al. Influence of donor condition on postoperative graft survival and function in human liver transplantation. *Transplant Proc* 2000; 32(2):322-326.
- (103) Rostron AJ, Avlonitis VS, Cork DM, Grenade DS, Kirby JA, Dark JH. Hemodynamic resuscitation with arginine vasopressin reduces lung injury after brain death in the transplant donor. *Transplantation* 2008; 85(4):597-606.

- (104) Zaroff JG, Babcock WD, Shiboski SC, Solinger LL, Rosengard BR. Temporal changes in left ventricular systolic function in heart donors: Results of serial echocardiography. *J Heart Lung Transplant* 2003; 22(4):383-388.
- (105) Zaroff JG, Babcock WD, Shiboski SC. The impact of left ventricular dysfunction on cardiac donor transplant rates. *J Heart Lung Transplant* 2003; 22(3):334-337.
- (106) Gilbert EM, Krueger SK, Murray JL, Renlund DG, O'Connell JB, Gay WA et al. Echocardiographic evaluation of potential cardiac transplant donors. *J Thorac Cardiovasc Surg* 1988; 95(6):1003-1007.
- (107) Stoddard MF, Longaker RA. The role of transesophageal echocardiography in cardiac donor screening. *Am Heart J* 1993; 125(6):1676-1681.
- (108) Lewandowski TJ, Aaronson KD, Pietroski RE, Pagani FD, Altschuler R, Higgins RS et al. Discordance in interpretation of potential donor echos. *J Heart Lung Transplant* 1998; 17(1(Supp-1)):S100.
- (109) Zaroff JG, Rordorf GA, Ogilvy CS, Picard MH. Regional patterns of left ventricular systolic dysfunction after subarachnoid hemorrhage: Evidence for neurally mediated cardiac injury. *J Am Soc Echocardiogr* 2000; 13(8):774-779.

- (110) Paul JJ, Tani LY, Shaddy RE, Minich LL. Spectrum of left ventricular dysfunction in potential pediatric heart transplant donors. *J Heart Lung Transplant* 2003; 22(5):548-552.
- (111) Dujardin KS, McCully RB, Wijdicks EFM, Tazelaar HD, Seward JB, McGregor CGA et al. Myocardial dysfunction associated with brain death: Clinical, echocardiographic, and pathologic features. *J Heart Lung Transplant* 2001; 20(3):350-357.
- (112) Seiler C, Laske A, Gallino A, Turina M, Jenni R. Echocardiographic evaluation of left ventricular wall motion before and after heart transplantation. *J Heart Lung Transplant* 1992; 11(5):867-874.
- (113) Boucek MM, Mathis CM, Kanakriyeh MS, McCormack J, Razzouk A, Gundry SR et al. Donor shortage: use of the dysfunctional donor heart. *J Heart Lung Transplant* 1993; 12(6 Pt 2):S186-S190.
- (114) Kass DA, Maughan WL, Zhong MG, Kono A, Sunagawa K, Sagawa K. Comparative influence of load versus inotropic states on indexes of ventricular contractility: Experimental and theoretical analysis based on pressure-volume relationships. *Circulation* 1987; 76(6):1422-1436.
- (115) Satchithananda DK, Stoica SC, White PA, Charman S, Luckraz H, Schofield PM et al. Systolic right ventricular dysfunction in 'good' donor hearts- a normal finding? *J Heart Lung Transplant* 2001; 20(2):243.

- (116) Tei C, Ling LH, Hodge DO, Bailey KR, Oh JK, Rodeheffer RJ et al. New index of combined systolic and diastolic myocardial performance: a simple and reproducible measure of cardiac function--a study in normals and dilated cardiomyopathy. *J Cardiol* 1995; 26(6):357-366.
- (117) Yotti R, Bermejo J, Desco MM, Antoranz JC, Rojo-Alvarez JL, Cortina C et al. Doppler-derived ejection intraventricular pressure gradients provide a reliable assessment of left ventricular systolic chamber function. *Circulation* 2005; 112(12):1771-1779.
- (118) Vogel M, Cheung MM, Li J, Kristiansen SB, Schmidt MR, White PA et al. Noninvasive assessment of left ventricular force-frequency relationships using tissue Doppler-derived isovolumic acceleration: validation in an animal model. *Circulation* 2003; 107(12):1647-1652.
- (119) Harjai KJ, Scott L, Vivekananthan K, Nunez E, Edupuganti R. The Tei index: a new prognostic index for patients with symptomatic heart failure. *J Am Soc Echocardiogr* 2002; 15(9):864-868.
- (120) Cheung MM, Redington AN. Assessment of myocardial ventricular function in donor hearts: is isovolumic acceleration measured by tissue Doppler the Holy Grail? *J Heart Lung Transplant* 2004; 23(9 Suppl):S253-S256.
- (121) Vogel M, Schmidt MR, Kristiansen SB, Cheung M, White PA, Sorensen K et al. Validation of myocardial acceleration during isovolumic contraction as a

novel noninvasive index of right ventricular contractility: comparison with ventricular pressure-volume relations in an animal model. *Circulation* 2002; 105(14):1693-1699.

- (122) Kono T, Nishina T, Morita H, Hirota Y, Kawamura K, Fujiwara A. Usefulness of low-dose dobutamine stress echocardiography for evaluating reversibility of brain death-induced myocardial dysfunction. *Am J Cardiol* 1999; 84(5):578-582.
- (123) Babcock WD, Menza RL, Zaroff JG. Serial echocardiography increase donor heart utilization. *J Heart Lung Transplant* 2003; 22(1 (Supp 1)):74.
- (124) Bock JC, Lewis FR. Clinical relevance of lung water measurement with the thermal-dye dilution technique. *J Surg Res* 1990; 48(3):254-265.
- (125) Lange NR, Schuster DP. The measurement of lung water. *Crit Care* 1999; 3(2):R19-R24.
- (126) Chu RY, Carlile PV, Jr., Basmadjian G. Dual-isotope measurement of lung water. *Int J Rad Appl Instrum* 1989; 16(4):419-421.
- (127) Cutillo AG, Goodrich KC, Ganesan K, Watanabe S, Ailion DC, Albertine KH et al. Lung water measurement by nuclear magnetic resonance: correlation with morphometry. *J Appl Physiol* 1995; 79(6):2163-2168.

- (128) Saluhke T.V, Wyncoll D.L. Volumetric haemodynamic monitoring and continuous pulse contour analysis – an untapped resource for coronary and high dependency care units? *Br J Cardiol* 2002; 9(1):A1C20-A1C25.
- (129) Sakka SG, Ruhl CC, Pfeiffer UJ, Beale R, McLuckie A, Reinhart K et al. Assessment of cardiac preload and extravascular lung water by single transpulmonary thermodilution. *Intensive Care Med* 2000; 26(2):180-187.
- (130) Kirov MY, Kuzkov VV, Kuklin VN, Waerhaug K, Bjertnaes LJ. Extravascular lung water assessed by transpulmonary single thermodilution and postmortem gravimetry in sheep. *Crit Care* 2004; 8(6):R451-R458.
- (131) Katzenelson R, Perel A, Berkenstadt H, Preisman S, Kogan S, Sternik L et al. Accuracy of transpulmonary thermodilution versus gravimetric measurement of extravascular lung water. *Crit Care Med* 2004; 32(7):1550-1554.
- (132) Mihm FG, Feeley TW, Jamieson SW. Thermal dye double indicator dilution measurement of lung water in man: comparison with gravimetric measurements. *Thorax* 1987; 42(1):72-76.
- (133) Sturm JA. Development and significance of lung water measurement in clinical and experimental practice. In: Lewis FR, Pfeiffer UJ, editors. *Practical applications of fibreoptics in critical care monitoring*. Berlin: Springer, 1990: 129-139.

- (134) Sakka SG, Klein M, Reinhart K, Meier-Hellmann A. Prognostic value of extravascular lung water in critically ill patients. *Chest* 2002; 122(6):2080-2086.
- (135) Khan S, Trof RJ, Groeneveld AJ. Transpulmonary dilution-derived extravascular lung water as a measure of lung edema. *Curr Opin Crit Care* 2007; 13(3):303-307.
- (136) Mitchell JP, Schuller D, Calandrino FS, Schuster DP. Improved outcome based on fluid management in critically ill patients requiring pulmonary artery catheterization. *Am Rev Respir Dis* 1992; 145(5):990-998.
- (137) Smith M. Physiologic changes during brain stem death--lessons for management of the organ donor. *J Heart Lung Transplant* 2004; 23(9 Suppl):S217-S222.
- (138) Klemperer JD, Klein I, Gomez M, Helm RE, Ojamaa K, Thomas SJ et al. Thyroid hormone treatment after coronary-artery bypass surgery. *N Engl J Med* 1995; 333(23):1522-1527.
- (139) Ranasinghe AM, Quinn DW, Pagano D, Edwards N, Farouqi M, Graham TR et al. Glucose-insulin-potassium and tri-iodothyronine individually improve hemodynamic performance and are associated with reduced troponin I release after on-pump coronary artery bypass grafting. *Circulation* 2006; 114(1 Suppl):I245-I250.

- (140) Wahlers T, Cremer J, Fieguth HG, Dammenhayn L, Albes J, Schafers HJ et al. Donor heart-related variables and early mortality after heart transplantation. *J Heart Lung Transplant* 1991; 10(1 Pt 1):22-27.
- (141) Stoica SC, Satchithananda DK, Atkinson C, White PA, Redington AN, Goddard M et al. The energy metabolism in the right and left ventricles of human donor hearts across transplantation. *Eur J Cardiothorac Surg* 2003; 23(4):503-512.
- (142) Novitzky D, Cooper DK, Rosendale JD, Kauffman HM. Hormonal therapy of the brain-dead organ donor: experimental and clinical studies. *Transplantation* 2006; 82(11):1396-1401.
- (143) Novitzky D, Cooper DK. Results of hormonal therapy in human brain-dead potential organ donors. *Transplant Proc* 1988; 20(5 Suppl 7):59-62.
- (144) Rosendale JD, Chabalewski FL, McBride MA, Garrity ER, Rosengard BR, Delmonico FL et al. Increased transplanted organs from the use of a standardized donor management protocol. *Am J Transplant* 2002; 2(8):761-768.
- (145) Timek T, Vahl CF, Bonz A, Schaffer L, Rosenberg M, Hagl S. Triiodothyronine reverses depressed contractile performance after excessive catecholamine stimulation. *Ann Thorac Surg* 1998; 66(5):1618-1625.

- (146) Taniguchi S, Kitamura S, Kawachi K, Doi Y, Aoyama N. Effects of hormonal supplements on the maintenance of cardiac function in potential donor patients after cerebral death. *Eur J Cardiothorac Surg* 1992; 6(2):96-101.
- (147) Zuppa AF, Nadkarni V, Davis L, Adamson PC, Helfaer MA, Elliott MR et al. The effect of a thyroid hormone infusion on vasopressor support in critically ill children with cessation of neurologic function. *Crit Care Med* 2004; 32(11):2318-2322.
- (148) Pennefather SH. Effects of triiodothyronine and vasopressin on cardiac function and myocardial blood flow after brain death. *J Heart Lung Transplant* 1993; 12(5):889-890.
- (149) Yoshioka T, Sugimoto H, Uenishi M, Sakamoto T, Sadamitsu D, Sakano T et al. Prolonged hemodynamic maintenance by the combined administration of vasopressin and epinephrine in brain death: a clinical study. *Neurosurg* 1986; 18(5):565-567.
- (150) Bosso FJ, Allman FD, Pilati CF. Myocardial work load is a major determinant of norepinephrine-induced left ventricular dysfunction. *Am J Physiol Heart Circ Physiol* 1994; 266(2):H531-H539.
- (151) Stoica SC, Satchithananda DK, White PA, Parameshwar J, Redington AN, Large SR. Noradrenaline use in the human donor and relationship with load-

independent right ventricular contractility. Transplantation 2004; 78(8):1193-1197.

- (152) Schnuelle P, Berger S, De Boer J, Persijn G, Van der Woude FJ, Hickman R. Effects of catecholamine application to brain-dead donors on graft survival in solid organ transplantation. Transplantation 2001; 72(3):362-363.

- (153) Kinoshita Y, Okamoto K, Yahata K, Yoshioka T, Sugimoto T, Kawaguchi N et al. Clinical and pathological changes of the heart in brain death maintained with vasopressin and epinephrine. Pathol Res Pract 1990; 186(1):173-179.

- (154) Wilhelm MJ, Pratschke J, Beato F, Taal M, Laskowski IA, Paz DM et al. Activation of proinflammatory mediators in heart transplants from brain-dead donors: evidence from a model of chronic rat cardiac allograft rejection. Transplant Proc 2002; 34(6):2359-2360.

- (155) Segel LD, vonHaag DW, Zhang J, Follette DM. Selective overexpression of inflammatory molecules in hearts from brain-dead rats. J Heart Lung Transplant 2002; 21(7):804-811.

- (156) Venkateswaran RV, Patchell VB, Wilson IC, Mascaro JG, Thompson RD, Quinn DW et al. Early donor management increases the retrieval rate of lungs for transplantation. Ann Thorac Surg 2008; 85(1):278-286.

- (157) Venkateswaran RV, Steeds RP, Quinn DW, Nightingale P, Wilson IC, Mascaro J et al. The haemodynamic effects of adjunctive hormone therapy in potential heart donors: A prospective randomized double blind factorially designed controlled trial. *Eur Heart J* 2009; 30(14):1771-1780.
- (158) Taylor DO, Edwards LB, Boucek MM, Trulock EP, Aurora P, Christie J et al. Registry of the International Society for Heart and Lung Transplantation: twenty-fourth official adult heart transplant report--2007. *J Heart Lung Transplant* 2007; 26(8):769-781.
- (159) Horwich TB, Patel J, MacLellan WR, Fonarow GC. Cardiac troponin I is associated with impaired hemodynamics, progressive left ventricular dysfunction, and increased mortality rates in advanced heart failure. *Circulation* 2003; 108(7):833-838.
- (160) Venkateswaran RV, Steeds RP, Wilson IC, Mascaro JG, Thompson RD, Townend JN et al. Early management and norepinephrine withdrawal but not hormonal therapy, improve donor heart function. *Circulation* 2006; S-II:709.
- (161) Darracott-Cankovic S, Stovin PG, Wheeldon D, Wallwork J, Wells F, English TA. Effect of donor heart damage on survival after transplantation. *Eur J Cardiothorac Surg* 1989; 3(6):525-532.

- (162) Sopko N, Shea KJ, Ludrosky K, Smedira N, Hoercher K, Taylor DO et al. Survival is not compromised in donor hearts with echocardiographic abnormalities. *J Surg Res* 2007; 143(1):141-144.
- (163) Goland S, Czer LSC, Kass RM, Siegel RJ, Mirocha J, De Robertis MA et al. Use of cardiac allografts with mild and moderate left ventricular hypertrophy can be safely used in heart transplantation to expand the donor pool. *J Am Coll Cardiol* 2008; 51(12):1214-1220.
- (164) Jeevanandam V, Furukawa S, Prendergast TW, Todd BA, Eisen HJ, McClurken JB. Standard criteria for an acceptable donor heart are restricting heart transplantation. *Ann Thorac Surg* 1996; 62(5):1268-75.
- (165) Milano A, Livi U, Casula R, Bortolotti U, Gambino A, Zenati M et al. Influence of marginal donors on early results after heart transplantation. *Transplant Proc* 1993; 25(6):3158-3159.
- (166) Lima B, Rajagopal K, Petersen RP, Shah AS, Soule B, Felker GM et al. Marginal cardiac allografts do not have increased primary graft dysfunction in alternate list transplantation. *Circulation* 2006; 114(1 Suppl):I27-I32.
- (167) Cheung MM, Smallhorn JF, Redington AN, Vogel M. The effects of changes in loading conditions and modulation of inotropic state on the myocardial performance index: comparison with conductance catheter measurements. *Eur Heart J* 2004; 25(24):2238-2242.

- (168) Venkateswaran RV, Ganesh JS, Thekkudan J, Steeds R, Wilson IC, Mascaro J et al. Donor cardiac troponin-I: a biochemical surrogate of heart function. *Eur J Cardiothorac Surg* 2009; 36(2):286-292.
- (169) Olszewski R, Timperley J, Szmigielski C, Monaghan M, Nihoyannopoulos P, Senior R et al. The clinical applications of contrast echocardiography. *Eur J Echocardiogr* 2007; 8(3):S13-S23.
- (170) Marelli D, Laks H, Bresson S, Ardehali A, Bresson J, Esmailian F et al. Results after transplantation using donor hearts with preexisting coronary artery disease. *J Thorac Cardiovasc Surg* 2003; 126(3):821-825.
- (171) Trulock EP, Edwards LB, Taylor DO, Boucek MM, Keck BM, Hertz MI. Registry of the International Society for Heart and Lung Transplantation: Twenty-third Official Adult Lung and Heart-Lung Transplantation Report--2006. *J Heart Lung Transplant* 2006; 25(8):880-892.
- (172) Anyanwu AC, Rogers CA, Murday AJ. Intrathoracic organ transplantation in the United Kingdom 1995-99: results from the UK cardiothoracic transplant audit. *Heart* 2002; 87(5):449-454.
- (173) Amado JA, Lopez-Espadas F, Vazquez-Barquero A, Salas E, Riancho JA, Lopez-Cordovilla JJ et al. Blood levels of cytokines in brain-dead patients: relationship with circulating hormones and acute-phase reactants. *Metabolism* 1995; 44(6):812-816.

- (174) Avlonitis VS, Fisher AJ, Kirby JA, Dark JH. Pulmonary transplantation: the role of brain death in donor lung injury. *Transplantation* 2003; 75(12):1928-1933.
- (175) Dupont E, Schandene L, Denys C, Wybran J. Differential in vitro actions of cyclosporin, methylprednisolone, and 6-mercaptopurine: implications for drugs' influence on lymphocyte activation mechanisms. *Clin Immunol Immunopathol* 1986; 40(3):422-428.
- (176) Straznicka M, Follette DM, Eisner MD, Roberts PF, Menza RL, Babcock WD. Aggressive management of lung donors classified as unacceptable: excellent recipient survival one year after transplantation. *J Thorac Cardiovasc Surg* 2002; 124(2):250-258.
- (177) Aigner C, Winkler G, Jaksch P, Seebacher G, Lang G, Taghavi S et al. Extended donor criteria for lung transplantation--a clinical reality. *Eur J Cardiothorac Surg* 2005; 27(5):757-761.
- (178) De Perrot M, Chaparro C, McRae K, Waddell TK, Hadjiliadis D, Singer LG et al. Twenty-year experience of lung transplantation at a single center: Influence of recipient diagnosis on long-term survival. *J Thorac Cardiovasc Surg* 2004; 127(5):1493-1501.

- (179) Pierre AF, Sekine Y, Hutcheon MA, Waddell TK, Keshavjee SH, Mulligan MS et al. Marginal donor lungs: A reassessment. *J Thorac Cardiovasc Surg* 2002; 123(3):421-428.
- (180) Riou B, Guesde R, Jacquens Y, Duranteau R, Viars P. Fiberoptic bronchoscopy in brain-dead organ donors. *Am J Respir Crit Care Med* 1994; 150(2):558-560.
- (181) Mukadam ME, Harrington DK, Wilson IC, Mascaro JG, Rooney SJ, Thompson RD et al. Does donor catecholamine administration affect early lung function after transplantation? *J Thorac Cardiovasc Surg* 2005; 130(3):926-927.
- (182) Hedlund LW, Putman CE. Methods for detecting pulmonary edema. *Toxicol Ind Health* 1985; 1(2):59-68.
- (183) Liebman PR, Philips E, Weisel R, Ali J, Hechtman HB. Diagnostic value of the portable chest x-ray technic in pulmonary edema. *Am J Surg* 1978; 135(4):604-606.
- (184) Ware LB, Wang Y, Fang X, Warnock M, Sakuma T, Hall TS et al. Assessment of lungs rejected for transplantation and implications for donor selection. *Lancet* 2002; 360(9333):619-620.

- (185) Matthay MA, Folkesson HG, Clerici C. Lung epithelial fluid transport and the resolution of pulmonary edema. *Physiol Rev* 2002; 82(3):569-600.
- (186) Lane SM, Maender KC, Awender NE, Maron MB. Adrenal epinephrine increases alveolar liquid clearance in a canine model of neurogenic pulmonary edema. *Am J Respir Crit Care Med* 1998; 158(3):760-768.
- (187) Perkins GD, McAuley DF, Thickett DR, Gao F. The beta-Agonist Lung Injury Trial (BALTI): A Randomized Placebo-controlled Clinical Trial. *Am J Respir Crit Care Med* 2006; 173(3):281-287.
- (188) Michard F. Bedside assessment of extravascular lung water by dilution methods: temptations and pitfalls. *Crit Care Med* 2007; 35(4):1186-1192.
- (189) Pearce Morton Lee, Yamashita J, Beazell J. Measurement of Pulmonary Edema. *Circ Res* 1965; 16(5):482-488.
- (190) Holcroft JW, Trunkey DD. Extravascular lung water following hemorrhagic shock in the baboon: Comparison between resuscitation with Ringer's lactate and Plasmanate. *Ann Surg* 1974; 180(4):408-417.
- (191) Chen HI. Hemodynamic mechanisms of neurogenic pulmonary edema. *Biol Signals* 1995; 4(3):186-192.

- (192) Atabai K, Ware L, Snider M, Koch P, Daniel B, Nuckton T et al. Aerosolized β_2 -adrenergic agonists achieve therapeutic levels in the pulmonary edema fluid of ventilated patients with acute respiratory failure. *Intensive Care Med* 2002; 28(6):705-711.