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POST OPERATIVE ATRIAL FIBRILLATION

A Thesis presented for the degree of Doctor of Medicine

By

Dr John Cosgrave, MB BCh BAO (NUI), LRCPI&SI, MRCP (UK)

To Dublin University, Trinity College

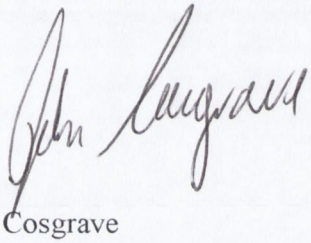
September 2006

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Dr John Cosgrave

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Summary of Thesis.

Post operative atrial fibrillation is a common occurrence following all forms of cardiac surgery apart from heart transplantation. Coronary artery bypass is the commonest cardiac surgical procedure with more than 800,000 cases performed per year worldwide. The incidence of post coronary artery bypass atrial fibrillation varies depending on the definitions used, but ranges from 10 to 40% of patients. This arrhythmia may terminate spontaneously and have no consequences however in some it causes significant morbidity and may even increase mortality. Thus it can be seen that with a potential incidence of up to 320,000 cases per year this arrhythmia imposes a significant burden on our health care systems. Despite many theories the precise aetiology remains unknown; this thesis will investigate some of the current theories for the development of atrial fibrillation.

There are some data to suggest an inflammatory basis for atrial fibrillation both in the surgical and non surgical settings. On pump cardiac surgery is associated with a significant inflammatory response. In light of this I enrolled 150 patients undergoing coronary artery bypass grafting and measured five different inflammatory markers at baseline and at a variety of timepoints following surgery. All patients were closely monitored for the development of atrial fibrillation. There was no association between the development of atrial fibrillation and the inflammatory response as assessed by the markers measured. In fact apart from sVCAM all the markers were lower in the group that developed atrial fibrillation.

Alterations in the refractory period of the atrial tissue may precipitate atrial fibrillation. Changes in the electrical properties of the atria could be caused by atrial

stretch secondary to increased atrial pressure. Left ventricular dysfunction can occur in the post-operative period and potentially lead to atrial pressure overload. As invasive haemodynamic monitoring is impractical for the time period when atrial fibrillation can develop we used brain natriuretic peptide as a surrogate marker for left ventricular end diastolic pressure. I studied 133 patients with good left ventricular function and normal pre operative BNP levels. All patients had a significant rise in their post-operative BNP levels and that in those that developed atrial fibrillation greater than 48 hours after surgery this rise was significantly different to those that remained in sinus rhythm.

Not only can the atrial electrical activity be altered acutely but patients with pre-existing atrial abnormalities may be at increased risk of developing atrial fibrillation. These structural changes are associated with advancing age and the average age of patients undergoing cardiac surgery is increasing. I collected atrial tissue samples from 92 patients prior to commencing cardiopulmonary bypass. We studied 10 different morphological features and found that none of them correlated with the development of atrial fibrillation.

On pump cardiac surgery involves arresting the heart and providing circulation via an extra-corporeal circuit. During this process the heart is at risk of ischaemia and potentially of infarction. Post operative infarction has been associated with atrial fibrillation in previous studies. I measured Troponin T a sensitive marker of myocardial necrosis in 100 patients and correlated the levels with post-operative outcomes including atrial fibrillation. While almost all the patients demonstrated some

degree of troponin elevation there was no correlation with any of the endpoints analysed.

Thus the data from this thesis do not show any association between the post-operative inflammatory response, the atrial histological substrate, markers of myonecrosis and post-operative atrial fibrillation. Following cardiac surgery brain natriuretic peptide levels rise significantly and that this elevation is associated with the onset of atrial fibrillation greater than 48 hours after surgery.

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I would like first to acknowledge the constant support of my supervisor Dr Brendan Foley. Despite being a busy clinician he always found time for discussion about the design and execution of this thesis. Without his enthusiasm and encouragement this project would never have been completed. I would also like to thank his colleagues in the Department of Cardiology Professor Michael Walsh and Dr Peter Crean who continue to foster an environment contributing to quality research.

Throughout this project I worked closely with all the staff of the Cardiac surgery unit. I received constant assistance and many useful suggestions and it was a pleasure to be allowed to work in such a professional and caring unit. It is a credit to all that such a complex procedure is carried out with such excellent results and such a high degree of patient satisfaction. The head of the department Ms Eilis McGovern and her colleagues Mr Michael Tolan and Mr Vincent Young deserve particular mention as does Dr Tom Ryan of the Department of Anaesthetics and Intensive Care.

Drs Sean O'Briain and Richard Flavin of the Department of Pathology were extremely helpful in analysing the tissue samples and in educating me in the intricacies of atrial histology. Dr Kathleen Bennet of the Department of Pharmacology and Therapeutics was always available to guide me through statistical analysis and to help convert vast amounts of data into clinically meaningful results.

I would also like to thank Professor Dermot Kelleher and all the staff of the Sir Patrick Duns laboratory. A particular mention is required for the patience of Dr Deirdre Ni Eidhin and Dr Anne Murphy for tolerating a clinician utilizing valuable laboratory resources and their vast knowledge. It is also important to mention Professor Con Feighery and Dr Alex Whelan of the Department of Immunology. Not only were they instrumental in the CRP analysis but were always available for advice on the many complexities of the inflammatory response.

Thanks are also due to the Board of The Royal City of Dublin Hospital Trust for the creation and maintenance of the research institute within the CREST Directorate. In particular this work was supported by a research grant from the Royal City of Dublin Trust without which this project would not have been possible.

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OBJECTIVES.

The objective of this thesis was to evaluate different theories for the development of post operative atrial fibrillation. The thesis has been divided into four different sections as follows:

1/ The primary aim of this section of the thesis was to determine if there was a relationship between the peri-operative inflammatory response, assessed by soluble forms of the adhesion molecules and C-reactive protein, and the development of atrial fibrillation following coronary artery bypass surgery.

2/ The objective of this study was to determine whether there was a relationship between atrial stretch and the development of post-operative atrial fibrillation. Brain natriuretic peptide was used as a surrogate marker for left ventricular end diastolic pressure and was measured 24 and 48 hours following the onset of cardiopulmonary bypass.

3/ The aim of this portion of the thesis was to determine whether there is a relationship between the pre-operative atrial morphology and the development of post-operative atrial fibrillation. I sought to determine if the pre-operative atrial substrate as determined by 10 histological features was associated with the development of atrial fibrillation.

4/ The objective of this study was to evaluate the association between post-operative troponin T elevation (a marker of myocardial necrosis) in a low risk group undergoing cardiac surgery and peri-operative variables and clinical outcomes including atrial fibrillation.

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Publications:

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2/ Cosgrave J, Foley JB, McGovern E, Bennett K, Young V, Tolan M, Crean P, Walsh M. Brain Natriuretic Peptide Elevation And The Development Of Atrial Fibrillation Following Coronary Artery Bypass Surgery. **Interactive Cardiovascular and Thoracic Surgery**. 2006; 5: 111-114.

3/ Cosgrave J, Foley JB, Flavin R, O'Briain DS, Fitzpatrick E, Bennett K, McGovern E, Young V, Tolan M, Crean P and Walsh M. Preoperative Atrial Histological Changes are not Associated with Postoperative Atrial Fibrillation. **Cardiovascular Pathology**. 2006; 15(4):213-217.

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9/ Cosgrave J, Foley JB, Bennett K, McGovern E, Young V, Tolan M, Crean P and Walsh M. The Association of Peri-operative BNP Levels in Patients Undergoing CABG and Post-operative Atrial Fibrillation. **Irish Cardiac Society.** 2003 Poster Presentation.

10/ Cosgrave J, Foley JB, Flavin R, O'Briain DS, Fitzpatrick E, Bennett K, McGovern E, Young V, Tolan M, Crean P and Walsh M. Atrial histological analysis does not predict atrial fibrillation in patients undergoing CABG. **Irish Cardiac Society**. 2004 Poster Presentation.

CHAPTER 1

Introduction

INTRODUCTION.

Post operative atrial fibrillation remains a common occurrence following all forms of cardiac surgery. While many patients recover spontaneously in others it is associated with prolongation of hospital stay and deleterious clinical events such as cerebro-vascular accidents. Despite many advances in the field of electrophysiology the precise aetiology of atrial fibrillation remains to be defined.

This introductory chapter will give a historical perspective on cardiac surgery and then review the current knowledge about the aetiology and clinical consequences of this arrhythmia. The post-operative inflammatory response will be discussed from the perspective of causation and key inflammatory markers and finally the natriuretic peptides and their potential clinical applications will be introduced.

Cardiac surgery a historical perspective.

The history of cardiac surgery began in Germany in 1896 when Ludwig Rehn successfully sutured a stab wound in the right ventricle (1). In the nineteen twenties Rehn performed the first pericardiectomy and in 1923 Elliot Cutler attempted the first mitral valvulotomy through the apex of the left ventricle (2). Two years later Souttar performed the first mitral valvulotomy by introducing his fingers through the left atrial appendage. Unfortunately, both procedures were abandoned for the next 25 years and the development of cardiac surgery stagnated during that time (3).

The next important advances occurred in the field of congenital heart disease when in 1938 Gross closed a patent ductus arteriosus, and in 1944 Blalock created the

first subclavian to pulmonary artery anastomosis for the treatment of Tetralogy of Fallot (4, 5) In 1945 both Gross and Crafoord independently performed the first successful resections of a coarctation of the aorta with end to end repair (6, 7).

The late forties and early fifties were notable for the development of many different techniques to successfully treat mitral stenosis; however, the modern era of open cardiac surgery began in 1952 with an ASD closure using hypothermia (8). John Gibbon used the first bypass pump to close an ASD in May of1953, but unfortunately the subsequent four patients died and the procedure was abandoned (9). This led to Lilleheis work on cross circulation using an adult donor with the same blood group to provide the circulation for a child having a congenital heart defect repaired (10). The first case to combine hypothermia with extra-corporeal circulation was performed in Duke University in 1956 and throughout the following decade many modifications were made which allowed treatments to be developed for a variety of congenital cardiac lesions (11).

Valve replacement surgery was pioneered by Charles Hufnagel who in 1952 implanted a prosthesis in the descending aorta of a patient with aortic incompetence. The first valves were implanted in the human heart in 1960 by Starr and Harken in independent operations (12, 13, 14).

The era of cardiac revascularisation began with the pioneering work of a Canadian surgeon Arthur Vineberg (15). In 1950 he dissected the internal mammary artery (IMA) and implanted it in a myocardial tunnel parallel to the left anterior descending coronary artery. The intention was that this vessel would arborise and develop collateral communications with the native coronary vessels. This procedure

was developed prior to the advent of selective coronary angiography (1959) and was initially not widely accepted. In 1965 Sones group demonstrated flow from the implanted IMA into the surrounding native vessels (16). Between 10 and 20,000 Vineberg operations were carried out worldwide however this procedure became obsolete following the development of coronary artery bypass as we now know it. This procedure utilising a saphenous vein as the conduit was initially performed in the Cleveland clinic in1967 by Favalaro and ushered in the modern era of cardiac revascularisation surgery (17). Currently in all its forms this is the most frequently performed cardiac operation, with more than 800,000 cases per year worldwide.

ATRIAL FIBRILLATION.

Atrial fibrillation (AF) is an irregular cardiac rhythm, which originates from above the atrio-ventricular node. It is characterised by rapid atrial depolarisation with chaotic fibrillary waves (18). The atrial cells can depolarise as rapidly as 400-600 times a minute, fortunately, not all these impulses are transmitted to the ventricles. This usually results in an irregular ventricular response. As the depolarisation of the atria is so rapid and chaotic the atria merely quiver and there is no functional atrial contraction. This leads to the increased embolic risk associated with this rhythm and may lead to haemodynamic compromise in some patients who are dependant on atrial transport to maintain adequate cardiac output.

Post-operative atrial fibrillation.

Atrial fibrillation can occur after any surgical intervention. In surgery, which does not involve a thoracotomy, the incidence is approximately 5%(19). If a thoracotomy is required for non-cardiac surgery the incidence of atrial fibrillation remains low at 12%, however following pneumonectomy the occurrence of this complication approaches 20-40% similar to the frequency seen following coronary artery surgery (20,21).

Atrial fibrillation following cardiac surgery.

The first reports of atrial fibrillation following cardiac surgery came from Favolaro's group in the Cleveland Clinic. In their review of their first 100 patients to undergo CABG between 1967and 1968, 12 patients developed atrial fibrillation (22). This relatively low incidence may be explained by the average age of the group, which at 50 was low compared to current practice.

In more recent times atrial fibrillation remains the commonest post-operative complication, it occurs in between 10 and 65% of patients depending on the type of surgery, the definition of the arrhythmia, the method of arrhythmia monitoring and the clinical characteristics of the patients studied (23,24). Patients undergoing valve replacement or repair have a much higher incidence of AF than patients who have isolated myocardial revascularisation (25,26) A meta-analysis of 24 studies of isolated coronary artery bypass grafting published in 1991 estimated the incidence at 26.7%. The age of patients referred for surgery has continued to increase since then and these data likely underestimate the current size of the problem (27).As coronary artery

bypass is such a common procedure in the western world any complication with an incidence of up to 40% has significant clinical and financial implications (28). A recent editorial in the Journal of the American College of Cardiology stated that post-operative atrial fibrillation is costing the American health care system one billion dollars per year (29).

There is a temporal relationship between surgery and the onset of atrial fibrillation. It is most likely to occur on days 2 and 3 following the operation, but may occur up to many weeks following the operation (23). In one large series of over 1500 patients undergoing cardiac surgery 28.4% developed AF. Of these only 4.7% of supraventricular arrhythmias occurred on the first post-operative day with a peak of 43% on day 2 and an incidence as low as 0.4% on day 9 or greater (30).

Aetiology.

The precise aetiology of atrial fibrillation, be it independent of, or related to surgery remains unclear, although there are a number of theories. Unlike non-surgical AF the relative electrophysiological importance of a rapidly firing focus has not been completely characterised (31, 32). This is not to say that atrial ectopic activity does not play a role in the pathogenesis of post-operative AF as it has been shown that atrial ectopics are more common in the minutes and hours prior to the onset of AF (33). These ectopics may precipitate AF in patients with a susceptible atrial electrical substrate.

The multiple wavelength hypotheses, which was first proposed by Moe in 1962, may also have a role to play in post-operative AF (34). This suggests that atrial

fibrillation is sustained by multiple re-entrant circuits and that random pathways are determined by atrial refractoriness, excitability and conduction properties. In most cases these re-entrant circuits are constantly arising, colliding and being extinguished. It requires a critical mass of atrial tissue to sustain the arrhythmia and adjacent atrial regions with different refractory periods may increase re-entry. Another important concept is the presence of "drivers", these are areas that generate a rapid rhythm of a very short cycle length, the atria are unable to respond to this in a one to one fashion and so the result is fibrillatory conduction. These atrial re-entry circuits are often referred to as "rotors" and can be self-sustaining. A vortex of electrical waves (spiral waves) can arise and precipitate and propagate AF. The pulmonary veins and indeed other structures such as the vena cava, ligament of Marshall and the thoracic veins have also been implicated in AF. A sleeve of atrial tissue often extends into these structures and a rapidly firing focus has been shown to precipitate AF. Slowing in atrial conduction may also increase re-entry and this is supported by the fact that an increase in pre-operative P-wave duration on signal averaged ECG is a risk factor for the development of post-operative AF (35,36). A short cycle wavelength and an increase in atrial size may enhance atrial vulnerability to AF (37). Atrial ischaemia, trauma, stretch, sympathetic activation or inflammation may alter atrial refractoriness, shorten cycle length or slow atrial conduction and hence precipitate the arrhythmia.

Modern cardioplegia, particularly with single atrial cannulae may result in inadequate atrial protection and subsequent ischaemia. The atrial septum remains warmer than the ventricular myocardium and often demonstrates electrical activity while the ventricle is in asystole. This factor has been shown to be associated with the

development of post-operative AF (38-40). A small retrospective angiographic study found that compared to controls, patients who developed post-operative AF were more likely to have impaired blood supply to either the SA or AV node (41). The situation is not however clear-cut as there is a low incidence of AF following cardiac transplantation, an operation that is usually associated with atrial ischaemia. In a study by Sato et al on a canine model augmented atrial hypothermia had no significant effect on atrial refractory periods or on the inducibility of arrhythmias (42).

Increased sympathetic activation occurs in the peri-operative period in response to the physical and psychological stresses the surgery imposes (43). In non-surgical AF an increase in sympathetic tone can shorten the atrial refractory period, promote atrial re-entry and increase triggered automaticity (37, 44). In an experimental model of isolated human atrial fibres epinephrine has been shown to cause arrhythmogenic changes (45). In accordance with this theory is the observation that withdrawal of pre-operative beta-blockers in the post-operative period leads to an increase in the incidence of AF, perhaps due to a combination of an up-regulation of beta-receptors and increased circulating catecholamines (23). Also a study by Kalman et al on 131 patients found that right atrial norepinephrine levels were independently associated with the occurrence of post-operative AF (46). Advancing age, which is a consistent predictor for post-operative AF, is also associated with increased levels of norepinephrine (23, 47). Despite this there are conflicting data in one study of patients administered exogenous catecholamines there was no difference in the incidence of post-operative AF (48).In another study, while the catecholamine levels were elevated for three days post-operatively there was no association with the development of AF

(43). Sympathetic activation is highest in the first 24 hours after surgery whereas AF is commoner later in the post-operative course (49).

Fluid volume shifts occur in the post-operative period either as a consequence of neuro-hormonal activation, sepsis, the post-operative inflammatory response or iatrogenic causes. The interstitial movement of fluid may alter the electrical properties of the atria or as shown in a study on patients undergoing pneumonectomy alter the intracardiac pressure without causing chamber dilation (50, 51). Such increase in intracardiac pressure may lead to atrial stretch and altered refractoriness.

As cardiac surgery is associated with both a high incidence of AF and a significant pro-inflammatory milieu many authors have hypothesised that AF is precipitated by inflammation. The theory that AF may be associated with inflammation is supported by a number of studies and observations, which will be discussed in detail later.

Clinical predictors of atrial fibrillation.

Pre-operative factors.

Many studies have attempted to define the risk factors for the development of atrial fibrillation; unfortunately these have yielded conflicting results. Only advancing age has consistently predicted a higher incidence of post-operative AF (23-25, 27, 28, 30). For each decade of increasing age the incidence is increased by approximately 50%. It is estimated that less than 5% of patients under 40 and more than a third of patients over 70 develop AF (52). This strong association may well be explained by age related structural changes such as increased fibrosis and delayed conduction (53).

Either could contribute to the formation of a suitable electrical substrate for the propagation of AF. The age of patients undergoing cardiac surgery is steadily rising and this may partially explain the rising incidence of this arrhythmia.

The presence of chronic obstructive airways disease is an independent predictor for post-operative AF perhaps due to right atrial enlargement secondary to pulmonary hypertension. A prior history of AF or congestive cardiac failure is also associated (24, 26, 30). Hypertension which is associated with AF in non-surgical populations also seems to play a role; perhaps due to increased atrial fibrosis and men are more likely to develop AF than women (23, 25).

Pre-operative digoxin use has been studied in two large trials (26, 30). In one of these digoxin use was associated with AF on univariate analysis but not when a multivariate model was used. In the other study the incidence of AF was 44% in those taking pre-operative digoxin and 32% in those who were free of digoxin therapy. As more elderly patients and those with poor LV function or a prior history of AF are likely to be taking digoxin its clinical utility as a predictive marker would seem to be limited.

Clinical parameters such as cardiovascular risk factors, severity and clinical presentation of coronary disease or haemodynamic parameters prior to surgery have not been shown to consistently predict AF (26, 54).

Intra-operative.

Again conflicting data exist on the relative importance of the intra-operative variables as predictors of AF. It seems reasonable that longer procedures with

prolonged cardiopulmonary bypass and aortic cross clamp times would lead to atrial ischaemia due to inadequate myocardial protection. Some studies support this hypothesis (26, 54, 55) while others disagree (30, 56).

The form and method of cardioplegia delivery does not seem to be a consistent predictor of AF. Comparisons between warm and cold cardioplegia and single atrial cannulation versus bicaval cannulation have yielded conflicting results.

In recent years there has been a lot of interest in less invasive surgery. It has been suggested that "off-pump" or beating heart surgery is associated with a lower incidence of AF perhaps due to less atrial handling. A number of small studies comparing the different techniques have not shown any difference in the incidence of AF (57-59). Subsequently two meta-analysis have been published which have included non-randomised data, these have demonstrated a significant reduction in AF for the off pump groups (60, 61). These data may have an inherent selection bias as many surgeons only perform "off-pump" surgery in cases with good LV function or in those only requiring a single graft. These authors conclude more data from prospective randomised trials are required to answer this question.

Post-operative.

As mentioned post-operative withdrawal of pre-operative beta-blockade is a strong risk factor for the development of AF (23). This is probably due to the increased levels of circulating endogenous catecholamines and an up-regulation of beta-receptors caused by chronic beta-blocker therapy. Respiratory compromise and the need for prolonged ventilation following surgery are associated with the

development of post-operative AF as are electrolyte abnormalities (23). In one prospective case control study of 2,402 patients the incidence of post-operative AF was 36%. A serum potassium level of <3.5mmol/l was a predictor for AF (OR 1.7, 95% confidence intervals 1.0-2.7) although the significance was borderline (62).

Clinical consequences of post-operative atrial fibrillation.

In the majority of patients that develop post-operative atrial fibrillation there are no significant sequelae. Many revert to sinus rhythm spontaneously within the first 24 hours. Despite this complications do occur, which include embolic events, haemodynamic compromise, persistent AF and increased length of hospital stay.

The incidence of cerebro-vascular accidents following cardiac surgery ranges from 1-3%; these data have been collected in large series of patients (63-65). Many risk factors such as advanced age, pre-existing cerebro-vascular disease, severe atherosclerosis of the aorta, prolonged cardio-pulmonary bypass and significant hypotension have been identified as predisposing to peri-operative stroke.

Despite the fact that post-operative AF is usually self-limiting a number of studies have shown an association between post-operative AF and embolic events. This may be explained by data from non-surgical AF, in a trans-oesophageal echo study of acute onset AF (i.e. less than three days) 14% demonstrated left atrial thrombus and 39% demonstrated spontaneous echo contrast, a strong predictor of embolic events (66). In a prospective study of 453 patients undergoing CABG cerebro-vascular events occurred in 10 subjects (2.2%). Stroke or TIA occurred in 6 of

86 patients (7%) with AF vs. 4 of 367 (1%) without post-operative AF (67). A case control study of 5195 patients gave similar results. In this study 54 patients with a cerebro-vascular event were selected and compared to 54 randomly chosen controls. AF occurred in 29 of the cases and 15 of the controls, which gave an odds ratio for stroke with post-operative AF of 3 (68). A retrospective study by Cresswell showed that AF after cardiac surgery increased the risk of post-operative stroke from 1.4% to 3.3% (24).

Significant haemodynamic compromise resulting in hypotension or congestive cardiac failure while rare can be caused by post-operative AF. In general patients with LV dysfunction, either systolic or diastolic are more dependant on atrial transport to maintain cardiac output and are more likely to be intolerant of AF. Rapid ventricular response rates and sustained arrhythmias are more likely to result in haemodynamic deterioration (69).

Between 15 and 30% of patients with post-operative AF revert spontaneously to sinus rhythm within 2 hours and between 25 and 80% revert to sinus within 24 hours of the onset of the arrhythmia (70-72) Persistence of AF to hospital discharge is rare and over 90% of patients with post-operative AF have reverted to sinus rhythm 6-8 weeks after their operation (73). Persistent AF while rare, can prolong hospital stay, result in increased morbidity and expose patients to iatrogenic side effects such as pro-arrhythmia from the anti-arrhythmic drugs used or bleeding problems secondary to the anticoagulants used (74).

Atrial fibrillation is consistently associated with prolonged hospital stays of 3-4 days (23, 24, 50, 75, 76), whether this is causally related to atrial fibrillation

independent of the other patient characteristics is difficult to elucidate. This prolongation in hospital stay significantly increases the cost of the surgical procedure with some estimates from the United States suggesting that AF results in an incremental cost of over one billion dollars per year (29). Some delay in hospital discharge may be as a consequence of therapeutic interventions such as anticoagulation and some of the delay maybe because some of the patients that develop AF have a more complicated post-operative course.

INFLAMMATORY MARKERS.

C-Reactive Protein (CRP).

CRP is one of a group of acute phase proteins, which are defined as those, whose concentration increases or decreases by at least 25% in response to inflammatory stimuli. They are predominately produced in the liver in response to Il-1, Il-6, tumour necrosis factor and leukocyte inhibiting factor (77). CRP was discovered in 1930 in the serum of patients with pneumonia and its name derives from the fact that it interacts with pneumococcal C-polysaccharide. CRP along with serum amyloid P are members of the pentraxin family of proteins. They consist of 5 identical non-glycosylated subunits of molecular mass 23027, bound non-covalently that are resistant to proteolysis. The gene, which encodes CRP, is on chromosome 1 and following up-regulation by Il-6 increased levels are secreted within 6 hours. Plasma levels can double every 8 hours and usually peak about 50 hours after the initial stimulus. The half-life is 19 hours and unlike the ESR levels can fall quickly after cessation of the pro-inflammatory stimulus. The normal median level is 0.8 mg/l and

90% of the normal population have levels < 3 mg/l. Levels < 10 mg/l are normal for 99% of the population but higher levels usually indicate the presence of organic disease.

In the presence of calcium CRP binds to microbial polysaccharides and phospholipids. This causes activation of the classical complement pathway and opsonizes bacteria to promote phagocytosis. The ability to recognise and bind to phosphocholine and phospholipids means CRP can facilitate phagocytosis of constituents of damaged cells. It can also induce tissue factor and cytokine expression by monocytes. The net effect of CRP seems to be anti-inflammatory, it neutralises the pro-inflammatory mediator platelet activating factor and has inhibitory effects on neutrophil function by decreasing the surface expression of L-selectin. CRP also stimulates mononuclear cells to produce IL-1 receptor antagonist, while its precise role is yet to be elucidated it does seem to be more than merely a marker of inflammation.

In contrast to other acute phase proteins the plasma half-life and catabolic rate are constant under virtually all conditions. While CRP elevation is non-specific it is not modified by therapeutic interventions unless they modify the underlying disease process. The only condition, which alters the normal CRP response, is severe synthetic liver failure, due to an inability to produce proteins normally. These features make CRP the ideal marker of the inflammatory process.

Adhesion molecules.

The cell adhesion molecules are a group of interrelated glyco-proteins expressed on leukocytes, platelets and activated endothelial cells, which mediate cell to cell and cell to extracellular matrix interactions. These molecules share a similar structure; with an extracellular component, with binding domains that interact with other adhesion molecules or components of the extracellular matrix, a hydrophobic transmembrane component and an intracytoplasmic component, which interacts with the cell cytoskeleton and intracellular signalling pathways (78). These molecules have an important role in embryogenesis, epithelial integrity, the immune response and inflammation.

A key part of the inflammatory response is the binding of leukocytes to the endothelial surface; the initial contact is made through rolling of the leukocytes along the endothelial surface. Subsequently a firm attachment is formed via specific endothelial ligands and the leukocytes transmigrate into the interstitium, where degranulation and phagocytosis occurs. Based on their structure and function the adhesion molecules are divided into 4 main families, which play different roles in this process. While largely membrane bound these molecules are also shed from the cell surface to form a soluble form in the plasma. The precise mechanism by which this occurs is unknown but much of our knowledge of the role of these molecules in the pathogenesis of cardiovascular disease is derived from measuring the soluble component (79).

Selectins.

The selectin family is comprised of three molecules named for the cell type on which they were originally identified: Endothelial (E-selectin), platelet (P-selectin) and leukocyte (L-selectin), but they are not exclusively located on these cells. They are composed of an N-terminal lectin domain, an epidermal growth factor like region, 2 to 9 complement regulatory repeats and a short cytoplasmic tail (80). Binding of selectins is calcium dependant and they bind to cell surface glycans that possess a specific sialyl-LewisX-type structure that is also found in blood group antigens (81). Selectins mediate the initial attachment of activated leukocytes to the blood vessel wall during the capture and rolling step of the inflammatory adhesion mechanism. To facilitate this process they can form rapid bonds to promote adherence, fast dissociation to facilitate rolling and they have a favourable free energy of association to resist shear forces in the vasculature (78). The binding affinity of selectins is low but it is enough to act as a biological brake that causes the leukocyte to decelerate and roll along the endothelium.

Accumulation of leukocytes is essential for host defence to infection and injury and the importance of these molecules can be seen in a rare disease caused by an enzyme defect, which prevents the production of the sialyl-Lewis structure. The selectins are produced normally but their ligand is not. Leukocytes from these patients are not adequately recruited from the circulation, as they cannot mediate the rolling phase. This results in a syndrome characterised by developmental defects, life threatening bacterial infections, poor wound healing and abnormalities in blood group profiles (82).

Endothelial-selectin (ELAM-1) (CD62).

This is a 115 kDa cell surface glycoprotein, which is expressed on activated endothelium and functions at an early stage of leukocyte binding (83). As it is only minimally expressed on resting endothelial cells its up-regulation and the subsequent production of the soluble form are good markers for endothelial activation (84). A wide range of inflammatory mediators including IL-1 and TNFα stimulate the production and transportation of E-selectin to the cell surface with maximal expression occurring 4-6 hours after stimulation (78).

The ligand for E-selectin is a sialylated fucosylated molecule which binds to the lectin domain of E-selectin and which is expressed on neutrophils, monocytes and a subset of memory T-cells (85). ELISA techniques have shown that detectable levels of soluble E-selectin (sE-selectin) are present in normal healthy individuals and sE-selectin levels have been found to be elevated in a number of inflammatory and malignant conditions (86).

Platelet-selectin (GMP-140) (PADGEM) (CD62).

P-selectin is a cell surface glycoprotein that unlike the other adhesion molecules is stored intracellularly in Weibel-Palade bodies of endothelial cells and in alpha granules of platelets. This storage function means that P-selectin can rapidly reach the cell surface within 10 minutes of activation (86-88). The expression of P-selectin can be induced by a variety of inflammatory stimuli including thrombin, histamine, complement factors, free radicals, Il-1 and TNFα (89, 90). The cell surface

half-life of P-selectin is short after which it is recycled into the intracellular compartments.

P-selectin is also involved in the adhesion of platelets to monocytes and neutrophils, playing a central role in neutrophil accumulation within thrombi. The adhesion of leukocytes and neutrophils to the endothelium is initiated by weak interactions with P-selectin that produce a characteristic rolling motion of the leukocytes and neutrophils on the endothelial surface. P-selectin is found in the plasma of normal individuals at ng/ml concentrations (91). Circulating P-selectin appears to be slightly smaller than native P-selectin. An alternatively spliced mRNA encoding a form of human P-selectin lacking the transmembrane anchoring domain has been reported for both megakaryocytes and endothelial cell, and evidence suggests that the majority of circulating soluble P-selectin (sP-selectin) arises in this manner (92). A number of studies have reported that levels of sP-selectin in biological fluids may be elevated in subjects with a variety of pathological conditions (93). A ligand for P-selectin is P-selectin Glycoprotein Ligand-1 (PSGL-1), which is expressed by all blood neutrophils, monocytes and lymphocytes, but specific glycosylation is required for ligand function.

Immunoglobulin superfamily.

This family of adhesion molecules has evolved to serve a wide variety of functions. They are classified together because they all contain one or more common immunoglobulin like repeats, characterised by 2 cysteines separated by 55-75 amino-acids (81). Most of theses molecules span the cell membrane and have a short

cytoplasmic tail, the Ig repeats are extracellular. They act as receptors for growth factors, receptors for the Fc region of immunoglobulins and are cellular counter receptors for integrins.

Intercellular adhesion molecule-1 (ICAM-1) (CD54).

This member of the immunoglobulin superfamily is a 95kDa cell surface glycoprotein, which is constitutively expressed on some tissues and induced on others by pro-inflammatory cytokines such as IL-1β, Interferon-γ (INF-γ) and TNF-α (94). ICAM-1 is involved in the binding of leukocytes through leukocyte function associated molecule-1 (LFA-1) (CD11a) and Mac-1 (CD11b) which are members of the β2 family of integrins (95). Blotting techniques and ELISA methods have shown that detectable levels of soluble ICAM-1 (sICAM-1) are present in serum from normal individuals (96).

Vascular cell adhesion molecule-1 (VCAM-1).

VCAM-1 is a 110kDa 715 amino acid, type I transmembrane glycoprotein typically characterised by the presence of seven C2-type immunoglobulin (Ig) domains (97). Cells known to express VCAM-1 include neurons, endothelial cells, smooth muscle cells, fibroblasts and macrophages (98). VCAM-1 is absent from resting endothelial cells but can be up-regulated by TNF-α, thrombin and lipopolysaccharide (78). Functionally, VCAM-1 binds to both $\alpha_4\beta_1$ (VLA-4, very late appearing antigen-4) and $\alpha_4\beta_7$ (LPAM-1) integrins. These integrin receptors are found on a variety of cells with VLA-4 found on all leukocytes with the exception of

neutrophils (99). VCAM-1 ligand interactions are key events in the rate and timing of leukocyte extravasation.

Integrins.

These are membrane glycoproteins with two principle subunits designated α and β, which traverse the cell membrane and are characterised by non-covalent interactions. The α subunit is between 120 and 180 kd and the β subunit is between 90 and 110 kd. Approximately 20 different integrins have been described and they are divided into subgroups based on their β subunit (78). An important feature of integrins is that they exist in both active and inactive forms. An activated cell can transmit a signal from its cytoplasm that modifies the confirmation of the extracellular domains of integrins on the cell membrane, increasing the affinity of integrins for their ligands. Individual cells may express more than one integrin. Thus, integrin display on a given cell type helps define a variety of receptor-counter receptor interactions. The β_1 or VLA (Very Late Antigen) subfamily is expressed by a variety of cell types; they bind to molecules in the extracellular matrix and the basement membrane (100). The $\beta 2$ subfamily of integrins consists of leukocyte receptors LFA-1 (leukocyte function associated antigen) and Mac-1, which are exclusively expressed on leukocytes and are the cellular counter receptors for ICAM-1 (84). The $\beta 3$ subfamily is expressed on endothelial cells and platelets and plays a key role in platelet activation and thrombosis, (101) this group includes the platelet glycoprotein IIb/IIIa receptor which is expressed by platelets and undergoes conformational change when platelets are activated promoting platelet aggregation by cross-linking fibrin.

Cadherins.

This family is composed of the "classic" cadherins (N, P, R, B and E) and some subfamilies of structurally related proteins (102,103). Their primary function is to bind to homologous proteins on adjacent cells but they can also bind to structurally heterogeneous cadherins (104). Structurally they are composed of an extracellular protein with an N-terminal binding domain and calcium dependant regions, which maintain the structural integrity of the protein (105). They also have a cytoplasmic tail, which mediates interactions with the cytoskeleton via intracellular proteins called α and β catenins. These molecules establish links between adjacent cells. They form zipper-like structures at zona adherens junctions, membrane regions where a cell makes contact with another cell. Through these junctions bundles of actin filaments run from cell to cell. Cadherins have a key role in segregating embryonic tissues. The expression of specific adhesion molecules in the embryo is crucial for the migration of cells and the differentiation of tissues. In some cancers disruption of the cadherin molecules has been implicated in modulating the metastatic phenotype (106) Pemphigus vulgaris is a blistering skin condition characterised by disruption of the normal epidermal structure. It is caused by breakdown of cell to cell adhesion and is associated with antibodies to cadherin family members (107).

INFLAMMATORY RESPONSE TO CARDIOPULMONARY BYPASS (CPB).

The inflammatory response to on-pump cardiac surgery is multifactorial and relates not only to surgical trauma but also contact with the extracorporeal circuit, reperfusion injury and endotoxaemia (108). While the inflammatory reactions are complex they are mainly mediated through the plasma protein systems of the contact and complement systems. Other crucial players are neutrophils, monocytes, cytokines and endothelial activation.

Contact system.

This is composed of a number of soluble plasma proteins many of which are components of the clotting cascade (109). Following contact with negatively charged surfaces Factor XII is activated and cleaved into Factor XIIa and XIIf. Factor XIIa activates Factor XIa, which initiates the intrinsic clotting cascade. Prekallikrein is converted to the active form Kallikrein by Factor XIIa; Kallikrein directly activates neutrophils and facilitates cleavage of Factor XII in a feedback loop. Factor XIIa also activates high molecular weight kinogen to form bradykinin.

Complement.

Complement activation occurs via both the classic and alternative pathway; however the alternative pathway is the most important. The classic pathway requires formation of antigen-antibody complexes, which is unusual following cardiac surgery, but does occur when preformed antibodies recognise some component of the heparin-

protamine complex (110). The alternative pathway is constantly laying down C3b on surfaces it comes in contact with. Surfaces with inhibitory proteins, such as decay accelerating factor prevent further complement activation. This is one of the ways the immune system differentiates self from non-self. As soon as blood comes in contact with the foreign surfaces of the cardiopulmonary bypass circuit C3b is laid down, this results in amplification of the alternative pathway and production of large quantities of C3a, C5a and the membrane attack complex C5b-9. Following return of C5a to the systemic circulation there is a dramatic fall in the white cell count, which is caused by pulmonary and other end-organ leukosequestration (110). This process seems to be mediated by activation of P-selectin, which can be rapidly released from endothelial storage granules (111). C5a induces neutrophil expression of the integrin Mac-1, which binds to ICAM-1 and causes platelets and monocytes to release cytokines. Therefore complement activation plays a crucial role in neutrophil-endothelial interactions with extravasation into the interstitium and subsequent phagocytosis and degranulation.

Neutrophils.

Neutrophils are responsible for much of the end-organ damage seen after CPB. As previously described interactions between the endothelium and the neutrophil mediated by adhesion molecules are crucial for the local host defence against disease. Unfortunately following CPB these interactions are so diffuse that tissue damage occurs. When activated which can occur within 20 seconds of exposure to complement they develop pseudopodia and can release the contents of their granules. Neutrophil

derived proteases break down elastin, collagen and fibronectin; this damages the extracellular matrix and contributes to capillary fluid leak that causes extracellular fluid overload and organ dysfunction (108). Activated neutrophils produce cytotoxic substances such as oxygen free radicals, hydrogen peroxide and hypobromous acid.

Monocytes.

Monocyte activation occurs within the bypass circuit but is slower than that of either complement or neutrophils; however within the surgical wound activation is prompt (109). Activated monocytes express Tissue factor and Mac-1 and are strongly pro-coagulant due to activation of the extrinsic coagulation pathway. They also produce numerous pro and anti-inflammatory cytokines such as Il-1, Il-2, Il-4, Il-6, Il-8, Il-10 and Il-12.

Endotoxaemia.

Endotoxin is a lipopolysaccharide, which is frequently detected, in high concentrations in the systemic circulation following CPB (112). The mechanism of its release is unclear although it is thought to be caused by translocation of gut bacteria secondary to splanchnic ischaemia and impaired Kupffer cell function. Endotoxin is a potent stimulant of complement activation, endothelial activation and macrophage tumour necrosis factor release.

Endothelial activation.

The endothelium was initially thought to be a non-thrombogenic passive barrier between the blood constituents and the interstitium. Recent research has revealed that the opposite is the case with the endothelium being highly biologically active. It plays an integral role in regulating membrane permeability, lipid transport, vasomotor tone, coagulation, fibrinolysis, inflammation and vascular wall structure (113). During CPB there are a host of potential insults to the endothelium including hypoxia, surgical trauma, endotoxin, reperfusion injury and inflammatory cytokines. These lead to endothelial activation, which is characterised, by vasoconstriction, abnormal coagulation, leukocyte adhesion and disruption of the normal barrier function (114).

The immediate response is to deploy stored inflammatory mediators such as P-selectin; this is followed by a delayed response mediated by transcription of NFκβ dependant genes leading to production of mediators such as E-selectin and ICAM-1. Vascular tone is normally controlled by a balance between relaxing factors such as nitric oxide, adenosine, prostacyclin and vasoconstrictors such as endothelin, leukotrienes and angiotensin II. Following endothelial injury the balance is tipped towards vasoconstriction, which can lead to vasospasm and disruption of normal small vessel perfusion. The normal endothelium prevents clotting by expressing heparin like substances to potentiate antithrombin III, by tissue plasminogen activator to stimulate plasmin production and by expressing thrombomodulin, which binds protein C. The activated endothelium on the other hand expresses tissue factor, which binds to Factor VIIa and initiates thrombin formation via activated Factor X. The combination of

fibrin production stimulated by thrombin and vasoconstriction often causes blockage of the microcirculation.

NATRIURETIC PEPTIDES.

These are a family of 3 related compounds that cause vasodilation and natriuresis. All these molecules are structurally similar and all share a 17 amino acid ring. Their principle role is to protect the body against volume overload (as in congestive cardiac failure) and hypertension. The first suggestion that the heart was in fact an endocrine organ came in 1956 when granules were detected in guinea pig atria with electron microscopy (115). Further evidence came in the same year when inflation of a balloon in the left atrium of a dog stimulated diuresis (116). The landmark discovery was made in 1981 when de Bold infused extracts of atrial tissue into rats (117). Following this in 1984 atrial natriuretic peptide (ANP) was isolated and cloned. Each member of the family is encoded by a different gene and usually expressed in different tissues.

Atrial Natriuretic Peptide (ANP).

ANP is produced primarily in the cardiac atria and its release is primarily stimulated by increases in atrial stretch secondary to increases in intravascular volume. Other hormones such as endothelin, arginine vasopressin, angiotensin II and catecholamines also stimulate ANP release (118). Normal cardiac ventricular tissue produces little ANP approximately 1% of the atrial contribution, but it is present in

foetal ventricular tissue, neonates and in hypertrophied ventricles. Non-cardiac sites such as the anterior lobe of the pituitary, lung, kidney and brain produce small amounts of ANP. The gene for ANP is located on the short arm of chromosome 1 and activation leads to production of a precursor protein (pro-ANP) 126 amino acids long, which is stored in atrial granules (119). This is cleaved into two components, the 28 amino acid carboxy terminal fragment is the mature ANP but there are some data to suggest that the remaining 98 amino acid fragment has similar biological properties to ANP.

Brain Natriuretic Peptide (BNP).

This was originally discovered in porcine brain (120) however while it is present in human brain, the cardiac ventricles are the primary source of BNP. It is produced as pro-BNP a 108 amino acid hormone that is cleaved to the active 32 amino-acid hormone. BNP is produced in response to elevation in end-diastolic pressure (121,122). BNP levels correlate closely with pulmonary capillary wedge pressure in left ventricular systolic dysfunction and it is used as a screening tool for the presence of ventricular dysfunction (123). It has a short half-life of 22 minutes as it is broken down by neutral endo-peptidases and binds to natriuretic peptide clearance receptors and reflects ventricular function in the preceding hours (124,125).

C-type Natriuretic Peptide (CNP).

This molecule is also formed from a precursor molecule but forms two different active peptides of 22 and 53 amino acids in length. The smaller molecule is more potent and predominates in the central nervous system, anterior pituitary, kidney and vascular endothelium. Immuno-histochemical staining has localised CNP to smooth muscle and endothelial cells of coronary arteries.

Urodilatin.

Urodilatin is a related compound produced in the kidney from the ANP gene via an alternative process. It is a 32 amino acid hormone that plays a role in sodium and water handling in the kidney.

Natriuretic peptide receptors.

All these molecules exert their biological action by binding to high affinity receptors on their target cells. Three different receptors have been identified in mammals called A, B and C. A and B receptors are structurally similar with 44% homology in the extracellular domain. They both activate cyclic GMP dependant signalling pathways to cause natriuresis and vasodilation. The A receptor can bind both ANP and BNP but has a preference for ANP and is the most abundant type of receptor in large blood vessels. The B receptor predominates in the brain but is also found in small quantities in large blood vessels, its preferred ligand is CNP. Both A and B receptors are found in the kidneys and adrenal glands. The C-type receptor binds all three peptides with equal affinity and is involved in the breakdown of these

compounds. Following binding to the receptor it is internalised and the natriuretic peptides are enzymatically degraded, the C receptor then returns to the cell surface. Another degradation pathway is via the neutral endopeptidases, which are present in renal tubular cells and vascular endothelium. These molecules cleave the natriuretic peptides opening the ring structure common to them all and rendering them inactive. The relative contribution of both these systems is unknown but both ensure that these peptides have a short half-life.

Natriuretic peptide actions.

Both ANP and BNP have almost identical actions; they dilate arteries and veins and promote natriuresis by inhibiting sodium transport in the collecting duct of the kidneys. They both have complex neuro-hormonal effects, which antagonise the deleterious hormonal activation, which occurs in heart failure. They decrease renin secretion from the macula densa, directly inhibit aldosterone secretion and attenuate the stimulatory effect of angiotensin II on aldosterone release. Both cause stimulation of vagal afferents and so inhibit sympathetic nervous system activation (118). CNP on the other hand has limited natriuretic properties and acts in a paracrine fashion to cause vasodilation and to inhibit vascular cell growth in response to mitogens.

Potential roles for natriuretic peptides.

BNP is the most widely studied member of the natriuretic peptide family; it has a broad potential both in the diagnosis and therapy of cardiovascular disorders. It has been evaluated as a prognostic indicator in acute coronary syndromes and congestive

cardiac failure and as method of therapeutic monitoring in heart failure. It has also been used as a screening tool for structural heart disease and as a useful tool in differentiating cardiac from non-cardiac dyspnoea.

Congestive cardiac failure.

As already described BNP is predominately produced by ventricular myocardium in response to elevations in left ventricular end diastolic pressure (LVEDP). This makes BNP a more sensitive and specific marker of ventricular dysfunction than the other natriuretic peptides. BNP correlates well with right atrial pressure, pulmonary capillary wedge pressure and left ventricular end diastolic pressure. The main symptom of heart failure is dyspnoea and the degree of dyspnoea correlates closely with the left ventricular end diastolic pressure, hence BNP correlates well with the NYHA functional classification (125).

While there have been significant advances in the treatment of heart failure it remains a condition with a poor prognosis and high readmission rates. There are some data that monitoring of BNP and more particularly the pro-hormone N terminal BNP allows more appropriate up titration of therapy with better long term clinical results. More intensive therapy guided by N-BNP has been shown to result in a greater reduction in this marker and less deaths or readmissions with heart failure (126).

BNP has also been shown to be a useful prognostic indicator. In one study of patients admitted with decompensated heart failure the BNP levels rose during the admission in the patients who subsequently died or were readmitted (127). In another

study of patients with EF < 35% the log BNP level was a significant independent predictor of sudden cardiac death (128).

Acute coronary syndromes.

This group of conditions comprises unstable angina, ST-segment elevation myocardial infarction and non-ST segment elevation myocardial infarction. There are multiple treatment options for these conditions and it is imperative to accurately risk stratify this group in order to target the more aggressive interventions appropriately (129). Current standard methods of risk stratification include clinical characteristics, ECG changes, markers of myocyte necrosis (Troponin) and non-invasive stress testing. In one large study of over 2,500 patients with all types of acute coronary syndromes, the degree of BNP elevation approximately 40 hours after the onset of ischaemic symptoms correlated with the risk of death, new or recurrent myocardial infarction and new or recurrent heart failure (130). This association was independent of clinical evidence of heart failure and the other known predictors of death following an acute coronary syndrome. In the sub-group of patients without ST elevation NT-proBNP levels on admission have also been shown to give independent prognostic information. In one study of 755 patients followed for a median of 40 months subjects in the highest quartile of BNP elevation had a relative risk of death of 26.6 compared with the lowest quartile (131).

Evaluation of dyspnoea.

In patients presenting to the emergency department the aetiology of acute dyspnoea can be difficult to elucidate as examination findings, chest radiographs and routine blood tests are often non-specific. A simple blood test with a high sensitivity and specificity for the diagnosis of heart failure would be extremely useful as it would allow rapid and accurate triage of patients and so reduce unnecessary investigations and therapies.

Alan Maisel's group from San Diego studied 321 patients presenting with dyspnoea. BNP was measured and two independent cardiologists who were blinded to the BNP levels gave the probability of heart failure as the final diagnosis (132). They found that patients with heart failure had BNP levels of 758pg/ml whereas those with lung disease had levels of 61pg/ml. One limitation of this study was that patients with cor pulmonale were included in the heart failure group however patients with right heart failure had consistently lower BNP levels than those with LV dysfunction, probably due to the lower mass of the right ventricle. In a more recent paper patients presenting with dyspnoea were randomised to two diagnostic strategies one using BNP and the other not. This study found that using BNP reduced hospital admission, ICU admission, length of hospital stay and total cost of admission (133).

Population screening.

In many areas of medicine population screening has become increasingly popular in an effort to identify disease at an early stage and alter long-term prognosis. In cardiovascular disease enthusiasm has been tempered by the high cost of

echocardiography for the detection of structural heart disease. This has led to much interest in the use of BNP as a screening tool.

Henry Dargie's group in Glasgow carried out an ECG, echocardiography, clinical questionnaires, NT-BNP and BNP levels in a random sample of the general population (134). They found that patients with either symptomatic or asymptomatic LV dysfunction had significantly higher BNP levels than those with normal systolic function. More recently it has been realised that patients with heart failure represent a heterogeneous group and that not all will have LV systolic dysfunction. A Japanese study of a health-screening programme enrolled 1098 subjects who underwent echocardiography, an ECG, CXR, physical exam and a clinical questionnaire (135). They found that BNP was an efficient and cost effective method for screening for a wide variety of cardiac disorders. These conditions included atrial fibrillation, hypertensive heart disease and valvular heart disease. These data are interesting as BNP elevation is really a marker of elevated intra-cardiac pressure not LV systolic function. Therefore BNP may be elevated in conditions, which cause diastolic dysfunction such as hypertension or aortic stenosis.

Conclusion.

Atrial fibrillation is a common occurrence following coronary artery bypass surgery, in most patients it is self limiting but some suffer deleterious consequences. The precise aetiology is unclear although there are many theories. The role of inflammation, cardiac neuro-hormonal activation, the underlying atrial substrate and myocardial necrosis will be explored in this thesis.

CHAPTER 2

Clinical and Laboratory Methods

INTRODUCTION.

This chapter will describe the inclusion and exclusion criteria for this thesis and the clinical methods and monitoring of the included patients. A brief outline of the surgical and anaesthetic technique will also be provided. The preparation of the samples and the principles and technique of the various assays used will also be explained.

Ethical approval.

Ethical approval was granted for all limbs of this research work by the Federated Dublin Voluntary Hospitals Ethics review committee under the title *"Post-operative Atrial Fibrillation A Potential inflammatory mechanism"*. Enrolled patients gave written informed consent. Individual participation did not interfere with the clinical management of the patients who agreed to participate in the study. The research protocol complied with the Declaration of Helsinki.

Definition of Atrial Fibrillation.

Atrial fibrillation is an irregular cardiac rhythm, which originates from above the AV node. It is characterised by rapid atrial depolarisation with chaotic fibrillary waves. The AV node demonstrates variable conduction, which results in a variable ventricular response rate.

The primary endpoint was defined as atrial fibrillation sustained for at least one hour, or atrial fibrillation requiring emergency therapy due to haemodynamic compromise or rapid clinical deterioration. The rhythm was defined as AF when there

were no consistent P waves before each QRS complex and the ventricular rate was irregular with a variable R-R interval.

Inclusion Criteria.

Patients with significant valvular heart disease are more likely to develop post-operative AF and those undergoing valvular surgery have the highest incidence of AF. These patients undergo a wide variety of procedures and consequently their hearts are exposed to significant haemodynamic stresses with the potential to confound our results. We attempted to recruit a homogenous population undergoing the same procedure to reduce the potential for confounding variables. The study population consisted of patients with the following characteristics;

- Patients undergoing elective on-pump isolated coronary artery bypass surgery.
- Patients in sinus rhythm prior to the surgery.
- Good LV systolic function (estimated EF>45%).
- Age between 18 and 80.
- Written informed consent.

Exclusion Criteria.

Pre-existing diseases with an inflammatory component would cause an increase in levels of soluble adhesion molecules and CRP; which could impact on the significance of post-operative elevation. Therefore, we attempted to control for this by excluding patients with any of the following conditions;

- Active malignancy.

- Collagen vascular disease.
- Chronic inflammatory illness.
- Concurrent infection.
- Diabetes Mellitus.
- Chronic renal failure (serum creatinine > 200μmol/l).
- Impaired LV systolic function (EF < 45%).
- Valvular heart disease.
- Pre-operative atrial fibrillation.
- Those taking steroids or other immunosuppressive drugs or pre-operative anti-arrhythmic drugs.

Clinical methods and monitoring.

Patients who satisfied the above criteria were enrolled the night prior to surgery and provided written informed consent. Baseline demographic data were recorded and the patients were then followed throughout their hospital stay and reviewed six weeks following discharge.

Following their operation patients were transferred to cardiac surgery Intensive Care Unit (ICU), if the initial course was uncomplicated they were extubated the night of their surgery and transferred to the high dependency unit the following morning. Arterial lines were removed prior to ICU discharge and central venous lines were removed on day three. All patients had epicardial pacing wires implanted as a routine and these were also removed on day three if there were no significant bradyarrhythmias.

Patients had continuous cardiac rhythm monitoring for 72 hours following surgery. This system has a 24 hour recall facility and was analysed on a daily basis. The prophylactic use of digoxin, amiodarone or pacing strategies to potentially reduce post-surgical atrial fibrillation was not employed during the study period. None of the patients were on digoxin or amiodarone before the surgery. The majority of patients (84%) were on beta-blockers pre-operatively and these were continued as soon as possible following surgery unless there was a contra-indication.

Cardiac Surgical and Anaesthetic technique.

Standard cardiac anaesthetic technique was used with low dose fentanyl supplemented by propofol for induction. Intra-operative anaesthesia was maintained with a combination of an inhalational agent (sevoflourane) and intravenous propofol. Neuromuscular blockade was achieved with pancuronium to facilitate intubation and post-operative sedation was maintained with propofol at 1-3 ng/kg/hr.

Cardiopulmonary bypass was achieved with a single right atrial cannulation technique using a Jostra HL 20 heart lung machine with an arterial multiflow roller pump connected to a Cobe Optima microporous hollow fibre oxygenator with a Cobe Sentry arterial filter.

St Thomas' cardioplegia concentrate in Ringers chloride solution was mixed in a 4:1 ratio with arterial blood and delivered at an induction dose of between 800 and 1000 mls. All surgeons used the alpha stat method of blood gas management. All patients were allowed to drift down to 32° Centigrade with active rewarming

commencing half way through the internal mammary artery anastomosis. Shed mediastinal blood was routinely re-transfused at the end of surgery.

C-Reactive Protein Estimation.

High sensitivity CRP (hsCRP) was measured using rate nephelometry with a Dade Behring apparatus. The investigator prepared the samples and the analysis was carried out following the standard lab techniques by the immunology department.

Soluble Cell Adhesion Molecule Estimation.

Preparation of samples.

Venous samples were taken from the central line where possible to minimise patient discomfort and from a peripheral vein if the line had been removed. Clotted samples were then centrifuged at 5,000 rpm for ten minutes. Serum was pipetted off and stored in 2ml aliquots at -70°C. Levels of soluble cell adhesion molecules were measured in batches from the frozen serum samples.

Principle of the Assay.

The enzyme linked immunosorbent assay kits were purchased from R&D systems, Abgindon, U.K. These assays utilise an immuno-enzymometric technique for quantification of the soluble cell adhesion molecule present in the sample. This involves the simultaneous reaction of the soluble cell adhesion molecule or the standard (supplied with the assay kit) to two antibodies directed against different epitopes on the specific soluble cell adhesion molecule. One antibody is coated onto

the walls of the microtitre wells and the other is conjugated to the enzyme horseradish peroxidase (HRP). Any soluble cell adhesion molecule present forms a bridge between the two antibodies. After removal of unbound material by aspiration and washing, the amount of conjugate bound to the well is detected by a substrate specific for the enzyme which yields a coloured product proportional to the amount of conjugate (and thus soluble cell adhesion molecule in the sample). The coloured product can be quantified photometrically. By analysing standards of soluble cell adhesion molecule concentration coincident with samples and plotting a curve of signal versus concentration, the concentration of unknowns can be determined.

Assay Procedure.

During each assay all the standards and 33% of the patient samples were assayed in duplicate to assess the variability of the measurement. A total of 60 samples were measured in each assay plate.

- All samples were thoroughly thawed and both reagents and samples were brought to room temperature prior to commencing the assay.
- All samples and controls were diluted 1 in 20 with sample diluent apart from the VCAM assays, which were diluted to 1 in 50.
- Control serum was reconstituted with 500µml of de-ionised water.
- Six standards of increasing concentration were reconstituted with 1ml of deionised water.
- 100 µl of diluted anti-cell adhesion molecule horseradish peroxidase was added to each well of a 96 well microtitre plate.

- 100 of standard, diluted sample or diluted parameter control serum were added to each well with sufficient force to ensure mixing.
- The microtitre plate was covered with a plate sealer and incubated at room temperature for 90 minutes.
- The contents from each well were then aspirated and then each well was washed by adding 300 µl of a prepared wash buffer. This process was repeated five times for a total of six washes. After the last wash the contents of each well were aspirated.
- Next 100 µl of a provided substrate was added to each well.
- The plate was again covered and incubated at room temperature for 30 minutes.
- Then 100 µl of stop solution was added to each well in the same order as the substrate.
- Finally the optical density (OD) of each well was determined at a wavelength of 450 nm and again at a correction wavelength of 620 nm.

These ELISA assays were carried out in the Sir Patrick Dun's Research Laboratory, St. James's Hospital, Dublin.

Brain Natriuretic Peptide estimation.

Preparation of samples

Venous samples were taken in EDTA tubes as above. Non-clotted samples were then centrifuged at 5,000 rpm for ten minutes. Plasma was pipetted off and

stored in 2ml aliquots at -20°C. BNP levels were measured in batches from the frozen plasma samples.

Assay Procedure.

The test device (Triage® meter) was provided by Biosyn Diagnostics a subsidiary of Biosite Incorporated San Diego California. The Triage® BNP test is a commercially available single use fluorescence immunoassay device which determines the concentration of BNP in plasma or whole blood. This system is designed for "point of care" testing and is easily portable. Following inoculation of the test cartridge with 250µml of plasma with a specially provided transfer pipette the cartridge is simply inserted into the test device. Results are available within 15 minutes and are expressed in pg/ml. The BNP system used in this study reported results between 5 and 1300 pg/ml, greater than 100pg/ml is considered to be abnormal and suggestive of LV dysfunction.

Assay Precision

Adhesion Molecules

Intra-assay Precision

Three or four serum samples of known concentration were assayed in replicates of 10 to assess intra-assay precision

Inter-assay Precision

Three or four serum samples of known concentration were assayed in 14 or 18 separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	18	18	18
Mean ng/ml	84	430	668	94	451	730
Standard Deviation	4.28	21.1	37.4	9.31	39.7	57.7
CV %	5.1	4.9	5.6	9.9	8.8	7.9

Table 2.1: Coefficient of Variability (CV) for sP-Selectin assay.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	18	18	18
Mean ng/ml	21.9	56.6	115	20.4	54.7	115
Standard Deviation	1.1	2.7	5.4	1.8	3.1	8.5
CV %	5.0	4.8	4.7	8.8	5.7	7.4

Table 2.2: Coefficient of Variability (CV) for sE-Selectin assay.

	Intra-assay Precision				Inter-assay Precision			
Sample	1	2	3	4	1	2	3	4
n	10	10	10	10	18	18	18	18
Mean ng/ml	66.1	126	281	476	64.2	117	290	453
Standard Deviation	3.2	6.1	9.3	21.9	6.5	8.7	17.4	27.6
CV %	4.8	4.8	3.3	4.6	10.1	7.4	6.0	6.1

Table 2.3: Coefficient of Variability (CV) for sICAM-1 assay.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	14	14	14
Mean ng/ml	11.7	28.6	55.1	9.8	24.9	49.6
Standard Deviation	0.69	1.23	2.7	1.0	2.12	4.41
CV %	5.9	4.3	4.9	10.2	8.5	8.9

Table 2.4: Coefficient of Variability (CV) for sVCAM-1 assay.

Brain Natriuretic Peptide

The average Intra-assay Precision and Inter-assay Precision were determined using the ANOVA model by testing control materials that had BNP added at concentrations near the decision points of the assay and throughout the range of the standard curve. The study was conducted over 12 days, testing each control 10 times per day. Each device was read on 5 Triage meters.

	Intra-assay Precision			Inter-assay Precision		
Mean pg/ml	71.3	629.9	4087.9	71.3	629.9	4087.9
Standard Deviation	6.3	69.1	475.5	7.0	75.5	500.1
CV %	8.8	11.0	11.6	9.9	12.0	12.2

Table 2.5: Coefficient of Variability (CV) for Brain Natriuretic Peptide assay.

Tissue Preparation for Atrial Histology.

Prior to commencing extracorporeal circulation two cannulae are inserted, the first is placed in the aortic root and receives blood returning from the bypass circuit. The second is placed in the right atrium and drains venous blood returning to the heart. This blood then passes through the bypass pump and is oxygenated. The atrial cannula

is usually placed in the right atrial appendage. The appendage is opened by a small incision and sutured around the cannula. For the purposes of this study a small section of atrial appendage was resected prior to suturing the cannula. Atrial tissue samples were then transected and placed in liquid nitrogen, 10% formalin and bouins fixative simultaneously. Tissue was embedded into paraffin in the routine fashion, cut into 4μm sections and stained with haematoxylin and eosin.

CHAPTER 3

Relationship Between Post-operative Atrial Fibrillation and the Degree of Inflammatory Response

INTRODUCTION.

Cardiac surgery is associated with an inflammatory response in almost all patients; this is multifactorial and has been previously been discussed in detail. The theory that AF may be precipitated by inflammation is supported by a number of studies and observations. Pericarditis is common in the post-operative period but as there are no hard diagnostic criteria some authors have evaluated pericardial effusions as a surrogate for pericardial inflammation. One echocardiographic study found that 63% of patients with an effusion developed AF compared to 11% without an effusion (136). Poor peri-operative echo windows limit the diagnosis of pericardial effusions and in one study utilising M-mode echocardiography 85% of patients had an effusion, which limits the predictive power of this association (137). In a canine model of sterile pericarditis caused by the introduction of talc, atrial arrhythmias can be induced in 56-71% of dogs (138). While atrial inflammation secondary to pericarditis may play a role it is almost impossible to diagnose local areas of atrial or pericardial inflammation with standard techniques.

The first study to look at components of the inflammatory cascade following surgery was carried out by Bruins et al on 19 patients (139). They found that cardiopulmonary bypass caused biphasic complement activation and that the second phase, which occurred within the first 5 days after surgery was associated with the development of arrhythmias. There are limited data to suggest that post-operative non-steroidals reduce the incidence of AF (140,141). In a study designed to show the effect of dexamethasone on post-operative shivering a post hoc analysis was carried out on 235 patients. The authors found that in the group treated with steroids there was

a significant reduction in the incidence of AF, 20% compared with 36.8% in the placebo group (142). All these studies have limitations, in one it was a post hoc analysis of a study not originally designed to look at AF, in the others the sample size was very small and, apart from Bruins paper, no analysis of inflammatory markers was under taken to explain their findings.

A more recent paper published in Circulation has examined the effect of different polymorphisms in the gene for Interleukin-6 (143). They evaluated 110 cases of which 26 developed post-operative atrial fibrillation. Of the three polymorphisms, 62 had a GG genotype, 38 had a GC genotype and 10 had a CC genotype. On univariate and multivariate analysis the GG genotype was the only independent predictor of AF. This occurred in 33.9% of GG cases compared with 10.4% of non-GG cases. The area under the curve of Interleukin-6 and Fibrinogen was also significantly higher in the AF group, although interestingly there was no significant difference between the degree of CRP elevation in the two groups. This study was limited by a short monitoring period of 48 hours and the fact that it is a post hoc analysis of a prospective randomised investigation. Nevertheless, they are the only convincing data for an inflammatory aetiology for post-operative atrial fibrillation.

In the area of non-surgical AF Frustaci et al postulated that inflammation might play a role in AF (144). They selected a group of 12 patients with symptomatic refractory AF and a control group of 11 patients with Wolff-Parkinson-White syndrome. Endomyocardial biopsies of the right atrial septum and apical segments of both ventricles were performed. Morphometric assessment by light microscopy and electron microscopy were carried out. The biopsies were all normal in the control and

all abnormal in the AF group. In 8 of the 12 patients with AF, inflammatory infiltrates consistent with a diagnosis of atrial myocarditis were found, two patients had areas of myocyte hypertrophy and vacuolar degeneration and the final two patients demonstrated extensive areas of atrial fibrosis. Most of the patients with AF had normal ventricular biopsies and the authors conclude that an atrial cardiomyopathic process was responsible for AF and that in the majority this was mediated by an inflammatory process.

In recent times there has been great interest in the association between high sensitivity CRP and the incidence of all forms of cardiovascular disease. Mina Chung's group from the Cleveland Clinic have analysed a cohort of 131 patients with atrial arrhythmias and 71 controls (145). They found that CRP levels in patients with AF were higher than controls and those patients with persistent AF had higher levels than those with paroxysmal AF. This study had some limitations; the control group was not matched for age, sex, hypertension, coronary artery disease, valvular heart disease or LV dysfunction, factors which limit the applicability of these results. The authors attempted to compensate for these limitations in their statistical analysis. The conclusion was that an inflammatory basis for AF couldn't be definitively supported by their work.

Data from the Cardiovascular Health Study published by Aviles et al also lends support to the hypothesis that inflammation plays a role in the pathogenesis of non-surgical AF (146). In this study CRP was measured at baseline in 5806 patients and the mean follow up was 6.9 ± 1.6 years. Cases of AF were identified by self-report, ECG analysis and hospital discharge diagnosis. At baseline mean age was 73±5 years,

5% of subjects had AF and their CRP levels were higher than the group in sinus rhythm. During follow-up 897 subjects developed AF, after adjustment for multiple factors the highest quartile of CRP at baseline remained an independent predictor for the development of AF. Both these groups concluded that a definite causal relationship between AF and inflammation could not be demonstrated.

Objectives.

The primary aim of this section of the thesis was to determine if there was a relationship between the peri-operative inflammatory response, assessed by soluble forms of the adhesion molecules and C-reactive protein, and the development of atrial fibrillation following coronary artery bypass surgery.

METHODS.

A total of 149 patients undergoing first time non-emergency on pump coronary artery bypass grafting were recruited. The recruitment period commenced in January 2001 and terminated in November 2002. The inclusion and exclusion criteria were previously described. Prior to surgery a trans-thoracic echocardiogram was performed and left ventricular function and left atrial size were measured. Patients had continuous cardiac rhythm monitoring for 72 hours following surgery, which was reviewed by a cardiologist on a daily basis. The majority of patients (84%) were on beta-blockers pre-operatively and these were continued as soon as possible following surgery unless there was a contra-indication.

During the initial phase of the study (100 patients) blood was drawn just prior to anaesthetic induction and 6, 24, 48 and 72 hours following the onset of cardiopulmonary bypass (CPB). In the second phase (49 patients) baseline samples were obtained as above and the post-operative samples were taken 72 hours following the onset of cardiopulmonary bypass. This time was chosen based on our initial data from the first 100 patients and the fact that post-operative atrial fibrillation is most common on the third day following surgery.

High sensitivity CRP was measured using rate nephelometry with a Dade Behring apparatus. The adhesion molecules sP-selectin, sE-selectin, sICAM-1 and sVCAM-1 were measured in stored sera using an enzyme linked immunosorbent assay technique. Results were corrected for haemodilution using haematocrit (Hct) by the following formula; result x baseline Hct / post-operative Hct

Statistical analysis.

Means and standard deviations are presented for normal data, medians and inter quartile ranges (IQR) for non-normal data, and proportions for categorical data. For the patients with multiple time points the post-operative inflammatory response was assessed by calculating the area under the curve for the inflammatory markers. Comparison between those who developed atrial fibrillation and those who did not was made using a Students T-test for the normally distributed continuous data, Wilcoxon rank sums or a Kruskal Wallis test for non-normal continuous data and Chi-squared test for the categorical data. A p value ≤ 0.05 was considered significant, with

two-sided tests. All analyses were performed using the JMPin software (SAS Institute Inc.).

RESULTS.

Patient demographics.

Baseline demographics are presented in table 3.1. Apart from the patients who developed atrial fibrillation being older (65.2 ± 8 years compared with 60.9 ± 10 years in the non AF group $p = 0.007$) there were no significant differences between the 55 patients who developed atrial fibrillation and the 94 who did not in their peri-operative variables and cardiac risk factors.

Patients who developed atrial fibrillation had longer post-operative hospital stay, which is consistent with the previous literature (8.8 ± 5.7 days compared with 7.04 ± 3.7 days $p = 0.02$).

Eighty-four percent of patients were on beta-blockers pre-operatively which were given the morning of surgery and as soon as possible following surgery usually on the first post-operative day. A small number of patients who were on pre-operative B-blockers did not receive them in the immediate post-operative period due to either hypotension or bradyarrhythmia. There was no difference between the group that developed atrial fibrillation and those that did not in B-blocker therapy either before or after the surgery.

Post-operative inflammatory response.

Surgery was associated with an inflammatory response in all patients as reflected by an increase in the inflammatory markers from baseline (tables 3.2-3.3).

In the initial group of 100 sP-selectin peaked first at 24 hours, sE-selectin, sICAM, sVCAM and CRP peaked at 72 hours. This is consistent with the fact that P-selectin is stored in intracellular granules while the other markers require transcriptional up-regulation. After analysing the data on the first 100 patients we continued enrolling patients, collecting samples at baseline and 72 hours, as these time points reflected the key changes observed.

	AF (n=55)	No AF (n=94)	P Value
Age (years)	65.1 ± 8	60.9 ± 10	0.007
Female	16.3% (9)	18% (17)	0.1
Hypertension	53% (29)	50% (47)	0.97
Current Smoker	13% (7)	11% (10)	0.94
Ex Smoker	55% (30)	68% (64)	0.51
Aspirin	91% (50)	98% (92)	0.86
Preop B-Blocker	85% (47)	83% (78)	0.1
Post B-Blocker	82% (45)	81% (76)	0.94
Statin	75% (41)	82% (77)	0.81
ACE Inhibitor	45% (25)	41% (39)	0.89
Ca Channel Blocker	40% (22)	36% (34)	0.88

Nitrate	58% (32)	62% (58)	0.94
Diuretic	15% (8)	12% (11)	0.85
No. of Grafts	2.98 ± 0.7	2.93 ± 0.9	0.72
Bypass Time*	95.5 ± 23	92.07 ± 30	0.47
Cross clamp*	52.9 ± 15.8	52.9 ± 18.4	1.0
CoreTemperature	32.8 ± 1.26	33.0 ± 1.3	0.36
Hospital stay†	8.9 ± 5.8	7.0 ± 3.7	0.02

Table 3.1: Demographic data in 149 patients, mean values and standard deviations are presented. *in minutes, †in days.

ng/ml	Baseline	6 Hours	24 Hours	48 Hours	72 Hours
sE-Selectin	44.4 (32-57)	47.9 (36-63)	54.3 (37-70)	57.0 (39-70)	57.0 (41-73)
sP-Selectin	94.2 (65-115)	119 (81-151)	133 (92-161)	109 (86-144)	110 (86-139)
sICAM-1	271(230-320)	324(262-377)	412(344-492)	481(397-593)	516(411-621)
sVCAM-1	461(367-596)	513(429-639)	674(525-828)	668(564-895)	740(619-917)
CRP (mg/dl)	3.4(1.5-5.6)	6.6(4.5-9.9)	154(105-186)	261(225-297)	260(219-299)

Table 3.2: Inflammatory markers in 100 patients at various time points following surgery medians and inter-quartile ranges are presented.

Development of atrial fibrillation.

In the initial phase of the 39 of the 100 subjects developed AF, in the second phase 16 of 49 (32.6%) subjects developed AF. None of the patients required urgent therapy for haemodynamic instability.

(ng/ml)	Baseline	72 hours
sE-Selectin	46.1(34-60.9)	58.6(41.2-74.5)
sP-Selectin	105.6(75.5-140.5)	124.8(93.8-175.6)
SICAM-1	283(236-338)	496(395-611)
SVCAM-1	453(347-577)	669(532-850)
CRP (mg/dl)	2.88(1.35-5.84)	257(182-301)

Table 3.3: Post-operative inflammatory response baseline and day three in all 149 patients data presented are medians and inter-quartile ranges.

Inflammatory markers (AF compared to non-AF).

When analysing the data in the first 100 patients comparison was made between the areas under the curve of the post-operative inflammatory markers. There was no difference between the AF and non-AF group for sE-Selectin ($p = 0.19$); sP-Selectin ($p = 0.43$); sICAM-1 ($p = 0.94$); sVCAM-1 ($p = 0.62$); CRP ($p = 0.79$).

Adjustment of these data by logistic regression for age and baseline values did not alter the results.

In the second phase of the study, 49 patients were recruited sixteen of these (33%) developed atrial fibrillation. We combined the data from the 2 phases and looked at the inflammatory markers at baseline and 72 hours post-operatively. The median values at baseline and on day three are presented with the data on day three corrected for baseline values (table 3.4 – 3.5). There was no difference between the 55 (37%) subjects who developed atrial fibrillation and the 94 who did not for the various inflammatory markers either in absolute terms or for the degree of change from baseline.

Baseline	AF (n=55)	Non-AF (n=94)	P-value
sE-Selectin	41.9(33.2-55.1)	48.5(34.6-63.9)	0.07
sP-Selectin	106.8(69-144)	105(77.5-133.7)	0.98
sICAM-1	284(239-322)	282(233-343)	0.73
sVCAM-1	465(367-596)	438(335-574)	0.29
CRP (mg/dl)	2.57(1.19-4.09)	3.35(1.45-6.28)	0.54
Day Three			
sE-Selectin	56.3(41.9-71.9)	58.7(41-76.6)	0.66
sP-Selectin	116(93.9-153.6)	130.3(93.2-179)	0.35
sICAM-1	486(399-597)	510(394-616)	0.80
sVCAM-1	706(538-869)	653(517-834)	0.17
CRP (mg/dl)	244(171-290)	260(186-307)	0.34

Table 3.4: Baseline and Day three inflammatory markers in 149 patients AF compared with non-AF. Data presented are medians and inter-quartile ranges comparison by a Wilcoxon Rank Sums Test.

Day Three(ng/ml)	AF (n=55)	Non-AF (n=94)	P-value
sE-Selectin (corrected)	10.9(0.153-24.8)	7.62(-2.7-20.5)	0.33
sP-Selectin (corrected)	20.3(-20.8-40.2)	17.63(-5.1-61.6)	0.37
sICAM-1 (corrected)	180(119-290)	201(131-297)	0.95
sVCAM-1 (corrected)	232(87-356)	197(92-344)	0.55
CRP (mg/dl) (corrected)	241(170-287)	254(183-306)	0.32

Table 3.5: Day three inflammatory markers in 149 patients AF compared with non-AF. Data presented are medians and inter-quartile ranges and have been corrected for the baseline values comparison by a Wilcoxon Rank Sums Test.

DISCUSSION.

We have studied the potential association between the peri-operative inflammatory response, assessed by inflammatory markers, and post-operative atrial fibrillation. Although coronary artery bypass surgery was associated with a marked inflammatory response with elevations in all the markers studied we found no difference in the degree of inflammatory response between those who did and did not develop atrial fibrillation. In fact apart from VCAM-1 all the markers were higher in the group that did not develop AF.

The peri-operative clinical situation is complex with many potential variables, which could influence the incidence of AF. These include the intra-operative determinants such as atrial ischaemia and trauma from surgical handling and post-operative events such as sepsis and prolonged inotrope or ventilatory requirements. Our study population was selected to reduce these potential confounding variables by deliberately recruiting patients undergoing the same operation with similar baseline characteristics. A number of pre-operative conditions that were exclusion criteria can cause an increase in inflammatory markers and potentially obscure the significance of any post-operative elevation. We also excluded patients having valve replacement or repair. These patients have a very high incidence of AF both pre and post-operatively, most have structurally abnormal hearts and during surgery undergo a wide variety of procedures that cause different haemodynamic stresses than does isolated coronary bypass surgery. We also excluded so called "off-pump" surgery, which is done without cardioplegia or the CPB circuit. This is a relatively new procedure, which is only suitable for a subset of patients, and the majority of surgery in our unit is "on-

pump". While all of the patients in this study had significant ischaemic heart disease, they had well preserved left ventricular function and normal left atrial size on echocardiography before the surgery. The mean age for the study population was 63 years, which is representative of patients undergoing surgery in our unit. We did observe that those who developed atrial fibrillation were older and the incidence of AF in our study was 37% both features consistent with the literature.

As we have discussed the role of inflammation in the pathogenesis of atrial fibrillation has been proposed with varying data to support it (147). Very little of the early surgical literature is convincing, either because it is observational or limited by small sample size and there are very little data on the degree of inflammation. The most interesting data comes from Gaudino's group, which looked at genetic polymorphisms of the interleukin-6 gene. This group also measured CRP, but similar to our results found no difference in CRP levels between those that did and did not develop AF (143). Non surgical AF may have a different pathogenesis to post-operative AF but again the data suggesting an inflammatory aetiology has some flaws. Mina Chung's control group is very different to her AF subjects, in some key characteristics, which may alter both the propensity to develop AF and also an individuals CRP level (145). The data from the Cardiovascular Health Study also has limitations as the patients that developed AF had higher baseline CRP levels, were older and more likely to have diabetes, LV dysfunction, hypertension, ischaemic heart disease and to be smokers (146). Perhaps the association seen could be explained by the association between CRP and ischaemic heart disease and that the group with ischaemic heart disease has a higher incidence of AF. The hazard ratio of 1.24 while

low was similar to that found with other traditional risk factors for AF such as hypertension and ischaemic heart disease. Again, while these are interesting data the authors concluded that a definite cause effect relationship could be established.

It can be seen that the data to support the hypothesis that AF may in part be mediated by inflammation is somewhat limited. We chose the model of coronary artery bypass surgery because of the high frequency of atrial fibrillation following cardiac surgery and the variety of inflammatory stimuli associated with the surgical undertaking, we felt that this setting was one where the potential importance of the role of inflammation in the pathogenesis of atrial fibrillation could be studied. Despite these advantages cardiac bypass surgery is associated with a host of other features that may also increase the potential development of atrial fibrillation. During the surgery these include atrial trauma from handling and cannulation for the extracorporeal bypass circuit, myocardial ischaemia during the period of extracorporeal perfusion and reperfusion injury following the return of myocardial perfusion. Following the surgery features that may contribute include haemodynamic alterations, neuro-hormonal perturbations, the pro-arrhythmic potential of inotropes, the deleterious effects of the acute withdrawal of anti-arrhythmic drugs including beta-blockers along with electrolyte imbalance, hypoxic and hypercarbic stimuli and post-operative infections (23, 24, 50).

The cellular adhesion molecules that we measured are up-regulated by a number of pro-inflammatory cytokines and are expressed on the activated endothelium. They play an integral role in neutrophil rolling and extravasation. Unfortunately it is impossible to measure these compounds directly in the clinical

setting and so we used the soluble forms as markers of endothelial activation. CRP is a more non-specific marker of inflammation, produced by the liver as a result of stimulation by interleukin-6. By measuring a number of different markers produced in response to different stimuli at different time-points we have attempted to accurately quantify the post-operative response.

Atrial fibrillation is potentially an electrophysiological response to a heterogeneous group of substrate related factors and triggering events. Atrial stretch and fibrosis is associated with an increased potential for atrial fibrillation. In those over the age of 50 years there is progressive replacement of normal cardiac tissue with fibrous tissue and amyloid deposition, which provides an important substrate for atrial fibrillation (148). Perhaps this explains the rising incidence of AF in modern surgical practice.

While we did not see a relationship between the inflammatory markers that we assessed and post-operative atrial fibrillation we cannot exclude that inflammation may still have an important role in its pathogenesis. Serum markers while of use are surrogates and do not provide exact information on the degree of atrial inflammation associated with the surgery and do not provide information about the site of the inflammation. The extent and regions of the atria experiencing inflammation may have relevance in the development of post-operative atrial fibrillation. Details with regard to the degree of pericardial inflammation, the extent of transmurality of the process and regional variability in inflammation with involvement of key arrhythmogenic areas such as the pulmonary veins cannot be assessed by the serum markers we used.

Radioactively labelled inflammatory cells may provide more concise information in this regard.

Conclusion.

Coronary artery bypass surgery was associated with a significant inflammatory response in all of the patients studied. Using serum inflammatory markers as surrogates of the inflammatory process we did not find a difference in the inflammatory response in patients who developed post-operative atrial fibrillation and those who did not.

CHAPTER 4

The Association Between Post-operative BNP Elevation and Atrial Fibrillation

INTRODUCTION.

There has been major progress in the understanding of the pathogenesis of AF in the non-surgical setting, but factors related to cardiac surgery associated AF are not well understood (21, 22, 149). Changes in left atrial pressure secondary to left ventricular (LV) dysfunction may have a role in the genesis of post cardiac surgery AF. Elevation in left ventricular end diastolic pressure (LVEDP) can be caused by either systolic or diastolic LV dysfunction. When the mitral and tricuspid valves are open this increased pressure will be transmitted to the atria. As the atria are relatively thin walled structures any increase in pressure is likely to cause atrial stretch. Stretching the atrial myocytes has the potential to alter the atrial refractory period and hence lower the threshold for arrhythmia induction.

Brain Natriuretic peptide (BNP) is produced by ventricular myocardium in response to elevation in end-diastolic pressure (121, 122). BNP levels correlate closely with pulmonary capillary wedge pressure (which is a surrogate for left atrial pressure) in left ventricular systolic dysfunction. It has a short half-life of 22 minutes as it is broken down by neutral endo-peptidases and binds to natriuretic peptide clearance receptors and reflects ventricular function in the preceding hours (124, 125). It has been demonstrated to be elevated following coronary artery bypass grafting (CABG), with levels elevated up to 24 hours following the surgery (150-152).

BNP in atrial fibrillation.

The initial data on BNP in atrial fibrillation were conflicting with some studies showing the elevation to be independent of left ventricular function and others

showing that the left ventricle was the primary determinant of BNP levels. A study from the Brigham and women's Hospital attempted to clarify this issue (153). They analysed 72 outpatients with chronic AF and compared them with controls in sinus rhythm, patients with signs, symptoms or a history of heart failure were excluded. They found that patients with AF had a higher median BNP and that they were more likely to be in the two highest quartiles of BNP values. A multivariate analysis which included LV mass and LV systolic function still found that AF was associated with BNP elevation. The effect of cardioversion on BNP has also been studied, 40 patients with chronic non-valvular AF with low ventricular rates were analysed (154). None of the subjects had clinical or echocardiographic evidence of LV dysfunction. Amongst the 36 that returned to sinus rhythm the BNP levels fell from 61pg/ml to 23pg/ml within 24 hours. In the group of 4 with a failed cardioversion there was no significant change in BNP levels.

Following the surgical maze procedure many patients return to sinus rhythm with atrial contraction. Interestingly BNP levels also fall as has been demonstrated by Albage et al (155). The study population consisted of 15 patients undergoing isolated maze (III) procedures. All the patients were free of AF at 6 months follow- up and both ANP and BNP levels were significantly lower than the pre-operative values. The cause of the elevation in BNP in AF remains unclear but it may be related to an alteration in the left ventricular filling pattern caused by the lack of atrial contraction. Another theory is that the irregular rate of ventricular contraction possibly with different diastolic filling times contributes to impairment of cardiac function.

BNP following cardiac surgery.

Cardiac surgery particularly when using a cardiopulmonary bypass circuit has the potential to cause significant myocardial injury and subsequent left ventricular dysfunction; this can be reversible or irreversible. The insult is multifactorial and can be related to ischaemia, reperfusion injury, trauma from surgical handling or the inflammatory response induced by surgery (156).

When compared to other patients following major surgery or trauma, cardiac surgical cases have a significantly higher post-operative BNP level (150). An initial study of 19 patients with good LV function undergoing isolated coronary bypass analysed BNP levels at baseline and 1, 5, 10 and 20 minutes after aortic declamping (151). The intra-operative BNP values were all significantly higher than the baseline value and correlated with the peak myocardial lactate production although there was no correlation with troponin levels. In 3 subjects myocardial lactate elevation persisted although none developed peri-operative infarction. These patients demonstrated a more marked BNP elevation. These data suggest that myocardial ischaemia and reperfusion are the primary stimuli for early post-operative BNP release, a hypothesis that is supported by increases in BNP following stress testing in patients with angina pectoris (157).

A subsequent study of 25 patients also undergoing isolated coronary artery bypass evaluated samples taken at baseline, before removal of the aortic clamp, 3 and 10 minutes following clamp removal and 5 minutes and 2 hours after termination of CPB (152). They demonstrated a small but significant difference between the BNP level taken prior to removal of the clamp and the sample taken 3 minutes after clamp

removal. Only at 2 hours after the termination of CPB was the BNP level significantly greater than baseline, there was no correlation between BNP and the length of time the aorta was cross-clamped. The authors postulated that while BNP release was stimulated by reperfusion the main stimulus was ventricular filling and increases in pre-load caused by iatrogenic volume loading.

While the precise stimulus for BNP release remains unclear there are some data to support its role as a prognostic indicator. Chello et al studied 31 patients with poor LV function (EF < 35%) undergoing CABG and 40 healthy controls (158). Echocardiography and natriuretic peptide estimation were carried out at baseline and approximately 10 months after surgery. The authors found that a lower pre-operative BNP level and a higher number of viable myocardial segments were associated with recovery of LV function. Preliminary data from the St George's hospital in London has shown that patients with a complicated post-operative course such as pulmonary oedema, prolonged inotropic support or ongoing ischaemia have NT-proBNP levels at least twice that of those with an uneventful recovery (159). Further research is required to explain the precise mechanism of BNP release and its role in peri-operative outcomes.

Natriuretic peptides in post-operative atrial fibrillation.

The role of ANP was initially studied in 80 patients undergoing CABG, 29 of this group developed AF. Atrial areas and ANP were measured both pre and post-operatively and the authors found no difference in either between those that developed AF and those that did not (160).

Another study of 88 patients with good LV function examined pre-operative atrial size, ANP and BNP at baseline, 18 and 28 hours post-operatively (161). Patients had continuous ECG monitoring during their hospital stay, with an episode of AF longer than 5 minutes the end-point. Thirty one of the subjects developed AF with a mean time from surgery to onset of 56 ± 22 hours. Pre-operative atrial areas and ANP were significantly higher in the group that developed AF as were post-operative ANP. Neither pre nor post-operative BNP levels were significantly different between the two groups. Only 2 variables were found to be independent predictors of AF on multivariate analysis, these were advancing age and left atrial cross sectional area. This negative result may be explained by the short half-life of BNP and potentially inappropriate early sampling time points (18 and 28 hours), which were not temporally related to the development of AF. The other limitation of this study was their definition of AF of just 5 minutes as self-limiting short episodes are of little clinical significance and may not be associated with any changes in neuro-hormonal markers.

Objectives.

The objective of this study was to determine whether there was a relationship between the degree of BNP elevation 24 and 48 hours following the onset of cardiopulmonary bypass and the development of post-operative AF. BNP was used as a surrogate marker for LVEDP.

METHODS.

One hundred and thirty three patients were entered into the study. This consisted of 65 cases of atrial fibrillation and 68 selected controls. The recruitment period commenced in December 2001 and terminated in February 2003. Patients who were in sinus rhythm undergoing first time non-emergency on pump coronary artery bypass grafting were asked to participate in the study. The exclusion criteria and endpoints have previously been described.

Post operative AF usually develops 48-72 hours following the operation (162). In order to determine the BNP levels in the period before the potential onset of AF, samples were taken in EDTA tubes prior to anaesthetic induction and 24 and 48 hours following the onset of cardiopulmonary bypass. Samples were centrifuged within 1 hour and stored at minus 20 degrees centigrade pending analysis. BNP levels were measured with a fluorescence immunoassay on freshly thawed samples (Biosite Diagnostics San Diego California). This assay has a detection range from 5pg/ml-1300pg/ml; in the setting of cardiac failure a decision threshold of 100pg/ml is usually recommended.

Statistical analysis.

Descriptive statistics are presented for the baseline characteristics of patients in the study. Means and standard deviations are presented for normal data, medians and inter quartile ranges (IQR) for non-normal data, and proportions for categorical data. BNP data were non-normal, neither a square root transformation nor a log transformation were able to normalise the data. Comparison was made between the

two groups using a Wilcoxon rank sums test for the non-normal BNP data and Chi-squared analysis for the descriptive data. A p value ≤ 0.05 was considered significant with two-sided tests. All analyses were performed using the JMPin software (SAS Institute Inc.).

RESULTS.

Onset of atrial fibrillation.

AF occurred in 65 of the 133 subjects. None of the patients required urgent therapy for haemodynamic instability. The mean time to onset of AF was 60 ± 27 hours, 4 patients developed AF within 24 hours of surgery, 26 patients between 24 and 48 hours, 16 between 48 and 72 hours, 13 between 72 and 96 hours and 6 patients 96 hours or greater after surgery.

Demographic data.

Baseline demographics are presented in table 4.1; there was little difference between the two groups in clinical variables. Patients that developed AF tended to be older and also to have a longer hospital stay, both features which are consistent with the previous literature in this area. Most patients (84%) were on beta-blockers prior to surgery; these were given the morning of surgery and on the day immediately following surgery, unless there was a contraindication such as haemodynamic compromise. When analysing the data we divided the subjects into groups depending on the time to onset of AF into those who developed it within the first 48 hours of the surgery or after the 48 hour time mark (table 4.2).

	AF (n = 65)	Non-AF (n=68)	P Value
Age (years)	65(60-71)	62(53-69)	0.007
Hypertension	56% (36)	51% (35)	0.92
Smoker	14% (9)	11% (7)	0.77
Ex Smoker	47% (30)	66% (45)	0.28
Aspirin	89% (58)	92% (63)	0.98
Preop B-Blocker	85% (55)	83% (56)	0.98
Postop B-Blocker	79% (51)	81% (55)	0.1
Statin	70% (46)	82% (56)	0.66
ACE Inhibitor	47% (31)	41% (28)	0.75
Ca Channel Blocker	40% (26)	37% (25)	0.93
Nitrate	57% (37)	62% (42)	0.89
Diuretic	10% (7)	13% (9)	0.9
No. of Grafts	3.0 ± 0.7	3.08 ± 0.8	0.54
Bypass Time*	91.3 ± 23	92.97 ± 29.6	0.72
Cross clamp*	51.01 ± 15	53.41 ± 16.2	0.38
Core Temperature	32.9 ± 1.16	33.0 ± 1.09	0.61
Hospital stay †	7(6-9)	6(5-7)	0.005

Table 4.1: Demographic data, mean values and standard deviations are presented for normal data, medians and IQR for non-normal data. *in minutes, †in days.

	AF<48hrs N = 30	AF>48hrs N = 35	No AF N = 68	P Value
Age	65(59-72)	64(60-72)	62(53-69)	0.08
No. of Grafts	2.96 ±0.75	3.02 ± 0.79	3.08 ± 0.8	0.78
Bypass Time*	89.2 ± 25	93.2 ± 22	92.9 ± 29	0.79
Cross clamp*	50.3 ± 15	51.7 ± 14.8	53.41 ± 16	0.64
Temperature	32.97 ± 1	32.8 ± 1.24	33.0 ± 1.09	0.68
Hospital stay†	6.5(5-9.3)	7(6-10)	6(5-7)	0.001

Table 4.2: Age and peri-operative variables of the three groups according to whether or not they developed AF and whether it occurred within or after 48 hours. *in minutes, † in days.

BNP elevation after surgery.

Baseline median BNP was 44.7pg/ml (IQR 17.9-101pg/ml) consistent with a selected group of patients with good LV function. Following cardiopulmonary bypass the median BNP level rose to 379pg/ml (IQR 253-548pg/ml) at 24 hours and 366pg/ml (IQR 223-482pg/ml) at 48 hours. This represents an eight-fold rise in BNP induced by surgery (figure 4.1).

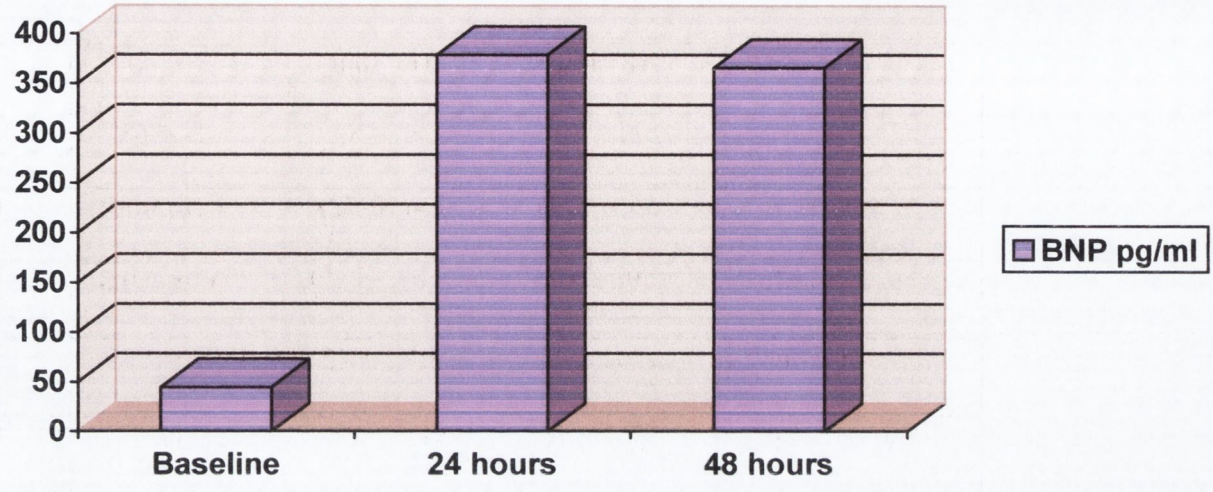


Figure 4.1: Post-operative BNP elevation in total cohort (pg/ml).

BNP Data: patients with AF compared to controls.

The pre-operative median BNP tended to be higher in the AF group than the non-AF group [49.4pg/ml (IQR 24.8-132pg/ml) and 40.15pg/ml (IQR 14.2-77.05pg/ml) respectively, p=0.07] (figure 4.2).There was a rise in median BNP in both the AF and non-AF groups, with similar median BNP levels at 24 hours in both AF and non-AF patients [384pg/ml (IQR 254-618pg/ml) and 365pg/ml (IQR 252-517pg/ml) respectively, p=0.29]. The median BNP at 48 hours was greater in the AF group than the non-AF group, [440pg/ml (IQR 250-606pg/ml) and 319pg/ml (IQR 169-443pg/ml) respectively p=0.001].

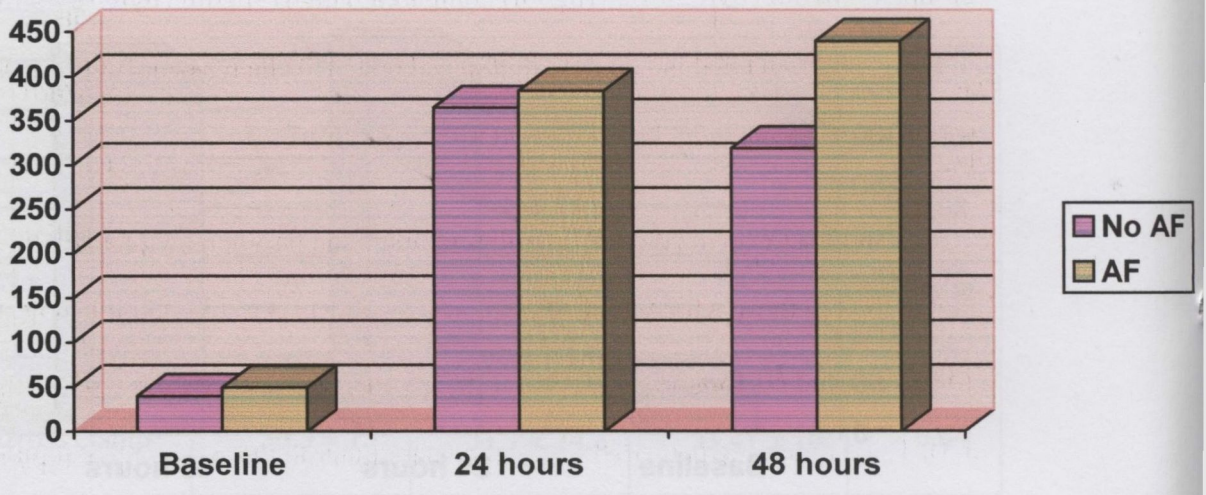


Figure 4.2: Post-operative BNP levels (pg/ml), AF compared to controls.

BNP Data: onset of AF before or after 48 hours.

As AF itself may impact on the BNP level the patients who developed AF were divided into those who developed AF before or after 48 hours (163). Of the 65 patients that developed AF; 30 (46%) developed AF in the first 48 hours following surgery and the remaining 35 (54%) developed AF greater than 48 hours after surgery. The mean time to onset of AF in the pre-48 hour group was 37 ± 8.6 hours and 79.6 ± 22 hours in the post-48 hour group.

BNP levels in both of these groups were compared with those of the 68 patients who remained in sinus rhythm. In those who developed AF in the first 48 hours baseline BNP levels were similar to those who remained in sinus rhythm [44.9pg/ml (IQR 13.8-134pg/ml) and 40.15pg/ml (IQR 14.2-80.75pg/ml) respectively, p=0.54] (figure 4.3). At 24 hours the BNP levels remained similar in the AF and non-AF groups [381pg/ml (IQR 246-516pg/ml) and 365pg/ml (IQR 252-517pg/ml) respectively, p=0.73]. At 48 hours there was a further increase in BNP levels in the AF

group, 454pg/ml (IQR 248-661pg/ml) compared with 319pg/ml (IQR 169-443pg/ml) in those who remained in sinus rhythm, p = 0.004. This increase in BNP was seen after the onset of AF and may be influenced by the AF itself.

In those who developed AF after 48 hours the baseline median BNP was higher than in those who remained in sinus rhythm (n = 68) [53.2pg/ml (IQR 32.6-138pg/ml) and 40.15pg/ml (IQR 14.2-80.75pg/ml) respectively, p = 0.056] (figure 4.3). The 24 hour median BNP was somewhat higher in the AF group, 440pg/ml (IQR 252-691pg/ml) compared with 365pg/ml (IQR 252-517pg/ml) for the non-AF group, p=0.19. The 48 hour median BNP level, which was before the onset of the AF, was increased in the AF group, 405pg/ml (IQR 248-583pg/ml) compared with 319pg/ml (IQR 169-443pg/ml) for those who remained in sinus rhythm, p = 0.02. As the baseline median BNP was higher in those who developed AF after 48 hours this difference was controlled for. The 48-hour BNP value in this later onset AF group remained elevated following correction for the baseline level (p = 0.05.

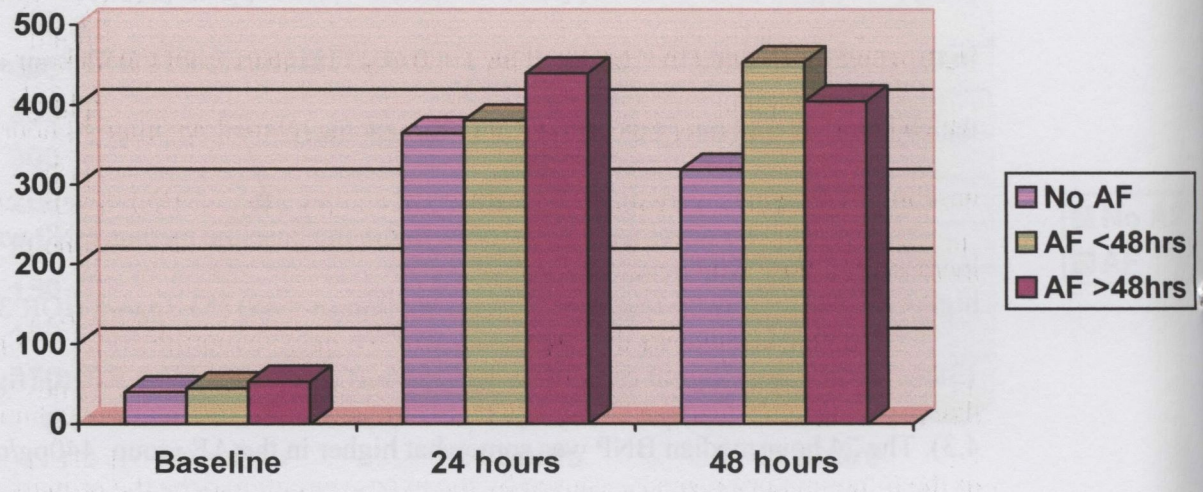


Figure 4.3: Post-operative BNP elevation (pg/ml) in those who developed AF before or after 48 hours.

DISCUSSION.

We have demonstrated that patients with well preserved pre-operative systolic function have a significant post-operative BNP elevation and that the degree of elevation at 48 hours following surgery was greater in those who developed subsequent AF. We postulated that the threshold for the development of AF might have been lowered by atrial stretch secondary to left ventricular dysfunction. We used BNP as a surrogate for left ventricular dysfunction and showed that the degree of elevation of BNP at 48 hours was significantly greater in those who subsequently developed AF.

As almost half of the patients developed AF prior to the 48-hour time-point this difference could occur as a result of the AF rather than being causative. Accordingly, we assessed the BNP levels in those who developed AF before and after

48 hours. The patients who developed AF within 48 hours of surgery did not have significantly different BNP levels to the control group apart from the 48 hour value, which could be secondary to the AF. In those who developed AF after 48 hours the baseline BNP values were marginally different and the 48-hour BNP levels were increased in those who developed AF.

These data suggests that the etiology of AF may be multifactorial. Perhaps in those who develop "early AF" i.e. less than 48 hours, atrial trauma from cannulation or the inflammatory response caused by the bypass circuit may be the primary stimuli for AF. In those who develop AF later atrial stretch may be an important feature in the pathogenesis of AF either directly or as facilitator for other triggers.

Our results demonstrate that despite having normal LV function pre-operatively most patients undergoing CABG develop LV dysfunction, which is often sub-clinical and in this study occurred up to 48 hours after the operation. The etiology of this dysfunction is unclear. It may be a residual effect of cardioplegia and cardiopulmonary bypass or it may be caused by ongoing ischaemia or myonecrosis. We excluded patients with LV systolic dysfunction and valvular surgery as we felt that in both these groups baseline BNP may be elevated and this may obscure the significance of the post-operative elevation. Also in valve surgery the procedures are more complex and the atria undergo different haemodynamic stresses. Accordingly, we recruited a homogeneous group in order to reduce any potential confounding variables.

As previously discussed there are limited data on BNP elevation following cardiac surgery. In the area of post-operative AF, Hakala et al. are the only group to

have studied the relationship with pre and post-CABG BNP levels (161). They measured BNP at baseline and 18 and 28 hours following surgery. They found no correlation between BNP and AF at any of the time points. The mean time to onset of AF was 56 hours. BNP has a short half-life and perhaps their negative result was due to sampling at an inappropriate time point unrelated to the development of AF. Our data are the first to show an association between BNP elevation as a surrogate for LVEDP and post-operative AF. Further work is required to explain the aetiology of this elevation.

Identifying patients most at risk for the development of AF remains difficult. Predictors of AF following CABG include age, a previous history of AF, P wave duration on signal averaged electrocardiogram and withdrawal of pre-operative beta-blockade. The ability to accurately predict a group at increase risk of post-CABG AF would facilitate targeting prophylactic therapies, such as amiodarone, biatrial overdrive pacing or potentially a diuretic to decrease left ventricular end-diastolic pressure.

Conclusion.

We have demonstrated that patients with normal pre-operative systolic function have a significant BNP rise, which remains elevated for 48 hours or more following the surgery. We have identified that those who developed AF following the operation had a greater rise in BNP than those who remained in sinus rhythm, suggesting that left ventricular dysfunction and subsequent left atrial stretch may have a role in its pathogenesis.

CHAPTER 5

Relationship of Pre-operative Atrial Histology with Post-operative Atrial Fibrillation

INTRODUCTION.

Atrial fibrillation is a complex arrhythmia that is characterised by rapid atrial depolarisation with chaotic fibrillary waves (18). Moe's hypothesis suggests that multiple re-entrant circuits cause AF and that these random pathways are determined by atrial refractoriness, excitability and conduction properties. In most cases these re-entrant circuits are constantly arising, colliding and being extinguished and so the arrhythmia is terminated (34). Areas of delayed conduction within the atria can cause these re-entrant circuits to propagate and lead to sustained arrhythmia. Some authors have postulated that while the onset of AF requires a trigger an abnormal atrial substrate is required for maintenance of the arrhythmia (164).

Before trying to analyse the atrial histological substrate it is important to try and define what is normal. The prevalence of AF increases with age and many "normal" changes occur in the ageing heart. Unfortunately it remains difficult to define what precisely is normal for age as many disease processes such as ischaemic heart disease are more common in the elderly. Biologic aging processes should be absent in the young, universally present in the very old, increase in proportion to age and have no correlation with specific diseases (165).

From middle age onwards there is progressive smooth muscle proliferation and an increase in interstitial collagen and elastin (166, 167). This is seen microscopically as foci of fibrosis distributed throughout the subendocardium and myocardium of both atria and ventricles. Dissolution of the distinct endocardial layers occurs with vacuolisation and atrophy of endocardial muscle and replacement with connective tissue. The sinus node is also affected by the ageing process; there is gradual loss of

the normal nodal fibres and an increase in fibrous tissue and fat. Amyloid deposition also appears to be more prevalent in the sinus node than in other myocardial cells.

With advancing age there is a decrease in the number and an increase in the size of the cardiac myocytes. The nuclei become enlarged and irregular and focal basophilic myocardial degeneration is extremely common (53). Lipofuscin pigment a dark pigment thought to be caused by breakdown products of cells increases in proportion to age and is ubiquitous in the elderly (165). Senile cardiac amyloid deposition may be a normal finding or related to an as yet undefined disease process. It is very rare before the age of 60 but is present in over 65% of hearts from subjects over the age of 90. It is usually an incidental autopsy finding of semi-translucent yellow deposits lining the left atrial endocardium. Focal deposition is common and seems to be of little significance however extensive deposition has been correlated with heart failure and atrial fibrillation (168).

Not only do microscopic changes occur there are also changes in chamber dimensions. As we grow older the long axis of the heart shortens, there is a slight decrease in the internal ventricular dimensions and there is dilation of the aortic root and left atrium. The atrial dilation may occur as a consequence of the decreased diastolic ventricular compliance seen in the elderly. All these "normal" changes may actually provide the abnormal substrate required and explain the strong association between AF and advancing age.

A number of studies have tried to define the atrial substrate by obtaining tissue during cardiac surgery or by endomyocardial biopsy. Frustaci's group recruited a highly select cohort of 12 patients with refractory paroxysmal lone AF and a control

group of 11 patients with Wolff-Parkinson-White syndrome who were undergoing invasive electrophysiological studies (144). All patients had structurally normal hearts on echocardiography and normal coronary angiograms. They analysed atrial and ventricular biopsies. Morphometric assessment by light microscopy showed that the biopsies were all normal in the control and all abnormal in the AF group. In 8 of the patients with AF, inflammatory lympho-mononuclear infiltrates with associated focal necrosis of myocytes were found. Five patients had interstitial fibrosis with focal replacement and 2 patients had myocyte hypertrophy with vacuolar degeneration. A further 2 subjects had extensive areas of atrial fibrosis without inflammatory infiltrates. Most of the patients with AF had normal ventricular biopsies and the authors conclude that an atrial cardiomyopathic process was responsible for AF and that in the majority this was mediated by an inflammatory process. During follow-up AF recurred in 9 patients despite anti-arrhythmic therapy and interestingly the 3 patients with a diagnosis of active atrial myocarditis who were discharged on steroid therapy had no recurrence of AF.

In another study of patients with therapy resistant AF Connelly and co-workers examined the atrial appendage of 19 patients obtained during the surgical maze procedure. They selected a control group of autopsy samples without AF some with coronary disease and some without (169). They found that the patients undergoing the maze procedure had a significant increase in the degree of vacuolar degeneration compared with controls. Interstitial adipose content was increased but did not quite reach statistical significance. There was an increase in myocyte hypertrophy and interstitial fibrosis when compared to the control group without coronary disease but

not when compared to the controls with ischaemic heart disease. Both these studies need to be interpreted cautiously as both selected patients with resistant AF, also it is unknown if AF itself may cause the changes described. Myocyte hypertrophy and interstitial fibrosis were also found to be significantly more common in the atria of patients with AF undergoing cardiac surgery by Aime-Sempe et al. They analysed 50 patients of whom 11 were in AF. In the AF group the myocyte nuclei were irregular and the sarcomeric apparatus was disrupted with replacement by glycogen granules (170).

The right atrial appendage was examined in a study of 245 subjects undergoing a variety of cardiac surgical procedures. Forty of the patients had amyloid deposition demonstrated by Congo red staining and this amyloid was further defined by staining with antibodies against AA amyloid, ANP, transthyretin, immunoglobulin light chains and β2 microglobulin. The heart can be infiltrated by 3 different forms of amyloidosis; AL amyloid associated with systemic disease is made up of immunoglobulin light chains. In senile cardiovascular amyloidosis, which is a normal ageing process, there is deposition of transthyretin in the interstitium of both atria and ventricles. Isolated atrial amyloidosis (IAA) is a strictly localised variant, which increases markedly with age. Over 90% of people in the ninth decade show some evidence of IAA where the fibrillary protein deposited is derived from ANP. All the cases with amyloid stained positive for ANP and the authors postulated that high local concentrations of ANP precursor proteins led to the amyloid deposition. Hence conditions that cause atrial stretch or volume overload and up-regulation of ANP production may lead to IAA. The 38 patients with persistent AF were compared to an age and sex matched control

group of patients in sinus rhythm, selected from the total cohort (171). The main findings were that amyloid was more common in older patients, those with AF and those undergoing mitral valve replacement. Surprisingly the amount of amyloid demonstrated an inverse correlation with the degree of atrial fibrosis, which is frequently thought to be the primary cause of an abnormal atrial electrical substrate. In those in sinus rhythm the P-wave duration, which has previously been shown to be associated with the development of AF, was an independent predictor for the presence of amyloid. The authors suggest that IAA can affect atrial conduction and provide the substrate for re-entry and the propagation of AF.

Analysing tissue samples from patients with AF must be done cautiously, as AF itself may be the cause of the abnormalities described. This concept is clinically relevant as the longer a patient remains in AF the more likely it is that the rhythm will become permanent. So called electrical remodelling was first described in 1995 when it was noticed that maintenance of AF in a goat model by atrial pacing, led to a significant shortening of the atrial refractory period and an increase in the susceptibility to sustained AF (172). This effect seems to be mediated by alterations in the atrial ion channels. Many animal studies using both light and electron-microscopy have demonstrated inducible atrial changes following periods of AF (173). These changes resemble those seen in ventricular myocardium subjected to chronic ischaemia. The most prominent feature is an increase in cell size with associated myolysis (central loss of sarcomeres) and perinuclear accumulation of glycogen. There are also alterations in connexin expression, changes in mitochondrial shape and fragmentation of the sarcoplasmic reticulum. The cells seem to dedifferentiate to a

more foetal stage of development with loss of desmin and re-expression of α-smooth muscle actin. These changes seem to occur as part of a physiological response to the chronic calcium overload associated with AF.

Two previous studies have attempted to define the atrial histological substrate in patients in sinus rhythm who developed post-operative AF. Goette et al found that the degree of atrial fibrosis combined with P-wave duration was associated with the development of post-operative AF (174). Patient age correlated with surface P-wave duration and with the amount of atrial fibrosis. Their series of 259 patients (20% incidence of AF) included those undergoing a wide variety of procedures including valve replacements and their definition of AF only required the arrhythmia to be sustained for greater than 5 minutes. In their discussion the authors comment that their low r-values may be partially explained by the multifactorial pathogenesis of post-operative AF. They also noted that there was a considerable overlap in the degree of fibrosis between the 2 groups.

Ad et al studied the right atrial appendage of 60 patients in sinus rhythm undergoing elective coronary artery bypass surgery (175). Similar to our study they concentrated on the samples before the onset of cardiopulmonary bypass and used samples fixed in formalin and stained with haematoxylin and eosin. The incidence of atrial fibrillation was low at only 25% and only 8 of the atrial samples were normal. They analysed a number of histological features but only found two that were associated with the development of atrial fibrillation. In their population 48 of the 60 had some degree of myolysis, 10 of these had severe myolysis of which 9 developed AF. On univariate analysis they found that lipofuscin pigmentation was associated

with AF ($p = 0.02$) but they felt that this was associated with the degree of myolysis so it could not be considered an independent predictor. They found no multivariate predictors of AF. Myolysis has been hypothesized to contribute to the loss of atrial contractile force, which in turn may enhance atrial dilatation and lower the threshold for persistence of AF (173).

As atrial fibrillation is such a common occurrence after cardiac surgery we felt that despite the limitations inherent in analysis of atrial tissue that this would be the best model for defining whether an abnormal atrial substrate contributes to the development of this arrhythmia. Following cardiac surgery many potential triggers such as inflammation, atrial stretch or catecholamine excess exist but the atrial substrate remains poorly defined.

Objectives.

The objective of our study was to determine whether there is a relationship between the pre-operative atrial morphology and the development of post-operative atrial fibrillation.

METHODS.

A cohort of 94 patients in sinus rhythm undergoing first time coronary artery bypass grafting was recruited. The study period commenced in December 2001 and terminated in November 2002. Patients with impaired left ventricular systolic function ($EF < 45\%$), valvular heart disease and diabetes were excluded. The atria were

examined for the following histological parameters modified from Ad et al, as set out in Table 5.1, using standard semi-quantitative morphological assessment.

Table 5.1: Histological parameters analysed.

Myolysis	Absent Mild Moderate Severe
Inflammation	Absent Mild Moderate Severe
Nuclear size	Grade 1 or Grade 2
Pericardial exudates (fibrinous)	Absent Present
Mesothelial hyperplasia	Absent Present
Lipofuscin pigment	Absent Mild Moderate Severe
Fibrosis	Absent Mild Moderate Severe
Diverticulae	Absent Present
Arteriolar hypertrophy	Absent Present
Contraction banding	Absent Present

Myolysis was defined as the loss of muscle striation within myocytes, together with cytoplasmic vacoulation. **Nuclear size** reflects myocyte size, and hence nuclear enlargement parallels myocyte hypertrophy. **Pericardial exudate** and **mesothelial hyperplasia** reflect a reactive pericardium. **Diverticulae** were defined as out-pouching of the epicardial wall with loss of the muscle coat between the epi and pericardium. **Contraction banding** reflects myocyte necrosis secondary to ischaemia.

Where applicable, results were graded into mild, moderate and severe. Histological features, which were absent or mild, were assigned a score of 0; presence

of a feature and if moderate or severe was assigned a score of 1. These scores were used for statistical analysis.

Slides were examined independently by two pathologists experienced in the assessment of cardiac histology. Each set of results was validated by examination of 50 random cases by both pathologists to ensure agreement. Inter-observer variability was calculated based on 50 random samples which were analyzed by both pathologists. Both phi and kappa values are represented with a value of 1 indicating perfect agreement and a value of 0.5 indicating no agreement and that the results could have occurred by chance (table 5.2).

Table 5.2: Inter-observer variability for the ten parameters analyzed. Percentage agreement is the number that both investigators agreed on.

Parameter	% agreement	Phi	Kappa	Standard Dev
Myolysis	96	0.96	0.93	0.05
Inflammation	100	1.0	1.0	0
Fibrosis	94	0.81	0.81	0.07
Nuclear Size	78	0.52	0.51	0.12
Pericardial Exudate	98	0.95	0.95	0.05
Mesothelial Hyperplasia	100	1.0	1.0	0
Lipofuscin	100	1.0	1.0	0
Diverticulae	90	0.79	0.79	0.09
Arteriolar Hypertrophy	92	0.85	0.79	0.09
Contraction Banding	98	0.95	0.95	0.05

Both investigators were blinded as to which patients developed post-operative atrial fibrillation. Univariate and multivariate analysis were carried out using the Chi-Square and Fischer's exact tests, with the Wilcoxon/Kruskal-Wallis test (rank sums) applied to the multivariate analysis.

Patients were continuously monitored for the development of atrial fibrillation post-operatively, for a total of three days. The primary endpoint was defined as atrial fibrillation sustained for at least one hour or requiring therapy due to haemodynamic instability.

RESULTS.

Of the 94 patients studied, 36 (38%) developed post-operative atrial fibrillation. All but 2 of the 94 patients had abnormal atrial histology with one or more of the above mentioned features identified.

On univariate analysis no correlation was found between the development of post-operative atrial fibrillation and the 10 morphological features assessed (Tables 5.3 and 5.4). Parameters found previously by other investigators to be independently associated with the development of post-operative AF, notably myolysis, lipofuscin pigment and atrial fibrosis where not found to be so in our study. Importantly, myolysis was found in 63% of the non-AF group and in 75.6% of the AF group. Of the total group of 94 patients, 54 had moderate myolysis and 10 had severe myolysis, of those with moderate myolysis 24 developed AF (44%), of the 10 with severe myolysis 4 (40%) developed AF. Lipofuscin pigment was found in 12.2% of the

control group and 5.4 % of the AF group. Similarly, fewer of the patients who developed AF had evidence of atrial fibrosis, as compared to the group that remained in sinus rhythm (13.5% compared to 22.4% respectively). Patients were assigned an aggregate score based on the presence or absence of the features studied. If a feature was absent or graded as mild it was scored as a zero, if a feature was graded as moderate or severe it was assigned a one. Using this aggregate score there was no significant difference between those that developed AF and those that did not (figure 5.1).

Table 5.3: Percentage of patients in each group with the features studied.

Variable	Non-AF Group n = 58	AF Group n = 36
Myolysis	63.7%	75%
Inflammation	12%	5.5%
Fibrosis	22.4%	13.8%
Nuclear size	46.5%	38.8%
Pericardial exudates	18.9%	19.4%
Mesothelial hyperplasia	41.4%	33.3%
Lipofuscin pigment	12%	5.5%
Diverticulae	58.6%	52.7%
Arteriolar hypertrophy	46.5%	30.5%
Contraction banding	74.1%	77.7%

Table 5.4: Correlations between histological features studied and AF.

Variable	Correlation coefficient (2tail) Prob> ChiSq	Fisher Exact test p-value
Myolysis	0.2	0.26
Inflammation	0.27	0.47
Fibrosis	0.26	0.30
Nuclear size	0.36	0.40
Pericardial exudate	0.62	0.79
Mesothelial hyperplasia	0.61	0.67
Lipofuscin pigment	0.27	0.47
Diverticulae	0.71	0.83
Arteriolar hypertrophy	0.06	0.08
Contraction banding	0.66	0.81

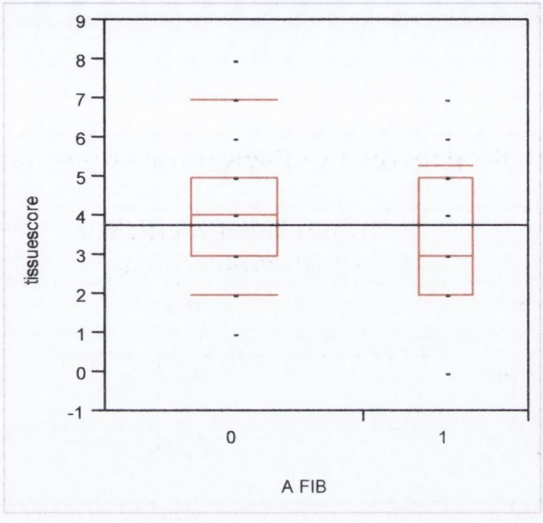


Figure 5.1. Tissue score plot v Atrial Fibrillation

0= non-fibrillating group, 1= fibrillating group

[Wilcoxon/Kruskal-Wallis tests (Rank sums) gave a p value of 0.37(1- way test, Chi-Square approximation)]

As previously discussed advancing age may be associated with changes in atrial histology. Accordingly we analysed the association between the features studied and the age of the subjects (Table 5.5). Apart from myolysis and arteriolar hypertrophy which were more common in younger patients there was no statistically significant difference in the age of patients in the histological parameters examined.

Table 5.5: Median age of patients presenting with various features.

Variable	Median Age if present	Number with feature	Median Age if absent	Number without feature	P value
Myolysis	62 yrs	64	70 yrs	30	0.001
Inflammation	63 yrs	9	64 yrs	85	0.79
Fibrosis	66 yrs	18	63 yrs	76	0.52
Nuclear size	64 yrs	41	64 yrs	53	0.66
Pericardial exudate	65.5 yrs	18	63.5 yrs	76	0.33
Mesothelial hyperplasia	65 yrs	36	63.5 yrs	58	0.74
Lipofuscin pigment	71 yrs	9	63 yrs	85	0.1
Diverticulae	63 yrs	53	65 yrs	41	0.89
Arteriolar hypertrophy	62.5 yrs	38	65 yrs	56	0.05
Contraction banding	64 yrs	71	63 yrs	23	0.63

DISCUSSION.

Our data have shown no significant difference between the group that developed AF and the group that did not in the histological features studied. In fact apart from myolysis and the presence of pericardial exudates all the features studied were more common in the group that did not develop AF (not statistically significant). Of note neither lipofuscin pigment nor myolysis were more common in those that developed AF, as previously found in a study by Ad et al (175).

As previously discussed one of the most consistent predictors of all forms of AF is advancing age and any interpretation of atrial histology must take account the

"normal" age related changes. While the group that developed AF was older we did not find that advancing age was associated with abnormal atrial histology. In fact myolysis and arteriolar hypertrophy were more common in the younger patients. Most of the data on age related atrial changes relates to quite elderly populations often in the ninth decade, in our study the mean age was 62.4 ± 9.8 years.

It has been proposed that atrial fibrosis may lead to intra-atrial conduction disturbances and to the promotion of the re-entrant circuits required for the propagation of AF (34, 173). In a previous study by Goette et al the degree of atrial fibrosis combined with P-wave duration was associated with the development of post-operative AF (174). This study has some limitations as their series included those undergoing valve replacements and their definition of AF only required the arrhythmia to be sustained for greater than 5 minutes. While reaching statistical significance the r values were low and the authors postulated that this may be partially explained by the multifactorial pathogenesis of post-operative AF. Interestingly, in our study, the group that developed AF had fewer patients with atrial fibrosis when compared with those who that remained in sinus rhythm. In data on acute onset of non-surgical AF from an autopsy series of 100 consecutive patients, those with AF for less than two weeks demonstrated little or no fibrosis while those with chronic AF demonstrated extensive fibrosis (176). It would seem that while fibrosis is important in persistent AF it does not seem to play a role in acute onset post-operative AF.

Ad et al studied the right atrial appendage of 60 patients in sinus rhythm undergoing elective coronary artery bypass surgery (175). In their population 48 of the 60 had some degree of myolysis, 10 of these had severe myolysis of which 9

developed AF. On univariate analysis they found that lipofuscin pigmentation was associated with AF ($p = 0.02$) but they felt that this was associated with the degree of myolysis so it could not be considered an independent predictor. They found no multivariate predictors of AF. In our group myolysis was a little more frequent in the fibrillating group (75% compared with 64%), however it was not found to be statistically significant ($p = 0.26$). Myolysis particularly should be interpreted with caution as it may be an artefact (177) In contrast to the study by Ad et al we found that lipofuscin pigment was more prevalent in the group that remained in sinus rhythm. Even though lipofuscin pigment is a marker of the 'wear and tear' within the muscle cell and reflects free radical generation it is an age related phenomenon and must be interpreted cautiously particularly in the presence of myolysis.

Conclusion.

In conclusion while sustained atrial fibrillation can cause electrical and structural remodelling our data shows no association between pre-operative atrial histology and post-operative atrial fibrillation. One limitation of our study is that the right atrial appendage may not be representative of the atrium as a whole, however atrial biopsies from other sites are not clinically practical in the setting of routine coronary artery bypass grafting.

CHAPTER 6

The Association Between Peri-operative Troponin T Elevation and Clinical Outcomes

INTRODUCTION.

When cardiac cells become necrotic the sarcolemmal membrane is disrupted; intracellular molecules can then diffuse into the interstitium and eventually via the lymphatics into the microvasculature and peripheral blood. The speed at which these molecules appear depends on the intracellular location, molecular weight, local blood and lymphatic flow and the rate of elimination from the peripheral blood. Myocardial necrosis can occur following trauma, ischaemia, toxin exposure, inflammation or infection. The ideal cardiac marker must be highly specific with high concentrations in the myocardium and negligible or undetectable levels in non cardiac tissue. To ensure high sensitivity a marker must be rapidly released into the blood following myocardial injury and there should be a direct relationship between the serum level and the degree of myocardial damage. For clinical applicability the marker must persist in the blood to allow an appropriate diagnostic window.

Cardiac Troponins.

These markers overcome many of the limitations of the traditional "cardiac enzymes" CK, AST and LDH. Muscle contraction occurs when actin and myosin filaments slide over each other to shorten the sarcomere, which is the fundamental contractile unit. Neither the actin nor myosin filaments shorten rather it is their movement relative to each other that causes contraction of the sarcomere. The troponin complex is attached to the thin filament (actin) via the tropomyosin molecule. It comprises three regulatory proteins, troponin C, I and T with different functions. When intracellular calcium is low the configuration of the tropomyosin molecule

prevents the myosin chain (thick filament) from binding to the actin. Calcium causes troponin C activation and subsequent binding to the inhibitory protein troponin I. This weakens the interaction between troponin T and tropomyosin and allows actin myosin binding. The majority of intracellular troponin is found tightly bound but up to 6% of troponin T and 3% of I are found in the cytosolic pool.

Troponin T and I are found in skeletal and cardiac muscle but they are encoded by different genes and have different amino-acid sequences. This has allowed the development of high affinity antibodies specific to the cardiac troponins and a number of commercial assays are now available. Cardiac troponin T is present in small amounts in skeletal muscle during foetal development and can be expressed in disease states that involve skeletal muscle regeneration such as Duchenne muscular dystrophy (178). In health neither are detectable and an abnormal value is one that exceeds 99% of a reference control group, levels usually rise greater than 20 times the reference range. These features mean that these assays are able to detect even minor degrees of myocardial necrosis (179). Elevation usually begins within three hours of myocardial infarction and release can be sustained for up to 14 days. Skeletal and cardiac troponin C have the same amino-acid sequence and so no assays have been developed to measure this compound (180).

Clinical roles for troponin.

Diagnosis of myocardial infarction.

The original WHO diagnostic criteria for AMI were based on three criteria of which at least 2 were required to make the diagnosis. These were elevation of the

traditional cardiac enzymes, a history of ischaemic chest pain and significant ECG changes. These criteria had limitations as up to 33% of patients with AMI have no chest pain and up to 40% have an inconclusive ECG. This low sensitivity is matched by the low specificity of the cardiac enzymes (181). These limitations led to the development of new guidelines by the European Society of Cardiology and the American College of Cardiology (182). This document states that the best biochemical indicator of myocardial necrosis is "a concentration of cardiac troponin exceeding the decision limit on at least one occasion during the first 24 hours after the onset of clinical event". One of the concerns about this strategy is that the higher sensitivity of troponin which can detect infarcts of less than 1 gram of cardiac muscle will lead to an increase in the incidence of MI with important social and economic impact (183). In the past many patients with normal CK levels were diagnosed as unstable angina, in the current era many of these patients have myocardial necrosis demonstrated by elevation in their troponin and correctly are diagnosed as a non-ST elevation MI. Even small elevations in troponin levels have been shown to be associated with a worse prognosis.

Prognostic indicator in acute coronary syndromes.

A number of studies have analysed the association between troponin elevation and adverse outcome in acute coronary syndromes. Data from the GUSTO IIa study of 855 patients within 12 hours of onset of symptoms analysed troponin T, CKMB and ECG criteria. The primary endpoint was a composite of death, infarction, bypass surgery or angioplasty within 30 days. 289 of 801 subjects with available data had an

elevated troponin, and the investigators found a linear and direct relationship between the degree of troponin elevation and death within 30 days. The group that often poses the most diagnostic difficulty is those without ST elevation, in this group 30 day mortality was 7.6% if the troponin was elevated compared with 1.2% if it was normal. The admission troponin level remained a significant predictor of 30 day mortality even when adjusted for the various ECG categories and troponin T was shown to be a better prognostic indicator than CKMB (184).

Similar data were also presented from the TIMI IIIB study by Eugene Braunwalds group. This was a study of 1404 subjects with unstable angina or non Q wave MI in which 573 (41%) had troponin I levels of at least 0.4ng/ml and these were compared to the 831 subjects with levels < 0.4ng/ml. Within 42 days there were 21 deaths in the group with an elevated troponin compared with 8 in the normal troponin group. They found that after adjusting for multiple variables that troponin I remained an independent risk factor for 42 day mortality. It was also noted that even when the CKMB was normal troponin conveyed prognostic information (185).

Chest pain is a frequent presenting complaint in emergency departments worldwide, many patients have a normal or non diagnostic ECG and the history is often vague or atypical. Some authorities estimate that up to 7% of patients with myocardial infarction are inappropriately discharged each year. Coupled with this are the economic consequences of unnecessary hospital admissions. Christian Hamm's group from Hamburg studied the effect of using bedside troponin assays to streamline patients in the emergency department. They enrolled 773 patients with chest pain and without ST elevation and measured both troponin I and T. Troponin T was positive in

123 (16%) of patients and I was positive in 171 (22%). Using troponin no patients with a subsequent diagnosis of MI were inappropriately discharged and they found that the 30 day event rate was very low in patients with a negative troponin T (1.1%) and troponin I (0.3%). The authors concluded that while these tests are not substitutes for the ECG and clinical assessment the risk of an acute coronary syndrome is very low in patients with negative troponin tests (186).

In the setting of ST segment elevation the admission troponin level is associated with a higher incidence of failed thrombolysis and adverse clinical events (184). Primary angioplasty is the most effective therapy for AMI but even when this is used the admission troponin predicts short and long term outcomes. In data on 140 consecutive patients treated with primary angioplasty the 30 day all cause mortality was significantly higher in the troponin positive group, 15.6% compared with 3.9%. At 9 months the difference remained significant 18.8% mortality in the troponin positive group compared to 3.9% in the troponin negative group. Initial procedural success was also strongly associated with troponin elevation; TIMI flow rates and myocardial reperfusion were much lower in the troponin positive group (187).

Prognostic indicator following percutaneous intervention.

Elevation of cardiac enzymes occurs in between 5 and 30% of interventional procedures and has been shown to be associated with adverse clinical events in both the short and long term. In the absence of obvious complications such as abrupt vessel closure or side branch occlusion these elevations seem to be caused by distal embolisation of thrombus or plaque material (187). A variety of studies have

examined troponin I and T elevation and have demonstrated an overall incidence of approximately 44%. Marty Leon's group studied 1,129 consecutive patients with normal pre-procedural Tn-I levels and found that levels >0.45ng/ml or greater than 3 times the upper limit of normal were a strong independent predictor of major in hospital complications. Interestingly there was no association with clinical events during the 8 month follow up period although development of a new Q wave was associated with late clinical outcomes (188). The same group also studied the significance of troponin re-elevation following PCI in a group of 132 patients with acute coronary syndromes and pre-procedural elevation of troponin. Patients with a post procedural re-elevation had a higher in hospital mortality and were more likely to develop Q waves. At 6 months troponin re-elevation, increasing age and diabetes mellitus were found to be associated with adverse clinical outcomes (189). These findings and others support the ESC/ACC statement that post-procedural troponin elevation represents a significant clinical event.

Prognostic indicator following cardiac surgery.

Since the first reports of coronary artery bypass surgery in 1969 there have been varying data on CK-MB elevation and its significance. Overall in the early studies between 51 and 93% of patients sustained CK-MB elevations but data on clinical outcomes were rare. The first large study of CABG was published in 1981, 9777 patients were enrolled and 5.7% had a peri-operative MI as defined by CK-MB elevation and ECG criteria. Patients with a peri-operative MI had significantly lower 5 year survival rates than those without an MI; 40% compared with 73% (190). These

findings were confirmed in the Guardian study of 2,394 patients in which the degree of CK-MB elevation was correlated with 6-month survival. Increases in CK of between 5 and 10 times the upper limit of normal were associated with an increase in mortality of 75 %, increases of between 10 and 20 times the upper limit of normal were associated with an increase in mortality of 2.5 times (191). It can be seen from these data that elevation in the traditional cardiac enzymes is almost inevitable after cardiac surgery and that marked elevations are associated with adverse outcomes.

Similarly troponin elevation after cardiac surgery is common with up to 40% of cases demonstrating a marked elevation but the precise significance of this elevation and cut off values to diagnose myocardial infarction have yet to be defined. Michel Carrier studied 590 patients who had CABG in a single institution; they recorded troponin I, T, clinical data, ECG changes and CK-MB levels. Their diagnosis of post-operative MI was based on the presence of 2 out of 3 of the following criteria; new Q wave, CK-MB > 100iu/l (normal 0-30) and a positive pyrophosphate scan. Of the 493 patients with troponin T data available 22 (4.5%) were considered to have had a post-operative MI, of the 97 with troponin I data available 6 (6%) were considered to have had a post-operative MI. The authors found that both troponins were significantly higher in those considered to have had an MI and that more patients with an elevated troponin died, required inotropic support and had longer ICU and hospital stays (192). Interestingly patients with an elevated troponin without new Q waves or CK elevation were considered to be false positives by the authors. This may partially be explained by the nature of the assay used. This was a first generation troponin T assay which

exhibits cross reactivity with skeletal muscle, a problem solved in more modern assays by refinements in the antibodies.

A more recent study analysed troponin T and CK-MB in 224 patients undergoing a variety of cardiac procedures of which only 135 were isolated coronary artery bypass operations. They found that patients with a complicated ICU course (n = 21) were more likely to have higher levels of both troponin T and CK-MB. In hospital death was also associated with higher troponin levels although not with higher CK-MB levels. The authors concluded that a cut off point of 1.58ng/ml, which is 15 times the upper limit of normal, was a useful predictor of adverse outcomes. They also found that ECG criteria were insensitive for diagnosing peri-operative injury (193). This study has a number of limitations, baseline values were not available in all subjects so it is unclear whether the troponin elevation was as a consequence of surgery or whether it was related to pre-operative infarction, a factor which could negatively impact on surgical outcome. Their study population was heterogeneous with their patients having undergone a variety of cardiac procedures with a variety of methods of myocardial protection. Despite these limitations this study seems to demonstrate that only large elevations in troponin have a negative impact.

Some of the troponin studies are limited by their definition of what constitutes significant myocardial injury (194). One study has looked at the hard endpoint of angiographically proven graft occlusion, which has been shown to correlate with prognosis (195). Complete data were available on 103 patients of whom 15 had occluded grafts, 2 of these were internal mammary arteries and the rest were saphenous vein grafts. One patient had two occluded vein grafts. The authors found

that on univariate analysis a peak troponin T > 3μg/l and more than two proximal anastomosis were associated with graft occlusion. Using troponin alone had a sensitivity of 75% for detecting graft occlusion compared to 20% for clinical means alone. Ten patients developed new Q waves or LBBB, of these three had occluded grafts and the others had significantly higher troponin and CK-MB levels than those without Q waves. Of the group of patients (n = 64) with an uncomplicated course and patent grafts the median troponin T was 0.9μg/l (IQR 0.4-2.0). The conclusion of this study was that troponin T and CK-MB mass could be used to identify patients at high risk of graft occlusion (195).

Another study if troponin I elevation examined the importance of early elevation in this marker. In a group of 540 patients undergoing CABG assays were performed at anaesthetic induction and immediately after the termination of cardiopulmonary bypass. Adverse events were defined as death or post-operative Q waves on the ECG. These endpoints occurred in 21 (3.9%) of subjects whose TnI levels were significantly higher than the control group, 0.91ng/l compared with 0.37ng/l. the authors concluded that this enabled surgeons to accurately identify patients at risk of complications and potentially perform specific therapeutic interventions (196).

Significance of troponin elevation.

It can be seen from these data that troponin is a sensitive and specific indicator for myocardial necrosis. In the setting of acute coronary syndromes minor degrees of elevation confers prognostic information. Following percutaneous intervention even if

there is a good angiographic result troponin elevation is associated with poor outcomes. The situation following cardiac surgery is more complex as there are many potential reasons for cardiac enzyme or troponin elevation. During surgery there is unavoidable manipulation of the heart and trauma from atrial cannulation or sutures. This probably means that some degree of cardiac damage is inevitable however both elevation in CK-MB and troponin has been shown to be associated with adverse events. The difficulty lies in deciding what degree of elevation relates to post-operative infarction and what can be attributed to minor degrees of surgical trauma. A rapid rise and fall in troponin level may indicate a wash out of the cytosolic component, without significant myocardial damage. This may represent reversible dysfunction caused by ischaemia or surgical trauma. A more sustained elevation seems to be associated with myocardial necrosis and the degree of elevation is related to the extent of necrosis as troponin continues to be released from the myofibrils of necrotic cells (197). In conclusion almost all patients have some degree of elevation of cardiac markers following surgery but the clinical relevance is unclear (198).

Objectives.

The objective of this study was to evaluate the association between post-operative troponin T elevation, peri-operative variables and clinical outcomes in a low risk group undergoing cardiac surgery.

METHODS.

The study was approved by the Hospital Ethics Committee in July 2001. The recruitment period commenced in December 2001 and terminated in November 2002. One hundred non-diabetic patients undergoing first time non-emergency on pump coronary artery bypass grafting were recruited. The exclusion criteria were poor LV function (EF < 40%), renal failure (creatinine > 200), malignancy, chronic inflammatory conditions and concurrent infection Subjects were recruited the night before surgery, baseline demographic data were recorded and the patients were followed throughout their hospital stay and reviewed six weeks post surgery.

The peri-operative data recorded were number and type of grafts, cardiopulmonary bypass time (CPB), aortic cross clamp time (AOC) and lowest core temperature (bladder). The post-operative data included death, ECG changes of myocardial infarction, new onset of atrial fibrillation, intra-aortic balloon pump use, inotrope requirements, time to extubation, ICU stay and length of hospital stay. An ECG was taken on admission and on day five following surgery. These were analysed by an experienced cardiologist who was blinded to both clinical outcomes and to troponin results. ECG criteria analysed were new pathological Q-waves, defined as > 40ms wide in at least two contiguous leads and with a magnitude of at least one third of the R wave.

Blood was drawn just prior to anaesthetic induction and twelve and twenty four hours following the onset of cardiopulmonary bypass. Troponin T was assayed immediately using a Cardiac T Diagnostic Reader (Roche Diagnostics, Lewes East Sussex England) a third generation troponin assay. Post-operative values were

measured at 12 and 24 hours and adjusted for the baseline level as a change score. Clinicians were blinded to results. Previous large studies of acute coronary syndromes have considered troponin T levels below 0.03 ng/ml as negative, between 0.03 ng/ml and 0.1 ng/ml to denote a low level of questionable significance, between 0.1ng/ml and 2ng/ml to indicate detectable myocardial damage and greater than 2ng/ml to reflect extensive myocardial damage (184, 199). Following cardiac surgery clinically significant myocardial damage has been considered at levels in excess of 1ng/ml (192).

In 76% of patients cardioplegia was cold and delivered via the anterograde route, in 21% it was tepid anterograde and in 3% it was anterograde and retrograde. All patients were allowed to drift down to 32° centigrade with active rewarming commencing half way through the IMA anastomosis. Shed mediastinal blood was routinely re-transfused at the end of surgery.

Statistical analysis.

Descriptive statistics are presented for the baseline characteristics of patients in the study. Means and standard deviations are presented for normal data, medians and inter quartile ranges (IQR) for non-normal data, and proportions for categorical data. The change in troponin levels at 12 and 24 hours from baseline were calculated. The association between clinical and post-operative variables and the change in troponin T levels were examined using a non-parametric Wilcoxon Rank Sum test (categorical clinical variables), or a Spearmans non-parametric correlation (continuous variables). Results are expressed as means ± standard deviation unless otherwise stated. A $p<0.05$

was considered statistically significant, with two-sided tests used. All analyses were performed using the JMP software (SAS Institute Inc.).

RESULTS.

Baseline demographics are presented in Table 6.1 with the majority of subjects male and presenting with stable Canadian Cardiovascular Association (CCS) class II or III angina. All patients had good left ventricular function. The mean number of grafts was 3 (range 1-5), 98% had an internal mammary artery (IMA) and only 3 patients had a single graft placed. Twenty four percent had more than one arterial grafts placed and two percent of the total cohort had three.

The mean cardiopulmonary bypass time was 94 ± 27 minutes with a mean aortic cross clamp time of 54 ± 15 minutes. Lowest core temperature was measured from the bladder and was 33 ± 1.16 degrees centigrade. Median time to extubation was 6 hours (Interquartile range 5-7) with a range of 3-495 hours. Median stay in intensive care (ICU) was 22 hours (IQR 20-24) with a range of 7-600 hours and hospital stay was 6 days (IQR 5.7-7) with a range of 4-39 days.

There were no in hospital deaths, 8 patients developed new pathological Q waves, 14 required inotropic support with adrenaline or noradrenaline and there was no requirement for intra-aortic balloon pumping. Forty patients developed new onset atrial fibrillation. Two patients died in the first month following discharge.

All patients had a negative troponin T prior to surgery except for one patient who was operated on following a recent myocardial infarction and had an elevated

troponin T of 2.1ng/ml (upper detection limit of assay) before the surgery. Troponin T elevation was seen in 95 of the 100 patients. The 4 patients without a troponin rise all had an uncomplicated post-operative course with an early hospital discharge of on average five days. Troponin values were not normally distributed and medians are presented (figure 6.1). Median 12 hour troponin T was 0.33 ng/ml and 24 hour troponin T was 0.19 ng/ml. Median peak troponin in the post-operative phase was 0.355 ng/ml with 82% of the peak values at 12 hours.

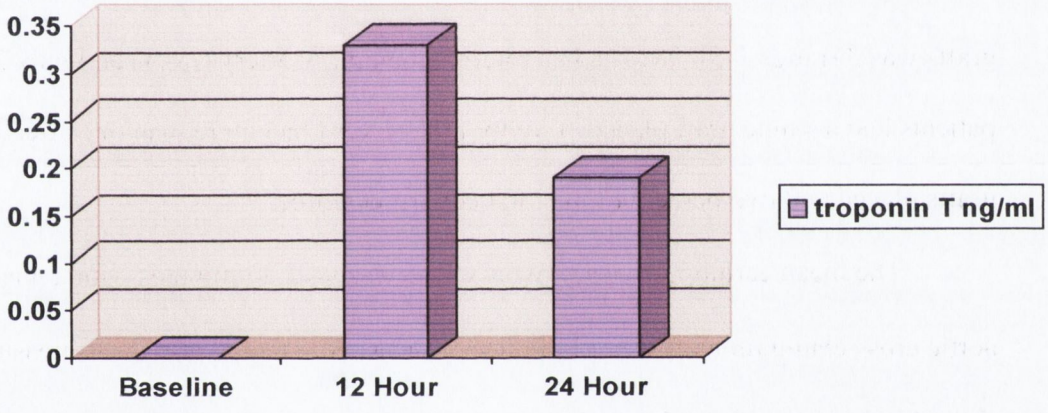


Figure 6.1: Median troponin values (change from baseline) in ng/ml for the whole cohort.

There were no post-mortems performed on the two patients who died in the first month following discharge. One occurred following an admission to another centre with acute pulmonary oedema and the other was a sudden death, presumed cardiac. Both patients had a relatively minor troponin T rise (0.28 ng/ml and 0.1 ng/ml at 12 and 24 hours respectively in the first patients, and 0.11 ng/ml and 0.19 ng/ml at 12 and 24 hours in the other). Neither developed surgery associated Q-waves or required inotropic support.

Table 6.1: Baseline Demographic Data on 100 patients.

Age (years ± standard deviation)	62 ± 9.8
Male	83%
Previous MI	30%
CCS I / II / III / IV	4% / 41% / 45% / 10%
Euroscore	3.7 ± 2.9
Risk factors:	
Hypertension	50%
Hyperlipidaemia	77%
Current / Ex Smoker	10% / 62%
Medications:	
Aspirin	91%
Beta-Blockers	84%
Statins	82%
ACE I	29%
Calcium Channel Blockers	33%
Nitrates	65%
Diuretics	11%

Where applicable data are shown as mean ± standard deviation

CCS = Canadian Cardiovascular Society; ACE I = Angiotensin Converting Enzyme Inhibitor

There was no difference in troponin levels between the 8 patients who developed new pathological Q-waves and the 92 that did not, 0.25 ng/ml and 0.33 ng/ml respectively at 12 hours ($p = 0.5$), and 0.14 ng/ml compared with 0.19 ng/ml at 24 hours ($p = 0.34$) (Table 6.2). None of the 8 required inotropic support and only one developed atrial fibrillation; their median hospital stay was 5 days (IQR 5-6.25). Five of the hundred patients had troponin T levels above a previously defined post-operative cut off value for clinically significant myocardial damage of 1ng/ml. Of these one developed a Q wave and only one other patient required inotropic support.

Fourteen patients required inotropic support with either adrenaline or noradrenaline. These patients had a median troponin T rise at 12 hours of 0.31 ng/ml compared with 0.33 ng/ml in those that did not require inotropes ($p = 0.73$), at 24 hours the median troponin T was equal in both groups.

The endpoints of death, new Q waves and inotrope use were analysed as a combined endpoint (figure 6.2). This occurred in twenty-four patients. Twelve hour troponin T in this group was 0.28 ng/ml compared to 0.33 ng/ml in the uncomplicated group ($p = 0.57$), and the 24 hour troponin T was similar 0.19 ng/ml compared with 0.2 ng/ml ($p = 0.56$).

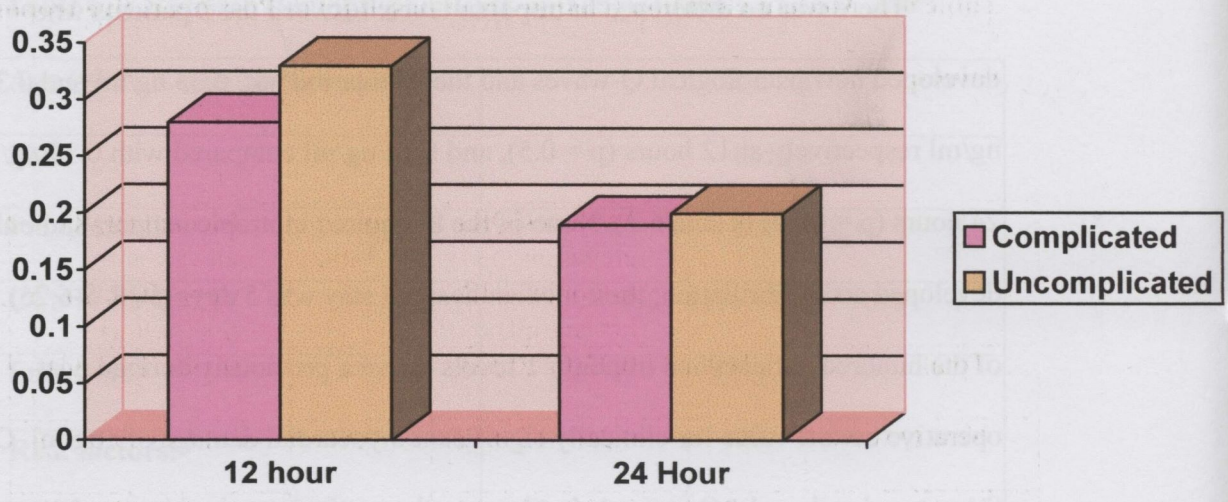


Figure 6.2: Median troponin values (change from baseline) in ng/ml for patients with post-operative complications compared to those without.

In the forty patients that developed atrial fibrillation 12 and 24 hour troponin levels were similar to the levels in the sixty patients that did not develop atrial fibrillation (0.3 ng/ml and 0.34 ng/ml respectively at 12 hours and 0.19 ng/ml in both groups at 24 hours).

When analysing the other clinical endpoints of time to extubation, time to ICU discharge and length of hospital stay we used a Spearmans non-parametric correlation. There was no significant correlation between either the 12 or 24 hour troponin values and these endpoints (Table 6.3).

Table 6.2: Median elevation (change from baseline) in Post-operative troponin values.

	N	Median (IQR) elevation in troponin at 12 hrs (ng/ml)	p-value	Median (IQR) elevation in troponin at 24 hrs (ng/ml)	p-value
Total	100	0.33(0.2,0.53)		0.19(0.13,0.34)	
New Q wave	8	0.25(0.15,0.52)	0.51	0.14 (0.09,0.38)	0.34
No Q wave	92	0.33(0.21,0.53)		0.19 (0.14,0.34)	
Inotropes	14	0.31(0.24,0.55)	0.74	0.19 (0.13,0.34)	0.71
No inotropes	86	0.33(0.19,0.51)		0.19(0.15,0.39)	
MACE	24	0.28(0.20,0.54)	0.57	0.19(0.11,0.37)	0.57
No MACE	76	0.33 (0.2,0.52)		0.2 (0.14,0.34)	
A Fib	40	0.30(0.16,0.50)	0.35	0.19(0.15,0.38)	0.61
No A Fib	60	0.34(0.23,0.54)		0.19(0.12,0.33)	

Troponin data were non-normal and is expressed as the median with interquartile ranges. MACE = combined endpoint of death, new pathological Q waves and Inotrope use; A. Fib = Atrial Fibrillation

Table 6.3: Spearmans correlation (p-value) between Clinical variables and Troponin elevation (change from baseline) at 12 and 24hrs.

Clinical variable	Troponin at 12 hrs	Troponin at 24 hrs
CPB time*	0.37 (0.0001)	0.24 (0.014)
Cross clamp*	0.35 (0.0003)	0.27 (0.007)
Core Temp	-0.41 (<0.0001)	-0.40 (<0.0001)
Intubation†	0.14 (0.17)	0.11 (0.30)
ICU discharge†	0.06 (0.56)	0.18 (0.07)
Hospital stay‡	0.05 (0.65)	0.05 (0.61)

CPB time = Cardiopulmonary Bypass time; Cross clamp = Aortic cross clamp time; ICU = intensive Care Unit; *in minutes, †in hours, ‡ in days.

There was a relationship between both cardiopulmonary bypass time and aortic cross clamp time at both time points but the R values were relatively low (Table 6.3). There was an inverse correlation with core temperature again at both time points (p = 0.0001) although patients were only moderately hypothermic (Fig 6.3-6.5).

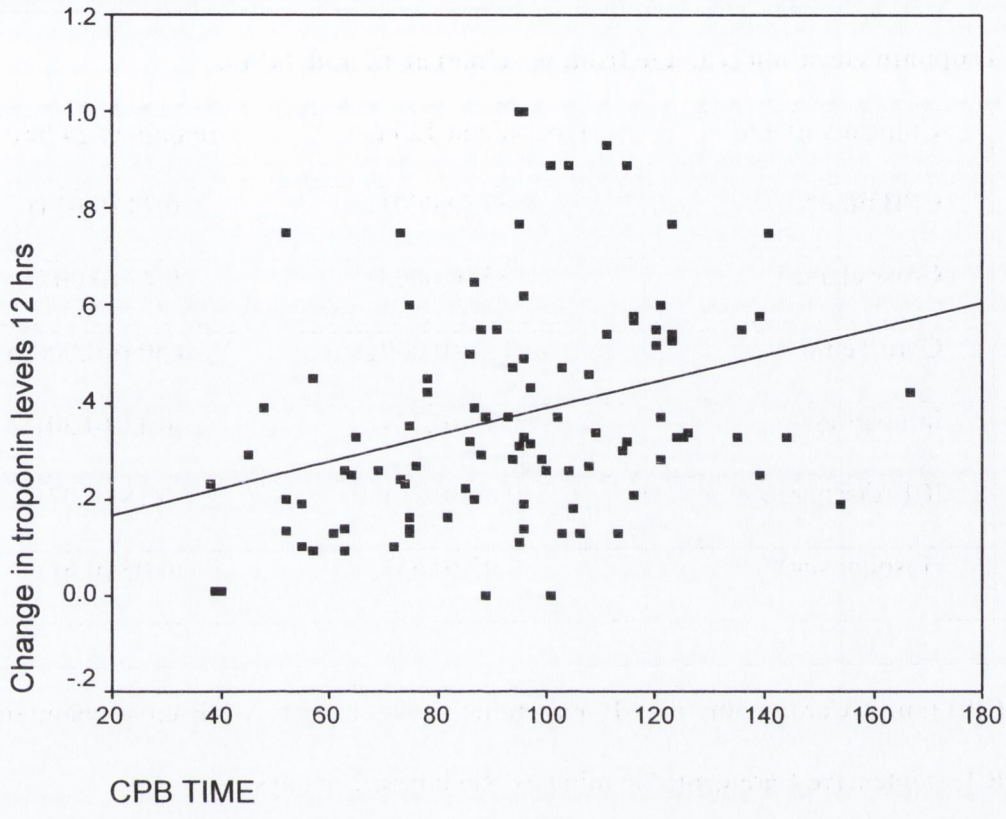


Fig. 6.3: Correlation between CPB time (mins) and 12 hour troponin T (change from baseline) (ng/ml), Spearmans correlation coefficient 0.37 (p = 0.0001).

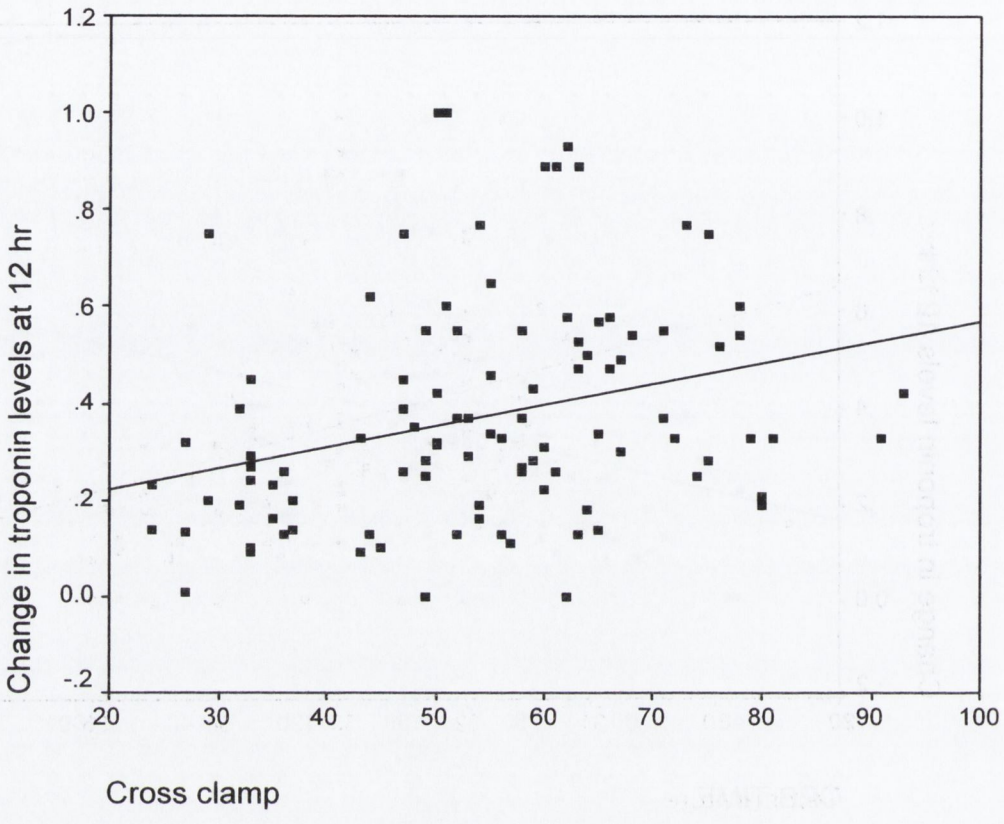


Fig. 6.4: Correlation between aortic cross clamp time (mins) and 12 hour troponin T (change from baseline) (ng/ml), Spearmans correlation coefficient 0.35 (p = 0.0003).

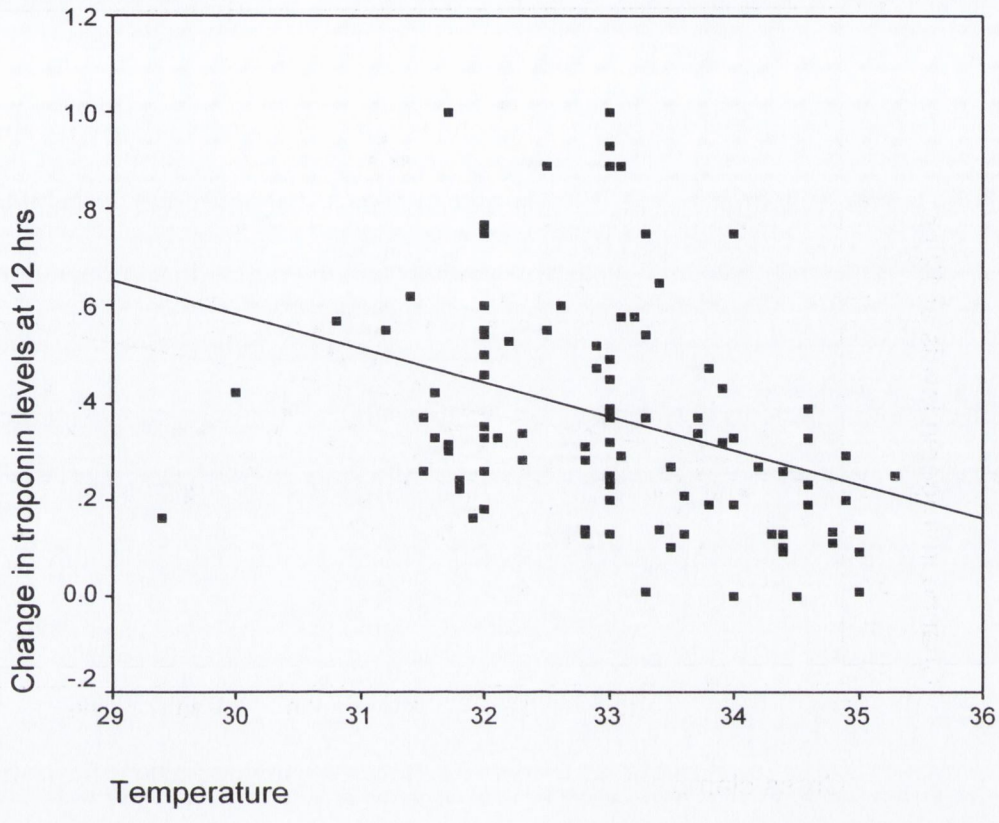


Fig. 6.5: Correlation between core temperature (degrees centigrade) and 12 hour troponin T (change from baseline) (ng/ml), Spearmans correlation coefficient minus 0.41 (p = 0.0001).

DISCUSSION.

In this study of 100 low risk patients undergoing first time on pump CABG 95% had a troponin T elevation. Post-operative troponin T elevation was associated with both cardiopulmonary bypass time and aortic cross clamp time and was inversely associated with the core temperature. Troponin levels were not associated with the clinical variables of time to extubation, length of ICU stay or length of hospital stay

and there was no association with death, new onset atrial fibrillation, new Q-wave formation or inotrope use.

The association of troponin T elevation with cardiopulmonary bypass time and aortic cross clamp time may reflect insufficient myocardial protection during the surgery or may be related to greater myocardial handling during longer operations. The association of troponin elevation with ischemic time without being related to adverse clinical events may represent reversible cell dysfunction without significant necrosis; this may be mediated by "wash out" of the cytosolic component.

The importance of CABG associated troponin elevation is relevant to current research interests, as the degree of troponin elevation has been cited as a marker of the extent of myocardial injury when comparing on-pump and off-pump surgical techniques (200). Furthermore, a randomised study with angiographic follow-up has shown lower graft patency with off-pump surgery despite a greater troponin elevation in on-pump CABG (201).

The detection of myocardial injury following cardiac surgery remains important as peri-operative infarction adversely affects prognosis. In the CASS study of 1340 patients the incidence of peri-operative Q-wave infarction was 4.8%. The in hospital mortality was 9.7% in those with Q-wave infarcts compared with 1% in those without (202). A smaller long-term study assessed 174 patients undergoing CABG with a 30 month follow-up. Patients with peri-operative myocardial infarction had a cardiac event rate of 31% compared with 12% in those without (203).

In the setting of CABG current techniques used to detect myocardial necrosis have limitations. New Q-waves are thought to indicate myocardial damage, however,

in a study of 302 patients there was no difference between those with and without Q-waves with regard to the degree of troponin elevation on day four (204). One potential reason for the lack of specificity of new Q-waves may be due to pre-existing cardiac damage that was not manifest on the initial ECG, but may become evident following the surgery due to improved perfusion and function of a contra-lateral segment. Alternatively, the Q-waves may be transitory and due to temporary alterations in ventricular depolarisation (197). Furthermore, Q-waves may not occur in the setting of myocardial necrosis if the infarct is small or if there is diffuse subendocardial injury. The lack of association between troponin T elevation and new Q-wave formation that we observed may relate to the relative lack of sensitivity and specificity of ECG criteria rather than any deficiency in troponin T as a marker of myonecrosis. The number of patients who developed Q-waves in our study however is small and it is not possible for us to reach meaningful conclusions in this regard.

Echocardiography while easily available has a high degree of inter and intra observer variability and because of associated chest discomfort following the surgery may be limited by sub-optimal examinations. Nuclear techniques are sensitive however they remain expensive and are not available in all centres and when available they may not be practical in the intensive care setting (205).

These limitations mean that a simple serum marker that could assess myocardial damage would be beneficial in the early risk stratification of patients. CK-MB is found in skeletal muscle and even though this is often considered the "gold standard" it has limited sensitivity and specificity (206). Troponin T is both sensitive and specific and in general cardiology practice and often confers prognostic

information. Our data suggest that in low risk patients post-operative troponin elevation does not adversely affect prognosis and as such cannot be used to risk stratify these patients.

The main limitation of this study is the relatively small sample size and low number of adverse events. The exclusion criteria also limit the general applicability of our results.

Conclusion.

Troponin T elevation occurred in the majority of patients undergoing on pump coronary artery bypass surgery. This elevation was related to the length of cardiopulmonary bypass time and aortic cross clamp time and inversely with core temperature. In this low risk group of patients the degree of elevation was not associated with post-operative outcomes and in particular was not associated with the development of new Q-waves or atrial fibrillation.

CHAPTER 7

CONCLUSIONS

CONCLUSIONS.

The research studies included in this thesis sought to further our knowledge of the aetiology of post-operative atrial fibrillation. There were four distinct experimental limbs to this work.

Objective 1.

This study was designed to determine whether the inflammatory response induced by cardiac surgery as assessed by serum inflammatory markers was associated with the development of post-operative atrial fibrillation.

Results.

Coronary artery bypass surgery was associated with an inflammatory response in all of the patients studied. 55 of 149 patients developed atrial fibrillation (37%) and there was no significant difference in the levels of the serum inflammatory markers measured between the 55 patients with atrial fibrillation and the 94 without: sE-Selectin (ng/ml): 56.3(41.9-71.9) compared with 58.7(41-76.6), sP-Selectin (ng/ml): 116(93.9-153.6) compared with 130.3(93.2-179), sICAM-1 (ng/ml): 486(399-597) compared with 510(394-616), sVCAM-1 (ng/ml): 706(538-869) compared with 653(517-834) and CRP(mg/dl): 244(171-290) compared with 260(186-307).

Conclusions.

We did not find a difference in the inflammatory response, assessed by serum inflammatory markers, in patients who developed post-operative atrial fibrillation and those who did not.

Objective 2.

The objective of this study was to determine whether there was a relationship between atrial stretch and the development of post-operative atrial fibrillation. The degree of BNP elevation 24 and 48 hours following the onset of cardiopulmonary bypass was used as a surrogate marker for left ventricular end diastolic pressure and hence atrial pressure.

Results.

Atrial fibrillation occurred in 65 patients. Median 48hour brain natriuretic peptide levels were greater in the atrial fibrillation group than the controls (440pg/ml (AF) and 319pg/ml respectively $p = 0.001$). As atrial fibrillation itself is associated with an increase in brain natriuretic peptide we divided the subjects into early atrial fibrillation (<48hrs) and late (>48hrs). In those who developed atrial fibrillation within 48 hours of surgery there was no difference in the 24 hour brain natriuretic peptide levels in the subjects and controls (381pg/ml and 365pg/ml respectively $p = 0.73$). In those that developed atrial fibrillation after 48hours the median 48 hour brain natriuretic peptide level was greater than in the control group (405pg/ml and 319pg/ml respectively $p = 0.02$).

Conclusions.

Patients with normal pre-operative systolic function demonstrate a significant rise in brain natriuretic peptide levels following coronary artery bypass surgery. This increase was more evident in those who develop atrial fibrillation more than 48 hours after the surgery.

Objective 3.

The objective of our study was to determine whether there is a relationship between the pre-operative atrial morphology and the development of post-operative atrial fibrillation.

Results.

36 (38%) of the patients developed atrial fibrillation. No correlation was found between the 10 features assessed including myolysis and lipofuscin pigmentation and the development of atrial fibrillation.

Conclusion.

Simple morphology changes of right atrial appendages do not predict the development of post-operative atrial fibrillation.

Objective 4.

The objective of this study was to evaluate the association between post-operative troponin T elevation, peri-operative variables and clinical outcomes in a low risk group undergoing cardiac surgery.

Results.

Post-operative Troponin elevation occurred in 95% of the cohort. Troponin T elevation was related to the duration of cardiopulmonary bypass ($p < 0.01$) and aortic cross clamp time ($p < 0.01$). There was also an inverse relationship with peri-operative core temperature ($p < 0.01$). There were no association between post-operative troponin elevation and the clinical outcomes.

Conclusion.

Post-operative troponin T elevation occurs in the majority of patients undergoing elective on pump coronary artery bypass surgery. In this low risk cohort troponin T elevation was associated with procedural duration but not with clinical outcomes and in particular was not associated with atrial fibrillation.

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