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# Changes in brain natriuretic peptide concentrations following open cardiac surgery with cardioplegic cardiac arrest

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**Abstract**

Elevated brain natriuretic peptide (BNP) concentration in peripheral blood reflects impaired cardiac ventricular function. We investigated the release pattern of BNP following cardioplegic cardiac arrest during heart surgery. In particular, we sought to discover whether there is an increase in peripheral BNP concentrations following reperfusion of the ischaemic heart. A secondary aim of the study was to investigate whether allopurinol, an anti-oxidant, has any effect on BNP release. A total of 29 patients scheduled for elective coronary artery bypass grafting were recruited, of whom 12 were randomly allocated to receive allopurinol with their pre-medication. Blood specimens were taken at six time points from the indwelling arterial catheter, the first before surgery and the last 2 h following the termination of cardiopulmonary bypass (CPB). BNP was found to decrease markedly when the aortic cross clamp was applied and the heart was isolated from circulation ($P=0.0001$). There was a slight increase in BNP following cross clamp release and myocardial reperfusion ($P=0.04$). A more substantial increase occurred with weaning from CPB when ventricular filling occurred ($P=0.0015$). Only the final BNP value, 2 h after CPB, was elevated compared with baseline ($P=0.0013$). Allopurinol had no demonstrable effect on changes in BNP. 

*Keywords:* Brain natriuretic peptide concentrations; Open cardiac surgery; Cardioplegic cardiac arrest

## 1. Introduction

One of the major challenges during cardiac surgery is to protect the heart from ischaemic and reperfusion injury. Brain natriuretic peptide (BNP) is thought to be a counter-regulatory hormone, which protects the heart from injury [1,2]. This protection may result from natriuresis, diuresis and vasorelaxation [2]. BNP is released predominantly by the cardiac ventricles and blood concentrations are inversely correlated with the ventricular ejection fraction [2,3]. Plasma BNP is also raised in patients who have impaired diastolic function [4,5], as occurs in aortic stenosis and ventricular hypertrophy. In a recent study, an acute rise in BNP in coronary sinus blood was observed during reperfusion following cardioplegic cardiopulmonary bypass (CPB) [6].

Cardiac troponin T (cTnT) is a structural peptide in cardiac myocytes. An increase in plasma cTnT suggests that myocardial damage has occured [7]. Troponin T is a more reliable marker of myocardial injury than creatine kinase MB [8], and is a sensitive and specific indicator of myocardial injury during coronary artery bypass surgery [7,9].

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Allopurinol has been shown to attenuate reperfusion injury following myocardial infarction and has been advocated as a cardioprotective agent for use in cardiac surgery [10]. Several studies have documented improved cardiac function following cardioplegic cardiopulmonary bypass when allopurinol has been given as part of the premedication [10–13]. The protective effects of allopurinol are thought to relate to xanthine oxidase inhibition [14,15], preservation of purines as energy substrates, and direct oxygen free radical scavenging properties [11,16–20]. Allopurinol is associated with decreased natriuretic peptide release following reperfusion in a rat model [21].

This study aimed to document the variations in plasma BNP concentrations during cardiac surgery involving cardioplegic CPB, particularly following aortic clamp removal and reperfusion of the ischaemic heart. We sought to discover whether there is an increase in plasma BNP coinciding with the decrease in blood pressure, which may occur following removal of the aortic clamp. An acute BNP release, possibly through vasorelaxation, may cause blood pressure to fall [2]. A secondary intention was to investigate whether preoperative allopurinol affects changes in BNP concentrations and blood pressure.

## 2. Materials and methods

### *2.1. Patients*

A total of 29 patients undergoing elective coronary artery bypass grafting gave informed consent for the study. Data from three patients was used in a pilot study and one patient was excluded from the comparative study as she had significant aortic stenosis, which resulted in markedly elevated baseline BNP concentrations [22]. Of the remaining 25 patients, none had signs of heart failure, and only one had a left ventricular ejection fraction less than 30%. Twelve patients were randomised to receive 600 mg allopurinol orally as part of their standard pre-medication comprising oral temazepam and ranitidine, and intramuscular papaveratum. Patients and investigators were blinded to the type of pre-medication used. Perioperative myocardial infarction was judged by new ECG changes or a post-operative rise in cTnT exceeding 1 μg/l [23–25]. The King's Healthcare Research Ethics Committee approved the study.

All patients had bilateral mammary artery grafts and radial artery grafts if there were more than two grafts. The majority had three bypass grafts. Three patients had two grafts and four had four. Anaesthesia was induced with propofol and fentanyl. A single dose of vecuronium was administered to facilitate tracheal intubation. Anaesthesia was maintained with nitrous oxide and isoflurane in oxygen before CPB and with a propofol infusion on CPB. Epinephrine was used if inotropic support was required, glyceral trinitrate was used as a venodilator, and phenylephrine was used to maintain mean arterial pressure on CPB. Anticoagulation was achieved with heparin 4 mg/kg and further increments were added to maintain an activated clotting time over 480 s. Reversal of anticoagulation was achieved with protamine. No patient received aprotinin, a protease inhibitor which may affect the physiological breakdown of BNP [26].

Cardiopulmonary bypass was achieved with a roller pump and a membrane oxygenator. Multiple doses of St Thomas' cardioplegia solution in warm (32°C) oxygenated blood were administered for myocardial preservation when the aortic clamp was applied. Patients were cooled to a core temperature of 32°C. Mean arterial pressure was maintained above 60 mmHg for the duration of CPB. Bypass times and duration of aortic cross clamping were noted. Mean arterial pressure (MAP), bypass pump flow rate, and the use of phenylephrine to maintain MAP was recorded before and after removal of the aortic clamp.

### *2.2. Blood sampling and laboratory analysis*

In three patients, blood was sampled from the coronary sinus, pulmonary artery, arterial and venous ports of the cardiopulmonary bypass circuit, and from the indwelling radial arterial cannula. Samples were taken only from the indwelling radial artery cannula in the 26 patients recruited to the comparative study after it was established from the first three patients that there was no difference in BNP concentrations in the pulmonary artery, bypass circuit (arterial and venous ports), and the radial artery.

Blood samples for BNP were taken before surgical incision, before removal of aortic cross clamp, 3 and 10 min following cross clamp removal, and 5 min and 2 h after termination of CPB. Samples for cTnT were taken before surgical incision and 2 h after CPB.

Blood samples for BNP were taken into EDTA tubes to which 90 μl of aprotinin had been added to inhibit breakdown of BNP. Samples for cTnT were taken into plain tubes with no additives. All samples were stored between 2 and 8°C and within 6 h were centrifuged at 1700 *g* for 20 min. Platelet poor plasma and serum were obtained and stored at −70°C until analysis. Plasma BNP concentrations were determined using a solid phase sandwich immunoradiometric assay (Shionoria, Cis UK Ltd., Bucks, UK), which has a co-efficient of variability of less than 10% in the range 3–100 ng/l. There is no cross reactivity with other peptides, specifically atrial natriuretic peptide. The detection limit of the assay is 3 ng/l.

Troponin is a structural protein which exists in different isoforms in different muscles. Cardiac TnT (cTnT) concentrations were measured using an automated enzyme immunoassay (ES300, Roche, Lewes, UK). This is both sensitive and specific for cTnT and there is less than 0.1% cross reactivity with skeletal muscle troponin. A preoperative cTnT in excess of 0.04 μg/l and post-operative concentration over 1 μg/l are suggestive of significant myocardial damage. The detection limit of the assay is 0.02 μg/l.

### 2.3. Statistics

Statistical analysis was carried out using the Mann Whitney U, Wilcoxon signed ranks, and paired *t*-tests for comparisons. Friedman ANOVA was used for repeated measures. Correlation was assessed with the Spearman rank-signed test. All the data collected were analysed using Analyse-It for Microsoft Excel (Leeds, UK).

## 3. Results

One patient had a pre-operative cTnT concentration suggestive of significant myocardial damage. The allopurinol and non-allopurinol groups were well matched in terms of pre-operative age, ejection fraction, aortic cross clamp and bypass times (Table 1). There were no significant differences in MAP and pump flow rates before and after aortic cross clamp removal in both groups, with only two patients requiring small increments of phenylephrine. Three patients required inotropic support with epinephrine to facilitate weaning from CPB. No patient had post-operative cTnT values exceeding those associated with uncomplicated cardiac surgery, apart from the patient in whom cTnT was elevated before surgery.

### 3.1. BNP

Baseline BNP values ranged between 4 and 202 ng/l. In the first three patients, there were no differences in BNP concentrations in the pulmonary artery blood, the arterial and venous reservoirs of the bypass circuit, or radial artery blood. All further samples were, therefore taken from the radial artery. BNP decreased when the aortic clamp was applied

Table 1
Median values (95% confidence intervals) in the allopurinol and non-allopurinol groups[a]

| Group | Allopurinol | Non-allopurinol |
|---|---|---|
| Age (years) | 66 (57–70) | 60 (58–71) |
| Cross clamp duration (min) | 56 (37–66) | 48 (38–62) |
| Bypass time (min) | 94 (71–110) | 82 (68–115) |
| Baseline cTnT (μg/l) | 0.02 (0.02–0.03) | 0.02 (0.02–0.02) |
| cTnT 2 h post CPB (μg/l) | 0.21 (0.12–0.42) | 0.26 (0.17–0.56) |
| Baseline BNP (ng/l) | 37 (20–43) | 26 (13–35) |
| Change in BNP from baseline value 2 h post CPB (ng/l) | 4 (1–19) | 1 (−2–12) |

[a] None of the differences between the two groups is significant.

and the heart was isolated from circulation ($P=0.0001$). There was a slight, but significant increase in BNP concentration (median=1.5 ng/l (95% CI=0–3 ng/l), $P=0.04$) from before the aortic clamp removal to 3 min following clamp removal. BNP increased further following termination of CPB ($P=0.0015$), although even at 5 min following CPB, BNP concentration was still lower than baseline ($P=0.0002$). Only at 2 h post CPB was BNP elevated compared with baseline ($P=0.0013$) (Fig. 1). At no time point was BNP concentration significantly different between the allopurinol and non-allopurinol groups. There was no correlation between change in BNP and aortic cross clamp times. There was a very strong correlation between baseline BNP and BNP values at all time points. The correlation between baseline concentrations and values two h post CPB was 0.92 (95% CI 0.83–0.97).

### *3.2. cTnT*

There was no significant difference in post-operative cTnT values between the allopurinol and non-allopurinol groups. There was no correlation between post-operative cTnT concentrations and aortic cross-clamp times ($r=0.07$; 95% CI=−0.34–0.45). There was a significant increase in cTnT 2 h post CPB (median=0.23 μg/l, 95% CI=0.17–0.42) compared with baseline CPB (median=0.02 μg/l, 95% CI=0.02–0.02) ($P<0.0001$). There was no correlation between BNP and cTnT in the magnitude of change from baseline to post-operative values ($r=0.07$; 95% CI=−0.34–0.45).

## 4. Discussion

### *4.1. BNP*

BNP has a short half life of about three min as it is rapidly removed from circulation by binding to natriuretic peptide clearance receptors (c-type receptors), which are located on endothelial cells, and it is inactivated through cleavage by neutral endopeptidases (NEP) present within renal tubular and vascular cells [2]. There was no difference in BNP concentrations measured in the pulmonary artery and the radial artery, suggesting that there is minimal decline in a single heart to arm circulation. The venous and arterial limbs of the CPB circuit had the same BNP concentrations. The lack of BNP breakdown in the CPB reservoir may relate to several factors, including the absence of endothelial cells and the low temperature, which may decrease NEP activity.

BNP concentrations decreased precipitously when the aortic clamp was applied and the heart, the major source of BNP, was excluded from the circulation. The significant differences amongst BNP concentrations at the various time points confirm that BNP is both rapidly released into circulation and also rapidly cleared from the blood. Ischaemia, reperfusion, diastolic dysfunction and ventricular distension

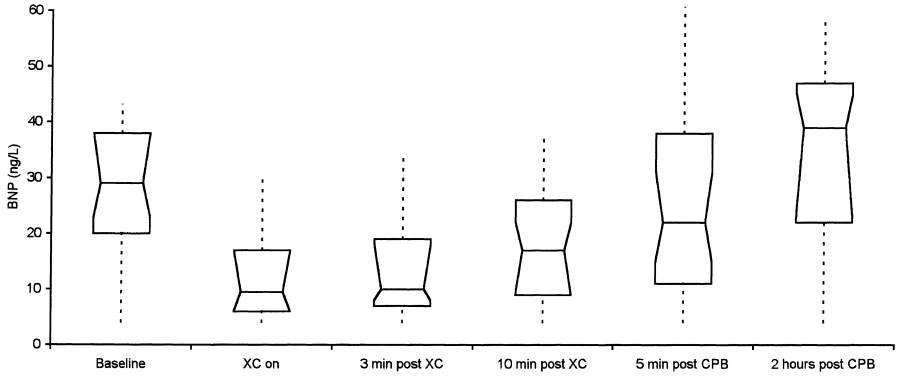


Fig. 1. Box and whisker plots of BNP values at various time points. XC=aortic clamp. Min=minutes. CPB=cardiopulmonary bypass. The plots show median values, inter quartile ranges, and 1.5 times inter quartile ranges. Friedman ANOVA: $P=0.0001$.

have all been postulated as stimuli to BNP release. The results of this study suggest that there is BNP release with reperfusion of the heart, but that a more significant stimulus is ventricular filling and ejection. Unlike a previous study, no spike in BNP was seen following reperfusion of the heart [6]. This may be because samples were not taken from the coronary sinus in our study. Coronary sinus blood was not used, as there is stasis of blood during CPB in the coronary circulation, which may artificially elevate the local BNP concentrations. This might explain the surge in the coronary sinus BNP concentrations when the clamp is removed. A similar surge in peripheral BNP was not seen in the study by Mair et al. or in our study [6]. Systemic BNP should reflect that produced by the heart as almost all peripheral BNP originates from the cardiac ventricles [27]. The reason for the increased BNP value at 2 h post CPB compared with baseline may relate to increased preload on the heart resulting from increased intravascular volume (patients had a median positive fluid balance of 0.9 litres post-operatively) or transient diastolic dysfunction [28]. Another study in which BNP was measured also found a post-operative increase, but this was only detected 6 h following bypass and remained high for several weeks [28]. The same study also found a weak correlation between BNP at 24 h post CPB and aortic cross clamp times [28]. There was no such correlation in our study, although we only measured BNP up to 2 h post CPB. We did not examine the time course of BNP release as the study was designed to show changes in peripheral BNP concentration in relation to reperfusion of the heart.

The lack of correlation between BNP and cTNT suggests that the degree of myocardial necrosis in these patients was insufficient to affect ventricular function and circulating BNP concentrations. The very strong correlation between BNP at all time points with baseline values implies that there is a similar process occurring in all patients. The pattern may be different when there is severe post-operative ventricular dysfunction.

### *4.2. cTnT*

Peak cTnT concentrations occur in peripheral blood between 12 and 48 h following cardiac surgery [24]. Even at 2 h following CPB, median cTnT concentrations were elevated ten fold compared with baseline concentrations. In this patient group, the increase in cTnT was in keeping with the trauma caused by surgery itself, and does not suggest significant myocardial necrosis [29]. This is supported by the absence of ECG changes suggesting myocardial infarction and favourable clinical outcome in all patients. There was no difference in the cTnT concentrations in the allopurinol and control groups. This could mean that allopurinol has no protective effect in this context or that the study was not powerful or sensitive enough to detect such an effect. Another possibility is that improved techniques of myocardial preservation, specifically the use of warm oxygenated blood cardioplegia [30–32], decrease the overall incidence of ischaemic and reperfusion injury. This is supported by the observation that there was no correlation between aortic cross clamp times and post-operative cTnT values, unlike findings in previous studies [33].

In summary, this study demonstrates that there is a consistent and predictable pattern of BNP release reflected by peripheral BNP concentrations during and following cardioplegic cardiopulmonary bypass. BNP decreases when the aortic clamp is applied and the heart is isolated from circulation. There is a minor increase in BNP concentrations following cardiac reperfusion. There is a more substantive increase in peripheral BNP following CPB when ventricular distension and ejection occur.

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