BNP AS A BIOMARKER IN TRAUMA PATIENTS

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INTRODUCTION

In 1988, a new natriuretic peptide was found, Brain Natriuretic peptide (BNP). BNP, together with the sympathetic nervous system and other hormones, regulate body fluid homeostasis and blood pressure [1]. In numerous cardiovascular diseases, the natriuretic peptides function as markers for diagnosis and prognosis. They have also been found to have some therapeutic importance.

Physicians are continuously searching for reliable biomarkers in the hope that this could simplify and improve accuracy of medical decisions. Interest and research in BNP has rapidly increased over recent years. Initially BNP was used primarily as a diagnostic aid for acute dyspnoea [1] .Newer research shows correlations between BNP levels and various pathological states, some of which are significant and have opened the door for more investigations to be done. This could lead to an increase in BNP's utility in clinical medicine.

THE ENDOCRINE HEART

The belief that the heart may be an endocrine organ started around sixty years ago when it was shown that dilation of the atrium produced natriuresis[2].To increase suspicions further, intracellular granules in the cells of the atrium, which seemed endocrine in nature, were visualised on electron microscopy. An experiment published in 1981 by deBold[3] and associates added some definitive proof, in which they injected rats with extracts from atrial cells and observed a rapid diuresis and natriuresis. Atrial natriuretic factor,now known as Atrial natriuretic peptide, was in fact the hormone shown to cause these effects.

A research group demonstrated a structure comparable to ANP from the porcine brain in 1988[2], Brain Natriuretic Peptide, also named B-Type Natriuretic Peptide (BNP). BNP was soon after shown to act on the same peripheral receptors as Atrial Natriuretic Peptide and it was produced in and secreted by cardiac myocytes[2] .A third peptide was also discovered: C-type natriuretic peptide (CNP). CNP is found in various places in the body including the brain, the pituitary gland, kidneys and vascular endothelial cells. Very little is present in the heart and circulation making it thus primarily a paracrine mediator.[4]

SYNTHESIS AND STRUCTURE OF BNP

BNP belongs to a group of natriuretic peptides. This group of peptides has a common ring structure, which consists of seventeen amino acids. It is thought that this structure is necessary for ligand receptor binding and for the peptides to produce their physiological effects.[5] BNP is a 32-amino acid chain held in a horse-shoe form by a disulfide bond linking cysteine molecules at positions 10 and 26.

It can cause a decrease in blood pressure which is regulated by stress in the wall of the heart. BNP has a half- life of approximately 15-20min. It has been isolated and a variety of species of BNP peptides and cDNA clones have been sequenced. ANP has a similar structure between species however the structure of BNP is not preserved. BNP in the rat and pig have a 26- and 45- amino acid peptide respectively. However in humans, the major circulating form is the 32-amino acid peptide.[5]

The BNP gene is positioned on the short arm of the human chromosome one. The BNP gene has three exons. BNP mRNA is translated to preproBNP which has 134 amino acids, subsequently a signal peptide of 26 amino acids is removed, giving us proBNP which consists of 108 amino acids[6]. The atria and ventricles contain different molecular structures. The atria have BNP-32 which is the main molecular form of around sixty percent, compared to the ventricle which the main molecular form is proBNP-108.

ProBNP is cleaved into N-terminal proBNP-76 and BNP-32 in the trans-golgi network by furin and corin. The physiological function of NT-pro BNP is still unidentified. BNP-32 and N-terminal pro-BNP are then released into the circulation via a constitutive pathway [6]. There have been recent trials that have shown that proBNP-108 is also seen in human circulation. In the event of severe heart failure the ratio of proBNP-108 to BNP-32 is increased [7].
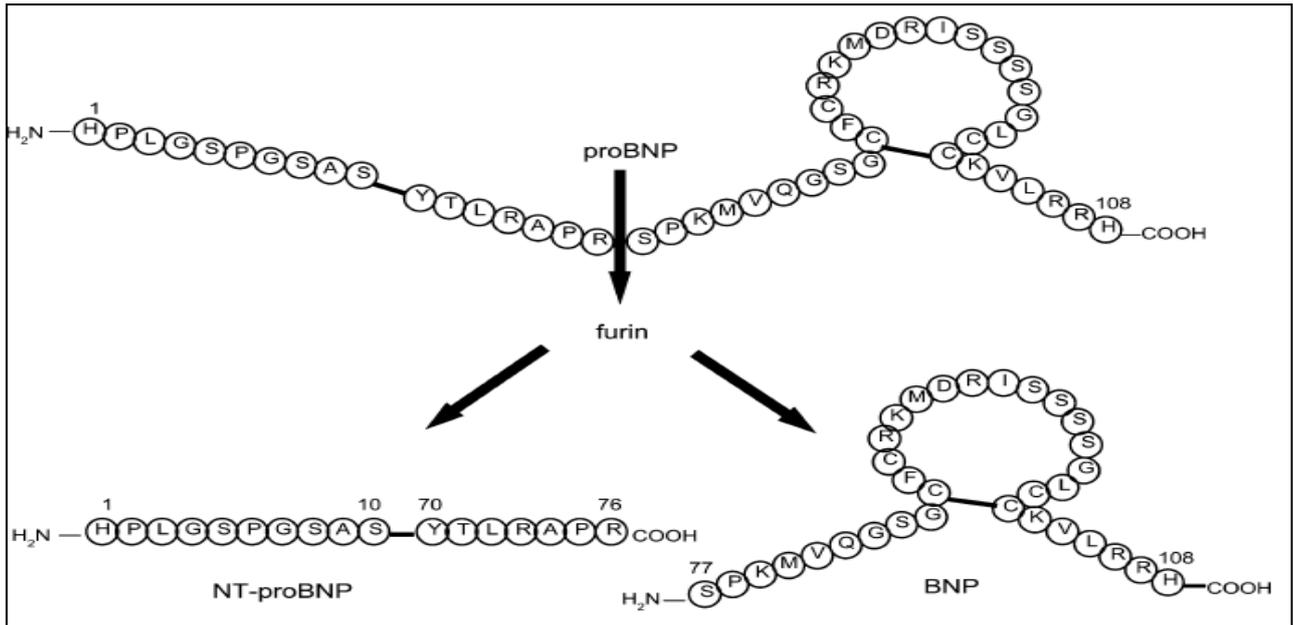
Figure 1 Structure of ProBNP, BNP, NT-ProBNP.[2]

Biochemical and physiological characteristics of BNP, NT-proBNP and proBNP peptides.

	BNP	NT-proBNP	proBNP
Molecular mass	3462 Da	8457 Da[a]	11900 Da[a]
Amino acids	32	76	108
Biological function	Active hormone	Inactive	Pro-hormone
Half life	15–20 min	>60 min	>60 min
Glycosylation	Not glycosylated	Highly glycosylated in vivo	Highly glycosylated in vivo

Figure 2 [8]

ACTIONS OF BNP

BNP produces most of its physiological effects by binding to natriuretic peptide receptor A (NPR-A) on the membrane of specific cells. BNP however has approximately ten times less affinity for NPR-A than ANP. BNP also binds Natriuretic Peptide receptor B(NPR-B) but to lesser extent. These receptors contain an intracellular guanylyl-cyclase area. Binding of BNP with the NPR-A causes an increase in levels of cyclic GMP (cGMP); which control biological actions such as natriuresis, diuresis, vasodilatation, inhibition of aldosterone synthesis and lipolysis.[5] Both ANP and BNP increase sodium excretion in the kidney.

This effect is caused by afferent arteriolar dilation and mesangial cellular relaxation, leading to an increased glomerular filtration. BNP also inhibits sodium reabsorption by acting on the renal tubules. It increases capillary permeability and relaxes vascular smooth muscle in capillaries. BNP also opposes the vasopressor effects of angiotensin II and catecholamines as well as inhibiting secretion of renin.[4] BNP itself and the BNP mRNA concentrations are lower in the ventricles as compared to the atria in the human heart.

However, as the mass of the ventricle is approximately seventy percent of cardiac tissue, majority of cardiac BNP and its mRNA are derived from the ventricles.[5] BNP secretion could actually serve as an emergency defence against ventricular overload in pathological conditions. This is apparent as we see that levels of BNP mRNA in the myocardium and BNP(including NT-proBNP) in circulation are significantly elevated compared with ANP in patients with congestive heart failure.[5]

SECRETION AND METABOLISM OF BNP

Stretch of the myocyte in the heart is considered to be the major stimulus for secretion of BNP. Despite this, certain hormones (endothelin I, angiotensin II and catecholamines) may also augment secretion through paracrine and perhaps other endocrine mechanisms[9]. Some studies have recently shown that hypoxia alone may stimulate natriuretic peptide release [10].

ANP and BNP are cleared by uptake from Natriuretic peptide clearance receptors (NPR-C). BNP and ANP may also be metabolically degraded by proteolytic enzymes such as neutral endopeptidase (NEP). This mechanism is believed to be less important with BNP than for ANP. Clearance of NT-ProBNP from plasma is not as well studied as its production by the heart[11]. NT-ProBNP clearance has been attributed as renal in nature, with other possible clearance pathways not yet known. The different pathways described above may be the reason why NT-ProBNP's(60-120min) half- life is longer than that of BNP(15-20min) along with ANP(10min)[11].

Renal dysfunction is a continuing problem in the interpretation of NT-ProBNP levels as this is possibly a source of error. This leads controversy of not having clear cut- off values when renal dysfunction is significant. We are still left with the question of whether BNP is influenced less by renal impairment than NT-ProBNP. Not much information is available to show a difference[11].
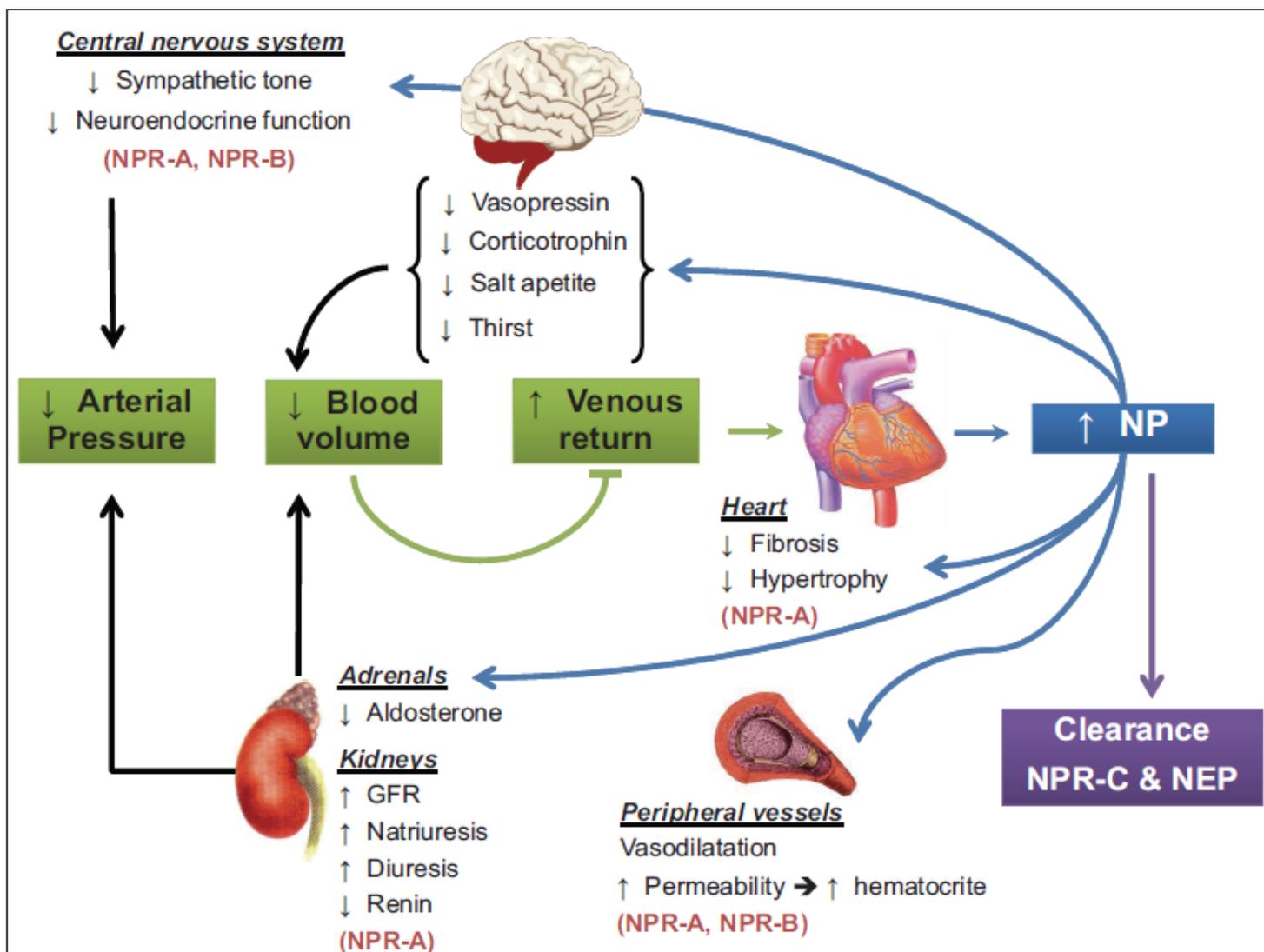

Figure 3 Main physiological effects of Natriuretic Peptides in response to acute change in blood volume [12]

ESTABLISHED USES OF BNP AS A BIOMARKER

In the emergency setting a patient that has acute dyspnoea, a normal level of BNP or NT-proBNP can rule out acute heart failure. In the event that it is greater than 500pg/ml makes it more likely to be heart failure. This is the principal use of measuring BNP [1].

Other clinical uses of BNP include:
- Screening for heart failure: Even in patients without symptoms, BNP and NT proBNP may be elevated if left ventricular dysfunction is present.
- Prognostic indicator for heart failure, cardiac surgery patients, vascular surgery patients(superior to revised cardiac risk index for risk stratification) [13], mortality in diabetic patients [14] and patients with acute coronary syndromes [15].
- Guides management of a patient with heart failure.

Interpreting results however does require that some factors be taken into account. BNP levels have a high sensitivity but lack specificity for heart failure. Elevated levels of BNP could be present in situations other than heart failure such as renal failure and numerous others (Figure 4), whereas obese patients could have lower levels. It must be used with other tools for an improved positive predictive value. Inappropriate ordering and interpretation of these results could be costly and lead to mismanagement of patients.

Figure 4: Causes for elevated Natriuretic peptides[8]

Selected causes of elevated natriuretic peptide concentrations (according to references [19–21]).

Cardiac
- Heart failure, including right ventricular syndromes
- Acute coronary syndrome
- Heart muscle disease, including LVH
- Valvular heart disease
- Pericardial disease
- Atrial fibrillation
- Myocarditis
- Cardiac surgery
- Cardioversion

Noncardiac
- Advancing age
- Anemia
- Renal failure
- Liver disease
- Pulmonary: obstructive sleep apnea, severe pneumonia, pulmonary hypertension
- Endocrine diseases (such as hyperthyroidism and primitive or secondary hyperaldosteronism)
- Chronic inflammatory diseases (such as amyloidosis)
- Critical illness
- Bacterial sepsis
- Severe burns
- Toxic-metabolic insults, including cancer chemotherapy and envenomation

BNP MEASUREMENT

Which B-type-related peptide should we measure?

There are at least 3 different peptides that could be measured in human plasma samples: BNP which is the active hormone, NT-proBNP which is the inactive terminal fragment and proBNP which is the pro-hormone. These 3 peptides have different biochemical characteristics and pathophysiological relevance.[8] From an investigative point of view, the inactive peptide NT-proBNP and proBNP are more stable in vivo and in vitro with a longer plasma half-life and a lower intra-individual biological variation than active peptide BNP.

According to the investigative characteristics and clinical results, we would assume that the inactive peptide NTproBNP and proBNP would be a better biomarker for the progression of Heart Failure (HF) than the active hormone BNP. However currently all the commercially available immunoassay methods considered specific to active peptide BNP significantly cross-react with proBNP.[8] All the international guidelines state that the commercially available BNP and NT-proBNP immunoassays usually give clinically comparable results when used for diagnosis, prognosis and follow-up of HF patients.

At present, we must realize that the commercially available immunoassay methods considered specific for the active form of B-type cardiac natriuretic peptides present an obvious paradox. From a pathophysiological point of view, it would be better to measure the active peptide BNP when we are interested in evaluating the current biological status of the cardiac endocrine function.[8] Currently none of the commercially available methods are able to provide such information accurately as these methods are greatly affected by inactive peptides, the concentrations of which are higher than active hormones in the blood samples of HF patients.

At the present time, all the commercially available immunoassay methods for BNP assay are not completely specific for the active form of the peptide.[8] BNP levels as well as NT-proBNP levels are measured by immunoassay [8].

- A BNP level lower than 100pg/ml has a sensitivity of 90% and a specificity of 76% [8]
- A BNP level lower than 50pg/ml has a sensitivity of 97% and a specificity of 62% [8]

Some laboratories report in units ng per Litre (ng/L), which is equivalent to pg/ml With patients in congestive cardiac failure, BNP levels are usually above 100pg/ml. There is a diagnostic grey area for values between 100pg/ml and 500pg/ml. However many studies address this and by using history and other available tools the correct diagnosis can be made.

BNP AND TRAUMA

From the above literature it can be seen that there are many associations between BNP levels and various pathological states. Based on that, assumptions can be made about the possibility of using BNP levels in the trauma patient either directly or indirectly due to injury. There has been research done in this regard but it is limited. Below we will describe some of the correlations that have been found.

BNP as a marker for acute blood loss/hypovolaemia

The elevation of BNP levels with increased intra vascular volume status has been well described, hence postulations have been made of the possibility that with decreased volume status, BNP levels may actually be lower than normal physiological levels. The number of studies done to evaluate decreased BNP levels in the human body is limited. Current techniques of clinical measurement for identifying acute blood loss in patients include; heart rate, mean arterial blood pressure, haemoglobin measurement, base excess, pH, more invasive cardiac output monitoring (eg Pulmonary artery catheter measurements) and central venous saturation.

Each of these has their limitations in that they may be excessively invasive or inaccurate for various reasons. A study done by Kia et al[16] in 2004 studied haemodynamically unstable trauma patients aged 18-45 years where consecutive BNP levels and haemoglobin (Hb) at admission, 8 hours and 24 hours and also every morning during the resuscitative period were measured. This was done to determine if BNP has a place in identifying clinically significant blood loss. Their exclusions included patients with cardiac injury, congestive cardiac failure and those known with cardiomyopathy.

Patients were then grouped as the Blood loss group if Hb dropped by more 3g/dl, or required blood or blood products in the initial 24hours since admission. The other group, termed the Non-blood loss group where the Hb did not drop more than 3g/dl in the first 24 hours[16]. The initial BNP levels showed a trend toward a considerable difference between the groups however this was not statistically significant. Fluid requirements between these 2 groups differed significantly, almost double for the Blood loss group. In the non- blood loss group it was found that all except one had normal BNP levels.

In this study[16] of 14 patients, 9 fell in the group for blood loss and all BNP values were below normal at admission. The results of this trial show that early recognition of blood loss can be shown by BNP measurement.[16] BNP may be decreased in any state that decreases pulmonary artery catheter wedge pressure, but in the acutely- injured patient it is usually assumed to indicate blood loss. This trial showed that BNP levels could possibly identify intravascular volume status at the initial trauma assessment. However trials with larger numbers need to be performed.

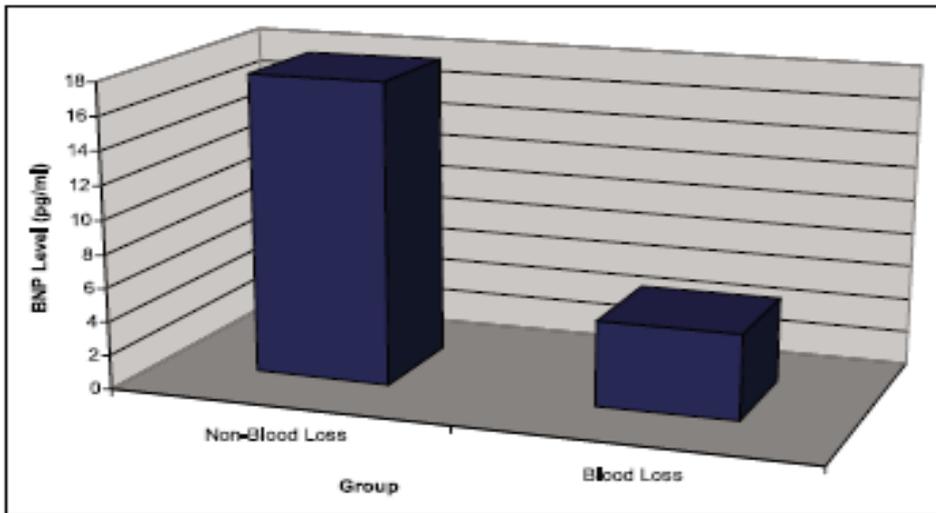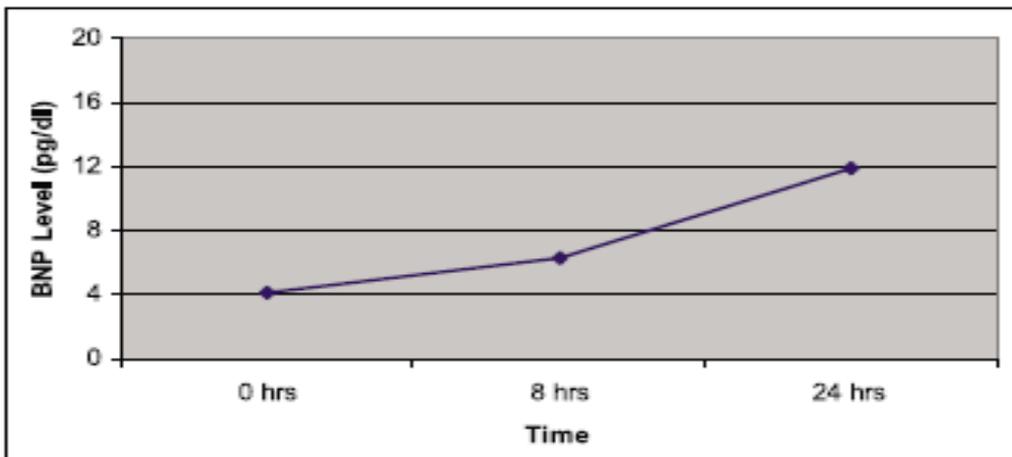**Figure 5. BNP level difference between groups in trial by Kia et al[16]**

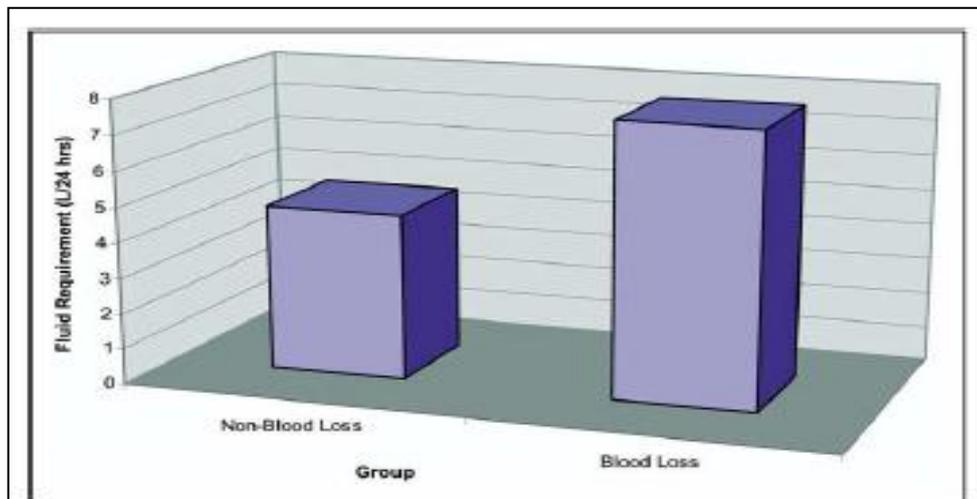**Figure 6. BNP levels during resuscitation. Kia et al [16]**

Figure 7. Bar graph representing the amount of resuscitation fluid required by each group. Kia et al [16]

BNP as a biomarker for fluid resuscitation in the trauma patient

Calls for more careful resuscitation are being made by many leaders in the trauma field, as the immediate enthusiastic post- injury fluid resuscitation has been related to severe complications such as [16, 17] :

- Persistent Haemorrhage
- Pulmonary oedema
- Abdominal compartment syndrome
- Extremity compartment syndrome
- Cerebral oedema
- Congestive heart failure

Laboratory markers currently used to measure adequacy of resuscitation include Lactate level normalisation and clearing of arterial base deficit. These are extremely helpful in evaluating response to fluid resuscitation however they also depend on liver and renal function. Other assessments of cardiac preload while resuscitating a patient include insertion of a pulmonary artery catheter to measure wedge pressure or insertion of a central venous line to measure pressure at the superior vena cava and right atrium.These do require invasive monitoring and are subject to inaccuracy if there is cardiopulmonary dysfunction. [17]

In the study mentioned earlier by Kia et al[16], they also noted that fluid resuscitation correlated with increasing BNP levels however results were not statistically significant and they recommended further trials with large study populations. [16] A study done by Friese[17] hypothesized that BNP values following trauma and fluid resuscitation will show a relationship with the diagnosis of volume overload. Here they prospectively followed 134 trauma ICU patients and BNP measurements were done at admission, 12, 24 and 48 hours. Chest X-rays, looking for evidence of pulmonary oedema, were taken of 45 patients at 24 hours by a radiologist that was unaware of the BNP levels. This study showed that at the time of admission the BNP values were low, but during the resuscitative period and as time progressed the measurements of the BNP values increased(p-value = 0.002).

 At 24 hours, pulmonary oedema was not present in 25 of the patients and 20 were noted to have pulmonary oedema. The patients that the radiologist found to have features of pulmonary oedema on chest x-ray had a increased mean BNP at 24hours compared to those that did not have radiological features. Based on this they consider BNP a possible marker of excessive volume resuscitation following injury. A marker such as BNP could assist when we need to decide between fluid resuscitation or the use of vasopressors for circulatory support, especially if this can be done at the bedside [17] This study still however has other confounders: many trauma patients may have concurrent myocardial infarcts or blunt cardiac injury and the features of pulmonary oedema on X-ray may not be adequate.

Recommendations are currently calling for more careful fluid resuscitation. Many new techniques to monitor the resuscitative processes are available however are not readily available to most and are expensive. For this reason, a non- invasive biomarker such as BNP for blood loss and resuscitation which can be done at the bedside, must be considered. There are obviously many confounders (eg Spinal shock, renal failure). Additional studies against higher standard techniques for measuring volume status and larger study populations can possibly lead to utilization of BNP as a marker for blood loss and resuscitation.[17]

BNP as a predictor for mechanical ventilation after injury

Research has shown an association between the amount of circulating BNP and various clinical results, one of which is the possibility of a correlation linking BNP levels and the need for mechanical ventilation. In a prospective observational study by Vander Werf et al in 2010 [18] BNP measurements were monitored in trauma patients admitted to a surgical ICU. BNP measurements were taken at admission, 12, 24 and 48 hours. Multiple outcome measures were looked at such as number of days ventilated, ICU length of stay, hospital length of stay and mortality. Forty-four patients were enrolled in this study, 30 of which required mechanical ventilation. The Median Injury Severity score was 19.5 with a mortality of fourteen percent.

The number of days they required mechanical ventilation was associated with the change in BNP levels at 24hours. Patients with increased levels of BNP in their plasma more than or equal to 75pg/ml at 24hours had three times the likelihood to be ventilated for more than three days. Change in BNP also significantly correlated with APACHE II score.[18] Based on the study above it could also be postulated that if BNP correlates with the APACHE II score, that the need for mechanical ventilation could just be associated with the severity of the injury rather than the increase in BNP levels.

Another factor we are drawn to is that the raised BNP could be due to over-resuscitation and lead to pulmonary dysfunction. However in this study no association was found between net resuscitation volume and BNP values at 24 and 48 hours.[18] Various confounders must be taken into account in these trials such as the small study populations, no standardised protocol for resuscitation and patients with head injuries. These are factors that must be considered in future trials.[7]
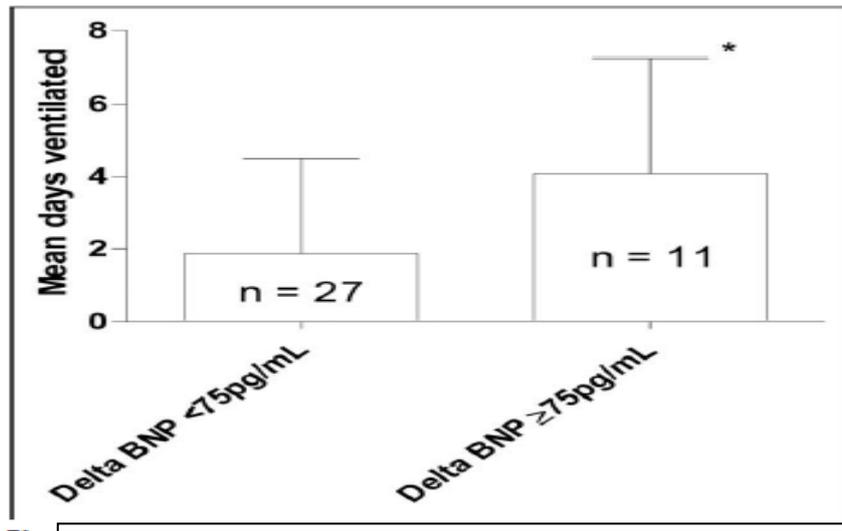
Figure 8: 'Mean ventilator days as a function of change in BNP'. Vander Werf et al [18]

BNP elevation in trauma patients without congestive heart failure

In patients that have impaired left ventricular diastolic and systolic function, BNP levels have been shown to be increased. Medical patients that present with acute dyspnoea can have the diagnosis of heart failure quickly excluded by BNP levels alhough none of the trials done included trauma patients. Tachypnoea, hypoxia and dyspnoea are frequently encountered in trauma patients. Early diagnosis of the cause is vital given that the management of a patient for cardiac failure rather than for pulmonary contusions, haemothorax, pneumonia, pulmonary embolus and fat embolism are very different.

A study by Stewart et al in 2007[19] which included 50 patients measured BNP at admission, 24 hours and 48 hours as well as a transthoracic echocardiograph done within 24 hours. In this study there wasn't a noteworthy correlation between BNP values and echocardiograph (p=0.149). Twenty-eight of the patients had intracranial bleeds of which 18 had raised BNP. Of the patients that had head injuries the echocardiographs were normal in 7 of them despite having elevated BNP (mean BNP= 182pg/ml).[19] Another study by Kirchoff et al in 2008 [7] questioned the validity of the results as they did not score the severity of injury to the patients and they included head- injured patients in which BNP is known to be increased irrespective of cardiovascular status.

The Kirchoff study also questioned the use of transthoracic echocardiograph to assess volume status as it is believed to be a less accurate measure of volume status than transoesphageal echo and transpulmonary thermodilution which was used by Kirchoff et al[7]. In the Kirchoff et al[7] study they included 26 polytrauma patients presenting with an Injury Severity Score higher than 16. Two groups were formed from the patients A: Minor signs of organ dysfunction (used Marshall Multiple Organ Dysfunction Score of less than or equal to 4) and B: Major signs of organ dysfunction and a MODS of greater than four.

They excluded head injuries from their study. In this trial, plasma NT-ProBNP values notably correlated with clinical signs of MODS 24hours after the injury. Additionally, significant correlations were made with elevated NT-ProBNP values and a decrease in cardiac index at 24 hours post injury. For the diagnosis of post traumatic cardiac dysfunction there could be some value in measuring NT-proBNP.[7]

Utility of BNP as a biomarker for acute lung injury

Acute lung injury is under diagnosed. Severe trauma is a well known cause of acute lung injury. A retrospective case study [20] that incorporated 192 patients from 2002-2006 compared those with acute lung injury(107) and those that did not have Acute lung injury(85). They measured 21 different biomarkers and, of these, it was found that 7 had a high diagnostic accuracy. Of these 7, BNP was rated in the top 3 panel performers of biomarkers. [21]

Summary of the final diagnostic model using seven biomarkers to diagnose ALI/ARDS				
Biomarker	Quartiles (25th – 75th)	Odds Ratio for ALI/ARDS^1	95% CI	P-value
RAGE	1216 – 3310	3.33	1.85 – 5.99	<0.001
PCP III	7.3 – 13.1	2.90	1.61 – 5.23	<0.001
BNP	0.17 – 0.45	0.45	0.26 – 0.77	0.004
ANG2	4659 – 12078	2.20	1.19 – 4.05	0.01
IL8	16.8 – 64.6	1.81	1.03 – 3.17	0.04
TNF-α	3.6 – 13.1	0.51	0.27 – 0.98	0.04
IL10	21.4 – 151.2	2.02	0.96 – 4.25	0.06

Figure 9 [21]

BNP is the major biomarker used to distinguish between hydrostatic and increased permeability pulmonary oedema. In this study, they established that BNP values were much lower in the sample with acute lung injuries as compared to the sample that did not have acute lung injury. This suggested the possibility of a higher occurrence of cardiac dysfunction and volume overload. BNP alone as a biomarker performed poorly. As part of a panel of biomarkers it is found to be useful for diagnosing acute lung injury.

BNP levels in the general intensive care unit (ICU) as a prognosticator

There is a lot of attention focused on the utility of BNP in ICU for detecting and differentiating conditions causing ventricular dysfunction and for discriminating between cardiogenic pulmonary oedema and various other prognostic indicators. Most studies are however limited by low patient numbers, limited admitting diagnoses and BNP testing done at only one point in time. Kotanidou et al[20] did a study between 2005-2007 which included all critically ill patients and excluded cardiac patients.

It was a prospective study of 233 mechanically ventilated patients. It included 118 medical, 83 surgical and 32 multiple trauma (comprising 20 with brain injury) patients. Elevated NT-ProBNP was markedly associated with organ failure, cardiac impairment, renal failure and thrombocytopaenia. Organ dysfunction and in particular, renal dysfunction, where predicted by NT-ProBNP values. In this study it was noted that elevated NT-ProBNP is also related to higher levels of mortality. Patients that had a NT-proBNP level of more than 941pg/ml on admission had 7 times more chance of dying in the intensive care unit than patients that had lower levels[20].
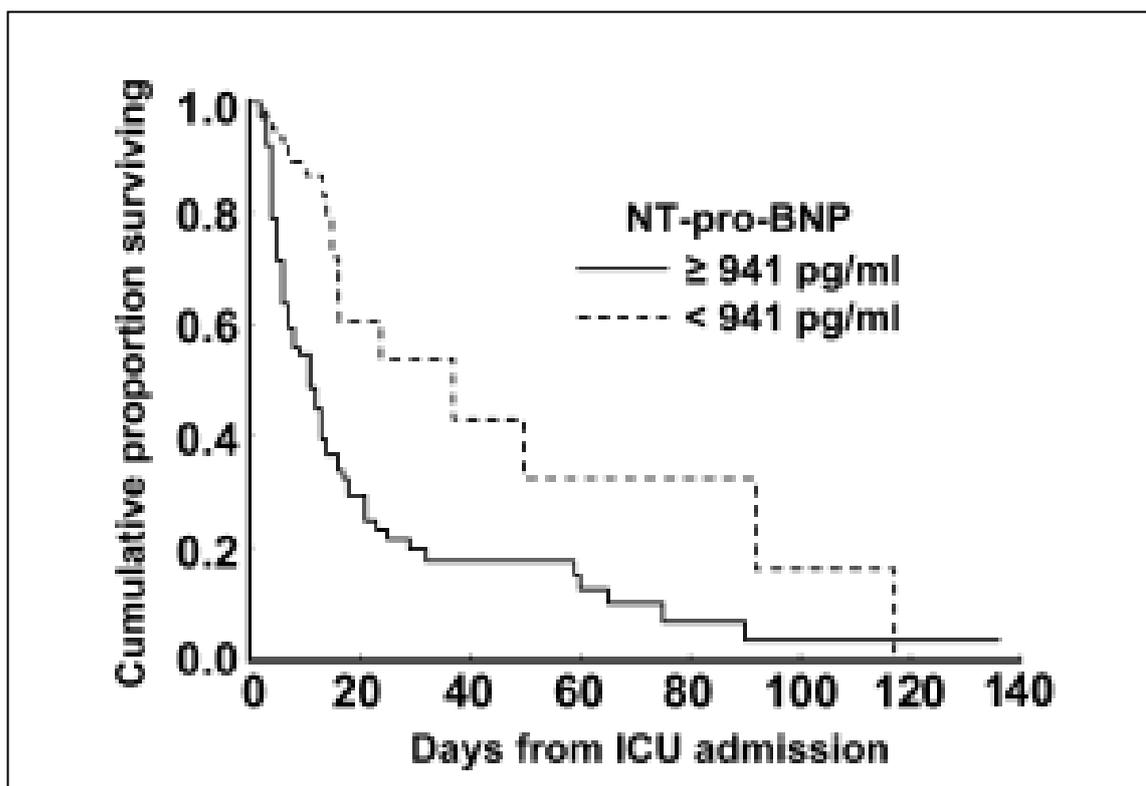

Figure 10: 'Kaplan- Meier survival curves in the critically ill'. Kotanidou et al [20]

CONCLUSION

In trauma patients, research on BNP levels and its correlation with clinical outcomes seems to be limited. Monitoring BNP levels has several limitations including gender, obesity, age, haemodynamics, brain injury, renal failure, certain humoral parameters as well as the variation of plasma BNP in individuals. These can affect circulating BNP levels in both pathological and physiological conditions. Despite the limitations there are a few trials of note as discussed in this talk. Further studies must be done to reveal if BNP can be a biomarker of significance in trauma. The trauma patient is not simple to manage and if a tool is available that could assist in diagnosing, triaging and finding precise resuscitation end points, effort should be made to explore all possible avenues.

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