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B-type natriuretic peptide signal peptide (BNPsp) in patients presenting with chest pain☆

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ABSTRACT

Objectives: We assessed the ability of B-type natriuretic peptide signal peptide (BNPsp) to assist with the identification of patients with myocardial infarction (MI) and unstable angina pectoris (UAP).

Design and methods: We studied 505 patients who presented to hospital within 4 h of onset of chest pain suspicious of ACS. Blood samples were drawn at 0, 1, 2 and 24 h from presentation and assayed for BNPsp, NT-proBNP, TnI and high sensitivity TnT. The ability of BNPsp and other markers to diagnose acute myocardial infarction (MI) and unstable angina pectoris (UAP) and predict subsequent events within one year was assessed. Statistical analysis was made using ROC AUC in SPSS, v.22.

Results: Receiver operator area under the curve (AUC) data for the discrimination of MI was 0.69 for BNPsp and 0.97 for troponin, with BNPsp failing to add to troponin. However, in non-MI patients, BNPsp had discriminative power for UAP ($p < 0.05$), and when combined with presentation values of NT-proBNP, white cell count and potassium into a unique parameter (UARatio), generated an AUC of 0.76 for UAP in patients with normal ECG results ($p < 0.001$). In non-MI patients, the UARatio was significantly predictive of subsequent stroke (AUC = 0.70, $p < 0.05$) and heart failure (AUC = 0.82, $p < 0.01$) within one year.

Conclusions: In patients with chest pain, BNPsp is predictive of MI but is not a useful adjunct to troponin. However, the ability of BNPsp, in conjunction with NT-proBNP and key analytes, to diagnose UAP and other ischemic syndromes merits further investigation.

1. Introduction

Suspected acute coronary syndromes (ACS) are frequent among hospital emergency department (ED) presentations and comprise between 5 and 15% of all attendances [1]. The rapid identification of those with genuine myocardial infarction (MI) has been enhanced via the use of highly sensitive cardiac troponin biomarker assays, [2–5] but biomarker assisted identification of those with non-infarction ischemia (e.g. unstable angina pectoris (UAP)) is an area of unmet clinical need. UAP is an important clinical substrate for subsequent cardiovascular events and its clear, early identification could help reduce related cardiovascular morbidity and mortality [6].

We have recently provided the first reports that fragments of the signal peptide (sp) regions of the natriuretic hormones B-type natriuretic peptide (BNPsp), A-type natriuretic peptide (ANPsp) and C-type natriuretic peptide (CNPsp) are present in the human circulation [7–9]. Both BNPsp and ANPsp display rapid rises in the circulation during ST-elevation MI. BNPsp also shows prompt and significant elevation within 30 min in the setting of dobutamine stress echocardiography [10]. Thus, given the need for markers that can discriminate cardiac ischemia, short of tissue necrosis, from other non-cardiac causes of chest pain, we sought to determine the potential of BNPsp – in combination with other markers such as troponin and NT-proBNP – to improve

Abbreviations: BNPsp, B-type natriuretic peptide signal peptide; STEMI, ST-elevation myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; UAP, unstable angina pectoris; ACS, acute coronary syndromes; hsTnT, highly sensitive troponin T assay; WCC, white cell count; ROC, receiver operating characteristic curve; AF, atrial fibrillation.

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the early identification of true cardiac ischemia in a prospective study of patients presenting with chest pain suspicious of ACS. As a secondary aim, we also assessed the prognostic ability of BNPsp alongside troponin and NT-proBNP in these patients.

2. Methods

2.1. Study population and design

Patients with chest pain suspicious of acute coronary syndromes (ACS) were prospectively enrolled into our ongoing observational study known as Signal Peptides in Acute Coronary Events (SPACE, http://www.anzctr.org.au, ACTRN12609000057280). All patients were enrolled in accord with protocols approved by the Health and Disabilities Ethics Committee of the Ministry of Health, New Zealand. All participants gave informed consent before recruitment and all investigations conformed to the principles of the Declaration of Helsinki. Between March 2009 and September 2013, 505 eligible patients aged 18 years or older with the primary complaint of acute chest pain clinically suspicious of ACS and ≤4 h from onset were recruited. More general/atypical symptoms (such as fatigue, nausea, vomiting, sweating and faintness) were not used as inclusion criteria. Patients with end stage renal disease on dialysis were excluded.

2.2. Adjudicated diagnosis

The adjudicated diagnosis of acute MI was made in accordance with published guidelines [1], by two independent cardiologists with access to all clinical data, but not BNPsp or hsTnT results. In the case of disagreement, an independent third cardiologist adjudicated to resolve this. The biochemical component of the diagnosis of MI was based on a contemporary TnI assay (not highly sensitive) with 1 value ≥99th URL (99th percentile = 0.03 μg/L) within 12 h of presentation. Atrial fibrillation (AF) during emergency department presentation was determined from the ECG, whereas the diagnosis of UAP was made on the basis of confirmatory provocative investigations (exercise tolerance testing (ETT) or dobutamine stress echocardiography testing (DSE)) or angiographic catheterisation findings. Other cardiac disorders were defined as non-ACS cardiac presentations comprising conduction disorders (sick sinus syndrome), arrhythmias (atrial fibrillation/flutter) and acute heart failure. Undifferentiated chest pain was defined as chest pain without definitive associated clinical findings or cardiac tests where doubt remains as to the aetiology. Non-cardiac chest pain was defined as present when a definite non-cardiac cause for symptoms was identified.

2.3. Follow up and prognostic end points

Within 365 days post-discharge, patients were followed up by telephone or in writing. Reported clinical events were identified from the patients themselves (or their primary physician) corroborated by the records of the treating institution or by the centralised New Zealand Ministry of Health database registry entries on mortality and events. The post-discharge end points considered were death, MI, acute decompensated heart failure and stroke. Events were analysed by ROC analysis for three groups; all patients (n = 505), MI patients (n = 115) and non-MI patients (n = 390).

2.4. Clinical assessment and sample collection

For all patients, initial assessment included clinical history, physical examination, ECG recordings, standard blood tests, pulse oximetry and chest radiography. Patient management was at the discretion of the attending physicians. Only standard clinical core lab TnI (Abbott Architect, non-high sensitive index test available at time of study initiation) and other standard blood test results were available to treating staff.

After consent was given, serial blood samples for measurement of BNPsp, NT-proBNP and hsTnT (EDTA tubes) and TnI and lipids (Heparin tubes) were taken at 0, 1, 2 and 12–24 h after presentation. Blood samples (10 ml) were drawn into EDTA tubes chilled on ice, centrifuged at 2500 ×*g* for 10 min and the plasma frozen at −80 °C prior to assays. Heparin samples were collected into 5 ml tubes and immediately sent to the hospital core biochemistry unit for measurement of cTnI and lipids.

2.5. BNPsp assay

BNPsp was measured using our previously reported assay [7–10]. Briefly, the assay has a sample detection limit of 5.0 ± 0.6 pmol/L, ED_{50} of 161 ± 8 pmol/L and a sample working range of 4–112 pmol/L in which the intra-assay CV is <10%. Inter-assay CVs are ~14% at 130 pmol/L and ~13% at 44 pmol/L respectively. The 99th percentile upper limit of the normal range for BNPsp is 25 pmol/L at which the intra-assay CV is 6.2%. Cross-reactivity assessment shows no detectable interference with other relevant peptides or with medications commonly used in cardiovascular disorders.

2.6. Cardiac and other marker assays

NT-proBNP and hsTnT were determined on a Cobas e411 analyser (Roche Diagnostics). The limit of detection (LOD) for the NT-proBNP assay was 5 ng/L and had an imprecision co-efficient of variation (CV) of 4.6% at 44 ng/L. The LOD for the hsTnT assay was 5 ng/L with an imprecision CV of <10% at 13 ng/L. For the purposes of this study, an hsTnT value of 14 ng/L was used as the upper limit of normal cut-off and the clinical threshold for the diagnosis of MI [11]. All hsTnT results were submitted to Penzberg during the worldwide reassessment of hsTnT by Roche and only 3 required adjustment, all of which were below 14 ng/L. TnI was determined by a contemporary assay (Abbott Architect) with a 99th percentile cut-off of 30 ng/L (0.03 μg/L). Cholesterol, HDL, LDL and triglycerides were determined by the core Christchurch hospital lab (Canterbury Health Laboratories) on an Abbott Series C analyser.

2.7. Statistical analysis

Continuous variables are presented as median (interquartile range, (IQR)) and categorical variables as numbers and percentages. Bivariate associations between patient outcomes and continuous variables were analysed using non-parametric Mann–Whitney U test and categorical variables using the Pearson χ^2 test. Analysis of plasma analyte results employed Spearman rank order correlation testing and receiver operator characteristic curve (ROC) analysis and diagnostic performance (sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV)) were carried out using SPSS v22 (IBM). For ROC curve generation and biomarker panel comparisons, biomarker data were analysed as standardised variables (z-scores). In all cases, the standardised variable was derived from the maximum biomarker value obtained from the t = 0, 1 and 2 h samples.

Individual biomarkers (BNPsp, NT-proBNP, TnI and hsTnT) were assessed by ROC analysis for the prediction of index MI and UAP. Combinatorial assessment of standardised biomarkers for the detection of index UAP, thus generating a ratio here termed “UARatio”, was made using analytes according to whether ROC analysis indicated a lower or higher value. Thus, the UARatio exploits lower ROC values which have increased separation from higher ROC values, compared with neutral performers (~0.5), to predict index UAP. Higher ROC analytes function as numerators, whereas lower ROC values function as denominators. Iterative analysis identified a minimum core set of best performing standardised markers, whose additive nature was confirmed by singular removal and addition, whilst consistency was assessed in 3 randomly selected study population halves.