Myotrophin is a more powerful predictor of major adverse cardiac events following acute coronary syndrome than N-terminal pro-B-type natriuretic peptide

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ABSTRACT

Myotrophin is a 12 kDa protein initially isolated from hypertrophied hearts of spontaneously hypertensive rats and acts by modulating NF-κB activity. We have reported previously the presence of myotrophin in patients with human systolic heart failure; however, its role as a predictor of MACE (major adverse cardiac events) in patients with ACS (acute coronary syndrome) is unclear. In the present study, we sought to investigate this and compared myotrophin with NTproBNP (N-terminal pro-B-type natriuretic peptide), a marker of MACE. We studied 356 patients with ACS {276 men; mean age, 63.0 ± 12.8 years; 80.6 % STEMI [ST segment elevation MI (myocardial infarction)]; and 19.4 % NSTEMI (non-STEMI)}. Blood measurement was made at 25–48 h after the onset of chest pain. The plasma concentration of myotrophin and NTproBNP was determined using in-house non-competitive immunoassays. Patients were followed-up for the combined end point of death, MI or need for urgent revascularization. Over the median follow-up period of 355 (range 0–645) days, there were 28 deaths, 27 non-fatal MIs and 73 patients required urgent revascularization. Myotrophin was raised in patients with MACE compared with survivors [510.7 (116.0–7445.6) fmol/ml compared with 371.5 (51.8–6990.4) fmol/ml respectively; P = 0.001; values are medians (range)]. Using a Cox proportional hazards model, myotrophin {HR (hazard ratio), 1.64 [95 % CI (confidence interval), 0.97–2.76]; P = 0.05} and Killip class above 1 [HR, 1.52 (95 % CI, 0.93–2.42); P = 0.10] were the only independent predictors of MACE. A Kaplan–Meier survival curve revealed a significantly better clinical outcome in patients with myotrophin below the median compared with those with myotrophin above the median (log rank, 7.63; P = 0.006). In conclusion, after an ACS, levels of myotrophin are more informative at predicting MACE than NTproBNP and may be useful to risk stratify patients.

INTRODUCTION

ACI [acute MI (myocardial infarction)] is a leading cause of mortality and morbidity. Recent advances in the treatment of AMI have improved patient survival; however, despite this, there is still an appreciable mortality associated with this condition. The challenge remains to try and identify those patients who are deemed...
to be at high risk of adverse clinical outcome. Circulating natriuretic peptides certainly provide some information with regard to prognosis following AMI [1,2]; however, a multimarker approach may be more informative [3].

Myotrophin is a 12 kDa protein initially isolated from hypertrophied hearts of spontaneously hypertensive rats [4]. Elevated levels have also been found in human cardiomyopathic hearts [5], and our group [6] has shown early activation of the myotrophin system in heart failure, which is more evident in males. The in vitro effects of myotrophin on cultured cardiomyocytes include an increase in protein synthesis, cellular hypertrophy, gap-junction formation, increased sarcomere number, induction of early response genes, such as c-myc, c-fos and c-jun, and, subsequently, induction of transcripts of skeletal α-actin, total myosin and ANP (atrial natriuretic peptide) [7]. These effects are thought to be mediated via PKC (protein kinase C) activation [8]. Myotrophin is thought to interact with NF-κB (nuclear factor κB) [9], disrupting the formation of the NF-κB p50–p65 trans-activating heterodimers while increasing the formation of repressive NF-κB p50–p50 homodimers [10]. Although there is evidence of increased activity of NF-κB in human heart failure, little is known about the role of NF-κB in AMI [11]. Human myotrophin has been cloned [12] and found to be highly homologous with the rat protein with its mRNA widely distributed in many tissues, including relatively high levels in heart and skeletal muscle.

Outside of the cardiovascular system, myotrophin has been predicted to be and validated as a target of the islet-specific microRNA mir-375. Overexpression of miR-375 suppresses glucose-induced insulin secretion. Thus myotrophin is a regulator of insulin secretion and may thereby constitute a novel pharmacological target for the treatment of diabetes [13].

The role of myotrophin in AMI and its use as a marker for prognostication of AMI is unknown. In the present study, we investigated whether myotrophin is activated post-AMI and whether it would be of benefit in determining the prognosis of AMI, which remains a leading cause of mortality and morbidity. We compared this with NTproBNP (N-terminal pro-BNP (B-type natriuretic peptide)), which has been shown to be of prognostic benefit in this group of patients [2].

**MATERIALS AND METHODS**

**Study population**

We studied 356 consecutive patients post-AMI, who were admitted to the Coronary Care Unit of Leicester Royal Infirmary. The study complied with the Declaration of Helsinki, was approved by the local Ethics Committee and written informed consent was obtained from patients. AMI was defined at presentation with at least two of three standard criteria, i.e. appropriate symptoms, acute ECG changes of infarction (ST elevation or depression, new left bundle branch block) and a rise in troponin T above the 99th centile for our population [14]. AMI was subcategorized into STEMI (ST segment elevation MI) or NSTEMI (non-STEMI). Primary treatment for STEMI in our institution is thrombolytic therapy and this was administered by the attending physician if the patient presented within 12 h of symptom onset. Exclusion criteria were known malignancy or surgery in the previous month. Control subjects were age- and gender-matched and recruited from University of Leicester, and had peptide measurements made once.

**Plasma samples**

Blood measurement was made at 25–48 h after the onset of chest pain for the determination of plasma myotrophin and NTproBNP. This time period was chosen as NTproBNP has been found to be useful at predicting MACE (major adverse cardiac events) in patients post-AMI with samples taken outside the first 24-h window [2]. After 15 min of bed rest, 20 ml of blood was collected into tubes containing EDTA and aprotinin. All plasma was stored at −70 °C until assayed in a single batch.

**Echocardiography**

Transsthoracic echocardiography was performed in patients using a Sonos 5500 instrument (Philips Medical Systems). A 16-segment LVWMI [LV (left ventricular) wall motion index], based on the American Society of Echocardiography model, was derived by scoring each LV segment [1 = normal, 2 = hypokinesis, 3 = akinesia and 4 = dyskinesis (Paradoxical Motion)] and dividing the total by the number of segments scored. LVEF (LV ejection fraction) was calculated using the biplane method of discs formula [15]. Inter- and intra-coefficients of variation were 9.3 and 11.4 % respectively.

**NTproBNP assay**

Our NTproBNP assay was based on a non-competitive assay [16]. Sheep antibodies were raised to the N-terminus of human NTproBNP and monoclonal mouse antibodies were raised to the C-terminus. The anti-(N-terminus NTproBNP) IgG was affinity-purified and biotinylated. Samples or NTproBNP standards were incubated in anti-(C-terminal NTproBNP) IgG-coated wells with the biotinylated antibody for 24 h at 4 °C. Detection was with MAE (methyl-acridinium ester)-labelled streptavidin. The lower limit of detection was 0.3 fmol/ml. There was no cross-reactivity with ANP, BNP or CNP (C-type natriuretic peptide). Inter- and intra-assay coefficients of variation were 2.3 and 4.8 % respectively. The results from this in-house assay were highly correlated \((r = 0.90, P < 0.0001; n = 86)\) with those obtained with the NTproBNP assay marketed by Roche Diagnostics.
Myotrophin assay

The myotrophin assay was based on an immunoluminometric non-competitive assay. ELISA plates were coated with 100 µl of anti-(mouse IgG) antibody (100 ng/well; Sigma Aldrich) in PBS. Wells were then blocked with 10% (v/v) foetal calf serum in PBS. A specific commercial monoclonal antibody (IgG2b, clone 49; Becton Dickinson Biosciences Pharmingen) served as the capture antibody. The detector antibody was a rabbit polyclonal antibody that had been described previously by us in immunoluminometric assays of myotrophin [6], but for the present assays was enriched further by affinity purification on a column of myotrophin peptide (LTAFEATDNQAI; corresponding to amino acids 102–113 in the C-terminal domain of human myotrophin) immobilized on to Affigel 10 (Bio-Rad Laboratories). Bound specific antibody was then eluted using 0.1 M glycine/HCl (pH 2.4) and was rapidly neutralized with Tris base. A total of 100 µl of immunoluminometric assay buffer (1.5 mmol/l NaH2PO4, 8 mmol/l Na2HPO4, 140 mmol/l NaCl, 1 mmol/l EDTA, 1 g/l BSA and 0.1 g/l azide) containing 10 ng of the Becton Dickinson monoclonal antibody was pipetted into the ELISA-plate wells, followed by 50 µl of plasma samples and standards. Plates were incubated overnight at 4°C. After washing, the detector affinity-purified rabbit antibody (20 ng/100 µl) was pipetted into the wells and the plates were incubated at room temperature for 3 h. Following washing, a goat biotinylated anti-(rabbit IgG) antibody (1:200,000 dilution; Rockland Immunochemicals), previously preadsorbed with human, rabbit and mouse serum proteins, was incubated in the wells for 1 h, followed by MAE-labelled streptavidin for a further 90 min. Chemiluminescence was elicited with sequential injections of H2O2 in nitric acid, followed by NaOH containing cetyl trimethylammonium bromide, as described previously [6,16]. Intra- and inter-assay coefficients of variation were < 10%.

End points

We assessed the value of both myotrophin and NT-proBNP for the prediction of death, MI or need for urgent revascularization as a combined primary end point. We also investigated the secondary end point of heart failure. Hospitalization for heart failure was defined as a hospital admission for which heart failure was the primary reason.

Hospitalization for AMI was defined as above. End points were obtained by reviewing the Office of National Statistics Registry, which records all hospital deaths and by contacting each patient. There was a minimum 30-day follow-up of all surviving patients.

Statistical analysis

Statistical analyses were performed on SPSS Version 12. The continuous variables in the two independent groups were compared using the Mann–Whitney U test. Spearman’s correlations were performed, and Cox proportional hazards analyses were conducted, which included baseline patient characteristics (age, sex, serum creatinine, Killip class, territory of AMI, LVWMI and whether the patient received thrombolysis or not) and peptide markers (including troponin I), to test the independent predictive power of the peptides above and below the median for death, non-fatal MI and need for urgent revascularization. NTproBNP and myotrophin were normalized by log-transformation. Thus HRs (hazard ratios) refer to a 10-fold rise in the levels of these markers. Kaplan–Meier survival curves were generated to visualize the relationship between the NTproBNP and myotrophin and the composite end points. A P value below 0.05 was deemed to be statistically significant.

RESULTS

Patient characteristics

The demographic features of the patient population are shown in Table 1. Median length of follow-up was 355 days, with a range of 0–645 days. Of the patients enrolled, 65.5% of the STEMI patients received
thrombolysis during the index admission. No patient was lost to follow-up. During follow-up, 28 patients died, 27 were re-admitted with AMI, 73 patients required urgent revascularization and there were 28 re-admissions with heart failure.

Echocardiographic data were available for 297 (83.4\%) of the 356 patients and was performed at a median of 3.5 (range 2–5) days after presentation with AMI. A total of 36 ECGs were unanalysable, and 22 patients did not receive an ECG.

**Myotrophin levels in patients and controls**

Plasma levels of myotrophin in patients with AMI ranged from 51.8–7445.5 fmol/ml. The time course of secretion of myotrophin was assessed in 50 patients who had daily samples taken, and this revealed a significant difference over the 5 days (\(P < 0.01\); Figure 1). Levels in patients with AMI were significantly higher than those observed in the control subjects [405.7 (51.8–7445.5) fmol/ml compared with 348.1 (34.1–3982.9) fmol/ml respectively; \(P = 0.044\); values are medians (range)] and was higher in patients who died compared with the survivors [595.3 (203.4–7445.6) fmol/ml compared with 397.1 (51.8–6990.4) fmol/ml respectively; \(P = 0.005\)].

There were weak, but significant, correlations of myotrophin with NTproBNP (\(r = 0.113, P = 0.034\)), LVWMI (\(r = 0.155, P = 0.007\)) and Killip class (\(r = 0.217, P = 0.001\)). Myotrophin did not differ significantly according to sex, age, the presence or absence of diabetes mellitus, hypertension, previous MI diagnosis, hypercholesterolaemia, troponin level or whether a patient received thrombolysis or not.

**NTproBNP levels in patients and controls**

NTproBNP was significantly elevated in patients with AMI compared with controls [1367.1 (0.3–12175.1) fmol/ml compared with 10.1 (0.3–134.4) fmol/ml respectively; \(P < 0.001\)] and was higher, although not significantly, in patients who had MACE compared with survivors [1846.7 (0.3–11906.5) compared with 1268.0 (0.30–12175.1) fmol/ml respectively; \(P = 0.061\)]. The time course of secretion of NTproBNP revealed a significant difference over the 5 days (\(P < 0.0001\); Figure 2).

**Relationship between myotrophin and echocardiographic parameters**

For the whole population, mean LVWMI was 1.53 (range, 1.08–2.75) and LVEF was 36 (range, 9–68). The LVWMI score in those subjects with anterior AMI was higher than in those with inferior AMI [1.69 (1.08–2.75) compared with 1.41 (1.00–2.60) respectively; \(P < 0.0001\)]. However, LVEF was not different between the two groups [35 (9–68) % compared with 37 (13–65) % respectively; \(P = 0.074\)]. NTproBNP correlated positively with LVWMI (\(r = 0.373, P < 0.0001\)) and negatively with LVEF (\(r = -0.30, P < 0.0001\)).

**Myotrophin and NTproBNP as predictors of MACE**

Myotrophin was raised in patients with MACE compared with survivors [510.7 (51.8–6990.4) fmol/ml compared with 371.5 (51.8–6990.4) fmol/ml respectively; \(P = 0.001\)]. No difference was observed in patients who mounted a larger myotrophin response with regard to territory of infarct, STEMI compared with NSTEMI, or background previous drug therapy [including previous use of aspirin, \(\beta\)-blockers, statins, ACE (angiotensin-converting enzyme) inhibitors or angiotensin II receptor blockers].

When clinical and demographic characteristics were entered into a Cox proportional hazards model, the only independent predictors of MACE were myotrophin (HR, 1.64 [95 % CI (confidence interval), 0.97–2.76]; \(P = 0.05\)) and Killip class above 1 [HR, 1.52 (95 % CI, 0.93–2.42); \(P = 0.10\)]. The Kaplan–Meier survival curve revealed a significantly better clinical outcome in patients with myotrophin below the median compared with those with myotrophin above the median (log rank, 7.63; \(P = 0.006\); Figure 3).
Myotrophin is a powerful predictor of MACE following ACS

In addition, there was a grading to the primary end point which increased as the levels of myotrophin or NTproBNP increased. Positive myotrophin and NTproBNP (i.e. both above their respective median values) was associated with a significantly higher rate of the primary endpoint than having either peptide level above their medians or both peptides below their medians (log rank, 3.93; \(P = 0.048\); Figure 4).

When patients were examined for one or more raised myotrophin or NTproBNP peptide levels, the receiver-operating curve for NTproBNP yielded an AUC (area under the curve) of 0.56 (95% CI, 0.49–0.62; \(P = 0.091\)); for myotrophin, the AUC was 0.60 (95% CI, 0.54–0.67; \(P = 0.002\)). The logistic model combining the two markers yielded an AUC of 0.62 (95% CI, 0.56–0.68; \(P < 0.001\)), which exceeded that of either peptide alone.

**Myotrophin as a predictor of heart failure**

Myotrophin was raised in patients with heart failure compared with those without [641.1 (113.6–7445.6) fmol/ml compared with 397.9 (51.8–6990.4) fmol/ml respectively; \(P = 0.05\)]. In a Cox proportional hazards model, however, only age (HR, 1.04; \(P = 0.04\)), Killip class (HR, 2.41; \(P = 0.08\)) and male gender (HR, 0.47; \(P = 0.06\)) were found to be independent predictors of heart failure.

**DISCUSSION**

The aim of the present study was to assess the utility of myotrophin and NTproBNP in determining the prognosis of patients with ACS (acute coronary syndrome). The results of the study confirm the independent prognostic value of myotrophin in determining MACE in patients who have ACE. The predictive value of myotrophin provides risk prediction independent of NTproBNP and other known clinical predictors of MACE. Our study showed only a weak correlation between myotrophin and LVWMI and no correlation between myotrophin and peak troponin I. Myotrophin may be initially released from the myocardium, but may not necessarily be a marker of myocardial necrosis. Both myotrophin and NTproBNP are raised after an AMI, but their secretion patterns differ over the 5 days following an AMI with significant differences noted for both peptides. Myotrophin is raised very early after an AMI with levels staying fairly constant, suggesting a possible extra-cardiac source of secretion as well.

Reperfusion therapy has improved mortality post-MI; however, despite this the outcome of patients is still poor [17]. For this reason risk stratification remains important and may be useful in the selection of treatment regimes in the future.

We compared myotrophin with NTproBNP, which is a more stable byproduct in the production of BNP [18]. We have shown that myotrophin is superior at predicting MACE than NTproBNP in a Cox proportional hazards model. In addition, myotrophin had predictive power.
even in the patients with NTproBNP levels above the median, suggesting that further risk stratification of this high-risk group is possible. Furthermore, using a combination of myotrophin and NTproBNP, elevation of both above their respective medians was associated with a significantly higher rate of MACE than having either peptide level above the median or both peptides below the median. This is the first study showing the benefits of myotrophin as a prognostic marker in patients with ACSs. Over 80% of the population consisted of STEMI. It would be interesting to see if the data could be replicated in both STEMI and NSTEMI groups. Currently, the numbers are too small to give us meaningful information about this.

In univariate analysis, myotrophin was significantly raised in patients who suffered MACE compared with survivors. On multivariate analysis, myotrophin retained independent prognostic information but not NTproBNP. This was independent of established clinical variables. Myotrophin was raised in patients re-admitted with heart failure; however, on multivariate analysis, myotrophin did not give independent prognostic information. One of the limitations of the present study may be the number of patients recruited. Also, it would be interesting to see if similar data could be obtained from blood samples taken on admission. A larger study may be appropriate to detect the utility of myotrophin in predicting death and MI individually. Nevertheless, the present study is the first, however, reporting the utility of myotrophin in combination with NTproBNP in patients with ACS.

In conclusion, the present study reveals that the myotrophin system is activated during AMI, and that myotrophin is an independent predictor of MACE in patients with ACS. Myotrophin may be useful for risk stratification in ACS patients.

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REFERENCES


