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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 kD protein initially isolated from hypertrophied hearts of spontaneously hypertensive rats and acts by modulating NF κ B activity. We have reported the presence of myotrophin in patients with human systolic heart failure. However its role as a predictor of major adverse cardiac events (MACE) in patients with acute coronary syndrome (ACS) is unclear. We sought to investigate this and compared it to N-terminal pro B type natriuretic peptide (NTproBNP), a marker of MACE. We studied 356 ACS patients (276 men, mean age 63.0 ± 12.8 years, 80.8% STEMI, 19.2% NSTEMI). Blood measurement was made at 25-48hrs 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 endpoint of death, MI or need for urgent revascularisation. Over the median follow up period of 355 days (range 0-645) there were 28 deaths, 27 non-fatal MI and 73 patients required urgent revascularisation. Myotrophin was raised in patients with MACE compared to survivors (Median [Range], fmol/ml, 510.7; [116.0–7445.6] vs. 371.5; [51.8– 6990.4] fmol/ml; $p=0.001$). Using a Cox proportional hazards model myotrophin (HR 1.64, 95% CI: 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. 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, $p=0.006$). After an ACS, levels of myotrophin are more informative at predicting MACE than NTproBNP and may be useful to risk stratify patients.

Key words: Acute coronary syndrome, myotrophin, NTproBNP, ACS prognosis

Short title: Myotrophin is a powerful predictor of MACE following ACS

Introduction

Acute myocardial Infarction (AMI) 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 (ACO). 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 kD protein initially isolated from hypertrophied hearts of spontaneously hypertensive rats [4]. Elevated, levels have also been found in human cardiomyopathic hearts⁵ and our group has shown early activation of the myotrophin system in heart failure, which is more evident in males [6]. 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, c-jun, and, subsequently, of transcripts of skeletal alpha-actin, total myosin, and atrial natriuretic peptide [7]. These effects are thought to be mediated via protein kinase C activation [8]. Myotrophin is thought to interact with nuclear factor kappa B (NFkB) [9] disrupting the formation of the NFkB p50-p65 transactivating heterodimers while increasing the formation of repressive NFkB p50-p50 homodimers [10] Although there is evidence of increased activity of NFkB in human heart failure little is known about the role of NFkB in acute MI [11]. Human myotrophin has been cloned [12] and found to be highly homologous to the rat protein with its messenger ribonucleic acid (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. Over expression 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 as a useful marker for prognostication of AMI is unknown. In this 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 N-terminal pro B type natriuretic peptide (NTproBNP), which has been shown to be of prognostic benefit in this group of patients [2].

Methods

Study population

We studied 356 consecutive post myocardial infarction patients 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 sub categorised into ST segment elevation myocardial infarction (STEMI) or non-ST segment myocardial infarction (NSTEMI). Primary treatment for STEMI in our institution is thrombolytic therapy and was administered by the attending physician if the patient presented within 12 hours 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 25-48hrs after the onset of chest pain for determination of plasma myotrophin and NTproBNP. This time period was chosen as NTproBNP has been found to be useful at predicting MACE in post AMI patients with samples taken outside the first 24-hour window [2]. After 15 minutes bed rest, 20mL blood was collected into tubes containing EDTA and aprotinin. All plasma was stored at -70°C until assayed in a single batch.

Echocardiography

Transthoracic echocardiography was performed in patients using a Sonos 5500 instrument (Philips Medical Systems, Reigate, UK). A 16-segment left ventricular wall motion index (LVWMI) based on the American Society of Echocardiography model was derived by scoring each LV segment (1=normal, 2=hypokinesis, 3=akinesis and 4=dyskinesis (Paradoxical Motion), and dividing the total by the number of segments scored. Left ventricular ejection fraction (LVEF) 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-terminal of human NTproBNP and monoclonal mouse antibodies

were raised to the C- terminal. The N-terminal IgG was affinity-purified and biotinylated. Samples or NTproBNP standards were incubated in C-terminal IgG-coated wells with the biotinylated antibody for 24 hours at 4°C. Detection was with methyl-acridinium ester (MAE)-labelled streptavidin. The lower limit of detection was 0.3 fmol/ml. There was no cross reactivity with atrial natriuretic peptide, BNP, or C-type natriuretic peptide. Inter and intra coefficients of variation were 2.3% and 4.8% respectively. The results from this in-house assay are highly correlated ($r=0.90$, $P<0.0001$, $n=86$) to those obtained on the NTproBNP assay marketed by Roche Diagnostics Ltd. (Lewes, East Sussex, UK).

Myotrophin assay

The myotrophin assay was based on an immunoluminometric non-competitive assay. ELISA plates were coated with 100 μ L of anti-mouse IgG (100 ng/well, Sigma Aldrich Co., Gillingham, UK) in PBS. Wells were then blocked with 10% foetal calf serum in PBS. A specific commercial monoclonal antibody (IgG_{2b}, clone 49, Becton Dickinson Biosciences Pharmingen, Oxford, UK) served as the capture antibody. The detector antibody was a rabbit polyclonal antibody that had been previously reported by us in immunoluminometric assays of myotrophin [6], but for the current assays, was further enriched by affinity purification on a column of myotrophin peptide (LTAFEATDNQAI, corresponding to amino acids 102-113 in the C-terminal domain of human myotrophin) immobilised onto Affigel 10 (Biorad Laboratories, Hemel Hempstead, UK). Bound specific antibody was then eluted using 0.1 M glycine-HCl (pH 2.4) and rapidly neutralised with Tris base. 100 μ L of immunoluminometric assay buffer containing 10ng of the Becton Dickinson monoclonal antibody was pipetted into the ELISA wells, followed by 50 μ L of plasma samples and standards.

Plates were incubated overnight at 4°C. After washes, the detector affinity purified rabbit antibody (20ng/100µL) was pipetted into the wells and plates were incubated at room temperature for 3 hours. Following washes, a goat biotinylated anti-rabbit IgG (Rockland Immunochemicals Inc., Gilbertsville, PA, USA, previously pre-adsorbed with human, rabbit, mouse serum proteins) at a dilution of 1:200000 was incubated within the wells for 1 h, followed by MAE-labelled streptavidin for another 1½ hours. Chemiluminescence was elicited with sequential injections of H₂O₂ in nitric acid, followed by sodium hydroxide containing cetyl trimethylammonium bromide, as described [6,16]. Intra and inter-assay coefficients of variation were found to be less than 10%.

End points

We assessed the value of both myotrophin and NTproBNP for the prediction of death, MI or need for urgent revascularisation as a combined primary endpoint. We also investigated the secondary endpoint of heart failure. Hospitalization for heart failure was defined as a hospital admission for which heart failure was the primary reason. Hospitalisation for AMI was defined as above. Endpoints 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 (SPSS Inc, Chicago, Illinois). 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 revascularisation. NTproBNP and myotrophin were normalised by log transformation. Thus, hazard ratios refer to a tenfold rise in the levels of these markers. Kaplan-Meier survival curves were generated to visualise the relationship between the peptides NTproBNP and myotrophin and the composite endpoints. 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 readmitted with AMI, 73 patients required urgent revascularisation and there were 28 readmissions with heart failure.

Echocardiographic data was available for 297 (83.6%) of the 356 patients and performed at a median of 3.5 days (range 2–5) after presentation with AMI. 36 echocardiograms were unanalysable and 22 patients did not receive an echocardiogram.

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 this revealed a significant difference over the 5 days ($p<0.01$) and is shown in figure 1. Levels in AMI patients were significantly higher than those observed in the control subjects (Median [Range], fmol/ml, 405.7; [51.8– 7445.5]; vs. 348.1; [34.1–3982.9]; $p=0.044$) and was higher in patients who died (Median [Range], fmol/ml, 595.3; [203.4–7445.6] vs. 397.1; [51.8– 6990.4]; $p=0.005$). There was 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, hypercholesterolemia, troponin level or whether a patient received thrombolysis or not.

NTproBNP levels in patients and controls

NTproBNP was significantly elevated in AMI compared with controls (Median [Range], fmol/ml, 1367.1; [0.3–12175.1] vs. 10.1; [0.3– 134.4]; $p<0.001$) and was higher in patients who had a MACE (1846.7; [0.3–11906.5] vs. 1268.0 [0.30– 12175.1] fmol/ml; $p=0.061$). The time course of secretion of NTproBNP revealed a significant difference over the 5 days ($p<0.0001$) and is shown in figure 2.

Relationship between myotrophin and echocardiographic parameters

For the whole population, mean LVWMI was 1.53 (range 1.08-2.75) and EF 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] vs. 1.41 [1.00-2.60], $p<0.0001$).

However LVEF was no different between the two groups (median [range] 35 [9-68] vs. 37 [13-65] %, $p=0.074$). There was correlation of myotrophin with LVWMI ($r=0.155$, $p=0.007$) NTproBNP also correlated positively with LVWMI ($r=0.373$, $p<0.0001$) and negatively with the EF ($r=-0.30$, $p<0.0001$).

Myotrophin and NTproBNP as predictors of MACE

Myotrophin was raised in patients with MACE compared to survivors (Median [Range], fmol/ml, 510.7; [116.0–7445.6] vs. 371.5; [51.8– 6990.4]; $p=0.001$). No difference was noted in patients who mounted a larger myotrophin response with regard to territory of infarct, STEMI vs. NSTEMI, or background previous drug therapy (including previous use of aspirin, beta-blockers, statins, ACE 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: 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).

In addition there was a grading to the primary endpoint, which increased as the levels of myotrophin or NTproBNP increased. A 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 area under the

curve (AUC) 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 2 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 (median [range] fmol/ml, 641.1[113.6-7445.6] vs. 397.9[51.8-6990.4], $p=0.05$). 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 sex (HR 0.47, $p=0.06$), were found to be independent predictors of heart failure.

Discussion

The aim of this study was to assess the utility of myotrophin and NTproBNP in determining the prognosis of ACS patients. The results of this study confirm the independent prognostic value of myotrophin in determining MACE in patients who have an acute coronary syndrome. The predictive value of myotrophin provides risk prediction independent of NTproBNP and other known clinical predictors of MACE. Our study showed only 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 and 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 the outcome of patients despite this is still poor [17]; for this reason risk stratification remains important and may be useful to select treatment regimes in the future.

We compared myotrophin with NTproBNP, which is a more stable by-product 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 MACE rate 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 acute coronary syndromes. Over 80% of the population consisted of STEMI. It would be interesting to see if the data can 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 to survivors. On multivariate analysis myotrophin retained independent prognostic information but not NTproBNP. This was independent of established clinical variables. Myotrophin is raised in patients readmitted with heart failure however on multivariate analysis myotrophin does not give independent prognostic information. One of the limitations of this study may be the number of patients recruited. Also it would be interesting to see if similar data can be obtained from admission bloods. A larger study may be appropriate to detect the utility of myotrophin in predicting death and MI individually.

This is the first study 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 an 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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Legends

Figure 1 Time dependent changes in myotrophin (mean \pm SEM) after onset of AMI

Figure 2 Time dependent changes in NTproBNP (mean \pm SEM) after onset of AMI

Figure 3 Kaplan-Meier Curve: Time to MACE related to above and below plasma myotrophin

Figure 4 Kaplan-Meier Curve: Time to Primary Outcome related to above and below median plasma myotrophin and NTproBNP levels. 1) Below median myotrophin and NTproBNP, 2) Below median myotrophin or NTproBNP 3) Above median myotrophin and NTproBNP

Table 1 Characteristics of Patients and Controls in the Study. Values are means (SD) or numbers (percentage)

	Controls	AMI Patients
Number	40	356
Age (in years)	60.4 ± 11.6	63.0 ± 12.8
Male Sex	25	276 (77.7)
Previous Medical History		
Myocardial infarction	None	51 (14.4)
Angina Pectoris	None	57 (16.1)
Hypertension	None	153 (43.1)
Diabetes mellitus	None	71 (20.0)
Hypercholesterolaemia	None	105 (29.6)
Obesity	None	43 (12.1)
Current/Ex-Smokers	None	125 (35.2)
ST-elevation AMI	None	287 (80.8)
Thrombolytic	None	188 (53)
Territory of Infarct		
Anterior	N/A	146 (41.1)
Inferior	N/A	142 (40.0)
Other/undetermined	N/A	66 (18.6)
Killip Class on Admission		
I	N/A	136 (38.3)
II	N/A	178 (50.1)
III	N/A	35 (9.9)

IV	N/A	6 (1.7)
Peak CK (I/U)	N/A	1313.5 ± 1453.9
Peak Troponin I (ng/ml)	N/A	22.4 ± 33.2
Creatinine (μmol/l)	N/A	101.0 ± 28.2

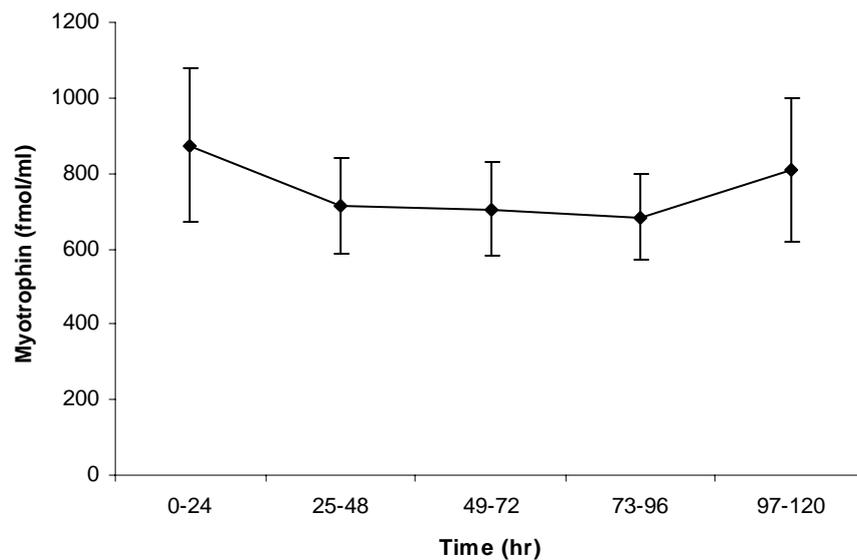


Figure 1

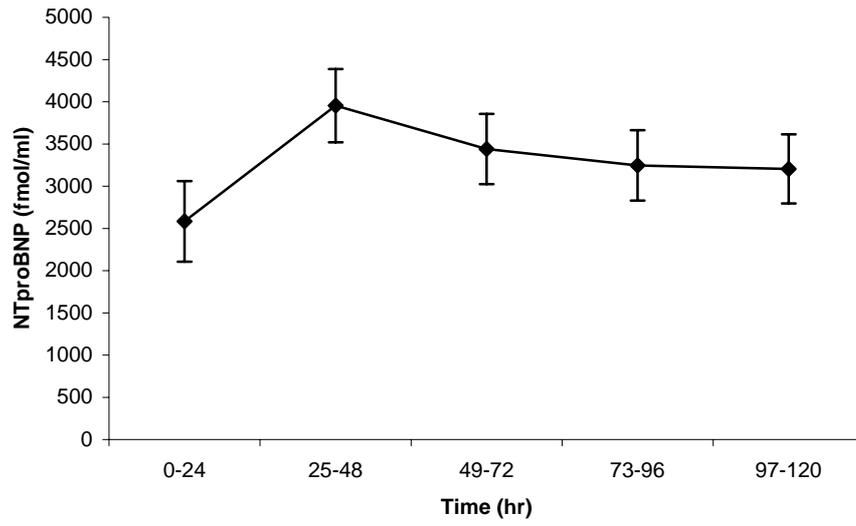


Figure 2

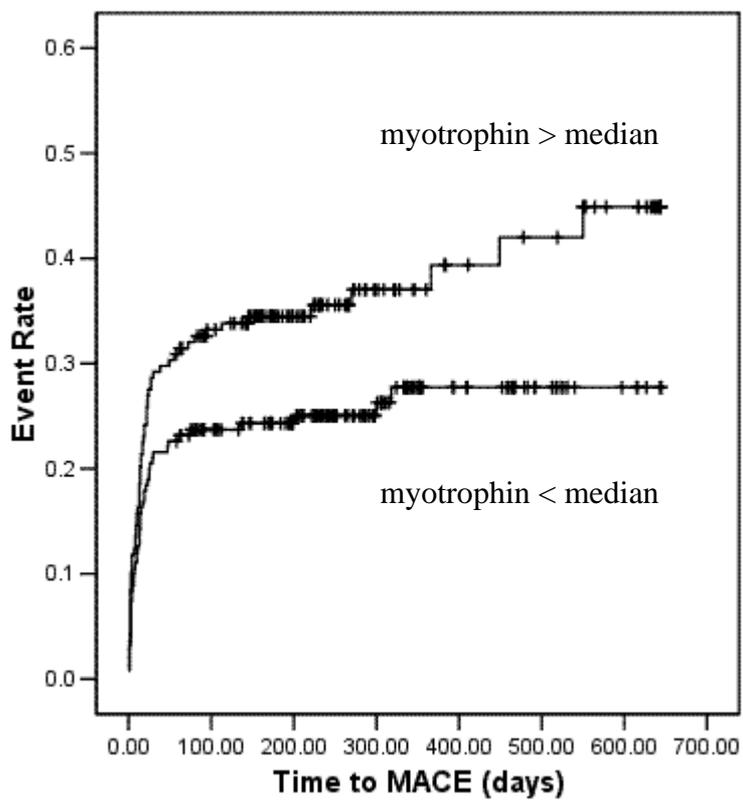


Figure 3

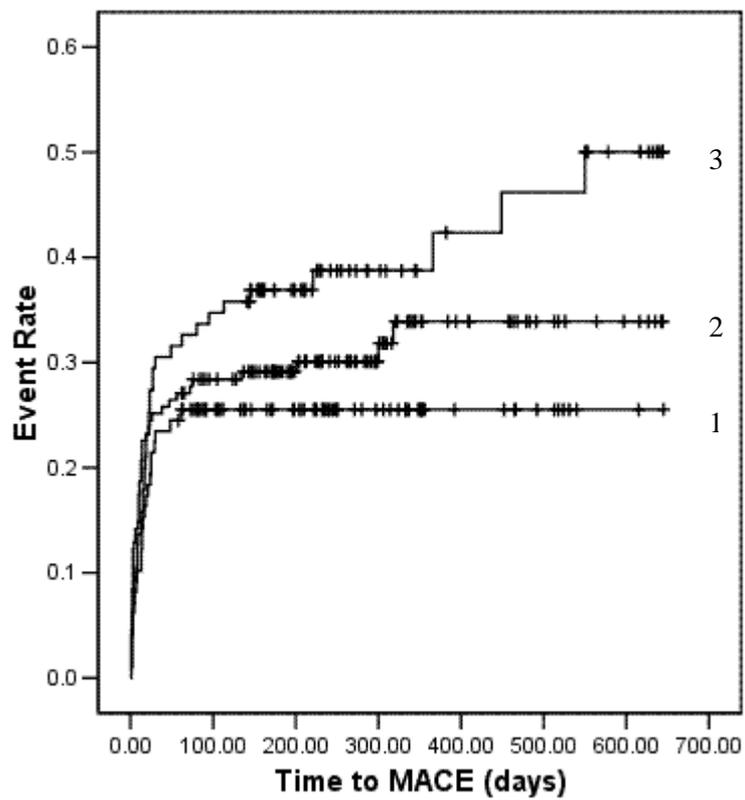


Figure 4