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Oxygen-Regulated Protein-150 and prognosis following myocardial infarction

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Prof. L. Ng has submitted patents for the University of Leicester on biomarkers in heart disease.

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ABSTRACT

Oxygen regulated protein (ORP150) is a chaperonin expressed in tissues undergoing hypoxic or endoplasmic reticulum stress. We investigated plasma levels of ORP150 in acute myocardial infarction (AMI) patients and its relationship to prognosis together with a known risk marker N-terminal pro-B-type natriuretic peptide(N-BNP). Plasma from 396 consecutive AMI patients was obtained for measurement of ORP150 and N-BNP. Mortality and cardiovascular morbidity (acute coronary syndromes/heart failure) was determined during follow-up. A specific ORP150 assay detected the 150kD protein in plasma extracts, including 3 and 7kD fragments. During follow-up (median 455 days) 43(10.9%) patients died. Both N-BNP and ORP150 levels were higher in those who died compared to survivors (median[range] N-BNP 724[14.5-28840] vs 6167[154.9-33884] pmol/L P<0.0005; ORP150 257[5.9-870.9] vs 331[93.3-831.8] pmol/L P<0.001). In a Cox regression model for mortality prediction, both N-BNP(odds ratio 5.06, P<0.001) and ORP150(odds ratio 2.39, P<0.01) added prognostic information beyond creatinine and use of thrombolytics. Kaplan-Meier survival analysis revealed ORP150 added prognostic information to N-BNP, especially in those with supramedian N-BNP levels. A simplified dual-marker approach with both markers below, either above or both above their respective medians effectively stratified mortality risk(log rank statistic for trend 32.7, P<0.00005). ORP150 levels were not predictive of other cardiovascular morbidity (acute coronary syndromes, heart failure). ORP150 and peptide fragments derived from it are secreted following AMI and provides independent prognostic information on mortality. High levels associated with endoplasmic reticulum/hypoxic stress predict a poor outcome.

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INTRODUCTION

The prognosis following acute myocardial infarction (AMI) varies widely, and recent evidence suggests that biochemical markers may be suitable for risk stratification. In particular, plasma levels of B-type natriuretic peptide (BNP) or the N-terminal portion derived from its precursor (N-BNP) obtained in the subacute phase have proved to be useful in predicting mortality [1,2]. These peptides may reflect changes in wall stress following AMI. An additional biochemical marker which reflects tissue hypoxia may confer improvements in risk assessment.

Recent work on tissue hypoxia and endoplasmic reticulum stress has led to the cloning of a stress protein called Oxygen Regulated Protein (ORP150), a 150 kD endoplasmic reticulum associated protein that functions as a chaperone for protein folding and maturation [3]. The induction of this protein in rat astrocytes, human aortic vascular myocytes and mononuclear leucocytes showed specificity for hypoxia but not other stressful stimuli [3,4]. In addition, tissue extracts prepared from human atherosclerotic lesions demonstrated increased expression of ORP150 mRNA and protein, with most of the mRNA found in macrophages [4]. Its functions include a protective effect against hypoxia-induced damage and endoplasmic reticulum stress, since reducing its expression with antisense oligonucleotides leads to enhanced susceptibility of mononuclear phagocytes to hypoxic damage [4]. Evidence for a function of ORP150 in vascular disease comes from observations on a stroke model in mice [5,6]. In a mouse model of cerebral ischaemia, there was rapid induction of ORP150 mRNA and protein in the hypoxic neurons, even within the ischaemic and energy depleted zones [5]. In ischaemic human brains, although ORP150 expression in neurons was only sparingly induced, there was a significant induction of ORP150 in astrocytes [6]. Neurones overexpressing ORP150 were resistant to hypoxic stress and mice genetically engineered to overexpress ORP150 in their neurons had smaller strokes under ischaemic stress [6]. Cytoprotection was associated with suppressed caspase-3-like activity and enhanced brain-derived neurotrophic factor (BDNF), indicating a role for ORP150 in cytoprotection under hypoxic conditions [6]. In cultured rat cardiomyocytes, hypoxia-reoxygenation induced cell death was enhanced or attenuated by reduced or increased ORP150 expression respectively within the cells [7]. Decreased ORP150 expression was associated with enhanced caspase-3 and -8 activation, cytochrome-c release and DNA fragmentation, reflecting increased apoptotic cell death. Increased ORP150 expression in rat hearts led to improved functional recovery after ischaemia [7]. There is also a suggestion that ORP150 may be secreted in diabetes, reflected in an increased auto-antibody titre to ORP150 [8].

In the present study, we investigated whether ORP150 was present in human plasma after cardiac tissue hypoxia (AMI), and whether its measurement following AMI improves the risk stratification provided by N-BNP, a recognised risk marker.

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MATERIALS AND METHODS

Study population

We studied 396 consecutive patients admitted to the Coronary Care Unit of Leicester Royal Infirmary with AMI between September 1999 and April 2001. Acute myocardial infarction was defined as presentation with at least two of three standard criteria, i.e. appropriate symptoms, acute ECG changes of infarction (ST elevation, new LBBB), and a rise in creatine kinase (CK) to at least twice the upper limit of normal, i.e. >400 IU/L. Relevant clinical information such as prior medical history, pharmacological therapy, Killip class, cardiac enzymes, renal function and lipid profile was collected. All patients were followed up and end-points of all-cause mortality or cardiovascular morbidity (such as further hospitalisation with acute coronary syndromes, heart failure or for revascularisation) were validated by review of hospital notes. Normal subjects (n=125, 95 male, mean±SD age 64.2±7.5 years) were recruited from the community and had no symptoms, signs, echocardiographic or ECG evidence of heart disease and were on no treatment. All subjects gave informed consent to participation in the study, which was approved by the local Ethics Committee. The study conforms to the principles of the Declaration of Helsinki.

Blood Sampling

A single blood sample for measurement of plasma ORP150 and N-BNP was taken between 72-96 hours after symptom onset, based on previous work from our group [2]. 20mls of peripheral venous blood was drawn into pre-chilled Na-EDTA (1.5mg/ml blood) tubes containing 500 IU/ml aprotinin. Samples were centrifuged at 3000 rpm at 4°C for 15 min, before the plasma was separated and stored at −70 °C until assay.

Assay for ORP150 and N-BNP

N-BNP was measured in 10-20µL of unextracted plasma using a validated in-house immunochemiluminometric assay, as previously described [2].

The ORP150 assay was a competitive assay, constructed using a polyclonal antibody raised in rabbits immunised with keyhole limpet haemocyanin conjugated with a peptide corresponding to the N-terminal domain (amino acids 33-45) of the human ORP150 sequence (LAVMSVDLGSESM). IgG from the sera was purified on protein A sepharose columns and then affinity-purified on an Affigel column with immobilised peptide. The biotinylated peptide tracer was purified on reverse-phase HPLC using an acetonitrile gradient. Prior to assay, plasma was extracted on C_{18} Sep-Pak columns and dried on a centrifugal evaporator. Plasma extracts and standards were reconstituted with ILMA (immunoluminometric assay) buffer consisting of (in

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mmol/l) NaH_2PO_4 1.5, Na_2HPO_4 8, NaCl 140, EDTA 1 and (in g/l) bovine serum albumin 1, azide 0.1. ELISA plates were coated with 100 ng of anti-rabbit IgG (Sigma Chemical Co., Poole, UK) in 100 µl of 0.1 mol/l sodium bicarbonate buffer, pH 9.6. Wells were then blocked with 0.5% bovine serum albumin in bicarbonate buffer. A competitive ILMA was set up by preincubating 20 ng of the IgG with standards or samples within the wells. After overnight incubation, 50 µl of the diluted biotinylated ORP150 peptide tracer (100 fmol) was added to the wells. Following another 24 h of incubation at 4°C, wells were washed 3 times with a wash buffer (NaH_2PO_4 1.5 mmol/l, Na_2HPO_4 8 mmol/l, NaCl 340 mmol/l, Tween 0.5 g/l, sodium azide 0.1 g/l). Detection was with streptavidin labeled with methyl-acridinium ester, as described [2]. Within assay coefficients of variation were 3.1, 4.3 and 5.9% for 2, 30, 500 fmol/tube respectively. There was no cross-reactivity with peptides previously demonstrated to be elevated in heart failure such as ANP, BNP, N-BNP or CNP. Markers of AMI such as troponin I, troponin T or creatine kinase would not be extracted by C_{18} Sep-Pak columns due to their large molecular masses. In addition, neither CKMB (35 ng/ml) or Troponin I (50 ng/ml) was detectable in the ORP150 assay. These levels of CKMB and Troponin I are similar to those described in AMI after 3-5 days [9].

Size exclusion chromatography and gel electrophoresis of plasma extracts

Plasma extracts were fractionated by isocratic size exclusion chromatography on a 300 x 7.8mm Bio-Sep SEC S2000 column (Phenomenex, Macclesfield, Cheshire, UK) using 50 mmol/l NaH_2PO_4 (pH 6.8) at a flow rate of 1 ml/min as the mobile phase. Standards used to establish molecular weights included IgG (150kD), BSA (68kD) , ovalbumin (44kD), soybean trypsin inhibitor (20kD), aprotinin (6.5kD) and tryptophan (204D) (from Sigma Chemical Co, Poole, UK.). Fractions collected every 20 sec were dried on a centrifugal evaporator before assaying for ORP150 as above.

Western Blotting of cell extracts

GM-1 monocytic cells (Roche Pharma, Basel [10]) were cultured in RPMI growth medium (containing 10% foetal calf serum). The GM-1 cell line is derived from the human U937 monocyte cell line and could be differentiated using 1µmol/L retinoic acid, 10nmol/L 1,25-dihydroxycholecalciferol and 200 U/ml interferon-γ (all from Sigma Chemical Co., Poole, UK). Following 4 days of culture with these differentiation-inducing agents, GM-1 cells were treated with 50µmol/L hydrogen peroxide for 3 h as this has been reported to upregulate ORP150 expression [11]. Cells were extracted in Laemlli-sample buffer, reduced with dithiothreitol, and 100µg protein samples resolved on 10% SDS-polyacrylamide gels. Following western blotting, nitrocellulose membranes were blocked overnight in 10% milk/tris buffered saline containing 0.1%

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Tween-20. Blots were incubated with 1μg/ml ORP150 antibodies (our in-house rabbit polyclonal antibody and a commercial monoclonal mouse ORP150 IgG2a antibody (clone 2F07) from Immuno-Biological Laboratories (IBL) Co. Ltd., Gunma, Japan which was raised against a recombinant protein containing aa 508-999 of the ORP150 sequence) for 2 h. The specificity of the IBL monoclonal antibody had been confirmed on ORP150 transgenic and knock-out mice. The second antibodies used were horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG respectively. Detection was by enhanced chemiluminescence.

Statistical analysis

Data were analysed on SPSS Version 10.1 (SPSS Inc., Chicago IL) and presented as mean (SD) or median (range) for data with non-Gaussian distribution, which were log transformed prior to analysis. For continuous variables, mean levels in normal subjects and those suffering or being spared each clinical event were compared by one-way analysis of variance (ANOVA, with Bonferroni correction for multiple comparisons) or the Kruskal-Wallis test (for non-parametric analyses). Mann-Whitney P values are reported for non-parametric analyses. Clinical variables included as potential predictors of outcome included age, Killip class (dichotomised as class 1 versus 2, 3 or 4), log plasma creatinine and history of prior AMI, heart failure, hypertension or diabetes. The independent predictive power of peptide levels and clinical factors was tested using Cox proportional hazards regression, using both forward and backward likelihood-ratio analysis. Kaplan-Meier survival curves were constructed for groups with ORP150 or N-BNP above and below their respective median values and the log rank statistic for trend with associated P values are quoted. Two tailed P values of <0.05 were considered significant.

RESULTS

A Western blot of GM-1 cell extracts is illustrated in figure 1a. Both the commercial ORP150 monoclonal antibody and our in-house rabbit polyclonal affinity-purified antibody detected a single band of approximately 147kD molecular weight, which resembles the reported molecular weight of ORP150 [3,4].

A typical standard curve for ORP150 peptide is illustrated in Figure 1b, showing a fall in chemiluminescence with increasing concentrations of the peptide. Dilutions of patients' plasma extracts confirmed parallelism with the standard curve. Isocratic size exclusion chromatography on human plasma extracts confirmed the presence of 150 kD protein (the predicted molecular weight of mature ORP150 protein), as well as 2 other immunoreactive fractions at about 3 and 7 kD

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(Figure 2), which are likely to be degradation products consisting of the N-terminal of ORP150 (the epitope to which the antiserum was directed).

ORP150 was detectable in the extracted plasma from 125 normal subjects, who were age and gender matched with the AMI patients. The median [range] of ORP150 was 138 [13-562] pmol/L, and N-BNP was 22.4 [5.7-631] pmol/L (figure 3). In normal subjects, ORP150 was not correlated with age, although there was a weak correlation with plasma creatinine ($r_s = 0.212$, $P<0.02$).

Table 1 shows the clinical characteristics of the patients. Patients who died (n=43) during the median follow up period of 455 days were older, had higher plasma creatinine levels and a higher incidence of previous MI. No patient was lost to follow-up. Those who died did not have a higher prevalence of anterior AMI, although they had a lower thrombolytic treatment rate ($P<0.0005$ by χ^2 test). Plasma levels (median [range] pmol/L) of N-BNP (851 [14.5-33884]) and ORP150 (266 [5.9-870.9]) were higher in the post-AMI patients compared to controls (figure 3, $P<0.001$ for both). Plasma levels of both N-BNP and ORP150 were significantly higher in post-AMI patients who died compared to survivors (Table 1 and figure 3, $P<0.0005$ and $P<0.004$ for N-BNP and ORP150 respectively, with Bonferroni correction). There was no difference in ORP150 levels in patients with a previous history of AMI, diabetes, heart failure or hypertension.

Receiver Operating Characteristic (ROC) curves for N-BNP and ORP150 in prediction of all-cause mortality are plotted in figure 4, the area under the curves being 0.808 (95% confidence interval 0.736-0.880) and 0.661 (95% confidence interval 0.575-0.747) respectively. The correlation between ORP150 levels and N-BNP ($r_s = 0.149$, $P<0.003$) or plasma creatinine ($r_s = 0.171$, $P<0.001$) was modest, with no significant correlation with age. Although plasma N-BNP was correlated with peak plasma creatine kinase levels ($r_s = 0.226$, $P<0.0005$), ORP150 showed no such correlation ($r_s = 0.02$, P=NS). ORP150 levels did not differ according to gender or site of infarction.

Patients with Killip class>1 had slightly higher ORP150 levels than those with Killip class 1, which did not achieve conventional levels of significance (281.8[5.9-870.9] vs 251.2[8.9-831.8] pmol/L, $P<0.07$). There were no significant differences in ORP150 levels between those patients with Killip classes 2, 3 or 4. This contrasts with N-BNP (median,[range]) where higher levels were demonstrated in patients with age>median 67 years (1412, [14.5-33884] vs 543, [14.5-14125] pmol/L, $P<0.0005$), in female gender (female 1445, [35.5-33884] vs male 691 [14.5-28840] pmol/L, $P<0.0005$), in those with anterior AMI (anterior AMI, 1096[14.5-16982] vs other site of AMI, 692[14.5-33884] pmol/L, $P<0.02$), and in those with Killip class>1 (Killip class>1,1244[14.5-33884] vs Killip class=1, 513[14.5-21877] pmol/L, $P<0.0005$).

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Plasma N-BNP, ORP150 and all-cause mortality

Kaplan-Meier survival curves for supra and inframedian peptide levels for N-BNP (median value 851 pmol/L) or ORP150 (median value 266 pmol/L) are presented in figure 5. Both plasma markers were significant predictors of mortality (log rank ?^{2} statistic for N-BNP 29.53, P<0.00005; for ORP150 8.62, P<0.003). The predictive value of ORP150 was examined in patients stratified for N-BNP levels (figure 6). The log rank statistic for supra- compared to infra-median ORP150 levels adjusted for supra- compared to infra-median N-BNP levels was 6.98 (P<0.008 for trend). However, the incremental value of ORP150 was most evident in the group with supramedian N-BNP levels >851 pmol/L (log rank ?^{2} statistic 5.26, P<0.02). ORP150 levels provided no incremental information on survival in those with inframedian N-BNP values (log rank ?^{2} statistic 2.04, P=NS).

The unadjusted odds ratios for mortality were 9.45 for N-BNP, 2.88 for ORP150. Using stepwise forward likelihood ratio logistic regression analysis for prediction of all-cause mortality with supra- and inframedian levels of ORP150, N-BNP, log creatinine, age, previous history of AMI, anterior site of AMI, use of thrombolysis and peak creatine kinase levels, a model with ORP150, N-BNP, log creatinine and use of thrombolysis as the only independent predictive factors accounted for a Nagelkerke r^2 of 0.33 (P<0.0005). Odds ratios were 2.98 (P<0.006), 5.87 (P<0.001), 61.29 (P<0.001) and 0.25 (P<0.0005) for supra- compared to inframedian values of ORP150, N-BNP, log creatinine and use of thrombolysis respectively. Killip class was not an independent predictor of death.

Cox proportional hazard modeling was employed to adjust for potential confounding factors in prediction of mortality. Table 2 shows that supra- compared to inframedian ORP150 levels maintained independent predictive value, together with supra- compared to inframedian N-BNP levels, use of thrombolysis and log creatinine levels.

In order to simplify application of this predictor, we utilised the ranks in both the N-BNP and ORP150 ranked groups to yield a prognostic index, with patients divided into 3 groups (both peptides below the medians, either peptide above the median and both peptides above the medians). Figure 7 shows the survival analysis using this new prognostic index, showing 1 death during the observational period in the group with both peptides below the medians (n=107), a high mortality rate in those with both peptides above the medians (27 deaths in 105 patients), and an intermediate mortality rate in those with either peptide above the medians (15 deaths in 184 patients) (log rank statistic for trend 32.7, P<0.0005).

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ORP150 and other cardiovascular morbidity

ORP150 measured in the subacute phase of AMI was not associated with other cardiovascular end-points such as rehospitalisation with heart failure (n=24(6.1%)), acute coronary syndromes (including unstable angina (n=62(15.7%)), non-fatal AMI (n=18(4.6%)) or revascularisation procedures (n=52(13.1%))). There was also no relationship with development of angina or positive exercise tests following AMI.

DISCUSSION

ORP150 was initially described as a chaperone protein localised to the endoplasmic reticulum with a role in protein folding and maturation [6]. Suppression of its expression led to larger cerebral infarct size[6] and enhanced susceptibility to hypoxic damage [4,7]. The specificity of our in-house antibody was similar to that of a commercially available ORP150 monoclonal antibody which had been validated in transgenic and knock-out mice models. Although the ORP150 protein is predominantly intracellular, damage to plasma membranes during AMI may permit leakage of proteins to the extracellular space. Thus, tissue ORP150 expression may be reflected in plasma levels, which we describe for the first time here. These levels may in turn reflect endoplasmic reticulum stress in hypoxic tissues.

Our data suggests that ORP150 in plasma was not solely the 150kD protein, but smaller fragments containing the N-terminal were also present. Fragmentation of the protein could have occurred from endoproteases present in necrotic tissue.

The levels of ORP150 following AMI were weakly correlated with N-BNP and both were higher in those who died during follow-up compared to survivors. However the factors that affected plasma N-BNP had little influence on ORP150 levels (for example, Killip class, site of infarction, age, creatine kinase levels, gender). Thus N-BNP and ORP-150 may reflect different processes in the pathophysiology of AMI, with N-BNP reflecting wall stress and ORP-150 endoplasmic reticulum stress. This was confirmed by the independent predictive value for death of ORP150 when used in conjunction with N-BNP in Cox proportional hazards analysis and in binary logistic regression analysis. From the physiology of the natriuretic peptide and ORP150 systems, they are likely to be counter-regulatory to ischaemic damage and the relationship with a poor prognosis may reflect the degree of tissue damage. However, a deleterious effect of excessive ORP150 expression cannot be discounted, as recent evidence suggests that transgenic mice overexpressing this protein suffer from age-related vacuolar degeneration of cardiac myocytes [12].

When patients were stratified for N-BNP levels, it was evident that ORP150 had incremental value in defining mortality risk in those with supramedian N-BNP levels, enabling even higher risk patients to be distinguished. Thus, those patients with both markers below the median have a good

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prognosis compared to those with both markers above the median. The effective identification of these high-risk patients early in the course of AMI may facilitate the focusing of therapy towards this group (whether pharmacological, interventional or device-related).

A limitation of our study is the uncertainty concerning whether modulating endoplasmic reticulum stress and ORP150 following AMI could influence the poor outcome, and this should be the subject of further research.

Conclusion

This is the first report of a relationship between high plasma levels of a chaperone hypoxic stress-related protein (ORP150) following AMI and increased mortality. ORP150 has incremental predictive value for all-cause mortality over an accepted risk marker such as N-BNP. Endoplasmic reticulum stress following AMI may indicate a poor prognosis.

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Figure Legends

Figure 1a. A western blot of GM-1 cell extracts. Both the in-house rabbit polyclonal antibody and the IBL ORP150 monoclonal antibody detected a single band of molecular weight of about 147 kD.

Figure 1b. A standard curve for the ORP150 peptide competitive immunoassay. 3 patients' plasma extracts (solid circles joined by solid line or hollow triangles joined by dotted lines) were diluted in two fold steps, showing parallelism with the standard curve.

Figure 2. Size exclusion chromatography with analysis of the fractions for ORP150. The points of elution of markers for 150 kd (IgG), 20 kD (soybean trypsin inhibitor) and 6.5 kD (aprotinin) are indicated by arrows. Three peaks of immunoreactivity for ORP150 are evident at 150, approximately 7 and approximately 3 kD.

Figure 3. Box and whisker plots showing plasma N-BNP and ORP150 levels in normal subjects, survivors post-AMI and patients who died post-AMI. Boxes represent interquartile ranges, with the median level marked. The whiskers represent the 97.5 percentiles of observed values.

Figure 4. Receiver Operating Characteristic curves for N-BNP and ORP150 in prediction of all-cause mortality.

Figure 5. Kaplan-Meier survival analysis of patients following AMI, stratifying patients as below or above the median values of plasma N-BNP (median value 851 pmol/L) or of ORP150 (median value 266 pmol/L).

Figure 6. Kaplan-Meier survival analysis of patients following AMI, illustrating the effect of stratifying patients as below or above the median value of plasma ORP150 (266 pmol/L) for the supra and inframedian N-BNP strata (median value 851 pmol/L).

Figure 7. Kaplan-Meier survival analysis of patients following AMI, stratifying patients as having both N-BNP and ORP150 levels below median, either marker above the median or both markers above the median (median values of N-BNP and ORP were 851 and 266 pmol/L respectively).

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Table 1. Clinical details of the post-AMI patients. Medians (range) are reported and P values are quoted for Mann-Whitney, Kruskal Wallis or χ^2 test.

	All Patients (N=396)	**Dead (n=43)**	**Survivors (n=353)**	**P-Value (Dead v Survivors)**
Age (years)	66.5 (32-95)	73 (55-89)	65 (32-95)	<0.0005
Male	295 (74.5%)	33 (76.7%)	262 (74.2%)	0.720
Follow Up (days)	465 (5-764)	67 (5-639)	479 (179-764)	<0.0005
Anterior AMI	143	18	125	
(thrombolysed)	(101)	(7)	(94)	<0.0005
Other site AMI	253	25	228	
(thrombolysed)	(164)	(8)	(156)	<0.0005
Previous History:				
(n, (%))				
AMI	78 (19.7%)	17 (39.5%)	61 (17.3%)	0.001
Hypertension	134 (33.8%)	19 (44.2%)	115 (32.6%)	0.129
Diabetes	88 (22.2%)	13 (30.2%)	75 (21.3%)	0.181
Heart Failure	20 (5.1%)	4 (9.3%)	16 (4.5%)	0.177
Highest Killip Class				
I	172 (43.7%)	9 (21.4%)	163 (46.3%)	<0.0005
II	160 (40.6%)	14 (33.3%)	146 (41.5%)	
III	53 (13.5%)	15 (35.7%)	38 (10.8%)	
IV	4 (9.5%)	4 (9.5%)	5 (1.42%)	
Plasma Measurements				
Creatinine	96	128	94	<0.0005
(μmol/L)	(35-346)	(61-346)	(35-265)	
Peak Creatine Kinase	1371 (75-	1191	1394	0.419
(mU/L)	15456)	(312-12652)	(75-15456)	
N-BNP	851	6167	724	P<0.0005
(pmol/L)	[14.5-33884]	[154.9-33884]	[14.5-28840]	
ORP150	266.1	331	257	P<0.004
(pmol/L)	[5.9-870.9]	[93.3-831.8]	[5.9-870.9]	

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Table 2 Cox Regression Model for survival post-AMI. B is the coefficient for the variable that is specified in the regression equation and the SEM is the standard error of that estimate.

	B	SEM	P value	Hazard Ratio
N-BNP > 851 pmol/L	1.622	.492	0.001	5.06
ORP150 > 266 pmol/L	0.87	.345	0.01	2.39
Log Plasma Creatinine	3.541	.0.931	0.001	34.50
Thrombolysis	-1.224	.324	0.001	0.29

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Figure 1a

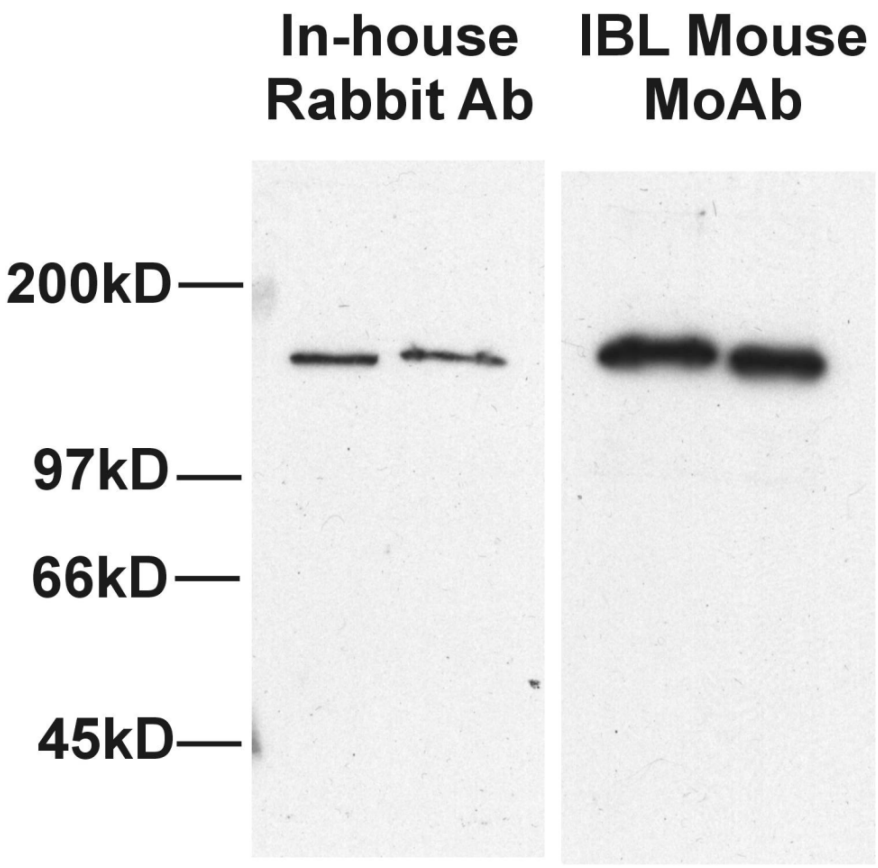

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Figure 1b

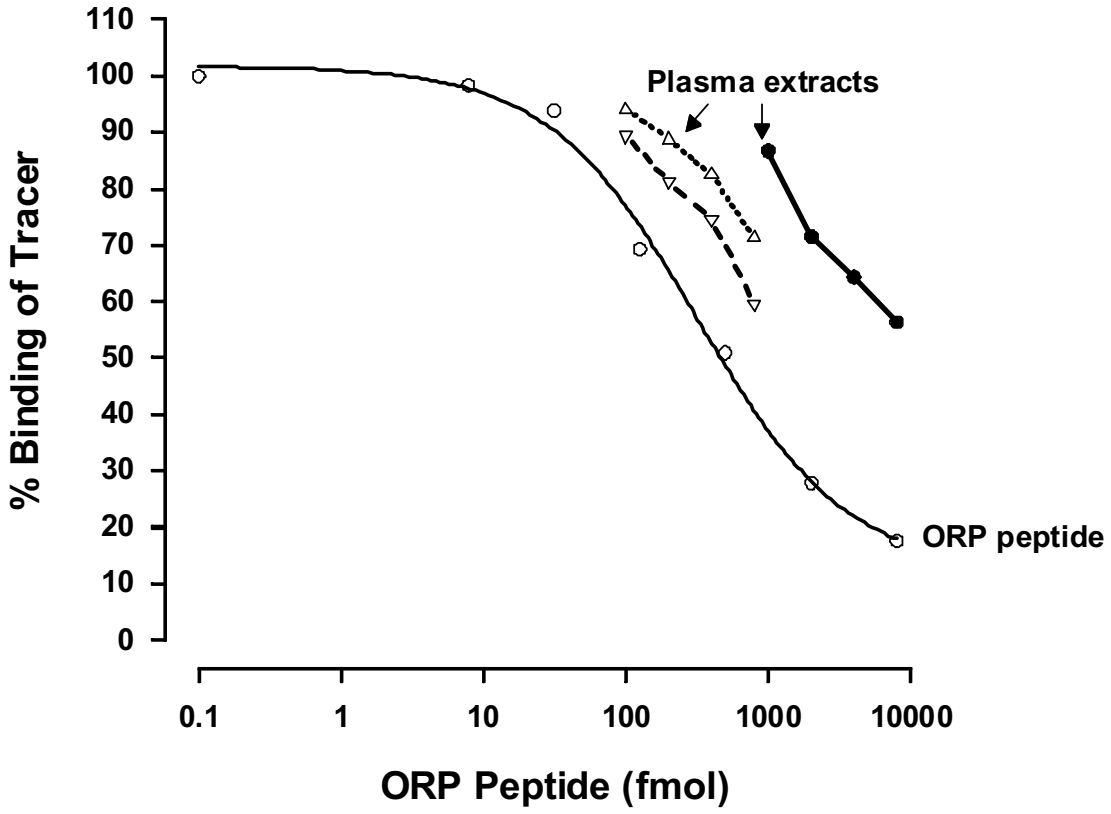

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Figure 2

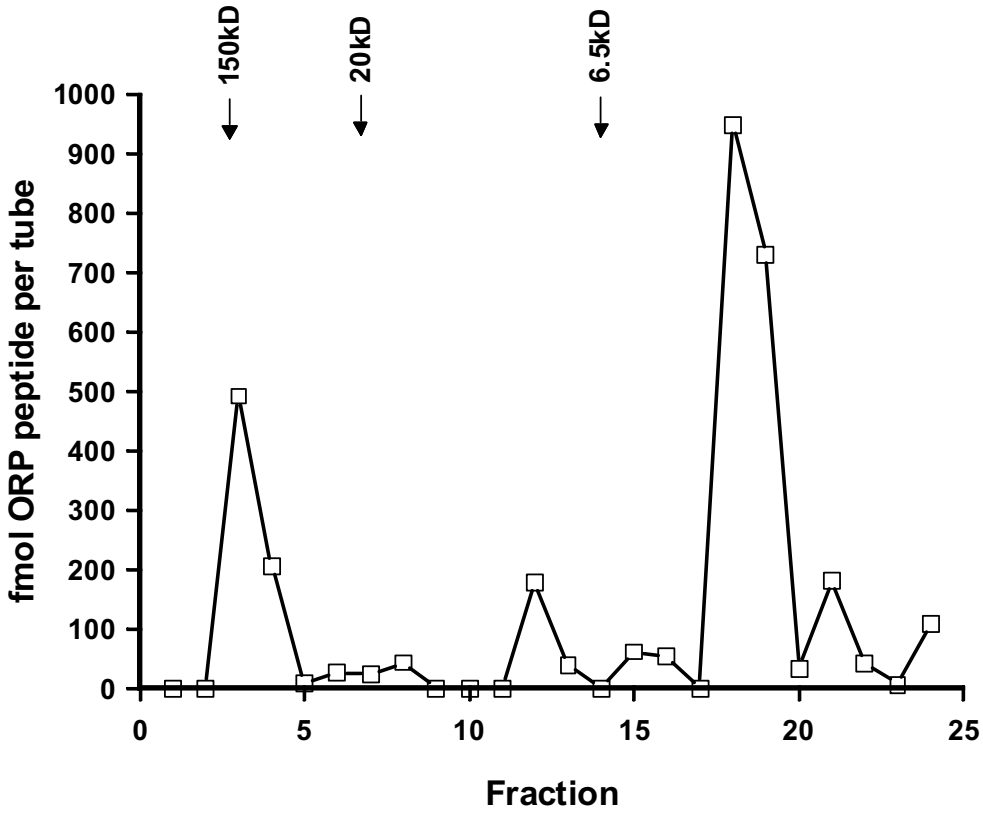

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Figure 3

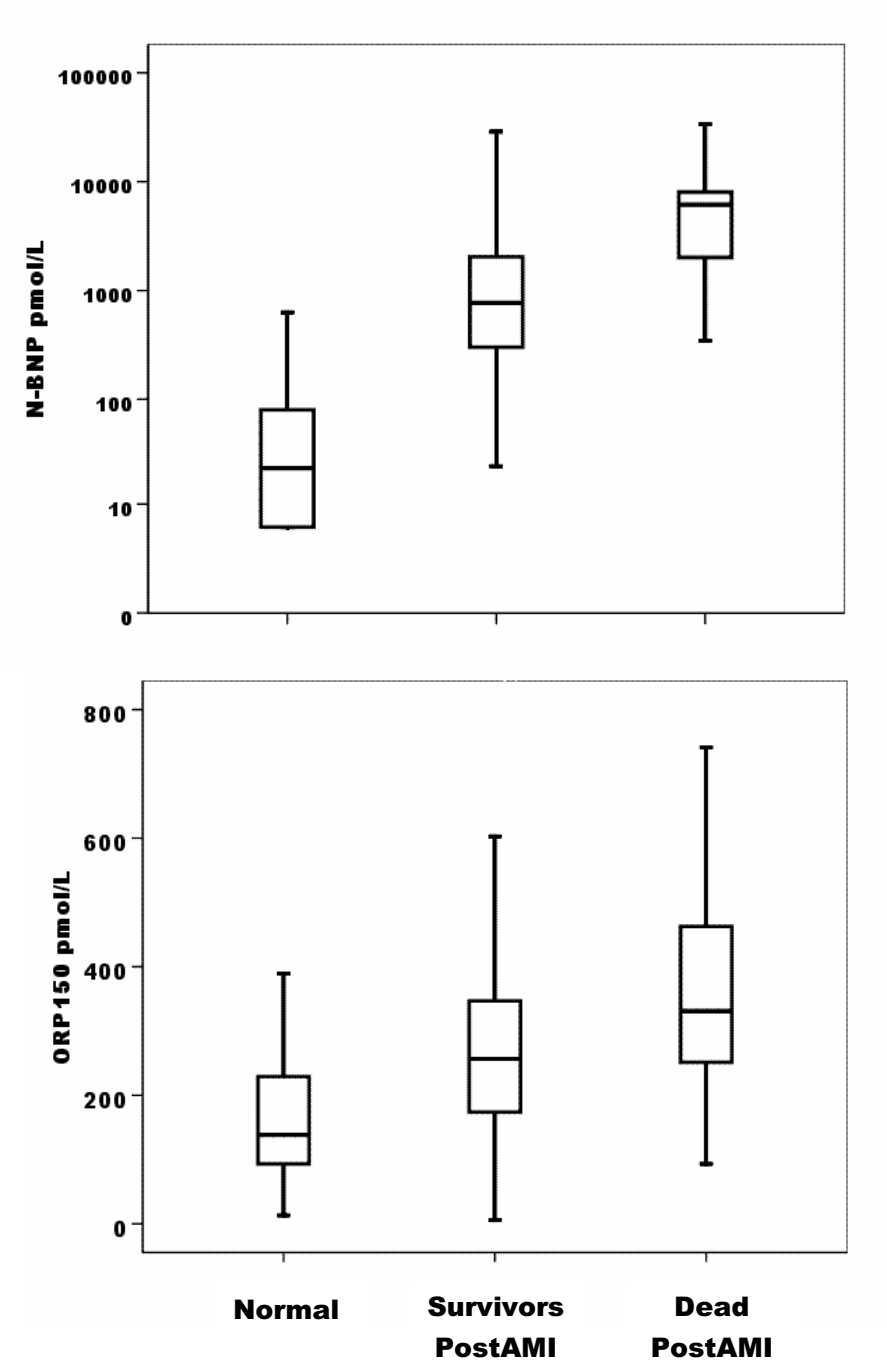

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Figure 4

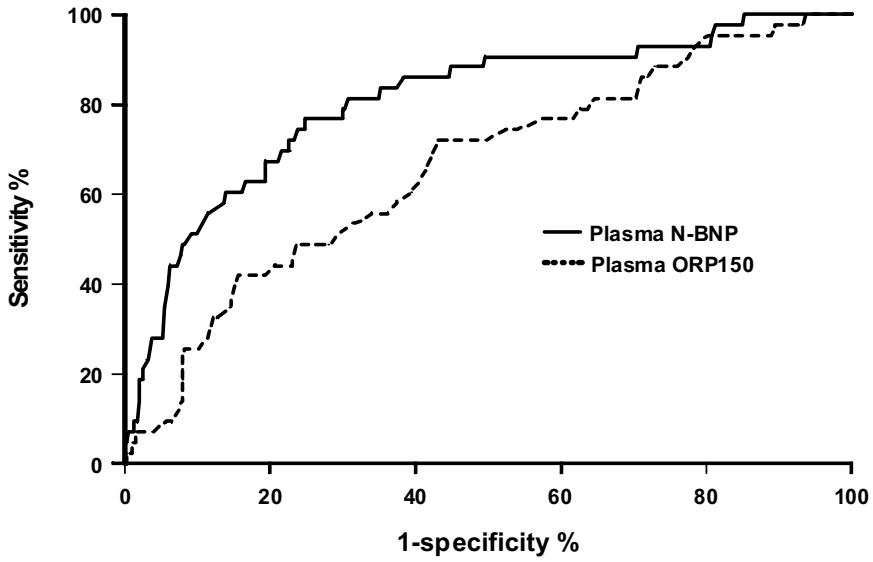

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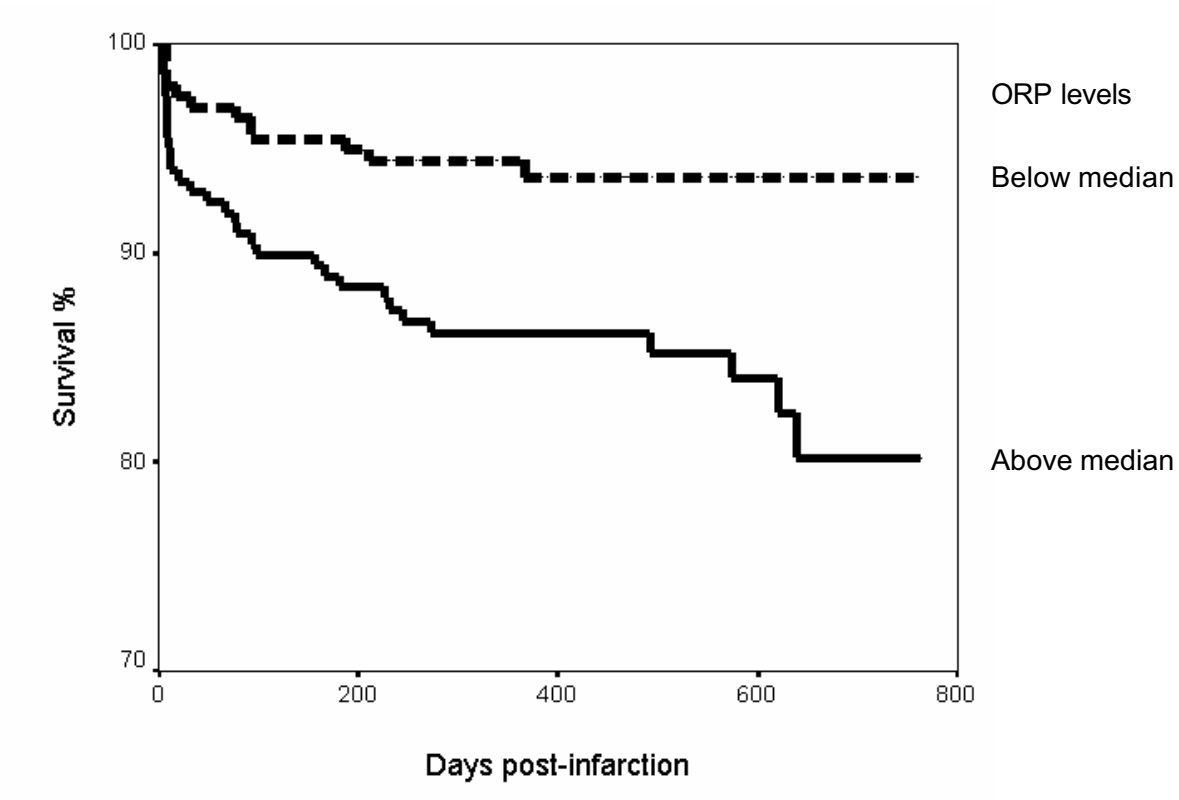
Figure 5



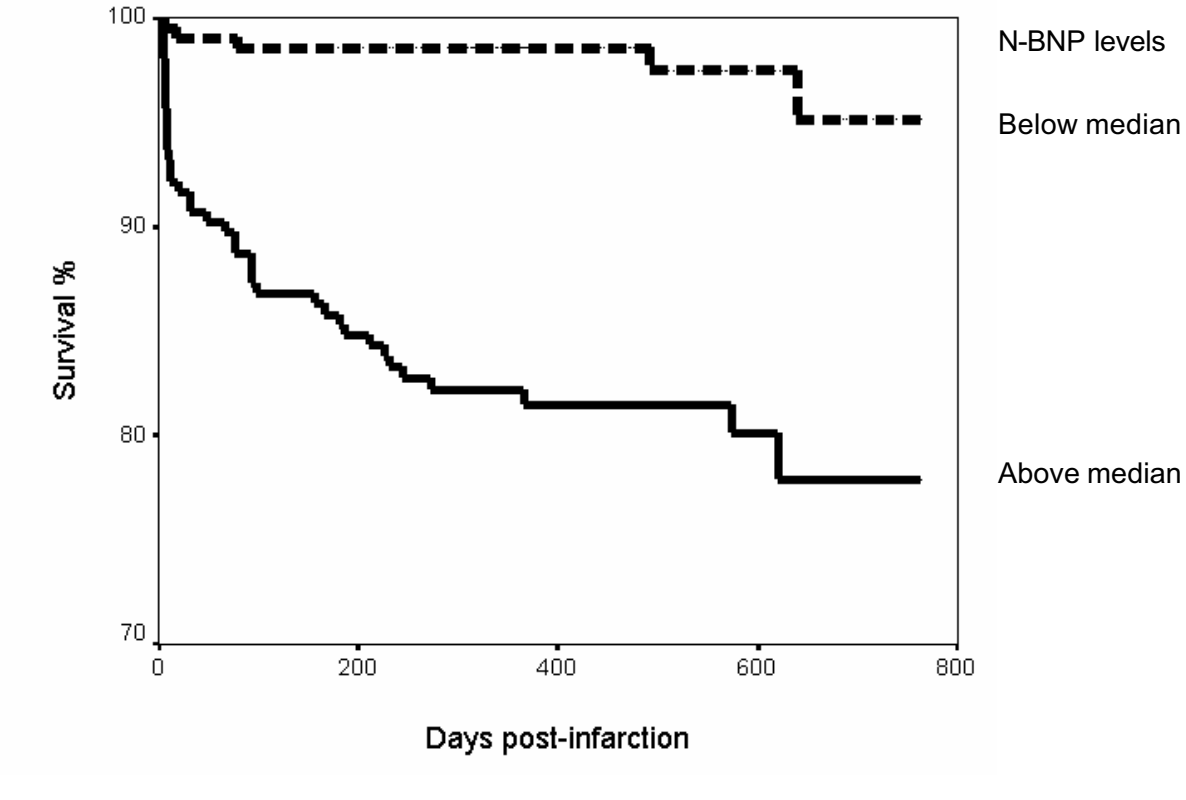


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Figure 6

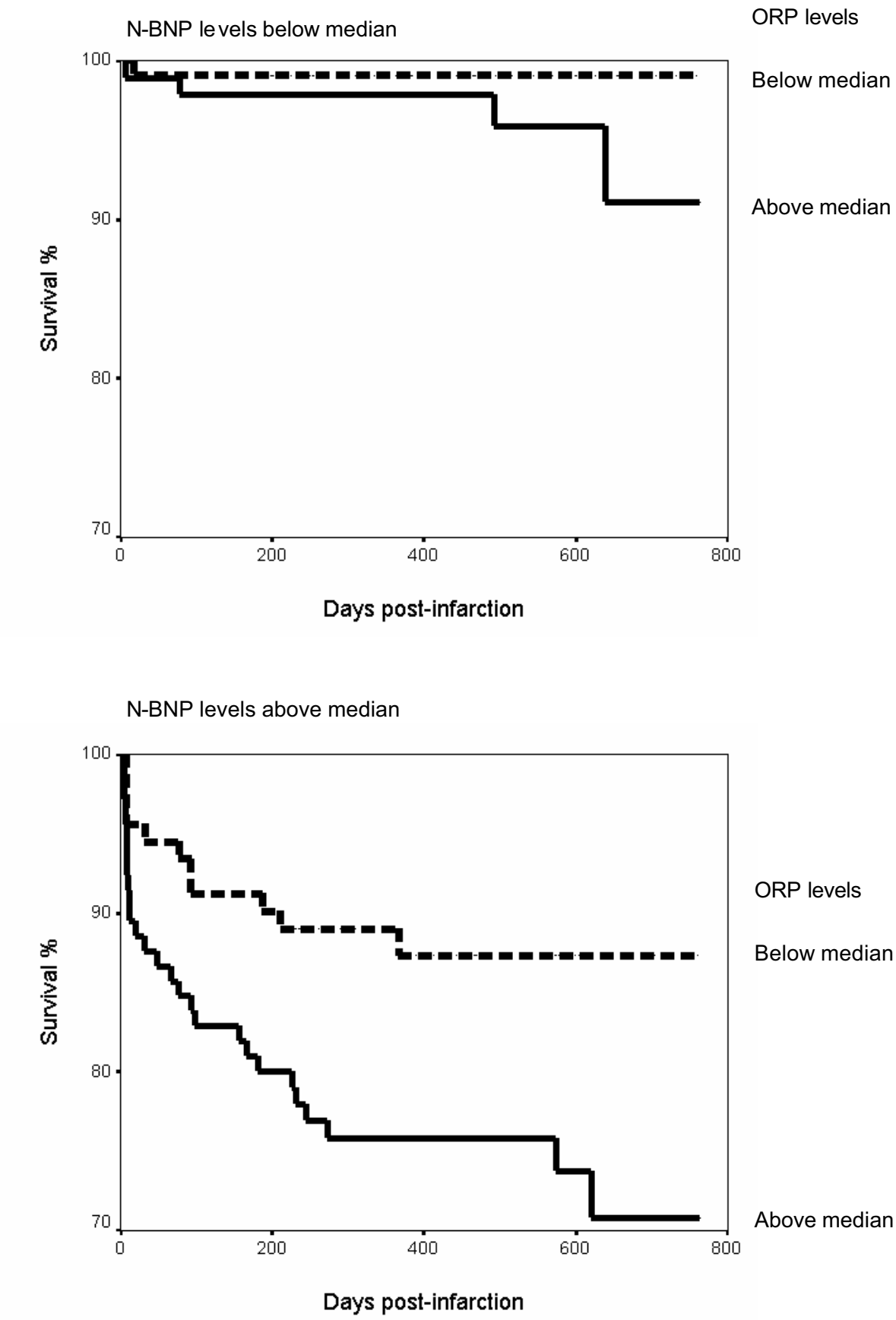

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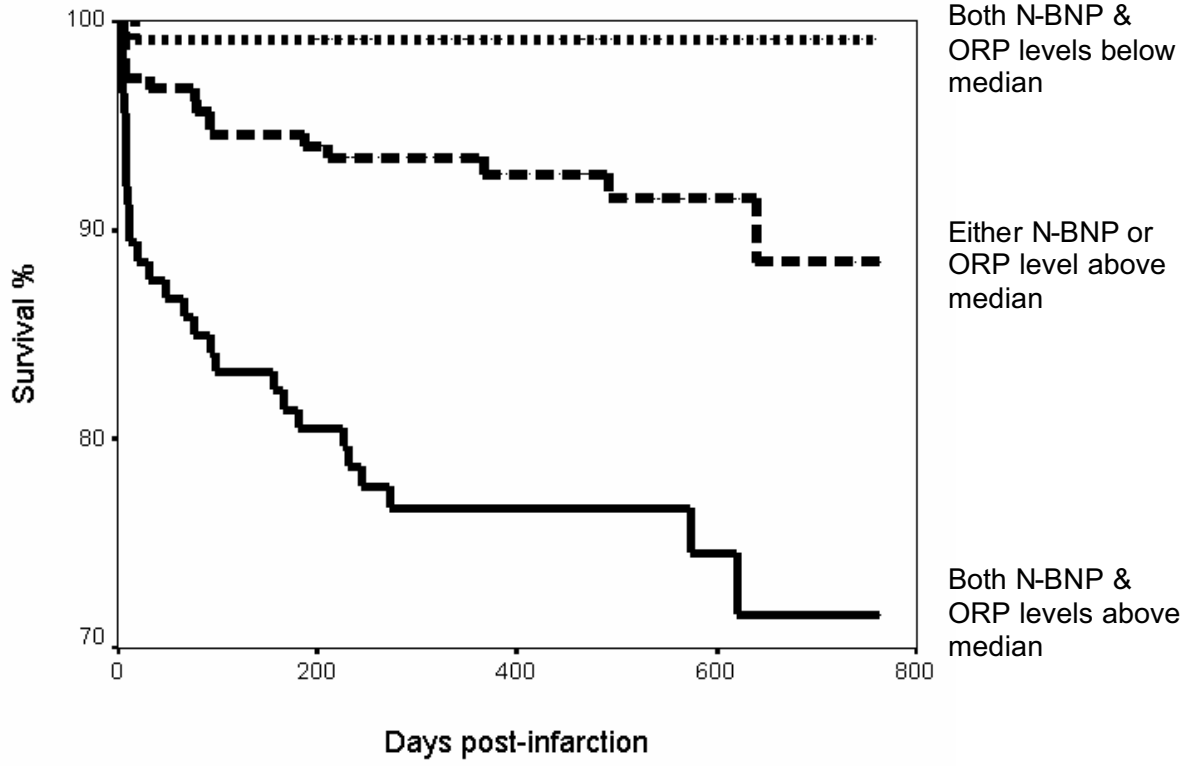
Figure 7



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