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(71) Demandeur/Applicant:
INVERNESS MEDICAL SWITZERLAND GMBH, CH
(72) Inventeur/Inventor:
NG, LEONG, GB
(74) Agent: BERESKIN & PARR

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(57) **Abrégé/Abstract:**

The present invention provides a method for screening, diagnosis or prognosis of acute coronary syndrome (ACS) in a mammalian subject, for determining the stage or severity of ACS in a mammalian subject, for identifying a mammalian subject at risk of developing ACS, or for monitoring the effect of therapy administered to a mammalian subject having ACS. The method comprises measuring the level of Urotensin II (UTN) in a sample of bodily fluid from the mammalian subject. The invention also provides a kit, immunoassay or device for carrying out the method.

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(71) Applicant (for all designated States except US): **INVER-
NESS MEDICAL SWITZERLAND GMBH** [CH/CH];
Bundesplatz 10, CH-6300 Zug (CH).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **NG, Leong** [GB/GB];
Department of Medicine, Level 4, Clinical Sciences Build-
ing, Leicester Royal Infirmary, Leicester LE2 7LX (GB).

(74) Agents: **LEE, Nicholas, John** et al.; Kilburn & Strode, 20
Red Lion Street, London WC1R 4PJ (GB).

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(54) Title: METHODS OF DIAGNOSING ACUTE CORONARY SYNDROME BY MEASURING UROTENSIN II

(57) Abstract: The present invention provides a method for screening, diagnosis or prognosis of acute coronary syndrome (ACS) in a mammalian subject, for determining the stage or severity of ACS in a mammalian subject, for identifying a mammalian subject at risk of developing ACS, or for monitoring the effect of therapy administered to a mammalian subject having ACS. The method comprises measuring the level of Urotensin II (UTN) in a sample of bodily fluid from the mammalian subject. The invention also provides a kit, immunoassay or device for carrying out the method.



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Methods

The present invention relates to methods for the diagnosis, *inter alia*, of acute coronary syndromes (ACS) in mammalian subjects, such as humans. In particular, it relates to such methods in which the level of urotensin II (UTN) in a sample is measured.

Ischaemic heart disease is a major health burden in developed countries, and its main aetiology is atherosclerosis. Accumulation of lipid, especially oxidised or modified LDL, together with macrophages and other cells, leads to plaque growth and instability. Rupture of these plaques leads to thrombosis, and a resulting occlusion of the coronary arterial lumen precipitates presentation as an acute coronary syndrome (ACS). Acute coronary syndromes include myocardial infarction (MI) and unstable angina (UA).

Acute coronary syndromes are major health problems in the world but there are limited means for effective diagnosis and risk stratification of patients at the moment. Biochemical techniques involving measurement of various blood analytes are currently utilised to achieve these objectives. For example, Brain natriuretic peptide (BNP) or its N-terminal precursor, N-terminal proBrain natriuretic peptide (N-BNP) are secreted during acute cardiac ischaemia, and may provide prognostic information about the patient (Omland, *et al.*, *Circulation* 2002, **106**: 2913-8; Richards, *et al.*, *Circulation* 2003, **107**: 2786-92).

In a first aspect, the present invention provides a method for screening, diagnosis or prognosis of acute coronary syndrome (ACS) in a mammalian subject, for determining the stage or severity of ACS in a mammalian subject, for identifying a mammalian subject at risk of developing ACS, or for monitoring the effect of therapy administered to a mammalian subject having ACS, said method comprising:

measuring the level of a first marker in a sample of bodily fluid from said mammalian subject, wherein said first marker is Urotensin II (UTN).

In a second aspect, the invention provides an immunoassay, kit or device for carrying out the method of the first aspect.

5 In a third aspect, the invention provides an immunoassay, kit or device for screening, diagnosis or prognosis of acute coronary syndrome (ACS) in a mammalian subject, for determining the stage or severity of ACS in a mammalian subject, for identifying a mammalian subject at risk of developing ACS, or for monitoring the effect of therapy administered to a mammalian subject having ACS, said immunoassay, kit or device comprising:

10 instructions for taking a sample of bodily fluid from said mammalian subject; and

one or more reagents for measuring the level of a first marker in the sample, wherein the first marker is Urotensin II (UTN).

15 The invention provides assays and kits for detecting an increased risk of ACS in a subject (i.e. a human or non-human mammal) by detecting the level of urotensin II, alone or in combination with another marker, in a bodily fluid sample whereby an elevated level of urotensin II relative to the normal level is indicative of an increased risk of ACS.

20

The terms "urotensin" or "UTN," as used herein, refer to urotensin II (see GenBank Accession Numbers NM_021995 and NM_006786) and fragments and variants thereof (e.g., allelic variants). Urotensin is derived from a prohormone precursor, pro-urotensin (GenBank Accession Number O95399), which is processed to mature urotensin and an N-terminal peptide. The term "urotensin," as used herein, further includes pro-urotensin, mature urotensin, its N-terminal derived peptide, its signal peptide, and a C-terminal peptide (Glu-Thr-Pro-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Val (disulphide bond between Cys⁵ and Cys¹⁰)) as well as fragments thereof. The term urotensin also refers to urotensin related peptide (URP) (Ala-Cys-Phe-Trp-Lys-Tyr-Cys-Val (disulphide bond between Cys² and Cys⁷)) as well as the proform of

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30 urotensin related peptide (GenBank Accession Number NM_198152).

Urotensin-II is a recently-cloned cardiovascular peptide homologous to the teleost hormone (Ames, *et al.*, *Nature* 1999, **401**: 282-286). This cyclic undecapeptide is the ligand for an orphan G-protein receptor (GPR14) and both peptide and receptors are distributed within the cardiovascular and nervous systems (Ames, *et al.*, *Nature* 1999, **401**: 282-286). Although a potent vasoconstrictor for certain vascular beds in rats and monkeys (Ames, *et al.*, *Nature* 1999, **401**: 282-286; Douglas, *et al.*, *J Cardiovasc Pharmacol* 2000, **36**: S163-6), it may be vasodilator in mesenteric resistance vessels (Bottrill, *et al.*, *Br J Pharm* 2000, **130**: 1865-1870). There are important species differences in the reactivity of different vessels to UTN. For example, UTN vasoconstricts monkey vessels, it vasodilates human pulmonary vasculature and mesenteric resistance vessels (Stirrat, *et al.*, *Am J Physiol* 2001, **280**: H925-928) and has no effect on human subcutaneous resistance vessels (Hillier, *et al.*, *Circulation* 2001, **103**: 1378-1381). UTN also causes vascular smooth muscle hypertrophy (Watanabe, *et al.*, *J Hypertens* 2001, **19**: 2191-2196). Direct effects on the monkey myocardium include myocardial depression (Ames, *et al.*, *Nature* 1999, **401**: 282-286) although in human myocardium, a positive inotropic effect was demonstrated also (Russell, *et al.*, *Br J Pharm* 2001, **132**: 5-9). In addition, hypertrophic effects and a possible role in ventricular remodeling have been described (Zou, *et al.*, *FEBS Lett* 2001, **508**: 57-60; Tzanidis, *et al.*, *Eur Heart J* 2000, **21**: 72). Its secretion may be therefore detrimental to cardiac function, from the cardiac-depressor, remodeling and hypertrophic effects.

It has been established that UTN is a marker for heart failure. Recent work has documented increased UTN and GPR14 expression in myocardium of heart failure patients (Douglas, *et al.*, *Lancet* 2002, **359**: 1990-1997). The secretion of UTN into the plasma in heart failure patients, especially even in mild cases of early disease, has also been demonstrated (Ng, *et al.*, *Circulation* 2002, **106**: 2877-2880).

UTN is secreted into the plasma in ACS and can be used as a marker of acute coronary syndromes, optionally in combination with other markers of acute coronary syndromes, such as troponins, creatine kinase MB isoform, myoglobin or natriuretic

peptides such as N-BNP and BNP. UTN can also be used a marker of myocardial damage, following balloon angioplasty and may complement assays of troponins.

5 The measured level of urotensin may be compared with a level of urotensin which is indicative of the absence of ACS. This level may be the level of urotensin from one or more mammalian subjects free from ACS, or a previously determined reference range or value for urotensin in such mammalian subjects free from ACS. In this way, the measured levels can be compared with reference levels determined from population studies of subjects free from the condition in question to provide a
10 diagnosis or prognosis. Such subjects may be matched for age and/or gender. In one embodiment, the reference or normal values of the levels of urotensin typically found in a sample of bodily fluid which is indicative of the absence of ACS may range from 3-10 fmol/ml. Levels of urotensin that are indicative of an increased risk of ACS may range from 10-15 fmol/ml, 15-20 fmol/ml, 20-25 fmol/ml, 25-30 fmol/ml or more.
15 Where the present invention is concerned with monitoring the effect of therapy administered to a mammalian subject having ACS, the measured level of urotensin can be compared with a base level for the subject. The base level may be determined prior to commencement of the therapy. Deviations from this base level indicate whether there an increase or decrease of ACS and hence whether the therapy is
20 effective. An increased level of urotensin indicates ACS and *vice versa*.

In the present invention, the term "bodily fluid" includes all fluids that can be obtained from a mammalian body, including, for example, blood, plasma, urine, lymph, gastric juices, bile, serum, saliva, sweat, and spinal and brain fluids.

25 Furthermore, the bodily fluids may be either processed (e.g., serum) or unprocessed. Methods of obtaining a bodily fluid sample from a subject are known to those skilled in the art.

30 A second marker or further markers indicative of ACS may be measured. These markers may be one or more of brain natriuretic peptide, troponin, creatine kinase MB isoform, and myoglobin, as well as fragments and precursors thereof.

As used herein, the term "brain natriuretic peptide" includes a native brain natriuretic peptide (BNP), N-terminal pro-BNP (N-BNP), as well as portions thereof, variants thereof, and chimeras thereof.

5 Myocardial stretch, myocardial tension, and myocardial injury triggers increased production of proBNP from cardiac myocytes in the left ventricle. proBNP is the intact precursor to the two circulating forms, BNP (the active peptide) and N-terminal proBNP (N-BNP – the inactive peptide). The level of N-BNP and/or BNP may be measured.

10

Troponins reflect myocardial damage, and elevations are useful in the risk stratification of ACS. In a recent report, N-BNP (>669 ng/L, or approximately 79 fmol/ml) and Troponin T levels (>0.01 $\mu\text{g/L}$) were independent predictors of mortality, and Troponin T levels (>0.01 $\mu\text{g/L}$) predicted further myocardial infarction events in the 30 days after presentation (James *et al*, *Circulation*, 2003, 108:275-81). Creatine kinase MB isoform (CKMB) and myoglobin are currently used for the diagnosis of myocardial infarction, as both are elevated more rapidly than the troponins.

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20 When measuring the levels of the second marker(s), corrections for age and gender may be necessary in order to improve the accuracy of diagnosis.

Urotensin may be useful in combination with brain natriuretic peptide (or any other second marker) in assessing the prognosis of patients with acute coronary syndromes; after myocardial infarction, the combination of peptides is useful in risk stratification of patients with respect to mortality.

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In a manner similar to the first marker, urotensin, the level of the or each second marker may be compared with a level of the or each second marker which is indicative of the absence of ACS. This level may be the level of the or each second marker from one or more mammalian subjects free from ACS, or with a previously determined reference range for the or each second marker in mammalian subjects free

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from ACS. Where measured, the normal value of N-BNP that is indicative of the absence of ACS may range from 10-50 fmol/ml. Levels of N-BNP that are indicative of an increased risk of ACS may range from 100-300, 300-600 fmol/ml, 600-1200 fmol/ml, 1200-1800 fmol/ml, 1800-2400 fmol/ml, 2400-3000 fmol/ml, 3000-3600 fmol/ml or more. The normal level of myoglobin that is indicative of the absence of ACS may vary according to the device used to measure the level (Le Moigne *et al*, *Clin Biochem.*, 2002, **35**(4):255-62). Thus, the normal level may be up to 92 µg/L for men and 76 µg/L for women if measured by Olympus, up to 46 µg/L if measured by Vidas or up to 70 µg/L if measured by Immulite Turbo. Another study suggests that the normal level may be up to 65 µg/L for men and 55 µg/L for women (Penttila *et al*, *Clin Biochem.* 2002, **35**(8):647-53). Levels of myoglobin which exceed the above levels may be indicative of ACS. For Troponin T, the cut off for AMI may be 0.05 µg/L (Collinson *et al*, *Heart.* 2003 **89**(3):280-6). The disclosure of James *et al*, *J Am Coll Cardiol.* 2003, **41**(6):916-24 is incorporated in this regard. For Troponin I, the level for diagnosis of ACS (including MI) may be 0.6 µg/L or above (Apple *et al*, *Clin Chem.* 2000, **46**(4):572-4). For CKMB, the level for diagnosis of ACS (including MI) may be 5 µg/L or more (Falahati *et al*. *Am Heart J.* 1999, **137**(2):332-7). Values below these respective levels may indicate the absence of ACS.

Marker levels may be provided in units of concentration, mass, moles, volume, concentration or other measure indicating the amount of marker present in the sample.

The respective levels of the first and second markers may be measured using an immunoassay, i.e. an assay that utilises an antibody to bind specifically to a marker.

Such assays may be competitive or non-competitive immunoassays. An antibody, or generally any molecule, "binds specifically" to an antigen (or other molecule) if the antibody binds preferentially to the antigen, and, e.g., has less than about 30%, preferably 20%, 10%, or 1% cross-reactivity with any other molecule. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen. The immunoglobulin molecules useful

in the invention can be of any class (e.g., IgG, IgE, IgM, IgD and IgA) or subclass of immunoglobulin molecule. Antibodies include, but are not limited to, polyclonal, monoclonal, bispecific, humanised and chimeric antibodies, single chain antibodies, Fab fragments and F(ab')₂ fragments, fragments produced by a Fab expression
5 library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Portions of antibodies include Fv and Fv' portions. Antibodies can be naturally-occurring antibodies, e.g., monoclonal antibodies obtained by the method of Koehler and Milstein and polyclonal antibodies obtained, for example, by injection of an antigen into an animal. Antibodies can also be partially or fully humanised.

10 Antibodies binding to urotensin can be obtained commercially. Examples of commercially-available antibodies binding to urotensin include anti-urotensin (Phoenix Pharmaceuticals), rabbit anti-urotensin (Biodesign International), rabbit anti-human urotensin (Immundiagnostik).

15 Antibodies binding to BNP can be obtained commercially. Examples of commercially-available antibodies binding to BNP are rabbit anti-human BNP polyclonal antibody (Biodesign International), rabbit anti-BNP amino acids 1-20 polyclonal antibody (Biodesign International), anti-human BNP monoclonal antibody
20 (Immundiagnostik), and rabbit anti-human BNP amino acids 1-10 polyclonal antibody (Immundiagnostik).

Antibodies binding to troponins, creatine kinase MB isoform and myoglobin can be obtained from Research Diagnostics Inc for example.

25 Depending on the assay used to diagnose ACS (see below), the antibodies specific to the markers of ACS may further comprise a label, e.g., a fluorescent or magnetic label, a latex or gold particle, a fluorescent moiety, an enzyme, an electrochemically active species, etc. In embodiments where the label is attached to the antibody, the
30 antibody is said to be "directly labelled." An antibody can also be "indirectly labelled," i.e., the label is attached to the antibody through one or more other molecules, e.g., biotin-streptavidin. Alternatively, the antibody is not labelled, but is

later contacted with a binding agent after the antibody is bound to a specific marker of ACS. For example, there may be a “primary antibody” and a second antibody or “secondary antibody” that binds to the Fc portion of the first antibody. Labels may be linked, preferably covalently, to antibodies according to methods known in the art. In the immunoassay, the presence or amount of analyte (or marker) present is determined by detection of the presence or concentration of the label.

Further depending on the assay used to diagnose ACS, antibodies may be linked to a solid surface. The solid surface can be selected from a variety of those known in the art including plastic tubes, beads, microtiter plates, latex particles, magnetic particles, cellulose beads, agarose beads, paper, dipsticks, and the like. Methods for direct chemical coupling of antibodies to the cell surface are known in the art, and may include, for example, coupling using glutaraldehyde- or maleimide-activated antibodies. Methods for chemical coupling using multiple step procedures include biotinylation, coupling of trinitrophenol (TNP) or digoxigenin using for example succinimide esters of these compounds. Biotinylation can be accomplished by, for example, the use of D-biotinyl-N-hydroxysuccinimide. Succinimide groups react effectively with amino groups at pH values above 7, and preferentially between about pH 8.0 and about pH 8.5. Biotinylation can be accomplished by, for example, treating the antibodies with dithiothreitol followed by the addition of biotin maleimide.

In certain embodiments of the invention, antibodies are contacted with the sample of bodily fluid obtained from a mammalian subject at least for a time sufficient for the antibody to bind to a marker used to diagnose ACS. For example, an antibody may be contacted with the sample of bodily fluid for at least about 10 minutes, 30 minutes, 1 hour, 3 hours, 5 hours, 7 hours, 10 hours, 15 hours, or 1 day.

In one embodiment, an immunoassay is performed by contacting a sample from a subject to be tested with an appropriate antibody under conditions such that immunospecific binding can occur if the marker is present, and detecting or measuring the amount of any immunospecific binding by the antibody. Any suitable immunoassay can be used, including, without limitation, competitive and

non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), “sandwich” immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays and protein A immunoassays.

For example, a marker can be detected in a fluid sample by means of a two-step sandwich assay. In the first step, a capture reagent (e.g., an anti-marker antibody) is used to capture the marker. The capture reagent can optionally be immobilised on a solid phase. In the second step, a directly- or indirectly-labelled detection reagent is used to detect the captured marker. In one embodiment, the detection reagent is an antibody. In another embodiment, the detection reagent is a lectin.

In one embodiment, a lateral flow immunoassay device may be used in the “sandwich” format wherein the presence of sufficient marker in a bodily fluid sample will cause the formation of a “sandwich” interaction at the capture zone in the lateral flow assay. The capture zone as used herein may contain capture reagents such as antibody molecules, antigens, nucleic acids, lectins, and enzymes suitable for capturing urotensin and other markers described herein. The device may also incorporate one or more luminescent labels suitable for capture in the capture zone, the extent of capture being determined by the presence of analyte. Suitable labels include fluorescent labels immobilised in polystyrene microspheres. Microspheres may be coated with immunoglobulins to allow capture in the capture zone. Such an assay may be carried out using the lateral flow immunoassay described in EP291194.

Other assays that may be used in the methods of the invention include, but are not limited to, flow-through devices. In a flow-through assay, one reagent (usually an antibody) is immobilized to a defined area on a membrane surface. This membrane is then overlaid on an absorbent layer that acts as a reservoir to pump sample volume through the device. Following immobilization, the remainder of the protein-binding

5 sites on the membrane are blocked to minimize nonspecific interactions. When the assay is used, a bodily fluid sample is added to the membrane and filters through the matrix, allowing any marker specific to the antibody in the sample to bind to the immobilised antibody. In an optional second step (in embodiments wherein the first reactant is an antibody), a tagged secondary antibody (an enzyme conjugate, an antibody coupled to a colored latex particle, or an antibody incorporated into a colored colloid) may be added or released that reacts with captured marker to complete the sandwich. Alternatively, the secondary antibody can be mixed with the sample and added in a single step. If a marker is present, a colored spot develops on the surface of the membrane.

10 The invention also provides an immunoassay, kit or device for detection of urotensin which may incorporate the method of the first aspect of the present invention. Such an immunoassay kit or device may comprise an antibody which binds specifically to urotensin and optionally an antibody which binds the second marker. In addition, such an immunoassay, kit or device may optionally comprise one or more of the following: (1) instructions for using the immunoassay, kit or device for diagnosis or prognosis of ACS; (2) a labelled binding partner to the antibody; (3) a solid phase (such as a reagent strip) upon which the or each antibody is immobilised; and (4) a label or insert indicating regulatory approval for diagnostic, prognostic or therapeutic use or any combination thereof. If no labelled binding partner to the or each antibody is provided, the or each antibody itself can be labelled with a detectable marker, e.g., a chemiluminescent, enzymatic, fluorescent, or radioactive moiety. Additional antibodies to other markers of ACS may be included.

25 As used herein, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise, and "comprise" and "comprising" are used in the inclusive, open sense, meaning that additional elements may be included.

30 Preferred features of each aspect of the invention are as for each of the other aspects mutatis mutandis. The prior art documents mentioned herein are incorporated to the fullest extent permitted by law.

EXAMPLES

The invention will now be described further in the following non-limiting examples.

5 Reference is made to the accompanying drawings in which:

Figures 1a and 1b show plasma levels of Urotensin and N-BNP, respectively, in patients with Myocardial Infarction [MI] or Unstable Angina [UA] compared to normal subjects without these.

10

Figures 2a and 2b show Receiver Operating Characteristic Curves (ROC Curves) for the diagnosis of Acute Coronary Syndromes using plasma urotensin and plasma N-BNP, respectively.

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Figures 3a and 3b show plasma levels of Urotensin and N-BNP, respectively, in patients with ACS who either had an uncomplicated clinical course or were subsequently rehospitalised with a further event (MI or UA).

20

Figures 4a and 4b show plasma levels of Urotensin and BNP, respectively, in patients with stable angina, undergoing coronary angiography and either having a balloon angioplasty or no intervention to their coronary arteries. Levels of both markers peak at 2 hours after the balloon angioplasty.

Example 1

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Study Populations

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486 patients admitted to the Leicester Royal Infirmary with acute coronary syndromes were studied. Acute myocardial infarction (MI) 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. Unstable angina (UA) patients were defined as having no acute ST elevation on their ECGs and CK was less than twice

the upper limit of normal. 372 patients had MI and 114 had UA. These patients were compared with 130 normal controls of similar age and gender, with no previous cardiac history.

5 *End Points in myocardial infarction and unstable angina patients*

End-points were defined cardiovascular morbidity (rehospitalisation with a further episode of myocardial infarction or unstable angina) following discharge from the index hospitalisation.

10 *Blood Sampling and plasma extraction*

In all subjects, 20ml of peripheral venous blood was drawn into pre-chilled Na-EDTA (1.5mg/ml blood) tubes containing 500 IU/ml aprotinin after a period of 15 min bed rest. In MI and UA patients, a single blood sample was taken between 72-96 hours after symptom onset. After centrifugation at 3000 rpm at 4°C for 15 min, plasma was separated and stored at -70 °C until assay. Prior to assay, plasma was extracted on C₁₈ Sep-Pak (Waters) columns and dried on a centrifugal evaporator.

Assay of UTN

This was carried out as previously described in Ng, *et al.*, *Circulation* 2002, 106: 2877-2880. In brief, antibody specific for UTN was obtained from Peninsular Laboratories, CA. Biotinylated UTN purified on reverse phase HPLC served as the tracer. A competitive assay using C₁₈ extracts of plasma was utilized, incubating 50ng of the antibody with extracts or standards in immunoassay buffer consisting of (in mmol/l) NaH₂PO₄ 1.5, Na₂HPO₄ 8, NaCl 140, EDTA 1 and (in g/l) bovine serum albumin 1, azide 0.1. After 24 h of incubation at 4°C, biotinylated UTN tracer was added (250 fmols/well). Immunoprecipitates were recovered in anti-rabbit IgG coated ELISA plates. Following washes and incubation with streptavidin-MAE, chemiluminescence was elicited as described in Ng, *et al.*, *Circulation* 2002, 106: 2877-2880 and Ng, *et al.*, *Clinical Science* 2002, 102: 411-416.

30 *Assay BNP and N-BNP*

Antibody specific for BNP was obtained from Peninsular Laboratories, CA. Biotinylated BNP purified on reverse phase HPLC served as the tracer. A competitive assay using C₁₈ extracts of plasma was utilized, incubating 25ng of the antibody with extracts or standards in immunoassay buffer, as detailed under assay for 5 UTN. The assay for N-BNP was a 2-site non-competitive assay, employing antibodies against the N- and C-terminal of human N-BNP, as described in Ng, *et al.*, *Circulation* 2002, **106**: 2877-2880 and Omland, *et al.*, *Circulation* 2002, **106**: 2913-8.

Statistical analysis

10 Statistical analysis was performed using SPSS Version 11.0 (SPSS Inc, Chicago, MI). Data are presented as mean $\pm$ SEM or median (range) for data with non-Gaussian distribution, which were log transformed prior to analysis. For continuous variables, one-way analysis of variance (ANOVA) was used and post-hoc comparisons sought with Bonferonni's test. The interaction of multiple independent variables was sought 15 using the univariate General Linear Model procedure with least significant difference P values reported. Spearman correlation analysis was performed (r_s are reported) and box plots were constructed consisting of medians, boxes representing interquartile ranges and the whiskers representing the 2.5th to the 97.5th centile. Receiver operating characteristic curves (ROC) were constructed for the detection of ACS compared to 20 normal subjects. P values below 0.05 were considered significant.

Results & Discussion

UTN in plasma of normal and ACS patients

25 UTN was detectable in plasma extracts of almost all subjects. Some extracts were below the detection limit of our assay (<3.1 fmol/ml). The levels of UTN in plasma of ACS patients (both MI and UA) were very significantly higher than that of normal controls (figure 1, ANOVA P<0.0005). Bonferonni's test confirmed UTN levels in UA (P<0.0005) or MI (P<0.0005) were significantly higher than normal controls. 30 The levels of N-BNP in plasma of ACS patients (both MI and UA) were very significantly higher than that of normal controls (figure 1, ANOVA P<0.0005). Bonferonni's test confirmed N-BNP levels in UA (P<0.0005) or MI (P<0.0005) were

significantly higher than normal controls. In addition, N-BNP levels in MI were higher than that in UA ($P < 0.0005$). Levels of UTN and N-BNP were correlated ($r_s = 0.354$, $P < 0.0005$).

5 Figure 2 illustrates a receiver operating characteristic curve for the diagnosis of ACS (MI and UA) using plasma UTN levels. The ROC area under the curve (ROC AUC) was 0.89 for UTN, significantly different ($P < 0.0005$) from the diagonal (AUC of 0.50), but similar to that of N-BNP (ROC AUC 0.93, figure 2). Both markers thus have utility in the identification of patients with ACS. At a level of UTN of 9.1
10 fmol/ml, there was a 94% sensitivity, 74% specificity for the diagnosis of ACS, with positive predictive values of 93% and a negative predictive value of 77%. These figures allow effective ruling-in of the diagnosis of ACS. Appropriately changing the cut-off values can also improve the utility of UTN to rule out an ACS event.

15 *Plasma UTN and prognosis of ACS events*

Figure 3 shows that levels of UTN in patients who had an index admission with ACS, but were subsequently either readmitted with MI or UA, or remained well and were not rehospitalised. Data was available on 447 patients. It can be seen UTN is elevated in the 98 patients who readmitted with MI or UA ($P < 0.005$), compared to the
20 349 patients who had an uncomplicated clinical course. In contrast, plasma N-BNP was not different in patients who had an uncomplicated course, compared to those subsequently rehospitalised with MI or UA (figure 3). UTN can be an effective marker for future ACS events in patients admitted to hospital with ACS, and this can assist in the risk stratification and planning of therapeutic strategies for such higher
25 risk patients.

Example 2

Study Population and blood sampling

30 Blood samples were obtained from 23 patients with stable angina undergoing coronary angiography. 13 of these patients had balloon angioplasty in addition to angiography.

Blood samples were taken before angiography (basal), 2 hours, 6 hours and 24 hours after angiography (or angioplasty). Samples were extracted on C₁₈ columns and assayed for UTN and BNP as described above.

5

Results & Discussion

Figure 4 shows the plasma levels of UTN during angiography with or without angioplasty. Levels change significantly with time ($P<0.0005$), peaked at 2 hours after the procedure ($P<0.0005$ compared to all other time points), and differed
10 between those with angioplasty compared to those without angioplasty ($P<0.011$).

The levels of BNP also changed significantly with time ($P<0.0005$), peaked at 2 hours after the procedure ($P<0.0005$ compared to all other time points), and also differed between those with angioplasty compared to those without angioplasty ($P<0.008$).

15

Although high levels of both markers are evident at 2 hours after the angioplasty, higher levels may be evident before 2 hours or between 2 and 6 hours, and the optimal timing of the blood sample has to be determined. It is likely to fall within the range 10 min to 6 hours after the procedure.

20

BNP is an established marker of cardiac damage, being released during cardiac ischaemia. However, the acute secretion of UTN during cardiac ischaemia (e.g. during balloon angioplasty) is unexpected. Cardiac ischaemia can be transient and mild during this procedure, but the median 10 fold rise in UTN suggests it could be a
25 very sensitive indicator of cardiac ischaemia and may provide information on the degree of cardiac damage incurred during angioplasty. This should complement information obtained from other peptide assays, e.g. troponins and BNP.

In addition, the acute response of UTN to balloon angioplasty suggests it may be a
30 marker for the early diagnosis of an acute coronary syndrome, since levels peak within 2 hours. Current markers of myocardial ischaemia which are released rapidly from the myocardium during an ACS event (such as myoglobin) may be non-specific

for cardiac muscle, and additional markers (such as UTN and BNP) can increase the specificity of myoglobin.

Claims

1. A method for screening, diagnosis or prognosis of acute coronary syndrome (ACS) in a mammalian subject, for determining the stage or severity of ACS in a mammalian subject, for identifying a mammalian subject at risk of developing ACS, or for monitoring the effect of therapy administered to a mammalian subject having ACS, said method comprising:

measuring the level of a first marker in a sample of bodily fluid from said mammalian subject, wherein said first marker is Urotensin II (UTN).

10

2. A method as claimed in claim 1, wherein the bodily fluid is plasma.

3. A method as claimed in claim 1 or claim 2, further comprising measuring the level of a second marker indicative of ACS.

15

4. A method as claimed in claim 3, wherein the second marker is one or more of brain natriuretic peptide, troponins, creatine kinase MB isoform, and myoglobin.

5. A method as claimed in any preceding claim, wherein the mammalian subject is a human.

20

6. A method as claimed in any preceding claim, wherein the measured level of the marker is compared with a level of marker which is indicative of the absence of ACS.

25

7. A method as claimed in claim 6, wherein the level of the marker which is indicative of the absence of ACS is the level of the marker from one or more mammalian subjects free from ACS, or a previously determined reference range for the marker in mammalian subjects free from ACS.

30

8. A method as claimed in any preceding claim, wherein the level of the marker is measured by contacting the sample with an antibody that is immunospecific for the

marker and measuring any binding that has occurred between the antibody and at least one species in the sample.

5 9. A method as claimed in claim 8, wherein the antibody is a monoclonal antibody.

10. A method as claimed in any preceding claim, wherein the acute coronary syndrome is myocardial infarction or unstable angina.

10 11. A kit, immunoassay or device for carrying out the method of any preceding claim.

12. A kit, immunoassay or device for screening, diagnosis or prognosis of acute coronary syndrome (ACS) in a mammalian subject, for determining the stage or
15 severity of ACS in a mammalian subject, for identifying a mammalian subject at risk of developing ACS, or for monitoring the effect of therapy administered to a mammalian subject having ACS, said kit, immunoassay or device comprising:

instructions for taking a sample of bodily fluid from said mammalian subject;
and

20 one or more reagents for measuring the level of a first marker in the sample, wherein the first marker is Urotensin II (UTN).

13. A kit, immunoassay or device as claimed in claim 12, wherein the bodily fluid is plasma.

25

14. A kit, immunoassay or device as claimed in claim 12 or claim 13, wherein the one or more reagents for measuring the level of the first marker comprise an antibody that binds specifically to urotensin.

30 15. A kit, immunoassay or device as claimed in claim 12, 13 or 14, further comprising one or more reagents for measuring the level a second marker indicative of ACS.

16. A kit, immunoassay or device as claimed in claim 15, wherein the one or more reagents for measuring the level of the second marker comprises an antibody that binds specifically to the second marker.

5

17. A kit, immunoassay or device as claimed in claim 14 or claim 16, wherein the antibody is a monoclonal antibody.

10

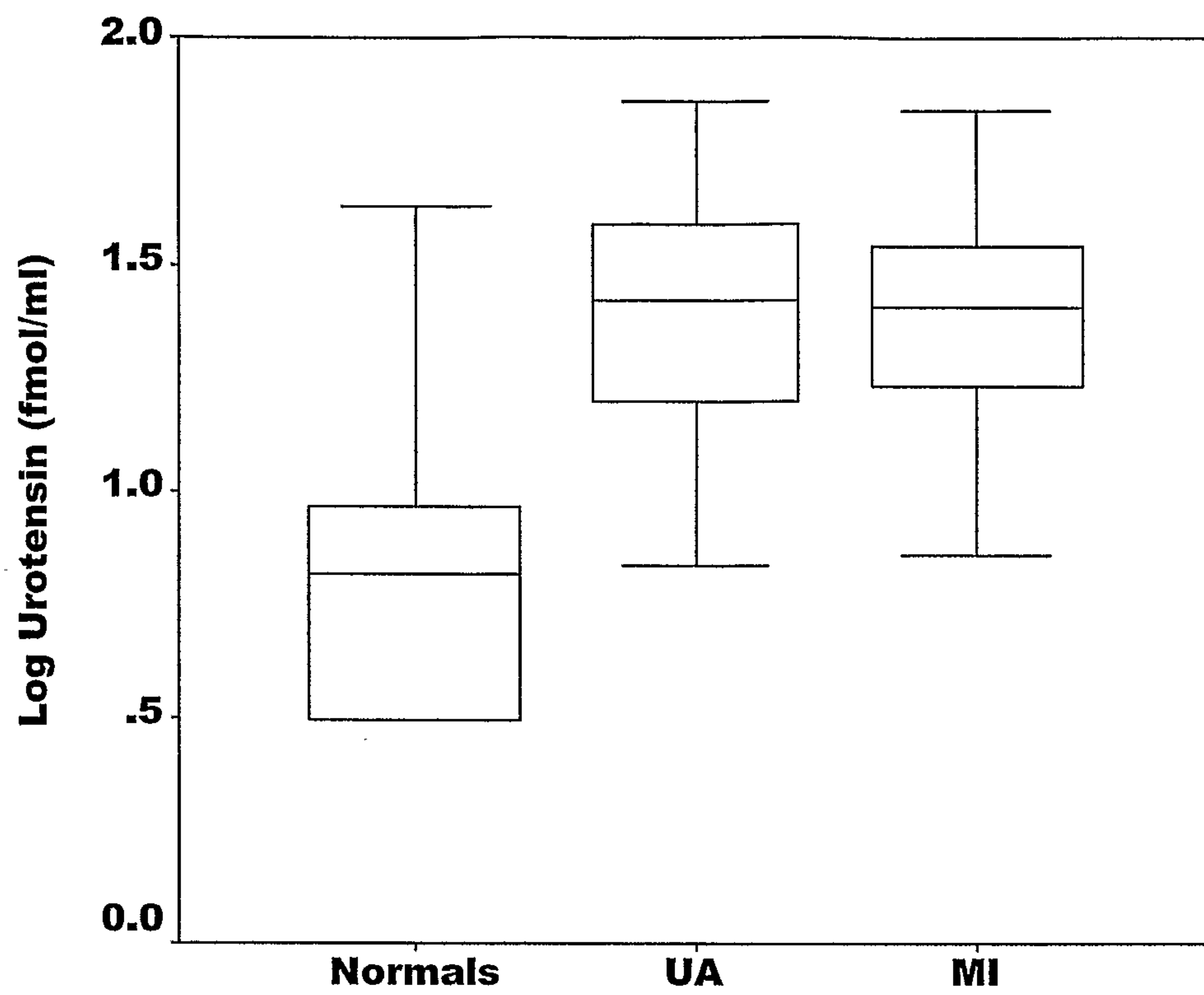
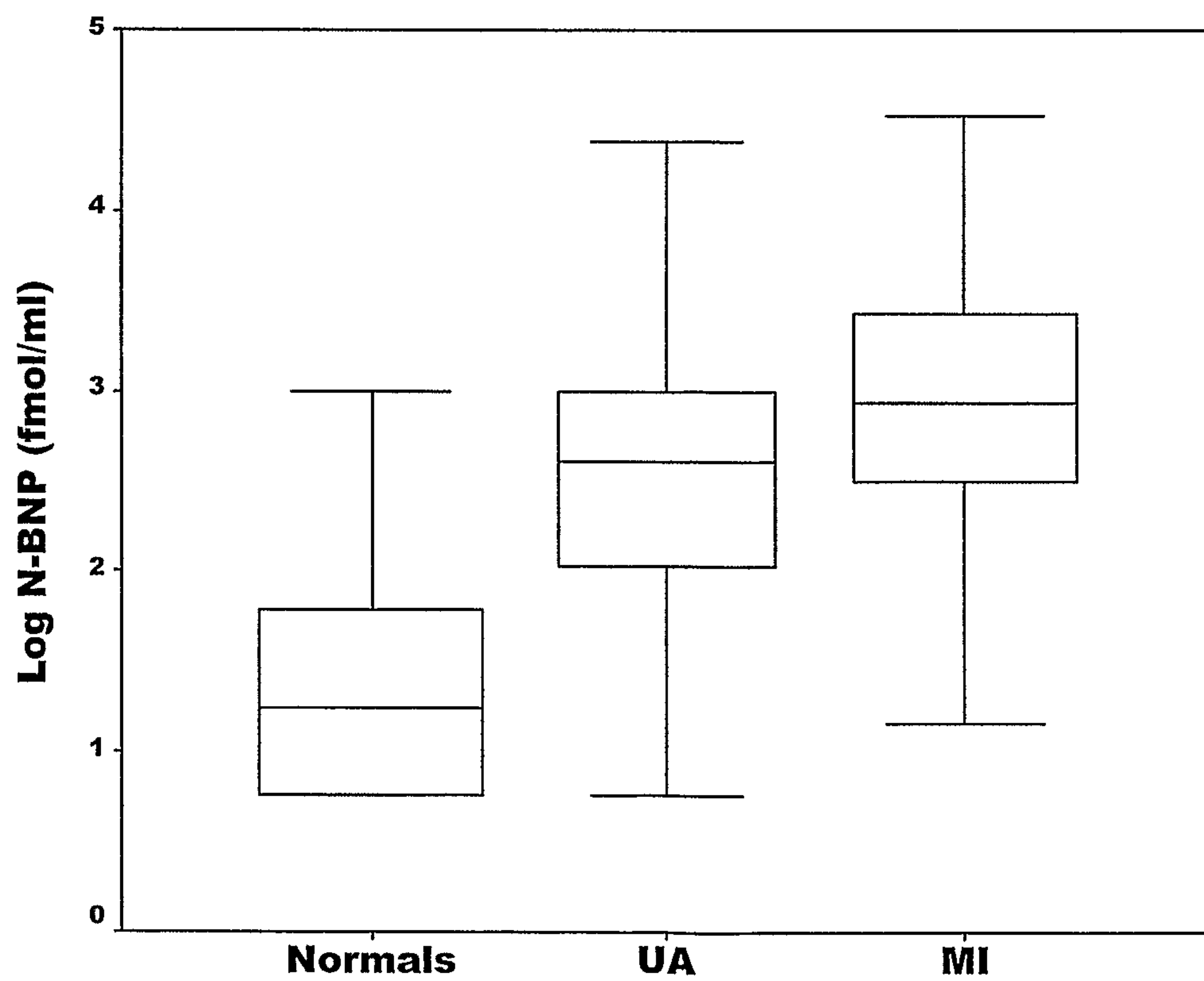
18. A kit, immunoassay or device as claimed in claim 15, 16 or 17, wherein the second marker is one or more of brain natriuretic peptide, troponins, creatine kinase MB isoform, and myoglobin.

15

19. A kit, immunoassay or device as claimed in any one of claims 12 to 18, wherein the mammalian subject is human.

20. A kit, immunoassay or device as claimed in any one of claims 12 to 19, wherein the acute coronary syndrome is myocardial infarction or unstable angina.

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Figure 1a**Figure 1b**

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Figure 2a
ROC Curve

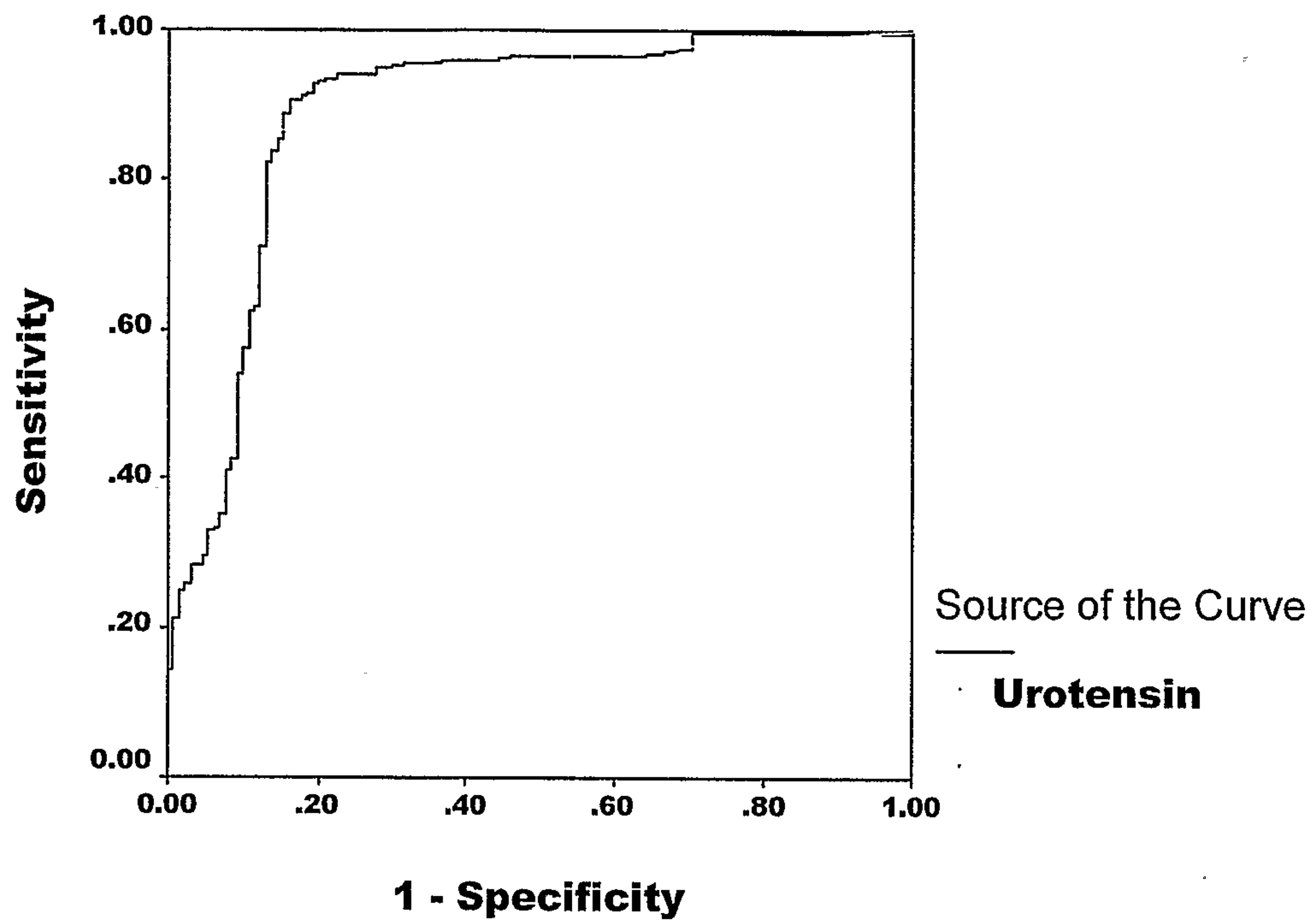
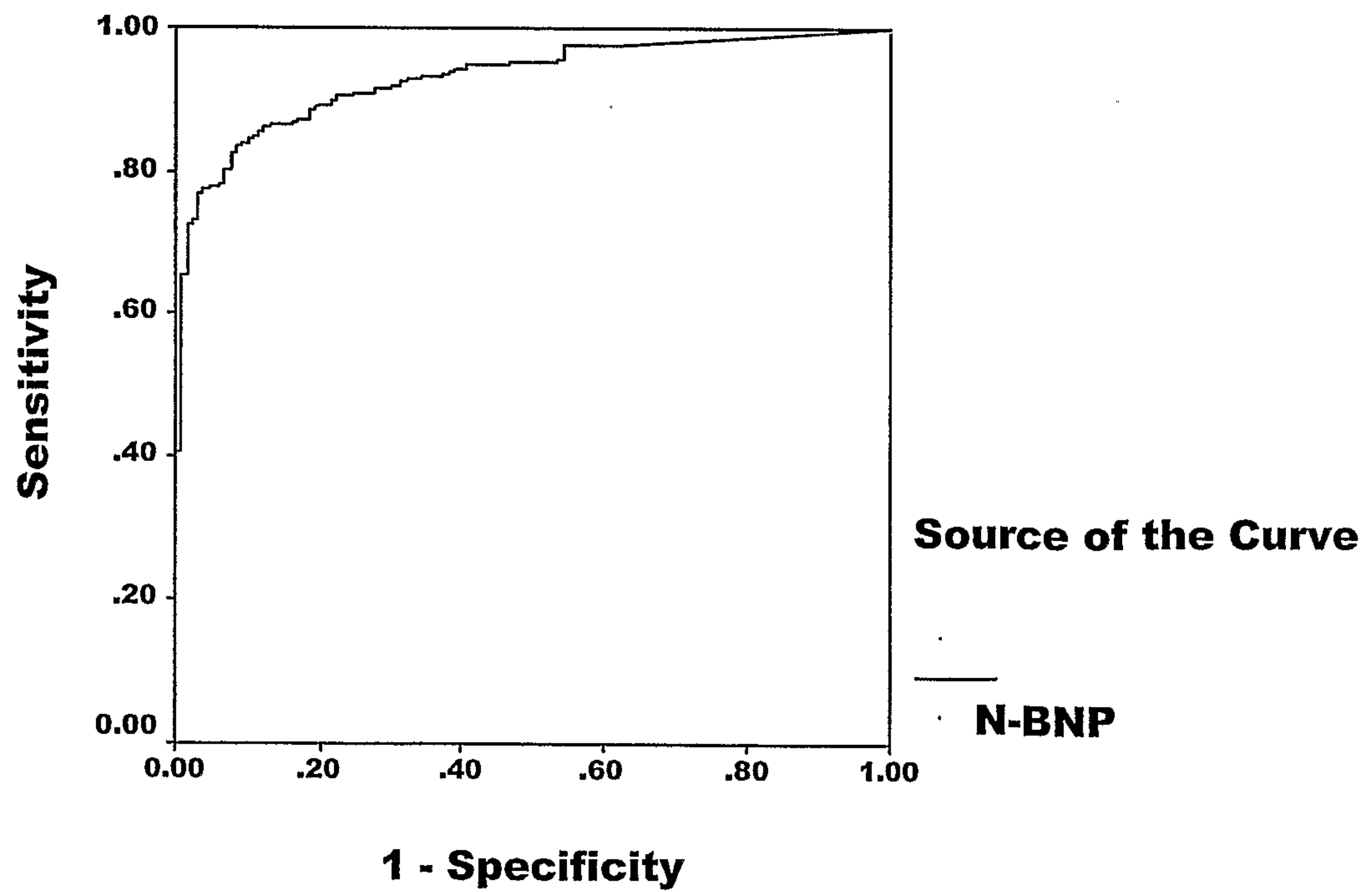
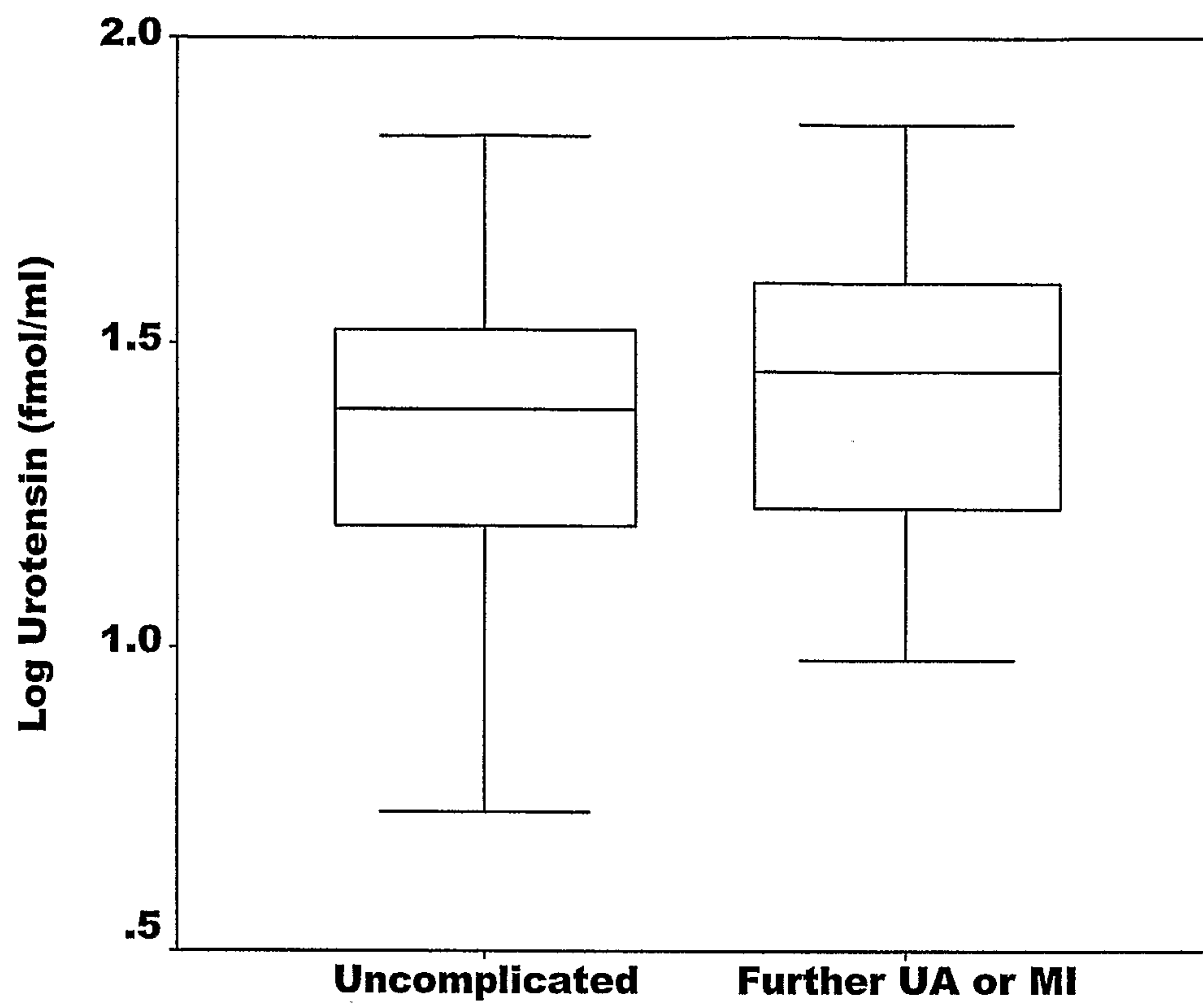
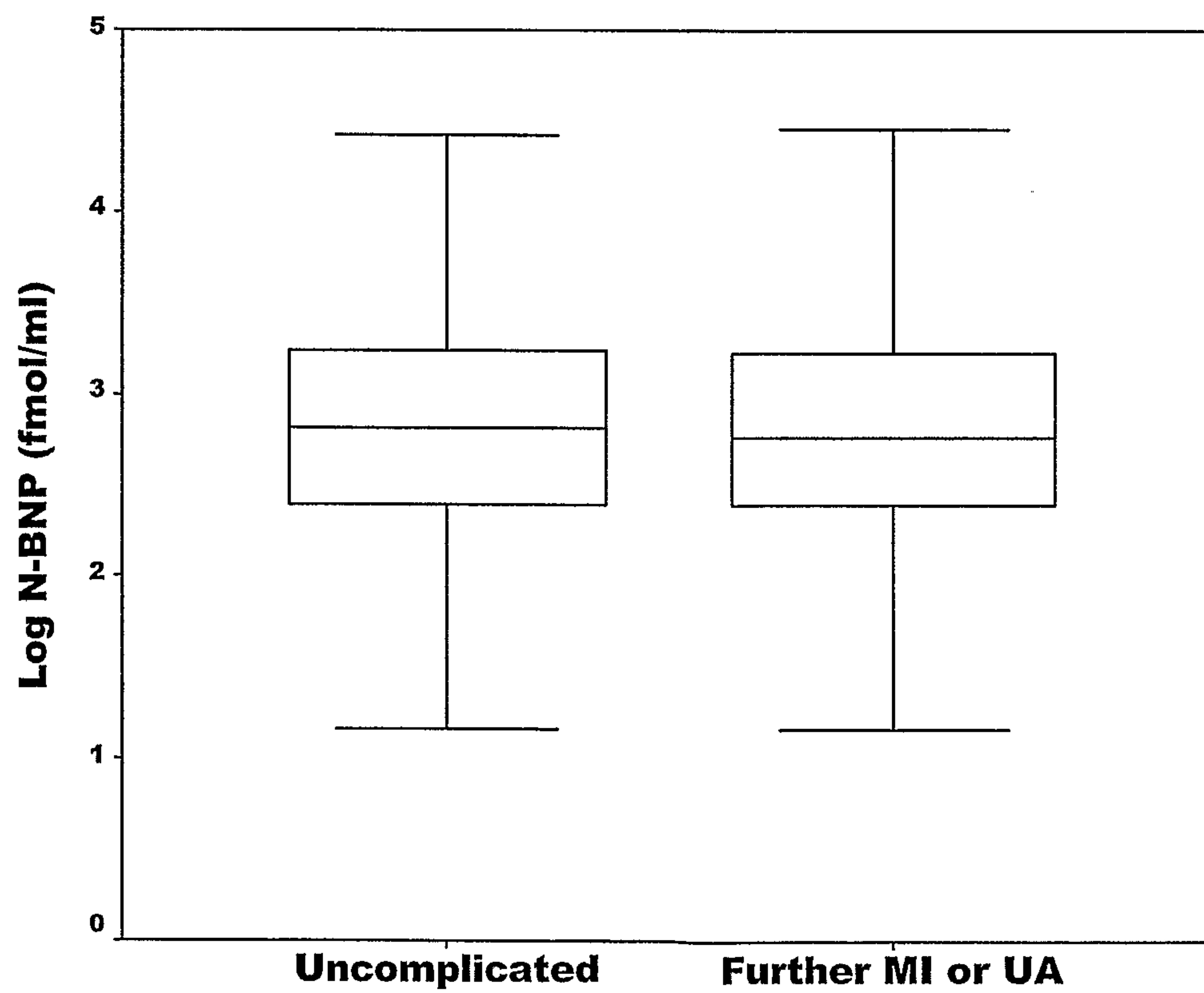


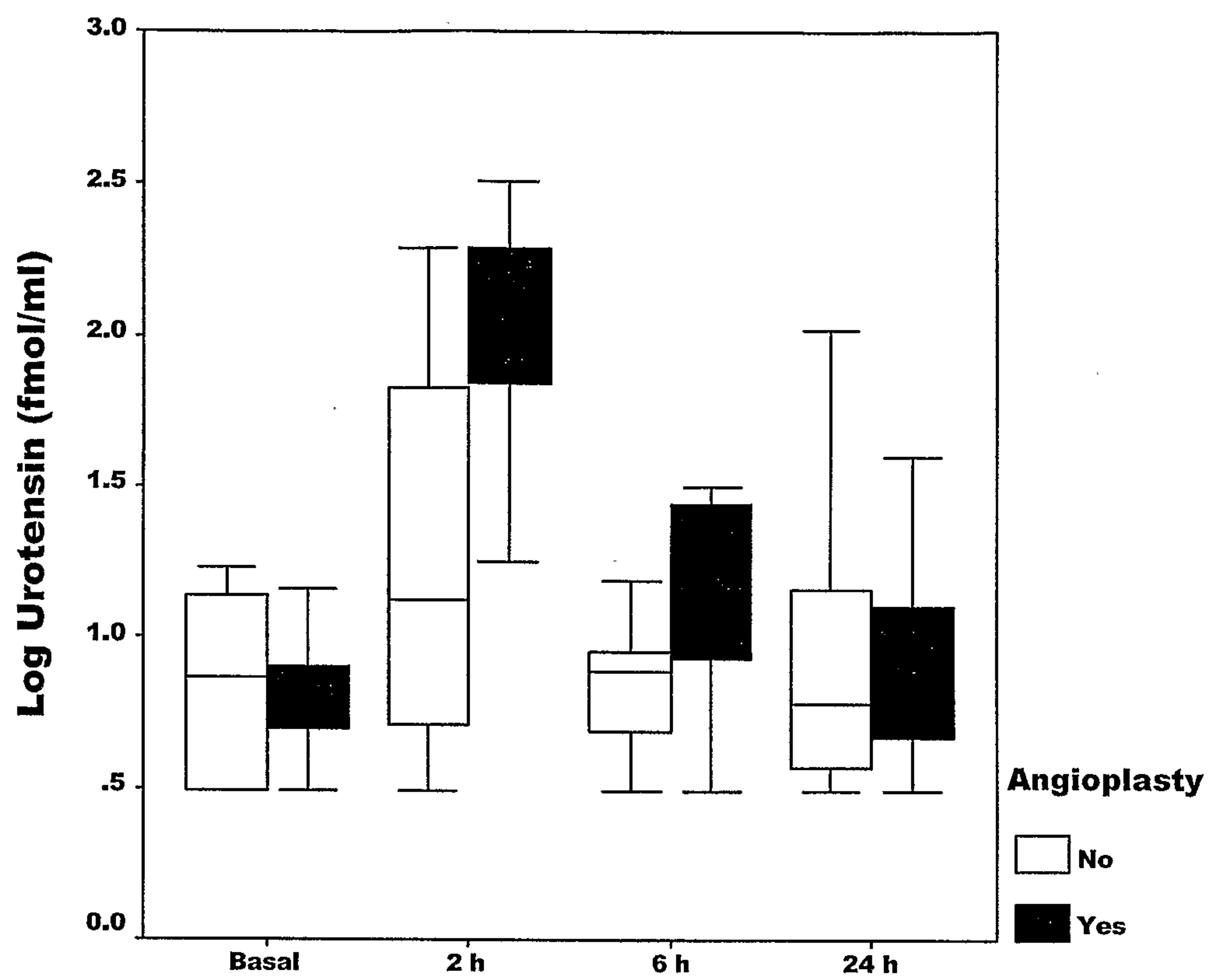
Figure 2b



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Figure 3a**Figure 3b**

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Figure 4a**Figure 4b**