

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 June 2007 (07.06.2007)

PCT

(10) International Publication Number
WO 2007/062676 A1

(51) International Patent Classification:

G01N 33/74 (2006.01) **A61P 9/00** (2006.01)
A61K 38/22 (2006.01)

(21) International Application Number:

PCT/EP2005/012844

(22) International Filing Date:

1 December 2005 (01.12.2005)

(25) Filing Language:

English

(26) Publication Language:

English

(71) Applicant (for all designated States except US):
B.R.A.H.M.S. AKTIENGESELLSCHAFT [DE/DE];
Neuendorfstrasse 25, 16761 Hennigsdorf (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BERGMANN, Andreas** [DE/DE]; Baumläuferweg 47, 12351 Berlin (DE). **STRUCK, Joachim** [DE/DE]; Zerndorfer Weg 52A, 13465 Berlin (DE). **MORGENTHALER, Nils, G.** [DE/DE]; Heiligenseestrasse 121 F, 13503 Berlin (DE).

(74) Agent: **ANDRAE, Steffen**; Andrae Flach Haug, Balanstrasse 55, 81541 München (DE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

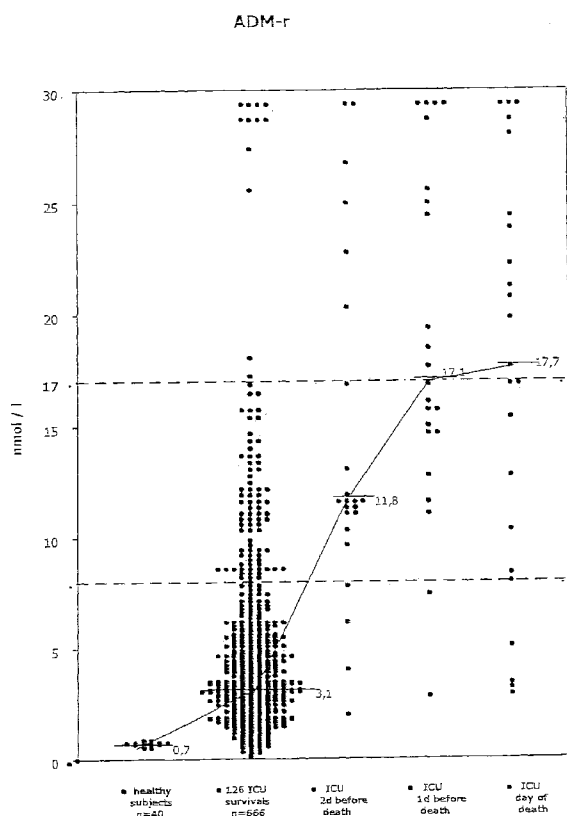
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv))

[Continued on next page]

(54) Title: METHODS FOR THE DIAGNOSIS AND TREATMENT OF CRITICALLY ILL PATIENTS WITH ENDOTHELIN, ENDOTHELIN AGONISTS AND ADRENOMEDULLIN ANTAGONISTS



(57) Abstract: The ratio of concentrations of pro-adrenomedullin (pro-ADM)/pro-endothelin (pro-END) immunoreactivity in body fluids of critically ill patients is used as for the diagnosis, course control and prognosis, including an assessment of the mortality risk, of severe life threatening diseases. Further, a treatment of critically ill patients having high levels of pro-ADM but insufficient levels of pro-END immunoreactivities with a medicament comprising vasoconstrictive endothelin or its precursors, and/or endothelin agonists or adrenomedullin antagonists is provided.



Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

- 1 -

METHODS FOR THE DIAGNOSIS AND TREATMENT OF CRITICALLY ILL PATIENTS WITH
ENDOTHELIN, ENDOTHELIN AGONISTS AND ADRENOMEDULLIN ANTAGONISTS

Methods for the diagnosis and treatment of critically ill patients, and the use of endothelin and endothelin agonists with vasoconstrictor activity for the preparation of medications for the treatment of critically ill patients

The present invention relates to novel diagnostic, and derived therapeutic, methods useful for the diagnosis and treatment of a group of severe, life threatening diseases. The invention can also be considered as relating to the field of intensive care medicine.

More specially, the invention is based on the surprising finding that the physiological release of two different vasoactive types of peptides, namely of peptides of the adrenomedullin and endothelin families, for which it was already reported that they can be found elevated during potentially life threatening conditions as e.g. sepsis and other severe conditions, if considered in combination, provides new and highly relevant information on the actual status of a critically ill patient and his prognosis. A detected imbalance of the peptides of said families, at least if determined by means of suitable methods providing information on the release of said peptides rather than on their actual level in a body fluid, above certain critical threshold concentration levels in the circulation of the patient, enables a physician to intervene and to restore said balance so that the patient gets a chance to recover

- 2 -

from an acute crisis which otherwise would lead to his death.

The diseases for which the invention is contemplated as being of high diagnostic and therapeutic value comprise sepsis, SIRS, septic shock, MOF (multiple organ failure), severe systemic or local infections, especially bacterial infections, peritonitis, pancreatitis, local and/or systemic inflammation, meningitis, trauma, ruptured aortic aneurysm, intoxication, endotoxemia, anuria, renal insufficiency, arterial hypertension, pulmonary hypertension, arteriosclerosis, several types of cancer, for example colorectal cancer, congestive heart failure, cardiovascular diseases, coronary artery disease, ischemia, antiarrhythmic effects, renal and/or heart failure, organ injury, and diseases related to growth, and others.

The invention is based on the surprising finding that high levels of vasoactive peptides of the endothelin family, which can be determined by measuring peptides indicating a high physiological release of the potent vasoconstrictors as endothelin-1, in critically ill patients with high levels of adrenomedullin can exert a beneficial, potentially life saving effect. In the scientific literature, in which occasionally speculations about an antagonistic behaviour of adrenomedullin and endothelin peptides can be found, high concentrations of the vasoconstrictors of the endothelin family are constantly considered as dangerous and undesirable. High levels of adrenomedullin, on the contrary, are regarded as positive. An example for such speculations is found in the publication of Takayuki Shindo et al., Hypotension and Resistance to Lipopolysaccharide-Induced Shock in Transgenic Mice Overexpressing Adrenomedullin in Their Vasculature, In: Circulation. 2000; 101:2309-2316.

The applicants have determined the formation or release of peptides of the adrenomedullin and endothelin families in

- 3 -

critically ill patients, using new assays measuring prohormone (propeptide) immunreactivities which provide information on the physiological release of both types of peptides rather than on the actual levels of the "mature" peptides in the circulation, and they found that under certain circumstances explained in more detail below in the examples in connection with the results of measurements of sera of critically ill patients, a dramatic decrease of measurable endothelin formation, while at the same time the levels of released adrenomedullin are critically high, precedes the death of such patients.

Based on said findings the applicants have defined a condition called "low endothelin syndrome" (in the following briefly LES) or "endothelin deficiency syndrome" which is characterized by a life threatening change in the release phase of endothelin (more specially of "endothelin-r" or "END-r" as it is defined below) in relation to the release of adrenomedullin (more specially "adrenomedullin-r" or "ADM-r" as it is defined below). Said syndrome is characterized by a high systemic concentration, or a high release rate, of the vasodilator adrenomedullin and, concomitantly, by an insufficient release rate of the vasoconstrictor endothelin (END-r). The consequence of such mismatch is an uncontrollable vasodilation and an insufficient supply of one or more organs with blood, i.e. a single or multiple organ failure.

In the present application the suffix "-r", used in terms as END-r or ADM-r, stands for "released". It means that the parameter in question is not the actual, more or less momentary or transient measurable serum or plasma concentration of the biologically active biomolecule (mature peptide) in question but rather reflects its cumulative release over a longer period of time. It can be directly measured e.g. by measuring a peptid fragment formed from the physiological precursor peptide (pre-pro- or pro-peptide or -hormone)

- 4 -

concomitantly with the formation of the active biomolecule, which fragment, however, has a considerably longer half life in the circulation (a considerably slower degradation or clearance rate) than the active biomolecule. Assays for measuring ADM-r are described in applicants' European patent EP 1 488 209 B1 „Bestimmung eines midregionalen Proadrenomedullin-Teilpeptids in biologischen Flüssigkeiten zu diagnostischen Zwecken, sowie Immunoassays für die Durchführung einer solchen Bestimmung“. Assays for measuring END-r are described in applicants' European patent EP 1 564 558 B1 „Verfahren zur Bestimmung der Bildung von Endothelinen zu Zwecken der medizinischen Diagnostik, sowie Antikörper und Kits für die Durchführung eines solchen Verfahrens“. The contents of both patents are hereby expressly included into the present application by reference. Examples 1 to 6 of the present application can be considered as excerpts from both patents to provide in the present application an enabling disclosure.

Both the measurable adrenomedullin and endothelin formation or release (ADM-r and END-r) are part of the relevant combined measurements according to the present invention. As relevant parameter a "ratio" of both can be defined. It is explained in more detail below.

The invention, according to one of its aspects, relates to the use of the "ratio" of ADM-r/END-r, in particular a ratio pro-adrenomedullin (pro-ADM)/pro-endothelin (pro-END) or fragments or combinations thereof, for the diagnosis of the low endothelin syndrome (LES) in critically ill patients affected by one of the diseases/conditions listed above. More specially the invention relates to the measurement of said ratio for the course control and prognosis of such disease, for the assessment of the mortality risk alone and/or in combination with a respective computer program.

In one of its preferred embodiments the invention relates to

- 5 -

further include clinical and/or other information for the evaluation of such risk.

LES may comprise but is not limited to the association of diseases like those mentioned above.

ADM-r and END-r are normally measured by determining in suitable body fluids, especially in the circulation (serum, plasma, whole blood) a relatively persistent fragment of their physiological precursor peptides (pre-pro- or pro-peptides or -hormones). What is measured, therefore, can also be regarded as pro-peptide immunoreactivity (pro-hormone immunoreactivity). The terms pro-ADM and pro-END relate to such measurable immunoreactivity and shall comprise also amino acid sequences showing at least 75 % homology, preferred at least 80 % homology, more preferred at least 90 % homology to pro-ADM or pro-END, their fragments or precursors.

The term diagnosis also comprises prognosis, early prognosis and course control.

The invention further relates to the use of endothelin forms having vasoconstrictor activity, more particular of END-1 and/or big-END, as well as optionally of END-agonists (molecules binding to the END receptor and exerting there the same or similar effects as the vasoactive endothelin) and/or ADM-antagonists (molecules binding to the ADM receptor and blocking there the effects as the vasoactive adrenomedullin) for the preparation of a medicament for the treatment of LES and/or diseases comprising those mentioned above. LES can be considered as an acute metabolic disorder involving an imbalance of certain potent effector molecules. By applying to a patient the active physiological molecule for re-establishing the required balance, similar to the administration of insulin to a diabetes patient, detrimental effects of a lack of said active physiological molecule can be outbalan-

- 6 -

ced. The molecules involved in LES are adrenomedullin (ADM) and endothelin (END). In sepsis patients the concentrations of endothelin and adrenomedullin are increased above normal, depending on the severity of disease (Fig. 1 and 2). In a very severe state of sepsis preceding death, according to the present invention it was surprisingly found that the concentration of endothelin decreases dramatically (Fig. 3 and 4). This, in view of persisting high concentrations of ADM, consequently lead to a dramatic decrease in blood pressure. That dramatic decrease in blood pressure caused by the lack of endothelin (LES) frequently leads to the death of the patient as is disclosed in the present invention.

With respect to the published knowledge concerning adrenomedullin and endothelin, reference is made to the two earlier applications mentioned above. In addition, reference also is made to applicants' earliest patent application relevant for the determination of pro-ADM and pro-END in sepsis, published inter alia as WO 00/22439. The following summarizes the most important facts.

Adrenomedullin (ADM) was first described in 1993 as a new hypotensive peptide containing 52 amino acids that was isolated from a pheochromocytoma. In the same year the precursor protein pre-pro-adrenomedullin (pre-pro-ADM) containing 185 amino acids was described. The precursor protein contains a signal sequence of 21 amino acids at its N-terminus. ADM comprises the positions 95-146 of the pre-pro-ADM amino acid sequence and is a splice product thereof. Another splice product is PAMP, a 20 amino acid peptide spliced from positions 22-41 that follow the 21 amino acids of the signal peptide. The terms ADM or pro-ADM used in the present invention comprise ADM, its precursors and fragments thereof.

So far ADM and PAMP are known to influence blood pressure. ADM is a potent vasodilator, the hypotensive effect is

- 7 -

supposed to be located in the C-terminal part of ADM. Peptide sequences at the N-terminus on the contrary show hypertensive effects. PAMP was also shown to have a hypotensive effect, even though the mechanism of action seems to be different.

In connection with the discussion of therapeutical aspects of the present application, the term "endothelin" (END) generally comprises endothelin forms with vasoconstrictor activity, especially endothelin-1 (END-1) and its precursor big-endothelin, but may also comprise related molecules as e.g. endothelin-2 (END-2) or endothelin-3 (END-3) as well as their precursors.

In connection with analytical determinations, "endothelin" generally means END-r as defined above. It is, however, within the scope of the invention to determine or monitor active forms of endothelin, i.e. especially END-1 or big-endothelin, because their decrease and finally disappearance from the circulation also is directly indicative of the onset of a critical, life-threatening crisis. Absolute values for measurable concentrations, and ratios of measurable concentrations, of course, are influenced by e.g. the molecular species actually measured, by the units in which the measurements are expressed, and by the calibration of the assays used. The absolute numerical values reported in the present application are relevant for the assays designs described herein only. Since they may have to be adapted if e.g. another analyte is measured or the particulars of its determination are changed, it is not practical to express the results of the present invention in absolute numerical values. However, by following the teachings of the present application, including the described scoring of the measured results, the skilled person can easily determine the numbers and ratios relevant for any modified determination method.

Endothelin-1 comprises 21 amino acids and is the most potent

- 8 -

vasoconstrictor known. 3 isoforms of END are existing: endothelin-1 (END-1), endothelin-2 (END-2) and endothelin-3 (END-3), END-1 is present in the highest concentrations and is the most potent. Endothelin-1 is synthesized in the endothelial cells, in lung, heart, kidney and brain. The primary translation product of the human endothelin-1 gene is pre-proendothelin-1, comprising 212 amino acids. In the secretory pathway the signal peptidase removes the short N-terminal signal sequence, comprising 17 amino acids. The resulting pro-endothelin is subsequently processed to a still biologically inactive big-endothelin, comprising 38 amino acids, by the protease furin, which can be found in the circulation. The mature biologically active endothelin-1 is formed from big-endothelin by endothelin converting enzyme. Endothelin exerts its biological effect by binding to specific receptors that are localized on muscle cells, myocytes and fibroblasts. This binding causes the efflux of calcium, activation of phospholipase C and inhibition of Na/K ATPase. Besides the vasoconstrictive effect, endothelin also regulates growth.

The concentration of ADM in the blood circulation and other biological fluids is increased significantly in a variety of diseases like congestive heart failure, myocardial infarction, renal diseases, high blood pressure, diabetes mellitus, in the acute phase of shock, sepsis, septic shock, multiple organ failure. PAMP concentrations are also increased in some of the above mentioned diseases/disorders, however the plasma level is decreased relatively to ADM. It is known that in sepsis and septic shock unusually high concentrations of ADM are observed. This finding is related to the hemodynamic changes that are typical for the course of disease in patients with sepsis and other severe diseases like SIRS.

Increased plasma concentrations of endothelin-1 and big-endothelin were shown for several diseases comprising car-

- 9 -

diovascular diseases comprising pulmonary hypertension, arteriosclerosis, congestive heart failure, cardiac infarction, sepsis, septic shock and cancer.

A major cause of mortality due to sepsis is the so-called multiple organ failure, which is caused by a systemic vasodilation, including a decrease of blood pressure and a loss of priority supply of single organs and tissues. Endothelin is thought to play a role in the degree of severity of sepsis and to play a role in the mortality of sepsis.

Analytical/diagnostic aspects of the invention

The present invention in one of its aspects relates to the diagnosis of LES and/or associated diseases comprising those mentioned above. The present invention further relates to the concomitant determination of the levels of END-r and ADM-r, more specially of pro-END and pro-ADM and/or their fragments. The combined measurement enables the calculation of a ratio and/or an algorithm and the use of such a derived ratio and/or algorithm for the diagnosis, prediction, prognosis of course of severity and the risk of mortality of LES and/or the above mentioned diseases (Fig. 1 and 2).

The invention is based on the surprising finding that both values of pro-ADM and pro-END are increasing in patients during course of disease, wherein the values of pro-ADM are increasing more than those of pro-END, leading to an increase of the overall ratio pro-ADM/pro-END until one day before exit.

More remarkably, however, the concentration of endothelin on the day of death decreases dramatically. Consequently the ratio and/or algorithm of pro-ADM/pro-END increases dramatically (Fig. 3 and 4).

The determination of ADM, more specially ADM-r, as well as

- 10 -

END, more specially END-r, therefore provides information on the health state of a critically ill patient, especially with respect to the development of a life threatening crisis which requires an intervention. This is explained more completely referring to the following analytical findings and the results shown in Table 1 (see Example 8) and the Figures:

149 patients were included in a study, who were admitted to an intensive care unit (ICU) and developed a sepsis during their stay at ICU.

EDTA-plasma of the patients was gained at each day of their stay at ICU (3-29 days). The plasma was stored at -20 °C until further use.

Pro-ADM and pro-END values were determined by using a coated tube assay as described in the earlier European patents of the applicant already mentioned above. For the assays, synthesized peptides were used to produce antigens and injected into animals to raise antibodies against the different peptides as described earlier. In a preferred embodiment the peptides were linked to the carrier protein keyhole limpet hemocyanin using MBS (m-maleimidobenzoyl-N-hydroxysuccinimid ester) and used for the immunisation of sheep.

More particular, amino acid sequences of partial peptides 83-94 [SSPDAAIRVKR= Sequence ID 2] and 69-86 [RPQDMKGASRPEDSSPD = Sequence ID 3] of pre-pro-ADM 1-185 [Sequence ID 1] as well as partial peptide 168-181 [RSSEEHLRQTRSET = Sequence ID 6] and 200-212 [SRERYVTHNRAHW = Sequence ID 7] of pre-pro-END 1-212 [Sequence ID 5] were synthesized. These sequences are comprised in the pre-pro-ADM 1-185 and pre-pro-END 1-212. An amino terminal cystein residue was added to each peptide. The peptides were conjugated to a hemocyanin and antibodies were produced to the above mentioned

- 11 -

peptides in sheep as described earlier. Polyclonal antibodies were raised against the above mentioned peptides. Antibodies were purified. In a preferred embodiment, this was achieved by ligand specific affinity chromatography by coupling the peptides via the amino terminal cysteine residue to SulfoLink-Gel of Pierce (Boston, USA) according to the methods of Pierce. The antibodies further were tagged with a marker to enable detection. The marker used is preferably a luminescent marker and yet more preferred a chemiluminescent marker as described earlier.

Methods for the detection of binding of the antibody to the respective molecule are known by the person skilled in the art comprising sandwich immuno assays, radio immuno assays, ELISA or point of care tests. Preferably, according to the invention the binding of the antibody to the target is detected by chemiluminescence.

The detection and the determination of the concentration of pro-ADM and pro-END can be conducted in various body fluids, tissues and other biomaterials but preferably is conducted in serum or plasma. It can take place in various states of all kinds of diseases as exemplified above which may lead to LES, sepsis, septic shock, SIRS and related severe conditions .

It is part of the invention to use the correlation of ADM-r and END-r, preferably of precursor proteins with pro-ADM and pro-END immunoreactivity, or, if feasible, also of the mature proteins like END and ADM in the state of disease/-disorder in body fluids, tissues or other biomaterials, blood, plasma and liquor in particular.

The invention further uses the significant change of precursor protein concentrations in body fluids, tissue and other biomaterials in disease or disorder, in particular in a severe state of disease accompanied by LES as acute com-

- 12 -

plication.

ADM-r, if measured as described herein, is present in plasma of healthy individuals at concentrations of < 1 nmol/l (median: 0,7). Critically diseased persons who survived a sepsis show a significantly increased ADM-r concentration (median 3,1). The median of dying patients is significantly increased as compared to the group of surviving patients (median 11,8/17,1/17,7; 2 days before exit/one day before exit/on the day of death respectively, Fig. 1).

The concentration of END-r as a putative antagonist of ADM, if measured as described herein, increases from a median of 0,035 nmol/l plasma to 0,054 nmol/l in surviving patients. The concentration in dying patients further increases to 0,096 and 0,133 nmol/l two days before exit and one day before exit respectively. But surprisingly the concentration of END-r decreases dramatically on the day of death (median 0,064 nmol/l, Fig. 2).

These findings can be correlated with the prognosis of survival in a severe state of LES or other diseases comprising those mentioned above by determining the concentration of ADM-r, END-r and combinations thereof and forming, as additional parameter, a ratio of the above mentioned proteins and/or an algorithm thereof.

Firstly, the concentration of ADM-r alone was used to determine the prognosis of survival (Table 1 and Fig. 1). Column 2 (specificity) shows the number of surviving patients having a concentration of less than 17 nmol/l serum/plasma of ADM-r, i.e. 117 of 126 patients have a concentration of less than 17 nmol/l, 102 patients a concentration of less than 8 nmol/l. Thus ADM-r shows a specificity of 93 % for survival at a cut off at 17 nmol/l and 81 % at a cut off at 8 nmol/l.

- 13 -

The sensitivity of mortality is determined in the following way: 23 patients died during their stay at ICU. At a cut off value of 17 nmol/l of ADM-r six patients (26 % = sensitivity of mortality) showed concentrations above 17 nmol/l two days before exit, 12 patients (52 %) one day before exit and 13 patients (57 %) on the day of death. At a cut off value at 8 nmol/l, 19 (83 %) of 23 patients showed concentrations above 8 nmol/l of ADM-r two days before exit, 21 patients (91 %) one day before exit and 19 patients (83 %) on the day of death (Table 1; see Example 8).

Thus a cut-off value at 8 nmol/l of ADM-r (i.e. excluding the patients having a concentration below 8 nmol/l of ADM-r) includes most of the patients who died during their stay at ICU.

Further, as has been found by the inventors, the ratio of ADM-r/END-r according to the present invention forms an additional parameter of high validity in connection with the prognosis of septic patients in ICUs.

Surprisingly said ratio increases during the course of disease and even more surprisingly the ratio increases dramatically on the day of death. According to the results, the specificity and sensitivity of survival and mortality respectively of LES and various other diseases comprising those mentioned above are disclosed, as well as the prognosis of severity of disease and mortality risk.

Measured ratios of AMD-r/END-r are shown in Table 1 and Fig. 3. The ADM-r/END-r ratio in healthy individuals has a maximum value of about 50 (median 20). The median of surviving patients at ICU is increased to 56. Significant further increases can be observed two days (median 135) and one day (median 129) before exit and a surprisingly dramatic increase on the day of death at a median of 336. The prognosis at a specificity of 76 % (Table 1, i.e. patients included

- 14 -

having a concentration of ADM-r of less than 43 nmol/l) is 91 % two days before exit, 96 % one day before exit and 87 % on the day of death (Table 1 and Fig. 3). At a higher specificity of 93 %, (i.e. patients included having a concentration of ADM-r of less than 18 nmol/l), a prognosis is only possible on the day of death having a sensitivity of 61 % (Table 1).

It can surprisingly be deduced from the results that the threatening of life in LES is only present at high ADM-concentrations, as a high potential of vasodilation must be reached. High concentrations of ADM are only tolerated if they are outbalanced by the respective END concentration.

For a further analysis of the results, the patients were further selected:

1. according to the ADM-r-concentration (>8 nmol/l) and
2. according to the ratio of ADM-r/END-r (Fig. 4).

It was shown that a high portion of surviving patients with an ADM-r concentration > 8 nmol/l at one day of their stay at the ICU, $N=24$ had a ratio of less than 90 ($N=15$) and thus their survival was assured. According to that assumption however also the dying patients would be included with a high probability 2 days before their death (sensitivity 87 % two days before exit / 91 % one day before exit / 78 % on the day of death). Taking into account the sum of patients included in the study (126 surviving patients) the specificity surprisingly is as high as 93 %.

The sequential analysis of ADM-r, END-r and the ratio and/or the algorithm of ADM-r/END-r at an equally high specificity (93 %) surprisingly shows a higher sensitivity for the prognosis (87 %/91%/78 %) than the analysis of ADM-r alone (26 %/52 %/57 %) or ratio alone (0/0/61) (values 2 days before death/1 day before death/day of death respectively).

- 15 -

The invention, consequently, also relates to the detection, early detection, determination of severity, course control and prognosis of the diseases/disorders mentioned above using said ratio or the determined algorithm.

In this context the invention contemplates the use of a computer program to use the data received from the ratio for the detection, early detection, determination of severity, course control and prognosis of LES and/or the diseases mentioned above.

For carrying out measurements as discussed above, assays for ADM-r and END-r, as they are described in applicants' European patents mentioned above, and the corresponding kits, can be used with advantage.

Therapeutical aspects of the invention

In contrast to the state of the art, the invention discloses the surprising finding that elevated concentrations of endothelin (especially if determined as END-r) are beneficial for a patient having highly elevated concentrations of ADM-r as they are typically found in patients in severe conditions as e.g. sepsis and the related diseases mentioned above. In other words, the results show that high levels of END-r enable a patient to tolerate high, otherwise deadly levels of ADM-r typical for conditions as sepsis so that death can be prevented. Shortly, high ADM levels apparently can be outbalanced by physiological END levels of sufficient height.

A measurable decrease of END-r, or serum END, in such critically ill patient constitutes a clear clinical sign indicating a high risk of death of patients having high levels of ADM-r. The observed sharp decrease of measurable END-r and END respectively may be interpreted as sign of an exhaustion of the cellular reservoirs of END precursors under the

- 16 -

physiological stress of a condition as sepsis, or of an inability of the physiological, molecular machinery producing END and its precursors to keep up with the parallel continuing production of ADM and/or to outbalance the continuing vasodilatory action of high ADM concentrations.

According to a first therapeutical aspect the invention contemplates the administration of vasoconstrictive endothelin forms, especially of END-1 and/or big-END, to critically ill patients having high measured levels of ADM-r to externally replenish the active END in such patients. "Vasoconstrictive endothelin forms" are intended to include directly acting endothelin forms as END-1, as well as precursors as big-END which as such are inactive but are readily converted to an active form when they are introduced into the circulation of a human patient.

The administration of endothelin, in clinical settings as e.g. in ICUs (intensive care units), can be preceded by an analysis of the actual concentrations of ADM-r, END-r and a determination of the "ratio" discussed above. However, such preceding measurement and analysis must not be considered as an indispensable pre-requisite for the administration of endothelin according to the present invention.

In health care settings, e.g. in an ICU, there may not be enough time to wait for results of measurements of biomolecules as e.g. ADM-r and END-r before a decision regarding a therapeutic intervention can be made. It is, therefore, within the scope of the present invention to administer active endothelin to critically ill patients in response to characteristic changes of other physiological parameters which are indicative of the underlying low endothelin syndrome (LES). Such parameters may be measurements of physiological variables as blood pressure, body temperature, pulse frequency and similar parameters as they are used to monitor the state of a septic patient. Knowing the beneficial effect

- 17 -

of an administration of endothelin, such administration can be effected on the basis of purely empirical clinical data.

It is, however, also within the scope of the present invention to administer endothelin in response to a quantitative or semi-quantitative measurement of ADM-r alone, i.e. when the ADM level gets critically high. In view of the requirement to take a quick decision, it may be preferable to conduct the determination of ADM-r in ICUs with POC (point-of care) measuring devices, e.g. devcies of the immunochromatographic type or devices with electrochemical probes quickly producing readings for the concentration of interest, to obtain an at least semiquantitative reading allowing an evaluation of the actual levels of ADM-r in a patient and to make a decision for the intravenous administration of endothelin. Suitable POC measuring devices are well known to the skilled person. An example for a suitable design is the rapid test device PCT-Q® obtainable from B.R.A.H.M.S Aktiengesellschaft for the rapid POC determination of procalcitonin (PCT) e.g. in a hospital setting.

It is further within the scope of the present invention to administer endothelin to patients which are continuously monitored for the concentration(s) on one or more of the relevant biomolecules, or other relevant clinical parameters.

As stated earlier, in a therapeutical context "endothelin" means endothelin forms with vasoconstrictor activity, especially endothelin-1 (END-1) and its precursor big-endothelin, but the term may also extend to related molecules of similar activity, as e.g. endothelin-2 (END-2) or endothelin-3 (END-3) as well as their precursors.

The administration of the most active, directly acting END-1 at the moment is regarded as being preferred in critical care situations. Preferably such administration is an in-

- 18 -

travenous administration by infusion or injection, using a liquid formulation (solution or dispersion) containing a suitable concentration of END-1 together with pharmaceutically acceptable carriers etc.. Such liquid formulation (medicament) can contain all pharmaceutically acceptable and useful excipients, stabilisers and other ingredients suitable for such type of administration and for maintaining a stable condition within the administered formulation for the time of infusion.

It is, further, within the scope of the invention to administer, or co-administer, slower acting forms of endothelins, for example admixtures of quick acting and slower acting forms. Slower acting forms, e.g. in the form of big-END, may provide a more uniform pharmacokinetic profile and a longer lasting action of endothelins. Such forms may be more suitable for injection, while directly acting forms are preferred for administration by infusion.

It is further within the scope of the present invention to administer agonists of endothelin in place of, or together with, vasoconstrictive endothelins to achieve special effects, e.g. a favourable pharmacokinetic profile, or to assist the patient in situations when the most critical acute phase has been survived.

Further, since the life-threatening influence for the patient is not the lack of endothelin per se but the vasodilating action of high concentrations of ADM (ADM-r), it is also within the scope of the invention to use, in place of endothelin and/or endothelin agonists, or in combination therewith, adrenomedullin antagonists, i.e. molecules which prevent or attenuate the vasodilating action of adrenomedullin, e.g. by blocking its relevant receptors, or substances preventing the binding of adrenomedullin to its receptor (e.g. specific binders as e.g. antibodies binding to adrenomedullin and blocking its receptor binding sites; "immuno-

- 19 -

logical neutralisation"). Such use, or combined use, including a subsequent or preceding separate use, may in certain cases be desirable for example to improve the therapeutic success, or to avoid undesirable physiological stress or side effects.

The invention further discloses the use of endothelin forms with vasoconstrictor activity, more specially END-1 and/or big-END, as well as END agonists, for the preparation of a medicament for the treatment of LES and other conditions and diseases comprising those mentioned above. In particular, endothelin-1, big-endothelin and/or any endothelin 1 receptor agonists are used as active ingredients to prepare medicaments for administering them to a critically ill patient. Preferably the medicament is a liquid formulation for intravenous administration by infusion, for example via a catheter, or by injection.

There do not seem to exist, in the available scientific literature, proposals to use vasoconstrictive endothelin for medical purposes. The claimed use, therefore, apparently can be considered as first medical use.

In medicaments as discussed above the mentioned active END may be used, as is customary in the art, in liquid formulations containing suitable concentrations of e.g. appropriate carriers, stabilizers, nutrients, electrolytes, dissolved salts, osmotic agents for providing isotonic conditions, buffers and/or solvents and other adjuvants and excipients suitable for administration in a patient.

Description of the Figures

Fig. 1 shows the concentration of ADM-r in plasma of 40 healthy control individuals, 126 surviving patients at ICU and 23 patients, two days and one day before death and on the day of death.

- 20 -

Fig. 2 shows the concentration of END-r in plasma of 40 healthy control individuals, 126 surviving patients at ICU and 23 patients, two days and one day before death and on the day of death.

Fig. 3 shows the ratio of ADM-r/END-r in plasma of 40 healthy control individuals, 126 surviving patients at ICU and 23 patients, two days and one day before death and on the day of death.

Fig. 4 shows the ratio of ADM-r/END-r including only patients having a ADM-r concentration > 8 nmol/l in plasma; 24 being surviving patients at ICU and 20 further patients, two days and one day before death and on the day of death.

Example 1 - Peptide Synthesis

An N-terminal cystein residue was added to synthesized Peptides of human pro-ADM and pro-END. They were purified using mass spectrometry and the quality controlled using reversed phase HPLC and lyophilized in aliquots (Jerini AG, Berlin, Germany) according to standard procedures known to the person skilled in the art.

Example 2 - Conjugation and Immunization

Peptides of Sequence IDs 1-4 were conjugated to the carrier protein KLH (keyhole limpet hemocyanin) by MBS (Maleimidobenzoyl-N-hydroxysuccinimid ester) according to the protocols for "NHS-esters-maleimide crosslinkers" by PIERCE, Rockford, IL, USA. Sheep were immunized receiving 100 μ g of conjugate (μ g according to the peptide content of the conjugate). Starting at month 4 after immunization every four weeks 700 ml of blood were withdrawn from every sheep and antiserum was gained by centrifugation. Conjugation, immunizations and production of antisera were done by MicroPharm, Carmarthenshire, UK.

- 21 -

Example 3 - Purification of Antibodies

The polyclonal antibodies from sheep were purified using ligand specific affinity purification. For that step the Cys(0)-peptides were linked to SulfoLink-Gel supplied by Pierce (Boston, USA). The binding occurred according to the protocol of Pierce. 5 mg of peptide were added per 5 ml of gel.

In summary, columns were washed three times with 10 ml elution buffer (50mM citric acid, pH 2.2) and binding buffer (100mM sodium phosphate, 0.1% Tween, pH 6.8). 100 ml of sheep antiserum were filtered using a filter diameter of 0.2 μ m and added to the column material. The gel was eluted quantitatively with 10ml binding buffer. The material was incubated over night at room temperature by gentle rotation. The material was transferred to empty columns (NAP 25, Pharmacia, emptied). The eluates were discarded. Subsequently the columns were washed with 250 ml protein-free binding buffer (protein content of washed eluate < 0.02 A280 nm). Elution buffer was added to the washed columns and fractions of 1 ml were collected. The protein content of each fraction was determined by the BCA-method (according to the protocol of PIERCE, Rockford, IL, USA). Fractions of a protein content > 0.8 mg/ml were pooled.

Example 4 - Tagging

500 μ l of affinity purified antibodies generated against the peptides of Sequence IDs 1-4 were rebuffered in 1 ml 100 mM potassium phosphate buffer (pH 8.0) was re-buffered via a NAP-5 gel filtration column (Pharmacia) according to the protocol of Pharmacia.

For the tagging with a chemiluminescent marker 10 μ l of MA70-Akridinium-NHS-ester (1mg/ml; Hoechst Behring) were added to 67 μ l of antibody solution and incubated for 15

- 22 -

minutes at room temperature. The solution was rebuffered in 1 ml solvent A (50mM potassium phosphate, 100 mM NaCl, pH 7.4) in a NAP-5 gel filtration column according to the protocols of Pharmacia, particles of low molecular weight were eluted. For elution of unbound labels a gel filtration HPLC was done (Column: Waters Protein Pak SW300). The sample was added and chromatographed at a flow rate of 1ml/minute in solvent A. The sample was measured at wave length of 280 and 368 nm to determine the degree of tagging. The absorption ratio 368/280 nm had a peak of 1.0. The fractions containing monomeric antibodies were collected (retention time 8-10 minutes) and taken up in 3 ml 100mM sodium phosphate, 150 mM NaCl, 5% bovine serum albumin, 0,1 % sodium azide, pH 7.4).

The tagged antibody was used in a sandwich assay as well as in different assays like SPALT-assays.

Example 5 - Coupling

For a sandwich-assay the purified antibodies against the peptides of Sequence IDs 1-4 were immobilized on irradiated polystyrol tubes (Greiner, Germany). For that procedure the antibody solutions were diluted to a protein concentration of 6.7 µg/ml with 50 mM Tris, 100 mM NaCl, pH 7.8. 300 µl of diluted protein solution per tube were pipetted. These were incubated for 20 hours at room temperature, the solution was removed. Then 4.2 ml of a 10 mM sodium phosphate, 2% Karion FP, 0.3 % bovine serum albumin, pH 6.5 solution were added to each tube. After 20 hours the solution was removed and the tubes dried in a vacuum drier. The procedure can also be done as inverse sandwich assay according to procedures known to a person skilled in the art.

Example 6 - Sandwich Immunoassay

The following assay buffer was used: 100 mM sodium phospho-

- 23 -

te, 150 mM NaCl, 5 % bovine serum albumin, 0.1 % unspecified sheep IgG, 0.1 % sodium azide, pH 7.4.

The protein content of EDTA-plasma of healthy individuals and patients of various diseases /diseases mentioned above was determined.

In the tubes containing the immobilized antibody EDTA-plasma of patients was pipetted (10 μ l ADM and 50 μ l END-r). 200 μ l of PBS (phosphate buffered saline), 0.1 % bovine serum albumin were added to each tube and incubated at room temperature for 2 hours. After washing 3 times with 4 ml PBS 200 μ l acridiniumester-tagged polyclonal sheep antibody (affinity purified, raised against the peptide 83-94 [Sequence ID 2] and 69-86 [Sequence ID 3] of pre-pro-ADM 1-185 as well as partial peptide 168-181 [RSSEEHLRQTRSET = Sequence ID 6] and 200-212 [SRERYVTHNRAHW = Sequence ID 7] of pre-pro-END 1-212, 20 ng each in PBS, 0.1 % bovine serum albumin) respectively. After further incubation of 2 hours at room temperature unbound tagged antibodies are removed by washing 5 times with 2 ml PBS each. Tagged antibody bound to the tube was quantified by measuring the luminescence in a luminometer (Berthold LB 952T/16).

The individual values measured were quantified according to a usual calibration curve that was established using defined dilutions of synthesized pre-pro-ADM 45-92 and pre-pro-END 169-212 as standard solutions.

Example 7 - Measurements

Concentrations of ADM-r and END-r in healthy individuals and state of disease

149 patients were included in the study who were at the intensive care unit for different reasons and who developed a sepsis during their stay at the intensive care unit (cri-

- 24 -

teria: www.talessin.de/scripte/medizin/sepsis1.html). Every day during their stay at the intensive care unit (3-29 days) EDTA-plasma of the patients was obtained and stored at -20°C until further use.

ADM-r is present in plasma of healthy individuals at concentrations of < 1 nmol/l (median: 0,7). Critically diseased persons who survived a sepsis show a significantly increased ADM-r concentration (median 3,1). The median of dying patients is significantly increased as compared to the group of surviving patients (median 11,8/17,1/17,7; 2 days before exit/one day before exit/on the day of death respectively, Fig. 1).

The concentration of END-r (pro-END) as a putative antagonist of ADM increases from a median of 0,035 nmol/l plasma to 0,054 nmol/l in surviving patients. The concentration further increases to 0,096 and 0,133 nmol/l two days before exit and one day before exit respectively. Surprisingly the concentration of END-r decreases dramatically on the day of death (median 0,064 nmol/l, Fig. 2).

Example 8 - Evaluations

ADM-r and the prognosis for severity of disease/disorder

The concentration of ADM-r was used to determine the prognosis of survival (Table 1 and Fig. 1). Column 2 of Table 1 (specificity) shows the number of surviving patients having a concentration of less than 17 nmol/l plasma of ADM-r, i.e. 117 of 126 patients have a concentration of less than 17 nmol/l, 102 patients a concentration of less than 8 nmol/l. Thus ADM-r shows a specificity of 93 % for survival at a cut off at 17 nmol/l and 81 % at a cut off at 8 nmol/l. The sensitivity of mortality is determined in the following way: 23 patients died during their stay at ICU. At a cut off value of 17 nmol/l of ADM-r six patients (26 % = sensitivity

- 25 -

of mortality) showed concentrations above 17 nmol/l two days before exit, 12 patients (52 %) one day before exit and 13 patients (57 %) on the day of death. At a cut off value at 8 nmol/l, 19 (83 %) of 23 patients showed concentrations above 8 nmol/l of ADM-r two days before exit, 21 patients (91 %) one day before exit and 19 patients (83 %) on the day of death (Table 1).

Thus a cut-off value at 8 nmol/l of ADM-r, i.e. excluding the patients having a concentration below 8 nmol/l of ADM-r, includes most of the patients who died during their stay at ICU.

- 26 -

Table 1:

	Specifi- city Survivals n=126	2nd day- before death n=23	Sensitivity Last day before death n=23	day of death n=23
ADM-r				
Cut off 17 nmol/l	117	6	12	13
Cut off 8 nmol/l	102	19	21	19
Ratio ADM-r/END-r				
Cut off 215	117	0	0	14
Cut off 90	96	21	22	20
Ratio ADM-r/END-r				
ADM-r >8 nmol/l				
Cut off 90	117	20	21	18
	Specifi- city Survivals	2nd day before death	Sensitivity Last day before death	day of death
ADM-r				
Cut off 17 nmol/l	93 %	26 %	52 %	57 %
Cut off 8 nmol/l	81 %	83 %	91 %	83 %
Ratio ADM- r/END-r				
Cut off 215	93 %	0 %	0 %	61 %
Cut off 90	76 %	91 %	96 %	87 %
Ratio ADM-r/END-r				
ADM-r >8 nmol/l				
Cut off 90	93 %	87 %	91 %	78 %

- 27 -

Example 9 - RatioRatio ADM-r/END-r

The ratio of ADM-r /END-r is shown in Table 1 and Fig. 3. The ADM-r/END-r ratio in healthy individuals has a maximum value of about 50 (median 20). The median of surviving patients at ICU is increased to 56. Significant further increases can be observed two days (median 135) and one day (median 129) before exit and a surprisingly dramatic increase on the day of death at a median of 336. The prognosis at a specificity of 76 % (Table 1, i.e. patients included having a concentration of ADM-r of less than 43 nmol/l is 91 % two days before exit, 96 % one day before exit and 87 % on the day of death (Table 1 and Fig. 3). At a higher specificity of 93 %, i.e. patients included having a concentration of ADM-r of less than 18 nmol/l, a prognosis is only possible on the day of death having a sensitivity of 61 % (Table 1).

Example 10Ratio ADM-r/END-r including patients having a concentration of ADM-r above 8 nmol/l

Thus the patients were further selected according to the ADM-concentration (>8 nmol/l) and according to the ratio of ADM-r/END-r (Fig. 4).

It was shown that a high portion of surviving patients with an ADM concentration > 8 nmol/l at one day of their stay at the ICU, $N=24$ had a ratio of less than 90 ($N=15$) and thus their survival was assured. According to that assumption however also the dying patients would be included with a high probability 2 days before their death (sensitivity 87 % two days

- 28 -

before exit /91 % one day before exit /78 % on the day of death). Taking into account the sum of patients included in the study (126 surviving patients) the specificity surprisingly is as high as 93 %.

Thus the sequential analysis of ADM-r, END-r and the ratio and or algorithm of ADM-r/END-r at an equally high specificity (93 %) surprisingly shows a higher sensitivity for the prognosis (87 %/91%/78 %) as the analysis of ADM (26 %/52 %/57 %) or ratio (0 /0 /61) in their own (values 2 days before death/one day before death/day of death respectively).

Claims:

1. *In vitro* method to diagnose in a critically ill patient the possible development or the presence of a life-threatening endothelin deficiency ("low endothelin syndrome" or "LES"), comprising

- determining *in vitro* in a sample of a body fluid of the patient the concentration of a pro-adrenomedullin (pro-ADM) molecule and/or one or more physiologically occurring fragments thereof,

- determining *in vitro* in a sample of a body fluid of the patient the concentration of a pro-endothelin (pro-END) molecule and/or one or more physiologically occurring fragments thereof,

- using the measured concentrations for calculating a ratio pro-ADM/pro-END,

- using the calculated ratio, optionally in combination with the measured concentration of pro-ADM alone, for an assessment of the development or presence of LES and of the severity of the disease and/or course and/or prognosis of course of the respective disease.

2. Method according to claim 1, wherein said ratio is used in the course of the assessment of a mortality risk.

3. Method according to claims 1 or 2, wherein the body fluid is plasma, serum, blood, liquor or urine.

4. Method according to any of claims 1 to 3, wherein the patient is a patient in an intensive care unit (ICU) admitted to the ICU with a diagnosis of one or more of sepsis, SIRS, septic shock, multiple organ failure, infections systemic or local, bacterial infections, heart disease, peritonitis, pancreatitis, local and/or systemic inflammation, meningitis, trauma, ruptured aortic aneurysm, intoxi-

- 30 -

cation, endotoxemia, anuria, renal insufficiency, arterial hypertension, pulmonary hypertension, arteriosclerosis, cancer, congestive heart failure, cardiovascular disease, coronary artery disease, ischemia, antiarrhythmic effects, cancer comprising colorectal cancer, renal and/or heart failure, organ injury.

5. Method according to any of claims 1 to 4, wherein the pro-ADM molecule and/or the pro-END molecule are determined using a selective immunodiagnostic assay recognizing a physiologically occurring fragment not comprising the mature vasoactive ADM and END respectively.

6. Method according to claim 6, wherein in the assays for the determination of pro-ADM and pro-END combinations of selective antibodies are used, and wherein at least one of the antibodies specifically binding to the physiologically occurring fragment is immobilized onto a solid phase and at least one second antibody specifically binding to another part of the same fragment is used for detection of said fragment attached to the solid phase.

7. Method according to claim 10 wherein the second antibody used for detection is tagged with a luminescent marker.

8. Method according to any of the preceding claims, wherein the determined pro-ADM fragment is a peptide having the amino acid sequence according to SEQ ID NOs 2 or 3, and the determined pro-END fragment is a peptide having the amino acid sequence according to SEQ ID NOs 6 or 7.

9. Method according to any of the preceding claims, wherein the determination of pro-adrenomedullin or its physiologically occurring fragment is conducted as semi-quantitative determination using an immunochromatographic POC measuring device.

- 31 -

10. Method according to any of the preceding claims, wherein for the calculation of the ratio, for the use of the ratio for diagnostic and prognostic purposes and for categorizing patients into groups of scores algorithms and suitable computer programmes respectively are used.

11. A computer device for processing the results of a method according to any of the preceding claims, comprising

- an input for receiving information from the method according to any of claims 1 to 9,
- means for analysing the received information and
- means for expressing the obtained results in terms of the severity of disease and/or as therapeutical advice.

12. Use of vasoactive endothelin and/or endothelin agonists and/or adrenomedullin antagonists or adrenomedullin neutralizing binders as active ingredient for the preparation of a medicament for the treatment of a critically ill patient developing a low endothelin syndrome.

13. Use according to claim 12, wherein the medicament is a liquid medicament for intravenous administration by infusion or injection.

14. Use according to claims 12 or 13, wherein the critically ill patient is a patient admitted to an intensive care unit (ICU) with a diagnosis of one or more of sepsis, SIRS, septic shock, multiple organ failure, infections systemic or local, bacterial infections, heart disease, peritonitis, pancreatitis, local and/or systemic inflammation, meningitis, trauma, ruptured aortic aneurysm, intoxication, endotoxemia, anuria, renal insufficiency, arterial hypertension, pulmonary hypertension, arteriosclerosis, cancer, congestive heart failure, cardiovascular disease, coronary artery disease, ischemia, antiarrhythmic effects, cancer comprising colorectal cancer, renal and/or heart failure, organ injury.

- 32 -

15. Use according to any of claims 12 to 14, wherein the vasoactive endothelin is selected from endothelin-1, big-endothelin, endothelin-2, endothelin-3 and any combination thereof.

16. Use according to any of claims 12 to 15, wherein the preparation further comprises a pharmaceutically acceptable liquid carrier and one or more of stabilizers, nutrients, electrolytes, dissolved salts, osmotic agents for providing isotonic conditions, buffers and/or solvents and other adjuvants and excipients suitable for intravenous administration into the patient.

1/4

ADM-r

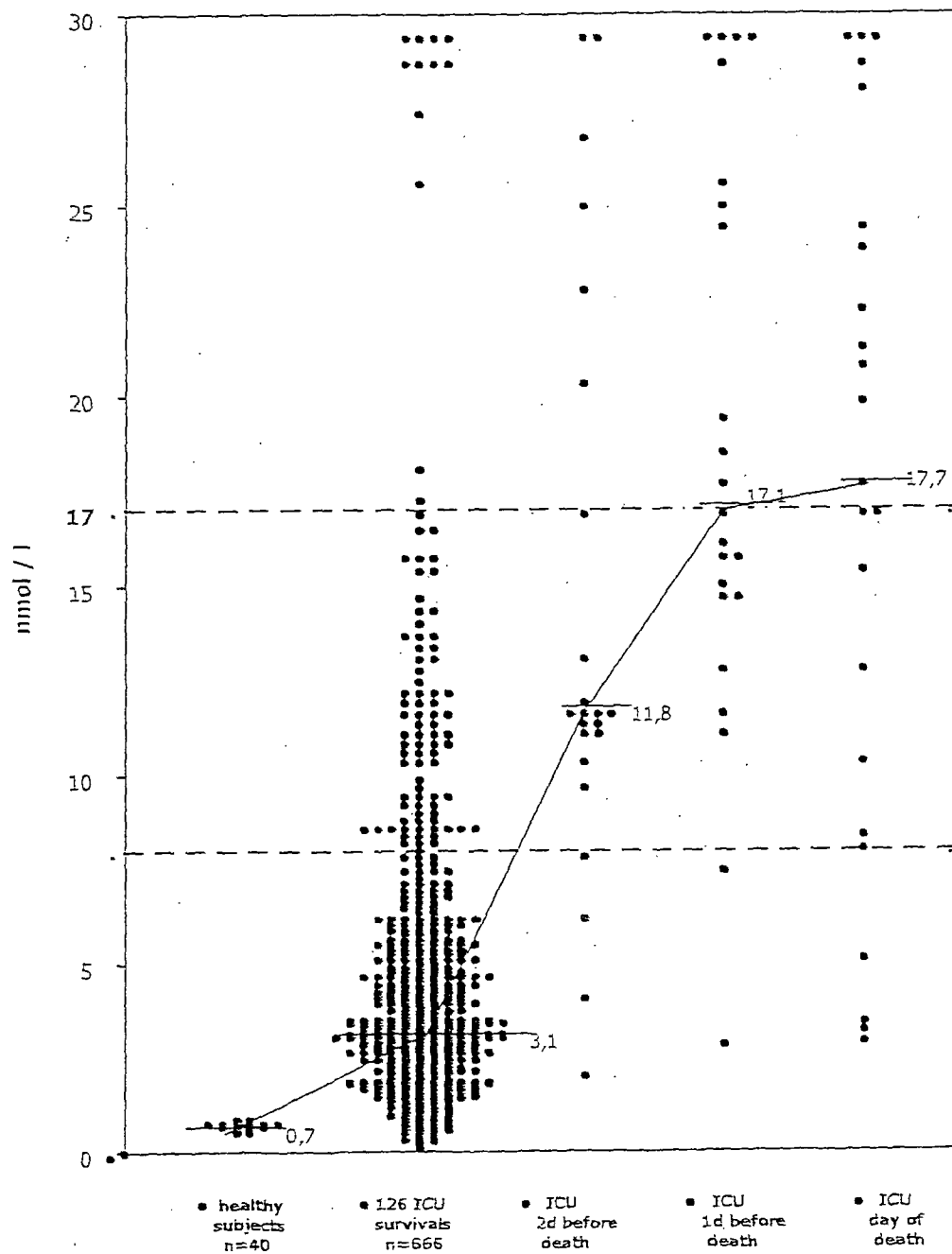


FIGURE 1

2/4

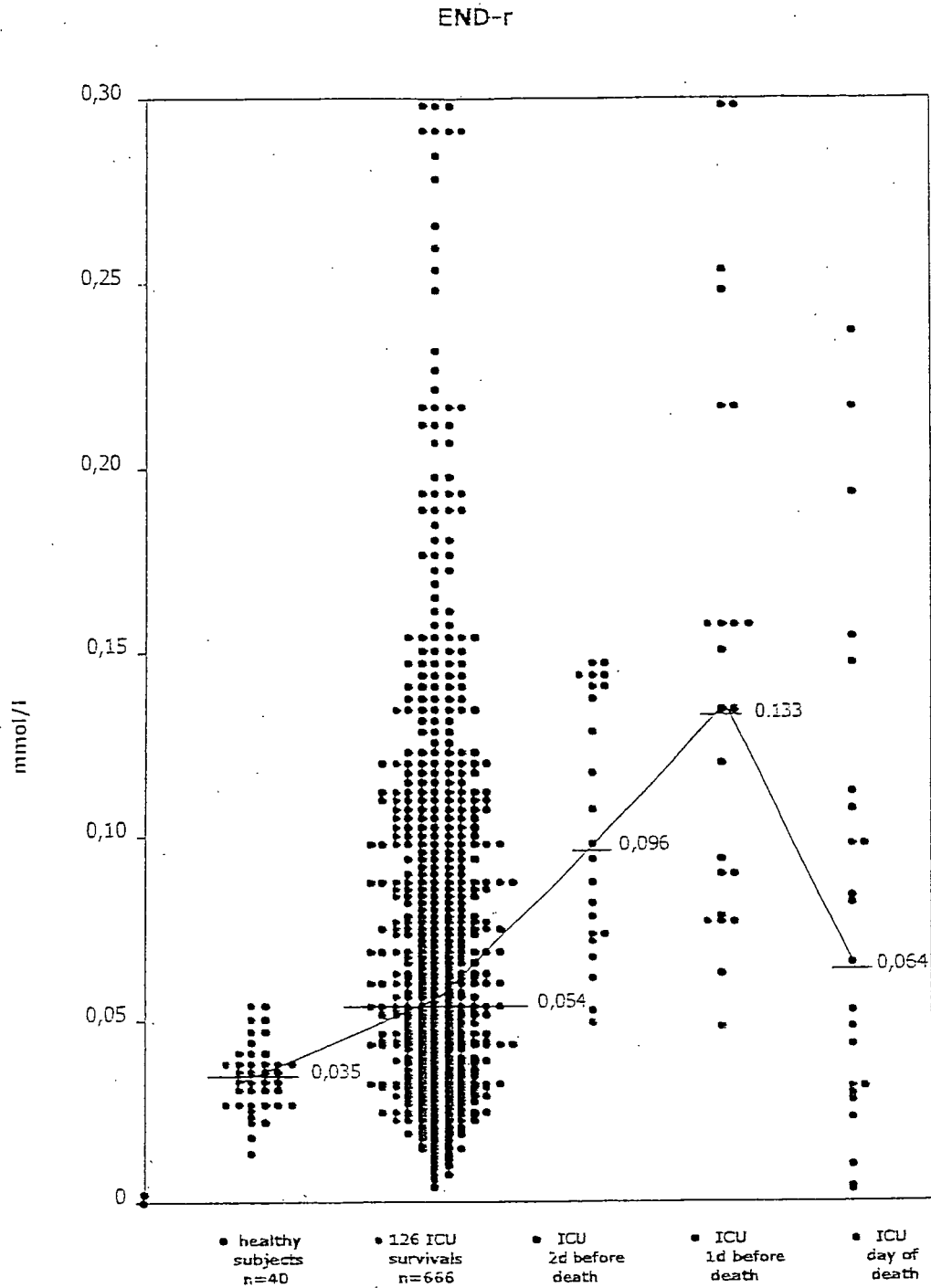


FIGURE 2

3/4

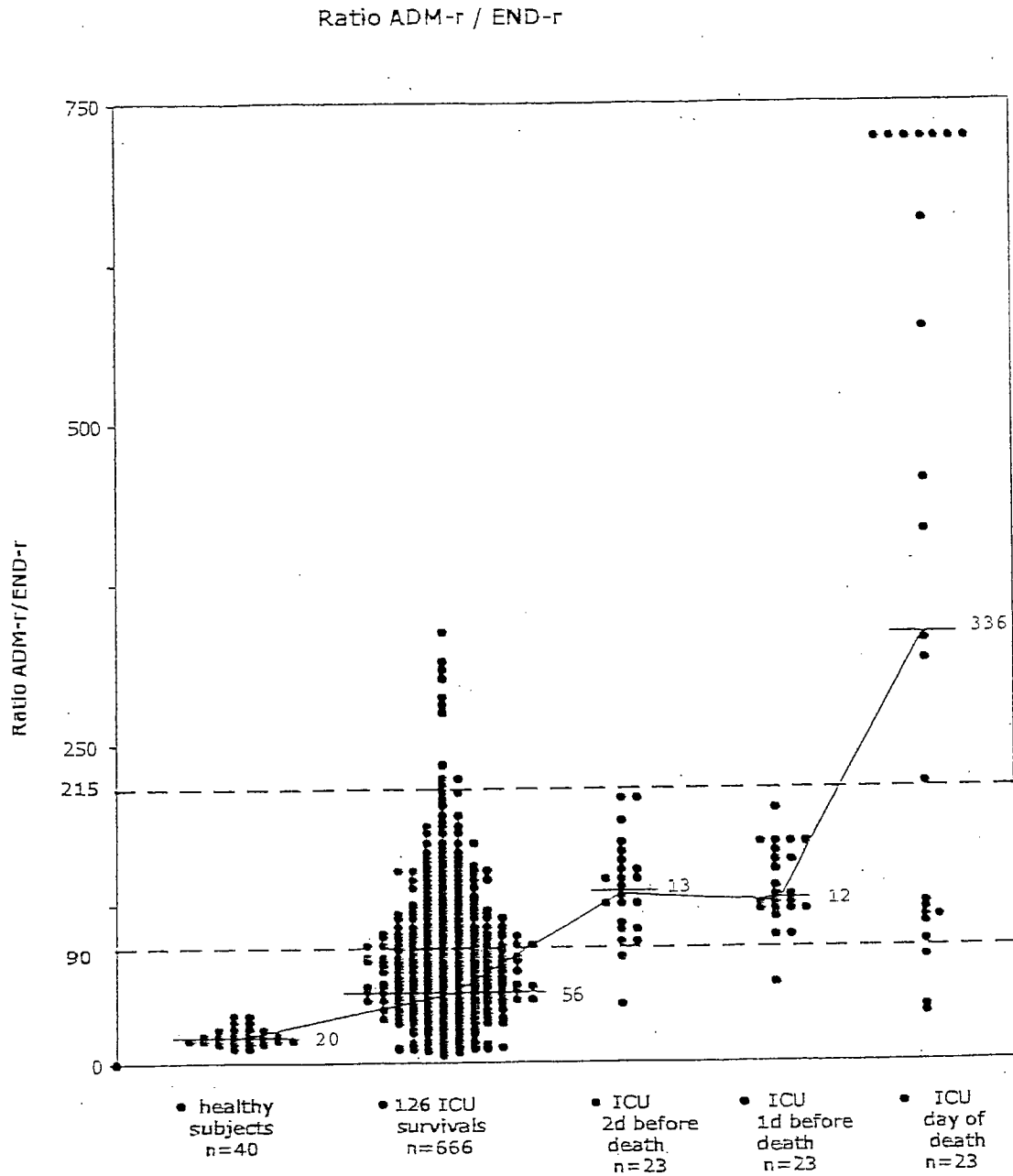


FIGURE 3

4 / 4

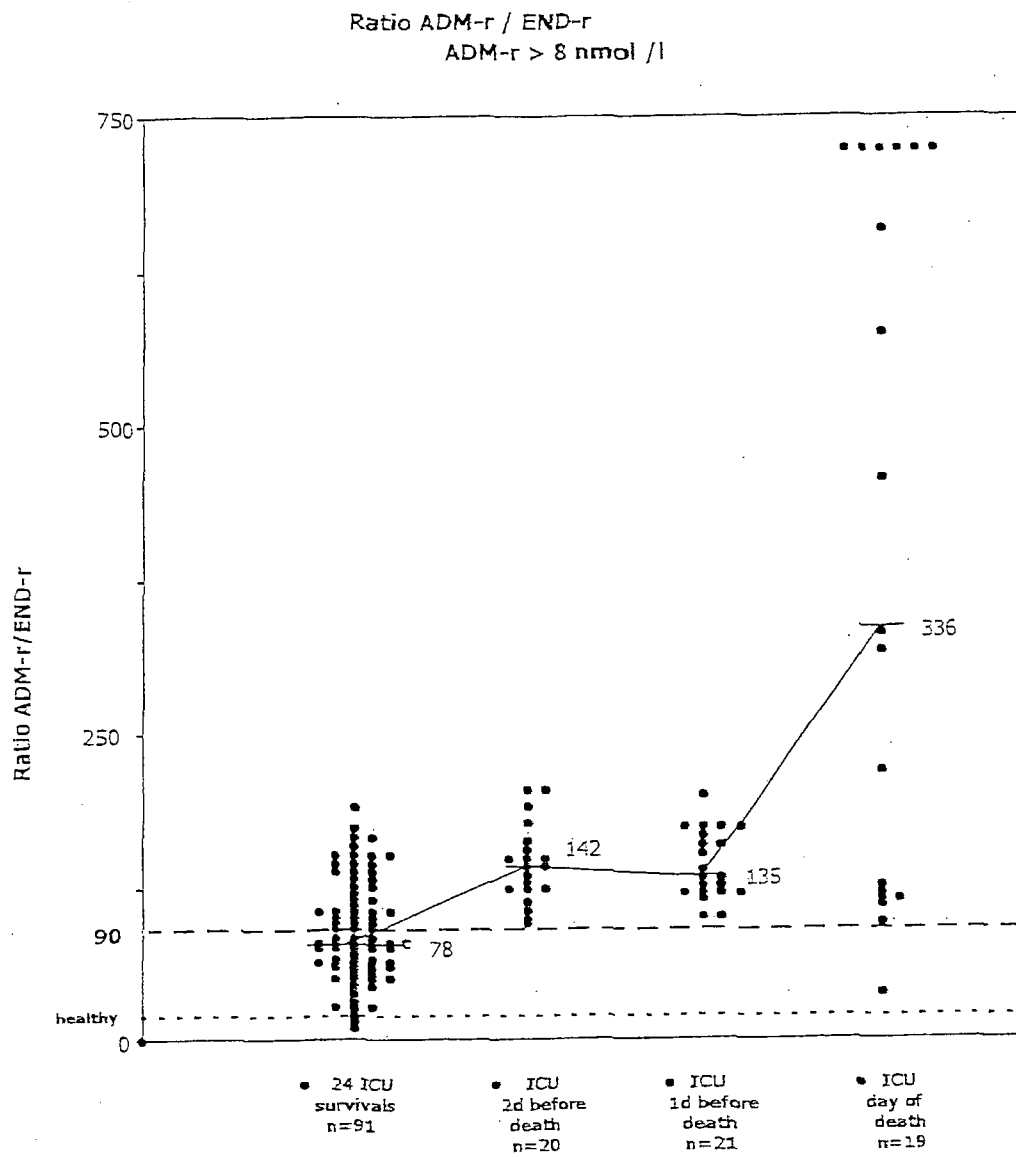


FIGURE 4

SEQUENCE LISTING

<110> B.R.A.H.M.S Aktiengesellschaft

<120> Methods for the diagnosis and treatment of critically ill patients, and the use of endothelin and endothelin agonists with vasoconstrictive activity for the preparation of medicaments for the treatment of critically ill patients

<130> 4278 PCT

<160> 8

<170> PatentIn version 3.2

<210> 1

<211> 185

<212> PRT

<213> Artificial

<220>

<223> synthetic sequence

<400> 1

Met Lys Leu Val Ser Val Ala Leu Met Tyr Leu Gly Ser Leu Ala Phe
1 5 10 15

Leu Gly Ala Asp Thr Ala Arg Leu Asp Val Ala Ser Glu Phe Arg Lys
20 25 30

Lys Trp Asn Lys Trp Ala Leu Ser Arg Gly Lys Arg Glu Leu Arg Met
35 40 45

Ser Ser Ser Tyr Pro Thr Gly Leu Ala Asp Val Lys Ala Gly Pro Ala
50 55 60

Gln Thr Leu Ile Arg Pro Gln Asp Met Lys Gly Ala Ser Arg Ser Pro
65 70 75 80

Glu Asp Ser Ser Pro Asp Ala Ala Arg Ile Arg Val Lys Arg Tyr Arg
85 90 95

Gln Ser Met Asn Asn Phe Gln Gly Leu Arg Ser Phe Gly Cys Arg Phe
100 105 110

Gly Thr Cys Thr Val Gln Lys Leu Ala His Gln Ile Tyr Gln Phe Thr
115 120 125

Asp Lys Asp Lys Asp Asn Val Ala Pro Arg Ser Lys Ile Ser Pro Gln
130 135 140

Gly Tyr Gly Arg Arg Arg Arg Arg Ser Leu Pro Glu Ala Gly Pro Gly
145 150 155 160

Arg Thr Leu Val Ser Ser Lys Pro Gln Ala His Gly Ala Pro Ala Pro
165 170 175

Pro Ser Gly Ser Ala Pro His Phe Leu
180 185

<210> 2

<211> 11

<212> PRT

<213> Artificial

<220>

<223> synthetic sequence

<400> 2

Ser Pro Asp Ala Ala Arg Ile Arg Val Lys Arg
1 5 10

<210> 3

<211> 16

<212> PRT

<213> Artificial

<220>

<223> synthetic sequence

<400> 3

Arg Pro Gln Asp Met Lys Gly Ala Ser Arg Ser Pro Glu Asp Ser Ser
1 5 10 15

<210> 4

<211> 58

<212> PRT

<213> Artificial

<220>

<223> synthetic sequence

<400> 4

Asn Lys Trp Ala Leu Ser Arg Gly Lys Arg Glu Leu Arg Met Ser Ser
1 5 10 15

Ser Tyr Pro Thr Gly Leu Ala Asp Val Lys Ala Gly Pro Ala Gln Thr
20 25 30

Leu Ile Arg Pro Gln Asp Met Lys Gly Ala Ser Arg Ser Pro Glu Asp
35 40 45

Ser Ser Pro Asp Ala Ala Arg Ile Arg Val
50 55

<210> 5

<211> 212

<212> PRT

<213> Artificial

<220>

<223> synthetic sequence

<400> 5

Met Asp Tyr Leu Leu Met Ile Phe Ser Leu Leu Phe Val Ala Cys Gln
1 5 10 15

Gly Ala Pro Glu Thr Ala Val Leu Gly Ala Glu Leu Ser Ala Val Gly
20 25 30

Glu Asn Gly Gly Glu Lys Pro Thr Pro Ser Pro Pro Trp Arg Leu Arg
35 40 45

Arg Ser Lys Arg Cys Ser Cys Ser Ser Leu Met Asp Lys Glu Cys Val
50 55 60

Tyr Phe Cys His Leu Asp Ile Ile Trp Val Asn Thr Pro Glu His Val
65 70 75 80

Val Pro Tyr Gly Leu Gly Ser Pro Arg Ser Lys Arg Ala Leu Glu Asn
85 90 95

Leu Leu Pro Thr Lys Ala Thr Asp Arg Glu Asn Arg Cys Gln Cys Ala
100 105 110

Ser Gln Lys Asp Lys Lys Cys Trp Asn Phe Cys Gln Ala Gly Lys Glu
115 120 125

Leu Arg Ala Glu Asp Ile Met Glu Lys Asp Trp Asn Asn His Lys Lys
130 135 140

Gly Lys Asp Cys Ser Lys Leu Gly Lys Lys Cys Ile Tyr Gln Gln Leu
145 150 155 160

Val Arg Gly Arg Lys Ile Arg Arg Ser Ser Glu Glu His Leu Arg Gln
165 170 175

Thr Arg Ser Glu Thr Met Arg Asn Ser Val Lys Ser Ser Phe His Asp
180 185 190

Pro Lys Leu Lys Gly Lys Pro Ser Arg Glu Arg Tyr Val Thr His Asn
195 200 205

Arg Ala His Trp
210

<210> 6
<211> 14
<212> PRT
<213> Artificial

<220>
<223> synthetic sequence

<400> 6

Arg Ser Ser Glu Glu His Leu Arg Gln Thr Arg Ser Glu Thr
1 5 10

<210> 7
<211> 13
<212> PRT
<213> Artificial

<220>
<223> synthetic sequence

<400> 7

Ser Arg Glu Arg Tyr Val Thr His Asn Arg Ala His Trp
1 5 10

<210> 8
<211> 120
<212> PRT
<213> Artificial

<220>

<223> synthetic sequence

<400> 8

Ala Leu Glu Asn Leu Leu Pro Thr Lys Ala Thr Asp Arg Glu Asn Arg
1 5 10 15

Cys Gln Cys Ala Ser Gln Lys Asp Lys Lys Cys Trp Asn Phe Cys Gln
20 25 30

Ala Gly Lys Glu Leu Arg Ala Glu Asp Ile Met Glu Lys Asp Trp Asn
35 40 45

Asn His Lys Lys Gly Lys Asp Cys Ser Lys Leu Gly Lys Lys Cys Ile
50 55 60

Tyr Gln Gln Leu Val Arg Gly Arg Lys Ile Arg Arg Ser Ser Glu Glu
65 70 75 80

His Leu Arg Gln Thr Arg Ser Glu Thr Met Arg Asn Ser Val Lys Ser
85 90 95

Ser Phe His Asp Pro Lys Leu Lys Gly Lys Pro Ser Arg Glu Arg Tyr
100 105 110

Val Thr His Asn Arg Ala His Trp
115 120

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2005/012844

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/74 A61K38/22 A61P9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; October 2001 (2001-10), ZHENG H ET AL: "[The change in the plasma contents of adrenomedullin and endothelin in burn patients during early postburn stage]" XP002352776 Database accession no. NLM11774814 abstract & ZHONGHUA SHAO SHANG ZA ZHI = ZHONGHUA SHAOSHANG ZAZHI = CHINESE JOURNAL OF BURNS. OCT 2001, vol. 17, no. 5, October 2001 (2001-10), pages 279-281, ISSN: 1009-2587 ----- -/--	1-10



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

23 May 2006

Date of mailing of the international search report

17.10.2006

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer:

Gundlach, Björn

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2005/012844

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FEI YU-XING ET AL: "Relationship of proadrenomedullin N terminal 20 peptide and the other vasoconstrictive substance in patients with chronic heart failure." ZHONGHUA XINXUEGUANBING ZAZHI, vol. 31, no. 8, August 2003 (2003-08), pages 600-602, XP008053669 ISSN: 0253-3758 abstract	1-10
A	----- WO 00/22439 A (B.R.A.H.M.S DIAGNOSTICA GMBH; BERGMANN, ANDREAS; STRUCK, JOACHIM; WEGL) 20 April 2000 (2000-04-20) cited in the application the whole document	1-10
A	----- US 2002/095041 A1 (CHAN MING FAI ET AL) 18 July 2002 (2002-07-18) the whole document	
A	----- EP 1 564 558 A (B.R.A.H.M.S AKTIENGESELLSCHAFT) 17 August 2005 (2005-08-17) sequences 4,5	1-10
A	----- WO 2004/090546 A (B.R.A.H.M.S AKTIENGESELLSCHAFT; BERGMANN, ANDREAS; STRUCK, JOACHIM) 21 October 2004 (2004-10-21) sequence 4	1-10
E	----- EP 1 619 505 A (BRAHMS AKTIENGESELLSCHAFT) 25 January 2006 (2006-01-25) the whole document -----	1-10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2005/012844

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-10

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: Claims 1-10

Method for diagnosing critically ill patients

2. claim: 11

Computer device

3. claims: 12-16

Use of endothelin, its agonists or adrenomedullin antagonists for the manufacture of medicaments.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2005/012844

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0022439	A	20-04-2000	AT 320607 T	15-04-2006
			DE 19847690 A1	20-04-2000
			EP 1121600 A2	08-08-2001
			JP 2002527753 T	27-08-2002
			US 6756483 B1	29-06-2004

US 2002095041	A1	18-07-2002	NONE	

EP 1564558	A	17-08-2005	AT 312351 T	15-12-2005
			WO 2005078456 A1	25-08-2005
			ES 2255003 T3	16-06-2006

WO 2004090546	A	21-10-2004	AT 312342 T	15-12-2005
			CN 1759319 A	12-04-2006
			DE 10316583 A1	28-10-2004
			EP 1488209 A1	22-12-2004
			ES 2250959 T3	16-04-2006
			MX PA05010827 A	09-03-2006

EP 1619505	A	25-01-2006	NONE	
