Homogeneous time-resolved fluoroimmunoassay for the measurement of midregional proadrenomedullin in plasma on the fully automated system B.R.A.H.M.S KRYPTOR (Caruhel P, Mazier C, Kunde J, Morgenthaler NG, Darbouret B.). As these peptides are generated in a stoichiometric ratio from the same precursor, their plasma levels are correlated to a certain extent.

Only in a few studies plasma ADM has been measured in patients with systemic inflammation, sepsis, severe sepsis or septic shock, and levels have been correlated with hemodynamic parameters:

In a study by Hirata *et al.* plasma ADM in septic patients was found to be correlated with heart rate, right arterial pressure, but not with mean arterial pressure (MAP) (Hirata Y, Mitaka C, Sato K, Nagura T, Tsunoda Y, Amaha K, Marumo F: Increased circulating adrenomedullin, a novel vasodilatory peptide, in sepsis. The Journal of clinical endocrinology and metabolism 1996, 81(4):1449-1453).

Nishio *et al.* reported that increased plasma concentrations of ADM were correlated with relaxation of vascular tone in patients with septic shock (correlation with cardiac index, stroke volume index, heart rate, decrease in diastolic blood pressure, systemic vascular resistance index and pulmonary vascular resistance index), however there was no significant correlation with mean blood pressure [19] .

In healthy subjects under exercise a significant negative correlation of plasma ADM and MAP was found [20].

Summary

In one aspect, there is provided an in vitro method for identifying a patient at risk to develop physiological shock states comprising the following steps:

- Determining the level of proadrenomedullin (proADM) (SEQ ID No. 1) and/or fragments thereof in a bodily fluid of said subject

- Correlating said level with the patient's risk to develop physiological shock states, wherein said subject is identified as having such a risk if the level of

- proADM (SEQ ID No. 1) and/or fragments thereof in the bodily fluid of said subject is above a threshold;

wherein said fragments of proADM (SEQ ID No. 1) are selected from the group consisting of mature adrenomedullin (ADM) (SEQ ID No. 4), mature ADM 1-52-Gly (SEQ ID No. 5), Midregional_proadrenomedullin (MR-proADM) (SEQ ID No. 3), C-terminal proadrenomedullin (CT-ADM) (SEQ ID No. 6) and proADM N-20 terminal peptide (SEQ ID No. 2); and

wherein the bodily fluid of said subject comprises a sample selected from the group consisting of whole blood, serum and plasma.

Detailed Description

Nothing is known about the association of circulating ADM or related peptide levels as pro-ADM or fragments thereof and the requirements for fluid resuscitation and vasopressors in patients developing shock. This is an unmet medical need as vasopressors are usually given very late when the condition of the patient is very serious. It is an unmet medical need to identify those patients in need of fluid resuscitation and vasopressors before the condition of the patient is very serious. It is an unmet medical need to predict the requirement for fluid resuscitation and vasopressor therapy earlier than by blood pressure measurement applying the cut-off value of 65 mm Hg, as recommended in the guidelines [21]. If blood pressure fells, this leads to diminished oxygen supply, organ dysfunction and death. It is thus an unmet need to early identify patients who are at risk to develop a blood pressure decline. If such patients

CLAIMS

1. An in vitro method for identifying a patient at risk to develop physiological shock states comprising the following steps:

- Determining the level of proadrenomedullin (proADM) (SEQ ID No. 1) and/or fragments thereof in a bodily fluid of said subject

- Correlating said level with the patient´s risk to develop physiological shock states, wherein said subject is identified as having such a risk if the level of proADM (SEQ ID No. 1) and/or fragments thereof in the bodily fluid of said subject is above a threshold;

wherein said fragments of proADM (SEQ ID No. 1) are selected from the group consisting of mature adrenomedullin (ADM) (SEQ ID No. 4), mature ADM 1-52-Gly (SEQ ID No. 5), Midregional_proadrenomedullin (MR-proADM) (SEQ ID No. 3), C-terminal proadrenomedullin (CT-ADM) (SEQ ID No. 6) and proADM N-20 terminal peptide (SEQ ID No. 2); and

wherein the bodily fluid of said subject comprises a sample selected from the group consisting of whole blood, serum and plasma.

2. An in vitro method for identifying a patient at risk to develop physiological shock states according to claim 1, wherein either the level of mature adrenomedullin (ADM) (SEQ ID No. 4) immunoreactivity and/or the level of mature ADM 1-52-Gly (SEQ ID No. 5) – immunoreactivity or the level of Midregional_proadrenomedullin (MR-proADM) (SEQ ID No. 3) immunoreactivity or the level of C-terminal proadrenomedullin (CT-ADM) (SEQ ID No. 6) immunoreactivity is determined and correlated with the risk of the patient to develop physiological shock states, wherein said patient is identified as at a risk to develop physiological shock states if the level of mature ADM (SEQ ID No. 4) immunoreactivity and/or the level of mature ADM 1-52-Gly (SEQ ID No. 5) – immunoreactivity or the level of MR-proADM (SEQ ID No. 3) immunoreactivity or the level of CT-ADM (SEQ ID No. 6) immunoreactivity in the bodily fluid of said subject is above a threshold.

3. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 1 or 2, wherein the level of proadrenomedullin (proADM) (SEQ ID No. 1) and/or fragments thereof is determined by using at least one binder selected from the group: a binder that binds to a region comprised within the following sequence of mature adrenomedullin (ADM) (SEQ ID No. 4) and/or mature ADM 1-52-Gly (SEQ ID No. 5) and a second binder that binds to a region comprised within the following sequence of mature ADM (SEQ ID No. 4) and/or mature ADM 1-52-Gly (SEQ ID No. 5).

4. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 1 or 2, wherein the level of proadrenomedullin (proADM) (SEQ ID No. 1) and/or fragments thereof is determined by using at least one binder selected from the group: a binder that binds to a region comprised within the following sequence of Midregional_proadrenomedullin (MR-proADM) (SEQ ID No. 3) and a second binder that binds to a region comprised within the following sequence of MR-proADM (SEQ ID No. 3).

5. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 1 or 2, wherein the level of proadrenomedullin (proADM) (SEQ ID No. 1) and/or fragments thereof is determined by using at least one binder selected from the group: a binder that binds to a region comprised within the following sequence of C-terminal proadrenomedullin (CT-proADM) (SEQ ID No. 6) and a second binder that binds to a region comprised within the following sequence of CT-proADM (SEQ ID No. 6).

6. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 1 to 5, wherein an assay is used for determining the level of proadrenomedullin (proADM) (SEQ ID No.1) and/or fragments thereof, wherein the assay sensitivity of said assay is able to quantify adrenomedullin (ADM) of healthy subjects and is < 70 pg/ml, preferably < 40 pg/ml and more preferably < 10 pg/ml.

7. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 3 to 5, wherein said binder exhibits a binding affinity to proadrenomedullin (proADM) (SEQ ID No. 1) and/or fragments thereof of at least 10^7 M^{-1}.

8. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 3 to 5 and 7, wherein said binder is selected from the group comprising an antibody or an antibody fragment or a non-Ig scaffold binding to proadrenomedullin (proADM) (SEQ ID No. 1) and/or fragments thereof.

9. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 1 to 8, wherein an assay is used for determining the level of proadrenomedullin (proADM) (SEQ ID No. 1) and/or fragments thereof, wherein such assay is a sandwich assay, preferably a fully automated assay.

10. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 3 to 9, wherein at least one of said two binders is labeled in order to be detected.

11. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 3 to 10, wherein at least one of said two binders is bound to a solid phase.

12. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 1 to 11, wherein a threshold for plasma adrenomedullin (ADM) of 90 pg/ml, preferably 70 pg/ml is applied.

13. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 1 to 12, wherein a threshold for plasma Midregional_proadrenomedullin (MR-proADM) (SEQ ID No. 3) of 0.9 nmol/L, preferably 0.7 nmol/L is applied.

14. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 1 to 13, wherein said patient is identified as having such a need if the level of proadrenomedullin (proADM) (SEQ ID No. 1)

and/or fragments thereof in the bodily fluid of said subject is above a threshold and if the patient has >66mmHg Mean Arterial Pressure (MAP), more preferably >70 mmHg MAP.

15. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 1 to 14 comprising the following steps:

- Determining the level of proadrenomedullin (proADM) (SEQ ID No. 1) and/or fragments thereof in a bodily fluid of said subject; and

- Correlating said level with the risk of said patient to develop physiological shock states independent from the patient's mean arterial pressure, wherein said patient is identified as having such a risk if the level of proADM (SEQ ID No. 1) and/or fragments thereof in the bodily fluid of said subject is above a threshold.

16. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 1 to 15 comprising the following steps:

- Determining the level of proadrenomedullin (proADM) (SEQ ID No. 1) and/or fragments thereof in a bodily fluid of said subject; and

- Correlating said level with the need to increase the intervention threshold value of the patient's mean arterial pressure to 70 mm Hg, more preferably to 75 mm Hg, even more preferably to 80 mm Hg, to initiate fluid resuscitation or a vasopressor therapy, wherein said patient is identified as having such a risk if the level of proADM (SEQ ID No. 1) and/or fragments thereof in the bodily fluid of said subject is above a threshold.

17. An in vitro method for identifying a patient at risk to develop physiological shock states according to any of claims 1 to 16, wherein said subject exhibits the following signs or suffers from the following conditions: infections, sepsis, heart failure, cardiopulmonary arrest, postoperative cardiac surgery, right ventricular infarction, bradyarrhythmias, polytrauma, burns, kidney injury.