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(54) Titre : PCT ET PRO-ADM UTILISEES COMME MARQUEURS DE CONTROLE DANS UN TRAITEMENT ANTIBIOTIQUE

(54) Title: PCT AND PRO-ADM AS MARKERS FOR MONITORING ANTIBIOTIC TREATMENT

(57) Abrégé/Abstract:
The invention relates to a method for antibiotic therapy guidance, stratification and/or control in a patient suffering from an infectious disease and receiving treatment with one or more antibiotic agents. In particular, the method comprises isolating a first sample from said patient, isolating a second sample from said patient after isolating the first sample and initiating antibiotic treatment, determining levels of procalcitonin (PCT) or fragment(s) thereof in the first and the second sample, and determining a level of proadrenomedullin (proADM) or fragment(s) thereof in at least the second sample, wherein the levels of PCT or fragment(s) thereof in said first and second samples, and the level of pro ADM or fragment(s) thereof in the second sample, are indicative of whether a change in the treatment with one or more antibiotic agents is required. In a preferred embodiment of the invention, the method comprises additionally determining a level of pro ADM or fragment(s) thereof in the first and second samples. In a preferred embodiment of the invention changes in the pro ADM and PCT levels between the first and second samples indicate a need for changing or maintaining the antibiotic treatment. Furthermore, the invention also relates to a kit for carrying out the method of the present invention.

Abstract

The invention relates to a method for antibiotic therapy guidance, stratification and/or control in a patient suffering from an infectious disease and receiving treatment with one or more antibiotic agents. In particular, the method comprises isolating a first sample from said patient, isolating a second sample from said patient after isolating the first sample and initiating antibiotic treatment, determining levels of procalcitonin (PCT) or fragment(s) thereof in the first and the second sample, and determining a level of proadrenomedullin (proADM) or fragment(s) thereof in at least the second sample, wherein the levels of PCT or fragment(s) thereof in said first and second samples, and the level of pro ADM or fragment(s) thereof in the second sample, are indicative of whether a change in the treatment with one or more antibiotic agents is required. In a preferred embodiment of the invention, the method comprises additionally determining a level of pro ADM or fragment(s) thereof in the first and second samples. In a preferred embodiment of the invention changes in the pro ADM and PCT levels between the first and second samples indicate a need for changing or maintaining the antibiotic treatment. Furthermore, the invention also relates to a kit for carrying out the method of the present invention.

PCT AND PRO-ADM AS MARKERS FOR MONITORING ANTIBIOTIC TREATMENT

DESCRIPTION

The invention relates to a method for antibiotic therapy guidance, stratification and/or control in a patient suffering from an infectious disease and receiving treatment with one or more antibiotic agents. In particular, the method comprises isolating a first sample from said patient, isolating a second sample from said patient after isolating the first sample and initiating antibiotic treatment, determining levels of procalcitonin (PCT) or fragment(s) thereof in the first and the second sample, and determining a level of proadrenomedullin (proADM) or fragment(s) thereof in at least the second sample, wherein the levels of PCT or fragment(s) thereof in said first and second samples, and the level of proADM or fragment(s) thereof in the second sample, are indicative of whether a change in the treatment with one or more antibiotic agents is required. In a preferred embodiment of the invention, the method comprises additionally determining a level of proADM or fragment(s) thereof in the first and second samples. In a preferred embodiment of the invention changes in the proADM and PCT levels between the first and second samples indicate a need for changing or maintaining the antibiotic treatment. Furthermore, the invention also relates to a kit for carrying out the method of the present invention.

BACKGROUND OF THE INVENTION

Despite significant improvements in diagnostic and preventative measures, the incidence of sepsis has continued to escalate rapidly in hospitalized patients (1), with mortality rates ranging between 10% and 54%, depending on the level of disease severity, definition of organ dysfunction used, and country specific incidence (2, 3). An early and accurate assessment of both the infectious load and disease severity, in terms of the overall pathophysiological host response, is therefore of crucial importance in the early stages of sepsis in order to make prompt and reliable decisions concerning diagnostic testing and treatment strategies, as well as in the later phase to reliably guide patient management, treatment monitoring, discharge decisions in the presence of clinical recovery.

It is therefore surprising that no gold standard diagnostic test for sepsis currently exists (4). The use of Procalcitonin (PCT) has partially filled this unmet need with regards to infectious load assessment, with observational and interventional data in the field of antibiotic guidance (5-7). However an accurate measure of disease severity has not yet been shown.

As such, numerous biomarkers and clinical scores have consequently been proposed, including the use of severity scores such as the Sequential Organ Failure Assessment (SOFA), Acute Physiological and Chronic Health Evaluation (APACHE) II and Simplified Acute Physiological (SAPS) II score, however these are rarely calculated on a daily basis in a routine manner due to the relatively high complexity and time resource requirements associated with each score. The use of novel biomarkers can satisfy this unmet clinical need, however few, if any, have successfully made it into widespread clinical routine (8).

Of these biomarkers, mid-regional pro-adrenomedullin (MR-proADM) – a peptide generated by multiple tissues in order to stabilise the microcirculation and protect against endothelial permeability and consequent organ failure (9-16) – has shown considerable promise, especially in the fields of sepsis (17), lower respiratory tract infections (18-21), lung transplantation (22) and

thoracic surgery (23). Indeed, the endothelium and microcirculation is widely acknowledged to play a significant role in the pathophysiological host response to sepsis (24, 25), with the regulation and distribution of blood flow within each organ of major importance (25), and may therefore provide an alternative indication as to the severity of the general host response, compared to scores of individual organ dysfunction.

The present invention therefore employs a range of biomarkers (PCT, lactate, C-reactive protein, MR-proADM) and clinical scores (SOFA, APACHE II and SAPS II) in order to (i) make an accurate assessment of disease severity within a short time after diagnosis, such as within 24 hours of diagnosis, and over the first ten days of ICU therapy, (ii) identify low risk patients who may be eligible for an early ICU discharge to a step-down setting, and (iii) identify patients who, despite decreasing infectious loads, remain at a high or increasing risk of mortality or other adverse events and may require urgent additional diagnostic and therapeutic interventions.

Accordingly, MR-proADM may be used as a tool to identify high severity patients who may require alternative diagnostic and therapeutic interventions, such as a change in the antibiotic therapy, and low severity patients who may potentially be eligible for an early ICU discharge in conjunction with an absence of ICU specific therapies and/or a continuation of treatment with the same antibiotic agents.

A need exists in the field of treating patients suffering from an infectious disease, such as sepsis patients, for additional means for therapy monitoring after initiating treatment, as well as means for antibiotic therapy guidance, stratification and/or control.

SUMMARY OF THE INVENTION

In light of the difficulties in the prior art, the technical problem underlying the present invention is the provision of means for therapy monitoring in a patient suffering from an infectious disease or a sepsis patient who is receiving antibiotic treatment, as well as means for antibiotic therapy guidance, stratification and/or control, in particular within a short time frame after initiating treatment.

The present invention therefore seeks to provide a method, kit and further means for therapy monitoring of patients suffering from an infectious disease, including means for antibiotic therapy guidance, stratification and/or control, as well as means for the prognosis, risk assessment and/or risk stratification of a subsequent adverse event in the health of such a patient, on the basis of proadrenomedullin (proADM) levels determined in a sample from a patient. One object of the invention is therefore the use of a biomarker or combination of biomarkers to distinguish critically ill patients and/or patients suffering from an infectious disease or sepsis, who have undergone or are undergoing treatment, in particular antibiotic treatment, who have a high risk of an adverse event, from critically ill patients and/or patients suffering from an infectious disease or sepsis who have a low risk of an adverse event.

The invention relates to a method for antibiotic therapy guidance, stratification and/or control in a patient suffering from an infectious disease and receiving treatment with one or more antibiotic agents, the method comprising

- isolating a first sample from said patient,

- isolating a second sample from said patient at a time point after isolating the first sample and after initiating antibiotic treatment,
- determining levels of procalcitonin (PCT) or fragment(s) thereof in the first and the second sample, and
- determining a level of proADM or fragment(s) thereof in at least the second sample,
- wherein the levels of PCT or fragment(s) thereof in said first and second samples, and the level of proADM or fragment(s) thereof in the second sample, are indicative of whether a change in the treatment with one or more antibiotic agents is required.

In a preferred embodiment of the method for antibiotic therapy guidance, stratification and/or control in a patient suffering from an infectious disease and receiving treatment with one or more antibiotic agents described herein, the first sample is isolated before, at the time point of or after determining symptoms of an infectious disease in said patient.

The patients of the method of the present invention have already been diagnosed as being suffering from an infectious disease and are already receiving antibiotic treatment. The method of the present invention can therefore be used for monitoring the success of the antibiotic treatment with the respective one or more antibiotic agents that has been initiated. The determined levels of PCT or fragments thereof and proADM or fragments thereof indicate whether a change of the ongoing antibiotic treatment is required. In other words, the levels of the markers PCT or fragments thereof and proADM or fragments thereof can be used as indicators of the likelihood of success of the respective antibiotic treatment and it can be decided, whether the treatment should be continued, because it is likely that it is working/improving the health state of the patient, or whether it should be changed.

In the context of the present invention, a change in the antibiotic treatment of a patient can involve a change in the dose, the administration route or regime or other parameters of antibiotic treatment, while the changed treatment may still encompass the same one or more antibiotic agents, which have been used initially. Furthermore, a change in the antibiotic treatment can also relate to a change in the one or more antibiotic agents use for treating the patient. A change can therefore relate to the addition of a further antibiotic agent or to the replacement of one or more antibiotic agents by one or more other agents.

In some embodiments, the antibiotic therapy guidance, stratification and/or control preferably involves the prognosis of the success or efficacy of the ongoing antibiotic therapy, also with respect to the likelihood of a future adverse event.

Physicians or medical personnel who are treating patients suffering from an infectious disease and who are receiving antibiotic therapy can employ the method of the present invention, preferably in a hospital setting, such as in a point of care format, or in an intensive care unit (ICU). The method is very useful to monitor the effect of a therapy that has been initiated on a patient with an infectious disease and can be used to judge whether a patient under treatment is a high risk patient that should be under intense medical observation, wherein the antibiotic treatment might be changed, or whether the patient is a low risk patient with a stable or even improving health state that might not require a change of the antibiotic treatment, possibly because the initiated treatment is successfully improving the state of the patient. Initial antibiotic

treatments of patients suffering from infectious diseases may have a direct effect on the likelihood of adverse events in the health of the patient. As such, the assessment of risk/prognosis of a future adverse event provides feedback on the efficacy of the therapy instigated.

The likelihood of the occurrence of a subsequent adverse event can be assessed on the comparison of the level of PCT or fragments thereof and/or the level of proADM or fragments thereof in the first and/or second sample in comparison to a reference level (such as a threshold or cut-off value and/or a population average), wherein the reference level may correspond to proADM or fragments thereof and/or PCT or fragments thereof in healthy patients, or in patients who have been diagnosed with an infectious disease.

Accordingly, the method of the present invention can help to predict the outcome of the antibiotic treatment and/or the likelihood of a subsequent adverse event in the health of the patient. This means, that the method of the invention can discriminate high risk patients, who are more likely to suffer from complications, or whose state will become more critical in the future despite the ongoing antibiotic therapy, from low risk patients, whose health state is stable or even improving, so that it is not expected that they will suffer from an adverse event, such as death of the patient or a deterioration of the patient's clinical symptoms or signs, which might require certain therapeutic measures.

A particular advantage of the method of the present invention is that an infectious disease patient that has been identified as a low risk patient by means of the method of the present invention, who does not require a change in the antibiotic therapy or at least no change of the one or more antibiotic agents, could be more rapidly discharged from an ICU. Also, for low risk patients, the intensity and/or frequency of the observation of the health status of the patient could be decreased. Accordingly, the hospital or other medical institution in charge of the patient could more efficiently decide which patients require intensive medical care and observation. Consequently, the respective hospital or institution could, for example, more efficiently occupy ICU beds with high-risk patients. This would lead to an improved medical care for the high-risk patients, since the medical personnel could focus of such patients, while low risk patients could be discharged from ICU. This would also lead to significant benefits from avoided costs for unnecessary measures that would otherwise be applied to low risk patients.

In preferred embodiments of the invention, said levels of PCT or fragment(s) thereof in said first and second samples, and the level of proADM or fragment(s) thereof in the second sample, are indicative of a subsequent adverse event in the health of said patient within 28 days after diagnosis and treatment initiation. In further preferred embodiments of the invention, said levels of PCT or fragment(s) thereof in said first and second samples, and the level of proADM or fragment(s) thereof in the second sample, are indicative of a subsequent adverse event in the health of said patient within 90 days after diagnosis and treatment initiation.

A preferred embodiment of the invention relates to a method for antibiotic therapy guidance, stratification and/or control in a patient suffering from an infectious disease and receiving treatment with one or more antibiotic agents, the method comprising

- isolating a first sample from said patient upon determining symptoms of an infectious disease in said patient,
- isolating a second sample from said patient after initially determining said symptoms and initiating antibiotic treatment,

- determining levels of procalcitonin (PCT) or fragment(s) thereof in the first and the second sample, and
- determining a level of proADM or fragment(s) thereof in at least the second sample,
- wherein the levels of PCT or fragment(s) thereof in said first and second samples, and the level of proADM or fragment(s) thereof in the second sample, are indicative of whether a change in the treatment with one or more antibiotic agents is required.

Isolation upon determining symptom of an infectious disease relates to an isolation shortly after determining symptoms of an infectious disease and might also be referred to as the time point of determining sympltoms of an infectious disease.

The time point when the patients have been diagnosed as being critically ill or when symptoms of an infectious disease are determined and the first treatment measures are initiated is defined as "time point 0" or the first sample, which may be the reference for the time point of isolation of the later second sample. If diagnosis of the patient or determination of the symptoms of an infectious disease and treatment initiation do not occur at the same time, time point 0 is the time point when the later of the two events of diagnosis/determination of symptoms and initiation of medical treatment occurs. Typically, diagnosis of critically ill patients and/or determination of symptoms of an infectious disease is immediately followed by or concomitant to initiation of therapy. The first sample is typically taken at or before this time.

It was entirely surprising that the level of proADM or fragments thereof in a later sample from the patient can provide critical information about the success of the ongoing antibiotic therapy and/or the likelihood of the occurrence of a subsequent adverse event in the health of said patients. There has been no indication that a single measurement of proADM or fragments thereof after diagnosis/determination of symptoms and treatment initiation of a patient suffering from an infectious disease could provide such relevant information with respect to the likelihood of success of the ongoing treatment and prognosis of the health status of the patient.

It is particularly advantageous to combine the determination of proADM or fragments thereof (in a second sample) with the determination of PCT or fragments thereof in an earlier sample (first sample) that is isolated from said patient and that might be used for diagnosing said patient as suffering from an infectious disease at time point 0 and determining the level of PCT or fragments thereof in a second sample isolated at a certain time point after diagnosis/determination of symptoms and treatment initiation, which is also preferably the same time point when proADM or fragments thereof are determined.

This allows to determine a difference in the level of PCT or fragment(s) thereof in the second sample in comparison to the level of PCT or fragment(s) thereof in the first sample. As indicated by the data below, determining a difference in the level of PCT or fragments thereof in the second sample in comparison to the first sample adds additional information to the information gained from the levels of proADM or fragments thereof in the second sample. Based on this combined information it might be possible to predict with a higher probability whether the ongoing antibiotic treatment will be successful with respect to stabilizing or improving the health state of the patient. This represents a surprising finding, as biomarkers for sepsis are typically not synergistic or complementary, but represent mere alternative diagnostic markers.

The combined determination of proADM or fragments thereof with the determination of PCT or fragments thereof, whether in the same sample or at samples obtained at different time points,

provides a synergistic effect with respect to the accuracy and reliability of determining the risk of a subsequent adverse event. These synergistic effects exist for the combined assessment of proADM or fragments thereof with other markers or clinical scores, such as lactate, CRP, SOFA, SAPS II, APACHE II, or other clinical assessments.

In a preferred embodiment of the present invention, the second sample is isolated from said patient within 30 minutes after determining symptoms of an infectious disease and initiating antibiotic treatment, or at least 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 days or 10 days after said diagnosis and initiating antibiotic treatment. In other embodiments the second sample is isolated from said patient 12-36 hours and/or 3-5 days after determining symptoms of an infectious disease and initiating antibiotic treatment.

In preferred embodiments of the method of the present invention said sample is isolated from said patient about 30 minutes, 1 hour, 2 hours, 3 hours, 4, hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours 22 hours, 24 hours, 30 hours, 36 hours, 42 hours, 48 hours, 60 hours, 72 hours, 84 hours, 4 days, 5 days, 6 days, 7 days, 8 days 9 days or 10 days after said diagnosis and initiating antibiotic treatment.

In other embodiments, the second sample is isolated at time points after said diagnosis and initiating antibiotic treatment of 30 minutes to 12 hours, 12-36 hours, 3-5 days, 7-14 days, 8-12 days, or 9-11 days.

Ranges between any given of the above values may be employed to define the time point of obtaining the sample.

According to a preferred embodiment of the present invention, the method comprises additionally determining a level of proADM or fragment(s) thereof in the first sample.

The fact that the level of proADM or fragments thereof at a time point as short as about 30 minutes after determining symptoms of an infectious disease and initiating treatment can provide such information was completely unexpected.

By determining the level of proADM or fragments thereof in the first and in the second sample it is possible to determine the difference in the level of proADM or fragment(s) thereof in the second sample in comparison to the level of proADM or fragment(s) thereof in the first sample. This allows the determination of a change of the level of proADM over the course of the antibiotic treatment.

It was surprising that the determination of the change of the levels of proADM or fragments thereof from the time point of diagnosis/determination of symptoms of an infectious disease and treatment initiation to a later time point can provide additional information with respect to the success of the antibiotic treatment and/or the occurrence of a future adverse event in the health of a patient. It is a great advantage of this embodiment of the present invention that the same sample that is used for the determining a level of PCT or fragments thereof at time point 0 can also be used for determining the baseline level of proADM or fragments thereof, which can be compared to the level of proADM or fragments thereof at a later time point after diagnosis and treatment initiation. By determining the change of the level of proADM or fragments thereof over the course of patient treatment the accuracy of predicting the success of the antibiotic treatment and/or the occurrence of an adverse event in the health of the patient can be further increased.

In a preferred embodiment of the present invention, an elevated level of proADM or fragment(s) thereof in the second sample compared to the first sample is indicative of a change of the one or more antibiotic agents being required.

According to the present invention, the term "indicate" in the context of "indicative that a change in the one or more antibiotic agents is required" is intended as a measure of likelihood of success of the ongoing antibiotic treatment with the respective one or more antibiotic agents. Preferably, the "indication" of the change in the one or more antibiotic agents being required is intended to refer to an increased likelihood that the ongoing antibiotic therapy with the respective one or more antibiotic agents is not successful in improving or stabilizing the health status of the patient. Therefore, a change in the antibiotic agents should be considered to prevent the occurrence of an adverse event in the patient.

In the context of the present invention, an indication of a requirement of changing one or more antibiotic agents used in the antibiotic therapy of a patient suffering from an infectious disease is associated with an increased likelihood of the occurrence of a future adverse event in the health of the patient. The indication of a change in the one or more antibiotic agents being required is intended as an assessment of antibiotic therapy efficacy, and is typically not to be construed in a limiting fashion as to point definitively to the absolute success or failure of antibiotic treatment.

Keeping the above in mind, the determination of severity levels of proADM and PCT is however very reliable with respect to determining whether an antibiotic change is required when using the cut-off values disclosed herein, such that the estimation of risk based on assessing a subsequent adverse event reliably enables an appropriate action by a medical professional.

It was surprising that based on the change of the level of proADM or fragments thereof it is possible to confidently predict the likelihood success of an ongoing antibiotic treatment and/or the occurrence of an adverse event in the health of the patient. An increase of the level or severity level of proADM or fragments thereof from the time point of determination of symptoms of an infectious disease and treatment initiation indicates that a change of antibiotic the one or more antibiotic agents might be required, potentially because it is likely that an adverse event will occur. Accordingly, based on the change of proADM or fragments thereof over the course of the treatment a physician can decide whether to change or modify the treatment of the patient or to stick to the initial treatment.

In the context of the present invention it is preferable that the patient is diagnosed as suffering from sepsis and/or septic shock. In light of the data presented herein, the prognostic value of proADM in samples of these patient groups is particularly accurate in predicting the likelihood of an adverse event in these patients and indicating whether a change in the ongoing antibiotic treatment is required.

Furthermore, the method of the present invention preferably comprises determining the level of MR-proADM. The employment of determining MR-proADM is preferred for any given embodiment described herein and may be considered in the context of each embodiment, accordingly. In preferred embodiments the "ADM fragment" may be considered to be MR-proADM.

In a preferred embodiment of the method described herein,

- an elevated level of PCT or fragment(s) thereof in the second sample compared to the first sample, and

- an intermediate or high severity level of proADM or fragment(s) thereof in the second sample, indicate that a change in the one or more antibiotic agents is required,
- wherein an intermediate severity level of proADM or fragment(s) thereof is above 4 nmol/l, preferably above 3 nmol/l, more preferably above 2.7 nmol/l, and below 6.5 nmol/l, preferably below 6.95 nmol/l, more preferably below 10.9 nmol/l, and
- a high severity level of proADM or fragment(s) thereof is above 6.5 nmol/l, preferably above 6.95 nmol/l, more preferably above 10.9 nmol/l.

In a preferred embodiment of the method described herein,

- a lower level of PCT or fragment(s) thereof in the second sample compared to the first sample, and
- a high severity level of proADM or fragments(s) thereof in the second sample, or an elevated severity level of proADM or fragment(s) thereof in the second sample compared to the first sample, such as an elevation from a low severity level to an intermediate or high severity level, or from an intermediate severity level to a high severity level,
- indicate that a change in the one or more antibiotic agents is required,
- wherein a low severity level of proADM or fragment(s) thereof is below 4 nmol/l, preferably below 3 nmol/l, more preferably below 2.7 nmol/l,
- an intermediate severity level of proADM or fragment(s) thereof is above 4 nmol/l, preferably above 3 nmol/l, more preferably above 2.7 nmol/l, and below 6.5 nmol/l, preferably below 6.95 nmol/l, more preferably below 10.9 nmol/l, and
- a high severity level of proADM or fragment(s) thereof is above 6.5 nmol/l, preferably above 6.95 nmol/l, more preferably above 10.9 nmol/l.

It was entirely surprising that a decrease of the level of PCT from the day of determination of symptoms and treatment initiation to a later time point after treatment has been initiated can be indicative of a requirement to change the antibiotic agents employed in an antibiotic treatment of a patient. In particular, this elevated risk is evident when a high severity level of proADM or fragments thereof has been determined.

It is believed that PCT is a marker for infectious diseases, such as sepsis. Accordingly, decreasing PCT values over the course of a treatment are considered to indicate an improvement of the health status of the patient. However, as disclosed herein, it has become evident that despite a decreasing PCT value the patient is at risk for a future adverse event, if the level of proADM or fragments thereof at the later time point is a high severity, or the severity level of proADM or fragments thereof is increasing over the course of the antibiotic treatment. Accordingly, the treating physician can adjust the treatment of such a patient that would have not been identified as a high-risk patient without determining proADM or fragments.

It was entirely surprising that a level of proADM or fragments thereof could be correlated with the requirement for a change in the one or more antibiotic agents used in the antibiotic treatment.

ProADM levels in samples from infectious disease patients of the present invention can preferably be assigned to 3 different severity levels of proADM. High levels of proADM indicate a high severity level, intermediate levels indicate an intermediate severity level and low levels indicate a low severity levels. The respective concentrations that determine the cut-off values for the respective severity levels depend on multiple parameters such as the time point of sample isolation after diagnosis and treatment initiation of the patient of the method of the present

invention and the method used for determining the level of proADM or fragments thereof in said sample.

The cut-off values disclosed herein refer preferably to measurements of the protein level of proADM or fragments thereof in a plasma sample obtained from a patient by means of the Thermo Scientific BRAHMS KRYPTOR assay. Accordingly, the values disclosed herein may vary to some extent depending on the detection/measurement method employed, and the specific values disclosed herein are intended to also read on the corresponding values determined by other methods.

In certain embodiments of the invention, a low severity level of proADM or fragment(s) thereof is below a cut-off value in the range of 1.5 nmol/l and 4 nmol/l. Any value within these ranges may be considered as an appropriate cut-off value for a low severity levels of proADM or fragments thereof. For example, 1.5, 1.55, 1.6, 1.65, 1.7, 1.75, 1.8, 1.85, 1.9, 1.95, 2.0, 2.05, 2.1, 2.15, 2.2, 2.25, 2.3, 2.35, 2.4, 2.45, 2.5, 2.55, 2.6, 2.65, 2.7, 2.75, 2.8, 2.85, 2.9, 2.95, 3.0, 3.05, 3.1, 3.15, 3.2, 3.25, 3.3, 3.35, 3.4, 3.45, 3.5, 3.55, 3.6, 3.65, 3.7, 3.75, 3.8, 3.85, 3.9, 3.95, 4.0 nmol/l.

In embodiments of the invention, a high severity level of proADM or fragment(s) is above a cut-off value in the range of 6.5 nmol/l to 12 nmol/l. Any value within these ranges may be considered as an appropriate cut-off value for a high severity levels of proADM or fragments thereof. For example, 6.5, 6.55, 6.6, 6.65, 6.7, 6.75, 6.8, 6.85, 6.9, 6.95, 7.0, 7.05, 7.1, 7.15, 7.2, 7.25, 7.3, 7.35, 7.4, 7.45, 7.5, 7.55, 7.6, 7.65, 7.7, 7.75, 7.8, 7.85, 7.9, 7.95, 8.0, 8.05, 8.1, 8.15, 8.2, 8.25, 8.3, 8.35, 8.4, 8.45, 8.5, 8.55, 8.6, 8.65, 8.7, 8.75, 8.8, 8.85, 8.9, 8.95, 9.0, 9.05, 9.1, 9.15, 9.2, 9.25, 9.3, 9.35, 9.4, 9.45, 9.5, 9.55, 9.6, 9.65, 9.7, 9.75, 9.8, 9.85, 9.9, 9.95, 10.0, 10.05, 10.1, 10.15, 10.2, 10.25, 10.3, 10.35, 10.4, 10.45, 10.5, 10.55, 10.6, 10.65, 10.7, 10.75, 10.8, 10.85, 10.9, 10.95, 11.0, 11.05, 11.1, 11.15, 11.2, 11.25, 11.3, 11.35, 11.4, 11.45, 11.5, 11.55, 11.6, 11.65, 11.7, 11.75, 11.8, 11.85, 11.9, 11.95, 12.0 nmol/l.

All cut-off values disclosed herein relating to the level of a marker or biomarker, such as proADM or PCT, are to be understood as "equal or above" a certain cut-off or "equal or below" a certain cut-off. For example, an embodiment relating to a level of proADM or fragment(s) thereof below 4 nmol/l, preferably below 3 nmol/l, more preferably below 2.7 nmol/l is to be understood as relating to a level of proADM or fragment(s) thereof equal or below 4 nmol/l, preferably equal or below 3 nmol/l, more preferably equal or below 2.7 nmol/l. Conversely, an embodiment relating to a level of proADM or fragment(s) thereof above 6.5 nmol/l, preferably above 6.95 nmol/l, more preferably above 10.9 nmol/l is to be understood as relating to a level of proADM or fragment(s) thereof equal or above 6.5 nmol/l, preferably equal or above 6.95 nmol/l, more preferably equal or above 10.9 nmol/l.

In the embodiments described herein, the severity levels are defined preferably by cut-off values, that represent boundaries between low, intermediate or high severity levels. Any embodiments that present cut-offs therefore may use the format of a single cut-off value as a boundary between two severity levels, or a single cutoff level for each severity level.

In some embodiments, the proADM cut-off value between low and intermediate severity levels is:

2.7 nmol/l ± 20%, or 2.7 nmol/l ± 15%, or ± 12%, ± 10%, ± 8%, or ± 5%,

and between intermediate and high severity levels:

10.9 nmol/l ± 20%, or 10.9 nmol/l ± 15%, or ± 12%, ± 10%, ± 8%, or ± 5%.

These cut-off values are preferably relevant for an assessment of proADM severity level at baseline, in other words upon diagnosis and/or therapy begin and/or hospitalization.

In some embodiments, the proADM cut-off value between low and intermediate severity levels is:

2.80 nmol/l ± 20%, or 2.80 nmol/l ± 15%, or ± 12%, ± 10%, ± 8%, or ± 5%,

and between intermediate and high severity levels:

9.5 nmol/l ± 20%, or 9.5 nmol/l ± 15%, or ± 12%, ± 10%, ± 8%, or ± 5%.

These cut-off values are preferably relevant for an assessment of proADM severity level after 1 day, in other words approx. 24 hours after baseline, in other words, approx. 1 day after diagnosis and/or therapy begin and/or hospitalization. For example, in embodiments where the proADM is measured one day after therapy begin, the cut-off values for day 1 may be employed. As is evident from the above, the cutoff between intermediate and high is somewhat lower than at baseline, i.e. as time progresses, even somewhat lower (but still relatively high) levels are associated with high risk and are classed in the high severity level.

In some embodiments, the proADM cut-off value between low and intermediate severity levels is:

2.80 nmol/l ± 20% or 2.80 nmol/l ± 15%, or ± 12%, ± 10%, ± 8%, or ± 5%,

and between intermediate and high severity levels:

7.7 nmol/l ± 20% or 7.7 nmol/l ± 15%, or ± 12%, ± 10%, ± 8%, or ± 5%.

These cut-off values are preferably relevant for an assessment of proADM severity level after 4 days, in other words approx. 4 days after baseline, in other words, approx. 4 days after diagnosis and/or therapy begin and/or hospitalization. For example, in embodiments where the proADM is measured 4 days after therapy begin, the cut-off values for day 4 may be employed. As is evident from the above, the cutoff between intermediate and high is somewhat lower than at baseline or at day 1, i.e. as time progresses, even somewhat lower (but still relatively high) levels are associated with high risk and are classed in the high severity level.

In some embodiments, the cutoff levels to be employed in the embodiments described above may be adjusted according to an appropriate level depending on the day the measurement is made. Each of the cut-off values is subject to some variation due to common variance as may be expected by the skilled person. The relevant cut-off levels are determined based on extensive data, as presented below, but are not intended in all possible embodiments to be final or exact values. By using a similar cut-off to those recited, i.e. within the ± 20%, ± 15%, ± 12%, ± 10%, ± 8%, or ± 5%, as can be determined by a skilled person, similar results may be expected.

Any embodiment reciting ± 20% of a given cut-off value, may be considered to also disclose ± 15%, ± 12%, ± 10%, ± 8%, or ± 5%.

Any embodiment reciting a particular cut-off value for baseline, day 1 or day 4, may be considered to also disclose the corresponding cut-off values for the other days, e.g. an embodiment reciting a baseline cut-off value may be considered to also relate to the same embodiment reciting the day 1 or day 4 cut-off value.

According to a preferred embodiment of the method of present invention,

- a more than 50 % lower level of PCT or fragment(s) thereof in the second sample compared to the first sample, and
- an intermediate severity level of proADM or fragment(s) thereof in the second sample compared to a low severity level of proADM or fragment(s) thereof in the first sample,

indicate that a change in the one or more antibiotic agents is required.

It was entirely surprising, that the degree of the relative change of the level of PCT in the first sample to the second sample can add a predictive value with respect to the efficacy and likelihood of success of the ongoing antibiotic treatment. As indicated in the data below, a decrease of the PCT level of over 50 % from time point 0 to the time point of isolation of the second sample together with an increase of the severity level of proADM or fragments thereof from a low to an intermediate severity level is likely to be associated with an unsuccessful ongoing antibiotic treatment. Accordingly, the treating physician or medical personnel should consider a change in one or more of the currently used antibiotic agents. This is particularly surprising, since even a strong/steep decrease of PCT levels of over 50 % can be associated with an unsuccessful treatment.

In preferred embodiments of the method of the present invention,

- a less than 50 % lower level of PCT or fragment(s) thereof in the second sample compared to the first sample, and
- a high severity level of proADM or fragment(s) thereof in the second sample compared to an intermediate severity level of proADM or fragment(s) thereof in the first sample, indicate that a change in the one or more antibiotic agents is required.

It was surprising that a decreasing level of PCT or fragments thereof of less than 50 % is indicative of an unsuccessful antibiotic treatment, if it is associated with an increase of the severity level of proADM or fragments thereof from a low or intermediate severity level to a high severity level.

Furthermore, in the context of the method of the present intervention it is preferable that an elevated level of PCT or fragment(s) thereof in the second sample compared to the first sample, and an intermediate or high severity level of proADM or fragment(s) thereof in the second sample, indicate that a change in the one or more antibiotic agents is required.

Surprisingly, if PCT levels are increasing over the course of the antibiotic treatment, a change in the antibiotic agents used might only be indicated, if the severity level of proADM or fragments thereof in the second sample are intermediate or high. In case of a low severity level of proADM, the person in charge of the antibiotic treatment should consider the possibility that the one or more antibiotic agents are effective in stabilizing or improving the state of the patient despite increasing PCT levels. Instead, a modification of the dose, route of administration or other modification of the antibiotic treatment without changing the combination of the one or more antibiotic agents used should be considered.

Preferably, the first and the second sample of the method of the present invention are selected from the group consisting of a blood sample, a serum sample, a plasma sample and/or a urine sample.

In one embodiment the invention additionally comprises informing the patient of the results of the diagnostic method described herein.

Further preferred embodiments of the method of the present invention additionally comprise

- determining a level of at least one additional biomarker or fragment(s) thereof in the first and the second sample, wherein the at least one additional biomarker preferably is lactate and/or C-reactive protein,
- wherein the levels of the at least one additional biomarker in said first and second samples, and the level of proADM or fragment(s) thereof in the second sample, is indicative of whether a change in the ongoing antibiotic treatment is required.

Preferred embodiments of the method for antibiotic therapy guidance, therapy stratification and/or control in a patient suffering from an infectious disease and receiving antibiotic treatment described herein additionally comprise

- determining at least one clinical score at the time point of isolation of the first sample and at the time point of isolation of the second sample, wherein the at least one clinical score preferably is SOFA, APACHE II and/or SAPS II,
- wherein the at least one clinical score at the time points of isolation of the first and second samples, and the level of proADM or fragment(s) thereof in the second sample, is indicative of whether a change in the ongoing antibiotic treatment is required.

In further embodiments of the method described herein, the method additionally comprises a molecular analysis of a sample from said patient for detecting an infection. The sample used for the molecular analysis for detecting an infection preferably is a blood sample. In a preferred embodiment the molecular analysis is a method aiming to detect one or more biomolecules derived from a pathogen. Said one or more biomolecule may be a nucleic acid, protein, sugar, carbohydrades, lipid and or a combination thereof such as glycosylated protein, preferably a nucleic acid. Said biomolecule preferably is specific for one or more pathogen(s). According to preferred embodiments, such biomolecules are detected by one or more methods for analysis of biomolecules selected from the group comprising nucleic acid amplification methods such as PCR, qPCR, RT-PCR, qRT-PCR or isothermal amplification, mass spectrometry, detection of enzymatic activity and immunoassay based detection methods. Further methods of molecular analysis are known to the person skilled in the art and are comprised by the method of the present invention.

The present invention further relates to a method for antibiotic therapy guidance, therapy stratification and/or control in a patient suffering from an infectious disease and receiving antibiotic treatment, comprising

- isolating a first sample from said patient upon determining symptoms of an infectious disease (time point 0) and isolating a second sample from said patient after initially determining said symptoms and initiating treatment,
- determining a level of at least one biomarker or fragment(s) thereof in the first and the second sample, and/or determining at least one clinical score at the time point of isolation of the first sample and at the time point of isolation of the second sample, wherein the at least one biomarker is procalcitonin (PCT), lactate and/or C-reactive protein, and the at least one clinical score is SOFA, APACHE II and/or SAPS II,
- determining a level of proADM or fragment(s) thereof in at least the second sample,
- wherein a change in the level of the at least one biomarker and/or the at least one clinical score between said first and second samples, and the level of proADM or

fragment(s) thereof in the second sample, are indicative of whether a change in the ongoing antibiotic treatment is required.

The present invention also relates to a kit for carrying out the method for antibiotic therapy guidance, therapy stratification and/or control in a patient suffering from an infectious disease and receiving antibiotic treatment described, wherein the kit comprises

- detection reagents for determining the level proADM or fragment(s) thereof, and optionally additionally for determining the level of PCT or fragment(s) thereof, in a sample from a subject, and
- reference data, such as a reference level, corresponding to proADM severity levels, and optionally PCT levels, wherein said reference data is preferably stored on a computer readable medium and/or employed in in the form of computer executable code configured for comparing the determined levels of proADM or fragment(s) thereof, and optionally additionally the determined levels of PCT or fragment(s) thereof, to said reference data.

The detection reagents for determining the level of proADM or fragment(s) thereof, and optionally for determining the level of PCT or fragment(s) thereof, are preferably selected from those necessary to perform the method, for example antibodies directed to proADM, suitable labels, such as fluorescent labels, preferably two separate fluorescent labels suitable for application in the KRYPTOR assay, sample collection tubes.

In one embodiment of the method described herein the level of proADM or fragment(s) thereof and optionally PCT or fragment(s) thereof is determined using a method selected from the group consisting of mass spectrometry (MS), luminescence immunoassay (LIA), radioimmunoassay (RIA), chemiluminescence- and fluorescence- immunoassays, enzyme immunoassay (EIA), Enzyme-linked immunoassays (ELISA), luminescence-based bead arrays, magnetic beads based arrays, protein microarray assays, rapid test formats such as for instance immunochromatographic strip tests, rare cryptate assay, and automated systems/analysers.

The method according to the present invention can furthermore be embodied as a homogeneous method, wherein the sandwich complexes formed by the antibody/antibodies and the marker, e.g., the proADM or a fragment thereof, which is to be detected remains suspended in the liquid phase. In this case it is preferred, that when two antibodies are used, both antibodies are labelled with parts of a detection system, which leads to generation of a signal or triggering of a signal if both antibodies are integrated into a single sandwich.

Such techniques are to be embodied in particular as fluorescence enhancing or fluorescence quenching detection methods. A particularly preferred aspect relates to the use of detection reagents which are to be used pair-wise, such as for example the ones which are described in US 4 882 733 A, EP-B1 0 180 492 or EP-B1 0 539 477 and the prior art cited therein. In this way, measurements in which only reaction products comprising both labelling components in a single immune-complex directly in the reaction mixture are detected, become possible.

For example, such technologies are offered under the brand names TRACE® (Time Resolved Amplified Cryptate Emission) or KRYPTOR®, implementing the teachings of the above-cited applications. Therefore, in particular preferred aspects, a diagnostic device is used to carry out the herein provided method. For example, the level of the proADM protein or a fragment thereof,

and/or the level of any further marker of the herein provided method are determined. In particular preferred aspects, the diagnostic device is KRYPTOR®.

In one embodiment of the method described herein the method is an immunoassay and wherein the assay is performed in homogeneous phase or in heterogeneous phase.

In one embodiment of the method described herein a first antibody and a second antibody are present dispersed in a liquid reaction mixture, and wherein a first labelling component which is part of a labelling system based on fluorescence or chemiluminescence extinction or amplification is bound to the first antibody, and a second labelling component of said labelling system is bound to the second antibody so that, after binding of both antibodies to said proADM or fragments thereof to be detected, a measurable signal which permits detection of the resulting sandwich complexes in the measuring solution is generated.

In one embodiment of the method described herein the labelling system comprises a rare earth cryptate or chelate in combination with a fluorescent or chemiluminescent dye, in particular of the cyanine type.

In one embodiment of the method described herein, the method additionally comprises comparing the determined level of proADM or fragment(s) thereof to a reference level, threshold value and/or a population average corresponding to proADM or fragments thereof in patients who have been diagnosed as being critically ill/suffering from an infectious disease and are under medical treatment, wherein said comparing is carried out in a computer processor using computer executable code.

The methods of the present invention may in part be computer-implemented. For example, the step of comparing the detected level of a marker, e.g. the proADM or fragments thereof, with a reference level can be performed in a computer system. In the computer-system, the determined level of the marker(s) can be combined with other marker levels and/or parameters of the subject in order to calculate a score, which is indicative for the diagnosis, prognosis, risk assessment and/or risk stratification. For example, the determined values may be entered (either manually by a health professional or automatically from the device(s) in which the respective marker level(s) has/have been determined) into the computer-system. The computer-system can be directly at the point-of-care (e.g. ICU or ED) or it can be at a remote location connected via a computer network (e.g. via the internet, or specialized medical cloud-systems, optionally combinable with other IT-systems or platforms such as hospital information systems (HIS)). Typically, the computer-system will store the values (e.g. marker level or parameters such as age, blood pressure, weight, sex, etc. or clinical scoring systems such as SOFA, qSOFA, BMI etc.) on a computer-readable medium and calculate the score based-on pre-defined and/or pre-stored reference levels or reference values. The resulting score will be displayed and/or printed for the user (typically a health professional such as a physician). Alternatively or in addition, the associated prognosis, diagnosis, assessment, treatment guidance, patient management guidance or stratification will be displayed and/or printed for the user (typically a health professional such as a physician).

In one embodiment of the invention, a software system can be employed, in which a machine learning algorithm is evident, preferably to identify hospitalized patients at risk for sepsis, severe sepsis and septic shock using data from electronic health records (EHRs). A machine learning approach can be trained on a random forest classifier using EHR data (such as labs, biomarker expression, vitals, and demographics) from patients. Machine learning is a type of artificial

intelligence that provides computers with the ability to learn complex patterns in data without being explicitly programmed, unlike simpler rule-based systems. Earlier studies have used electronic health record data to trigger alerts to detect clinical deterioration in general. In one embodiment of the invention the processing of proADM levels may be incorporated into appropriate software for comparison to existing data sets, for example proADM levels may also be processed in machine learning software to assist in diagnosing or prognosing the occurrence of an adverse event.

The combined employment of proADM or fragments thereof in combination with another biomarker such as PCT or CRP may be realised either in a single multiplex assay, or in two separate assays conducted on a sample form the patient. The sample may relate to the same sample, or to different samples. The assay employed for the detection and determination of proADM and for example PCT may also be the same or different, for example an immunoassay may be employed for the determination of one of the above markers. More detailed descriptions of suitable assays are provided below.

Cut-off values and other reference levels of proADM or fragments thereof in patients who have been diagnosed as being critically ill and are under treatment may be determined by previously described methods. For example, methods are known to a skilled person for using the Coefficient of variation in assessing variability of quantitative assays in order to establish reference values and/or cut-offs (George F. Reed et al., Clin Diagn Lab Immunol.2002; 9(6):1235-1239).

Additionally, functional assay sensitivity can be determined in order to indicate statistically significant values for use as reference levels or cut-offs according to established techniques. Laboratories are capable of independently establishing an assay's functional sensitivity by a clinically relevant protocol. “Functional sensitivity” can be considered as the concentration that results in a coefficient of variation (CV) of 20% (or some other predetermined % CV), and is thus a measure of an assay's precision at low analyte levels. The CV is therefore a standardization of the standard deviation (SD) that allows comparison of variability estimates regardless of the magnitude of analyte concentration, at least throughout most of the working range of the assay.

Furthermore, methods based on ROC analysis can be used to determine statistically significant differences between two clinical patient groups. Receiver Operating Characteristic (ROC) curves measure the sorting efficiency of the model's fitted probabilities to sort the response levels. ROC curves can also aid in setting criterion points in diagnostic tests. The higher the curve from the diagonal, the better the fit. If the logistic fit has more than two response levels, it produces a generalized ROC curve. In such a plot, there is a curve for each response level, which is the ROC curve of that level versus all other levels. Software capable of enabling this kind of analysis in order to establish suitable reference levels and cut-offs is available, for example JMP 12, JMP 13, Statistical Discovery, from SAS.

Cut off values may similarly be determined for PCT. Literature is available to a skilled person for determining an appropriate cut-off, for example Philipp Schuetz et al. (BMC Medicine. 2011; 9:107) describe that at a cut-off of 0.1 ng/mL, PCT had a very high sensitivity to exclude infection. Terence Chan et al. (Expert Rev.Mol.Diagn.2011; 11(5), 487.496) described that indicators such as the positive and negative likelihood ratios, which are calculated based on sensitivity and specificity, are also useful for assessing the strength of a diagnostic test. Values are commonly graphed for multiple cut-off values (CVs) as a receiver operating characteristic curve. The area under the curve value is used to determine the best diagnostically relevant CV. This literature

describes the variation of CVs (cut-off values, that is dependent on the assay and study design), and suitable methods for determining cut-off values.

Population averages levels of proADM or fragments thereof may also be used as reference values, for example mean proADM population values, whereby patients that are diagnosed as critically ill may be compared to a control population, wherein the control group preferably comprises more than 10, 20, 30, 40, 50 or more subjects.

In one embodiment of the invention, the cut-off level for PCT may be a value in the range of 0.01 to 100.00 ng/mL in a serum sample, when using for example a Luminex MAC Pix E-Bioscience Assay or the BRAHMS PCT-Kryptor Assay. In a preferred embodiment the cut-off level of PCT may be in the range of 0.01 to 100, 0.05 to 50, 0.1 to 20, or 0.1 to 2 ng/mL, and most preferably >0,05 to 0,5 ng/mL. Any value within these ranges may be considered as an appropriate cut-off value. For example, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 ng/mL may be employed. In some embodiments, PCT levels for healthy subjects are approximately 0.05 ng/mL.

The invention further relates to methods of treatment for the medical indications described herein, wherein the patient population to be treated is identified, stratified, monitoired, prognosed, diagnosed or otherwise assessed using the methods described herein. Suitable treatments for the methods are disclosed herein. The present invention is therefore particularly advantageous in identifying patients with increased risk of having an adverse event and initiating preventative or risk-reducing treatments, or initiating treatments to address the presence of any given medical condition, preferably those understood as critical illness.

Embodiments of the invention relating to determining the level of proADM or fragment(s) thereof in one or more samples of critically ill patients, such as those with sepsis

Furthermore, the invention relates to a method for therapy monitoring, comprising the prognosis, risk assessment and/or risk stratification of a subsequent adverse event in the health of a patient, comprising

- providing a sample of said patient, wherein the patient has been diagnosed as being critically ill and medical treatment has been initiated, wherein the sample is isolated from the patient after diagnosis and treatment initiation,
- determining a level of proADM or fragment(s) thereof in said sample,
- wherein said level of proADM or fragment(s) thereof correlates with the likelihood of a subsequent adverse event in the health of said patient.

The patients of the method of the present inventions have already been diagnosed as being critically ill and are already receiving treatment. The method of the present invention can therefore be used for monitoring the success of the treatment or therapy that has been initiated, on the basis of determining the likelihood of a subsequent adverse event. The therapy monitoring preferably involves the prognosis of an adverse event and/or the risk stratification or risk assessment of the patient with respect to a future adverse event, wherein this risk assessment and the determination of said risk is to be considered as a means of monitoring the initiated therapy.

In embodiments of the invention the term critically ill may be used interchangeably with a patient suffering with an infectious disease.

The use of proADM or fragments thereof as a single parameter in embodiments of the present invention is advantageous over the use of other single parameters, such as biomarkers or clinical scores, since proADM is more precise in the prediction of an adverse event as compared to other markers such as for the PCT, CRP, lactate or clinical scores such as SOFA, SAPS II or APACHE II. In certain embodiments the present invention comprises the determination of additional parameters, such as markers, biomarkers, clinical scores or the like. In another preferred embodiment of the present invention, the patient has been diagnosed using at least one of the biomarkers procalcitonin (PCT), lactate and C-reactive protein and/or at least one of the clinical scores SOFA, APACHE II and SAPS II.

In one embodiment of the invention, the critically ill patient is a patient diagnosed with an infectious disease, a patient diagnosed with an infectious disease and one or more existing organ failure(s), a patient diagnosed with sepsis, severe sepsis or septic shock and/or a posttraumatic or postsurgical patient. In light of the data presented herein, the prognostic value of proADM in samples of these patient groups is particularly accurate in predicting the likelihood of an adverse event in these patients.

In preferred embodiments of the present invention, the adverse event in the health of said patient is death, preferably death within 28-90 days after diagnosis and treatment initiation, a new infection, organ failure and/or a deterioration of clinical symptoms requiring a focus cleaning procedure, transfusion of blood products, infusion of colloids, emergency surgery, invasive mechanical ventilation and/or renal or liver replacement.

In preferred embodiments of the invention, said level of proADM or fragment(s) thereof correlates with the likelihood of a subsequent adverse event in the health of said patient within 28 days after diagnosis and treatment initiation. In further preferred embodiments of the invention, said level of proADM or fragment(s) thereof correlates with the likelihood of a subsequent adverse event in the health of said patient within 90 days after diagnosis and treatment initiation.

In certain embodiments of the invention, the treatment received by the patient comprises one or more of antibiotic treatment, invasive mechanical ventilation, non-invasive mechanical ventilation, renal replacement therapy, vasopressor use, fluid therapy, apheresis and/or organ protection.

In preferred embodiments of the invention, the sample is selected from the group consisting of a blood sample, a serum sample, a plasma sample and/or a urine sample.

Preferably, the method is carried out in some embodiments by determining a level of proADM or fragment(s) thereof, wherein said determining of proADM comprises determining a level of MR-proADM in the sample. The employment of determining MR-proADM is preferred for any given embodiment described herein and may be considered in the context of each embodiment, accordingly. In preferred embodiments the “ADM fragment” may be considered to be MR-proADM.

In further embodiments of the invention the level of proADM or fragment(s) thereof correlates with the likelihood of a subsequent adverse event in the health of said patient. In a preferred embodiment the level of proADM or fragment(s) thereof positively correlates with the likelihood of a subsequent adverse event in the health of said patient. In other words, the higher the level of proADM determined, the greater the likelihood of a subsequent adverse event.

According to a preferred embodiment of the present invention,

- a low severity level of proADM or fragment(s) thereof is indicative of the absence of a subsequent adverse event, or indicates a low risk of a subsequend adverse event, wherein the low severity level is below 4 nmol/l, preferably below 3 nmol/l, more preferably below 2.7 nmol/l, or
- a high severity level of proADM or fragment(s) thereof is indicative of a subsequent adverse event, or indicates a high risk of a subsequent adverse event, wherein the high severity level is above 6.5 nmol/l, preferably above 6.95 nmol/l, more preferably above 10.9 nmol/l.

According to a preferred embodiment of the present invention,

- a level of proADM or fragment(s) thereof below 4 nmol/l, preferably below 3 nmol/l, more preferably below 2.7 nmol/l, is indicative of the absence of a subsequent adverse event, or indicates a low risk of a subsequend adverse event, or
- a level of proADM or fragment(s) thereof above 6.5 nmol/l, preferably above 6.95 nmol/l, more preferably above 10.9 nmol/l, is indicative of a subsequent adverse event, or indicates a high risk of a subsequent adverse event.

According to a preferred embodiment of the present invention,

- a low severity level of proADM or fragment(s) thereof is indicative of the absence of a subsequent adverse event, wherein the low severity level is below 2.7 nmol/l, or
- a high severity level of proADM or fragment(s) thereof is indicative of a subsequent adverse event, wherein the high severity level is above 10.9 nmol/l.

This embodiment of the present invention is particularly advantageous when levels of proADM or fragments thereof are determined in a sample that has been isolated on the day of diagnosis and treatment initiation of the patient, particularly about 30 minutes after diagnosis and treatment initiation. This is evident from the analysis provided in example 3.

According to a preferred embodiment of the present invention,

- a low severity level of proADM or fragment(s) thereof is indicative of the absence of a subsequent adverse event, wherein the low severity level is below 2.7 nmol/l, or
- a high severity level of proADM or fragment(s) thereof is indicative of a subsequent adverse event, wherein the high severity level is above 10.9 nmol/l,
- wherein the level of proADM or fragments thereof is determined in a sample that has been isolated on the day of diagnosis and treatment initiation.

According to a preferred embodiment of the present invention,

- a low severity level of proADM or fragment(s) thereof is indicative of the absence of a subsequent adverse event, wherein the low severity level is below 2.8 nmol/l, or
- a high severity level of proADM or fragment(s) thereof is indicative of a subsequent adverse event, wherein the high severity level is above 9.5 nmol/l.

This embodiment of the present invention is particularly advantageous when levels of proADM or fragments thereof are determined in a sample that has been isolated on 1 day after said diagnosis and treatment initiation, as is evident from Table 12.

According to a preferred embodiment of the present invention,

- a low severity level of proADM or fragment(s) thereof is indicative of the absence of a subsequent adverse event, wherein the low severity level is below 2.8 nmol/l, or
- a high severity level of proADM or fragment(s) thereof is indicative of a subsequent adverse event, wherein the high severity level is above 9.5 nmol/l,
- wherein the level of proADM or fragments thereof is determined in a sample that has been isolated 1 day after of diagnosis and treatment initiation.

According to a preferred embodiment of the present invention,

- a low severity level of proADM or fragment(s) thereof is indicative of the absence of a subsequent adverse event, wherein the low severity level is below 2.25 nmol/l.

This cut-off value for the low severity level is particularly advantageous, because it has been determined in some embodiments as the optimal cut-off value, between the low and intermediate severity level of proADM or fragments thereof when the of the sample is isolated from the patient on day 4, day 7 or day 10 after said diagnosis and treatment initiation. This is evident from the analysis provided in Table 12.

According to a preferred embodiment of the present invention,

- a low severity level of proADM or fragment(s) thereof is indicative of the absence of a subsequent adverse event, wherein the low severity level is below 2.25 nmol/l,
- wherein the level of proADM or fragments thereof is determined in a sample that has been isolated 4 or 7 days after of diagnosis and treatment initiation.

According to a preferred embodiment of the present invention,

- a high severity level of proADM or fragment(s) thereof is indicative of a subsequent adverse event, wherein the high severity level is above 7.7 nmol/l.

This cut-off value for the high severity level is particularly advantageous, because it has been determined in some embodiments as the optimal cut-off value, between the intermediate and high severity level of proADM or fragments thereof when the of the sample is isolated from the patient on day 4 after said diagnosis and treatment initiation. This is evident from the analysis provided in Table 12.

According to a preferred embodiment of the present invention,

- a high severity level of proADM or fragment(s) thereof is indicative of a subsequent adverse event, wherein the high severity level is above 7.7 nmol/l,
- wherein the level of proADM or fragments thereof is determined in a sample that has been isolated 4 days after of diagnosis and treatment initiation.

According to a preferred embodiment of the present invention,

- a high severity level of proADM or fragment(s) thereof is indicative of a subsequent adverse event, wherein the high severity level is above 6.95 nmol/l.

This cut-off value for the high severity level is particularly advantageous, because it has been determined in some embodiments as the optimal cut-off value, between the intermediate and high severity level of proADM or fragments thereof when the of the sample is isolated from the patient on day 7 after said diagnosis and treatment initiation. This is evident from the analysis provided in Table 12.

According to a preferred embodiment of the present invention,

- a high severity level of proADM or fragment(s) thereof is indicative of a subsequent adverse event, wherein the high severity level is above 6.95 nmol/l
- wherein the level of proADM or fragments thereof is determined in a sample that has been isolated 7 days after of diagnosis and treatment initiation.

According to a preferred embodiment of the present invention,

- a high severity level of proADM or fragment(s) thereof is indicative of a subsequent adverse event, wherein the high severity level is above 7.45 nmol/l.

This cut-off value for the high severity level is particularly advantageous, because it has been determined as the optimal cut-off value, between the intermediate and high severity level of proADM or fragments thereof when the of the sample is isolated from the patient on day 10 after said diagnosis and treatment initiation. This is evident from the analysis provided in Table 12.

According to a preferred embodiment of the present invention,

- a high severity level of proADM or fragment(s) thereof is indicative of a subsequent adverse event, wherein the high severity level is above 7.45 nmol/l
- wherein the level of proADM or fragments thereof is determined in a sample that has been isolated 10 days after of diagnosis and treatment initiation.

Preferably, the patients of the present invention are intensive care unit (ICU)-patients, wherein:

- the low severity level of proADM or fragment(s) thereof indicates discharging of said patient from ICU, or
- the high severity level of proADM or fragment(s) thereof indicates modifying the treatment of the patient in the ICU.

It is a particular advantage of the present invention that based on the classification of the determined levels of proADM or fragments thereof it is possible to assess the probability of the occurrence of a future adverse event in the health of an ICU patient. Based on this assessment it is possible to adjust the next treatment options and decisions.

For example, if the level of proADM or fragments thereof falls into the category of a low severity level of proADM, the treating physician can decide with more confidence to discharge said patient from ICU, because it is unlikely that an adverse event in the health of said patient would occur, preferably, within the next 28 days, more preferably within the next 90 days. Accordingly, it might not be necessary to keep this patient on the ICU. It might also be possible to conclude that the

ongoing treatment is successfully improving the health state of the patient, as assessed by a measurement of risk of an adverse event.

In contrast, if the determination of the level of proADM or fragments thereof of said ICU patient indicates a high severity level of proADM or fragments thereof, the treating physician should keep the patient on the ICU. Additionally, it should be considered to adjust the treatment of the patient, because it is likely that the current treatment is not improving the health state of the patient, which is why the patient is more likely to suffer form an adverse event in the future.

A treatment modification in the sense of the present invention would include, without limitation, an adjustment of the dose or administration regime of the ongoing medication. Change of the ongoing treatment to a different treatment, addition of a further treatment option to the ongoing treatment or stop of an ongoing treatment. Different treatments that can be applied to patients in the context of the present invention have been disclosed in the detailed description of this patent application.

According to a particularly preferred embodiment of the present invention the low severity level is below 2.25 nmol/l, said sample is isolated from the ICU-patient 1 day or more after said diagnosis and treatment initiation, and the low severity level of proADM or fragment(s) thereof indicates discharging of said patient from ICU.

The present invention further relates to a method for for therapy monitoring, comprising the prognosis, risk assessment and/or risk stratification of a subsequent adverse event in the health of a patient, comprising

- providing a sample of said patient, wherein the patient is an intensive care unit (ICU)-patient and medical treatment has been initiated, wherein the sample is isolated from the patient after admission to ICU and treatment initiation,
- determining a level of adrenomedullin (ADM) or fragment(s) thereof in said sample,
- wherein said level of proADM or fragment(s) thereof correlates with the likelihood of a subsequent adverse event in the health of said patient.

In the context of the method of the present invention relating to ICU-patients (instead of patients that have been diagnosed as being critically ill), the reference for the time point of isolation of the sample used for determining proADM or fragments thereof is the time point when the patients are admitted to the ICU and the first treatment measures are initiated (time point 0). This time point corresponds to the time point of diagnosis and treatment initiation in the method of the present invention relating to patients that have been diagnosed as being critically ill.

All preferred embodiment of the method of the present invention relating to patients that have been diagnosed as being critically ill are herewith also disclosed in the context of the method of the present invention relating to ICU-patients.

Embodiments of the invention relating to additionally determining a level of PCT and/or other biomarkers (or clinical scores) in a first and a second sample (or at the time point of isolation of a first and a second sample) in critically ill patients

A preferred embodiment of the present invention comprises additionally determining a level of PCT or fragment(s) thereof in a sample isolated from the patient. In a preferred embodiment, the sample for determining a level of PCT or fragment(s) thereof is isolated before, at or after the time point of diagnosis and treatment initiation.

It is particularly advantageous to combine the determination of proADM or fragments thereof with the determination of PCT or fragments thereof in a sample, wherein the sample used for determining proADM may be the same or a different sample used for detecting PCT.

The combined determination of proADM or fragments thereof with the determination of PCT or fragments thereof, whether in the same sample or at samples obtained at different time points, provides a synergistic effect with respect to the accuracy and reliability of determining the risk of a subsequent adverse event. These synergistic effects exist for the combined assessment of proADM or fragments thereof with other markers or clinical scores, such as lactate, CRP, SOFA, SAPS II, APACHE II, or other clinical assessments.

According to a further preferred embodiment of the present invention, the method described herein comprises additionally

- determining a level of PCT or fragment(s) thereof in a first sample isolated from the patient, wherein said first sample is isolated before, at or after the time point of diagnosis and treatment initiation,
- determining a level of PCT or fragment(s) thereof in a second sample isolated from said patient, wherein the second sample has been isolated after the first sample, preferably within 30 minutes after isolation of the first sample or 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after isolation of the first sample, and
- determining a difference in the level of PCT or fragment(s) thereof in the second sample in comparison to the level of PCT or fragment(s) thereof in the first sample.

It is particularly advantageous to combine the determination of proADM or fragments thereof in a sample isolated from a patient with the determination of PCT or fragments thereof in a first sample and determining the level of PCT or fragments thereof in a second sample isolated after the first sample, wherein the sample used for the determination of proADM or fragments thereof may be the same of different than the first sample or the second sample used for determining PCT or fragments thereof.

In a preferred embodiment of the method described herein comprises additionally

- determining a level of PCT or fragment(s) thereof in a first sample isolated from the patient, wherein said first sample is isolated at or before the time point of diagnosis and treatment initiation (time point 0),
- determining a level of PCT or fragment(s) thereof in a second sample isolated from said patient after diagnosis and treatment initiation, preferably within 30 minutes after said diagnosis and treatment initiation or 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after said diagnosis and treatment initiation, and
- determining a difference in the level of PCT or fragment(s) thereof in the second sample in comparison to the level of PCT or fragment(s) thereof in the first sample.

It is particularly advantageous to combine the determination of proADM or fragments thereof (in a second sample) with the determination of PCT or fragments thereof in an earlier sample (first sample) that is isolated from said patient and that may be used for diagnosing said patient as being critically ill at time point 0 and determining the level of PCT or fragments thereof in a

second sample isolated at a certain time point after diagnosis and treatment initiation, which is also preferably the same time point when proADM or fragments thereof are determined. As indicated by the data below, determining a difference in the level of PCT or fragments thereof in the second sample in comparison to the first sample adds additional information to the information gained from the levels of proADM or fragments thereof in the second sample. Based on this combined information it might be possible to predict with a higher probability whether an adverse event in the health of said patient will occur as compared to predicting the likelihood of an adverse event purely on the information about the level of proADM or fragments thereof in the second sample. This represents a surprising finding, as biomarkers for sepsis are typically not synergistic or complementary, but represent mere alternative diagnostic markers.

In a preferred embodiment of the method described herein comprises additionally

- determining a level of lactate in a first sample isolated from the patient, wherein said first sample is isolated at or before the time point of diagnosis and treatment initiation (time point 0),
- determining a level of lactate in a second sample isolated from said patient after said diagnosis and treatment initiation, preferably within 30 minutes or at least 30 minutes, preferably 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after said diagnosis and treatment initiation, and
- determining a difference in the level of lactate in the second sample in comparison to the level of lactate in the first sample.

A preferred embodiment of the present invention comprises additionally determining a level of CRP or fragment(s) thereof in a sample isolated from the patient. In a preferred embodiment, the sample for determining a level of CRP or fragment(s) thereof is isolated before, at or after the time point of diagnosis and treatment initiation.

It is particularly advantageous to combine the determination of proADM or fragments thereof with the determination of CRP or fragment(s) thereof in a sample, wherein the sample used for determining proADM may be the same or a different sample used for detecting CRP or fragment(s) thereof.

According to a further preferred embodiment of the present invention, the method described herein comprises additionally

- determining a level of CRP or fragment(s) thereof in a first sample isolated from the patient, wherein said first sample is isolated before, at or after the time point of diagnosis and treatment initiation,
- determining a level of CRP or fragment(s) thereof in a second sample isolated from said patient, wherein the second sample has been isolated after the first sample, preferably within 30 minutes after isolation of the first sample or 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after isolation of the first sample, and
- determining a difference in the level of CRP or fragment(s) thereof in the second sample in comparison to the level of lactate in the first sample.

In a preferred embodiment of the method described herein comprises additionally

- determining a level of CRP or fragment(s) thereof in a first sample isolated from the patient, wherein said first sample is isolated at or before the time point of diagnosis and treatment initiation (time point 0),
- determining a level of CRP or fragment(s) thereof in a second sample isolated from said patient after said diagnosis and treatment initiation, preferably within 30 minutes or at least 30 minutes, preferably 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after said diagnosis and treatment initiation, and
- determining a difference in the level of CRP or fragment(s) thereof in the second sample in comparison to the level of CRP or fragment(s) thereof in the first sample.

A preferred embodiment of the present invention comprises additionally determining SOFA. In a preferred embodiment, SOFA is deterimned before, at or after the time point of diagnosis and treatment initiation.

It is particularly advantageous to combine the determination of proADM or fragments thereof with the determination SOFA, wherein the time point of sample isolation for determining proADM may be the same or a different from the time point of determining SOFA.

According to a further preferred embodiment of the present invention, the method described herein comprises additionally

- determining a first SOFA before, at or after the time point of diagnosis and treatment initiation,
- determining a second SOFA within 30 minutes after deteriminig the first SOFA or 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after determining the first SOFA, and
- determining a difference in the two determined SOFA.

In a preferred embodiment of the method described herein comprises additionally

- determining SOFA at or before the time point of diagnosis and treatment initiation (time point 0),
- determining SOFA after said diagnosis and treatment initiation, preferably within 30 minutes, or at least 30 minutes, preferably 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after said diagnosis and treatment initiation, and
- determining a difference in SOFA determined after said diagnosis and treatment initiation and SOFA determined at time point 0.

A preferred embodiment of the present invention comprises additionally determining SAPS II. In a preferred embodiment, SAPS II is deterimned before, at or after the time point of diagnosis and treatment initiation.

It is particularly advantageous to combine the determination of proADM or fragments thereof with the determination SAPS II, wherein the time point of sample isolation for determining proADM may be the same or a different from the time point of determining SAPS II.

According to a further preferred embodiment of the present invention, the method described herein comprises additionally

- determining a first SAPS II before, at or after the time point of diagnosis and treatment initiation,
- determining a second SAPS II within 30 minutes after deteriminig the first SOFA or 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after determining the first SAPS II, and
- determining a difference in the two determined SAPS II.

In a preferred embodiment of the method described herein comprises additionally

- determining SAPS II at or before the time point of diagnosis and treatment initiation (time point 0),
- determining SAPS II after said diagnosis and treatment initiation, preferably within 30 minutes, or at least 30 minutes, preferably 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after said diagnosis and treatment initiation, and
- determining a difference in SAPS II determined after said diagnosis and treatment initiation and SAPS II determined at time point 0.

A preferred embodiment of the present invention comprises additionally determining APACHE II. In a preferred embodiment, APACHE II is deterimned before, at or after the time point of diagnosis and treatment initiation.

It is particularly advantageous to combine the determination of proADM or fragments thereof with the determination APACHE II, wherein the time point of sample isolation for determining proADM may be the same or a different from the time point of determining APACHE II.

According to a further preferred embodiment of the present invention, the method described herein comprises additionally

- determining a first APACHE II before, at or after the time point of diagnosis and treatment initiation,
- determining a second APACHE II within 30 minutes after deteriminig the first APACHE II or 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after determining the first APACHE II, and
- determining a difference in the two determined APACHE II.

In a preferred embodiment of the method described herein comprises additionally

- determining APACHE II at or before the time point of diagnosis and treatment initiation (time point 0),
- determining APACHE II after said diagnosis and treatment initiation, preferably within 30 minutes, or at least 30 minutes, preferably 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after said diagnosis and treatment initiation, and
- determining a difference in APACHE II determined after said diagnosis and treatment initiation and APACHE II determined at time point 0.

In a preferred embodiment of the present invention,

- a lower level of PCT or fragment(s) thereof in the second sample compared to the first sample, and a low severity level of proADM or fragment(s) thereof, wherein the low severity level is below 4 nmol/l, preferably below 3 nmol/l, more preferably below 2.7 nmol/l, is indicative of the absence of a subsequent adverse event, or
- a lower level of PCT or fragment(s) thereof in the second sample compared to the first sample, and a high severity level of proADM or fragment(s) thereof, wherein the high severity level is above 6.5 nmol/l, preferably above 6.95 nmol/l, more preferably above 10.9 nmol/l, is indicative of a subsequent adverse event.

In the context of the present invention, determining a lower level of a marker in the second sample as compared to the first sample can be indicative of decreasing levels of the respective marker in the patient over the course of the initiated treatment. Conversely, elevated levels in the second sample as compared to the first sample might indicate increasing levels of the marker over the course of the treatment.

It is believed that PCT is a marker for critical illness of a patient, in particular for a sepsis patient. Accordingly, decreasing PCT values over the course of a treatment are considered to indicate an improvement of the health status of the patient. However, as disclosed herein, it has become evident that a decreasing PCT value could be indicative of the occurrence of a future adverse event, if the level of proADM or fragments thereof at the later time point is a high severity level. Accordingly, the treating physician can adjust the treatment of such a patient that would have not been identified as a high-risk patient without determining proADM or fragments thereof in the second sample.

On the other hand, a physician can be confident that an adverse event is unlikely to occur when the level of PCT is decreasing over the course of the treatment while the level of proADM or fragments thereof in the second sample is a low severity level of proADM or fragments thereof. Accordingly, such patients can be identified to be low-risk patients. It was entirely surprising that the combination of the determination of the change of PCT levels over the course of the treatment of a critically ill patient with the determination of proADM levels at the later time point leads to an improved treatment monitoring, prognosis and risk assessment for the occurrence of a future adverse event in the health of a patient.

According to another preferred embodiment of the method described herein,

- an elevated level of PCT or fragment(s) thereof in the second sample compared to the first sample, and a low severity level proADM or fragment(s) thereof, wherein the low severity level is below 4 nmol/l, preferably below 3 nmol/l, more preferably below 2.7 nmol/l, is indicative of the absence of a subsequent adverse event, or
- an elevated level of PCT or fragment(s) thereof in the second sample compared to the first sample, and a high severity level of proADM or fragment(s) thereof, wherein the high severity level is above 6.5 nmol/l, preferably above 6.95 nmol/l, more preferably above 10.9 nmol/l, is indicative of a subsequent adverse event.

In one embodiment the invention additionally comprises informing the patient of the results of the diagnostic method described herein.

Embodiments of the present invention relating to determining a level of proADM or fragment(s) thereof in a first and a second sample in critically ill patients

A preferred embodiment of the method of the present invention comprises additionally

- determining a level of proADM or fragment(s) thereof in a first sample isolated from the patient, wherein said first sample is isolated before, at or after the time point of diagnosis and treatment initiation, and
- determining a level of proADM or fragment(s) thereof in a second sample isolated from said patient, wherein said second sample has been isolated after the first sample and after the time point of diagnsosis and treatment inititation, preferably within 30 minutes after isolation of the first sample or 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after isolation of the first sample, and
- determining whether a difference in the level of proADM or fragment(s) thereof in the second sample in comparison to the level of proADM or fragment(s) thereof in the first sample is evident.

The first and the second sample used for deteriming a level of proADM or fragment(s) thereof may be the same of different from the first and the second sample used for determining a level of PCT or fragment(s) thereof.

A preferred embodiment of the method of the present invention comprises additionally

- determining a level of proADM or fragment(s) thereof in a first sample isolated from the patient, wherein said first sample is isolated at or before the time point of diagnosis and treatment initiation (time point 0), and
- determining a level of proADM or fragment(s) thereof in a second sample isolated after diagnosis and treatment initiation, preferably within 30 minutes, or after 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after said diagnosis and treatment initiation, and
- determining whether a difference in the level of proADM or fragment(s) thereof in the second sample in comparison to the level of proADM or fragment(s) thereof in the first sample is evident.

A preferred embodiment of the method of the present invention comprises additionally

- determining a level of proADM or fragment(s) thereof in a first sample isolated from the patient, wherein said first sample is used for diagnosing said patient as being critically ill (time point 0), and
- determining a level of proADM or fragment(s) thereof in a second sample isolated from said patient after diagnosis and treatment initiation, preferably within 30 minutes, or 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after said diagnosis and treatment initiation, and
- determining a difference in the level of proADM or fragment(s) thereof in the second sample in comparison to the level of proADM or fragment(s) thereof in the first sample.

A further preferred embodiment of the method of the present invention comprises additionally

- determining a level of proADM or fragment(s) thereof and optionally PCT or fragment(s) thereof in a first sample isolated from the patient, wherein said first sample is isolated at or before the time point of diagnosis and treatment initiation (time point 0), and
- determining a level of proADM or fragment(s) thereof and optionally PCT or fragment(s) thereof in a second sample isolated from said patient after said diagnosis and treatment initiation, preferably within 30 minutes, or 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after said diagnosis and treatment initiation, and
- determining a difference in the level of proADM or fragment(s) thereof in the second sample in comparison to the level of proADM or fragment(s) thereof in the first sample.

A further preferred embodiment of the method of the present invention comprises additionally

- determining a level of proADM or fragment(s) thereof and optionally PCT or fragment(s) thereof in a first sample isolated from the patient, wherein said first sample is used for diagnosing said patient as being critically ill (time point 0), and
- determining a level of proADM or fragment(s) thereof and optionally PCT or fragment(s) thereof in a second sample isolated from said patient after said diagnosis and treatment initiation, preferably within 30 minutes, or 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 4 days, 7 or 10 days after said diagnosis and treatment initiation, and

determining a difference in the level of proADM or fragment(s) thereof in the second sample in comparison to the level of proADM or fragment(s) thereof in the first sample. In one embodiment of the method described herein, an elevated level of proADM or fragment(s) thereof in the second sample compared to the first sample is indicative of a subsequent adverse event.

In a preferred embodiment of the method of the present invention

- an elevated level of proADM or fragment(s) thereof and an elevated level of PCT or fragment(s) thereof in the second sample compared to the first sample is indicative of a subsequent adverse event, and/or
- an elevated level of proADM or fragment(s) thereof and a lower level of PCT or fragment(s) thereof in the second sample compared to the first sample is indicative of a subsequent adverse event.

In preferred embodiments of the present invention an elevated level of proADM or fragments thereof in the second sample as compared to the first sample relates to an elevated severity level of proADM or fragments thereof. Conversely, in preferred embodiments of the present invention a lower level of proADM or fragments thereof in the second sample as compared to the first sample refer to a lower severity level of proADM or fragments thereof in the second sample as compared to the first sample.

Further embodiments of the present invention in critically ill patients

According to a preferred embodiment of the present invention, the patients are intensive care unit (ICU)-patients, and

- a lower level of PCT or fragment(s) thereof in the second sample compared to the first sample, and a low severity level of proADM or fragment(s) thereof, wherein the low severity level is below 4 nmol/l, preferably below 3 nmol/l, more preferably below 2.7 nmol/l, indicates discharging of said patient from ICU;
- a lower level of PCT or fragment(s) thereof in the second sample compared to the first sample, and a high severity level of proADM or fragment(s) thereof, wherein the high severity level is above 6.5 nmol/l, preferably above 6.95 nmol/l, more preferably above 10.9 nmol/l, indicates modifying the treatment of the patient in the ICU;
- an elevated level of PCT or fragment(s) thereof in the second sample compared to the first sample, and a low severity level proADM or fragment(s) thereof, wherein the low severity level is below 4 nmol/l, preferably below 3 nmol/l, more preferably below 2.7 nmol/l, indicates discharging of said patient from ICU; or
- an elevated level of PCT or fragment(s) thereof in the second sample compared to the first sample, and a high severity level of proADM or fragment(s) thereof, wherein the high severity level is above 6.5 nmol/l, preferably above 6.95 nmol/l, more preferably above 10.9 nmol/l, indicates modifying the treatment of the patient in the ICU.

In a further embodiment, the present invention relates to a kit for carrying out the method of the present invention, wherein the kit comprises

- detection reagents for determining the level proADM or fragment(s) thereof, and optionally additionally for determining the level of PCT, lactate and/or C-reactive protein or fragment(s) thereof, in a sample from a subject, and
- reference data, such as a reference level, corresponding to high and/or low severity levels of proADM, wherein the low severity level is below 4 nmol/l, preferably below 3 nmol/l, more preferably below 2.7 nmol/l, and the high severity level is above 6.5 nmol/l, preferably above 6.95 nmol/l, more preferably above 10.9 nmol/l, and optionally PCT, lactate and/or C-reactive protein levels, wherein said reference data is preferably stored on a computer readable medium and/or employed in the form of computer executable code configured for comparing the determined levels of proADM or fragment(s) thereof, and optionally additionally the determined levels of PCT, lactate and/or C-reactive protein or fragment(s) thereof, to said reference data.

In a further embodiment, the present invention relates to a kit for carrying out the method of the present invention, wherein the kit comprises

- detection reagents for determining the level proADM or fragment(s) thereof, and optionally additionally for determining the level of PCT or fragment(s) thereof, in a sample from a subject, and
- reference data, such as a reference level, corresponding to high and/or low severity levels of proADM, wherein the low severity level is below 4 nmol/l, preferably below 3 nmol/l, more preferably below 2.7 nmol/l, and the high severity level is above 6.5 nmol/l, preferably above 6.95 nmol/l, more preferably above 10.9 nmol/l, and optionally PCT levels, wherein said reference data is preferably stored on a computer readable medium and/or employed in the form of computer executable code

configured for comparing the determined levels of proADM or fragment(s) thereof, and optionally additionally the determined levels of PCT or fragment(s) thereof, to said reference data.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to a method for antibiotic therapy guidance, stratification and/or control in a patient suffering from an infectious disease and receiving treatment with one or more antibiotic agents. As is evident from the data presented herein, the likelihood of presence or absence of an adverse event in the health of a patient is indicated by the level of proADM or fragment(s) thereof, which provides information on potentially changing an antibiotic treatment.

The present invention has the following advantages over the conventional methods: the inventive methods and the kits are fast, objective, easy to use and precise for therapy monitoring of critically ill patients. The methods and kits of the invention relate to markers and clinical scores that are easily measurable in routine methods in hospitals, because the levels of proADM, PCT, lactate, c-reactive protein, SOFA, APACHE II, SAPS II can be determined in routinely obtained blood samples or further biological fluids or samples obtained from a subject.

As used herein, the “patient” or "subject" may be a vertebrate. In the context of the present invention, the term "subject" includes both humans and animals, particularly mammals, and other organisms.

In the context of the present invention, an “adverse event in the health of a patient” relates to events that indicate complications or worsening of the health state of the patient. Such adverse events include, without limitation, death of the patient, death of a patient within 28-90 days after diagnosis and treatment initiation, occurrence of an infection or a new infection, organ failure and deterioration of the patient’s general clinical signs or symptoms, such as hypotension or hypertension, tachycardia or bradycardia. Furthermore, examples of adverse events include situations where a deterioration of clinical symptoms indicates the requirement for therapeutic measures, such as a focus cleaning procedure, transfusion of blood products, infusion of colloids, invasive mechanical ventilation, non-invasive mechanical ventilation, emergency surgery, organ replacement therapy, such as renal or liver replacement, and vasopressor therapy.

The patient described herein who has been diagnosed as being “critically ill” can be diagnosed as an intensive care unit (ICU) patient, a patient who requires constant and/or intense observation of his health state, a patient diagnosed with sepsis, severe sepsis or septic shock, a patient diagnosed with an infectious disease and one or more existing organ failure(s), a pre- or post-surgical patient, a posttraumatic patient, a trauma patient, such as an accident patient, a burn patient, a patient with one or more open lesions. The subject described herein can be at the emergency department or intensive care unit, or in other point of care settings, such as in an emergency transporter, such as an ambulance, or at a general practitioner, who is confronted with a patient with said symptoms. Patients that are suspected to suffer from SIRS are not necessarily considered to be critically ill.

The term “ICU-patient” patient relates, without limitation, a patient who has been admitted to an intensive care unit. An intensive care unit can also be termed an intensive therapy unit or intensive treatment unit (ITU) or critical care unit (CCU), is a special department of a hospital or health care facility that provides intensive treatment medicine. ICU-patients usually suffer from severe and life-threatening illnesses and injuries, which require constant, close monitoring and

support from specialist equipment and medications in order to ensure normal bodily functions. Common conditions that are treated within ICUs include, without limitation, acute or adult respiratory distress syndrome (ARDS), trauma, organ failure and sepsis.

As used herein, "diagnosis" in the context of the present invention relates to the recognition and (early) detection of a clinical condition of a subject linked to an infectious disease. Also the assessment of the severity of the infectious disease may be encompassed by the term "diagnosis".

"Prognosis" relates to the prediction of an outcome or a specific risk for a subject based on an infectious disease. This may also include an estimation of the chance of recovery or the chance of an adverse outcome for said subject.

The methods of the invention may also be used for monitoring. "Monitoring" relates to keeping track of an already diagnosed infectious disease, disorder, complication or risk, e.g. to analyze the progression of the disease or the influence of a particular treatment or therapy on the disease progression of the disease of a critically ill patient or an infectious disease in a patient.

The term "therapy monitoring" or "therapy control" in the context of the present invention refers to the monitoring and/or adjustment of a therapeutic treatment of said subject, for example by obtaining feedback on the efficacy of the therapy.

In the present invention, the terms "risk assessment" and "risk stratification" relate to the grouping of subjects into different risk groups according to their further prognosis. Risk assessment also relates to stratification for applying preventive and/or therapeutic measures. Examples of the risk stratification are the low, intermediate and high risk levels disclosed herein.

As used herein, the term "therapy guidance" refers to application of certain therapies or medical interventions based on the value of one or more biomarkers and/or clinical parameter and/or clinical scores.

It is understood that in the context of the present invention "determining the level of proADM or fragment(s) thereof" or the like refers to any means of determining proADM or a fragment thereof. The fragment can have any length, e.g. at least about 5, 10, 20, 30, 40, 50 or 100 amino acids, so long as the fragment allows the unambiguous determination of the level of proADM or fragment thereof. In particular preferred aspects of the invention, "determining the level of proADM" refers to determining the level of midregional proadrenomedullin (MR-proADM). MR-proADM is a fragment and/or region of proADM.

The peptide adrenomedullin (ADM) was discovered as a hypotensive peptide comprising 52 amino acids, which had been isolated from a human phenochromocytome (Kitamura et al., 1993). Adrenomedullin (ADM) is encoded as a precursor peptide comprising 185 amino acids ("preproadrenomedullin" or "pre proADM"). An exemplary amino acid sequence of ADM is given in SEQ ID NO: 1.

SEQ ID NO:1: amino acid sequence of pre-pro-ADM:

1 MKLVSVALMY LGSLAFLGAD TARLDVASEF RKKWNKWALS RGKRELRMSS

51 SYPTGLADVK AGPAQTLIRP QDMKGASRSP EDSSPDAARI RVKRYRQSMN

101 NFQGLRSFGC RFGTCTVQKL AHQIYQFTDK DKDNVAPRSK ISPQGYGRRR

151 RRSLPEAGPG RTLVSSKPQA HGAPAPPSGS APHFL

ADM comprises the positions 95-146 of the pre-proADM amino acid sequence and is a splice product thereof. "Proadrenomedullin" ("proADM") refers to pre-proADM without the signal sequence (amino acids 1 to 21), i.e. to amino acid residues 22 to 185 of pre-proADM. "Midregional proadrenomedullin" ("MR-proADM") refers to the amino acids 42 to 95 of pre-proADM. An exemplary amino acid sequence of MR-proADM is given in SEQ ID NO: 2.

SEQ ID NO:2: amino acid sequence of MR-pro-ADM (AS 45-92 of pre-pro-ADM):

ELRMSSSYPT GLADVKAGPA QTLIRPQDMK GASRSPEDSS PDAARIRV

It is also envisaged herein that a peptide and fragment thereof of pre-proADM or MR-proADM can be used for the herein described methods. For example, the peptide or the fragment thereof can comprise the amino acids 22-41 of pre-proADM (PAMP peptide) or amino acids 95-146 of pre-proADM (mature adrenomedullin, including the biologically active form, also known as bio-ADM). A C-terminal fragment of proADM (amino acids 153 to 185 of pre proADM) is called adrenotensin. Fragments of the proADM peptides or fragments of the MR-proADM can comprise, for example, at least about 5, 10, 20, 30 or more amino acids. Accordingly, the fragment of proADM may, for example, be selected from the group consisting of MR-proADM, PAMP, adrenotensin and mature adrenomedullin, preferably herein the fragment is MR-proADM.

The determination of these various forms of ADM or proADM and fragments thereof also encompass measuring and/or detecting specific sub-regions of these molecules, for example by employing antibodies or other affinity reagents directed against a particular portion of the molecules, or by determining the presence and/or quantity of the molecules by measuring a portion of the protein using mass spectrometry.

Any one or more of the "ADM peptides or fragments" described herein may be employed in the present invention. Accordingly, the methods and kits of the present invention can also comprise determining at least one further biomarker, marker, clinical score and/or parameter in addition to proADM.

As used herein, a parameter is a characteristic, feature, or measurable factor that can help in defining a particular system. A parameter is an important element for health- and physiology-related assessments, such as a disease/disorder/clinical condition risk, preferably organ dysfunction(s). Furthermore, a parameter is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. An exemplary parameter can be selected from the group consisting of Acute Physiology and Chronic Health Evaluation II (APACHE II), the simplified acute physiology score (SAPSII score), quick sequential organ failure assessment score (qSOFA), sequential organ failure assessment score (SOFA score), body mass index, weight, age, sex, IGS II, liquid intake, white blood cell count, sodium, potassium, temperature, blood pressure, dopamine, bilirubin, respiratory rate, partial pressure of oxygen, World Federation of Neurosurgical Societies (WFNS) grading, and Glasgow Coma Scale (GCS).

As used herein, terms such as "marker", "surrogate", "prognostic marker", "factor" or "biomarker" or "biological marker" are used interchangeably and relate to measurable and quantifiable biological markers (e.g., specific protein or enzyme concentration or a fragment thereof, specific hormone concentration or a fragment thereof, or presence of biological substances or a fragment thereof) which serve as indices for health- and physiology-related assessments, such as a

disease/disorder/clinical condition risk, preferably an adverse event. A marker or biomarker is defined as a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers may be measured in a sample (as a blood, plasma, urine, or tissue test).

The at least one further marker and/or parameter of said subject can be selected from the group consisting of a level of lactate in said sample, a level of procalcitonin (PCT) in said sample, the sequential organ failure assessment score (SOFA score) of said subject, the simplified acute physiology score (SAPSII) of said subject, the Acute Physiology and Chronic Health Evaluation II (APACHE II) score of said subject and a level of the soluble fms-like tyrosine kinase-1 (sFlt-1), Histone H2A, Histone H2B, Histone H3, Histone H4, calcitonin, Endothelin-1 (ET-1), Arginine Vasopressin (AVP), Atrial Natriuretic Peptide (ANP), Neutrophil Gelatinase-Associated Lipocalin (NGAL), Troponin, Brain Natriuretic Peptide (BNP), C-Reactive Protein (CRP), Pancreatic Stone Protein (PSP), Triggering Receptor Expressed on Myeloid Cells 1 (TREM1), Interleukin-6 (IL-6), Interleukin-1, Interleukin-24 (IL-24), Interleukin-22 (IL-22), Interleukin (IL-20) other ILs, Presepsin (sCD14-ST), Lipopolysaccharide Binding Protein (LBP), Alpha-1-Antitrypsin, Matrix Metalloproteinase 2 (MMP2), Metalloproteinase 2 (MMP8), Matrix Metalloproteinase 9 (MMP9), Matrix Metalloproteinase 7 (MMP7, Placental growth factor (PlGF), Chromogranin A, S100A protein, S100B protein and Tumor Necrosis Factor α (TNFα), Neopterin, Alpha-1-Antitrypsin, pro-arginine vasopressin (AVP, proAVP or Copeptin), procalcitonin, atrial natriuretic peptide (ANP, pro-ANP), Endothelin-1, CCL1/TCA3, CCL11, CCL12/MCP-5, CCL13/MCP-4, CCL14, CCL15, CCL16, CCL17/TARC, CCL18, CCL19, CCL2/MCP-1, CCL20, CCL21, CCL22/MDC, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL3L3, CCL4, CCL4L1/LAG-1, CCL5, CCL6, CCL7, CCL8, CCL9, CX3CL1, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, CXCL2/MIP-2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7/Ppbp, CXCL9, IL8/CXCL8, XCL1, XCL2, FAM19A1, FAM19A2, FAM19A3, FAM19A4, FAM19A5, CLCF1, CNTF, IL11, IL31, IL6, Leptin, LIF, OSM, IFNA1, IFNA10, IFNA13, IFNA14, IFNA2, IFNA4, IFNA7, IFNB1, IFNE, IFNG, IFNZ, IFNA8, IFNA5/IFNaG, IFNω/IFNW1, BAFF, 4-1BBL, TNFSF8, CD40LG, CD70, CD95L/CD178, EDA-A1, TNFSF14, LTA/TNFB, LTB, TNFa, TNFSF10, TNFSF11, TNFSF12, TNFSF13, TNFSF15, TNFSF4, IL18, IL18BP, IL1A, IL1B, IL1F10, IL1F3/IL1RA, IL1F5, IL1F6, IL1F7, IL1F8, IL1RL2, IL1F9, IL33 or a fragment thereof.

As used herein, "procalcitonin" or "PCT" relates to a peptide spanning amino acid residues 1-116, 2-116, 3-116, or fragments thereof, of the procalcitonin peptide. PCT is a peptide precursor of the hormone calcitonin. Thus the length of procalcitonin fragments is at least 12 amino acids, preferably more than 50 amino acids, more preferably more than 110 amino acids. PCT may comprise post-translational modifications such as glycosylation, liposidation or derivatisation. Procalcitonin is a precursor of calcitonin and katacalcin. Thus, under normal conditions the PCT levels in the circulation are very low (< about 0.05 ng/ml).

The level of PCT in the sample of the subject can be determined by immunoassays as described herein. As used herein, the level of ribonucleic acid or deoxyribonucleic acids encoding "procalcitonin" or "PCT" can also be determined. Methods for the determination of PCT are known to a skilled person, for example by using products obtained from Thermo Fisher Scientific / B·R·A·H·M·S GmbH.

Lactate, or lactic acid, is an organic compound with the formula $CH_3CH(OH)COOH$, which occurs in bodily fluids including blood. Blood tests for lactate are performed to determine the status of the acid base homeostasis in the body. Lactic acid is a product of cell metabolism that can

accumulate when cells lack sufficient oxygen (hypoxia) and must turn to a less efficient means of energy production, or when a condition causes excess production or impaired clearance of lactate. Lactic acidosis can be caused by an inadequate amount of oxygen in cells and tissues (hypoxia), for example if someone has a condition that may lead to a decreased amount of oxygen delivered to cells and tissues, such as shock, septic shock or congestive heart failure, the lactate test can be used to help detect and evaluate the severity of hypoxia and lactic acidosis.

C-reactive protein (CRP) is a pentameric protein, which can be found in bodily fluids such as blood plasma. CRP levels can rise in response to inflammation. Measuring and charting CRP values can prove useful in determining disease progress or the effectiveness of treatments.

As used herein, the "sequential organ failure assessment score" or "SOFA score" is one score used to track a patient's status during the stay in an intensive care unit (ICU). The SOFA score is a scoring system to determine the extent of a person's organ function or rate of failure. The score is based on six different scores, one each for the respiratory, cardiovascular, hepatic, coagulation, renal and neurological systems. Both the mean and highest SOFA scores being predictors of outcome. An increase in SOFA score during the first 24 to 48 hours in the ICU predicts a mortality rate of at least 50% up to 95%. Scores less than 9 give predictive mortality at 33% while above 14 can be close to or above 95%.

As used herein, the quick SOFA score (qSOFA) is a scoring system that indicates a patient´s organ dysfunction or mortality risk. The score is based on three criteria: 1) an alteration in mental status, 2) a decrease in systolic blood pressure of less than 100 mm Hg, 3) a respiration rate greater than 22 breaths per minute.Patients with two or more of these conditions are at greater risk of having an organ dysfunction or to die.

As used herein, "APACHE II" or "Acute Physiology and Chronic Health Evaluation II" is a severity-of-disease classification scoring system (Knaus et al., 1985). It can be applied within 24 hours of admission of a patient to an intensive care unit (ICU) and may be determined based on 12 different physiologic parameters: AaDO2 or PaO2 (depending on FiO2), temperature (rectal), mean arterial pressure, pH arterial, heart rate, respiratory rate, sodium (serum), potassium (serum), creatinine, hematocrit, white blood cell count and Glasgow Coma Scale.

As used herein, "SAPS II" or "Simplified Acute Physiology Score II" relates to a system for classifying the severity of a disease or disorder (see Le Gall JR et al., A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA. 1993;270(24):2957-63.). The SAPS II score is made of 12 physiological variables and 3 disease-related variables. The point score is calculated from 12 routine physiological measurements, information about previous health status and some information obtained at admission to the ICU. The SAPS II score can be determined at any time, preferably, at day 2. The "worst" measurement is defined as the measure that correlates to the highest number of points. The SAPS II score ranges from 0 to 163 points. The classification system includes the followings parameters: Age, Heart Rate, Systolic Blood Pressure, Temperature, Glasgow Coma Scale, Mechanical Ventilation or CPAP, PaO2, FiO2, Urine Output, Blood Urea Nitrogen, Sodium, Potassium, Bicarbonate, Bilirubin, White Blood Cell, Chronic diseases and Type of admission. There is a sigmoidal relationship between mortality and the total SAPS II score. The mortality of a subject is 10% at a SAPSII score of 29 points, the mortality is 25% at a SAPSII score of 40 points, the mortality is 50% at a SAPSII score of 52 points, the mortality is 75% at a SAPSII score of 64 points, the mortality is 90% at a SAPSII score of 77 points (Le Gall loc. cit.).

As used herein, the term "sample" is a biological sample that is obtained or isolated from the patient or subject. "Sample" as used herein may, e.g., refer to a sample of bodily fluid or tissue obtained for the purpose of diagnosis, prognosis, or evaluation of a subject of interest, such as a patient. Preferably herein, the sample is a sample of a bodily fluid, such as blood, serum, plasma, cerebrospinal fluid, urine, saliva, sputum, pleural effusions, cells, a cellular extract, a tissue sample, a tissue biopsy, a stool sample and the like. Particularly, the sample is blood, blood plasma, blood serum, or urine.

Embodiments of the present invention refer to the isolation of a first sample and the isolation of a second sample. In the context of the method of the present invention, the terms "first sample" and "second sample" relate to the relative determination of the order of isolation of the samples employed in the method of the present invention. When the terms first sample and second sample are used in specifiying the present method, these samples are not to be considered as absolute determinations of the number of samples taken. Therefore, additional samples may be isolated from the patient before, during or after isolation of the first and/or the second sample, or between the first or second samples, wherein these additional samples may or may not be used in the method of the present invention. The first sample may therefore be considered as any previously obtained sample. The second sample may be considered as any further or subsequent sample.

"Plasma" in the context of the present invention is the virtually cell-free supernatant of blood containing anticoagulant obtained after centrifugation. Exemplary anticoagulants include calcium ion binding compounds such as EDTA or citrate and thrombin inhibitors such as heparinates or hirudin. Cell-free plasma can be obtained by centrifugation of the anticoagulated blood (e.g. citrated, EDTA or heparinized blood), for example for at least 15 minutes at 2000 to 3000 g.

"Serum" in the context of the present invention is the liquid fraction of whole blood that is collected after the blood is allowed to clot. When coagulated blood (clotted blood) is centrifuged serum can be obtained as supernatant.

As used herein, "urine" is a liquid product of the body secreted by the kidneys through a process called urination (or micturition) and excreted through the urethra.

In preferred embodiments of the present invention the patient has been diagnosed as suffering from sepsis. More particularly, the patient may have been diagnosed as suffering from severe sepsis and/or septic shock.

"Sepsis" in the context of the invention refers to a systemic response to infection. Alternatively, sepsis may be seen as the combination of SIRS with a confirmed infectious process or an infection. Sepsis may be characterized as clinical syndrome defined by the presence of both infection and a systemic inflammatory response (Levy MM et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med. 2003 Apr;31(4):1250-6). The term "sepsis" used herein includes, but is not limited to, sepsis, severe sepsis, septic shock.

The term "sepsis" used herein includes, but is not limited to, sepsis, severe sepsis, septic shock. Severe sepsis in refers to sepsis associated with organ dysfunction, hypoperfusion abnormality, or sepsis-induced hypotension. Hypoperfusion abnormalities include lactic acidosis, oliguria and acute alteration of mental status. Sepsis-induced hypotension is defined by the presence of a systolic blood pressure of less than about 90 mm Hg or its reduction by about 40 mm Hg or more

from baseline in the absence of other causes for hypotension (e.g. cardiogenic shock). Septic shock is defined as severe sepsis with sepsis-induced hypotension persisting despite adequate fluid resuscitation, along with the presence of hypoperfusion abnormalities or organ dysfunction (Bone et al., CHEST 101(6): 1644-55, 1992).

The term sepsis may alternatively be defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. For clinical operationalization, organ dysfunction can preferably be represented by an increase in the Sequential Organ Failure Assessment (SOFA) score of 2 points or more, which is associated with an in-hospital mortality greater than 10%. Septic shock may be defined as a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone. Patients with septic shock can be clinically identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and serum lactate level greater than 2 mmol/L (>18 mg/dL) in the absence of hypovolemia.

The term "sepsis" used herein relates to all possible stages in the development of sepsis.

The term "sepsis" also includes severe sepsis or septic shock based on the SEPSIS-2 definition (Bone et al., 2009). The term "sepsis" also includes subjects falling within the SEPSIS-3 definition (Singer et al., 2016). The term "sepsis" used herein relates to all possible stages in the development of sepsis.

As used herein, "infection" within the scope of the invention means a pathological process caused by the invasion of normally sterile tissue or fluid by pathogenic or potentially pathogenic agents/ pathogens, organisms and/or microorganisms, and relates preferably to infection(s) by bacteria, viruses, fungi, and/or parasites. Accordingly, the infection can be a bacterial infection, viral infection, and/or fungal infection. The infection can be a local or systemic infection. For the purposes of the invention, a viral infection may be considered as infection by a microorganism.

Further, the subject suffering from an infection can suffer from more than one source(s) of infection simultaneously. For example, the subject suffering from an infection can suffer from a bacterial infection and viral infection; from a viral infection and fungal infection; from a bacterial and fungal infection, and from a bacterial infection, fungal infection and viral infection, or suffer from a mixed infection comprising one or more of the infections listed herein, including potentially a superinfection, for example one or more bacterial infections in addition to one or more viral infections and/or one or more fungal infections.

As used herein „infectious disease" comprises all diseases or disorders that are associated with bacterial and/or viral and/or fungal infections.

In one embodiment the infection to be detected or to be tested for may be selected from species of Bordetella, such as Bordetella pertussis, Borrelia, such as Borrelia burgdorferi, Brucella, such as Brucella abortus, Brucella canis, Brucella melitensis or Brucella suis, Campylobacter, such as Campylobacter jejuni, Chlamydia and Chlamydophila, such as Chlamydia pneumonia, Chlamydia trachomatis, Chlamydophila psittaci, Clostridium, such as Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium tetani, Corynebacterium, such as Corynebacterium diphtheria, Enterococcus, such as Enterococcus faecalis, Enterococcus faecium, Escherichia, such as Escherichia coli, Francisella, such as Francisella tularensis, Haemophilus, such as Haemophilus influenza, Helicobacter, such as Helicobacter pylori, Legionella, such as Legionella pneumophila, Leptospira, such as Leptospira interrogans, Listeria, such as Listeria

monocytogenes, Mycobacterium, such as Mycobacterium leprae, Mycobacterium tuberculosis, Mycobacterium ulcerans, Mycoplasma, such as Mycoplasma pneumonia, Neisseria, such as Neisseria gonorrhoeae, Neisseria meningitides, Pseudomonas, such as Pseudomonas aeruginosa, Rickettsia, such as Rickettsia rickettsia, Salmonella, such as Salmonella typhi, Salmonella typhimurium, Shigella, such as Shigella sonnei, Staphylococcus, such as Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus, such as Streptococcus agalactiae, Streptococcus pneumonia, Streptococcus pyogenes, Treponema, such as Treponema pallidum, Vibrio, such as Vibrio cholera, Yersinia, such as Yersinia pestis, Yersinia enterocolitica or Yersinia pseudotuberculosis.

Pathogenic fungi are fungi that cause disease in humans or other organisms. Candida species are important human pathogens that are best known for causing opportunist infections in immunocompromised hosts (e.g. transplant patients, AIDS sufferers, cancer patients). Infections are difficult to treat and can be very serious: 30-40% of systemic infections result in death. Aspergillosis is another potential fungal pathogen. Aspergillus can cause disease in three major ways: through the production of mycotoxins; through induction of allergenic responses; and through localized or systemic infections. With the latter two categories, the immune status of the host is pivotal. The most common pathogenic species are Aspergillus fumigatus and Aspergillus flavus. Aspergillus flavus produces aflatoxin which is both a toxin and a carcinogen and which can potentially contaminate foods. Aspergillus fumigatus and Aspergillus clavatus can cause disease. Cryptococcus neoformans can cause disease in humans. Cryptococcus neoformans is the major human and animal pathogen. Cryptococcus laurentii and Cryptococcus albidus have been known to occasionally cause moderate-to-severe disease in human patients with compromised immunity. Cryptococcus gattii is endemic to tropical parts of the continent of Africa and Australia and can cause disease. Histoplasma capsulatum can cause histoplasmosis in humans, dogs and cats. Pneumocystis jirovecii (or Pneumocystis carinii) can cause a form of pneumonia in people with weakened immune systems, such as premature children, the elderly, and AIDS patients. Stachybotrys chartarum or "black mould" can cause respiratory damage and severe headaches.

In one embodiment the infection to be detected or to be tested for may be selected from Acinetobacter baumannii, Klebsiella pneumoniae, Acinetobacter lwoffii,Listeria monocytogenes, Aeromonas caviae, Morganella morganii,Aeromonas hydrophila, Neisseria gonorrhoeae, Aspergillus flavus,Neisseria meningitidis, Aspergillus nidulans, Pasteurella multocida, Aspergillus niger, Pasteurella pneumotropica, Aspergillus terreus,Propionibacterium acnes, Bacillus anthracis, Proteus mirabillis, Bacillus cereus, Providencia rettgeri, Bacillus subtilis, Pseudomonas aeruginosa, Bacteroides fragilis, Salmonella choleraesuis, Brucella melitensis, Serratia liquefaciens, Burkholderia cepacia, Serratia marcescens, Candida albicans, Staphylococcus aureus, Candida dubliniensis, Staphylococcus epidermidis, Candida glabrata, Staphylococcus haemolyticus, Candida krusei, Staphylococcus hominis, Candida parapsilosis, Staphylococcus saccharolyticus, Candida tropicalis, Staphylococcus warn-eri,Capnocytophaga canimorsus, Stenotrophomonas maltophilia, Citrobacter braakii, Streptococcus agalactiae, Citrobacter freundii, Streptococcus anginosus, Clostridium perfringens, Streptococcus bovis,Corynebacterium jeikeium, Streptococcus constellatus, Enterobacter aerogenes, Streptococcus dysgalactiae, Enterobacter cloacae,Streptococcus mutans, Enterobacter sakazakii, Streptococcus pneumoniae, Enterococcus faecalis, Streptococcus pyogenes,Enterococcus faecium, Streptococcus salivarius, Escherichia coli,Streptococcus sanguinis, Shigella sp., Streptococcus suis, Gemella haemolysans, Vibrio vulnificus, Gemella morbillorum, Yersinia enterocolitica,

Haemophilus influenzae, Yersinia pestis, Kingella kingae,Yersinia pseudotuberculosis and; Klebsiella oxytoca.

According to the present invention, critically ill patients, such as septic patients may need a very strict control, with respect of vital functions and/or monitoring of organ protection and may be under medical treatment.

In the context of the present invention, the term "medical treatment" or "treatment" comprises various treatments and therapeutic strategies, which comprise, without limitation, anti-inflammatory strategies, administration of ADM-antagonists such as therapeutic antibodies, si-RNA or DNA, the extracorporal blood purification or the removal of harmful substances via apheresis, dialyses, adsorbers to prevent the cytokine storm, removal of inflammatory mediators, plasma apheresis, administration of vitamines such as vitamin C, ventilation like mechanical ventilation and non-mechanical ventilation, to provide the body with sufficient oxygen, for example, focus cleaning procedures, transfusion of blood products, infusion of colloids, renal or liver replacement, antibiotic treatment, invasive mechanical ventilation, non-invasive mechanical ventilation, renal replacement therapy, vasopressor use, fluid therapy, apheresis and measures for organ protection.

Further treatments of the present invention comprise the administration of cells or cell products like stem cells, blood or plasma, and the stabilization of the patients circulation and the protection of endothelial glycocalyx, for example via optimal fluid management strategies, for example to reach normovolemia and prevent or treat hypervolemia or hypovolemia. Moreover, vasopressors or e.g. catecholamine as well as albumin or heparanase inhibition via unfractionated heparin or N-desulfated re-N-acetylated heparin are useful treatments to support the circulation and endothelial layer.

Additionally, medical treatments of the present invention comprise, without limitation, stabilization of the blood clotting, iNOS inhibitors, anti-inflammatory agents like hydrocortisone, sedatives and analgetics as well as insuline.

"Renal replacement therapy" (RRT) relates to a therapy that is employed to replace the normal blood-filtering function of the kidneys. Renal replacement therapy may refer to dialysis (e.g. hemodialysis or peritoneal dialysis), hemofiltration, and hemodiafiltration. Such techniques are various ways of diverting the blood into a machine, cleaning it, and then returning it to the body. Renal replacement therapy may also refer to kidney transplantation, which is the ultimate form of replacement in that the old kidney is replaced by a donor kidney. The hemodialysis, hemofiltration, and hemodiafiltration may be continuous or intermittent and can use an arteriovenous route (in which blood leaves from an artery and returns via a vein) or a venovenous route (in which blood leaves from a vein and returns via a vein). This results in various types of RRT. For example, the renal replacement therapy may be selected from the group of, but not limited to continuous renal replacement therapy (CRRT), continuous hemodialysis (CHD), continuous arteriovenous hemodialysis (CAVHD), continuous venovenous hemodialysis (CVVHD), continuous hemofiltration (CHF), continuous arteriovenous hemofiltration (CAVH or CAVHF), continuous venovenous hemofiltration (CVVH or CVVHF), continuous hemodiafiltration (CHDF), continuous arteriovenous hemodiafiltration (CAVHDF), continuous venovenous hemodiafiltration (CVVHDF), intermittent renal replacement therapy (IRRT), intermittent hemodialysis (IHD), intermittent venovenous hemodialysis (IVVHD), intermittent hemofiltration

(IHF), intermittent venovenous hemofiltration (IVVH or IVVHF), intermittent hemodiafiltration (IHDF) and intermittent venovenous hemodiafiltration (IVVHDF).

Artificial and mechanical ventilation are effective approaches to enhance proper gas exchange and ventilation and aim to save life during severe hypoxemia. Artificial ventilation relates to assisting or stimulating respiration of the subject. Artificial ventilation may be selected from the group consisting of mechanical ventilation, manual ventilation, extracorporeal membrane oxygenation (ECMO) and noninvasive ventilation (NIV). Mechanical ventilation relates to a method to mechanically assist or replace spontaneous breathing. This may involve a machine called a ventilator. Mechanical ventilation may be High-Frequency Oscillatory Ventilation or Partial Liquid Ventilation.

"Fluid management" refers to the monitoring and controlling of the fluid status of a subject and the administration of fluids to stabilize the circulation or organ vitality, by e.g. oral, enteral or intravenous fluid administration. It comprises the stabilization of the fluid and electrolyte balance or the prevention or correction of hyer- or hypovolemia as well as the supply of blood products.

Surgical emergencies/ Emergency surgery are needed if a subject has a medical emergency and an immediate surgical intervention may be required to preserve survival or health status. The subject in need of emergency surgery may be selected from the group consisting of subjects suffering from acute trauma, an active uncontrolled infection, organ transplantation, organ-preventive or organ-stabilizing surgery or cancer.

Cleaning Procedures are hygienic methods to prevent subjects from infections, especially nosocomial infections, comprising desinfection of all organic and anorganic surfaces that could get in contact with a patient, such as for example, skin, objects in the patient's room, medical devices, diagnostic devices, or room air. Cleaining procedures include the use of protective clothes and units, such as mouthguards, gowns, gloves or hygiene lock, and actions like restricted patient visits. Furthermore, cleaning procedures comprise the cleaning of the patient itself and the clothes or the patient.

In the case of critical illness, such as sepsis or severe infections it is very important to have an early diagnosis as well a prognosis and risk assessment for the outcome of a patient to find the optimal therapy and management. The therapeutic approaches need to be very individual and vary from case to case. A therapeutic monitoring is needed for a best practice therapy and is influenced by the timing of treatment, the use of combined therapies and the optimization of drug dosing. A wrong or omitted therapy or management will increase the mortality rate hourly.

A medical treatment of the present invention may be an antibiotic treatment, wherein one or more "antibiotics" or "antibiotic agents" may be administered if an infection has been diagnosed or symptoms of an infectious disease have been determined.

Antibiotics or antibiotic agents according to the present invention also encompass potentially the anti-fungal or anti-viral compounds used to treat a diagnosed infection or sepsis. The antibiotic agents commonly applied in the treatment of any given infection, as separated into the classes of pathogen are:

Gram positive coverage: Penicillins, (ampicillin, amoxicillin), penicillinase resistant, (Dicloxacillin, Oxacillin), Cephalosporins (1st and 2nd generation), Macrolides (Erythromycin, Clarithromycin, Azithromycin), Quinolones (gatifloxacin, moxifloxacin, levofloxacin), Vancomycin, Sulfonamide/trimethoprim, Clindamycin, Tetracyclines, Chloramphenicol, Linezolid, Synercid.

Gram negative coverage: Broad spectrum penicillins (Ticarcillin, clavulanate, piperacillin, tazobactam), Cephalosporins (2nd, 3rd, and 4th generation), Aminoglycosides, Macrolides, Azithromycin, Quinolones (Ciprofloxacin), Monobactams (Azetreonam), Sulfonamide/trimethoprim, Carbapenems (Imipenem), Chloramphenicol.

Pseudomonas coverage: Ciprofloxacin, Aminoglycosides, Some 3rd generation cephalosporins, 4th generation cephalosporins, Broad spectrum penicillins, Carbapenems.

Fungal treatments: Allyamines, Amphotericin B, Fluconazole and other Azoles, itraconazole, voriconazole, posaconazole, ravuconazole, echinocandins, Flucytosine, sordarins, chitin synthetase inhibitors, topoisomerase inhibitors, lipopeptides, pradimycins, Liposomal nystatin, Voriconazole, Echinocanidins, Imidazole, Triazole, Thiazole, Polyene.

Anti-viral treatments: Abacavir, Acyclovir (Aciclovir) , activated caspase oligomerizer, Adefovir , Amantadine , Amprenavir(Agenerase) , Ampligen, Arbidol, Atazanavir, Atripla , Balavir, Cidofovir, Combivir , Dolutegravir, Darunavir, Delavirdine, Didanosine, Double-stranded RNA, Docosanol, Edoxudine, Efavirenz, Emtricitabine, Enfuvirtide, Entecavir, Ecoliever, Famciclovir, Fixed dose combination (antiretroviral), Fomivirsen, Fosamprenavir, Foscarnet, Fosfonet, Fusion inhibitor, Ganciclovir, Ibacitabine, Imunovir, Idoxuridine, Imiquimod, Indinavir, Inosine, Integrase inhibitor, Interferon type III, Interferon type II, Interferon type I, Interferon, Lamivudine, Lopinavir, Loviride, Maraviroc, Moroxydine, Methisazone, Morpholinos, Nelfinavir, Nevirapine, Nexavir, Nitazoxanide, Nucleoside analogues, Novir, Oseltamivir (Tamiflu), Peginterferon alfa-2a, Penciclovir, Peramivir, Pleconaril, Podophyllotoxin, Protease inhibitor (pharmacology), Raltegravir, Reverse transcriptase inhibitor, Ribavirin, Ribozymes, Rifampicin, Rimantadine, Ritonavir, RNase H, protease inhibitors, Pyramidine, Saquinavir, Sofosbuvir, Stavudine, Synergistic enhancer (antiretroviral), Telaprevir, Tenofovir, Tenofovir disoproxil, Tipranavir, Trifluridine, Trizivir, Tromantadine, Truvada, Valaciclovir (Valtrex), Valganciclovir, Vicriviroc, Vidarabine, Viramidine, Zalcitabine, Zanamivir (Relenza), Zidovudine.

Furthermore, antibiotic agents comprise bacteriophages for treatment of bacterial infections, synthetic antimicrobial peptides or iron-antagonists/iron chelator can be used. Also, therapeutic antibodies or antagonist against pathogenic structures like anti-VAP-antibodies, anti-resistant clone vaccination, administration of immune cells, such as in vitro primed or modulated T-effector cells, are antibiotic agents that represent treatment options for critically ill patients, such as sepsis patients. Further antibiotic agents/treatments or therapeutic strategies against infection or for the prevention of new infections include the use of antiseptics, decontamination products, anti-virulence agents like liposomes, sanitation, wound care, surgery.

It is also possible to combine several of the aforementioned antibiotic agents or treatments strategies.

According to the present invention proADM and optionally PCT and/or other markers or clinical scores are employed as markers for therapy monitoring, comprising prognosis, prognosis, risk assessment and risk stratification of a subsequent adverse event in the health of a patient which has been diagnosed as being critically ill.

A skilled person is capable of obtaining or developing means for the identification, measurement, determination and/or quantification of any one of the above proADM molecules, or fragments or variants thereof, as well as the other markers of the present invention according to standard molecular biological practice.

The level of proADM or fragments thereof as well as the levels of other markers of the present invention can be determined by any assay that reliably determines the concentration of the marker. Particularly, mass spectrometry (MS) and/or immunoassays can be employed as exemplified in the appended examples. As used herein, an immunoassay is a biochemical test that measures the presence or concentration of a macromolecule/polypeptide in a solution through the use of an antibody or antibody binding fragment or immunoglobulin.

Methods of determining proADM or other the markers such as PCT used in the context of the present invention are intended in the present invention. By way of example, a method may be employed selected from the group consisting of mass spectrometry (MS), luminescence immunoassay (LIA), radioimmunoassay (RIA), chemiluminescence- and fluorescence-immunoassays, enzyme immunoassay (EIA), Enzyme-linked immunoassays (ELISA), luminescence-based bead arrays, magnetic beads based arrays, protein microarray assays, rapid test formats such as for instance immunochromatographic strip tests, rare cryptate assay, and automated systems/analyzers.

Determination of proADM and optionally other markers based on antibody recognition is a preferred embodiment of the invention. As used herein, the term, "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen binding site that specifically binds (immuno reacts with) an antigen. According to the invention, the antibodies may be monoclonal as well as polyclonal antibodies. Particularly, antibodies that are specifically binding to at lest proADM or fragments thereof are used.

An antibody is considered to be specific, if its affinity towards the molecule of interest, e.g. ADM, or the fragment thereof is at least 50-fold higher, preferably 100-fold higher, most preferably at least 1000-fold higher than towards other molecules comprised in a sample containing the molecule of interest. It is well known in the art how to develop and to select antibodies with a given specificity. In the context of the invention, monoclonal antibodies are preferred. The antibody or the antibody binding fragment binds specifically to the herein defined markers or fragments thereof. In particular, the antibody or the antibody binding fragment binds to the herein defined peptides of ADM. Thus, the herein defined peptides can also be epitopes to which the antibodies specifically bind. Further, an antibody or an antibody binding fragment is used in the methods and kits of the invention that binds specifically to ADM or proADM, particularly to MR-proADM.

Further, an antibody or an antibody binding fragment is used in the methods and kits of the invention that binds specifically to proADM or fragments thereof and optionally to other markers of the present inventions such as PCT. Exemplary immunoassays can be luminescence immunoassay (LIA), radioimmunoassay (RIA), chemiluminescence- and fluorescence-immunoassays, enzyme immunoassay (EIA), Enzyme-linked immunoassays (ELISA), luminescence-based bead arrays, magnetic beads based arrays, protein microarray assays, rapid test formats, rare cryptate assay. Further, assays suitable for point-of-care testing and rapid test formats such as for instance immune-chromatographic strip tests can be employed. Automated immunoassays are also intended, such as the KRYPTOR assay.

Alternatively, instead of antibodies, other capture molecules or molecular scaffolds that specifically and/or selectively recognize proADM may be encompassed by the scope of the present invention. Herein, the term “capture molecules” or “molecular scaffolds” comprises

molecules which may be used to bind target molecules or molecules of interest, i.e. analytes (e.g. proADM, proADM, MR-proADM, and PCT), from a sample. Capture molecules must thus be shaped adequately, both spatially and in terms of surface features, such as surface charge, hydrophobicity, hydrophilicity, presence or absence of lewis donors and/or acceptors, to specifically bind the target molecules or molecules of interest. Hereby, the binding may, for instance, be mediated by ionic, van-der-Waals, pi-pi, sigma-pi, hydrophobic or hydrogen bond interactions or a combination of two or more of the aforementioned interactions or covalent interactions between the capture molecules or molecular scaffold and the target molecules or molecules of interest. In the context of the present invention, capture molecules or molecular scaffolds may for instance be selected from the group consisting of a nucleic acid molecule, a carbohydrate molecule, a PNA molecule, a protein, a peptide and a glycoprotein. Capture molecules or molecular scaffolds include, for example, aptamers, DARpins (Designed Ankyrin Repeat Proteins). Affimers and the like are included.

In certain aspects of the invention, the method is an immunoassay comprising the steps of:

a) contacting the sample with

i. a first antibody or an antigen-binding fragment or derivative thereof specific for a first epitope of said proADM, and
ii. a second antibody or an antigen-binding fragment or derivative thereof specific for a second epitope of said proADM; and

b) detecting the binding of the two antibodies or antigen-binding fragments or derivates thereof to said proADM.

Preferably, one of the antibodies can be labeled and the other antibody can be bound to a solid phase or can be bound selectively to a solid phase. In a particularly preferred aspect of the assay, one of the antibodies is labeled while the other is either bound to a solid phase or can be bound selectively to a solid phase. The first antibody and the second antibody can be present dispersed in a liquid reaction mixture, and wherein a first labeling component which is part of a labeling system based on fluorescence or chemiluminescence extinction or amplification is bound to the first antibody, and a second labeling component of said labeling system is bound to the second antibody so that, after binding of both antibodies to said proADM or fragments thereof to be detected, a measurable signal which permits detection of the resulting sandwich complexes in the measuring solution is generated. The labeling system can comprise a rare earth cryptate or chelate in combination with a fluorescent or chemiluminescent dye, in particular of the cyanine type.

In a preferred embodiment, the method is executed as heterogeneous sandwich immunoassay, wherein one of the antibodies is immobilized on an arbitrarily chosen solid phase, for example, the walls of coated test tubes (e.g. polystyrol test tubes; coated tubes; CT) or microtiter plates, for example composed of polystyrol, or to particles, such as for instance magnetic particles, whereby the other antibody has a group resembling a detectable label or enabling for selective attachment to a label, and which serves the detection of the formed sandwich structures. A temporarily delayed or subsequent immobilization using suitable solid phases is also possible.

The method according to the present invention can furthermore be embodied as a homogeneous method, wherein the sandwich complexes formed by the antibody/antibodies and the marker, proADM or a fragment thereof, which is to be detected remains suspended in the liquid phase. In

this case it is preferred, that when two antibodies are used, both antibodies are labeled with parts of a detection system, which leads to generation of a signal or triggering of a signal if both antibodies are integrated into a single sandwich. Such techniques are to be embodied in particular as fluorescence enhancing or fluorescence quenching detection methods. A particularly preferred aspect relates to the use of detection reagents which are to be used pair-wise, such as for example the ones which are described in US4882733, EP0180492 or EP0539477 and the prior art cited therein. In this way, measurements in which only reaction products comprising both labeling components in a single immune-complex directly in the reaction mixture are detected, become possible. For example, such technologies are offered under the brand names TRACE® (Time Resolved Amplified Cryptate Emission) or KRYPTOR®, implementing the teachings of the above-cited applications. Therefore, in particular preferred aspects, a diagnostic device is used to carry out the herein provided method. For example, the level of proADM or fragments thereof and/or the level of any further marker of the herein provided method, such as PCT, is determined. In particular preferred aspects, the diagnostic device is KRYPTOR®.

The level of the marker of the present invention, e.g. the proADM or fragments thereof, PCT or fragements thereof, or other markers, can also be determined by a mass spectrometric (MS) based methods. Such a method may comprise detecting the presence, amount or concentration of one or more modified or unmodified fragment peptides of e.g. proADM or the PCT in said biological sample or a protein digest (e.g. tryptic digest) from said sample, and optionally separating the sample with chromatographic methods, and subjecting the prepared and optionally separated sample to MS analysis. For example, selected reaction monitoring (SRM), multiple reaction monitoring (MRM) or parallel reaction monitoring (PRM) mass spectrometry may be used in the MS analysis, particularly to determine the amounts of proADM or fragments thereof.

Herein, the term "mass spectrometry" or "MS" refers to an analytical technique to identify compounds by their mass. In order to enhance the mass resolving and mass determining capabilities of mass spectrometry, the samples can be processed prior to MS analysis. Accordingly, the invention relates to MS detection methods that can be combined with immuno-enrichment technologies, methods related to sample preparation and/or chromatographic methods, preferably with liquid chromatography (LC), more preferably with high performance liquid chromatography (HPLC) or ultra high performance liquid chromatography (UHPLC). Sample preparation methods comprise techniques for lysis, fractionation, digestion of the sample into peptides, depletion, enrichment, dialysis, desalting, alkylation and/or peptide reduction. However, these steps are optional. The selective detection of analyte ions may be conducted with tandem mass spectrometry (MS/MS). Tandem mass spectrometry is characterized by mass selection step (as used herein, the term “mass selection” denotes isolation of ions having a specified m/z or narrow range of m/z´s), followed by fragmentation of the selected ions and mass analysis of the resultant product (fragment) ions.

The skilled person is aware how quantify the level of a marker in the sample by mass spectrometric methods. For example, relative quantification “rSRM” or absolute quantification can be employed as described above.

Moreover, the levels (including reference levels) can be determined by mass spectrometric based methods, such as methods determining the relative quantification or determining the absolute quantification of the protein or fragment thereof of interest.

Relative quantification “rSRM” may be achieved by:

1. Determining increased or decreased presence of the target protein by comparing the SRM (Selected reaction monitoring) signature peak area from a given target fragment peptide detected in the sample to the same SRM signature peak area of the target fragment peptide in at least a second, third, fourth or more biological samples.

2. Determining increased or decreased presence of target protein by comparing the SRM signature peak area from a given target peptide detected in the sample to SRM signature peak areas developed from fragment peptides from other proteins, in other samples derived from different and separate biological sources, where the SRM signature peak area comparison between the two samples for a peptide fragment are normalized for e.g to amount of protein analyzed in each sample.

3. Determining increased or decreased presence of the target protein by comparing the SRM signature peak area for a given target peptide to the SRM signature peak areas from other fragment peptides derived from different proteins within the same biological sample in order to normalize changing levels of histones protein to levels of other proteins that do not change their levels of expression under various cellular conditions.

4. These assays can be applied to both unmodified fragment peptides and to modified fragment peptides of the target proteins, where the modifications include, but are not limited to phosphorylation and/or glycosylation, acetylation, methylation (mono, di, tri), citrullination, ubiquitinylation and where the relative levels of modified peptides are determined in the same manner as determining relative amounts of unmodified peptides.

Absolute quantification of a given peptide may be achieved by:

1. Comparing the SRM/MRM signature peak area for a given fragment peptide from the target proteins in an individual biological sample to the SRM/MRM signature peak area of an internal fragment peptide standard spiked into the protein lysate from the biological sample. The internal standard may be a labeled synthetic version of the fragment peptide from the target protein that is being interrogated or the labeled recombinant protein. This standard is spiked into a sample in known amounts before (mandatory for the recombinant protein) or after digestion, and the SRM/MRM signature peak area can be determined for both the internal fragment peptide standard and the native fragment peptide in the biological sample separately, followed by comparison of both peak areas. This can be applied to unmodified fragment peptides and modified fragment peptides, where the modifications include but are not limited to phosphorylation and/or glycosylation, acetylation, methylation (e.g. mono-, di-, or tri-methylation), citrullination, ubiquitinylation, and where the absolute levels of modified peptides can be determined in the same manner as determining absolute levels of unmodified peptides.

2. Peptides can also be quantified using external calibration curves. The normal curve approach uses a constant amount of a heavy peptide as an internal standard and a varying amount of light synthetic peptide spiked into the sample. A representative matrix similar to that of the test samples needs to be used to construct standard curves to account for a matrix effect. Besides, reverse curve method circumvents the issue of endogenous analyte in the matrix, where a constant amount of light peptide is spiked on top of the endogenous analyte to create an internal standard and varying amounts of heavy peptide are spiked to create a set of concentration standards. Test samples to be compared with either the normal or reverse curves are spiked with the same amount of standard peptide as the internal standard spiked into the matrix used to create the calibration curve.

The invention further relates to kits, the use of the kits and methods wherein such kits are used. The invention relates to kits for carrying out the herein above and below provided methods. The herein provided definitions, e.g. provided in relation to the methods, also apply to the kits of the invention. In particular, the invention relates to kits for therapy monitoring, comprising the prognosis, risk assessment or risk stratification of a subsequent adverse event in the health of a patient, wherein said kit comprises

- detection reagents for determining the level proADM or fragment(s) thereof, and optionally additionally for determining the level of PCT, lactate and/or C-reactive protein or fragment(s) thereof, in a sample from a subject, and - detection reagents for determining said level of proADM in said sample of said subject, and
- reference data, such as a reference level, corresponding to high and/or low severity levels of proADM, wherein the low severity level is below 4 nmol/l, preferably below 3 nmol/l, more preferably below 2.7 nmol/l, and the high severity level is above 6.5 nmol/l, preferably above 6.95 nmol/l, more preferably above 10.9 nmol/l, and optionally PCT, lactate and/or C-reactive protein levels, wherein said reference data is preferably stored on a computer readable medium and/or employed in the form of computer executable code configured for comparing the determined levels of proADM or fragment(s) thereof, and optionally additionally the determined levels of PCT, lactate and/or C-reactive protein or fragment(s) thereof, to said reference data.

As used herein, "reference data" comprise reference level(s) of proADM and optionally PCT, lactate and/or C-reactive protein. The levels of proADM and optionally PCT, lactate and/or C-reactive protein in the sample of the subject can be compared to the reference levels comprised in the reference data of the kit. The reference levels are herein described above and are exemplified also in the appended examples. The reference data can also include a reference sample to which the level of proADM and optionally PCT, lactate and/or C-reactive protein is compared. The reference data can also include an instruction manual how to use the kits of the invention.

The kit may additionally comprise items useful for obtaining a sample, such as a blood sample, for example the kit may comprise a container, wherein said container comprises a device for attachment of said container to a canula or syringe, is a syringe suitable for blood isolation, exhibits an internal pressure less than atmospheric pressure, such as is suitable for drawing a pre-determined volume of sample into said container, and/or comprises additionally detergents, chaotropic salts, ribonuclease inhibitors, chelating agents, such as guanidinium isothiocyanate, guanidinium hydrochloride, sodium dodecylsulfate, polyoxyethylene sorbitan monolaurate, RNAse inhibitor proteins, and mixtures thereof, and/or A filter system containing nitro-cellulose, silica matrix, ferromagnetic spheres, a cup retrieve spill over, trehalose, fructose, lactose, mannose, poly-ethylen-glycol, glycerol, EDTA, TRIS, limonene, xylene, benzoyl, phenol, mineral oil, anilin, pyrol, citrate, and mixtures thereof.

As used herein, the "detection reagent" or the like are reagents that are suitable to determine the herein described marker(s), e.g. of proADM, PCT, lactate and/or C-reactive protein. Such exemplary detection reagents are, for example, ligands, e.g. antibodies or fragments thereof, which specifically bind to the peptide or epitopes of the herein described marker(s). Such ligands might be used in immunoassays as described above. Further reagents that are employed in the immunoassays to determine the level of the marker(s) may also be comprised in the kit and are herein considered as detection reagents. Detection reagents can also relate to reagents that are

employed to detect the markers or fragments thereof by MS based methods. Such detection reagent can thus also be reagents, e.g. enzymes, chemicals, buffers, etc, that are used to prepare the sample for the MS analysis. A mass spectrometer can also be considered as a detection reagent. Detection reagents according to the invention can also be calibration solution(s), e.g. which can be employed to determine and compare the level of the marker(s).

The sensitivity and specificity of a diagnostic and/or prognostic test depends on more than just the analytical "quality" of the test, they also depend on the definition of what constitutes an abnormal result. In practice, Receiver Operating Characteristic curves (ROC curves), are typically calculated by plotting the value of a variable versus its relative frequency in "normal" (i.e. apparently healthy individuals not having an infection and "disease" populations, e.g. subjects having an infection. For any particular marker (like ADM), a distribution of marker levels for subjects with and without a disease/condition will likely overlap. Under such conditions, a test does not absolutely distinguish normal from disease with 100% accuracy, and the area of overlap might indicate where the test cannot distinguish normal from disease. A threshold is selected, below which the test is considered to be abnormal and above which the test is considered to be normal or below or above which the test indicates a specific condition, e.g. infection. The area under the ROC curve is a measure of the probability that the perceived measurement will allow correct identification of a condition. ROC curves can be used even when test results do not necessarily give an accurate number. As long as one can rank results, one can create a ROC curve. For example, results of a test on "disease" samples might be ranked according to degree (e.g. 1=low, 2=normal, and 3=high). This ranking can be correlated to results in the "normal" population, and a ROC curve created. These methods are well known in the art; see, e.g., Hanley et al. 1982. Radiology 143: 29-36. Preferably, a threshold is selected to provide a ROC curve area of greater than about 0.5, more preferably greater than about 0.7, still more preferably greater than about 0.8, even more preferably greater than about 0.85, and most preferably greater than about 0.9. The term "about" in this context refers to +/- 5% of a given measurement.

The horizontal axis of the ROC curve represents (1-specificity), which increases with the rate of false positives. The vertical axis of the curve represents sensitivity, which increases with the rate of true positives. Thus, for a particular cut-off selected, the value of (1-specificity) may be determined, and a corresponding sensitivity may be obtained. The area under the ROC curve is a measure of the probability that the measured marker level will allow correct identification of a disease or condition. Thus, the area under the ROC curve can be used to determine the effectiveness of the test.

Accordingly, the invention comprises the administration of an antibiotic suitable for treatment on the basis of the information obtained by the method described herein.

As used herein, the terms “comprising” and “including” or grammatical variants thereof are to be taken as specifying the stated features, integers, steps or components but do not preclude the addition of one or more additional features, integers, steps, components or groups thereof. This term encompasses the terms “consisting of” and “consisting essentially of”.

Thus, the terms “comprising”/“including”/”having” mean that any further component (or likewise features, integers, steps and the like) can/may be present. The term “consisting of” means that no further component (or likewise features, integers, steps and the like) is present.

The term “consisting essentially of” or grammatical variants thereof when used herein are to be taken as specifying the stated features, integers, steps or components but do not preclude the

addition of one or more additional features, integers, steps, components or groups thereof but only if the additional features, integers, steps, components or groups thereof do not materially alter the basic and novel characteristics of the described composition, device or method.

Thus, the term "consisting essentially of" means those specific further components (or likewise features, integers, steps and the like) can be present, namely those not materially affecting the essential characteristics of the composition, device or method. In other words, the term "consisting essentially of" (which can be interchangeably used herein with the term "comprising substantially"), allows the presence of other components in the composition, device or method in addition to the mandatory components (or likewise features, integers, steps and the like), provided that the essential characteristics of the device or method are not materially affected by the presence of other components.

The term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, biological and biophysical arts.

The present invention is further described by reference to the following non-limiting examples.

EXAMPLES

Methods of the examples:

Study Design and Patients:

This study is a secondary analysis of the Placebo-Controlled Trial of Sodium Selenite and Procalcitonin Guided Antimicrobial Therapy in Severe Sepsis (SISPCT), which was performed across 33 multidisciplinary intensive care units (ICUs) throughout Germany from November 2009 until February 2013 (26). Eligibility criteria included adult patients ≥18 years presenting with new onset severe sepsis or septic shock (≤24 hours), according to the SEPSIS-1 definition of the ACCP/SCCM Consensus Conference Committee, and further classified according to the 2016 definitions (sepsis-3 and septic shock-3) (4). Details of the study design, data collection and management were described previously (26). The ethics committee of Jena University Hospital and all other centres approved the study and written informed consent was obtained whenever necessary.

Biomarker Measurements:

Patients were enrolled up to 24 hours after diagnosis of severe sepsis or septic shock and PCT, CRP and lactate measured immediately thereafter. PCT was measured on devices with a measuring range of 0.02 - 5000 ng/ml, and a functional assay sensitivity and lower detection limit of at least 0.06 ng/ml and 0.02 ng/ml, respectively. Additional blood samples from all patients were collected and stored at the central study laboratory in Jena at -80°C. MR-proADM plasma concentrations were measured retrospectively (Kryptor®, Thermo Fisher Scientific, Germany) with a limit of detection of 0.05 nmol/L. Clinical severity scores including the Sequential Organ Failure Assessment (SOFA), Acute Physiological and Chronic Health Evaluation (APACHE) II and Simplified Acute Physiological (SAPS) II score were taken upon study enrollment.

Statistical Analysis:

Differences in demographic and clinical characteristics with regards to 28 day mortality were assessed using the $\chi 2$ test for categorical variables, and Student's t-test or Mann-Whitney U test for continuous variables, depending on distribution normality. Normally and non-normally distributed variables were expressed as mean (standard deviation) and median [first quartile - third quartile], respectively. The association between mortality and each biomarker and clinical score at all time points was assessed using area under the receiver operating characteristic curves (AUROC) and Cox regression analysis, with multivariate analysis corrected for age and the presence of comorbidities and septic shock. Patients were further classified into three severity subgroups (low, intermediate and high) based on the calculation of two AUROC cut-offs across the total population for each biomarker and clinical score at each time point, with a predefined sensitivity and specificity of close to 90%. A subgroup clinically stable patients was subsequently identified with an absence of any ICU associated procedures or complications (including focus cleaning procedures, emergency surgery, the emergence of new infections, transfusion of blood products, infusion of colloids, invasive mechanical ventilation, renal/liver replacement or vasopressor therapy and a deterioration in the patient's general clinical signs and symptoms), and a further group identified with corresponding low MR-proADM concentrations which had not shown any increase since the previous measurement. Mortality rates and average lengths of stay were calculated in both groups and compared against the patient group who were discharged at each specific time point.

Finally, two models stratifying patients with PCT changes of 20% (baseline to day 1, based on average PCT decreases observed over this time period) and 50% (baseline to day four, based on a previously constructed model (26)) were constructed. Patient subgroups were subsequently identified based on MR-proADM severity levels, and respective mortality rates calculated. The risk of mortality within each subgroup was calculated by Cox regression analysis and illustrated by Kaplan-Meier curves. The predicted risk of developing new infections and the requirement for focus cleaning procedures and emergency surgery over days 4 to 7 were subsequently investigated in the baseline to day 4 model. All data were analysed using the statistics software R (version 3.1.2).

Example 1: Patient Characteristics

Patient characteristics upon study enrollment are summarized in **Table 1**.

A total of 1089 patients with either severe sepsis (13.0%) or septic shock (87.0%) were analysed, with 445 (41.3%) and 633 (58.7%) patients also satisfying the criteria for sepsis-3 and septic shock-3, respectively. Enrolled patients had an average age of 65.7 (13.7) years and a mean SOFA score of 10.0 (3.3) points. The 28 day all-cause mortality rate (N = 1076) was 26.9% (sepsis-3: 20.0%; septic shock-3: 32.1%), with a hospital mortality rate of 33.4% (sepsis-3: 24.4%; septic shock-3: 40.4%). Infections originating from a single focus were found in 836 patients (77.7%), with pneumological (N = 324; 30.1%), intra-abdominal (N = 252; 23.4%), urogenital (N = 57; 5.3%) and bone/soft tissue (N = 50; 4.6%) origins most prevalent. Corresponding mortality rates were 26.5%, 24.6%, 22.8% and 28.0%, respectively. Multiple origins of infection were found in 240 (22.3%) patients. The most common causes of mortality included sepsis induced multiple organ failure (N = 132; 45.7%), refractory septic shock (N = 54; 18.7%), death due to pre-existing illness (N = 35; 12.1%) and acute respiratory insufficiency (N = 17; 5.9%). Other causes such as cardiogenic and hemorrhagic shock, pulmonary embolism, cerebral oedema, myocardial infarction and cardiac arrhythmia accounted for a combined mortality rate of 8.6%. A limitation of therapy was applied to 3.4% of patients.

Example 2: Association of baseline biomarkers and clinical scores with mortality

Univariate and multivariate Cox regression analysis found that MR-proADM had the strongest association with 28 day mortality across the total patient population, as well as within the sepsis-3 and septic shock-3 subgroups (**Table 2**). Corresponding AUROC analysis found significant differences in all biomarker and clinical score comparisons with MR-proADM, apart from APACHE II (sepsis-3 patient subgroup).

Similar results were also found for 7 day, 90 day, ICU and hospital mortality prediction (**Table 3**), with the addition of MR-proADM to all potential biomarkers and clinical score combinations (N = 63) significantly increasing prognostic capability (**Table 4**).

Example 3: Identification of high-risk patients

The total patient population was further stratified according to existing SOFA severity levels, and biomarker and clinical score performance in predicting 28 day mortality assessed in each subgroup. MR-proADM showed the highest accuracy of all parameters in the low (SOFA ≤7) and moderate (8≤ SOFA ≤13) severity SOFA subgroups (**Table 5; Table 6**).

Two corresponding MR-proADM cut-offs were subsequently calculated to identify low (≤2.7 nmol/L) and high (>10.9 nmol/L) severity subgroups at baseline. Compared to SOFA, a more accurate reclassification could be made at both low (MR-proADM vs. SOFA: N = 265 vs. 232; 9.8% vs. 13.8% mortality) and high (MR-proADM vs. SOFA: N = 161 vs. 155; 55.9% vs. 41.3%) severity cut-offs (**Table 7**).

A subgroup of 94 patients (9.3%) with high MR-proADM concentrations and corresponding low or intermediate SOFA had 28 and 90 day mortality rates of 57.4% and 68.9%, respectively, compared to 19.8% and 30.8% in the remaining patient population with low and intermediate SOFA values. Similar patterns could be found for SAPS II, APACHE II and lactate, respectively (**Tables 8-10**).

Example 4: Identification of low risk patients throughout ICU stay

The study cohort comprises a subset of clinically stable patients that did not face ICU related procedures or complications, such as focus cleaning procedures, emergency surgery, new infections, transfusion of blood products, infusion of colloids, invasive mechanical ventilation, renal/liver replacement, deterioration in the patient´s general clinical signs and symptoms. This group of clinically stable patients was categorized as low risk patients.

MR-proADM showed the strongest association with 28 day mortality across all subsequent time points (**Table 11**), and could provide a stable cut-off of ≤2.25 nmol/L in identifying a low risk patient population, resulting in the classification of greater patient numbers with lower mortality rates compared to other biomarkers and clinical scores (**Table 12**). Accordingly, 290 low MR-proADM severity patients could be identified on day 4, of which 79 (27.2%) were clinically stable and had no increase in MR-proADM concentrations from the last measurement (**Table 13**). A continuously low MR-proADM concentration could be found in 51 (64.6%) patients, whilst a decrease from an intermediate to low level severity level could be observed in 28 (35.4%) patients. The average ICU length of stay was 8 [7 - 10] days, with a 28 and 90 day mortality rate of 0.0% and 1.4%, respectively. In comparison, only 43 patients were actually discharged from the ICU on day 4, with a 28 and 90 day mortality rate of 2.3% and 10.0%. Analysis of the MR-proADM concentrations within this group of patients indicated a range of values, with 20 (52.6%),

16 (42.1%) and 2 (5.3%) patients having low, intermediate and high severity concentrations, respectively. Similar results were found for patients remaining on the ICU on days 7 and 10.

MR-proADM with a stable cut-off of ≤2.25 nmol/L could identify a greater number of low risk patients with lower mortality rates compared to other biomarkers and clinical scores. Based on that finding more patients could be discharged from the ICU compared to classifications without using ADM. By discharging more patients, the hospital can more efficiently occupy ICU beds and benefits from avoided costs.

Example 5: Additional impact of MR-proADM on procalcitonin guided therapy

Time-dependent Cox regression analysis indicated that the earliest significant additional increase in prognostic information to MR-proADM baseline values could be observed on day 1, with subsequent single or cumulative measurements resulting in significantly stronger associations with 28 day mortality (**Table 14**). Hence two PCT guided algorithm models were constructed investigating PCT changes from baseline to either day 1 or day 4, with corresponding subgroup analysis based on MR-proADM severity classifications.

Patients with decreasing PCT concentrations of ≥20% from baseline to day 1 (**Table 15** and **Table 16**) or ≥50% from baseline to day 4 (**Table 17** and **Table 18**) were found to have 28 day mortality rates of 18.3% (N = 458) and 17.1% (N = 557), respectively. This decreased to 5.6% (N = 125) and 1.8% (N = 111) when patients had continuously low levels of MR-proADM, although increased to 66.7% (N = 27) and 52.8% (N = 39) in patients with continuously high MR-proADM values (HR [95% CI]: 19.1 [8.0 - 45.9] and 43.1 [10.1 - 184.0]).

Furthermore, patients with decreasing PCT values of ≥50% (baseline to day 4), but continuously high or intermediate MR-proADM concentrations, had a significantly greater risk of developing subsequent nosocomial infections (HR [95% CI]: high concentrations: 3.9 [1.5 - 10.5]; intermediate concentrations: 2.4 [1.1 - 5.1] vs. patients with continuously low concentrations; intermediate concentrations: 2.9 [1.2 - 6.8]) vs. decreasing intermediate to low concentrations), or requiring emergency surgery (HR [95% CI]: intermediate concentrations: 2.0 [1.1 - 3.7] vs. decreasing intermediate to low concentrations). Conversely, patients with increasing intermediate to high concentrations were more likely to require cleaning of the infectious origin compared to those with continuously intermediate (HR [95% CI]: 3.2 [1.3 - 7.6]), or decreasing (HR [95% CI]: intermediate to low: 8.7 [3.1 - 24.8]); high to intermediate: 4.6 [1.4 - 14.5]) values. When PCT levels failed to decrease by ≥50%, a significantly increased risk of requiring emergency surgery was observed if MR-proADM concentrations were either at a continuously high (HR [95% CI]: 5.7 [1.5 - 21.9]) or intermediate (HR [95% CI]: 4.2 [1.3 - 13.2]) level, as opposed to being continuously low.

Example 6: Association of baseline biomarkers and clinical scores with mortality

MR-proADM showed the strongest association in patients with pneumological and intra-abdominal infections, as well as in patients with Gram positive infections, irrespective of the infectious origin (**Tables 19-20**). When patients were grouped according to operative emergency, non-operative emergency and elective surgery history resulting in admission to the ICU, MR-proADM provided the strongest and most balanced association with 28 day mortality across all groups (**Table 21**).

Example 7: Correlation of biomarkers and clinical scores with SOFA at baseline and day 1

MR-proADM had the greatest correlation of all biomarkers with the SOFA score at baseline, which was significantly increased when baseline values were correlated with day 1 SOFA scores. The greatest correlation could be found between MR-proADM and SOFA on day 10, with differences between individual SOFA subscores found throughout (**Tables 22-24**).

Example 8: Identification of high-risk patients

Similar results could be found in a subgroup of 124 patients (12.0%) with high MR-proADM concentrations and either low or intermediate SAPS II values (High MR-proADM subgroup: [54.8% and 65.6% mortality]; remaining SAPS II population [19.7% and 30.0% mortality]), as well as in 109 (10.6%) patients with either low or intermediate APACHE II values (High MR-proADM subgroup: [56.9% and 66.7% mortality]; remaining APACHE II population: [19.5% and 30.3% mortality]).

Example 9: Improved procalcitonin (PCT) guided therapy by combining PCT and ADM

Two PCT guided algorithm models were constructed investigating PCT changes from baseline to either day 1 or day 4, with corresponding subgroup analysis based on MR-proADM severity classifications (Tables 25-30).

The previous examples show an add-on value for ADM in patients having a PCT decrease at <20% or <50%, as well as in patients where PCT decreased by ≥20% or ≥50%. However, additional analysis demonstrates that ADM can be an add-on regardless of % of decrease or even increase of PCT. Decreasing PCT values could reflect patients where the antibiotic treatment appears to be working, therefore the clinician thinks they are on a good way to survival (i.e. kill the root cause of the sepsis – the bacteria – should result in the patient getting better).

For example, some patients have decreasing PCT levels from baseline (day of admission) to day 1 with a 28d mortality rate of 19%. By additionally measuring ADM, you can conclude from patients with low ADM a much higher chance of survival or much lower probability to die (**Table 25**; compare 19% mortality rate decreasing PCT only vs. 5% mortality rate PCT +low ADM). By having a reduced risk of dying, patients could be discharged from ICU with more confidence, or fewer diagnostic tests are required (i.e. you know they are on a good path to recovery).

On the other hand, new measures need to be considered for those with a high ADM value. They are at a much higher risk with regard to mortality (compare 19% mortality rate decreasing PCT only vs 58.8% mortality rate PCT +high ADM). The physician thinks the patient is getting better due to the decrease in PCT value, but in fact the ADM concentration remains the same. It can be therefore concluded that treatment ISNT working, and needs to be adapted as soon as possible).

In a similar way, ADM can help to stratify those patients with increasing PCT values (**Table 25)**.

Development of new infections

PCT and MR-proADM changes were analyzed in two models, either from baseline to day 1, or from baseline to day 4. Patients were grouped according to overall PCT changes and MR-proADM severity levels.

The number of new infections over days 1, 2, 3 and 4 (**Table 26**) and over days 4, 5, 6 and 7 (**Table 27**) were subsequently calculated in each patient who was present on day 1 or day 4 respectively. In some cases, patients were discharged during the observation period. It is

assumed that no new infections were developed after release. Patients with multiple infections over the observation days were counted as a single new infection.

As a clinical consequence, patients with high MR-proADM concentrations should potentially be treated with a broad-spectrum antibiotic on ICU admission, in conjunction with others, in order to stop the development on new infections. Special care should be taken with these patients due to their high susceptibility to pick up new infections.

Requirement for focus cleaning

PCT and MR-proADM changes were analyzed in two models, either from baseline to day 1, or from baseline to day 4. Patients were grouped according to overall PCT changes and MR-proADM severity levels.

The number of focus cleaning events over days 1, 2, 3 and 4 (**Table 28**) and over days 4, 5, 6 and 7 (**Table 29**) were subsequently calculated in each patient who was present on day 1 or day 4 respectively. In some cases, patients were discharged during the observation period.

Requirement of emergency surgery

PCT and MR-proADM changes were analyzed in two models, either from baseline to day 1, or from baseline to day 4. Patients were grouped according to overall PCT changes and MR-proADM severity levels.

The number of emergency surgery requirements/events over days 1, 2, 3 and 4 (**Table 30**) were subsequently calculated in each patient who was present on day 1. In some cases, patients were discharged during the observation period.

Example 10: Requirement for antibiotic change or modification

When combined within a PCT guided antibiotic algorithm, MR-proADM can stratify those patients who will require a future change or modification in antibiotic therapy, from those who will not.

PCT and MR-proADM changes were analyzed in two models, either from baseline to day 1, or from baseline to day 4. Patients were grouped according to overall PCT changes and MR-proADM severity levels.

The percentage of antibiotic changes on day 4 required for each patient group was subsequently calculated (**Tables 31 and 32**).

In patients with decreasing PCT values ≥50%

Patients with increasing MR-proADM concentrations, from a low to intermediate severity level, were more likely to require a modification in antibiotic therapy on day 4 than those who had continuously low levels (Odds Ration [95% CI]: 1.5 [0.6 - 4.1]).

In patients with decreasing PCT values <50%

Patients with either increasing MR-proADM concentrations, from an intermediate to high severity level, or continuously high concentrations, were also more likely to require changes in their antibiotic therapy on day 4 than patients with continuously low MR-proADM concentrations (Odds Ratio [95% CI]: 5.9 [1.9 - 18.1] and 2.9 [0.8 – 10.4], respectively).

Conclusion

Despite increasing PCT concentrations, either from baseline to day 1, or baseline to day 4, patients with continuously low MR-proADM concentrations had significantly lower modifications made to their prescribed antibiotic treatment than those with continuously intermediate or high concentrations.

As a clinical consequence, when faced with increasing PCT concentrations, a physician should check the patient's MR-proADM levels before deciding on changing antibiotics. Those with low MR-proADM concentrations should be considered for either an increased dose or increased strength of the same antibiotic before changes are considered. Those with higher MR-proADM concentrations should be considered for earlier antibiotic changes (i.e. on days 1 to 3, as opposed to day 4).

Discussion of examples

An accurate and rapid assessment of disease severity is crucial in order to initiate the most appropriate treatment at the earliest opportunity. Indeed, delayed or insufficient treatment may lead to a general deterioration in the patient's clinical condition, resulting in further treatment becoming less effective and a greater probability of a poorer overall outcome (8, 27). As a result, numerous biomarkers and clinical severity scores have been proposed to fulfil this unmet clinical need, with the Sequential Organ Failure Assessment (SOFA) score currently highlighted as the most appropriate tool, resulting in its central role in the 2016 sepsis-3 definition (4). This secondary analysis of the SISPCT trial (26), for the first time, compared sequential measurements of conventional biomarkers and clinical scores, such as lactate, procalcitonin (PCT) and SOFA, with those of the microcirculatory dysfunction marker, MR-proADM, in a large patient population with severe sepsis and septic shock.

Our results indicate that the initial use of MR-proADM within the first 24 hours after sepsis diagnosis resulted in the strongest association with short, mid and long-term mortality compared to all other biomarkers or scores. Previous studies largely confirm our findings (17, 28, 29), however conflicting results (30) may be explained in part by the smaller sample sizes analysed, as well as other factors highlighted within this study, such as microbial species, origin of infection and previous surgical history preceding sepsis development, all of which may influence biomarker performance, thus adding to the potential variability of results in small study populations. Furthermore, our study also closely confirms the results of a previous investigation (17), highlighting the superior performance of MR-proADM in low and intermediate organ dysfunction severity patients. Indeed, Andaluz-Ojeda et al. (17) place significant importance on the patient group with low levels of organ dysfunction, since "this group represents either the earliest presentation in the clinical course of sepsis and/or the less severe form of the disease". Nevertheless, a reasonable performance could be maintained across all severity groups with respect to mortality prediction, which was also the case across both patient groups defined according to the sepsis-3 and septic shock-3 criteria.

Analysis of the sequential measurements taken after onset of sepsis allowed for the identification of specific patients groups based on disease severity. The identification of both low and high-risk patients was of significant interest in our analysis. In many ICUs, the demand for ICU beds can periodically exceed availability, which may lead to an inadequate triage, a rationing of resources, and a subsequent decrease in the likelihood of correct ICU admission (32-35). Consequently, an accurate assessment of patients with a low risk of hospital mortality that may be eligible for an early ICU discharge to a step down unit may be of significant benefit. At each time point

measured within our study, MR-proADM could identify a higher number of low severity patients with the lowest ICU, hospital and 28 day mortality rates. Further analysis of the patient group with a low severity and no further ICU specific therapies indicated that an additional 4 days of ICU stay were observed at each time point after biomarker measurements were taken. When compared to the patient population who were actually discharged at each time point, a biomarker driven approach to accurately identify low severity patients resulted in decreased 28 and 90 day mortality rates. Indeed, patients who were discharged had a variety of low, intermediate and high severity MR-proADM concentrations, which was subsequently reflected in a higher mortality rate. It is, however, unknown whether a number of patients within this group still required further ICU treatment for non-microcirculatory, non-life threatening issues, or that beds in a step down unit were available. Nevertheless, such a biomarker driven approach to ICU discharge in addition to clinician judgement may improve correct stratification of the patient, with accompanied clinical benefits and potential cost savings.

Conversely, the identification of high-risk patients who may require early and targeted treatment to prevent a subsequent clinical deterioration may be of even greater clinical relevance. Substantial cost savings and reductions in antibiotic use have already been observed following a PCT guided algorithm in the SISPCT study and other trials (26, 36, 37), however relatively high mortality rates can still be observed even when PCT values appear to be decreasing steadily. Our study revealed that the addition of MR-proADM to the model of PCT decreases over subsequent ICU days allowed the identification of low, intermediate and high risk patient groups, with increasing and decreasing MR-proADM severity levels from baseline to day 1 providing a sensitive and early indication as to treatment success. In addition, the prediction of the requirement for future focus cleaning or emergency surgery, as well as the susceptibility for the development of new infections, may be of substantial benefit in initiating additional therapeutic and interventional strategies, thus attempting to prevent any future clinical complications at an early stage.

The strength of our study includes the thorough examination of several different subgroups with low and high disease severities from a randomized trial database, adjusting for potential confounders and including the largest sample size of patients with sepsis, characterized by both SEPSIS 1 and 3 definitions, and information on MR-proADM kinetics.

In conclusion, MR-proADM outperforms other biomarkers and clinical severity scores in the ability to identify mortality risk in patients with sepsis, both on initial diagnosis and over the course of ICU treatment. Accordingly, MR-proADM may be used as a tool to identify high severity patients who may require alternative diagnostic and therapeutic interventions, and low severity patients who may potentially be eligible for an early ICU discharge in conjunction with an absence of ICU specific therapies.

Tables

Table 1. Patient characteristics at baseline for survival up to 28 days

	Total (N = 1076)	Survivors (N = 787)	Non-Survivors (N = 289)	*P* value
Age (years) (mean, S.D.)	65.7 (13.7)	64.3 (14.0)	69.5 (12.0)	<0.0001
Male gender (n, %)	681 (63.3%)	510 (64.8%)	171 (59.2%)	0.0907
Definitions of sepsis and length of stay				
Severe sepsis (n, %)	139 (12.9%)	109 (13.9%)	30 (10.4%)	0.1251
Septic shock (n, %)	937 (87.1%)	678 (86.2%)	259 (89.6%)	0.1251
Sepsis-3 (n, %)	444 (41.3%)	356 (45.4%)	88 (30.4%)	<0.0001
Septic shock-3 (n, %)	630 (58.7%)	429 (54.6%)	201 (69.6%)	<0.0001
ICU length of stay (days) (median, IQR)	12 [6 - 23]	13 [7 - 26]	8 [4 - 15]	<0.0001
Hospital length of stay (days) (median, IQR)	28 [17 - 45]	34 [22 - 51]	14 [7 - 23]	<0.0001
Pre-existing comorbidities				
History of diabetes (n, %)	280 (26.0%)	188 (23.9%)	92 (31.8%)	0.0094
Heart failure (n, %)	230 (21.4%)	150 (19.1%)	80 (27.7%)	0.0027
Renal dysfunction (n, %)	217 (20.2%)	135 (17.2%)	82 (28.4%)	<0.0001
COPD (n, %)	131 (12.2%)	90 (11.4%)	41 (14.2%)	0.2277
Liver cirrhosis (n, %)	50 (4.7%)	27 (3.4%)	23 (8.0%)	0.0030
History of cancer (n, %)	319 (29.7%)	224 (28.5%)	95 (32.9%)	0.1630
Immunosuppression (n, %)	46 (4.3%)	30 (3.8%)	16 (5.5%)	0.2271
Microbiology				
Gram positive (n, %)	146 (13.6%)	113 (14.4%)	33 (11.4%)	0.2050
Gram negative (n, %)	132 (12.3%)	95 (12.1%)	37 (12.8%)	0.7467
Fungal (n, %)	51 (4.7%)	37 (4.7%)	14 (4.8%)	0.9223
Gram positive and negative (n, %)	183 (17.0%)	133 (16.9%)	50 (17.3%)	0.8767
Gram positive and fungal (n, %)	92 (8.6%)	68 (8.6%)	24 (8.3%)	0.8610
Gram negative and fungal (n, %)	51 (4.7%)	35 (4.5%)	16 (5.5%)	0.4631
Gram positive and negative and fungal (n, %)	115 (10.7%)	81 (10.3%)	34 (11.8%)	0.4922
Origin of infection				
Pneumonia (n, %)	453 (43.7%)	327 (42.9%)	126 (46.0%)	0.3798
Upper or lower respiratory (n, %)	44 (4.3%)	29 (3.8%)	15 (5.5%)	0.2523
Thoracic (n, %)	44 (4.3%)	35 (4.6%)	9 (3.3%)	0.3444
Bones/soft tissue (n, %)	78 (7.5%)	56 (7.4%)	22 (8.0%)	0.7161
Gastrointestinal (n, %)	80 (7.7%)	68 (8.9%)	12 (4.4%)	0.0107
Catheter associated (n, %)	30 (2.9%)	18 (2.4%)	12 (4.4%)	0.1015
Surgical wound (n, %)	41 (4.0%)	31 (4.1%)	10 (3.7%)	0.7586
Intraabdominal (n, %)	375 (36.2%)	276 (36.2%)	99 (36.1%)	0.9790
Cardiovascular (n, %)	6 (0.6%)	4 (0.5%)	2 (0.7%)	0.7082
Urogenital (n, %)	99 (9.6%)	70 (9.2%)	29 (10.6%)	0.5039
Central nervous system (n, %)	3 (0.3%)	2 (0.3%)	1 (0.4%)	0.7916
Bacteremia (n, %)	31 (3.0%)	20 (2.6%)	11 (4.0%)	0.2611
Organ dysfunction				
Neurological (n, %)	348 (32.3%)	240 (30.5%)	108 (37.4%)	0.0340
Respiratory (n, %)	486 (45.2%)	350 (44.5%)	136 (47.1%)	0.4502
Cardiovascular (n, %)	829 (77.0%)	584 (74.2%)	245 (84.8%)	0.0002
Renal dysfunction (n, %)	382 (35.5%)	249 (31.6%)	133 (46.0%)	<0.0001
Haematological (n, %)	156 (14.5%)	89 (11.3%)	67 (23.2%)	<0.0001
Gastrointestinal (n, %)	387 (36.0%)	271 (34.4%)	116 (40.1%)	0.0855
Metabolic dysfunction (n, %)	718 (66.7%)	504 (64.0%)	214 (74.1%)	0.0017
Other organ dysfunction (n, %)	499 (46.4%)	380 (48.3%)	119 (41.2%)	0.0378
Treatment upon ICU admission				
Invasive mechanical ventilation (n, %)	789 (73.3%)	567 (72.1%)	222 (76.8%)	0.1133
Non-invasive mechanical ventilation (n, %)	64 (5.9%)	46 (5.8%)	18 (6.2%)	0.8145
Renal replacement therapy (n, %)	326 (30.8%)	158 (20.5%)	168 (58.1%)	<0.0001
Vasopressor use (n, %)	980 (91.1%)	712 (90.5%)	268 (92.7%)	0.2391

Biomarker and severity scores				
MR-proADM (nmol/L) (median, IQR)	5.0 [2.6 - 8.8]	4.0 [2.3 - 7.2]	8.2 [5.2 - 12.6]	<0.0001
PCT (ng/mL) (median, IQR)	7.4 [1.6 - 26.9]	6.6 [1.4 - 25.1]	9.3 [2.6 - 31.8]	0.0325
Lactate (mmol/L) (median, IQR)	2.7 [1.6 - 4.7]	2.4 [1.5 - 4.0]	3.7 [2.1 - 7.2]	<0.0001
CRP (mg/L) (median, IQR)	188 [120.9 - 282]	189 [120.5 - 277.4]	188 [122 - 287]	0.7727
SOFA (points) (mean, S.D.)	10.02 (3.33)	9.58 (3.18)	11.22 (3.43)	<0.0001
SAPS II (points) (mean, S.D.)	63.27 (14.18)	61.08 (13.71)	69.24 (13.74)	<0.0001
APACHE II (points) (mean, S.D.)	24.24 (7.60)	23.05 (7.37)	27.49 (7.28)	<0.0001

ICU: Intensive Care Unit; COPD: chronic obstructive pulmonary disease; MR-proADM, mid-regional proadrenomedullin; PCT: procalcitonin; CRP: C-reactive protein; SOFA: Sequential Organ Failure Assessment; SAPS II: Simplified Acute Physiological score; APACHE II: Acute Physiological and Chronic Health Evaluation. Data are presented as absolute number and percentages in brackets, indicating the proportion of surviving and non-surviving patients at 28 days.

Table 2. Prediction of 28 day mortality following sepsis diagnosis

					Univariate				Multivariate		
		N	Events	AUROC	LR χ^2	C-index	HR IQR [95%]	p	LR χ^2	C-index	HR IQR [95%]
All patients	MR-proADM	1030	275	0.73	142.7	0.71	3.2 [2.6 - 3.9]	<0.0001	161.69	0.72	2.9 [2.4 - 3.6]
	PCT	1031	275	0.56	12.2	0.56	1.4 [1.2 - 1.7]	0.0005	70.28	0.64	1.4 [1.1 - 1.7]
	CRP	936	251	0.49	0.12	0.51	1.0 [0.9 - 1.2]	0.7304	50.54	0.62	1.1 [0.9 - 1.2]
	Lactate	1066	289	0.65	78.3	0.64	2.2 [1.8 - 2.5]	<0.0001	122.72	0.69	2.1 [1.7 - 2.5]
	SOFA	1051	282	0.64	47.3	0.62	1.6 [1.4 - 1.8]	<0.0001	96.05	0.67	1.6 [1.4 - 1.8]
	SAPS II	1076	289	0.67	70.5	0.65	1.8 [1.6 - 2.0]	<0.0001	100.3	0.67	1.6 [1.4 - 1.9]
	APACHE II	1076	289	0.67	69.9	0.65	1.9 [1.6 - 2.2]	<0.0001	99.21	0.67	1.7 [1.4 - 2.0]
Sepsis-3	MR-proADM	425	83	0.73	40.9	0.71	2.8 [2.0 - 3.8]	<0.0001	61.4	0.74	2.6 [1.8 - 3.7]
	PCT	425	83	0.56	4.6	0.56	1.4 [1.0 - 1.9]	0.0312	40.6	0.70	1.5 [1.1 - 2.1]
	CRP	382	81	0.55	2.1	0.54	0.9 [0.7 - 1.1]	0.1505	36.7	0.69	0.9 [0.7 - 1.1]
	Lactate	439	88	0.57	7.7	0.56	1.3 [1.1 - 1.6]	0.0057	45.0	0.69	1.3 [1.1 - 1.7]
	SOFA	428	86	0.58	3.2	0.56	1.2 [1.0 - 1.5]	0.0745	40.8	0.69	1.2 [1.0 - 1.5]
	SAPS II	439	88	0.62	14.5	0.61	1.7 [1.3 - 2.3]	0.0001	45.0	0.69	1.5 [1.1 - 2.0]
	APACHE II	439	88	0.70	30.8	0.68	2.1 [1.6 - 2.6]	<0.0001	52.6	0.71	1.7 [1.3 - 2.3]
Septic shock-3	MR-proADM	597	192	0.72	77.4	0.69	2.4 [2.0 - 3.0]	<0.0001	93.5	0.71	2.3 [1.8 - 2.9]
	PCT	597	192	0.50	0.4	0.51	1.1 [0.9 - 1.3]	0.5264	35.7	0.62	1.1 [0.9 - 1.4]
	CRP	545	170	0.53	2.1	0.53	1.1 [1.0 - 1.3]	0.1498	31.7	0.63	1.1 [1.0 - 1.4]
	Lactate	627	201	0.64	52.2	0.64	2.0 [1.7 - 2.4]	<0.0001	79.4	0.68	2.0 [1.7 - 2.4]
	SOFA	616	196	0.65	31.1	0.62	1.6 [1.4 - 1.9]	<0.0001	56.5	0.66	1.6 [1.3 - 1.9]
	SAPS II	627	201	0.67	42.2	0.65	1.7 [1.4 - 1.9]	<0.0001	59.8	0.66	1.6 [1.3 - 1.8]
	APACHE II	627	201	0.63	28.3	0.61	1.6 [1.3 - 1.9]	<0.0001	50.7	0.65	1.5 [1.3 - 1.8]

N: Number; AUROC: Area under the Receiver Operating Curve; LR χ2: HR: Hazard Ratio; IQR: Interquartile range. All multivariate analyses were associated by $p < 0.0001$ to 28 day mortality.

Table 3. Survival analysis for 7 day, 90 day, ICU and hospital mortality

				Univariate					Multivariate		
		Patients (N)	Mortality (N)	AUROC	LR x^2	C-index	HR IQR [95% CI]	p-value	LR x^2	C-index	HR IQR [95% CI]
7 day	MR-proADM	1037	131	0.72	71.6	0.71	3.3 [2.4 - 4.3]	<0.0001	82.1	0.73	3.4 [2.5 - 4.6]
	PCT	1038	131	0.58	9.7	0.58	1.5 [1.2 - 2.0]	0.0019	28.4	0.64	1.6 [1.2 - 2.1]
	CRP	943	111	0.55	1.2	0.55	1.1 [0.9 - 1.4]	0.2843	16.6	0.62	1.2 [0.9 - 1.4]
	Lactate	1074	135	0.72	86.0	0.71	3.1 [2.4 - 3.9]	<0.0001	99.1	0.73	3.1 [2.4 - 4.0]
	SOFA	1059	130	0.63	25.5	0.63	1.7 [1.4 - 2.0]	<0.0001	41.0	0.67	1.7 [1.4 - 2.1]
	SAPS II	1085	135	0.66	38.5	0.66	1.8 [1.5 - 2.2]	<0.0001	50.1	0.67	1.8 [1.5 - 2.2]
	APACHE II	1085	135	0.63	24.4	0.63	1.7 [1.4 - 2.1]	<0.0001	37.8	0.65	1.7 [1.4 - 2.1]
90 day	MR-proADM	1000	379	0.71	146.2	0.68	2.7 [2.3 - 3.2]	<0.0001	194.1	0.71	2.4 [2.0 - 2.8]
	PCT	1000	379	0.55	11.8	0.55	1.3 [1.1 - 1.5]	0.0006	113.5	0.65	1.3 [1.1 - 1.5]
	CRP	909	348	0.51	0.2	0.51	1.0 [0.9 - 1.2]	0.6641	92.3	0.64	1.1 [0.9 - 1.2]
	Lactate	1037	399	0.64	83.2	0.63	2.0 [1.7 - 2.3]	<0.0001	168.8	0.68	1.9 [1.6 - 2.2]
	SOFA	1021	388	0.62	48.1	0.61	1.5 [1.4 - 1.7]	<0.0001	143.7	0.67	1.5 [1.3 - 1.7]
	SAPS II	1045	399	0.66	81.1	0.64	1.7 [1.5 - 1.9]	<0.0001	144.4	0.67	1.5 [1.3 - 1.7]
	APACHE II	1045	399	0.67	86.4	0.64	1.8 [1.6 - 2.1]	<0.0001	146.8	0.67	1.6 [1.4 - 1.8]
ICU	MR-proADM	1023	264	0.73	136.4	0.73	4.0 [3.1 - 5.2]	<0.0001	158.3	0.75	3.7 [2.8 - 4.9]
	PCT	1024	264	0.58	18.0	0.58	1.6 [1.3 - 2.0]	<0.0001	73.0	0.67	1.6 [1.3 - 2.1]
	CRP	928	237	0.54	2.5	0.54	1.1 [1.0 - 1.3]	0.1108	51.4	0.65	1.2 [1.0 - 1.4]
	Lactate	1059	277	0.66	75.2	0.66	2.4 [2.0 - 3.0]	<0.0001	115.5	0.71	2.4 [1.9 - 2.9]
	SOFA	1044	270	0.64	48.6	0.64	1.8 [1.5 - 2.2]	<0.0001	95.2	0.69	1.8 [1.5 - 2.2]
	SAPS II	1070	277	0.65	58.7	0.65	1.9 [1.6 - 2.3]	<0.0001	91.2	0.68	1.8 [1.5 - 2.2]
	APACHE II	1070	277	0.66	62.5	0.66	2.1 [1.7 - 2.6]	<0.0001	91.6	0.69	1.9 [1.5 - 2.3]
Hospital	MR-proADM	980	323	0.73	152.0	0.74	4.0 [3.1 - 5.2]	<0.0001	186.8	0.76	3.6 [2.7 - 4.6]
	PCT	981	323	0.57	15.0	0.57	1.5 [1.2 - 1.9]	0.0001	96.2	0.68	1.5 [1.2 - 1.9]
	CRP	891	299	0.52	0.9	0.52	1.1 [0.9 - 1.3]	0.3480	76.0	0.67	1.1 [1.0 - 1.3]
	Lactate	1016	342	0.66	77.8	0.66	2.4 [2.0 - 2.9]	<0.0001	146.2	0.72	2.3 [1.9 - 2.9]
	SOFA	1001	333	0.63	41.3	0.63	1.7 [1.4 - 2.0]	<0.0001	118.9	0.70	1.7 [1.4 - 2.0]
	SAPS II	1027	342	0.65	59.1	0.65	1.9 [1.6 - 2.2]	<0.0001	115.9	0.69	1.7 [1.4 - 2.0]
	APACHE II	1027	342	0.67	76.7	0.67	2.2 [1.9 - 2.7]	<0.0001	127.1	0.71	1.9 [1.6 - 2.4]

All multivariate p values < 0.0001 apart from PCT and CRP for 7 day mortality (0.0015 and 0.0843, respectively).

Table 4. Survival analysis for MR-proADM when added to individual biomarkers or clinical scores

				Bivariate			Added value		Multivariate			Added value	
		Patients (N)	Mortality (N)	LR χ^2	C-index	HR IQR [95% CI]	LR χ^2	p-value	LR χ^2	C-index	HR IQR [95% CI]	LR χ^2	p-value
7 day	PCT	1037	131	76.5	0.72	4.0 [2.9 - 5.6]	66.8	<0.0001	86.2	0.73	4.2 [2.9 - 6.1]	57.8	<0.0001
	CRP	904	108	56.9	0.71	3.2 [2.3 - 4.3]	55.0	<0.0001	67.7	0.73	3.3 [2.3 - 4.7]	49.4	<0.0001
	Lactate	1029	131	112.5	0.75	2.3 [1.7 - 3.1]	28.1	<0.0001	125.1	0.76	2.4 [1.7 - 3.3]	26.4	<0.0001
	SOFA	1014	126	77.8	0.72	3.3 [2.3 - 4.6]	53.5	<0.0001	86.9	0.74	3.3 [2.3 - 4.7]	46.6	<0.0001
	SAPS II	1037	131	83.1	0.73	2.8 [2.0 - 3.7]	48.1	<0.0001	93.5	0.74	2.9 [2.1 - 4.0]	46.7	<0.0001
	APACHE II	1037	131	73.3	0.71	3.0 [2.2 - 4.1]	50.9	<0.0001	84.5	0.73	3.1 [2.2 - 4.2]	48.6	<0.0001
28 day	PCT	1030	275	163.0	0.73	4.3 [3.4 - 5.5]	150.7	<0.0001	174.9	0.73	3.9 [3.0 - 5.1]	105.0	<0.0001
	CRP	898	239	114.4	0.70	3.0 [2.5 - 3.8]	114.2	<0.0001	132.4	0.72	2.8 [2.2 - 3.6]	80.5	<0.0001
	Lactate	1022	275	163.8	0.72	2.7 [2.2 - 3.3]	85.9	<0.0001	184.5	0.73	2.5 [2.0 - 3.1]	61.4	<0.0001
	SOFA	1007	268	150.6	0.72	3.1 [2.5 - 3.9]	104.1	<0.0001	169.9	0.73	2.8 [2.2 - 3.6]	74.4	<0.0001
	SAPS II	1030	275	163.4	0.72	2.7 [2.2 - 3.3]	97.1	<0.0001	176.5	0.73	2.6 [2.1 - 3.3]	79.1	<0.0001
	APACHE II	1030	275	153.6	0.72	2.7 [2.2 - 3.4]	88.8	<0.0001	169.1	0.73	2.6 [2.1 - 3.3]	74.1	<0.0001
90 day	PCT	1000	379	170.8	0.70	3.6 [3.0 - 4.4]	159.0	<0.0001	208.2	0.71	3.1 [2.5 - 3.9]	94.8	<0.0001
	CRP	872	331	116.0	0.68	2.6 [2.2 - 3.1]	116.0	<0.0001	160.3	0.70	2.3 [1.9 - 2.8]	68.8	<0.0001
	Lactate	993	379	169.4	0.69	2.3 [1.9 - 2.7]	86.6	<0.0001	217.5	0.71	2.0 [1.7 - 2.4]	50.2	<0.0001
	SOFA	977	368	151.0	0.69	2.6 [2.1 - 3.1]	103.1	<0.0001	200.6	0.71	2.2 [1.8 - 2.7]	59.9	<0.0001
	SAPS II	1000	379	173.7	0.70	2.3 [1.9 - 2.7]	94.7	<0.0001	208.4	0.71	2.2 [1.8 - 2.6]	67.6	<0.0001
	APACHE II	1000	379	165.0	0.70	2.3 [1.9 - 2.7]	83.3	<0.0001	202.9	0.71	2.1 [1.8 - 2.6]	62.5	<0.0001
ICU	PCT	1023	264	149.5	0.75	5.7 [4.1 - 7.9]	131.4	<0.0001	165.3	0.76	4.9 [3.5 - 7.0]	92.6	<0.0001
	CRP	889	226	104.6	0.72	3.7 [2.8 - 4.8]	102.5	<0.0001	127.4	0.74	3.4 [2.5 - 4.6]	75.6	<0.0001
	Lactate	1015	264	153.5	0.74	3.2 [2.4 - 4.2]	78.9	<0.0001	175.6	0.76	2.9 [2.2 - 3.9]	57.5	<0.0001
	SOFA	1000	257	140.7	0.74	3.6 [2.7 - 4.8]	91.8	<0.0001	163.8	0.76	3.2 [2.4 - 4.4]	65.8	<0.0001
	SAPS II	1023	264	152.5	0.75	3.4 [2.6 - 4.4]	94.4	<0.0001	169.2	0.76	3.3 [2.5 - 4.3]	77.7	<0.0001
	APACHE II	1023	264	148.2	0.74	3.3 [2.5 - 4.4]	87.9	<0.0001	165.7	0.76	3.3 [2.5 - 4.3]	75.6	<0.0001
Hospital	PCT	980	323	174.7	0.76	6.4 [4.6 - 8.8]	159.5	<0.0001	198.9	0.77	5.2 [3.6 - 7.3]	103.2	<0.0001
	CRP	852	283	117.9	0.72	3.7 [2.9 - 4.8]	117.3	<0.0001	150.1	0.75	3.3 [2.5 - 4.3]	77.7	<0.0001
	Lactate	972	323	167.4	0.75	3.3 [2.5 - 4.3]	89.2	<0.0001	202.5	0.76	2.8 [2.1 - 3.8]	57.6	<0.0001
	SOFA	957	314	155.5	0.74	3.9 [3.0 - 5.2]	113.7	<0.0001	191.3	0.76	3.4 [2.5 - 4.5]	74.6	<0.0001
	SAPS II	980	323	165.8	0.75	3.5 [2.7 - 4.5]	107.7	<0.0001	194.2	0.76	3.2 [2.4 - 4.2]	81.3	<0.0001
	APACHE II	980	323	169.7	0.75	3.3 [2.6 - 4.3]	95.4	<0.0001	197.2	0.76	3.1 [2.4 - 4.1]	75.1	<0.0001

HR IQR [95% CI] indicates the hazard ratio for MR-proADM in each bivariate or multivariate model. 2 degrees of freedom in each bivariate model, compared to 11 in each multivariate model.

Table 5. AUROC analysis for 28 day mortality prediction based on SOFA severity levels

					Univariate				Multivariate			
		N	Events	AUROC	LR χ^2	C-index	HR IQR [95%]	p	LR χ^2	C-index	HR IQR [95%]	p
SOFA ≤7	MR-proADM	232	32	0.74	25.1	0.72	3.6 [2.2 - 6.0]	<0.0001	37.6	0.77	3.1 [1.7-5.6]	<0.0001
	PCT	232	32	0.55	0.9	0.55	1.3 [0.8 - 2.2]	0.3519	22.4	0.72	1.2 [0.7-2.1]	0.0134
	CRP	210	32	0.45	1.1	0.55	1.3 [0.8 - 2.0]	0.2881	17.5	0.69	1.3 [0.8-2.1]	0.0647
	Lactate	236	35	0.62	5.5	0.61	1.8 [1.1 - 3.0]	0.0186	24.3	0.71	1.7 [1.0-2.8]	0.0069
	SAPS II	240	35	0.65	9.3	0.50	2.0 [1.3 - 3.0]	0.0023	22.5	0.71	1.4 [0.8-2.5]	0.013
	APACHE II	240	35	0.69	14.3	0.64	2.4 [1.5 - 3.9]	0.0002	24.6	0.71	1.7 [1.0-3.0]	0.0061
SOFA 8 – 13	MR-proADM	620	172	0.72	74.3	0.70	2.7 [2.1 - 3.3]	<0.0001	89.3	0.72	2.3 [1.8-3.0]	<0.0001
	PCT	620	172	0.54	3.9	0.54	1.3 [1.0 - 1.6]	0.0482	46.3	0.65	1.3 [1.0-1.6]	<0.0001
	CRP	572	161	0.51	0.1	0.52	1.0 [0.9 - 1.2]	0.7932	39.3	0.64	1.0 [0.9-1.2]	<0.0001
	Lactate	650	181	0.61	26.9	0.61	1.7 [1.4 - 2.0]	<0.0001	61.6	0.67	1.6 [1.3-2.0]	<0.0001
	SAPS II	653	181	0.64	27.7	0.57	1.6 [1.3 - 1.9]	0.0014	53.9	0.64	1.4 [1.2-1.7]	<0.0001
	APACHE II	653	181	0.63	22.1	0.62	1.5 [1.3 - 1.8]	<0.0001	49.3	0.65	1.3 [1.1-1.6]	<0.0001
SOFA ≥ 14	MR-proADM	155	64	0.67	14.9	0.65	2.0 [1.4 - 3.0]	0.0001	25.6	0.69	2.2 [1.4-3.3]	0.0043
	PCT	155	64	0.49	0.2	0.52	1.1 [0.8 - 1.5]	0.6944	11.5	0.62	1.2 [0.8-1.7]	0.3169
	CRP	136	53	0.57	2.0	0.55	0.9 [0.7 - 1.1]	0.1569	14.9	0.64	2.6 [1.7-3.8]	0.0004
	Lactate	158	66	0.69	22.6	0.68	2.5 [1.7 - 3.6]	<0.0001	32.3	0.71	0.9 [0.7-1.1]	0.1370
	SAPS II	158	66	0.54	2.8	0.56	1.3 [0.9 - 1.8]	0.0930	15.3	0.63	1.2 [0.8-1.7]	0.2958
	APACHE II	158	66	0.54	1.8	0.54	1.3 [0.9 - 1.7]	0.1754	11.8	0.62	1.2 [0.9-1.7]	0.2487

N: Number; AUROC: Area under the Receiver Operating Curve; LR χ2: HR: Hazard Ratio; IQR: Interquartile range.

Table 6. Survival analysis for MR-proADM within different organ dysfunction severity groups when combined with individual biomarkers or clinical scores

				Univariate				Multivariate			
		Patients (N)	Mortality (N)	LR χ^2	C-index	HR IQR [95% CI]	p-value	LR χ^2	C-index	HR IQR [95% CI]	p-value
SOFA ≤7	PCT	232	32	30.0	0.75	5.3 [2.8 - 10.1]	<0.0001	41.8	0.78	5.0 [2.3 - 10.8]	<0.0001
	CRP	204	29	20.1	0.71	3.1 [1.8 - 5.3]	<0.0001	30.5	0.75	2.7 [1.4 - 5.0]	0.0013
	Lactate	229	32	25.1	0.72	3.5 [2.0 - 5.9]	<0.0001	37.2	0.77	3.1 [1.7 - 5.7]	0.0001
	SOFA	232	32	27.3	0.73	3.9 [2.3 - 6.7]	<0.0001	40.4	0.78	3.5 [1.9 - 6.5]	<0.0001
	SAPS II	232	32	28.9	0.74	3.2 [1.9 - 5.4]	<0.0001	38.4	0.78	3.1 [1.7 - 5.5]	0.0001
	APACHE II	232	32	34.2	0.77	2.9 [1.7 - 4.9]	<0.0001	41.4	0.79	3.0 [1.7 - 5.5]	<0.0001
SOFA 8 - 13	PCT	620	172	90.4	0.72	3.8 [2.8 - 5.0]	<0.0001	98.0	0.72	3.2 [2.3 - 4.4]	<0.0001
	CRP	544	153	63.1	0.69	2.6 [2.0 - 3.3]	<0.0001	78.6	0.71	2.4 [1.7 - 2.9]	<0.0001
	Lactate	617	172	81.4	0.70	2.4 [1.9 - 3.1]	<0.0001	97.0	0.72	2.1 [1.6 - 2.7]	<0.0001
	SOFA	620	172	76.2	0.70	2.6 [2.0 - 3.2]	<0.0001	90.7	0.72	2.3 [1.8 - 2.9]	<0.0001
	SAPS II	620	172	87.2	0.71	2.4 [1.9 - 3.1]	<0.0001	97.2	0.72	2.3 [1.8 - 2.9]	<0.0001
	APACHE II	620	172	79.0	0.70	2.5 [1.9 - 3.1]	<0.0001	90.9	0.72	2.3 [1.8 - 2.9]	<0.0001
SOFA ≥14	PCT	155	64	16.3	0.66	2.2 [1.5 - 3.2]	0.0001	27.1	0.69	2.4 [1.5 - 3.9]	0.0001
	CRP	134	52	13.4	0.65	1.9 [1.3 - 2.9]	0.0007	26.9	0.70	2.1 [1.3 - 3.3]	0.0007
	Lactate	155	64	28.9	0.69	1.7 [1.1 - 2.5]	0.0063	38.1	0.71	1.8 [1.1 - 2.8]	0.0068
	SOFA	155	64	15.3	0.65	2.0 [1.3 - 2.9]	0.0004	26.7	0.69	2.1 [1.3 - 3.2]	0.0004
	SAPS II	155	64	17.0	0.65	2.1 [1.4 - 3.1]	0.0001	26.2	0.69	2.2 [1.4 - 3.3]	0.0001
	APACHE II	155	64	15.1	0.64	2.0 [1.3 - 2.9]	0.0002	25.7	0.69	2.1 [1.4 - 3.3]	0.0002

Table 7. Corresponding 28 day SOFA and MR-proADM disease severity groups

	SOFA severity groups			
		Low severity (≤7 points) N = 232, 13.8% mortality	Intermediate severity (≤8 points ≤13) N = 620, 27.7% mortality	High severity (≥14 points) N = 155, 41.3% mortality
MR-proADM severity groups	Low severity (≤2.7 nmol/L) N = 265, 9.8% mortality	N = 111 (41.9%) 7.2% mortality	N = 139 (52.8%) 10.8% mortality	N = 15 (5.7%) 20.0% mortality
	Intermediate severity (<2.7 nmol/L ≤10.9) N = 581, 26.2% mortality	N = 114 (19.6%) 15.8% mortality	N = 394 (68.0%) 27.7% mortality	N = 73 (12.6%) 34.2% mortality
	High severity (>10.9 nmol/L) N = 161 55.9% mortality	N = 7 (4.3%) 85.7% mortality	N = 87 (53.4%) 55.2% mortality	N = 67 (41.6%) 53.7% mortality

MR-proADM: mid-regional proadrenomedullin; SOFA: Sequential Organ Failure Assessment

Table 8. Corresponding 28 day SAPS II and MR-proADM disease severity groups

	SAPS II severity groups			
MR-proADM severity groups		Low severity (≤53 points) N = 235, 11.5% mortality	Intermediate severity (≤54 points ≤79) N = 656, 29.3% mortality	High severity (≥80 points) N = 139, 40.3% mortality
	Low severity (≤2.7 nmol/L) N = 271, 10.3% mortality	N = 108 (39.9%) 7.4% mortality	N = 143 (52.8%) 11.2% mortality	N = 20 (7.4%) 20.0% mortality
	Intermediate severity (<2.7 nmol/L ≤10.9) N = 594, 26.4% mortality	N = 118 (19.9%) 13.6% morality	N = 398 (67.0%) 27.9% mortality	N = 78 (13.1%) 38.5% mortality
	High severity (>10.9 nmol/L) N = 165, 54.5% mortality	N = 9 (5.5%) 33.3% mortality	N = 115 (69.7%) 56.5% mortality	N = 41 (24.8%) 53.7% mortality

MR-proADM: mid-regional proadrenomedullin; SAPS II: Simplified Acute Physiological II

Table 9. Corresponding 28 day APACHE II and MR-proADM disease severity groups

	APACHE II severity groups			
MR-proADM severity groups		Low severity (≤19 points) N = 287, 11.5% mortality	Intermediate severity (≤20 points ≤32) N = 591, 30.3% mortality	High severity (≥33 points) N = 152, 41.4% mortality
	Low severity (≤2.7 nmol/L) N = 271, 10.3% mortality	N = 122 (45.0%) 7.4% mortality	N = 137 (50.6%) 10.9% mortality	N = 12 (4.4%) 33.3% mortality
	Intermediate severity (<2.7 nmol/L ≤10.9) N = 594, 26.4% mortality	N = 154 (25.9%) 12.3% mortality	N = 356 (59.9%) 30.1% mortality	N = 84 (14.1%) 36.9% mortality
	High severity (>10.9 nmol/L) N = 165, 54.5% mortality	N = 11 (6.7%) 45.5% mortality	N = 98 (59.4%) 58.2% mortality	N = 56 (33.9%) 50.0% mortality

MR-proADM: mid-regional proadrenomedullin; APACHE II: Acute Physiological and Chronic Health Evaluation II

Table 10. Corresponding 28 day lactate and MR-proADM disease severity groups

	Lactate severity groups			
MR-proADM severity groups		Low severity (≤1.4 mmol/L) N = 196, 15.8% mortality	Intermediate severity (<1.4 mmol/L ≤6.4) N = 668, 24.1% mortality	High severity (>6.4 mmol/L) N = 158, 52.5% mortality
	Low severity (≤2.7 nmol/L) N = 267, 10.5% mortality	N = 99 (37.1%) 8.1% mortality	N = 154 (57.7%) 9.1% mortality	N = 14 (5.2%) 42.9% mortality
	Intermediate severity (<2.7 nmol/L ≤10.9) N = 591, 26.6% mortality	N = 90 (15.2%) 21.1% mortality	N = 421 (71.2%) 25.2% mortality	N = 80 (13.5%) 40.0% mortality
	High severity (>10.9 nmol/L) N = 164, 54.9% mortality	N = 7 (4.3%) 57.1% mortality	N = 93 (56.7%) 44.1% mortality	N = 64 (39.0%) 70.3% mortality

MR-proADM: mid-regional proadrenomedullin

Table 11. Biomarker and SOFA association with 28 day mortality at days 1, 4, 7 and 10

		Patients (N)	Mortality (N)	AUROC	LR X^2	C-index	HR IQR [95% CI]	p-value	LR X^2	C-index	HR IQR [95% CI]	p-value
Day 1	MR-proADM	993	242	0.76	152.5	0.73	3.3 [2.8 - 4.0]	<0.0001	173.2	0.74	3.2 [2.6 - 4.0]	<0.0001
	PCT	993	242	0.59	23.1	0.59	1.6 [1.3 - 2.0]	<0.0001	74.6	0.65	1.6 [1.3 - 2.0]	<0.0001
	CRP	919	226	0.54	6.2	0.54	0.9 [0.8 - 1.0]	0.0128	61.2	0.65	0.9 [0.8 - 1.0]	<0.0001
	Lactate	1041	265	0.73	206.4	0.72	2.4 [2.2 - 2.7]	<0.0001	253.9	0.75	2.5 [2.2 - 2.8]	<0.0001
	SOFA	1011	260	0.74	143.8	0.72	2.5 [2.2 - 2.9]	<0.0001	192.8	0.75	2.6 [2.2 - 3.0]	<0.0001
Day 4	MR-proADM	777	158	0.76	100.5	0.73	3.2 [2.5 - 4.0]	<0.0001	123.7	0.75	3.0 [2.3 - 3.8]	<0.0001
	PCT	777	158	0.62	22.6	0.61	1.7 [1.4 - 2.1]	<0.0001	69.3	0.68	1.8 [1.4 - 2.2]	<0.0001
	CRP	708	146	0.48	0.7	0.52	1.1 [0.9 - 1.3]	0.3925	45.8	0.65	1.1 [0.9 - 1.4]	<0.0001
	Lactate	803	166	0.69	60.6	0.68	1.8 [1.6 - 2.0]	<0.0001	100.9	0.71	1.7 [1.5 - 2.0]	<0.0001
	SOFA	767	162	0.75	111.5	0.72	3.0 [2.4 - 3.6]	<0.0001	155.9	0.76	3.1 [2.5 - 3.8]	<0.0001
Day 7	MR-proADM	630	127	0.78	93.7	0.76	3.4 [2.6 - 4.3]	<0.0001	117.8	0.76	3.3 [2.5 - 4.3]	<0.0001
	PCT	631	128	0.72	62.3	0.70	2.6 [2.1 - 3.3]	<0.0001	101.6	0.74	2.7 [2.1 - 3.4]	<0.0001
	CRP	583	121	0.56	3.5	0.55	1.3 [1.0 - 1.6]	0.0606	47.1	0.67	1.3 [1.0 - 1.7]	<0.0001
	Lactate	658	138	0.68	69.4	0.68	2.0 [1.7 - 2.3]	<0.0001	112.2	0.73	2.0 [1.7 - 2.4]	<0.0001
	SOFA	617	128	0.75	107.7	0.73	2.7 [2.3 - 3.3]	<0.0001	140.2	0.77	2.8 [2.3 - 3.4]	<0.0001
Day 10	MR-proADM	503	82	0.78	72.6	0.76	4.3 [3.0 - 6.1]	<0.0001	90.9	0.78	3.8 [2.6 - 5.5]	<0.0001
	PCT	503	82	0.75	52.0	0.74	2.8 [2.2 - 3.7]	<0.0001	90.4	0.78	3.1 [2.3 - 4.2]	<0.0001
	CRP	457	80	0.61	10.0	0.60	1.6 [1.2 - 2.2]	<0.0001	51.2	0.71	1.8 [1.3 - 2.6]	<0.0001
	Lactate	516	88	0.61	19.8	0.61	1.6 [1.3 - 2.0]	<0.0001	54.7	0.70	1.6 [1.3 - 2.0]	<0.0001
	SOFA	490	84	0.76	85.8	0.75	3.3 [2.6 - 4.3]	<0.0001	107.8	0.78	3.1 [2.4 - 4.1]	<0.0001

Table 12. Low and high risk severity groups and corresponding mortality rates throughout ICU treatment

		Low severity patient population					High severity patient population				
		Patients (N)	Mortality (N, %)	Optimal cut-off	Sensitivity	Specificity	Patients (N)	Mortality (N, %)	Optimal cut-off	Sensitivity	Specificity
Day 1	MR-proADM	304	24 (7.9%)	2.80	0.90	0.37	162	87 (53.7%)	9.5	0.36	0.90
	PCT	203	25 (12.3%)	1.02	0.90	0.24	115	40 (34.8%)	47.6	0.17	0.90
	CRP	101	32 (31.7%)	99	0.90	0.14	88	18 (4.8%)	373	0.08	0.90
	Lactate	310	33 (10.6%)	1.22	0.88	0.36	185	109 (58.9%)	3.5	0.43	0.89
	SOFA	435	49 (11.3%)	8.0	0.88	0.40	165	87 (52.7%)	14	0.33	0.90
Day 4	MR-proADM	290	16 (5.5%)	2.25	0.90	0.44	120	58 (48.3%)	7.7	0.37	0.90
	PCT	147	16 (10.9%)	0.33	0.90	0.21	87	25 (28.7%)	14.08	0.16	0.90
	CRP	65	9 (13.8%)	32.7	0.90	0.06	51	15 (29.4%)	276.5	0.06	0.90
	Lactate	124	15 (12.1%)	0.89	0.91	0.17	136	65 (47.8%)	2.15	0.39	0.89
	SOFA	213	15 (7.0%)	5.5	0.91	0.33	137	67 (48.9%)	12.75	0.41	0.88
Day 7	MR-proADM	252	14 (5.6%)	2.25	0.89	0.47	104	54 (51.9%)	6.95	0.43	0.90
	PCT	184	14 (7.6%)	0.31	0.89	0.34	85	35 (41.2%)	4.67	0.27	0.90
	CRP	62	12 (19.4%)	27.4	0.90	0.11	69	23 (37.7%)	207	0.19	0.90
	Lactate	104	15 (14.4%)	0.84	0.89	0.17	102	51 (50.0%)	2.10	0.37	0.90
	SOFA	207	16 (7.7%)	5.5	0.88	0.39	91	48 (52.7%)	12.5	0.38	0.91
Day 10	MR-proADM	213	8 (3.8%)	2.25	0.90	0.49	78	35 (44.9%)	7.45	0.43	0.90
	PCT	177	9 (5.1%)	0.30	0.89	0.40	74	32 (43.2%)	2.845	0.39	0.90
	CRP	69	8 (11.6%)	32.1	0.90	0.16	52	14 (26.9%)	204	0.18	0.90
	Lactate	47	7 (14.9%)	0.68	0.92	0.09	65	24 (36.9%)	2.15	0.27	0.90
	SOFA	116	9 (7.8%)	4.5	0.89	0.26	85	42 (49.4%)	11.5	0.50	0.89

Table 13. Mortality and duration of ICU therapy based on MR-proADM concentrations and ICU specific therapies

	Patient severity group	N	SOFA	Length of stay (days)	28 day mortality (N, %)	90 day mortality (N, %)
Day 4	Total patient population	777	8.4 (4.3)	16 [10 - 27]	158 (20.3%)	256 (33.9%)
	Clinically stable	145	4.5 (2.4)	8 [6 - 11]	10 (6.9%)	22 (15.8%)
	Clinically stable and low MR-proADM	79	3.6 (1.5)	8 [7 - 10]	0 (0.0%)	1 (1.4%)
	Actual day 4 discharges*	43	3.6 (2.1)	-	1 (2.3%)	4 (10.0%)
Day 7	Total patient population	630	8.0 (4.2)	19 [13 - 31]	127 (20.2%)	214 (34.9%)
	Clinically stable	124	3.9 (1.7)	11.5 [9 - 16]	9 (7.3%)	17 (13.9%)
	Clinically stable and low MR-proADM	78	3.4 (1.6)	11 [9 - 14]	1 (1.3%)	4 (5.3%)
	Actual day 7 discharges*	36	3.6 (2.6)	-	2 (5.6%)	5 (13.9%)
Day 10	Total patient population	503	7.6 (4.0)	23.5 [17 - 34.25]	82 (16.3%)	159 (32.6%)
	Clinically stable	85	3.5 (1.8)	15 [13 - 22]	9 (10.6%)	14 (17.3%)
	Clinically stable and low MR-proADM	57	3.2 (1.3)	14 [12.25 - 19]	1 (1.8%)	2 (3.8%)
	Actual day 10 discharges*	29	4.0 (2.6)	-	5 (17.2%)	7 (24.1%)

* excludes same or next day mortalities

Table 14. Time dependent Cox regressions for single and cumulative additions of MR-proADM

	Univariate model					Multivariate model				
Addition of single days to baseline values	LR χ^2	DF	Added LR χ^2	Added DF	p-value	LR χ^2	DF	Added LR χ^2	Added DF	p-value
MR-proADM baseline	144.2	1	Reference			163.0	10	Reference		
+ Day 1	169.8	2	25.6	1	<0.001	190.6	11	27.6	1	<0.001
+ Day 4	161.9	2	17.7	1	<0.001	180.4	11	17.4	1	<0.001
+ Day 7	175.7	2	31.5	1	<0.001	195.1	11	32.1	1	<0.001
+ Day 10	179.8	2	35.6	1	<0.001	197.9	11	34.9	1	<0.001
Addition of consecutive days to baseline values										
MR-proADM baseline	144.2	1	Reference			163.0	10	Reference		
+ Day 1	169.8	2	25.6	1	<0.001	190.6	11	27.6	1	<0.001
+ Day 1 + Day 4	174.9	3	5.1	1	0.0243	195.4	12	4.8	1	0.0280
+ Day 1 + Day 4 + Day 7	188.7	4	13.9	1	<0.001	210.4	13	15.0	1	<0.001
+ Day 1 + Day 4 + Day 7 + Day 10	195.2	5	6.5	1	0.0111	216.6	14	6.2	1	0.0134

MR-proADM: mid-regional proadrenomedullin; DF: Degrees of Freedom

Table 15. 28 and 90 day mortality rates following PCT and MR-proADM kinetics

	Biomarker Kinetics		28 day mortality			90 day mortality		
	Baseline	Day 1	N	%	HR IQR [95% CI]	N	%	HR IQR [95% CI]
PCT decrease ≥20%			458	18.3%		447	28.2%	
MR-proADM severity level	Low	Low	125	5.6%	3.6 [1.6 - 8.1]*	121	13.2%	2.7 [1.6 - 4.8]*
	Intermediate	Intermediate	204	19.1%	5.3 [3.0 - 9.3]**	201	32.3%	3.8 [2.3 – 6.3]**
	High	High	27	66.7%	19.1 [8.0 - 45.9]***	27	70.4%	10.4 [5.3 - 20.2]***
	Increasing							
	Low	Intermediate	2	50.0%	-	2	50.0%	-
	Intermediate	High	10	40.0%	2.5 [0.9 - 7.0]††	10	50.0%	1.9 [0.8 - 4.8]††
	Decreasing							
	High	Intermediate	30	36.7%	0.4 [0.2 - 0.9]‡	29	44.8%	0.5 [0.2 - 0.9]‡
	High	Low	-	-	-	-	-	-
	Intermediate	Low	60	8.3%	0.4 [0.2 - 1.0]‡‡	57	12.3%	0.3 [0.2 - 0.7]‡‡
PCT decrease <20%			522	29.7%		508	42.5%	
MR-proADM severity level	Low	Low	106	10.4%	3.1 [1.7 - 5.9]*	105	16.2%	3.2 [1.9 - 5.3]*
	Intermediate	Intermediate	229	29.7%	2.0 [1.3 - 2.9]**	221	43.4%	1.9 [1.3 - 2.6]**
	High	High	77	49.4%	6.2 [3.2 - 12.2]***	75	64.0%	5.9 [3.4 - 10.3]***
	Increasing							
	Low	Intermediate	29	17.2%	1.8 [0.6 - 5.2]†	27	44.4%	3.2 [1.5 - 6.7]†
	Intermediate	High	45	53.3%	2.3 [1.4 - 3.6]††	45	68.9%	2.1 [1.4 - 3.2]††
	Decreasing							
	High	Intermediate	11	54.5%	-	11	72.7%	-
	High	Low	1	0.0%	-	1	100.0 %	-
	Intermediate	Low	24	12.5%	0.4 [0.1 - 1.2]‡‡	23	13.0%	0.2 [0.1 - 0.8]‡‡

Hazard ratios for patients with: * continuously intermediate vs. low values; ** continuously high vs. intermediate values *** continuously high vs. low values; † Increasing low to intermediate vs. continuously low values; †† Increasing intermediate to high vs. continuously intermediate values; ‡ decreasing high to intermediate vs. continuously high values; ‡‡ Decreasing intermediate to low vs. increasing intermediate to high values. Kaplan Meier plots illustrate either individual patient subgroups, or grouped increasing or decreasing subgroups.

Table 16. Mortality rates following changes in PCT concentrations and MR-proADM severity levels

			7 day mortality			ICU mortality			Hospital mortality		
	Baseline	Day 1	N	%	HR IQR [95% CI]	N	%	HR IQR [95% CI]	N	%	HR IQR [95% CI]
PCT decrease ≥20%			461	6.1 %		456	16.7 %		439	24.1 %	
MR-proADM severity level	Low	Low	126	2.4 %	1.9 [0.5 – 6.9]*	126	4.8 %	3.9 [1.6 – 9.6]*	123	7.3 %	4.9 [2.3 - 10.3]*
	Intermediate	Intermediate	205	4.4 %	8.2 [3.4 - 21.2]**	202	16.3 %	8.7 [3.7 - 20.7]**	194	27.8 %	6.2 [2.5 - 14.9]**
	High	High	27	29.6 %	15.2 [4.0 - 57.3]***	27	63.0 %	34.0 [11.0 - 105.5]***	27	70.4 %	30.1 [10.3 - 87.6]***
	Increasing										
	Low	Intermediate	3	0.0 %	-	2	0.0 %	-	2	0.0 %	-
	Intermediate	High	10	20.0 %	4.7 [1.0 - 21.6]††	10	30.0 %	2.2 [0.5 – 8.9]††	10	50.0 %	2.6 [0.7 - 9.3]††
	Decreasing										
	High	Intermediate	30	16.7 %	0.5 [0.2 - 1.6]‡	29	37.9 %	0.4 [0.1 - 1.1]‡	28	46.4 %	0.4 [0.1 - 1.1]‡
	Intermediate	Low	60	1.7 %	0.4 [0.0 - 3.0]‡‡	59	10.2 %	0.6 [0.1 – 1.5]‡‡	55	10.9 %	0.3 [0.1 - 0.8]‡‡
PCT decrease <20%			526	13.7 %		517	30.2 %		493	36.9 %	
MR-proADM severity level	Low	Low	107	5.6 %	2.0 [0.8 - 4.9]*	107	10.3 %	3.4 [1.7 - 6.8]*	102	13.7 %	3.6 [1.9 - 6.8]*
	Intermediate	Intermediate	230	10.9 %	2.6 [1.5 – 4.7]**	225	28.0 %	3.0 [1.8 - 5.2]**	216	36.6 %	2.4 [1.4 - 4.2]**
	High	High	77	26.0 %	5.3 [2.1 - 13.2]***	74	54.1 %	10.3 [4.7 - 22.3]***	72	58.3 %	8.8 [4.2 - 18.3]***
	Increasing										
	Low	Intermediate	30	13.3 %	2.5 [0.7 – 8.9]†	29	31.0 %	3.9 [1.4 - 10.7]†	27	37.0 %	3.7 [1.4 - 9.7]†
	Intermediate	High	46	28.3 %	3.0 [1.5 – 5.8]††	45	57.8 %	3.3 [1.7 - 6.4]††	43	65.1 %	3.2 [1.6 - 6.4]††
	Decreasing										
	High	Intermediate	11	36.6 %	0.5 [0.2 - 1.6]‡	11	54.5 %	1.0 [0.3 - 3.7]‡	10	80.0 %	-
	High	Low	1	0.0 %	-	1	0.0 %	-	1	0.0 %	-
	Intermediate	Low	24	0.0 %	?	24	4.2 %	0.1 [0.0 - 0.8]‡‡	22	4.5 %	0.1 [0.0 - 0.6]‡‡

Hazard ratios for patients with: * continuously intermediate vs. low values; ** continuously high vs. intermediate values *** continuously high vs. low values; † increasing low to intermediate vs. continuously low values; †† increasing intermediate to high vs. continuously intermediate values; ‡ decreasing high to intermediate vs. continuously high values; ‡‡ decreasing intermediate to low vs. continuously intermediate values

Table 17. 28 and 90 day mortality rates following changes in PCT concentrations and MR-proADM severity levels

	Biomarker Kinetics		28 day mortality			90 day mortality		
	Baseline	Day 4	N	%	HR IQR [95% CI]	N	%	HR IQR [95% CI]
PCT decrease ≥ 50%			557	17.1%		542	29.3%	
MR-proADM severity level	Low	Low	111	1.8%	11.2 [2.7 - 46.4]*	107	7.5%	5.3 [2.5 - 10.9]*
	Intermediate	Intermediate	209	18.7%	3.8 [2.3 - 6.5]**	206	33.5%	3.3 [2.1 - 5.1]**
	High	High	39	53.8%	43.1 [10.1 - 184.0]***	39	71.8%	17.4 [7.9 - 38.2]***
	Increasing							
	Low	Intermediate	24	25.0%	15.6 [3.1 - 77.2]†	24	41.7%	7.1 [2.8 - 17.9]†
	Intermediate	High	23	43.5%	2.6 [1.3 - 5.3]††	23	65.2%	2.6 [1.5 - 4.5]††
	Decreasing							
	High	Intermediate	42	21.4%	0.3 [0.1 - 0.7]‡	41	36.6%	0.3 [0.2 - 0.6]‡
	High	Low	3	0.0%	-	2	50.0%	-
	Intermediate	Low	105	7.6%	0.4 [0.2 - 0.8]‡‡	100	13.0%	0.3 [0.2 - 0.6]‡‡
PCT decrease < 50%			210	29.5%		203	45.5%	
MR-proADM severity level	Low	Low	56	7.1%	6.3 [2.2 - 18.1]*	55	12.7%	6.2 [2.8 - 13.9]*
	Intermediate	Intermediate	70	38.6%	1.5 [0.8 - 3.0]**	68	57.4%	1.3 [0.7 - 2.3]**
	High	High	23	52.2%	9.5 [3.1 - 29.5]***	22	63.6%	7.9 [3.2 - 19.5]***
	Increasing							
	Low	Intermediate	17	17.6%	2.8 [0.6 - 12.5]†	15	53.3%	5.5 [2.0 - 15.2]†
	Low	High	4	0.0%	-	4	25.0%	-
	Intermediate	High	30	46.7%	1.4 [0.7 - 2.6]††	30	66.7%	1.3 [0.8 - 2.2]††
	Decreasing							
	High	Intermediate	-	-	-	-	-	-
	High	Low	-	-	-	-	-	-
	Intermediate	Low	10	20.0%	-	9	33.4%	-

Hazard ratios for patients with: * continuously intermediate vs. low values; ** continuously high vs. intermediate values *** continuously high vs. low values; † Increasing low to intermediate vs. continuously low values; †† Increasing intermediate to high vs. continuously intermediate values; ‡ decreasing high to intermediate vs. continuously high values; ‡‡ Decreasing intermediate to low vs. continuously intermediate values

Table 18. ICU and hospital mortality rates following changes in PCT concentrations and MR-proADM severity levels

			ICU mortality			Hospital mortality		
	Baseline	Day 4	N	%	HR IQR [95% CI]	N	%	HR IQR [95% CI]
PCT decrease ≥ 50%			555	16.8 %		532	24.1 %	
MR-proADM severity level	Low	Low	114	2.6%	6.9 [2.1 - 23.1]*	109	2.8%	13.3 [4.1 - 43.8]*
	Intermediate	Intermediate	208	15.9 %	8.1 [3.8 - 17.2]**	197	27.4 %	5.1 [2.4 - 10.7]**
	High	High	38	60.5 %	56.2 [15.0 - 210.2]***	38	65.8 %	67.9 [18.0 - 256.6]***
	Low	Intermediate	24	29.2 %	15.1 [3.6 - 64.1]†	24	33.3 %	17.7 [4.2 - 73.6]†
	Intermediate	High	23	43.5 %	4.1 [1.7 - 10.0]††	23	56.5 %	3.4 [1.4 - 8.3]††
	High	Intermediate	41	22.0 %	0.2 [0.1 - 0.5]‡	39	33.3 %	1.3 [0.6 - 2.7]‡
	High	Low	3	0.0%	-	2	50.0 %	-
	Intermediate	Low	103	8.7%	0.5 [0.2 - 1.0]‡‡	99	11.1 %	0.3 [0.2 - 0.7]‡‡
PCT decrease < 50%			204	28.9 %		194	30.4 %	
MR-proADM severity level	Low	Low	56	1.8%	28.1 [3.7 - 216.3]*	54	7.4%	10.1 [3.3 - 31.2]*
	Intermediate	Intermediate	68	33.8 %	1.8 [0.7 - 4.8]**	65	44.6 %	1.9 [0.7 - 5.2]**
	High	High	21	47.6 %	50.0 [5.8 - 431.5]***	20	60.0 %	18.8 [4.8 - 72.7]***
	Low	Intermediate	16	43.7 %	42.8 [4.7 - 390.2]†	14	57.1 %	16.7 [3.8 - 72.4]†
	Low	High	4	0.0%	-	4	25.0 %	-
	Intermediate	High	29	58.6 %	2.8 [1.1 - 6.8]††	28	64.3 %	2.2 [0.9 - 5.6]††
	High	Intermediate	-	-		-	-	-
	High	Low	-	-		-	-	-
	Intermediate	Low	10	10.0 %	-	9	33.3 %	-

Hazard ratios for patients with: * continuously intermediate vs. low values; ** continuously high vs. intermediate values *** continuously high vs. low values; † Increasing low to intermediate vs. continuously low values; †† Increasing intermediate to high vs. continuously intermediate values; ‡ decreasing high to intermediate vs. continuously high values; ‡‡ Decreasing intermediate to low vs. continuously intermediate values

Table 19. Influence of infectious origin on 28 day mortality prediction

					Univariate				Multivariate			
		Patients (N)	Mortality (N)	AUROC	LR χ^2	C-index	HR IQR [95% CI]	p-value	LR χ^2	C-index	HR IQR [95% CI]	p-value
Pneumological	MR-proADM	313	83	0.72	37.9	0.69	2.7 [2.0 - 3.7]	<0.0001	45.1	0.71	2.5 [1.7 - 3.6]	<0.0001
	PCT	313	83	0.59	6.4	0.58	1.6 [1.1 - 2.2]	0.0112	26.0	0.66	1.5 [1.1 - 2.2]	0.0038
	CRP	267	65	0.46	0.8	0.53	0.9 [0.7 - 1.1]	0.3754	14.7	0.63	0.9 [0.7 - 1.1]	0.1422
	Lactate	322	86	0.61	12.6	0.61	1.6 [1.2 - 2.1]	0.0004	30.1	0.67	1.5 [1.1 - 2.0]	0.0008
	SOFA	315	83	0.63	12.4	0.62	1.7 [1.3 - 2.3]	0.0004	29.6	0.68	1.6 [1.1 - 2.2]	0.0010
	SAPS II	324	86	0.63	13.2	0.62	1.6 [1.3 - 2.1]	0.0003	28.8	0.67	1.5 [1.1 - 1.9]	0.0014
	APACHE II	324	86	0.63	19.5	0.64	1.9 [1.4 - 2.5]	<0.0001	33.4	0.68	1.7 [1.3 - 2.3]	0.0002
Intraabdominal	MR-proADM	238	58	0.78	47.4	0.75	4.5 [2.9 - 7.1]	<0.0001	55.7	0.76	4.8 [2.9 - 8.0]	<0.0001
	PCT	238	58	0.52	0.4	0.52	1.1 [0.8 - 1.7]	0.5249	15.0	0.64	1.2 [0.8 - 1.9]	0.1312
	CRP	233	59	0.48	0.1	0.53	1.0 [0.8 - 1.3]	0.7807	12.0	0.62	1.1 [0.8 - 1.4]	0.2864
	Lactate	249	62	0.67	18.0	0.66	2.2 [1.5 - 3.0]	<0.0001	28.2	0.70	2.1 [1.5 - 3.0]	0.0017
	SOFA	248	62	0.66	8.9	0.63	1.5 [1.2 - 2.0]	0.0029	18.3	0.64	1.5 [1.1 - 2.0]	0.0494
	SAPS II	252	62	0.68	17.9	0.66	1.9 [1.4 - 2.6]	<0.0001	24.3	0.67	1.9 [1.3 - 2.6]	0.0069
	APACHE II	252	62	0.68	14.6	0.65	1.8 [1.3 - 2.3]	0.0001	20.6	0.66	1.6 [1.2 - 2.2]	0.0241

MR-proADM AUROC values are significantly greater than all other parameters apart from APACHE II in pneumological origins of infection.

Table 20. Influence of microbial species on 28 day mortality prediction

					Univariate				Multivariate			
		Patients (N)	Mortality (N)	AUROC	LR χ^2	C-index	HR IQR [95% CI]	p-value	LR χ^2	C-index	HR IQR [95% CI]	p-value
Gram positive	MR-proADM	141	33	0.82	37.2	0.81	5.0 [2.9 - 8.6]	<0.0001	50.0	0.84	5.0 [2.7 - 9.2]	<0.0001
	PCT	142	33	0.64	7.9	0.64	2.4 [1.3 - 4.4]	0.0050	30.3	0.76	3.0 [1.5 - 5.7]	0.0008
	CRP	131	31	0.54	0.2	0.51	0.9 [0.7 - 1.3]	0.6561	19.8	0.71	1.0 [0.7 - 1.4]	0.0309
	Lactate	143	33	0.75	28.9	0.74	4.6 [2.6 - 8.1]	<0.0001	44.9	0.83	5.0 [2.6 - 9.7]	<0.0001
	SOFA	143	32	0.66	8.8	0.65	1.9 [1.3 - 2.8]	0.0031	31.8	0.76	2.7 [1.6 - 4.6]	0.0004
	SAPS II	146	33	0.72	16.8	0.71	2.9 [1.7 - 4.7]	<0.0001	28.4	0.76	2.7 [1.5 - 4.9]	0.0016
	APACHE II	146	33	0.73	17.3	0.71	2.4 [1.6 - 3.5]	<0.0001	33.1	0.77	2.8 [1.7 - 4.7]	0.0003
Gram negative	MR-proADM	124	35	0.69	12.1	0.68	2.3 [1.4 - 3.8]	0.0005	26.0	0.75	2.2 [1.2 - 3.8]	0.0037
	PCT	124	35	0.54	0.6	0.54	1.2 [0.7 - 2.1]	0.4580	17.8	0.67	1.2 [0.7 - 2.3]	0.0580
	CRP	110	30	0.57	0.4	0.56	1.2 [0.7 - 1.8]	0.5255	17.1	0.68	1.4 [0.9 - 2.2]	0.0727
	Lactate	131	37	0.65	10.0	0.64	1.9 [1.3 - 2.8]	0.0016	23.4	0.71	1.7 [1.1 - 2.7]	0.0093
	SOFA	129	37	0.65	9.0	0.64	1.8 [1.2 - 2.7]	0.0027	25.5	0.72	1.9 [1.2 - 2.9]	0.0045
	SAPS II	132	37	0.67	9.9	0.65	1.9 [1.3 - 2.8]	0.0017	25.1	0.71	1.9 [1.2 - 3.0]	0.0051
	APACHE II	132	37	0.69	7.9	0.66	1.7 [1.2 - 2.4]	0.0049	22.3	0.70	1.7 [1.1 - 2.6]	0.0139
Fungal	MR-proADM	50	14	0.74	7.9	0.69	2.5 [1.3 - 4.9]	0.0051	14.4	0.78	3.4 [1.1 - 10.7]	0.1548
	PCT	50	14	0.46	0.3	0.52	1.3 [0.5 - 3.0]	0.6104	8.5	0.72	1.1 [0.4 - 3.0]	0.5792
	CRP	43	12	0.65	0.6	0.65	0.8 [0.5 - 1.3]	0.4404	14.7	0.81	0.5 [0.2 - 1.2]	0.1427
	Lactate	51	14	0.60	2.7	0.59	2.0 [0.9 - 4.7]	0.1032	13.2	0.74	3.3 [1.0 - 11.0]	0.2128
	SOFA	49	12	0.54	0.8	0.54	1.4 [0.7 - 2.8]	0.3668	7.1	0.73	1.1 [0.5 - 2.8]	0.7164
	SAPS II	51	14	0.60	2.2	0.60	1.5 [0.9 - 2.6]	0.1412	10.0	0.75	1.4 [0.7 - 2.8]	0.4427
	APACHE II	51	14	0.62	1.6	0.62	1.6 [0.8 - 3.3]	0.2053	10.1	0.76	1.7 [0.7 - 4.4]	0.4321

Table 21. Influence of mode of ICU entry on 28 day mortality prediction

					Univariate				Multivariate			
		Patients (N)	Mortality (N)	AUROC	LR χ^2	C-index	HR IQR [95% CI]	p-value	LR χ^2	C-index	HR IQR [95% CI]	p-value
Operative	MR-proADM	466	113	0.77	87.4	0.75	4.1 [3.0 - 5.6]	<0.0001	106.4	0.77	3.8 [2.8 - 5.3]	<0.0001
	PCT	466	113	0.60	11.8	0.59	1.6 [1.2 - 2.2]	0.0006	53.1	0.70	1.7 [1.3 - 2.4]	<0.0001
	CRP	421	106	0.48	1.2	0.52	1.1 [0.9 - 1.4]	0.2696	39.7	0.68	1.2 [0.9 - 1.4]	<0.0001
	Lactate	483	120	0.68	46.4	0.67	2.4 [1.9 - 3.1]	<0.0001	73.7	0.71	2.3 [1.8 - 3.0]	<0.0001
	SOFA	482	118	0.68	34.9	0.65	2.0 [1.6 - 2.4]	<0.0001	65.7	0.71	2.0 [1.6 - 2.5]	<0.0001
	SAPS II	489	120	0.71	50.5	0.68	2.2 [1.8 - 2.7]	<0.0001	65.9	0.70	2.0 [1.6 - 2.5]	<0.0001
	APACHE II	489	120	0.71	47.8	0.68	2.3 [1.8 - 2.8]	<0.0001	64.8	0.71	2.0 [1.6 - 2.5]	<0.0001
Non-operative	MR-proADM	448	132	0.70	48.6	0.68	2.6 [2.0 - 3.4]	<0.0001	56.5	0.69	2.4 [1.8 - 3.3]	<0.0001
	PCT	449	132	0.52	0.8	0.52	1.1 [0.9 - 1.5]	0.3644	24.4	0.62	1.1 [0.8 - 1.4]	0.0066
	CRP	424	121	0.50	0.2	0.49	1.0 [0.8 - 1.2]	0.6280	23.6	0.62	1.0 [0.8 - 1.2]	0.0088
	Lactate	462	137	0.62	24.5	0.62	1.9 [1.5 - 2.4]	<0.0001	43.7	0.67	1.8 [1.4 - 2.3]	<0.0001
	SOFA	450	132	0.62	15.9	0.61	1.7 [1.3 - 2.1]	0.0001	39.5	0.66	1.7 [1.3 - 2.2]	<0.0001
	SAPS II	466	137	0.65	25.4	0.64	1.6 [1.3 - 1.9]	<0.0001	43.4	0.66	1.5 [1.3 - 1.8]	<0.0001
	APACHE II	466	137	0.64	23.9	0.63	1.7 [1.4 - 2.1]	<0.0001	40.2	0.66	1.6 [1.3 - 2.0]	<0.0001
Elective	MR-proADM	116	30	0.71	12.1	0.69	2.8 [1.6 - 5.2]	0.0005	17.3	0.72	2.3 [1.2 - 4.5]	0.0440
	PCT	116	30	0.59	3.3	0.59	1.6 [1.0 - 2.6]	0.0675	15.1	0.70	1.7 [1.0 - 2.8]	0.0873
	CRP	91	24	0.51	0.0	0.50	1.0 [0.7 - 1.4]	0.8650	11.5	0.70	0.8 [0.5 - 1.3]	0.3219
	Lactate	121	32	0.63	9.5	0.63	2.2 [1.4 - 3.6]	0.0020	21.0	0.72	2.2 [1.3 - 3.6]	0.0211
	SOFA	119	32	0.58	0.9	0.56	1.2 [0.9 - 1.6]	0.3476	13.7	0.69	1.0 [0.7 - 1.3]	0.1860
	SAPS II	121	32	0.60	1.4	0.59	1.3 [0.9 - 1.9]	0.2333	13.1	0.68	0.9 [0.6 - 1.5]	0.2177
	APACHE II	121	32	0.57	1.1	0.57	1.3 [0.8 - 1.9]	0.2945	13.1	0.69	0.9 [0.6 - 1.5]	0.2164

Table 22. Baseline biomarker and clinical score correlation with SOFA at baseline and day 1

	Baseline SOFA			Day 1 SOFA		
	Patients (N)	Correlation [95% CI]	p-value	Patients (N)	Correlation [95% CI]	p-value
MR-proADM	1007	0.46 [0.41 - 0.51]	<0.0001			
MR-proADM*	969	0.47 [0.41 - 0.51]	<0.0001	969	0.57 [0.52 - 0.61]	<0.0001
PCT	1007	0.23 [0.17 - 0.29]	<0.0001	969	0.22 [0.16 - 0.28]	<0.0001
CRP	918	0.06 [0.00 - 0.13]	0.0059	885	0.04 [0.00 - 0.12]	0.2709
Lactate	1044	0.33 [0.27 - 0.38]	<0.0001	1005	0.40 [0.35 - 0.45]	<0.0001
SAPS II	1051	0.60 [0.56 - 0.64]	<0.0001	1011	0.50 [0.45 - 0.54]	<0.0001
APACHE II	1051	0.62 [0.58 - 0.65]	<0.0001	1011	0.53 [0.48 - 0.57]	<0.0001

* using the same patients on baseline as on day 1

Table 23. Baseline MR-proADM correlations with SOFA subscores on baseline and day 1

	Baseline SOFA			Day 1 SOFA		
SOFA subscore	Patients (N)	Correlation [95% CI]	p-value	Patients (N)	Correlation [95% CI]	p-value
Circulation	1022	0.18 [0.12 - 0.23]	<0.0001	995	0.23 [0.17 - 0.29]	<0.0001
Pulmonary	1025	0.12 [0.06 - 0.18]	<0.0001	994	0.15 [0.09 - 0.21]	<0.0001
Coagulation	1028	0.30 [0.25 - 0.36]	<0.0001	1002	0.40 [0.35 - 0.45]	<0.0001
Renal	1030	0.50 [0.45 - 0.54]	<0.0001	1001	0.62 [0.58 - 0.66]	<0.0001
Liver	1014	0.20 [0.14 - 0.26]	<0.0001	993	0.36 [0.30 - 0.40]	<0.0001
CNS	1030	0.03 [-0.03 - 0.09]	0.3856	1003	0.08 [0.02 - 0.14]	0.0089

Table 24. Biomarker correlations with SOFA scores throughout ICU treatment

		MR-proADM	PCT	CRP	Lactate
Day 1	Patients (N)	960	960	894	1008
	Correlation [95% CI]	0.51 [0.46 - 0.55]	0.24 [0.18 - 0.30]	-0.04 [-0.10 - 0.03]	0.48 [0.43 - 0.53]
	p-value	<0.0001	<0.0001	<0.0001	<0.0001
Day 4	Patients (N)	729	729	667	754
	Correlation [95% CI]	0.58 [0.53 - 0.63]	0.13 [0.06 - 0.20]	0.14 [0.06 - 0.21]	0.36 [0.29 - 0.42]
	p-value	<0.0001	0.0003	0.0004	<0.0001
Day 7	Patients (N)	580	581	547	612
	Correlation [95% CI]	0.58 [0.53 - 0.64]	0.05 [-0.03 - 0.13]	0.15 [0.07 - 0.23]	0.43 [0.37 - 0.50]
	p-value	<0.0001	0.2368	0.0004	<0.0001
Day 10	Patients (N)	473	473	429	483
	Correlation [95% CI]	0.65 [0.59 - 0.70]	0.28 [0.20 - 0.37]	0.13 [0.03 - 0.22]	0.34 [0.26 - 0.42]
	p-value	<0.0001	<0.0001	0.0076	<0.0001

Table 25. Mortalities based on MR-proADM severities and increasing or decreasing PCT concentrations - Baseline to day 1

			28 day mortality		90 day mortality		7 day mortality		ICU mortality		Hospital mortality	
	Baseline	Day 1	N	%	N	%	N	%	N	%	N	%
Decreasing PCT			657	19.0 %	636	28.9%	657	6.4%	650	11.6 %	623	25.2
MR-proADM severity level	Low	Low	161	5.0%	157	14.0%	163	2.5%	162	5.6%	157	8.3%
	Intermediate	Intermediate	314	19.1 %	308	31.8%	316	4.7%	310	17.1 %	299	27.8 %
	High	High	51	58.8 %	50	64.0%	51	23.5 %	51	54.9 %	49	63.3 %
	Increasing											
	Low	Intermediate	10	20.0 %	10	30.0%	11	0.0%	11	18.2 %	10	20.0 %
	Intermediate	High	17	35.3 %	17	41.2%	17	17.6 %	17	29.4 %	17	41.2 %
	Decreasing											
	High	Intermediate	35	40.0 %	34	47.1%	35	20.0 %	34	41.2 %	32	50.0 %
	High	Low	-	-	-	-	-	-	-	-	-	-
	Intermediate	Low	63	7.9%	60	10.0%	63	1.6%	63	7.9%	58	8.6%
Increasing PCT			329	35.0 %	319	46.6%	331	17.5 %	324	35.8 %	31	42.3 %
MR-proADM severity level	Low	Low	66	13.6 %	65	15.4%	66	7.6%	66	10.6 %	64	14.1 %
	Intermediate	Intermediate	131	36.6 %	126	51.6%	131	14.5 %	128	35.2 %	122	42.6 %
	High	High	53	49.1 %	52	67.3%	53	20.2 %	50	58.0 %	50	60.0 %
	Increasing											
	Low	Intermediate	25	20.0 %	23	47.8%	26	15.4 %	25	32.0 %	23	39.1 %
	Low	High	-	-	-	-	-	-	-	-	-	-
	Intermediate	High	38	57.9 %	38	76.3%	39	30.8 %	39	61.5 %	36	72.2 %
	Decreasing											
	High	Intermediate	6	50.0 %	6	66.7%	6	33.3 %	6	50.0 %	6	83.3 %
	High	Low	1	0.0%	1	100.0 %	1	0.0%	1	0.0%	1	0.0%
	Intermediate	Low	9	22.2 %	8	25.0%	9	0.0%	9	0.0%	8	0.0%

Table 26. PCT kinetics from baseline to day 1 – development of new infections over days 1,2,3,4.

			New infections over Days 1, 2, 3, 4	
	Baseline	Day 1	N	%
Decreasing PCT			652	9.7%
MR-proADM severity level	Low	Low	161	6.8%
	Intermediate	Intermediate	315	11.7%
	High	High	51	11.8%
	Increasing			
	Low	Intermediate	10	0.0%
	Intermediate	High	17	5.9%
	Decreasing			
	High	Intermediate	34	8.8%
	High	Low	-	-
	Intermediate	Low	63	7.9%
Increasing PCT			329	18.5%
MR-proADM severity level	Low	Low	66	9.1%
	Intermediate	Intermediate	131	18.3%
	High	High	53	22.6%
	Increasing			
	Low	Intermediate	25	24.0%
	Low	High	-	-
	Intermediate	High	38	18.4%
	Decreasing			
	High	Intermediate	6	50.0%
	High	Low	1	0.0%
	Intermediate	Low	9	33.3%

Table 27. PCT kinetics from baseline to day 4 – development of new infections over days 4,5,6,7.

			New infections over Days 4, 5, 6, 7	
	Baseline	Day 4	N	%
Decreasing PCT			681	14.5%
MR-proADM severity level	Low	Low	144	8.3%
	Intermediate	Intermediate	256	17.6%
	High	High	57	28.1%
	Increasing			
	Low	Intermediate	31	22.6%
	Intermediate	High	36	13.9%
	Decreasing			
	High	Intermediate	42	11.9%
	High	Low	3	0.0%
	Intermediate	Low	111	8.1%

Table 28. PCT kinetics from baseline to day 1 – requirement for focus cleaning over days 1,2,3,4.

			Focus cleaning events over days 1, 2, 3, 4	
	Baseline	Day 1	N	%
Increasing PCT			329	21.0%
MR-proADM severity level	Low	Low	57	10.5%
	Intermediate	Intermediate	113	20.4%
	High	High	58	19.0%
	Increasing			
	Low	Intermediate	31	32.3%
	Low	High	3	33.3%
	Intermediate	High	59	28.8%
	Decreasing			
	High	Intermediate	1	0.0%
	High	Low	1	100.0%
	Intermediate	Low	6	0.0%

Table 29. PCT kinetics from baseline to day 4 – requirement for focus cleaning over days 4,5,6,7.

			Focus cleaning events over days 4, 5, 6, 7	
	Baseline	Day 4	N	%
Decreasing PCT			681	22.0%
MR-proADM severity level	Low	Low	144	16.7%
	Intermediate	Intermediate	256	24.2%
	High	High	57	31.6%
	Increasing			
	Low	Intermediate	31	32.3%
	Intermediate	High	36	50.0%
	Decreasing			
	High	Intermediate	42	16.7%
	High	Low	3	0.0%
	Intermediate	Low	111	9.9%

Table 30. PCT kinetics from baseline to day 1 – requirement of emergency surgery over days 1,2,3,4.

			Emergency surgery requirement over days 1, 2, 3, 4	
	Baseline	Day 1	N	%
Increasing PCT			329	23.7%
MR-proADM severity level	Low	Low	66	18.2%
	Intermediate	Intermediate	131	26.0%
	High	High	53	28.3%
	Increasing			
	Low	Intermediate	25	16.0%
	Low	High	-	-
	Intermediate	High	38	31.6%
	Decreasing			
	High	Intermediate	6	0.0%
	High	Low	1	100.0%
	Intermediate	Low	9	0.0%

Table 31. Increasing PCT from baseline to day 1 – antibiotic changes on day 4

Increasing PCT			259	21.6%
MR-proADM severity level	Low	Low	55	5.5%
	Intermediate	Intermediate	106	27.4%
	High	High	39	25.6%
	Increasing			
	Low	Intermediate	20	25.0%
	Intermediate	High	26	26.9%
	Decreasing			
	High	Intermediate	5	20.0%
	High	Low	1	100.0%
	Intermediate	Low	7	0.0%

Table 32. Increasing PCT from baseline to day 4 – antibiotic changes on day 4

Increasing PCT			85	23.5%
MR-proADM severity level	Low	Low	23	8.7%
	Intermediate	Intermediate	22	36.4%
	High	High	5	20.0%
	Increasing			
	Low	Intermediate	10	20.0%
	Intermediate	High	17	41.2%
	Low	High	4	0.0%
	Decreasing			
	High	Intermediate	-	-
	High	Low	-	-
	Intermediate	Low	4	0.0%

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CLAIMS

1. A method for antibiotic therapy guidance in a patient diagnosed as being critically ill with sepsis, severe sepsis, septic shock, or both sepsis and septic shock and receiving treatment with one or more antibiotic agents, the method comprising
   - isolating a first blood, serum, or plasma sample from said patient,
   - isolating a second blood, serum, or plasma sample from said patient at a time point after isolating the first sample and initiating antibiotic treatment,
   - determining levels of procalcitonin (PCT) or fragment(s) thereof comprising at least 12 amino acids in the first and the second sample, and
   - determining a level of proadrenomedullin (proADM) or fragment(s) thereof in at least the second sample,
   - wherein

     the levels of PCT or fragment(s) thereof comprising at least 12 amino acids in said first and second samples are compared to a reference level and/or compared to each other to determine a change in the level of PCT or fragment(s) thereof comprising at least 12 amino acids in the second sample in comparison to the first sample, and

     i. the level of proADM or fragment(s) thereof in the second sample is compared to a reference level, or

     ii. the method comprises additionally determining a level of proADM or fragment(s) thereof in the first sample, and the level of proADM or fragment(s) thereof in the second sample is compared to the level of proADM or fragment(s) thereof in the first sample to determine a change in the level of proADM or fragment(s) thereof in the second sample in comparison to the first sample,
   - wherein the levels of PCT or fragment(s) thereof comprising at least 12 amino acids in said first and second samples, and the level of proADM or fragment(s) thereof in the second sample or the change in the level of proADM or fragment(s) thereof in the second sample in comparison to the first sample are indicative of whether a change in the treatment with one or more antibiotic agents is required.

2. The method according to claim 1, wherein the first sample is isolated when symptoms of sepsis, severe sepsis, septic shock, or both sepsis and septic shock have been determined in said patient, and the second sample is isolated after said symptoms have been initially determined and antibiotic treatment has been initiated.

3. The method according to claim 2, wherein said second sample is isolated from said patient within 30 minutes after symptoms of sepsis, severe sepsis, septic shock, or both sepsis and septic shock have been determined and antibiotic treatment has been initiated, or at least 30 minutes, 1 hour, 2 hours, 6 hours or 12 hours after symptoms of sepsis, severe sepsis, septic shock, or both sepsis and septic shock have been determined and antibiotic treatment has been initiated.

Date Reçue/Date Received 2023-06-06

4. The method according to claim 2, wherein said second sample is isolated from said patient 12-36 hours or 3-5 days after symptoms of sepsis, severe sepsis, septic shock, or both sepsis and septic shock have been determined and antibiotic treatment has been initiated.

5. The method according to any one of claims 1 to 4, comprising determining the level of mid-regional proADM (MR-proADM).

6. The method according to any one of claims 1 to 5, comprising additionally determining a level of proADM or fragment(s) thereof in the first sample.

7. The method according to claim 6, wherein an elevated level of proADM or fragment(s) thereof in the second sample compared to the first sample is indicative of a change of the one or more antibiotic agents being required.

8. The method according to any one of claims 1 to 7, wherein

   - an elevated level of PCT or fragment(s) thereof comprising at least 12 amino acids in the second sample compared to the first sample, and
   - an intermediate or high severity level of proADM or fragment(s) thereof in the second sample,

   indicate that a change in the one or more antibiotic agents is required,

   - wherein an intermediate severity level of proADM or fragment(s) thereof is equal to or above 2.7 nmol/l, and below 6.5 nmol/l, and
   - a high severity level of proADM or fragment(s) thereof is equal to or above 6.5 nmol/l.

9. The method according to claim 8 wherein the intermediate severity level of proADM or fragment(s) thereof is equal to or above 3 nmol/l.

10. The method according to claim 8 wherein the intermediate severity level of proADM or fragment(s) thereof is equal to or above 4 nmol/l.

11. The method according to any one of claims 8 to 10 wherein the intermediate severity level of proADM or fragment(s) thereof is below 6.95 nmol/l and the high severity level of proADM or fragment(s) thereof is equal to or above 6.95 nmol/l.

12. The method according to any one of claims 8 to 10 wherein the intermediate severity level of proADM or fragment(s) thereof is below 10.9 nmol/l and the high severity level of proADM or fragment(s) thereof is equal to or above 10.9 nmol/l.

13. The method according to any one of claims 1 to 7, wherein

   - a lower level of PCT or fragment(s) thereof comprising at least 12 amino acids in the second sample compared to the first sample, and

- a high severity level of proADM or fragments(s) thereof in the second sample, or an elevated severity level of proADM or fragment(s) thereof in the second sample compared to the first sample,

indicate that a change in the one or more antibiotic agents is required,

- wherein a low severity level of proADM or fragment(s) thereof is below 4 nmol/l,
- an intermediate severity level of proADM or fragment(s) thereof is equal to or above 4 nmol/l and below 6.5 nmol/l, and
- a high severity level of proADM or fragment(s) thereof is equal to or above 6.5 nmol/l.

14. The method according to claim 13 wherein the elevated severity level of proADM or fragment(s) thereof in the second sample compared to the first sample is an elevation from the low severity level to the intermediate or high severity level, or an elevation from the intermediate severity level to the high severity level.

15. The method according to claim 13 or 14 wherein the low severity level of proADM or fragment(s) thereof is below 3 nmol/l and the intermediate severity level of proADM or fragment(s) thereof is equal to or above 3 nmol/l.

16. The method according to claim 13 or 14 wherein the low severity level of proADM or fragment(s) thereof is below 2.7 nmol/l and the intermediate severity level of proADM or fragment(s) thereof is equal to or above 2.7 nmol/l.

17. The method according to any one of claims 13 to 16 wherein the intermediate severity level of proADM or fragment(s) thereof is below 6.95 nmol/l and the high severity level of proADM or fragment(s) thereof is equal to or above 6.95 nmol/l.

18. The method according to any one of claims 13 to 16 wherein the intermediate severity level of proADM or fragment(s) thereof is below 10.9 nmol/l and the high severity level of proADM or fragment(s) thereof is equal to or above 10.9 nmol/l.

19. The method according to any one of claims 1 to 18, wherein

- A more than 50 % lower level of PCT or fragment(s) thereof comprising at least 12 amino acids in the second sample compared to the first sample, and
- an intermediate severity level of proADM or fragment(s) thereof in the second sample compared to a low severity level of proADM or fragment(s) thereof in the first sample,

indicate that a change in the one or more antibiotic agents is required;

- wherein a low severity level of proADM or fragment(s) thereof is below 4 nmol/l, and
- an intermediate severity level of proADM or fragment(s) thereof is equal to or above 4 nmol/l and below 6.5 nmol/l.

20. The method according to any one of claims 1 to 11, wherein

- a less than 50 % lower level of PCT or fragment(s) thereof comprising at least 12 amino acids in the second sample compared to the first sample, and
- a high severity level of proADM or fragment(s) thereof in the second sample compared to an intermediate severity level of proADM or fragment(s) thereof in the first sample,

indicate that a change in the one or more antibiotic agents is required;

- wherein an intermediate severity level of proADM or fragment(s) thereof is equal to or above 4 nmol/l and below 6.5 nmol/l, and
- a high severity level of proADM or fragment(s) thereof is equal to or above 6.5 nmol/l.

21. The method for antibiotic therapy guidance in a patient diagnosed as being critically ill with sepsis, severe sepsis, septic shock, or both sepsis and septic shock and receiving treatment with one or more antibiotic agents, according to any one of claims 1 to 20, additionally comprising

- determining a level of at least one additional biomarker or fragment(s) thereof in the first and the second sample, wherein the at least one additional biomarker is selected from the group consisting of lactate, C-reactive protein, soluble fms-like tyrosine kinase-1 (sFlt-1), Histone H2A, Histone H2B, Histone H3, Histone H4, calcitonin, Endothelin-1 (ET-1), Arginine Vasopressin (AVP), Atrial Natriuretic Peptide (ANP), Neutrophil Gelatinase-Associated Lipocalin (NGAL), Troponin, Brain Natriuretic Peptide (BNP), Pancreatic Stone Protein (PSP), Triggering Receptor Expressed on Myeloid Cells 1 (TREM1), Interleukin-6 (IL-6), Interleukin-1, Interleukin-24 (IL-24), Interleukin-22 (IL-22), Interleukin (IL-20), interleukins, Presepsin (sCD14-ST), Lipopolysaccharide Binding Protein (LBP), Alpha-1-Antitrypsin, Matrix Metalloproteinase 2 (MMP2), Metalloproteinase 2 (MMP8), Matrix Metalloproteinase 9 (MMP9), Matrix Metalloproteinase 7 (MMP7), Placental growth factor (PlGF), Chromogranin A, S100A protein, S100B protein,Tumor Necrosis Factor α (TNFα), Neopterin, Alpha-1-Antitrypsin, pro-arginine vasopressin (proAVP), Copeptin, atrial natriuretic peptide (ANP, pro-ANP), Endothelin-1, CCL1/TCA3, CCL11, CCL12/MCP-5, CCL13/MCP-4, CCL14, CCL15, CCL16, CCL17/TARC, CCL18, CCL19, CCL2/MCP-1, CCL20, CCL21, CCL22/MDC, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL3L3, CCL4, CCL4L1/LAG-1, CCL5, CCL6, CCL7, CCL8, CCL9, CX3CL1, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, CXCL2/MIP-2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7/Ppbp, CXCL9, IL8/CXCL8, XCL1, XCL2, FAM19A1, FAM19A2, FAM19A3, FAM19A4, FAM19A5, CLCF1, CNTF, IL11, IL31, IL6, Leptin, LIF, OSM, IFNA1, IFNA10, IFNA13, IFNA14, IFNA2, IFNA4, IFNA7, IFNB1, IFNE, IFNG, IFNZ, IFNA8, IFNA5/IFNaG, IFNω/IFNW1, BAFF, 4-1BBL, TNFSF8, CD40LG, CD70, CD95L/CD178, EDA-A1, TNFSF14, LTA/TNFB, LTB, TNFa, TNFSF10, TNFSF11, TNFSF12, TNFSF13,

TNFSF15, TNFSF4, IL18, IL18BP, IL1A, IL1B, IL1F10, IL1F3/IL1RA, IL1F5, IL1F6, IL1F7, IL1F8, IL1RL2, IL1F9 and IL33,

- wherein the levels of the at least one additional biomarker or fragment(s) thereof in said first and second samples, and the level of proADM or fragment(s) thereof in the second sample, are indicative of whether a change in the ongoing antibiotic treatment is required.

22. The method according to claim 22 wherein the at least one additional biomarker is lactate or C-reactive protein.

23. The method for antibiotic therapy guidance in a patient diagnosed as being critically ill with sepsis, severe sepsis, septic shock, or both sepsis and septic shock and receiving treatment with one or more antibiotic agents, according to any one of claims 1 to 22, additionally comprising

- determining at least one clinical score selected from the group consisting of Sequential Organ Failure Assessment (SOFA), Acute Physiological and Chronic Health Evaluation II (APACHE II) and Simplified Acute Physiology Score II (SAPS II) at the time point of isolation of the first sample and at the time point of isolation of the second sample,
- wherein the at least one clinical score at the time points of isolation of the first and second samples, and the level of proADM or fragment(s) thereof in the second sample, are indicative of whether a change in the ongoing antibiotic treatment is required.

24. A kit for carrying out the method of any one of claims 1 to 23, comprising:

- detection reagents for determining a level of proADM or fragment(s) thereof, and additionally detection reagents for determining a level of PCT or fragment(s) thereof comprising at least 12 amino acids, in a blood, serum, or plasma sample from a subject, and
- a computer-readable medium storing reference data, comprising a reference level corresponding to high and/or low severity levels of proADM, wherein the low severity level is below 4 nmol/l and the high severity level is equal to or above 6.5 nmol/l and reference data corresponding to diagnostically relevant PCT levels,
- wherein said reference data is employed in the form of computer executable code configured for comparing the determined levels of proADM or fragment(s) thereof, and additionally the determined levels of PCT or fragment(s) thereof comprising at least 12 amino acids, to said reference data.

25. The kit according to claim 24 wherein the low severity level is below 3 nmol/l.

26. The kit according to claim 24 wherein the low severity level is below 2.7 nmol/l.

27. The kit according to any one of claims 24 to 26 wherein the high severity level is equal to or above 6.95 nmol/l.

28. The kit according to any one of claims 24 to 26 wherein the high severity level is equal to or above 10.9 nmol/l.

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