Title: IL-6 DETECTION BASED EARLY DIAGNOSIS AND PREDICTION OF SYSTEMIC INFLAMMATORY RESPONSE SYNDROME AND SEPSIS IN ASYMPTOMATIC PATIENTS

Abstract: The present invention relates to the technical field of early diagnosis and prediction of systemic inflammatory response syndrome (SIRS) including sepsis in asymptomatic patients, preferably in patients undergoing a surgical intervention. In particular, it provides a method for detection or diagnosis of SIRS, or for detection or diagnosis of a risk to suffer from or develop SIRS, in an asymptomatic patient, comprising the steps of: a) determining the level of IL-6 or a variant thereof in a sample from the patient; b) comparing the level of IL-6 or a variant thereof determined in step a) to a reference level; c) detecting or diagnosing SIRS, or detecting or diagnosing a risk to suffer from or develop SIRS; wherein the sample is isolated at least 2 times at short intervals and steps a) and b) are repeated for each sample. Corresponding therapy monitoring and mortality prediction methods, kit of parts are also provided.
IL-6 detection based early diagnosis and prediction of systemic inflammatory response syndrome and sepsis in asymptomatic patients

Field of the Invention

The present invention relates to the technical field of early diagnosis and prediction of systemic inflammatory response syndrome (SIRS) or sepsis in asymptomatic patients, preferably in patients undergoing a surgical intervention, using 11-6 as a diagnostic and predictive biomarker. Corresponding therapy monitoring and mortality prediction methods, kit of parts are also provided.

In spite of the advances of modern medicine SIRS and sepsis represents a common and devastating syndrome that is increasing in frequency around the world. It belongs to the most frequent causes of death in intensive care patients.

Epidemiological evaluations found that a number of conditions can be described which define patients to be at risk to develop sepsis and severe sepsis. The described risk factors are gender (men), race (black), ethnicity (Hispanic), advanced age, and the following co-morbidities: Diabetes mellitus, malignancy, alcoholism, HIV infection, treatment with immunosuppressive agents (Hodgin KE, Moss M. The epidemiology of Sepsis. Current Pharm Design 2008;14: 1833-1839). In addition, an actual event e.g. major surgery, trauma, or burns which result in large area wounds represents an additional risk. SIRS without infection can occur in events such as pancreatitis, shock, ischemia, and polytrauma.

From 1991 on when the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) provided a conceptual and practical framework to define the systemic inflammatory response to infection (American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med. 1992 Jun;20(6):864-74) Earlier, various terminologies were used interchangeably, leading to much confusion. The major contribution from the conference was the development of uniform definitions for the various
stages of sepsis that could be universally and uniformly applied to patients suffering from these disorders.

At the time point when the clinical signs appear, the development and aggravation of the SIRS has already been started.

IL-6 belongs to a family of gp130 cytokines. All family members share a four-helical protein structure and they exert their signal via a receptor complex containing at least one subunit of the signal transducing receptor glycoprotein gp130. IL-6 binds to the IL-6 receptor (IL-6R) first and this IL-6/IL-6R complex binds to gp130 leading to a homodimerization and subsequent activation of the Jak/Stat- and Ras/Map/Akt-signal transduction pathway (Taga T, Kishimoto T. Gp130 and the Interleukin-6 Family of Cytokines. Annu. Rev. Immunol. 1997; 15: 797-819; Drucker C, Gewiese J, Malchow S, Scheller J, Rose-John S. Impact of Interleukin-6 Classic- and Trans-signaling on Liver Damage and Regeneration. J Autimm 2009 in press). There are two different signaling pathways described, one in which IL-6 binds to the membrane bound IL-6 receptor which leads to dimerization and activation of the signal transducing protein gp13. This pathway is restricted to cells which express the IL-6 receptor on their surface, which is only the case in some cell populations. However an alternative pathway exists in which IL-6 binds to a naturally occurring soluble IL-6R (sIL-6R) and this IL-6/sIL-6R complex activates gp130. This way, cells lacking the membrane bound IL-6-receptor can respond to IL-6. This second so called trans-signaling pathway also affects cells which express membrane-bound IL-6 receptors, e.g. hepatocytes. In this setting an activation of IL-6 trans-signaling can enhance stimulatory effects of IL-6. In 1988 it was proposed to name the cytokine IL-6 as further studies demonstrated that the protein shows activities not only on B-cells but also on T-cells, hematopoietic stem cells, hepatocytes and brain cells (Kishimoto T, Hirano T. A New Interleukin with Pleitropic Activities. Bio Essays 1988 Jul; 9(1): 11-15).

Already in 1989 it was reported that body fluids of patients with local acute infection and serum of patients with gram-negative and gram-positive bacteremia contain elevated levels of biologically active IL-6. It was assumed that the presence of IL-6 in serum during acute infection suggests that this cytokine is likely to participate in a cascade of local and systemic events that help to limit tissue damage (Helfgott DC, Tatter SB, Santhanam U, Clarick RH, Bhardwaj N, May LT, Sehgal PB. Multiple Forms of IFN-p2/IL-6 in Serum and Body Fluids during Acute Bacterial Infection. J Immunology 1989; 142:948-953.)

Mokart D, et al. (Br J Anaesth. 2005 Jun;94(6):767-77) discloses that based on PCT and IL-6 detection in patients undergoing cancer surgery, wherein IL-6 is sampled in the morning prior to as well as the morning following the surgery, i.e. at a time interval exceeding at least 20 h. Based on the PCT and IL-6 measurements are used to predict sepsis in patients displaying SIRS and day 1 post-surgery, i.e. the IL-6 levels do not allow to diagnose or predict the risk to suffer from SIRS or sepsis in patients who do not display SIRS symptoms day 1 post-surgery.

Mokart D, et al. (World J Surgery, 2009, 33: 558-566) discloses IL-6 and PCT based method of identifying patients at risk of developing sepsis based on a once-per-day collected blood sample.

In summary, none of the abovementioned publications allows for a diagnosis of SIRS and sepsis, or for predicting the risk to suffer from or to develop SIRS and sepsis, before the onset of generally recognized clinical signs and symptoms of SIRS and sepsis, i.e. in an asymptomatic patient. As a result, there is a need for a diagnostic method and a treatment monitoring method allowing for a sensitive and early detection of SIRS or sepsis and for predicting the risk to suffer from or develop SIRS and sepsis, before the onset of clinical signs and symptoms of SIRS.

Accordingly, one of the objects underlying the present invention lies in the provision of a means and method which solves at least some of the shortcomings of the hitherto known approaches to diagnosing SIRS and sepsis. Moreover, it is an object to provide a method and a treatment monitoring method, both of which allow for an early detection of the risk to suffer from or develop SIRS and sepsis before the onset of clinical symptoms. It is also an object to provide a kit and a computer program adapted for carrying out these methods.
Summary of the Invention

At least one of these objectives is accomplished by the provision of the subject matter defined in the claims and herein below.

In a first aspect it is provided a method for detection or diagnosis of a systemic inflammatory response syndrome (SIRS) or sepsis, or for detection or diagnosis of a risk to develop or suffer from SIRS or sepsis, in an asymptomatic patient, comprising the steps of

a) determining the level of IL-6 or a variant thereof in a sample from the patient;
b) comparing the level of IL-6 or a variant thereof determined in step a) to a reference level;
c) detecting or diagnosing SIRS, or detecting or diagnosing a risk to develop or suffer from SIRS or sepsis;

wherein the sample is isolated at least 2 times at short intervals and steps a) and b) are repeated for each sample.

It is also provided a method for detecting the level of IL-6 in an asymptomatic patient for detection or diagnosis of a risk to develop or suffer from SIRS or sepsis, comprising the steps of

a) determining the level of IL-6 or a variant thereof in a sample from the patient; and
b) comparing the level of IL-6 or a variant thereof determined in step a) to a reference level; preferably based on the comparison it is detected or diagnosed if the patients is at risk to develop or suffer from SIRS or sepsis;

wherein the sample is isolated at least 2 times at short intervals and steps a) and b) are repeated for each sample.

It is also provided a method for detection or diagnosis of a risk to develop or suffer from SIRS or sepsis, in an asymptomatic patient selected from a trauma patient, a patient with burns, a patient undergoing an invasive treatment, a patient undergoing a surgery, comprising the steps of

a) determining the level of IL-6 or a variant thereof in a sample from the patient;
b) comparing the level of IL-6 or a variant thereof determined in step a) to a reference level;
c) detecting or diagnosing SIRS, or detecting or diagnosing a risk to develop or suffer from SIRS;
wherein a sample is isolated at least 2 times at short intervals and steps a) and b) are repeated for each sample, preferably at least one sample is isolated upon admission of the patient and at least one sample is isolated after a treatment has been initiated or terminated.

For example, if the asymptomatic patient will be subjected to a invasive treatment at least one sample is isolated before the surgical intervention or to obtain a baseline IL-6 level, then at least one further sample is isolated and analyzed for IL-6 levels at short intervals after the completion of the invasive treatment.

Preferably, IL-6 or a means for detecting IL-6 is used to detect or diagnose the risk to develop or suffer from SIRS or sepsis, in an asymptomatic patient, wherein the level of IL-6 is determined at least 2 times at short intervals.

To the surprise of the present inventors, the method of the present invention showed that the close-meshed consecutive measurements of IL-6, preferably beginning before a treatment, for example a therapy associated with a significant risk of resulting in SIRS or sepsis, such as a major surgery, allows for a clear identification of asymptomatic patients at risk of suffering from or developing SIRS including sepsis well before the onset of clinical signs or pathological changes of laboratory parameters conventionally used to diagnose SIRS/sepsis (see e.g. Examples). Specifically, the present invention describes for the first time the IL-6 kinetics in asymptomatic patients, some of which could be identified as developing SIRS. It was unraveled that a large rise in IL-6 concentration when compared to the baseline level indicates a high risk to develop or suffer from SIRS or sepsis, well ahead of the display of clinical symptoms or signs supporting the diagnosis of SIRS and sepsis. Thus, contrary to what was known before, rather than analyzing the absolute level of IL-6 in the sample, the diagnosis of the risk to suffer from or develop SIRS or sepsis is essentially based on determining the increase of the IL-6 level over time. Owing to the early response properties of the method of the present invention prior to the appearance of clinical signs and symptoms of SIRS and sepsis, it is now possible to initiate appropriate treatments at an earlier point in time, when compared to hitherto known method of diagnosing SIRS and sepsis. Accordingly, the likeliness of a successful therapy of SIRS or the prevention of SIRS or sepsis increases and patients can be saved from dying as a result of SIRS or sepsis.

As used herein, the term "systemic inflammatory response syndrome (SIRS)" is generally known to the skilled worker. The term preferably encompasses SIRS as defined on the ACCP/SCCM
Consensus Conference Definitions (1992/2003) (see e.g. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med. 1992 Jun;20(6): 864-74). Preferably, a patient is considered to suffer from SIRS, if the patient displays at least 2 symptoms of the following a) to d), preferably of the following a) to e):

a) white blood cell count (WBC) > about 12,000/µL or < about 4000/µL,

b) body temperature > about 38 °C or < about 36 °C,

c) heart rate > about 90 beats/minute,

d) a respiratory rate > about 20 breaths/minute or a partial pressure of CO₂ of less than about 32 mm Hg, and

e) more than 10% immature white blood cells among the counted white blood cells.

The white blood cell count is usually determined by automated counting devices.

As far as children are concerned, the consensus criteria for diagnosing SIRS in a child are disclosed in Goldstein et al. (Pediatr. Crit Care Med 2005, 6(1), 2 - 8, see in particular Tables 2 and 3). An asymptomatic child patient in the sense of the present invention is a patient displaying less than 2, preferably less than 1 symptom of the ones described in Goldstein et al. (supra) which is incorporated herein by reference.

Preferably, the risk to suffer from or develop SIRS may be diagnosed or detected on the basis of an IL-6 level detected in a sample of a patient which is above the reference level. In this case it is preferably not required for the diagnosis or detection of the risk to suffer from or develop SIRS or sepsis that the patient displays at least two of the above SIRS symptoms (a) to (e). Preferably, the risk to suffer from or develop SIRS or sepsis may be diagnosed or detected solely on the basis of a determined IL-6 level which is above the reference level. It is preferred that the reference value is determined by multiplying an earlier determined IL-6 level (e.g. a baseline IL-6 level) in a given patient by a factor defined elsewhere herein (e.g. a factor of at least about 50, or at least about 100, or at least about 500 or at least about 1000). Any IL-6 level determined in a sample that was isolated from the given patient after the earlier sample(s) (which preferably provides the baseline level) was collected, and which IL-6 level is above the reference level is indicative of the patient to be at a high risk to develop or suffer from SIRS. It was the surprising contribution of the present inventors to unravel that based on IL-6 determination early detection and diagnosis of
the risk to suffer from or develop SIRS is possible, preferably before the patient displays two or more of the above SIRS symptoms (a) to (e).

In case the patient additionally displays a diagnosed infection, the patient is considered to be at risk to develop or suffer from sepsis.

As used herein, for "sepsis" the diagnostic criteria mentioned for "SIRS" above applies mutatis mutandis, however, in sepsis, a diagnosed infection is a mandatory additional diagnostic parameter. Methods for the detection or diagnosis of an infection are generally known in the field. As a result of the present invention, the risk to suffer from or develop sepsis may now preferably be detected or diagnosed on the basis of only two parameters, i.e. a level of IL-6 above the reference level and a diagnosed infection.

An "infection" in the sense of the present invention preferably is a viral, fungus or bacterial infection, preferably a bacterial infection associated with bacteria selected from E coli, staphylococcus aureus, Klebsiella pneumoniae, Streptococci or Pseudomonas aeruginosa. The infection may as well be an infection by a fungus selected from Candida albicans, Candida tropicalis or Aspergillus fumigatus.

An infection is diagnosed on the basis of assays and criteria generally known to the physician. Preferably, the infection is diagnosed on the basis of a bacterial culture assay, e.g. a culture medium inoculated with a sample from the patient, or based on molecular diagnostic methods. A fungus infection may for example be determined based on the generally known test assays such as Septifast.

The term "patient" as used herein relates to animals, preferably mammals, preferably dogs, cats, horses, cattle and most preferably humans, preferably male or female, preferably prenatal, perinatal, postnatal or neonatal children. Preferably the patient is an adult. Preferably the patient is not a prenatal, perinatal, postnatal or neonatal child.

As used herein, the expression "asymptomatic patient" is meant to encompass a patient who does not display clinical signs and symptoms generally considered to establish diagnosis of SIRS. Preferably, the asymptomatic patient displays less than 2 symptoms, preferably less than 1 symptom of the following:

a) white blood cell count (WBC) > about 12,000/µL or < about 4000/µL,

b) body temperature > about 38 °C or < about 36 °C,
c) heart rate > about 90 beats/minute,

d) a respiratory rate > about 20 breaths/minute or a partial pressure of CO₂ of less than about 32 mm Hg, and

e) more than 10% immature white blood cells among the counted white blood cells.

Preferably, the asymptomatic patient encompasses a patient selected from a trauma patient, a patient with burns, a patient undergoing an invasive treatment, a patient undergoing a surgical intervention, preferably a surgical intervention selected from an endoscopic intervention, or selected from findings on CT-scan or PET-CT-scan, or a patient at risk of developing SIRS or sepsis such as a patient meeting at least one of the following criteria:

- genetic disposition for sepsis,
- premature (perinatal, neonatal) or advanced age,
- sex is male,
- race is african american,
- medical co-morbidities including a chronic illness (like diabetes, congestive heart failure), pre-existing organ dysfunction (like cirrhosis or renal failure), physical or mental impairment,
- previous clinical interventions (like major surgery, endotracheal intubation, antibiotics), and
- social, religious or cultural factors;

all of the aforementioned criteria are described in further detail in Marshall JC, "Predisposition to Sepsis", Anaesthesia, Pain, Intensive Care and Emergency A.P.I.C.E., Springer Verlag, ISBN 88-470-0772-0. It is generally known that these factors may preferably be taken into account when practicing the present invention.

Optionally, the asymptomatic patients of the present invention excludes a patient, where the metabolization of IL-6 is impaired, such as in patients where IL-6 clearance via the splanchnic and kidney organ is impaired (Garibotta et al. (Cytokine, 2007, 37, 51-54).

In the context of the present invention, "Interleukin-6 (IL-6)" is preferably meant to encompass IL-6, as it is known in the art. Preferably, IL-6 encompasses interferon-β2, plasmacytoma growth
factor, hepatocyte stimulating factor and human B-cell-stimulating factor 2 (BSF2). IL-6 is preferably a protein produced from a single gene encoding a product of 212 amino acids, more preferably the 184 amino acid IL-6 peptide which is cleaved at the N-terminus of the 212 amino acid peptide (see Song M, Kellum JA. Interleukin-6. Crit Care Med 2005; 33 (Suppl2): 463-465 and NCBI sequence for the 212 amino acid long IL-6 precursor, accession number NP_000591). Preferably, IL-6 encompasses free IL-6 which is not bonded to its receptor IL-6R. Moreover, IL-6 may also encompass IL-6 in the state of the IL-6/IL-6R complex (see Taga T, Kishimoto T, Gpl30 and the Interleukin-6 Family of Cytokines. Annu. Rev. Immunol. 1997; 15: 797-819; Drucker C, Gewiese J, Malchow S, Scheller J, Rose-John S. Impact of Interleukin-6 Classic- and Trans-signaling on Liver Damage and Regeneration. J Autimm 2009 in press). Preferably, IL-6 is the IL-6 protein which can be bound or which is bound by the monoclonal anti-IL6 antibody M-BE8 (as defined in EP0430193, i.e. an antibody produced by the cell line BE-8, or in KLEIN, B., et al. 1991, Murine anti-interleukin 6 monoclonal antibody therapy for a patient with plasma cell leukemia, Blood 78, 1198-1204) or M-23C7. Preferably, IL-6 is the IL-6 which can be bound or which is bound by the antibody of Roche's IL-6 assay for use on Elecsys and cobas immunoassay systems (Roche). The term IL-6 also preferably encompasses a variant of the aforementioned IL-6, preferably of human IL-6. The variant encompasses a protein or peptide substantially similar to the specific reference IL-6 molecule, preferably to the human IL-6. The term substantially similar is well understood by the person skilled in the art. In particular, a IL-6 variant may be an isoform or allele which shows at least one amino acid exchange (and preferably up to about 25, more preferably up to about 15, more preferably up to about 10, more preferably up to about 5, most preferably up to about 3 amino acid exchanges) compared to the amino acid sequence of the specific reference IL-6 molecule. Preferably, such a IL-6 variant has a sequence identity to the specific reference IL-6 molecule of at least about 80%, preferably at least about 85%, more preferably at least about 90%, most preferably at least about 95%, most preferably at least about 98%, preferably with respect to human IL-6, even more preferably over the entire length of the human IL-6. The degree of identity between two amino acid sequences can be determined by algorithms well known in the art. Preferably, the degree of identity is to be determined by comparing two optimally aligned sequences over a comparison window, where the fragment of amino acid sequence in the comparison window may comprise additions or deletions (e.g., gaps or overhangs) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment. The percentage is calculated by determining the
number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman Add. APL. Math. 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman Proc. Natl. Acad Sci. (USA) 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, PASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by visual inspection. Given that two sequences have been identified for comparison, GAP and BESTFIT are preferably employed to determine their optimal alignment and, thus, the degree of identity. Preferably, the default values of 5.00 for gap weight and 0.30 for gap weight length are used. Variants referred to above may be allelic variants or any other species specific homologs, paralogs, or orthologs. The expression variant also encompasses also degradation products, e.g. proteolytic degradation products, which are still recognized by the diagnostic means or by ligands directed against the respective full-length protein or peptide. The term "variants" is also meant to cover splice variants. The term "variant" also relates to a post-translationally modified peptide such as glycosylated peptide. A "variant" is also a peptide which has been modified after collection of the sample, for example by covalent or non-covalent attachment of a label, particularly a radioactive or fluorescent label, to the peptide. Preferably, the IL-6 variant possesses essentially the same immunological and/or biological properties of the specific reference peptide, preferably the same immunological and/or biological properties as human IL-6, most preferably the same biological and/or immunological properties as human IL-6. Preferably the IL-6 variant displays at least about 70%, preferably at least about 80%, preferably at least about 90%, preferably at least about 95%, preferably at least about 98% of the human IL-6 activity. The IL-6 activity, is preferably the IL-6 receptor binding activity (see Taga T, Kishimoto T. Gpl30 and the Interleukin-6 Family of Cytokines. Annu. Rev. Immunol. 1997; 15: 797-819; Drucker C, Gewiese J, Malchow S, Scheller J, Rose-John S. Impact of Interleukin-6 Classic- and Trans-signaling on Liver Damage and Regeneration. J Autimm 2009 in press.). Preferably the IL-6 variant displays a human IL-6 receptor binding activity of at least about 80%, preferably at least about 90%, preferably at least about 95% of human IL-6.
According to a preferred embodiment of the present invention at least one additional marker or parameter indicative of SIRS or sepsis is determined including at least one parameter selected from an inflammation marker like CRP, another interleukin like IL-1 and/or IL-8, procalcitonin, white blood cell count, body temperature, heart rate, respiratory rate, and/or diagnosis of an infection, preferably a bacterial and/or fungus infection.

Moreover, the method of the present invention may comprise steps in addition to those explicitly mentioned above. For example, further steps may relate to sample collection, sample pre-treatments or evaluation of the results obtained by the method. The method of the present invention may be also used for monitoring, confirmation, sub-classification and risk assessment of developing or suffering from SIRS and for therapeutic monitoring the diseases of the present invention. It is also envisioned that in a preferred embodiment at least one additional marker or parameter may be determined apart from the two mentioned in step a), preferably for the purpose of obtaining additional diagnostic information beyond the detection or diagnosis of SIRS or sepsis. Such additional parameter may for example be the estimated glomerular filtration rate, the level of NGAL or creatinine which allows to additionally diagnose if the patient suffers from an impaired renal clearance.

The method may be carried out manually and/or assisted by automation. Preferably, steps (a), (b), and/or (c) may in total or in part be assisted by automation, e.g., by a suitable robotic and sensory equipment for the determination in step (a) or a computer-implemented comparison in step (b) and/or (c).

The term "sample" refers to a sample of a body fluid, to a sample of separated cells or to a sample from a tissue or an organ. Samples of body fluids can be obtained by well known techniques and include, preferably, samples of blood, plasma, serum, liquor or urine, more preferably, samples of blood, plasma or serum. Body fluid samples are preferably obtained by venepuncture, arterial puncture or ventricular puncture. Tissue or organ samples may be obtained from any tissue or organ by, e.g., biopsy. Separated cells may be obtained from the body fluids or the tissues or organs by separating techniques such as centrifugation or cell sorting. Preferably, cell-, tissue- or organ samples are obtained from those cells, tissues or organs which express or produce the peptides referred to herein.

The methods of the present invention may encompass a step of collecting a sample, which optionally may be an invasive step. The sample may be collected by way of an invasive step,
preferably a minimal invasive step, such as by venopuncture. The minimal invasive collection also encompasses the case where the sample is collected by use of a needle (lancette) which when applied to the skin, preferably the skin of a finger, elicits outflow of a small volume of blood which may then be collected for determining the amount of the markers in the sample. The sample is preferably collected invasively or minimal-invasively by way of a safe routine procedure, preferably by persons that do not need to have a strong medical training and the sample collection preferably poses no significant health risk for the person subjected to the sample collection.

Preferably the method of the present invention is an ex vivo or in vitro method.

The samples isolated at short intervals are obtained from a patient. As used herein, "short intervals" encompass an interval ranging from about 15 minutes to about 12 hours, preferably from about 15 minutes to 6 hours, preferably from about 15 minutes to about 3 hours, preferably from about 1 hour to about 12 hours, preferably from about 1 hour to 6 hours, preferably from about 1 hour to about 3 hours. More preferably, the short interval is an interval of about 15 minutes, preferably about 30 minutes, preferably about 1 hour, preferably about 2 hours, preferably about 3 hours, preferably about 4 hours, preferably about 5 hours, preferably about 6 hours.

Preferably, the samples are isolated from the patient for a period of up to about 10 days, preferably for a period of up to about 7 days, preferably for a period of up to about 5 days, preferably for a period of up to about 3 days. Depending on the condition and development of the condition of the patient the isolation of the samples and IL-6 detection may be further prolonged, for example, if the patient develops SIRS or sepsis the sampling may be extended until the SIRS or sepsis therapy has been successfully completed or even a few days beyond that point in time.

Preferably the samples are taken at least about once, preferably at least about twice before a treatment is carried out, and at least once, preferably at least about twice after the treatment. Preferably, the treatment is an invasive treatment as defined above. For example, before the treatment is carried out, one or two IL-6 levels are taken. Following the treatment a number of samples are taken at regular intervals of, e.g., 3-6 hours over a period of, e.g., 3 to 10 days, where IL-6 and preferably also the clinical status of the patient is determined.

The term "detecting and diagnosing SIRS" as used herein means assessing, identifying, evaluating, or classifying if an asymptomatic patient suffers from SIRS, preferably from sepsis.
The term "detecting and diagnosing a risk to develop or suffer from SIRS" is meant to encompass the prediction of the risk in an asymptomatic patient to develop or to suffer from SIRS, including sepsis, within a defined time window (predictive window) in the future. The predictive window is an interval in which the subject will develop a SIRS or optionally will die according to the predicted probability. Preferably, however, the predictive window is an interval of up to 20 days, preferably up to about 10 days, preferably up to about 7 days, preferably up to about 5 days, preferably up to about 5 days, preferably up to about 4 days, preferably up to about 3 days, preferably up to about 2 days, (i) after the method of the present invention has been carried out or (ii) after the first sample was obtained in which the detected IL-6 level was above the reference level or (iii) after admittance of the patient or (iv) after the first baseline level for IL-6 has been obtained/isolated or (v) after the treatment, e.g. the invasive treatment, has been initiated or terminated or (vi) after the first post-treatment sample has been isolated for determination of the IL-6 level.

Preferably, the patients identified as being at risk of developing or suffering from SIRS possesses a high probability of developing or suffering from SIRS. Preferably, the high probability is at least about 30%, preferably at least about 40%, preferably at least about 50%, preferably at least about 60%, preferably at least about 70%, preferably at least about 80%, preferably at least about 90%, preferably at least about 95%. Optionally, a high probability of developing or suffering from SIRS or sepsis, encompasses a probability of 100%, i.e. the patient will develop or does suffer from SIRS or sepsis.

Alternatively, the patient has a low probability of suffering from or developing SIRS or sepsis. Preferably, the low probability is up to about or less than about 30%, preferably up to about 20%, preferably up to about 10%, preferably up to about 5%. Optionally, a low probability of developing or suffering from SIRS or sepsis, encompasses a probability of 0%, i.e. the patient will not develop or does not suffer from SIRS or sepsis.

Methods for the determination of the % risk of suffering from or developing SIRS or sepsis are generally known. Preferably the % risk prediction is carried out within the predictive window mentioned above.

Preferably, the extent of the risk to develop or suffer from SIRS or sepsis correlates with the extent the detected IL-6 level is above the reference value. The risk to developing or suffering from SIRS or sepsis is preferably low in case the detected IL-6 level is lower than the reference
level. Preferably, the risk of developing or suffering from SIRS or sepsis is high in case the
detected IL-6 level is equal to or above the reference value.

As will be understood by those skilled in the art, such an assessment is usually not intended to be
correct for 100% of the subjects to be analyzed. The term, however, requires that the assessment
will be valid for a statistically significant portion of the subjects to be analyzed. Whether a
portion is statistically significant can be determined without further ado by the person skilled in
the art using various well known statistical evaluation tools, e.g., determination of confidence
intervals, p-value determination, Student's t-test, Mann-Whitney test, etc.. Details are found in
confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99 %.
The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Preferably, the probability
envisioned by the present invention allows that the prediction will be correct for preferably at least
60%, at least 70%, at least 80%, or at least 90% of the subjects of a given cohort.

Determining the level of IL-6 or a variant thereof, or of any other proteinaceous biomarker
according to the invention, relates to measuring the amount or concentration, preferably semi-
quantitatively or quantitatively. Measuring can be done directly or indirectly. Direct measuring
relates to measuring the amount or concentration of the peptide or polypeptide based on a signal
which is obtained from the peptide or polypeptide itself and the intensity of which directly
correlates with the number of molecules of the peptide present in the sample. Such a signal -
sometimes referred to herein as intensity signal -may be obtained, e.g., by measuring an intensity
value of a specific physical or chemical property of the peptide or polypeptide. Indirect
measuring includes measuring of a signal obtained from a secondary component (i.e. a
component not being the peptide or polypeptide itself) or a biological read out system, e.g.,
measurable cellular responses, ligands, labels, or enzymatic reaction products.

In accordance with the present invention, determining the amount of an IL-6 peptide or
polypeptide can be achieved by all known means for determining the amount of a peptide in a
sample. Said means comprise immunoassay devices and methods which may utilize labeled
molecules in various sandwich, competition, or other assay formats. Said assays will develop a
signal which is indicative for the presence or absence of the peptide or polypeptide. Moreover,
the signal strength can, preferably, be correlated directly or indirectly (e.g. reverse- proportional)
to the amount of polypeptide present in a sample. Further suitable methods comprise measuring a
physical or chemical property specific for the peptide or polypeptide such as its precise molecular mass or NMR spectrum. Said methods comprise, preferably, biosensors, optical devices coupled to immunoasays, biochips, analytical devices such as mass- spectrometers, NMR- analyzers, or chromatography devices. Further, methods include micro-plate ELISA-based methods, fully-automated or robotic immunoasays (available for example on Roche's ElecsysTM analyzers), CBA (an enzymatic Cobalt Binding Assay, available for example on Roche-HitachiTM analyzers), and latex agglutination assays (available for example on Roche-HitachiTM analyzers), homogenous and heterogeneous immune assays, competitive and non-competitive immune assays.

Preferably, determining the amount of a IL-6 peptide or polypeptide comprises the steps of (a) contacting a cell capable of eliciting a cellular response the intensity of which is indicative of the amount of the peptide or polypeptide with the said peptide or polypeptide for an adequate period of time, (b) measuring the cellular response. For measuring cellular responses, the sample or processed sample is, preferably, added to a cell culture and an internal or external cellular response is measured. The cellular response may include the measurable expression of a reporter gene or the secretion of a substance, e.g. a peptide, polypeptide, or a small molecule. The expression or substance shall generate an intensity signal which correlates to the amount of the peptide or polypeptide.

Also preferably, determining the amount of a IL-6 peptide or polypeptide comprises the step of measuring a specific intensity signal obtainable from the peptide or polypeptide in the sample, preferably in a sample selected from blood, serum, plasma or liquor. As described above, such a signal may be the signal intensity observed at an m/z variable specific for the peptide or polypeptide observed in mass spectra or a NMR spectrum specific for the peptide or polypeptide.

Determining the amount of a IL-6 peptide or polypeptide may, preferably, comprises the steps of (a) contacting the peptide with a specific ligand, (b) (optionally) removing non-bound ligand, (c) measuring the amount of bound ligand. The bound ligand will generate an intensity signal.

Binding according to the present invention includes both covalent and non-covalent binding.

A ligand according to the present invention can be any compound, e.g., a peptide, polypeptide, nucleic acid, or small molecule, binding to the peptide or polypeptide described herein. Preferred ligands include antibodies, nucleic acids, peptides or polypeptides such as receptors or binding partners for the peptide or polypeptide and fragments thereof comprising the binding domains for
the IL-6 peptides, and aptamers, e.g. nucleic acid or peptide aptamers. Methods to prepare such ligands are well-known in the art. For example, identification and production of suitable antibodies or aptamers is also offered by commercial suppliers. The person skilled in the art is familiar with methods to develop derivatives of such ligands with higher affinity or specificity. For example, random mutations can be introduced into the nucleic acids, peptides or polypeptides. These derivatives can then be tested for binding according to screening procedures known in the art, e.g. phage display.

Means for the detection of IL-6 are generally known in the art and preferably include anti-IL-6 antibodies, including polyclonal and monoclonal antibodies, as well as fragments thereof, such as Fv, Fab and F(ab)2 fragments that are capable of binding IL-6 antigen or hapten. The means for the detection of IL-6 of the present invention also include single chain antibodies, chimeric, humanized hybrid antibodies wherein amino acid sequences of a non-human donor antibody exhibiting a desired antigen-specificity are combined with sequences of a human acceptor antibody. Also included is an anti-IL-6 antibody from a mammalian species, preferably an antibody selected from human, rat, mouse, goat, sheep, cattle, and camel. Preferably, the anti-IL-6 antibody is the M-BE8 or M-23C7 anti-IL-6 antibody described above or an antibody binding to the IL-6 epitope recognized by the M-BE8 and/or M-23C7 antibody. The donor sequences will usually include at least the antigen-binding amino acid residues of the donor but may comprise other structurally and/or functionally relevant amino acid residues of the donor antibody as well. Such hybrids can be prepared by several methods well known in the art. Preferably, the ligand or agent binds specifically to the IL-6 peptide or polypeptide. Specific binding according to the present invention means that the ligand or agent should not bind substantially to ("cross-react" with) another peptide, polypeptide or substance present in the sample to be analyzed. Preferably, the specifically bound IL-6 peptide or polypeptide should be bound with at least 3 times higher, more preferably at least 10 times higher and even more preferably at least 50 times higher affinity than any other relevant peptide or polypeptide. Non-specific binding may be tolerable, if it can still be distinguished and measured unequivocally, e.g. according to its size on a Western Blot, or by its relatively higher abundance in the sample. Binding of the ligand can be measured by any method known in the art. Preferably, said method is semi-quantitative or quantitative. Suitable methods are described in the following.

First, binding of a ligand may be measured directly, e.g. by NMR or surface plasmon resonance.
Second, if the ligand also serves as a substrate of an enzymatic activity of the peptide or polypeptide of interest, an enzymatic reaction product may be measured (e.g. the amount of a protease can be measured by measuring the amount of cleaved substrate, e.g. on a Western Blot). Alternatively, the ligand may exhibit enzymatic properties itself and the "ligand/peptide or polypeptide" complex or the ligand which was bound by the peptide or polypeptide, respectively, may be contacted with a suitable substrate allowing detection by the generation of an intensity signal. For measurement of enzymatic reaction products, preferably the amount of substrate is saturating. The substrate may also be labeled with a detectable label prior to the reaction. Preferably, the sample is contacted with the substrate for an adequate period of time. An adequate period of time refers to the time necessary for a detectable, preferably measurable, amount of product to be produced. Instead of measuring the amount of product, the time necessary for appearance of a given (e.g. detectable) amount of product can be measured.

Third, the ligand may be coupled covalently or non-covalently to a label allowing detection and measurement of the ligand. Labeling may be done by direct or indirect methods. Direct labeling involves coupling of the label directly (covalently or non-covalently) to the ligand. Indirect labeling involves binding (covalently or non-covalently) of a secondary ligand to the first ligand. The secondary ligand should specifically bind to the first ligand. Said secondary ligand may be coupled with a suitable label and/or be the target (receptor) of tertiary ligand binding to the secondary ligand. The use of secondary, tertiary or even higher order ligands is often used to increase the signal. Suitable secondary and higher order ligands may include antibodies, secondary antibodies, and the well-known streptavidin-biotin system (Vector Laboratories, Inc.). The ligand or substrate may also be "tagged" with one or more tags as known in the art. Such tags may then be targets for higher order ligands. Suitable tags include biotin, digoxygenin, His-Tag, Glutathion-S-Transferase, FLAG, GFP, myc-tag, influenza A virus haemagglutinin (HA), maltose binding protein, and the like. In the case of a peptide or polypeptide, the tag is preferably at the N-terminus and/or C-terminus. Suitable labels are any labels detectable by an appropriate detection method. Typical labels include gold particles, latex beads, acridan ester, luminol, ruthenium, enzymatically active labels, radioactive labels, magnetic labels ("e.g. magnetic beads", including paramagnetic and superparamagnetic labels), and fluorescent labels. Enzymatically active labels include e.g. horseradish peroxidase, alkaline phosphatase, beta-Galactosidase, Luciferase, and derivatives thereof. Suitable substrates for detection include di-amino-benzidine (DAB), 3,3’-5,5’-tetramethylbenzidine, NBT-BCIP (4-nitro blue tetrazolium
chloride and 5-bromo-4-chloro-3-indolyl-phosphate, available as ready-made stock solution from Roche Diagnostics), CDP-Star™ (Amersham Biosciences), ECF™ (Amersham Biosciences). A suitable enzyme-substrate combination may result in a colored reaction product, fluorescence or chemoluminescence, which can be measured according to methods known in the art (e.g. using a light-sensitive film or a suitable camera system). As for measuring the enzymatic reaction, the criteria given above apply analogously. Typical fluorescent labels include fluorescent proteins (such as GFP and its derivatives), Cy3, Cy5, Texas Red, Fluorescein, and the Alexa dyes (e.g. Alexa 568). Further fluorescent labels are available e.g. from Molecular Probes (Oregon). Also the use of quantum dots as fluorescent labels is contemplated. Typical radioactive labels include 35S, 125I, 32P, 33P and the like. A radioactive label can be detected by any method known and appropriate, e.g. a light-sensitive film or a phosphor imager. Suitable measurement methods according to the present invention also include precipitation (particularly immunoprecipitation), electrochemiluminescence (electro-generated chemiluminescence), RIA (radioimmunoassay), ELISA (enzyme-linked immunosorbent assay), sandwich enzyme immune tests, electrochemiluminescence sandwich immunoassays (ECLIA), dissociation-enhanced lanthanide fluoro immuno assay (DELFIA), scintillation proximity assay (SPA), turbidimetry, nephelometry, latex-enhanced turbidimetry or nephelometry, or solid phase immune tests. Further methods known in the art (such as gel electrophoresis, 2D gel electrophoresis, SDS polyacrylamid gel electrophoresis (SDS-PAGE), Western Blotting, and mass spectrometry), can be used alone or in combination with labeling or other detection methods as described above.

More preferably the amount of IL-6 is determined by mass spectrometry method, preferably by isotope-dilution micro-HPLC-tandem mass spectrometry method, preferably by a method as described in the Examples and in Kobold U et al. (Clin Chem 2008; 54: 1584-6).

The amount of an IL-6 peptide or polypeptide may be, also preferably, determined as follows: (a) contacting a solid support comprising a ligand for the peptide or polypeptide as specified above with a sample comprising the peptide or polypeptide and (b) measuring the amount peptide or polypeptide which is bound to the support. The ligand, preferably chosen from the group consisting of nucleic acids, peptides, polypeptides, antibodies and aptamers, is preferably present on a solid support in immobilized form. Materials for manufacturing solid supports are well known in the art and include, inter alia, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and
surfaces, nitrocellulose strips, membranes, sheets, duracytes, wells and walls of reaction trays, plastic tubes etc. The ligand or agent may be bound to many different carriers. Examples of well-known carriers include glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for the purposes of the invention. Suitable methods for fixing/immobilizing said ligand are well known and include, but are not limited to ionic, hydrophobic, covalent interactions and the like. It is also contemplated to use "suspension arrays" as arrays according to the present invention (Nolan 2002, Trends Biotechnol. 20(1):9-12). In such suspension arrays, the carrier, e.g. a microbead or microsphere, is present in suspension. The array consists of different microbeads or microspheres, possibly labeled, carrying different ligands. Methods of producing such arrays, for example based on solid-phase chemistry and photo-labile protective groups, are generally known (US 5,744,305).

The term "about" as used herein encompasses a range of + and - 20% relative to the specific value, amount, concentration, level, etc, e.g. indication of a value of "about 100" is meant to encompass a value of a numerical range of 100 +/- 20%, i.e. a value range from 80 to 120. Preferably the term "about" encompasses a range of + and - 10% relative to the specific value, amount, concentration, level, etc, most preferably a range of + and - 5% relative to the specific value, amount, concentration, level, etc.

The term "comparing" as used herein encompasses comparing the level of the IL-6 peptide or polypeptide comprised by the sample to be analyzed with a level of a suitable reference source specified elsewhere in this description. It is to be understood that comparing as used herein refers to a comparison of corresponding parameters or values, e.g., an absolute amount is compared to an absolute reference level while a concentration is compared to a reference concentration or an intensity signal obtained from a test sample is compared to the same type of intensity signal of a reference sample. The comparison referred to in step (b) of the method of the present invention may be carried out manually or computer assisted. For a computer assisted comparison, the value of the determined level may be compared to values corresponding to suitable references which are stored in a database by a computer program. The computer program may further evaluate the result of the comparison, i.e. automatically provide the desired assessment in a suitable output format. Based on the comparison of the level determined in step a) and the reference level, the
diagnosis of SIRS in a patient is determined. Therefore, the reference level is to be chosen so that either a difference or a similarity in the compared levels allows allocation of subjects in to SIRS or not suffering from SIRS.

Accordingly, the term "reference level", refers to an amount, concentration or value which defines a cut-off. An amount, concentration or value of the parameter above the cut-off results in a different diagnosis when compared to patients displaying a determined amount, level or value of the parameter below the cut-off. Thus, by comparing the actually determined amount, concentration or value of the parameters IL-6, to the respective cut-offs it is possible to diagnose or detect the risk in patients to develop or suffer from SIRS or sepsis.

Of course, the reference level applicable for an individual subject may vary depending on various physiological parameters such as age, gender, subpopulation, alcohol intake, recent infections as well as on the means used for the determination of the polypeptide or peptide referred to herein. A suitable reference level may be determined by the method of the present invention from a reference sample to be analyzed together, i.e. simultaneously or subsequently, with the test sample. The reference levels of the present invention were confirmed in the Examples.

As will be understood by those skilled in the art, such a diagnostic assessment is usually not intended to be correct for all (i.e. 100%) of the patients to be identified. The term, however, requires that a statistically significant portion of patients can be identified (e.g. a cohort in a cohort study). Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student's t-test, Mann-Whitney test etc. Details are found in Dowdy and Wearden, Statistics for Research, John Wiley & Sons, New York 1983. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99%. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. More preferably, at least 60%, at least 70%, at least 80% or at least 90% of the patients of a population can be properly identified by the method of the present invention.

In general, for determining the respective levels allowing to establish the desired diagnosis in accordance with the respective embodiment of the present invention, ("threshold", "reference level"), the amount(s)/level(s) or amount ratios of the respective peptide or peptides are determined in appropriate patient groups. As stated before, the reference value is preferably determined by multiplying an earlier determined IL-6 level (e.g. a baseline IL-6 level) in a given
patient, preferably in the subject patient, by a factor defined elsewhere herein (e.g. a factor of at least about 50 or at least about 100, or at least about 500 or at least about 1000). E.g. based on retrospective clinical studies, like the one described in the Examples, where the outcome (no SIRS vs. SIRS vs. sepsis) is analyzed and compared to the changes of the level of IL-6 in patients over time, it is easily possible to statistically verify which "factors" are to be multiplied with a subject patient's baseline IL-6 level in order to establish a reference value which is associated with a specific risk probability of developing or suffering from SIRS or sepsis.

Preferably, the reference level is calculated by multiplying the IL-6 baseline level, by a factor of at least about 50, preferably by a factor of at least about 100, more preferably by a factor of at least about 500, most preferably by a factor of at least about 1000. For example, if the asymptomatic patient presenting to the physician or the hospital with a baseline IL-6 level of 10 pg/ml, the calculated reference level is preferably 500 pg/ml (50-fold increase), preferably 1 ng/ml (100-fold increase), preferably 5 ng/ml (500-fold increase), or preferably 10 ng (1000-fold increase). In such a patient, a level of IL-6 detected after subjecting the patient to (e.g. an invasive) a treatment (e.g. a severe surgery) above the indicated reference levels indicates that the patient is at high risk to develop or suffer from SIRS.

As used herein, the "baseline level" encompasses at least one IL-6 level obtained (i) when the patient presents to the physician, the emergency unit, the hospital, the intensive care unit, the surgeon or the anesthesiologist (ii) before and the therapy is initiated, e.g. before surgery, (iii) during the therapy, e.g. during surgery, and/or (iv) after the treatment has been completed, e.g. after the surgery has been completed. Also encompassed by the term is a baseline level which is determined after subjecting the patient to (e.g. an invasive) a treatment, e.g. within about 24 hours, preferably within about 15 hours, preferably within about 12 hours, preferably within about 6 hours after treatment. Alternatively, the baseline level is calculated by determining the median or average IL-6 level among the samples taken up to but not including a sample where the IL-6 level increased by more than about 20-fold, by more than about 30-fold, by more than about 50-fold, by more than about 50-fold, by more than about 100-fold, or by more than about 500-fold, relative to said median or average IL-6 level.

The diagnosis and detection of the risk of suffering from or developing SIRS or sepsis can be carried out by determining the respective parameters, preferably by measuring the level of IL-6,
using validated analytical methods. The results which are obtained are collected and analyzed by statistical methods known to the person skilled in the art.

Optionally, the reference values are established in accordance with the desired probability of suffering from or being at risk to suffer or develop the disease. For example, it may be useful to choose the median factor to be multiplied with the baseline level from the 60th, 70th, 80th, 90th, 95th or even the 99th percentile of the healthy and/or non-healthy patient collective, in order to establish the reference level(s).

A preferred reference level serving as a threshold may be derived from the upper limit of normal (ULN), i.e. the upper limit of the physiological amount to be found in a population. The ULN for a given population of subjects can be determined by various well known techniques. A suitable technique may be to determine the median of the population for the peptide or polypeptide amounts to be determined in the method of the present invention.

A reference level of a diagnostic marker can be established and confirmed, and the level of the marker in a patient sample can simply be compared to the reference level. 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, or "ROC" curves, are typically calculated by plotting the value of a variable versus its relative frequency in "normal" and "disease" populations. For any particular parameter of the invention, a distribution of marker levels for subjects with and without a disease will likely overlap. Under such conditions, a test does not absolutely distinguish normal from disease with 100% accuracy, and the area of overlap indicates where the test cannot distinguish normal from disease or high risk from low risk to suffer from or develop the disease. A threshold (cut-off, reference level) is selected, above which (or below which, depending on how a marker changes with the disease) the test is considered to be abnormal and below which the test is considered to be normal. The area under the ROC curve is a measure of the probability that the perceived measurement will allow correct identification or prediction of the risk of onset or development 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 an ROC curve. For example, results of a test on "disease" samples might be ranked according to degree of the disease (say 1=low, 2=normal, and 3=high) or probability of suffering from or developing the disease (say 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, Radiology 1982;143: 29-36.

In certain embodiments, markers and/or marker panels are selected to exhibit at least about 70% sensitivity, more preferably at least about 80% sensitivity, even more preferably at least about 85% sensitivity, still more preferably at least about 90% sensitivity, and most preferably at least about 95% sensitivity, combined with at least about 70% specificity, more preferably at least about 80% specificity, even more preferably at least about 85% specificity, still more preferably at least about 90% specificity, and most preferably at least about 95% specificity. In particularly preferred embodiments, both the sensitivity and specificity are at least about 75%, more preferably at least about 80%, even more preferably at least about 85%, still more preferably at least about 90%, and most preferably at least about 95%.

In other embodiments, a positive likelihood ratio, negative likelihood ratio, or odds ratio is used as a measure of a test's ability to predict risk or diagnose a disease. In the case of a positive likelihood ratio, a value of 1 indicates that a positive result is equally likely among subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a positive result is more likely in the diseased group; and a value less than 1 indicates that a positive result is more likely in the control group. In the case of a negative likelihood ratio, a value of 1 indicates that a negative result is equally likely among subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a negative result is more likely in the test group; and a value less than 1 indicates that a negative result is more likely in the control group. In certain preferred embodiments, markers and/or marker panels are preferably selected to exhibit a positive or negative likelihood ratio of at least about 1.5 or more or about 0.67 or less, more preferably at least about 2 or more or about 0.5 or less, still more preferably at least about 5 or more or about 0.2 or less, even more preferably at least about 10 or more or about 0.1 or less, and most preferably at least about 20 or more or about 0.05 or less. The term "about" in this context refers to +/- 5% of a given measurement.

In the case of an odds ratio, a value of 1 indicates that a positive result is equally likely among subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a positive result is more likely in the diseased group; and a value less than 1 indicates that a positive result is more likely in the control group. In certain preferred embodiments, markers and/or marker panels are preferably selected to exhibit an odds ratio of at least about 2 or more or
about 0.5 or less, more preferably at least about 3 or more or about 0.33 or less, still more preferably at least about 4 or more or about 0.25 or less, even more preferably at least about 5 or more or about 0.2 or less, and most preferably at least about 10 or more or about 0.1 or less. The term "about" in this context refers to +/- 5% of a given measurement.

Panels may comprise at least one additional marker; both specific markers of a disease (e.g., markers that are increased or decreased in bacterial infection, but not in other disease states) and/or non-specific markers (e.g., markers that are increased or decreased due to inflammation, regardless of the cause; markers that are increased or decreased due to changes in hemostasis, regardless of the cause, etc.). While certain markers may not be individually definitive in the methods described herein, a particular "fingerprint" pattern of changes may, in effect, act as a specific indicator of disease state. As discussed above, that pattern of changes may be obtained from a single sample, or may optionally consider temporal changes in one or more members of the panel (or temporal changes in a panel response value).

In a preferred embodiment of the methods of the present invention,

i) a level of IL-6 or a variant thereof determined in step a) at or above to the reference level is indicative of the patient to be at a high risk to suffering or developing SIRS; and

ii) a level of IL-6 or a variant thereof determined in step a) below the reference level is indicative of the patient to be at a low risk to suffering or developing SIRS.

It has been observed that the IL-6 concentration differs significantly among asymptomatic patients. For example, the following factors contribute to this inter-individual differences: recent alcohol consumption, physical exercise, stress, recent history of infections, injury and in acute hyperglycaemia. Accordingly, in a preferred embodiment of the method of the present invention, the IL-6 reference level is determined on a patient-by-patient basis.

Preferably, patients displaying IL-6 levels above the reference level are closely monitored for the onset of clinical signs and symptoms of SIRS or sepsis. More preferably, the patients are subjected to monitoring of the following parameters: IL-6 levels, white blood cell count (WBC) and determination of immature leucocyte forms, body temperature, heart-rate, respiratory rate, collection of microbiological specimens from drainages, serum, tracheobronchial secretions and urine and daily chest roentgenogram. Control endoscopies and radiological workup by ultrasound and CT-scan are warranted.
More preferably, the patients are treated as follows: Initiation of broad spectrum antibiotic treatment, antifungal treatment. In case of doubt, redo surgery shall be scheduled liberally.

In another aspect of the present invention it is provided a method of monitoring an asymptomatic patient the risk to develop or suffer from SIRS or sepsis, comprising the steps of

a) determining the level of IL-6 or a variant thereof in a sample from the patient;

b) comparing the level of IL-6 or a variant thereof determined in step a) to a reference level; and

c) recommending, deciding on, initiating, continuing, modulating or discontinuing the SIRS or sepsis therapy for the patient based on comparison in step c).

Diagnostic and therapeutic consequences of the above monitoring include: Microbiological specimens from serum, drainages, tracheobronchial secretions, and urine, endoscopical controls, radiological imaging procedures as are ultrasound, chest roentgenograms and CT-scans, initiation of broad spectrum antibiotic treatment or change of preexistent antibiotic treatment to a more efficient one, antifungal treatment. In case of doubt, redo surgery shall be scheduled liberally.

In case the level of IL-6 or a variant thereof is higher than the reference level, the sample or an additionally collected sample is subjected to an assay to identify the infectious organism, e.g. the bacteria, fungus, etc., contained in the sample. Such assays are generally known in the field and are relevant to the diagnosis of sepsis.

In another aspect of the present invention it is provided a method of predicting the risk of mortality in an asymptomatic patient, comprising the steps of

a) determining the level of IL-6 or a variant thereof in a sample from the patient;

b) comparing the level of IL-6 or a variant thereof determined in step a) to a reference level;

c) predicting the risk of mortality for the patient based on comparison in step b).

Unless specified differently, the definitions and preferred embodiments described with respect to the method for detection or diagnosis of a risk to suffer from or develop SIRS, above, also apply mutatis mutandis to the present aspect of the invention.
According to yet another aspect of the invention it is provided a device adapted for detection or
diagnosis of a risk to suffer from or develop SIRS, preferably according to a method described
above, comprising:

a) a first analyzing unit comprising a detection means for IL-6 or a variant thereof, wherein
the analyzing unit is adapted for determining the level of the IL-6 detected by the
detection means;

b) an evaluation unit comprising a computer comprising tangibly embedded a computer
program code for carrying out the comparison of the determined amount obtained from
the first analyzing unit with a suitable data base comprising a corresponding reference
level as specified above; preferably the first analyzing unit and preferably also the
evaluation units are operatively linked to each other.

Preferably, the device further comprises means for outputting the required diagnosis and
treatment and/or prevention on the basis of the diagnosis or risk prediction of the patient to suffer
from or develop SIRS or sepsis. More preferably the device further comprises means for
outputting the progress and/or response to a treatment and/or therapy of SIRS or sepsis.

According to yet another aspect of the invention it is provided a kit adapted for carrying out the
method describe above, comprising:

means for determining the level of IL-6 or a variant thereof;

means for comparing the determined level of IL-6 or a variant thereof with reference level;

instructions for carrying out the method.

The term "kit" as used herein refers to a collection of the aforementioned compounds, means or
reagents of the present invention which may or may not be packaged together. The components
of the kit may be comprised by separate vials (i.e. as a kit of separate parts) or provided in a
single vial. Moreover, it is to be understood that the kit of the present invention is to be used for
practising the methods referred to herein above. It is, preferably, envisaged that all components
are provided in a ready-to-use manner for practising the methods referred to above. Further, the
kit preferably contains instructions for carrying out the said methods. The instructions can be
provided by a users manual in paper- or electronic form. For example, the manual may comprise
instructions for interpreting the results obtained when carrying out the aforementioned methods
using the kit of the present invention.
According to yet another aspect of the invention it is provided a computer program comprising computer program code which is suitable for carrying out a method of the invention when the computer program is run on a computer.

In another aspect of the invention it is provided a computer readable medium with a computer program of the invention stored thereon.

In another aspect of the invention it is provided a computer program product with a computer program of the invention stored thereon. Preferably, the computer program further comprises means for outputting the required prevention and/or therapy on the basis of the diagnosed disease, the means being stored on a computer readable medium.

Unless specified differently, the definitions and preferred embodiments described with respect to the method detection or diagnosis of a systemic inflammatory response syndrome (SIRS), or for detection or diagnosis of a risk to suffer from or develop SIRS, above, also apply to the present aspect of the invention.

In another aspect of the invention it is provided a kit adapted for carrying out the method of the present invention, comprising:

i) means for determining the level of IL-6 or a variant thereof;

ii) means for comparing the determined level of IL-6 or a variant thereof with reference levels; and optionally

iii) instructions for carrying out the method.

In another aspect of the invention, it is provided a method for detection or diagnosis of a systemic inflammatory response syndrome (SIRS) or sepsis, or for detection or diagnosis of a risk to suffer from or develop SIRS, in an asymptomatic patient, comprising the steps of

a) determining the level of CRP in a sample from the patient;

b) comparing the level of CRP determined in step a) to a reference level;

c) detecting or diagnosing SIRS, or detecting or diagnosing a risk to develop SIRS;

wherein the sample is isolated at least 2 times at short intervals and steps a) and b) are repeated for each sample.
In another aspect of the present invention it is provided a method for detection or diagnosis of the risk to develop or suffer from a systemic inflammatory response syndrome (SIRS) or sepsis, in an asymptomatic patient, comprising the steps of

a) determining the level of procalcitonin in a sample from the patient;

b) comparing the level of procalcitonin determined in step a) to a reference level;

c) detecting or diagnosing the risk to develop or suffer from a systemic inflammatory response syndrome (SIRS) or sepsis;

wherein a sample is isolated at least 2 times at short intervals and steps a) and b) are repeated for each sample.

In another aspect of the invention, it is provided an antibody selected from an anti-IL-2 antibody, an anti-IL-3 antibody, an anti-IL-4 antibody, an anti-IL-5 antibody, and an anti-IL-6 antibody.

Brief description of the drawings

Figure 1 IL-6 (pg/ml) kinetics in asymptomatic patients pre- and post-surgery. IL-6 levels are plotted for baseline 1 (pre-surgery), baseline 2 (during surgery), and post-surgery (day 1, 2, 3 and 4; samples were taken at 6h intervals). The Figure shows a comparison between pooled patients who developed SIRS/sepsis and those who did not. Median and the percentiles are indicated.

Figure 2 IL-6 concentration (pg/ml) plotted over time for patients No. 1 to 7. The triangle indicates the time point clinical SIRS signs were diagnosed.

Figure 3 Characteristics of non-SIRS patients. IL-6 concentration (pg/ml) was plotted over time for non-SIRS patients.

Figure 4 CRP (mg/L) kinetics in asymptomatic patients pre- and post-surgery. CRP levels are plotted for baseline 1 (pre-surgery), baseline 2 (during surgery), and post-surgery (day 1, 2, 3 and 4; samples were taken at 6h intervals). The Figure shows a comparison between pooled patients who developed SIRS/sepsis and those who did not. Median and the percentiles are indicated.
Figure 5: CRP vs. IL-6 kinetics in asymptomatic patients pre- and post-surgery. The Figure shows a comparison between pooled patients who developed SIRS/sepsis and those who did not. Medians are indicated.

All references cited in this specification are herewith incorporated by reference with respect to their entire disclosure content and the disclosure content specifically mentioned in this specification.

The following Examples shall merely illustrate the invention. They shall not be construed, whatsoever, to limit the scope of the invention.

**Examples**

**Patient characteristics**

48 patients, (36 males and 12 females), were included into the study. The mean age of the patients was 61 years, ranging from 19 to 87. All patients were admitted to a thoracic surgical division of the Medizinische Universitat Graz, Austria, for elective surgery. 37 patients underwent lung surgery due to various carcinoma types, 9 patients had oesophagoectomy because of an oesophagus carcinoma, 1 patient underwent gastrectomy, and 1 patient underwent gastrectomy plus oesophagoectomy due to chemical burn.

In all patients an antibiotic treatment was initiated in the morning of the day of surgery.

All patients had two baseline level measurements before surgery to determine, both, IL-6 and routine laboratory parameters.

Systemic Inflammatory Response Syndrome (SIRS) was diagnosed based on the ACCP/SCCM Consensus Conference Definitions (1992/2003), i.e. a SIRS diagnosed patients displayed at least 2 symptoms of the following:

a) white blood cell count of more than about 12,000/µL or less than about 4000/*i/L,

b) a body temperature of more than about 38°C or less than about 36°C,

c) a heart rate of more than about 90 beats/minute or a partial pressure of CO₂ of less than about 32 mm Hg, and
d) a respiratory rate of more than about 20 breaths/minute,

e) more than about 10% immature white blood cells among the counted white blood cells.

Out of the 48 patients 10 (21%) developed SIRS within the time window of monitoring of the study. Out of the 10 patients showing clinical signs of SIRS three had in addition a positive blood culture which confirmed sepsis. Two of the cases responded to a change of antibiotic treatment though only in one patient the infection was confirmed by a positive blood culture. 5 out of the 10 patients showed purely SIRS signs without infection during the study period. One of the patients developed SIRS shortly after the end of the window of monitoring. In light of the results discussed below this shows that IL-6 based diagnosis and prediction is not limited to sepsis but is also applicable to SIRS.

Material and methods

IL-6 was determined using a sandwich ELISA immunoassay based on the Roche Cobas Elecsys IL-6 assay (Roche, Mannheim, Germany). In brief, 30 μL of the plasma or serum sample were incubated with a biotinylated monoclonal IL-6-specific antibody. After addition of a monoclonal IL-6-specific antibody labeled with a ruthenium complex and streptavidin-coated microparticles, the antibodies formed a sandwich complex with the antigen of the sample. The reaction mixture was then aspirated into the measuring cell of the Elecsys electrochemiluminescence device where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were then removed with ProCell.

Application of a voltage to the electrode then induced chemiluminescent emission which was measured by a photomultiplier. Results were determined via a calibration curve which was instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

The determined IL-6 concentration was statistically analyzed using standard statistical tests. According to the study protocol the enrolled patients were monitored regarding clinical and laboratory signs of SIRS starting with 2 baseline levels taken at two time points before and during surgery. After surgery, the patients were surveyed closely by taking blood samples twice
before and every 6 hours during 6 days after surgery. At every time point when blood was taken the clinical status was recorded according to the established SIRS and sepsis criteria.

Results

Surprisingly, the IL-6 concentration values of the SIRS/sepsis patients differed significantly from the median values of the patients who did not develop SIRS. Even though the IL-6 levels increased in all patients after surgery, only the patients of the SIRS group displayed a large increase in IL-6 concentration relative to their respective baseline levels, whereas the non-SIRS patients only showed an increase in IL-6 concentration by up to 10-fold when compared to the respective baseline levels (See figures).

**Characteristics of some of the SIRSpatients (see Figure 1 and 2):**

The patient characteristics of patient no. 1 were as follows: male, age 62, oesophageal carcinoma, oesophagectomy. Routine laboratory paramaters and IL-6 values before surgery were inconspicuous (IL-6 baseline levels 14.3 and 2.9 pg/ml). On the first day after surgery a baseline level of 6.4 pg/ml IL-6 was measured at noon. The IL-6 level 6 hours later was 2.279.00 pg/ml. Though the values were decreasing over time, they stayed high until day 6 with values around 500 pg/ml. Clinical SIRS/sepsis signs were only recognized first time on day 4, when a positive blood culture indicating a bacterial infection and an increased body temperature > 38 0C was documented. On day 6 the heart rate was >90 beats/minute. This case convincingly shows that close and frequent IL-6 monitoring allowed for early diagnosis and detection of a high risk to develop or suffer from SIRS well ahead of the onset of clinical signs and symptoms supporting a diagnosis of SIRS or sepsis.

The patient characteristics of patient no. 2 were as follows: male, age 81, carcinoma at the esophagogastric junction. Before surgery the patient had a low leucocyte count. The second baseline level was actually < 4000 white blood cells. On day 1 after surgery the patient had a body temperature < 36 0C and a breathing rate of >20 /minute. The two signs were taken as suggestive of SIRS. At the same time the IL-6 values which were at baseline 1 1.6 pg/ml and at baseline 2 37.9 pg/ml increased to 1400.00 pg/ml. Apart from the blood cell count which was increased to > 12 from day 2 until day 6 only the IL-6 values remained high (up to 500 pg/ml) until day 4 and then slowly decreased. No positive blood culture result was obtained, i.e. no
sepsis relevant infection was detected. This case shows that a level of IL-6 above the reference level allows for early diagnosis and detection of a high risk to develop or suffer from SIRS.

The patient characteristics of patient no. 3 were as follows: male, age 70, lung cancer, pneumonectomy. The patient had initially (baseline 1) an increased white blood cell count of > 12,000 but a normalized value at baseline 2 time point one week later. The baseline IL-6 levels were also within the normal range of 23.5 and 16.5 pg/ml. During all time points after surgery no clinical signs other than an elevated IL-6 level appeared which pointed to SIRS or sepsis. After day 2 the white blood cell count increased to levels of about 13,000 and did not decrease. IL-6 increased after surgery to values of 200 to 300 pg/ml with interim peaks of 600 pg/ml and even 850 pg/ml at one measuring point. On day 5 in the evening the IL-6 value suddenly increased to 3994.00 pg/ml after a value of 148.6 pg/ml 6 hours ago. Two days later, beyond the study protocol time line, the patient developed SIRS criteria and developed a critical condition but could be stabilized. No other laboratory or clinical parameter beside IL-6 had reflected the acute worsening of the patient. Accordingly, the tight monitoring of IL-6 surprisingly allowed for an early detection of a high risk to develop SIRS or sepsis which allowed for an early therapeutic intervention.

The patient characteristics of patient no. 4 were as follows: female, age 50, chondrosarcoma, metastases in the lung, pneumonectomy. The WBC count at baseline and during the entire study period of 6 days were in the normal range with a maximum value of 11,000 at day 4. IL-6 increased already during surgery (baseline 2) to a value of 282.7 pg/ml. At the first measurement after surgery (day 1/1) IL-6 increased to 1079 pg/ml and 6 hours later peaked at 2771 pg/ml. From that time point on the values decreased continuously to values of about 70 pg/ml at the end of the study period. SIRS criteria temperature > 38°C and HR > 90/ min appeared in this patient at the same time points as IL-6 had its highest values which confirms that IL-6 is a reliable early marker of SIRS.

Patient characteristics of patient number 5: male, 19 years, chemical burn of oesophagus and stomach. At baseline before surgery all parameter were in a normal range. IL-6 increased slightly up to 67.4 pg/ml during surgery when the second baseline blood sample was taken. The next blood sample taken 6 hours later (day 1/1) already showed an IL-6 value of more than 700 pg/ml which further increases to more than 1000 pg/ml during the next days (day 1/1- 3/1). Clinical
signs of SIRS (heart rate > 100/min and breath rate > 20/min) were registered from day 5/2 until the end of the study period (day 6/2).

Characteristics of patient number 6: female, 60 years old, lobectomy after lung cancer. At both baseline levels all parameter were in the normal range. The first IL-6 value after surgery (day 1/1) showed a rise to almost 500 pg/ml. IL-6 levels were increased over the full study period showing levels between 550 and 150 pg/ml. Leucocytes increased significantly and reached levels of > 20x10^9 from day 1/4 on. In conjunction to an increase of body temperature > 38°C it was registered as SIRS. After two time points body temperature went normal again but increased once more at day 6/2.

Characteristics of patient number 7: male, age 48 years, lung cancer, pneumectomy. Initially at baseline all parameters were in a normal range. Already at the second baseline timepoint the patient showed an increased heart rate of > 100/min which persists at almost all study time points. IL-6 increased significantly at day 1/1 to a value of almost 400 pg/ml and fell afterwards to moderately increased values of around 70 pg/ml during the next 7 sampling timepoints. At day 4/1 IL-6 started to increase again reaching values of more than 500 pg/ml. Clinical signs of SIRS (heart rate > 100/min and breath rate > 20/min) were registered from day 5/2 on until study end at day 6/2.

Characteristics of non-SIRSpatients (See Figures 1 and 3 to 5).

38 out of the 48 patients who did not develop SIRS or sepsis according to the definitions displayed up to one single symptom of SIRS during the observation period at a certain time point but never two or more and accordingly, these patients were diagnosed as not suffering from SIRS. The baseline IL-6 values were comparable to the one of the SIRS patients. After surgery all non-SIRS patients also had a slight increase in the IL-6 values some up to about 100 pg/ml, some up to 400, one with a single peak of 900 pg/ml, but they did not show the drastic elevation of IL-6 levels observed in the SIRS patient group. The observed small increase in IL-6 levels in the non-SIRS patients is within the range that one would expect, considering the severeness of the conducted surgery. Based on the outcome (no onset of SIRS or sepsis) in these patients the data confirm that the patients were at a low risk to suffer or develop SIRS or sepsis.
Literature:


Claims

1. A method for detection or diagnosis of the risk to develop or suffer from a systemic inflammatory response syndrome (SIRS) or sepsis, in an asymptomatic patient, comprising the steps of
   a) determining the level of IL-6 or a variant thereof in a sample from the patient;
   b) comparing the level of IL-6 or a variant thereof determined in step a) to a reference level;
   c) detecting or diagnosing the risk to develop or suffer from a systemic inflammatory response syndrome (SIRS) or sepsis;

   wherein a sample is isolated at least 2 times at short intervals ranging from about 15 minutes to about 12 hours and steps a) and b) are repeated for each sample.

2. The method of claim 1, wherein the asymptomatic patient is a patient who displays less than 2, preferably less than 1 symptom of the following a) to d), preferably of the following a) to e):
   a) white blood cell count of more than about 12,000/µL or less than about 4000/µL,
   b) a body temperature of more than about 38°C or less than about 36°C,
   c) a heart rate of more than about 90 beats/minute or a partial pressure of CO₂ of less than about 32 mm Hg, and
   d) a respiratory rate of more than about 20 breaths/minute,
   e) more than about 10% immature white blood cells among the counted white blood cells,

   optionally the patient does not display a diagnosed infection.

3. The method of claim 1 or 2, wherein the short interval ranges from about 1 to 6 hours, preferably from about 1 hour to about 3 hours.

4. The method according to any one of claims 1 to 3, wherein
i) a level of IL-6 or a variant thereof determined in step a) above to the reference level is indicative of the patient to be at high risk of suffering or developing SIRS or sepsis; and

ii) a level of IL-6 or a variant thereof determined in step a) below the reference level is indicative of the patient to be at low risk of suffering or developing SIRS or sepsis.

5. The method according to any one of claims 1 to 4, wherein the reference level is obtained by multiplying a baseline level of IL-6 or a variant thereof by a factor of at least about 50, preferably by a factor of at least about 100, more preferably by a factor of at least about 500, most preferably by a factor of at least about 1000.

6. The method of any one of claims 1 to 5, wherein IL-6 is an IL-6 which can be bound by murine anti-IL-6 monoclonal antibody M-BE8 or M-23C7.

7. The method of any one of claims 1 to 6, wherein the a level of IL-6 or a variant thereof in the sample is determined by

i) an antibody which binds to IL-6, or by a fragment or variant thereof;

ii) an antibody which specifically binds to IL-6, or a fragment or variant thereof;

iii) an antibody which binds to IL-6 which can be bound by murine anti-IL-6 monoclonal antibody M-BE8 or M-23C7, or by a fragment, or variant thereof; or

iv) a murine anti-IL-6 monoclonal antibody M-BE8 or M-23C7, or by a fragment or variant thereof.

8. The method of any one of claim 1, wherein CRP is determined instead of IL-6 and the method is used to diagnose SIRS or sepsis.

9. The method of any one of claims 1 to 8, wherein the asymptomatic patient is a patient selected from the group of a trauma patient, a patient with burns, a patient undergoing
an treatment, a patient undergoing an invasive treatment, a patient undergoing a surgical intervention.

10. The method of claim 9, wherein the patient is undergoing an invasive treatment and the sample is taken at least once before the treatment and at least once after the treatment.

11. The method according to any one of claims 1 to 10, wherein the method
   i) further comprises a step of collecting a sample from the patient by a minimal-invasive step,
   ii) excludes a surgical step of collecting a sample, or
   iii) is an *in vitro* method.

12. A method of monitoring an asymptomatic patient for the onset of SIRS or for assessing the risk to develop or suffer from SIRS, comprising the steps of
   a) determining the level of IL-6 or a variant thereof in a sample from the patient;
   b) comparing the level of IL-6 or a variant thereof determined in step a) to a reference level; and
   c) recommending, deciding on, initiating, continuing, modulating or discontinuing a SIRS therapy for the patient based on comparison in step b).

13. A kit adapted for carrying out the method of any of claims 1 to 12, comprising:
   i) a means for determining the level of IL-6 or a variant thereof;
   ii) a means for comparing the determined level of IL-6 or a variant thereof with reference levels; and optionally
   iii) an instruction for carrying out the method.
14. Use of a means for detecting IL-6 or a variant thereof in a sample of an asymptomatic patient, for early detecting or diagnosing the risk to develop or suffer from SIRS or sepsis, or for early detecting or diagnosing SIRS or sepsis.
IL-6 (pg/ml) kinetics in unsymptomatic patients pre- and post surgery. Comparison between patients who develop SIRS/sepsis and those who don't.
Figure 1 continued

IL-6 (pg/ml) kinetics in unsymptomatic patients pre- and post surgery. Comparison between patients who develop SIRS/sepsis and those who don't.
Figure 1 continued

IL-6 (pg/ml) kinetics in unsymptomatic patients pre- and post surgery. Comparison between patients who develop SIRS/sepsis and those who don't.

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| 75th perc. | 95.400 | 522.500 | 81.650 | 348.200 | 93.250 | 367.200 | 76.200 | 286.600 |
| 95th perc. | 269.500 | 643.100 | 358.400 | 438.600 | 525.800 | 424.150 | 336.650 | 329.050 |
| 50th perc. | 29.450 | 47.250 | 26.600 | 112.800 | 22.850 | 36.800 | 16.650 | 46.050 |
| 25th perc. | 45.800 | 150.700 | 41.750 | 198.750 | 37.600 | 136.400 | 29.450 | 109.100 |
| Median | 49.200 | 222.900 | 57.350 | 245.000 | 48.650 | 170.400 | 40.150 | 143.800 |
Figure 1 continued

IL-6 (pg/ml) kinetics in unsymptomatic patients pre- and post surgery. Comparison between patients who develop SIRS/sepsis and those who don't.

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IL-6 (pg/ml) kinetics in unsymptomatic patients pre- and post surgery. Comparison between patients who develop SIRS/sepsis and those who don't.

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Median: 52.000 139.200 24.100 202.700 33.300 244.900
IL-6 (pg/ml) kinetics in unsymptomatic patients pre- and post surgery. Comparison between patients who develop SIRS/sepsis and those who don't.

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Figure 4

CRP Levels

CRP kinetics in unsymptomatic patients pre- and post surgery. Comparison between patients who develop SIRS/sepsis and those who don't.

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<td>87.400</td>
<td>87.400</td>
<td>87.400</td>
<td>87.400</td>
<td>87.400</td>
</tr>
<tr>
<td>25th perc</td>
<td>71.700</td>
<td>71.700</td>
<td>71.700</td>
<td>71.700</td>
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<tr>
<td>75th perc</td>
<td>95.300</td>
<td>95.300</td>
<td>95.300</td>
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</tr>
<tr>
<td>Mean</td>
<td>87.400</td>
<td>87.400</td>
<td>87.400</td>
<td>87.400</td>
<td>87.400</td>
</tr>
</tbody>
</table>
**Figure 4 continued**

**CRP Levels**

CRP kinetics in unsymptomatic patients pre- and post surgery. Comparison between patients who develop SIRS/sepsis and those who don't.

<table>
<thead>
<tr>
<th>Day 1/4</th>
<th>Day 2/1</th>
<th>Day 2/2</th>
<th>Day 2/3</th>
<th>Day 2/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=32 Non-SIRS</td>
<td>n=2 SIRS</td>
<td>n=35 Non-SIRS</td>
<td>n=35 Non-SIRS</td>
<td>n=7 SIRS</td>
</tr>
</tbody>
</table>

**Values:**
- 75th percentile: 157.500, 184.600, 238.400, 238.500, 205.150, 287.530, 240.240, 273.300
- 5th percentile: 42.350, 52.600, 42.450, 36.500, 132.150, 53.600, 133.550, 77.600
- 25th percentile: 62.850, 71.500, 83.500, 91.500, 164.250, 95.100, 187.600, 98.600
- Median: 120.550, 261.600, 123.600, 211.200, 239.800, 186.100, 133.900, 237.600
Figure 4 continued

CRP Levels

CRP kinetics in unsymptomatic patients pre- and post surgery. Comparison between patients who develop SIRS/sepsis and those who don't.

<table>
<thead>
<tr>
<th></th>
<th>Day 3/2</th>
<th>Day 3/4</th>
<th>Day 3/3</th>
<th>Day 3/4</th>
</tr>
</thead>
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<tr>
<td>1000</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>100</td>
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<td>10</td>
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<tr>
<td>1.0</td>
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</table>

Table:

<table>
<thead>
<tr>
<th></th>
<th>Non-SIRS Baseline</th>
<th>≤ 8 SIRS</th>
<th>≤ 31 Non-SIRS</th>
<th>≤ 31 SIRS</th>
<th>n = 6 SIRS</th>
<th>n = 30 Non-SIRS</th>
<th>n = 30 SIRS</th>
<th>n = 32 Non-SIRS</th>
<th>n = 32 SIRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>75th percentile</td>
<td>293.400</td>
<td>324.300</td>
<td>207.600</td>
<td>290.700</td>
<td>216.900</td>
<td>320.350</td>
<td>173.800</td>
<td>321.850</td>
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<tr>
<td>95th percentile</td>
<td>972.050</td>
<td>145.750</td>
<td>210.600</td>
<td>316.750</td>
<td>266.550</td>
<td>346.600</td>
<td>295.100</td>
<td>352.920</td>
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<tr>
<td>Mean</td>
<td>45.000</td>
<td>163.650</td>
<td>207.750</td>
<td>119.400</td>
<td>26.200</td>
<td>104.550</td>
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</tr>
<tr>
<td>25th percentile</td>
<td>226.200</td>
<td>88.000</td>
<td>226.000</td>
<td>82.200</td>
<td>151.350</td>
<td>64.150</td>
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</tr>
<tr>
<td>Median</td>
<td>118.700</td>
<td>269.800</td>
<td>128.600</td>
<td>266.200</td>
<td>113.300</td>
<td>258.400</td>
<td>111.550</td>
<td>257.650</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4 continued

CRP Levels

IL-6 (pg/ml) kinetics in unsymptomatic patients pre- and post surgery. Comparison between patients who develop SIRS/sepsis and those who don't.

<table>
<thead>
<tr>
<th></th>
<th>Day 0/1</th>
<th>Day 0/2</th>
<th>Day 0/3</th>
<th>Day 0/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>n= 30 Non-SIRS</td>
<td>287.650</td>
<td>300.250</td>
<td>113.650</td>
<td>281.250</td>
</tr>
<tr>
<td>n= 8 SIRS</td>
<td>259.500</td>
<td>325.400</td>
<td>259.850</td>
<td>344.400</td>
</tr>
<tr>
<td></td>
<td>281.450</td>
<td>281.450</td>
<td>134.600</td>
<td>271.950</td>
</tr>
</tbody>
</table>

75th p<.05

50th p<.05

25th p<.05

Mean ± 2 sd
Figure 5 continued

SIRS/Sepsis
Non-SIRS

IL-6 pg/ml

Blood Samples

1400,000 1200,000 1000,000 800,000 600,000 400,000 200,000 0,000

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N 33/68

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

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
G01N

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

Electronic database consulted during the international search (name of database and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

**A** document member of the same patent family

Date of the actual completion of the international search
5 May 2011

Date of mailing of the international search report
04/07/2011

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-2016

Authorized officer
Behrens, Ralf

Form PCT/ISA/210 (second sheet) (April 2005)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>MOKART D ET AL: &quot;Early postoperative compensatory anti-inflammatory response syndrome is associated with septic complications after major surgical trauma in patients with cancer&quot;, BRITISH JOURNAL OF SURGERY, JOHN WRIGHT &amp; SONS, BRISTOL, GB, vol. 89, 1 January 2002 (2002-01-01), pages 1450-1456, XP007913941, ISSN: 0007-1323 abstract; materials and methods; figure 1; discussion on p. 1454 col. 1</td>
<td>1,2,4-7, 9-14</td>
</tr>
<tr>
<td>X</td>
<td>KUSTER H ET AL: &quot;Interleukin-1 receptor antagonists and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestations&quot;, LANCET THE, LANCET LIMITED, LONDON, GB LNKD-D01:10.1016/0140-6736(98)08148-3, vol. 352, no. 9136, 17 October 1998 (1998-10-17), pages 1271-1277, XP004738197, ISSN: 0140-6736 abstract; materials and methods; table 2; discussion on p. 1274 col. 1</td>
<td>1,2,4-7, 9-14</td>
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<td>X</td>
<td>BENDER L ET AL: &quot;Early and late markers for the detection of early-onset neonatal sepsis&quot;, DANISH MEDICAL BULLETIN, ALMINDELIGE DANSKE LAEGFORENING, DK, vol. 55, no. 4, 1 November 2008 (2008-11-01), pages 219-223, XP009136205, ISSN: 1603-9629 abstract; materials and methods; table 3</td>
<td>1-7, 9-14</td>
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<td>Relevant to claim No.</td>
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<td>REDL H ET AL: &quot;Procalci toni n release patterns in a baboon model of trauma and sepsis: relationship to cytokines and neopterin n&quot;. CRITICAL CARE MEDICINE, WILLS AND WILKINGS COMPANY, BALTIMORE, MA, US, vol. 28, no. 11, 1 November 2000 (2000-11-01), pages 3659-3663, XP009136213, ISSN: 0090-3493 abstract; materials and methods; figure 2</td>
<td>1-7,9-14</td>
</tr>
</tbody>
</table>
## Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

- see additional sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

   2-7, 12, Incompletely) ; 1, 9-11, 13(partial ly)

### Remark on Protest

- The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 2-7, 12, (incompletely); 1, 9-11, 13 (partially)

   Detecting the risk of developing SIRS in an asymptomatic patient employing IL-6 as biomarker. Kit therefore.

2. Claims: 8 (completely); 1, 9-11, 13 (partially)

   Diagnosis of SIRS or sepsis in an asymptomatic patient employing CRP as biomarker. Kit therefore.