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(54) Titre : PROCEDE POUR PREDIRE L'EVOLUTION INDIVIDUELLE D'UNE MALADIE EN CAS DE SEPSIS
(54) Title: METHOD FOR PROGNOSIS OF THE INDIVIDUAL COURSE OF DISEASE IN THE CASE OF SEPSIS

(57) Abrégé/Abstract:
The invention relates to the use of in-vitro gene expression profiles, obtained from a patient, for the generation of criteria for the prediction of an individual disease course in sepsis. The invention is further of use for determining the probability of survival in sepsis, the course evaluation of sepsis during treatment and for the classification of sepsis patients.

Abstract

The invention relates to the use of gene expression profiles, obtained in vitro from a patient sample, for the generation of criteria for the prediction of an individual course of disease in sepsis. The invention is further of use for determining the probability of survival in sepsis, the assessment of the course of disease in sepsis during treatment and for the classification of sepsis patients.

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JUMBO APPLICATIONS / PATENTS

THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE THAN ONE VOLUME.

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Description

Method for prognosis of the individual course of disease in the case of sepsis.

The present invention relates to the use of gene expression profiles obtained from a patient sample in vitro for setting up criteria for the prognosis of the individual course of disease in sepsis, a method for in vitro measurement of such gene expression profiles according to claim 10, as well the use of the gene expressions profiles and/or of probes used therefore for switching of and/or for changing the activity of target genes and/or for determining the gene activity for screening of active agents against sepsis and/or for evaluating the effect in the treatment of sepsis and/or the quality of the active agent and/or the integrity of the active agent in cellular and cell-free sepsis model systems and in sepsis animal models.

The present invention further relates to new possibilities of predicting the probability of surviving and the development of lethal complications in sepsis patients which can be derived from experimentally verified insights in conjunction with the occurrence of changes in gene activity (transcription) in patients with sepsis.

Despite advances in pathophysiological understanding and the supportive treatment of intensive care patients, generalised inflammatory conditions as SIRS and sepsis as defined according to American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (ACCP/SCCM) of 1992 [1] frequently occur in patients in intensive care facilities and contribute substantially to mortality of infections [2-3]. The mortality rate is approximately 20 % in the case of SIRS, approximately 40% in the case of sepsis, and increases to up to 70-80 % in the case of development of multiple organ dysfunctions [4-6]. The contribution of SIRS

and sepsis to morbidity and lethality is of multidisciplinary interest, as it increasingly puts the success of the most advanced or experimental treatment methods of many medicinal fields (e.g. traumatology, neurosurgery, cardiac/pulmonary surgery, visceral surgery, transplantation medicine, hematology/oncology, etc.) at a risk, as they all, without exception, are threatened by an increased risk of the development of SIRS and sepsis. This also becomes apparent from the continous increase of the occurence of sepsis, namely by 139% from 73.6 to 176 cases per 100,000 hospital patients from 1979 and 1977, for example [7]. Thus, the decrease of morbidity and lethality of many seriously ill patients goes along with the concurrent progress in prevention and treatment and especially detection and monitoring of the progression of the sepsis and severe sepsis.

On a molecular basis, sepsis is defined as a clinical picture caused by pathogenic microorganisms. On the basis of an exhaustion of molecular control and regulation mechanisms near the infection site, a generalised inflammatory reaction develops that affects the whole organism. This infection is responsible for the clinical symptoms/criteria for diagnosis/SIRS-criteria according to [1] confirmed by the physician. This generalised inflammatory condition (defined as sepsis according to [1]) goes along with signs of the activation of various cell systems (endothelial cells, but also all leucocyte-like cell systems and in particular of the monocyte/macrophage system). Finally, molecular mechanisms, the normal task of which it is to protect the host against invasive microorganisms, harm the host's own organs/tissues and, thus, essentially contribute to the development of the organ dysfunctions dreaded by the physicians [8-11].

The meaning of the term sepsis has changed considerably in the course of time. An infection, or the strong suspicion of an infection, are still an essential part of the current definition of sepsis. Of particular consideration is, however, the description of organ failure functions remote from the location of infection

in the framework of the inflammatory host reaction. The criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (ACCP/SCCM) of 1992 are the ones that became most accepted in international literature as definition of the term sepsis [1]. According to these criteria [1] distinctions are made between the clinically defined degrees of severity „systemic inflammatory response syndrome" (SIRS), „sepsis", „severe sepsis" and „septic shock". According to this definition, SIRS is defined as the systemic response of the inflammatory system to an infectious or non-infectious stimulus. At least two of the following clinical criteria must be satisfied in this context: Fever >38°C or hypothermia <36°C, a leukocytosis >12G/l or a leukopenia <4G/l or, as the case may be, a shift to the left in the differential haemogram, heart rate >90/min, tachypnoea >20 breaths/min or PaCO2 (partial pressure of carbon dioxide in arterial blood) <4.3 kPa. Those clinical conditions which satisfy the SIRS criteria and for which causatively an infection can be confirmed our at least is very probable are defined as sepsis. A severe sepsis is characterized by the additional occurrence of organ dysfunctions. Common organ dysfunctions include changes in the state of consciousness, oliguria, lactate acidosis or sepsis-induced hypotension with a systolic blood pressure lower than 90 mmHg, or drop in pressure of more than 40 mmHg from the initial value, respectively. If such a hypotension cannot be treated by the administration of crystalloids and/or colloids and the patient further needs treatment with catecholamines, then one refers to this as septic shock. Such a septic shock is detected in about 20% of all sepsis patients.

Sepsis is the clinical result of complex and highly heterogeneous molecular processes, which are characterized by the involvement of many components and their interactions on every organizational level of the human body: genes, cells, tissues, organs. The complexity of the underlying biological and immunological processes resulted in many kinds of studies comprising a wide range of clinical aspects. One of the results from these studies was that

the evaluation of new sepsis therapies is rendered more difficult due to the presently used criteria which are quite unspecific and clinical based and which do not sufficiently show the molecular mechanisms [12]. Due to a lack of specificity of the currently used diagnosis of sepsis and SIRS, the clinicians are actually not sure from which point of time a patient is to be treated with a specialised therapy, for example with antibiotics, which for their part can have substantial side effects [12]. A survey carried out by the European Society of Intensive Care Medicine (ESICM) showed that 71 % of the physicians questioned were unsure regarding the diagnosing of sepsis, despite many years of clinical experience [22].

Ground-breaking discoveries in molecular biology and immunology during the last two decades have allowed the development of a deepened understanding of sepsis that is oriented more towards the basic mechanisms. The knowledge resulting therefrom regarding relevant targets has formed the basis for the development of targeted and adjuvant therapeutic concepts basing primarily on the neutralisation of essential sepsis mediators [13-16]. One cause for the failure of almost all immunomodulatory approaches of therapy in clinical trials - despite effectivity in animal experiments - is considered to be the merely the poor correlation between the clinical diagnostic criteria, that are rather symptom oriented, and the basic mechanisms of a gerneralised immuno response [12, 17-18].

This is not surprising in retrospect, as even healthy humans may show changes of the heart rate and the respiratory rate, respectively, in the course of their daily activities, which per definitionem already would allow the diagnosis of SIRS. In consideration of today's biomedical possibilities, it seems anachronistic that 751 000 patients in the USA are diagnosed, classified and treated according to the above mentioned ACCP/SCCM criteria per year. Prominent authors thus have already long criticized that, at the expense of an improved sepsis diagnosis, in the past decade too much

energy and financial resources have been lavished on the search for a „magic bullet" for sepsis therapy [19]. Further, recently published expert's opinions also indicate that for a better pathophysiologic understanding of sepsis a modification of the consensus criteria according to [1] is necessary [20-21]. Furthermore, many physicians agree that the consensus criteria according to [1] do not correspond to a specific definition of sepsis. A survey carried out by the European Society of Intensive Care Medicine (ESICM) showed that 71 % of the physicians questioned are not sure about when to diagnose a sepsis, despite long-term experience in the clinical field [22].

Due to the above mentioned problems in the application of the consensus criteria according to [1], critical care physicians discuss various proposals for a more sensitive and specific definition of the various degrees of severity of sepsis [2,23]. The new feature here is the fact that molecular changes should be taken into account directly in the evaluation of the severity of a sepsis as well as in the inclusion in innovative methods of treatment of sepsis (as for example the therapy with activated recombinant protein C). This consensus process [23], which currently is being carried backed by five international professional associations, is at the current time long from completion. It is the object to establish a system for the evaluation of the severity of sepsis. By means of this system it should become possible to classify the reactions of patients on the basis of their predisposed conditions, the kind and extend of the infection, the kind and severity of the host response as well as the degree of the accompanying organ dysfunction. The system described is referred to as PIRO, the abbreviation of the English terms „predisposition", „insult infection", „response" and „organ dysfunction". From this, the individual chance of survival as well as the potential responding to the therapy may be derived [23]. Likewise, non-infectious conditions, which are currently subsumed under the concept of SIRS according to [1] are to be classified more precisely corresponding to the individual degree severity of SIRS.

Studies are carried out to find biomarkers which reflect the severity of the SIRS on the molecular level, as well, and render a clear discrimination of infectious conditions possible (currently classified as sepsis according to [1]). Similar classifications of the stages are already successfully used in other medical fields, for example for the classification of the different disease in oncology (TNM System, [24]).

In comparison to the consensus criteria according to [1], additional molecular parameters are to be included into the diagnosis [23] in order to render an improved correlation of the molecular inflammatory/immunologic host response with a degree of severity of the sepsis, and additionally, the making of statements on the individual prognosis possible. At the present time various scientific and commercial groups are intensively searching for such molecular biomarkers, since the conventional parameters, such as, for example, the determination of the C-reactive protein or the procalcitonin do not meet all clinical requirements and are, in particular, only insufficiently in the position to differentiate between surviving and nonsurviving sepsis patients [25]. Due to the insufficient specificity and sensitivity of the consensus criteria according to [1] and due to the faulty or delayed detection of the cause of infection, there is need for innovative diagnostic processes that are supposed to improve the capability of the person skilled in the art to prognosticate the course of disease with sepsis at an early point of time, to make it comparable in the clinical course and to make statements on the individual prognosis and the response to specific treatments.

Technological advances, in particular the development of microarray technology, make now it possible for the person skilled in the art to simultaneously compare 10 000 or more genes and their gene products. The use of such microarray technologies can now provide information regarding the status of health, regulatory mechanisms, biochemical interactions and signal transmitter networks. As the comprehension how an organism reacts

to infections is improved this way, this should facilitate the development of enhanced modalities of detection, diagnosis and therapy of sepsis disorders.

Microarrays have their origin in „southern blotting“ [10], which represented the first approach to immobilizing DNA-molecules so that it can be addressed three-dimensionally on a solid matrix. The first microarrays consisted of DNA-fragments, frequently with unknown sequence, and were applied dotwise onto a porous membrane (normally nylon). Routinely, cDNA, genomic DNA or plasmid libraries were employed and the hybridized material was labelled with a radioactive group [27-29].

Recently, the use of glass as substrate and fluorescence for detection together with the development of new technologies for the synthesis and for the application of nucleic acids in very high densities made it possible to miniaturize the nucleic acid arrays. At the same time, the experimental throughput and the information content were increased [30-32].

Further, it is known from WO 03/002763 that microarrays basically can be used for the diagnosis of sepsis and sepsis-like conditions.

The first explanation for the applicability of microarray technology was obtained through clinical trials in the field of cancer research. Here, expression profiles proofed to be valuable with regard to identification of activities of individual genes or groups of genes, which correlate with certain clinical phenotypes [33]. Many samples of individuals with or without acute leukaemia or diffuse B-cell lymphoma were analyzed and gene expression labels (RNA) were found and subsequently employed for the clinically relevant classification of these types of cancer [33,34]. Golub et al. found out that an individual gene is not enough to make reliable predictions, while, however, predictions based on the change in transcription of 53 genes

(selected from more than 6000 genes, which were present on the arrays) are highly accurate [33].

Alisadeh et al. [34] examined large B-cell lymphomas (DLBCL). Expression profiles were worked up by the authors with a "lympochip", a microarray bearing 18 000 clones of complementary DNA that was developed to monitor genes that are involved in normal and abnormal development of lymphocytes. By using cluster analysis, they managed to classify DILBCL in two categories that showed great differences with regard to the survival chance of patients. The gene expression profiles of these subtypes correlated to two significant stages of the B-cell differentiation.

Yeoh E. et al. described the use of gene expression profiles established by means of microarrays for the prognosis of the course of leukaemia [35]. From various other studies with cancer patients, further examples for the use of gene expression profiles for the prediction of the chance of survival are known [36-38]. These studies show that the probability of recurrence can be predicted by the measurement of the gene expression profiles. This would be of high clinical-medicinal importance as, on the one hand, it would be possible to pay more attention to these patients, for example by means of more appointments for consultation during tumor after-treatment. This way, an early diagnosis of relapse as well as a systematic treatment of same would be possible. On the other hand, fewer appointments would be necessary for patients with a gene expression profile that does not point to an increased risk of relapse. Further, such gene expressions would also be usable in the decision whether a tumor has to be treated more or less aggressively. As a result, the authors suggested to regularly creating gene expression profiles from cancer patients for an early identification of the individual risk for therapy-related complications in the patient [35].

The use of gene expression profiles for the prediction of the individual course of disease in sepsis and in particular for the prediction of the individual risk of development of lethal complications have not yet been described.

The German Patent Applications DE 103 36 511.7, DE 103 150 31.5 and 10 2004 009 952.9 which have not yet been prepublished, describe that gene expression profiles are, in principle, usable, for example by means of microarray technology for the diagnosis of SIRS, generalised inflammatory inflammations, sepsis and severe sepsis. These applications are herein incorporated by reference.

The invention disclosed in the present application is basing on the perception that the gene activities of surviving sepsis patients differs from the gene activities of nonsurviving sepsis patients. Thus, these differences in the gene activities allow for predictions for the chance of survival und the future development of lethal complications in sepsis patients by means of gene expression. The conventionally used protein markers (e.g. Procalcitonin (PCT) or C-reactive Protein (CRP)) and clinical parameters do not allow such a differentiation, but it is very important for the initiation of a specialised therapy in intensive care and, thus, for the improvement of the individual prognosis of survival.

Thus, it is the object of the present invention to render a prediction of the future course of,disease in sepsis possible by using gene expression profiles.

This object is solved by the features of claims 1, 10 and 23.

The present invention relates in particular to the use of gene expression profiles that have been obtained in vitro from a patient sample for the creation of criteria for the prediction of the individual course of disease in sepsis.

One preferred embodiment of the invention relates to the use of the gene expression profiles obtained for the determination of the chance of survival in sepsis.

Further, the present invention is usable for assessing the course of disease during therapy as well as the classification of sepsis patients.

The present invention is further usable as inclusion or exclusion criterion of patients with sepsis in clinical trials of the stages 2-4.

A preferred embodiment of the present invention is the creation of gene activity data for further electronic processing as well as for the production of software for the description of the individual prognosis for a sepsis patient, for diagnosis and/or patient data management systems.

The present invention may also be used for the creation of expert systems and/or for modulation of cellular ways of signal transfer.

For the creation of gene expression profiles according to the present invention, a majority of specific genes and/or gene fragments is used, selected from the group consisting of SEQ-ID No. 1 to SEQ-ID No. 247, as well as gene fragments thereof with 5-2000 or more, preferably 20-200, more preferably 20-80 nucleotides.

These sequences with the sequence ID 1 to SEQ-ID No. 247 are incorporated by the scope of the present invention and they are in detail disclosed in the enclosed sequence listing of 56 pages und 247 sequences which is, thus, part of the description of the present invention and, therefore, also part of the disclosure of the invention. In the sequence listing the single

sequences are further assigned to the SEQ-ID No. 1 to SEQ-ID No. 247 to their GenBank Accession No. (website: http://www.ncbi.nlm.nih.gov/).

The present invention further relates to the use of gene expression profiles, which are obtained in vitro from a patient sample, and/or of probes used for this purpose, selected from the group consisting of SEQ-ID No. 1 to SEQ-ID No. 247 as well as gene fragments therefrom with at least 5-2000, preferably 20-200, more preferably 20-80 nucleotides, for switching off and/or for changing the activity of target genes and/or the determination of the gene activity for the screening of active agents for sepsis and/or for assessing the effect on sepsis and/or the quality of the active agent and/or the integrity of the active agent in cellular and cell-free sepsis model systems and in sepsis animal models.

In this context, also hybridisable synthetic analogues of the listed probes may be used.

Further, the gene activities in sepsis patients can be determined in a biologic fluid and from this „value" conclusions may be drawn with regard to the course of disease, the chance of survival, the course of therapy or the possibility to include or exclude the sepsis patients in clinical trials.

Another embodiment of the invention is characterized in that a specific gene and/or gene fragment is selected from the group consisting of SEQ-ID No. 1 to SEQ-ID No. 247, as well as gene fragments thereof with 5-2000 or more, preferably 20-200, more preferably 20-80 nucleotides.

Another embodiment of the present invention is characterized in that at least 2 to 100 different cDNAs are used.

Another embodiment of the present invention is characterized in that at least 200 different cDNAs are used.

Another embodiment of the present invention is characterized in that at least 200 to 500 different cDNAs are used.

Another embodiment of the present invention is characterized in that at least 500 to 1000 different cDNAs are used.

Another embodiment of the present invention is characterized in that at least 1000 to 2000 different cDNAs are used.

Another embodiment of the present invention is characterized in that the genes or gene fragments listed in claim 11 and/or the sequences derived from their RNA are replaced by synthetic analogues, aptamers, as well as peptide nucleic acids.

Another embodiment of the invention is characterized in that the synthetic analogues of the genes comprise 5-100, in particular approx. 70 base pairs.

Another embodiment of the present invention is characterized in that the gene activity is determined by means of hybridisation methods.

Another embodiment of the present invention is characterized in that the gene activity is determined by means of microarrays.

Another embodiment of the invention is characterized in that the gene activity is determined by hybridisation-independent methods, in particular by enzymatic and/or chemical hydrolysis and/or amplification methods, preferably PCR, subsequent quantification of nucleic acids and/or of derivates and/or fragments of same.

Another embodiment of the present invention is characterized in that the sample is selected from: body fluids, in particular blood, liquor, urine, ascitic fluid, seminal fluid, saliva, puncture fluid, cell content, or a mixture thereof.

Another embodiment of the present invention is characterized in that cell samples are optionally subjected a lytic treatment, in order to free their cell contents.

It is obvious to the person skilled in the art that the individual features of the present invention shown in the claims can be combined with each other in any desired way.

The term marker genes as used in the present invention encompasses all derived DNA-sequences, partial sequences and synthetic analogues (for example peptido-nucleic acids, PNA). The description of the invention referring to the determination of the gene expression on RNA level is not supposed to be a restriction but only an exemplary application of the present invention.

The description of the invention referring to blood is only an exemplary embodiment of the present invention. The term biological liquids as used in the present invention encompasses all human body fluids.

Further advantages and features of the present invention will become apparent from the description of an embodiment.

Embodiment

Studies on differential gene expression in sepsis for differentiation between nonsurviving and surviving patients.

Whole blood samples of 28 patients who were under the care of a surgical intensive care unit were examined for the measurement of the differential

gene expression in connection with sepsis in order to differentiate between nonsurviving and surviving patients.

Whole blood samples of 12 surviving (9 male and 3 female patients) and 16 deceased patients (13 male und 3 female patients) were drawn during their whole stay in intensive care (patient samples). In the time period in the intensive care unit, each of these patients developed a sepsis the degree of severity being different. Gene expression profiles were analysed from those patients samples that have been drawn at the first day of treatment with the most severe degree of sepsis according to [1] during the first sceptical complications (some patients in intensive care suffer from more than one sepsis during the course of treatment).

A range of characteristics of the patients suffering from sepsis is shown in table 1. In the table, information regarding age, sex, cause of sepsis (cp. diagnosis) as well as clinical severity on the date of admission to the intensive care unit, measured according to in clinical literature well documented APACHE-II-Scores, as well as on the date of first treatment, measured according to in clinical literature well documented SOFA-Scores (both scores respectively in dots) were given. Likewise, the plasma protein level of procalcitonin (PCT), a more recent sepsis marker, from the samples of which the gene expression profiles were drawn at the date of treatment and the individual survival status are indicated.

As control samples, the total RNA from the cell lines SIG-M5 were used. All of the patient samples were co-hybridised with the control sample on one microarray each.

Table 1: Data of the group of patients

Patient	**Age**	**Gender**	Diagnosis	**Classification according to [1]**	**APACHE-II Score [Points]**	SOFA Score [Punkte]	**PCT [ng/ml]**	**Survival State**
1	48	female	acute cholezystitis (gangrenous)	severe sepsis	17	8	0.76	survived
2	71	male	other aspergillosis of the lung	sepsis	13	6	1.69	survived
3	57	male	insufficiency of the mitral valve III	septical shock	11	11	186	survived
4	77	female	peritonitis	severe sepsis	11	10	4.1	survived
5	50	female	infection of patchplastic prothesis AIC on both sides	severe sepsis	9	9	1.71	survived
6	33	male	polytrauma	septical shock	9	9	0.65	survived
7	83	male	stenosis of the aortic valve	septical shock	33	14	1.15	survived
8	60	male	prolapse of an intervertebral disc c6/7	severe sepsis	21	11	0.86	survived
9	45	male	polytrauma at traffic accident	severe sepsis	11	6	1.18	survived
10	20	male	polytrauma after traffic accident	septical shock	29	16	202	survived
11	67	male	catheter sepsis	severe sepsis	22	7	94.49	survived
12	42	male	epidural bleeding	severe sepsis	15	14	0.46	survived
13	53	female	acute pancreatitis (necrot.)	severe sepsis	20	11	2.16	died
14	56	male	acute posterior transmural myocardial infarction	septical shock	21	9	2.16	died
15	64	female	intracranial bleeding (non-traumatic)	septical shock	14	12	500	died
16	70	male	septical shock	septical shock	27	17	9.42	died
17	78	female	acute peritonitis	septical shock	27	10	29.2	died
18	70	male	septical shock	septical shock	10	15	1.02	died
19	72	male	malignant neoformation of the cardia	septical shock	10	12	4.92	died
20	88	male	choledochal bile stone without cholangitis or cholezystitis, bile-duct obstruction not stated	septical shock	18	7	11.6	died
21	63	male	intestinal perforation (non-traumatic)	septical shock	29	10	9.17	died

CA 02561817 2006-09-29

Table 1: Date of the group of patients (continued)

Patient	**Age**	**Gender**	Diagnosis	**Classification according to [1]**	**APACHE-II Score [Points]**	SOFA Score [Punkte]	**PCT [ng/ml]**	**Survival State**
22	74	male	respiratory insufficiency, not specified	septical shock	22	13	12	died
23	66	male	acute peritonitis in intestinal perforation (non-traumatic)	septical shock	13	8	9.05	died
24	64	male	cardiac disease, not specified, cardiac arrest with successful reanimation	severe sepsis	35	9	405	died
25	73	male	dissection of the aorta, thoracic	septical shock	19	12	123	died
26	61	male	pleural empyema, right side	septical shock	11	6	1.64	died
27	62	female	acute peritonitis	septical shock	27	16	16.1	died
28	73	male	intestinal pneumonia, respiratory insuffiency, not specified, acute myocardial infarction	septical shock	32	11	10.9	died

CA 02561817 2006-09-29

Experimental description:

After drawing whole blood, the total RNA of the samples was isolated using the PAXGene Blood RNA kit according to the manufacturer's (Qiagen) instructions.

Cell cultivation

For cell cultivation (control samples) 19 cryo cell cultures (SIGM5) (frozen in liquid nitrogen) were used. The cells were each inoculated with 2 ml Iscove's medium (Biochrom AG) supplemented with 20% fetal calf serum (FCS). Subsequently, the cell cultures were incubated in 12 well plates for 24 hours at 37°C in 5% CO2. Subsequently, the content of the 18 wells was parted in 2 parts with the same volume so that finally 3 plates of the same format (36 wells in total) were available. Afterwards, the cultivation was continued under the same conditions for 24 hours. Afterwards, the resulting cultures of 11 wells of each plate were combined and centrifuged (1000 x g, 5 min, ambient temperature). The supernatant was removed and the cell pellet was dissolved in 40 ml of the above mentioned medium. These 40 ml of dissolved cells were distributed in equal shares in two 250 ml flasks and incubated after adding 5 ml of the above-mentioned medium. 80 µl of the remaining 2 ml of the two remaining plates were placed in empty wells of the same plates that had previously been prepared with 1 ml of the above-mentioned medium. After 48 hours of incubations, only one of the 12 well plates was processed as follows: 500 µl were extracted from each well and combined. The resulting 6 ml were introduced into a 250 ml flask comprising approximately 10 ml of fresh medium. This mixture was centrifuged for 5 minutes with 1000 x g at ambient temperature and dissolved in 10 ml of the above-mentioned medium. The following results were obtained by the subsequent cell counting: 1,5 x 107 cells per ml, 10 ml total volume, total number of cells: 1.5 x 108. As the number of cells was not yet sufficient, 2.5 ml of the above-mentioned cell suspension was introduced into 30 ml of the above-mentioned medium in a 250 ml (75 cm2) flask (4 flasks in total). After 72 hours of incubation 20 ml of fresh medium were added to each flask. After the subsequent incubation of 24

hours, the cells were counted as described above. The total amount of cells was 3.8 x 10^8 cells. In order to obtain the desired number of cells of 2 x 106 cells, the cells were resuspended in 47.5 ml of the above mentioned medium in 4 flasks. After the incubation time of 24 hours, the cells were centrifuged and washed two times with phosphate buffer in absence of Ca^{2+} and Mg^{2+} (Biochrom AG).

The isolation of the total RNA is performed by means of NucleoSpin RNA L Kits (Machery&Nagel) according to the manufacturer's instructions. The above described process was repeated until the necessary number of cells was obtained. This was necessary to obtain the necessary amount of 6 mg total RNA corresponding to an efficiency of 600 µg RNA per 108 cells.

Reverse transcription / labelling / hybridisation

Subsequently, the complementary cDNA was prepared from the total RNA of the patient and control samples by means of reverse transcription under substitution of the dTTP-Fraktion by synthesized aminoallyl-deoxyuridintriphosphate (AA-dUTP). The RNA/cDNA complex was transformed into single strand cDNA by means of RNA hydrolysis. Subsequently, the cDNA samples were labelled with the fluorescencent dyes Cy3 and Cy5 (Amersham) by means of chemical binding to AA-dUTP.

A microarray from the company SIRS-Lab was used for the hybridisation of the samples. On the microarray used, 5308 different polynucleotides with lengths of 55 to 70 base pairs were immobilised. Each of the polynucleotides represents a human gene. The spots were immobilised with a multitude of different control spots within 28 subarrays, each of the subarrays being arranged in a grid of 15x15 spots. The hybridisation was carried out using the hybridisation station HS 400 (Tecan) according to the manufacturer's instructions. The hybridisation solution was composed of 3.5 x SSC (1xSSC comprises 150 mM NaCl and 15 mM sodiumcitrate), 0.3% SDS, 25% formamide, 0.8 µg/µl of each cot-1 DNA, yeast tRNA and polyA and the respective cDNA-samples. The arrays were hybridised for 10.5 hours at 42°C. The subsequent washing procedure is carried out as follows:

Addition of washing buffer I (2xSSC, 0.003%SDS) to the hybridisation chamber, washing at ambient temperature for 1.5 minutes, addition of washing buffer II (1xSSC) to the hybridisation chamber, washing with washing buffer for 1.5 minutes at ambient temperature, adding of washing buffer III (0.2xSSC) to the hybridisation chamber, washing with washing buffer III for 1.5 minutes at ambient temperature. Subsequently, the surfaces of the microarrays were dried with nitrogen at a pressure of 2.5 bar for 2.5 minutes at 30°C.

The hybridisation signals of the processed microarrays were subsequently read by means of the GenePix 4000B (Axon) scanner and the expression ratios of the different expressed genes were determined by means of the GenePix Pro 4.0 (Axon) software.

Analysis

For the analysis, the average intensity of one spot was determined as median value of the corresponding spot pixel.

Correction of Systemic Errors:

The median of the pixel of the local background was subtracted from the median of the spot pixel. For all further computations, the signal was transformed by means of arcus sinus hyperbolicus. The normalization occurred according to the approach of Huber et al. [39]. According to this approach, the additive and the multiplicative bias in a microarray was estimated on the basis of 70% of the gene samples present. For the analysis, the transformed relative ratios of the signals of the patients samples were calculated with respect to the control. This means that the calculation for the gene no. j (j=1,...,5308) of the patient no. n (n=1,...,28) revealed the data $G_{j,n}=\operatorname{arcsinh}(Scy5(j,n))- \operatorname{arcsinh}(Scy3(j,n))$, wherein [SCy3(j,n), SCy5(j,n)] is the associated signal pair. When a spot could not be analyses for a patient (no detectable signal intensity in both channels), the associated value was marked as „missing value“.

Statistical Comparison:

For comparison the paired random student test was employed per gene. Both random samples contained the values of the patient groups of surviving and nonsurviving patients, respectively. For choosing the differentially expressed genes, the associated p-value and the number of missing values were evaluated.

Results:

It applied for the group of the selected genes that the associated p-value was smaller than 0.05, with at least 5 detectable signals received per patient group.

The criterion for the grading of the examined genes was the level of the expression ratio of each gene. The most overexpressed or underexpressed genes, respectively, in the surviving and nonsurviving patients were the interesting ones.

Table 2 shows that 65 genes of the patient sample were found, which were significantly overexpressed in the nonsurviving patients, if compared with the surviving patients. Furthermore, Table 3 shows that 182 genes of the nonsurviving patients were significantly under-expressed, if compared with the surviving patients. From the results it is clear that the gene activities listed in Table 2 and Table 3 distinguish between surviving and nonsurviving sepsis patients (corresponding to sepsis according to [1]). Thus, the listed gene activities provide markers for the prediction of the chance of survival and the development of lethal complications in sepsis patients.

Table 2: Significant elevated gene activities in samples of patients with sepsis according to [1], indicated as their relative relationship to the corresponding gene activities of nonsurviving patients suffering from sepsis according to [1].

GenBank Accession No.	p-value	mean normalised and transformed expression value		Standard deviation		Difference of mean values	Seq-Id.
		Group of surviving patients	Group of non-surviving patients	Group of surviving patients	Group of nonsurviving patients		
AI560533	0.036	-0.42	-0.24	0.24	0.17	0.17	84
D49410	0.042	-0.40	-0.22	0.14	0.24	0.18	125
AI263527	0.047	-0.49	-0.32	0.12	0.26	0.18	67
AI732958	0.028	-0.25	-0.06	0.10	0.25	0.18	102
AI679923	0.033	-0.45	-0.27	0.19	0.23	0.19	97
R56877	0.034	-0.30	-0.10	0.19	0.23	0.20	215
AA478621	0.008	-0.30	-0.10	0.16	0.19	0.20	24
R93174	0.038	-0.36	-0.15	0.25	0.24	0.21	222
AA935872	0.030	-0.35	-0.14	0.14	0.28	0.21	46
H22921	0.016	-0.32	-0.10	0.16	0.24	0.21	132
R61687	0.046	-0.67	-0.46	0.13	0.31	0.22	218
N73694	0.040	-0.38	-0.16	0.25	0.26	0.22	153
H28119	0.040	-0.25	-0.03	0.22	0.27	0.22	134
AI697430	0.046	-0.46	-0.24	0.24	0.29	0.22	99
AI631076	0.043	-0.81	-0.58	0.19	0.31	0.23	92
N63777	0.038	-0.51	-0.28	0.28	0.26	0.23	150
AA889648	0.045	-0.36	-0.13	0.19	0.32	0.23	40
AA679067	0.039	-0.30	-0.07	0.33	0.22	0.23	30
AI865298	0.012	-0.34	-0.10	0.15	0.26	0.24	113
H14986	0.028	-0.06	0.18	0.19	0.31	0.24	129
AI033361	0.014	-0.36	-0.11	0.17	0.27	0.24	54
AI149817	0.012	-0.42	-0.17	0.30	0.15	0.25	60
AI269981	0.043	-0.22	0.03	0.27	0.31	0.25	68
NM_003082	0.043	-0.70	-0.45	0.20	0.33	0.25	168
AI620645	0.040	-0.41	-0.16	0.26	0.32	0.25	91
R06585	0.022	-0.48	-0.22	0.22	0.29	0.25	204
H61046	0.023	-0.41	-0.15	0.31	0.24	0.26	137
AI803880	0.022	-0.46	-0.20	0.22	0.30	0.26	106
AI862880	0.047	-0.71	-0.45	0.26	0.35	0.26	111
AI185721	0.033	-0.58	-0.32	0.32	0.28	0.26	62
AI888234	0.040	-0.53	-0.27	0.23	0.35	0.26	114
AA278821	0.048	-0.28	-0.01	0.31	0.32	0.26	7
AI933967	0.042	-0.66	-0.40	0.16	0.39	0.27	121
AI635650	0.026	-0.63	-0.37	0.24	0.31	0.27	93
R89075	0.032	-0.59	-0.32	0.29	0.31	0.27	221
H14100	0.018	-0.53	-0.25	0.20	0.31	0.27	128
AA699356	0.022	-0.22	0.06	0.21	0.33	0.27	32
NM_000678	0.012	-0.66	-0.39	0.17	0.29	0.27	186
AI579907	0.003	-0.41	-0.13	0.23	0.21	0.28	86
AA284108	0.009	-0.46	-0.17	0.29	0.24	0.29	9
AA406037	0.030	-0.42	-0.13	0.16	0.33	0.29	14
AA441939	0.007	-0.44	-0.14	0.24	0.25	0.30	18
H11718	0.020	-0.10	0.20	0.15	0.27	0.31	127
AA621333	0.036	-0.46	-0.14	0.23	0.40	0.33	29

Table 2 – Continuation

GenBank Accession No.	p-value	Mean normalised and transformed expression value		Standard deviation		Difference in mean values	SeqId.
		Group of surviving patients	Group of nonsurviving patients	Group of surviving patients	Group of nonsurviving patients		
AI864919	0.035	-0.51	-0.18	0.25	0.42	0.33	112
AI184594	0.032	-0.51	-0.18	0.43	0.32	0.34	61
NM-001288	0.048	-0.28	0.07	0.45	0.37	0.35	188
XM-043864	0.014	-0.54	-0.20	0.22	0.40	0.35	243
AI475085	0.026	-0.37	-0.01	0.31	0.31	0.36	80
H79760	0.027	-0.51	-0.15	0.33	0.34	0.36	139
XM-012039	0.047	-1.22	-0.84	0.33	0.52	0.37	239
AI377802	0.021	-0.49	-0.11	0.39	0.39	0.37	75
NM_021972	0.038	-0.94	-0.56	0.46	0.37	0.38	183
AI936462	0.022	-0.61	-0.23	0.33	0.45	0.38	123
AA846117	0.040	0.00	0.40	0.45	0.40	0.40	39
AI732878	0.032	-0.54	-0.13	0.61	0.31	0.41	101
M61199	0.031	-0.72	-0.29	0.36	0.48	0.43	143
NM_004513	0.007	-0.54	-0.11	0.35	0.38	0.43	176
NM-012068	0.014	-1.17	-0.70	0.16	0.55	0.47	194
NM_000228	0.032	-1.21	-0.68	0.61	0.55	0.53	156
NM_000629	0.014	-0.93	-0.39	0.41	0.51	0.54	158
AI655693	0.040	-0.50	0.04	0.48	0.44	0.55	94
AA017133	0.030	-0.26	0.29	0.42	0.37	0.55	5
H16999	0.027	-0.55	0.07	0.43	0.45	0.62	130
AI888493	0.024	-0.56	0.25	0.78	0.42	0.81	115

Table 3: Significant reduced gene activities in samples of patients with sepsis according to [1], indicated as their relative relationship to the corresponding gene activities of nonsurviving patients suffering from sepsis according to [1].

GenBank Accession No.	p-value	Mean normalised and transformed expression value		Standard deviation		Difference in mean values	Seq-Id.
		Group of surviving patients	Group of nonsurviving patients	Group of surviving patients	Group of nonsurviving patients		
AI147932	0.031	-0.02	-0.18	0.13	0.20	-0.17	58
R88267	0.036	0.08	-0.12	0.31	0.18	-0.21	220
AI039866	0.039	0.06	-0.15	0.34	0.14	-0.21	55
AI583425	0.023	0.22	0.00	0.22	0.22	-0.22	87
AA845475	0.032	0.13	-0.10	0.28	0.22	-0.22	38
AA992381	0.049	-0.24	-0.47	0.27	0.29	-0.23	48
NM_022740	0.047	0.21	-0.02	0.34	0.22	-0.23	184
R43258	0.025	0.11	-0.12	0.10	0.28	-0.23	208
N32057	0.022	-0.02	-0.25	0.20	0.25	-0.23	145
AI277856	0.026	0.13	-0.10	0.18	0.24	-0.23	70
AA454150	0.028	-0.03	-0.26	0.30	0.21	-0.23	21
AI597793	0.002	0.15	-0.09	0.10	0.19	-0.24	89
AA012911	0.009	0.32	0.08	0.20	0.19	-0.24	4
AA960982	0.037	0.28	0.03	0.37	0.20	-0.24	47
AA906962	0.037	-0.27	-0.52	0.34	0.27	-0.26	43
AI933013	0.042	0.40	0.14	0.38	0.24	-0.26	120
AA704293	0.012	0.37	0.11	0.30	0.18	-0.26	33
AA682521	0.022	0.10	-0.16	0.31	0.25	-0.26	31
AI142427	0.013	0.24	-0.03	0.20	0.29	-0.27	57
R54393	0.007	0.11	-0.15	0.32	0.12	-0.27	214
NM_015318	0.012	0.22	-0.04	0.22	0.25	-0.27	196
AA528169	0.050	0.45	0.18	0.40	0.22	-0.27	27
XM-048792	0.049	0.21	-0.06	0.23	0.36	-0.27	244
AI343613	0.039	-0.07	-0.34	0.34	0.27	-0.27	73
N91341	0.024	0.10	-0.18	0.27	0.27	-0.27	154
AI023785	0.020	0.06	-0.21	0.24	0.27	-0.27	53
NM_032721	0.021	0.22	-0.05	0.26	0.25	-0.28	201
AA035159	0.044	0.08	-0.20	0.36	0.21	-0.28	6
AI091302	0.012	0.32	0.04	0.21	0.26	-0.28	56
AA845372	0.040	0.56	0.28	0.29	0.35	-0.28	37
AI657063	0.005	0.19	-0.09	0.28	0.16	-0.28	95
AI187962	0.005	0.55	0.27	0.22	0.23	-0.28	64
AA845015	0.018	0.02	-0.27	0.31	0.27	-0.29	36
AA436651	0.042	0.28	0.00	0.46	0.23	-0.29	17
NM_147180	0.028	0.22	-0.07	0.36	0.26	-0.29	202
AI421397	0.030	0.42	0.13	0.37	0.25	-0.29	77
W15233	0.034	0.35	0.05	0.22	0.31	-0.30	230
AA906278	0.009	-0.16	-0.46	0.22	0.22	-0.30	42
AI187401	0.044	-0.07	-0.37	0.42	0.31	-0.30	63
AI492493	0.038	-0.16	-0.46	0.35	0.36	-0.30	81
AA496969	0.001	0.16	-0.15	0.22	0.18	-0.31	26
AI936300	0.049	0.61	0.30	0.31	0.36	-0.31	122
NM_002983	0.046	0.27	-0.04	0.38	0.34	-0.31	165

Table 3- Continuation 1

GenBank Accession No.	p-value	Mean normalised and transformed expression value		Standard deviation		Difference in mean values	Seq-Id
		Group of surviving patients	Group of nonsurviving patients	Group of surviving patients	Group of nonsurviving patients		
N53480	0.016	-0.18	-0.49	0.16	0.29	-0.31	149
AI400066	0.009	-0.14	-0.45	0.34	0.24	-0.32	76
AI799547	0.022	0.24	-0.08	0.22	0.35	-0.32	105
R94509	0.015	0.28	-0.04	0.32	0.27	-0.32	223
AI539457	0.036	0.27	-0.05	0.37	0.38	-0.33	83
AI921468	0.003	0.24	-0.09	0.30	0.22	-0.33	118
NM_005368	0.016	0.34	0.00	0.38	0.23	-0.34	247
NM_000597	0.026	0.12	-0.22	0.42	0.31	-0.34	185
NM_005658	0.045	0.29	-0.05	0.59	0.22	-0.34	178
NM_014211	0.003	0.21	-0.14	0.31	0.22	-0.34	195
AI342905	0.039	0.66	0.32	0.50	0.32	-0.35	72
NM_006273	0.013	0.49	0.14	0.34	0.29	-0.35	179
H28769	0.010	0.34	-0.01	0.39	0.25	-0.35	135
AA460188	0.040	0.23	-0.11	0.48	0.31	-0.35	23
R42778	0.022	0.43	0.08	0.48	0.27	-0.35	206
NM_004740	0.027	0.21	-0.15	0.50	0.27	-0.36	177
AA432083	0.011	0.14	-0.22	0.48	0.18	-0.36	16
AA397913	0.026	0.86	0.50	0.33	0.42	-0.36	10
AI473446	0.041	0.40	0.04	0.39	0.29	-0.36	79
AI289206	0.010	0.36	0.00	0.44	0.20	-0.36	71
NM_000074	0.018	0.43	0.06	0.42	0.30	-0.37	155
AA458848	0.012	0.07	-0.30	0.38	0.32	-0.37	22
R61395	0.008	0.47	0.10	0.30	0.28	-0.37	217
N39164	0.027	0.22	-0.15	0.41	0.24	-0.37	147
T91937	0.032	0.54	0.16	0.42	0.31	-0.38	226
AA707013	0.025	0.01	-0.37	0.51	0.29	-0.39	34
H20320	0.024	0.64	0.26	0.43	0.40	-0.39	131
K03195	0.045	0.21	-0.18	0.41	0.46	-0.39	141
NM_001710	0.049	0.63	0.24	0.69	0.23	-0.39	189
AA281330	0.030	0.69	0.30	0.54	0.35	-0.39	8
AI825890	0.049	0.32	-0.07	0.31	0.58	-0.39	109
AI270372	0.016	0.38	-0.01	0.51	0.24	-0.40	69
T97352	0.037	0.39	-0.01	0.46	0.46	-0.40	229
AA002267	0.040	0.42	0.02	0.64	0.30	-0.40	1
AI561302	0.040	0.09	-0.31	0.42	0.37	-0.40	85
AI741506	0.002	0.38	-0.03	0.14	0.28	-0.40	103
XM-012608	0.009	0.37	-0.03	0.45	0.29	-0.40	240
AA935686	0.012	0.39	-0.02	0.55	0.21	-0.41	45
R53564	0.015	0.46	0.06	0.40	0.26	-0.41	213
AI744597	0.016	0.28	-0.13	0.40	0.35	-0.41	104
AI148036	0.035	0.30	-0.11	0.60	0.17	-0.41	59
AI925267	0.036	0.41	0.00	0.63	0.34	-0.41	119
AA731720	0.007	0.31	-0.11	0.50	0.21	-0.42	35
NM_021132	0.006	0.21	-0.21	0.34	0.34	-0.42	200

Table 3- Continuation 2

GenBank Accession No.	p-value	Mean normalised and transformed expression value		Standard deviation		Difference in mean values	Seq-Id
		Group of surviving patients	Group of nonsurviving patients	Group of surviving patients	Group of nonsurviving patients		
NM_000590	0.010	1.00	0.58	0.37	0.36	-0.42	157
AF458059	0.048	0.58	0.15	0.59	0.39	-0.43	51
NM_003811	0.047	-0.48	-0.91	0.50	0.51	-0.43	171
AI492835	0.005	0.54	0.11	0.35	0.32	-0.43	82
NM_003010	0.016	0.41	-0.02	0.58	0.24	-0.43	167
R73518	0.026	0.22	-0.21	0.41	0.31	-0.43	219
AI221524	0.012	0.75	0.31	0.53	0.28	-0.44	66
NM_015645	0.043	-0.22	-0.66	0.60	0.40	-0.44	197
R00742	0.004	0.42	-0.02	0.43	0.28	-0.44	203
T95327	0.003	0.57	0.13	0.22	0.28	-0.44	227
NM_000823	0.002	0.73	0.29	0.27	0.34	-0.44	187
AA889842	0.032	0.58	0.14	0.41	0.55	-0.44	41
AI732314	0.021	0.43	-0.01	0.46	0.33	-0.44	100
AA011562	0.009	0.66	0.21	0.39	0.41	-0.45	3
H48923	0.011	0.09	-0.36	0.40	0.38	-0.45	136
AA479964	0.017	0.46	0.00	0.67	0.20	-0.46	25
N49899	0.011	0.47	-0.01	0.51	0.23	-0.48	148
H26279	0.021	0.58	0.09	0.59	0.39	-0.48	133
AI804425	0.044	0.81	0.32	0.69	0.42	-0.49	107
R38931	0.021	0.60	0.10	0.44	0.46	-0.49	205
W32791	0.050	0.70	0.20	0.68	0.37	-0.49	231
R97095	0.038	0.70	0.19	0.81	0.37	-0.50	224
R45218	0.025	0.88	0.37	0.61	0.43	-0.51	210
R49120	0.017	0.76	0.25	0.41	0.44	-0.51	211
AI435179	0.046	0.78	0.26	0.39	0.40	-0.52	78
AI831532	0.036	0.38	-0.15	0.55	0.33	-0.53	110
NM_006682	0.038	-0.25	-0.78	0.66	0.46	-0.53	181
NM_004324	0.017	0.40	-0.13	0.67	0.40	-0.54	174
XM_001754	0.019	0.56	0.01	0.80	0.26	-0.54	237
W96107	0.008	0.40	-0.14	0.59	0.32	-0.55	234
AA910846	0.025	1.09	0.54	0.73	0.45	-0.55	44
AA995114	0.030	1.33	0.78	0.77	0.47	-0.55	49
H98244	0.005	0.20	-0.36	0.59	0.33	-0.56	140
AA443712	0.032	1.00	0.44	0.77	0.49	-0.56	19
AA452134	0.037	1.08	0.51	0.78	0.54	-0.57	20
NM_003806	0.020	0.54	-0.04	0.83	0.34	-0.58	170
NM-018643	0.032	1.35	0.77	0.43	0.77	-0.58	198
XM-043467	0.042	1.03	0.45	0.42	0.48	-0.58	242
T80669	0.042	0.66	0.07	0.68	0.30	-0.59	225
AA398611	0.037	1.11	0.52	0.91	0.50	-0.60	11
N64374	0.023	0.81	0.22	0.67	0.50	-0.60	151
AI807985	0.032	0.86	0.27	0.78	0.53	-0.60	108
AI220247	0.031	0.74	0.13	0.98	0.25	-0.61	65
NM_000758	0.018	1.33	0.72	0.78	0.45	-0.61	159
N66205	0.037	1.45	0.83	0.82	0.60	-0.62	152

Table 3- Continuation 3

GenBank Accession No.	p-value	Mean normalised and transformed expression value		Standard deviation		Difference in mean values	Seq-Id
		Group of surviving patients	Group of nonsurviving patients	Group of surviving patients	Group of nonsurviving patients		
AA011511	0.009	0.22	-0.40	0.57	0.33	-0.63	2
NM_006419	0.017	0.60	-0.05	0.64	0.60	-0.65	180
AI016806	0.042	0.73	0.07	1.15	0.36	-0.65	52
NM-003327	0.027	1.58	0.92	0.94	0.51	-0.65	191
AA427522	0.046	0.85	0.19	1.10	0.37	-0.66	15
NM-019846	0.015	0.88	0.20	0.88	0.42	-0.68	199
AI915031	0.024	1.46	0.77	0.59	0.78	-0.69	117
R51857	0.002	1.18	0.48	0.72	0.32	-0.70	212
AI612918	0.024	1.51	0.81	0.71	0.77	-0.70	90
AA535107	0.045	0.65	-0.05	1.14	0.26	-0.70	28
H74219	0.015	0.79	0.08	0.49	0.49	-0.71	138
NM_004221	0.013	1.93	1.22	0.52	0.73	-0.72	173
XM-049959	0.020	2.09	1.37	0.58	0.81	-0.72	245
AA400488	0.037	0.60	-0.12	1.15	0.36	-0.72	12
AI668693	0.005	0.54	-0.18	0.79	0.33	-0.72	96
NM_021057	0.005	0.75	0.03	0.51	0.52	-0.72	182
NM_003946	0.014	0.99	0.27	0.69	0.15	-0.72	172
NM_002923	0.044	1.66	0.93	0.59	0.97	-0.73	164
NM_001671	0.046	1.29	0.55	1.09	0.51	-0.73	160
W74724	0.045	0.68	-0.05	0.62	0.48	-0.73	232
AI689080	0.010	1.59	0.85	0.57	0.73	-0.74	98
NM_002416	0.033	0.99	0.24	0.74	0.63	-0.75	162
R43203	0.036	2.13	1.38	0.86	0.81	-0.75	207
N32857	0.015	-0.16	-0.91	0.86	0.54	-0.75	146
AI591136	0.009	1.85	1.10	0.63	0.71	-0.76	88
XM_027216	0.018	0.34	-0.42	0.90	0.53	-0.76	241
NM_003651	0.038	0.95	0.17	0.62	1.01	-0.78	169
NM-007289	0.006	1.37	0.56	0.40	0.82	-0.81	193
NM_004437	0.000	1.25	0.44	0.40	0.50	-0.82	175
NM-006018	0.021	1.78	0.96	0.79	0.88	-0.82	192
XM-051535	0.027	2.12	1.28	0.76	0.98	-0.84	246
R59591	0.032	0.65	-0.21	0.87	0.37	-0.86	216
X00457	0.045	1.40	0.50	0.79	0.61	-0.90	235
AB007950	0.010	1.16	0.26	0.59	0.86	-0.90	50
AI361290	0.006	0.96	0.05	0.57	0.47	-0.91	74
NM_002185	0.004	0.61	-0.31	0.94	0.36	-0.92	161
AI910882	0.012	0.93	0.00	0.90	0.34	-0.93	116
AA405180	0.047	0.79	-0.16	1.55	0.38	-0.95	13
W86242	0.008	1.83	0.80	1.15	0.71	-1.03	233
BC018761	0.042	2.45	1.39	1.09	1.35	-1.06	124
NM-002133	0.029	1.83	0.76	1.10	0.86	-1.07	190
R43910	0.021	2.64	1.51	1.34	1.04	-1.13	209
T97328	0.020	2.48	1.34	0.78	1.12	-1.14	228
XM-007189	0.013	1.03	-0.13	0.98	0.60	-1.17	238
L39062	0.010	3.26	2.05	0.66	1.19	-1.21	142

Tabelle 3- Continuation 4

GenBank Accession No.	p-value	Mean normalised and transformed expression value		Standard deviation		Difference in mean values	Seq-Id.
		Group of surviving patients	Group of nonsurviving patients	Group of surviving patients	Group of nonsurviving patients		
H07058	0.030	1.05	-0.17	1.66	0.71	-1.22	126
X64641	0.024	2.37	1.11	1.10	1.26	-1.26	236
M90746	0.039	3.69	2.36	0.79	1.82	-1.33	144
NM_002984	0.006	2.32	0.97	1.50	0.79	-1.35	166
NM_002704	0.041	5.24	3.20	2.96	1.88	-2.04	163

These changes characterized in Tables 2 and 3 can be used for the inventive process according to claim 1.

The GenBank Acccession Numbers indicated in Tables 2 and 3 (Internet-access via http://www.ncbi.nlm.nih.gov/) of the individual sequences are associated with the attached 56-page sequence listing, itemized or in detail with respectively one sequence (Sequence ID: 1 up through Sequence ID: 247).

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DEMANDES OU BREVETS VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS COMPREND PLUS D'UN TOME.

CECI EST LE TOME __1___ DE __2___

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JUMBO APPLICATIONS / PATENTS

THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE THAN ONE VOLUME.

THIS IS VOLUME __1___ OF __2___

NOTE: For additional volumes please contact the Canadian Patent Office.

Claims

1. Use of gene expression profiles, obtained from a patient sample in vitro, for the generation of criteria for the prediction of an individual course of disease in sepsis.

2. Use according to claim 1 for determining the probability of survival in sepsis.

3. Use according to claim 1 for assessing the course of disease in sepsis during therapy.

4. Use according to claim 1 for classification of sepsis patients.

5. Use according to claim 4 as inclusion criterion or exclusion criterion of patients with sepsis in clinical trials of the stages 2-4.

6. Use according to one of claims 1 to 5, for the generation of gene activity data for further electronic processing.

7. Use according to claim 6, wherein the gene activity data is used for the production of software for the description of the individual prognosis of a sepsis patient, for diagnostic purposes and/or for patient data management systems.

8. Use according to claim 6, wherein the gene activity data is used for the production of expert systems and/or for modelling of cellular signal transmission paths.

9. Use according to one of claims 1 to 8, wherein a specific gene and/or gene fragment is used for the generation of gene expression profiles, the gene and/or gene fragment being selected from a group consisting of SEQ-ID No. 1 to SEQ-ID No. 247 as well as gene fragments therefrom with at least 5-2000, preferably 20-200, more preferably 20-80 nucleotides.

10. Method for in vitro measurement of gene expression profiles for the use of at least one of claims 1 to 9, characterized in that the gene activity of various certain genes associated with sepsis is determined in a patient sample, whereby the sepsis-specific genes and/or gene fragments are selected from the group consisting of: SEQUENCE-ID No. 1 to SEQ-ID No. 247, as well as gene fragments thereof with 5-2000 or more, preferably 20-200, more preferably 20-80 nucleotides.

11. Method according to claim 10, characterized in that at least 2 to 100 different cDNAs are used.

12. Method according to one of claims 10 or 11, characterized in that at least 200 different cDNAs are used.

13. Method according to one of claims 10 to 12, characterized in that at least 200 to 500 different cDNAs are used.

14. Method according to one of claims 10 to 13, characterized in that at least 500 to 1000 different cDNAs are used.

15. Method according to one of claims 10 to 14, characterized in that at least 1000 to 2000 different cDNAs are used.

16. Method according to one of claims 10 to 15, characterized in that the genes or gene fragments and/or the sequences derived from their RNA listed in claim 10 are replaced by synthetic analogues, aptamers, mirrormeres as well as peptide- and morpholine nucleic acids.

17. Method according to claim 16, characterized in that the synthetic analogues of the genes comprise 5-100, in particular approx. 70 base pairs.

18. Method according to claim 10 to 17, characterized in that the gene activities are determined by means of hybridisation methods.

19. Method according to claim 18, characterized in that the gene activity is determined by means of microarrays.

20. Method according to one of claims 10 to 19, characterized in that the gene activity is determined by hybridisation-independent methods, in particular by enzymatic and/or chemical hydrolysis and/or amplification methods, preferably PCR, subsequent quantification of nucleic acids and/or of derivates and/or of fragments of same.

21. Method according to one of claims 10 to 20, characterized in that the sample is selected from the group consisting of body fluids, in particular blood, liquor, urine, ascitic fluid, seminal fluid, saliva, puncture fluid, cell content, or a mixture thereof.

22. Method according to one of claims 10 to 22, characterized in that cell samples are optionally subjected to lytic treatment, in order to free their cell contents.

23. Use of gene expression profiles that are obtained in vitro from a patient sample and/or of probes used therefore, selected from the group consisting of SEQ-ID No. 1 to SEQ-ID No. 247 as well as gene fragments therefrom with at least 5-2000, preferably 20-80 nucleotides, for switching off and/or for changing the activity of target genes and/or the determination of the gene activity for the screening of active agents for sepsis and/or for assessing the effect on sepsis and/or the quality of the active agent and/or the integrity of the active agent in cellular and cell-free sepsis model systems and in sepsis animal models.

24. Use according to claim 23, characterized in that hybridisable synthetic analogues of the probes listed in claim 23 are used.