(19)
Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 295 127 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
09.11.2005 Bulletin 2005/45

(21) Application number: **01934830.9**

(22) Date of filing: **06.02.2001**

(51) Int Cl.[7]: **G01N 33/68**, A61K 38/00, G01N 33/573

(86) International application number:
PCT/US2001/003827

(87) International publication number:
WO 2001/063280 (30.08.2001 Gazette 2001/35)

(54) **INTER-ALPHA-TRYPSIN AS A MARKER FOR SEPSIS**

INTER-ALPHA-TRYPSIN ALS MARKER FÜR SEPSIS

L'INTER-ALPHA-TRYPSINE, MARQUEUR DE SEPSIES

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

(30) Priority: **28.02.2000 US 514450**

(43) Date of publication of application:
26.03.2003 Bulletin 2003/13

(73) Proprietor: **RHODE ISLAND HOSPITAL**
Providence, Rhode Island 02903 (US)

(72) Inventors:
- **LIM, Yow-Pin**
  East Providence, RI 02914 (US)
- **HIXSON, Douglas, C.**
  Barrington, RI 02806 (US)

(74) Representative: **Crump, Julian Richard John**
Mintz Levin Cohn Ferris Glovsky and Popeo Intellectual Property LLP
The Rectory
9, Ironmonger Lane
London EC2V 8EY (GB)

(56) References cited:
WO-A-92/18160

- **BALDUYCK M ET AL: "INFLAMMATION-INDUCED SYSTEMIC PROTEOLYSIS OF INTER-ALPHA-INHIBITOR IN PLASMA FROM PATIENTS WITH SEPSIS" JOURNAL OF LABORATORY AND CLINICAL MEDICINE, ST. LOUIS, MO, US, vol. 135, no. 2, February 2000 (2000-02), pages 188-198, XP001003028 ISSN: 0022-2143**
- **CARRETTE O ET AL: "PIG IALPHAI APPEARS UNMODIFIED IN PLASMA IN CASE OF ENDOTOXIN-INDUCED DISSEMINATED INTRAVASCULAR COAGULATION" BIOCHIMIE, PARIS, FR, vol. 79, no. 12, December 1997 (1997-12), pages 749-755, XP000994502 ISSN: 0300-9084**
- **ALBANI DALILA ET AL: "Inter-alpha-inhibitor as marker for neutrophil proteinase activity: An in vitro investigation." JOURNAL OF LABORATORY AND CLINICAL MEDICINE, vol. 130, no. 3, 1997, pages 339-347, XP008007194 ISSN: 0022-2143**
- **JOURDAIN M ET AL: "Effects of inter-alpha-inhibitor in experimental endotoxic shock and disseminated intravascular coagulation." AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE. UNITED STATES DEC 1997, vol. 156, no. 6, December 1997 (1997-12), pages 1825-1833, XP008007195 ISSN: 1073-449X**
- **FRITZ H: "PROTEINASE INHIBITORS IN SEVERE INFLAMMATORY PROCESSES (SEPTIC SHOCK AND EXPERIMENTAL ENDOTOXAEMIA): BIOCHEMICAL, PATHOPHYSIOLOGICAL AND THERAPEUTIC ASPECTS" CIBA FOUNDATION SYMPOSIUM, AMSTERDAM, NL, 1980, pages 351-379, XP002014033 ISSN: 0300-5208**
- **WITTE J ET AL: "Disturbances of selected plasma proteins in hyperdynamic septic shock." INTENSIVE CARE MEDICINE. UNITED STATES 1982, vol. 8, no. 5, 1982, pages 215-222, XP008007197 ISSN: 0342-4642**

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 1 295 127 B1

- **LIM YOW-PIN ET AL: "Monoclonal antibody 69.31 blocks the inhibitory activity of Inter-alpha inhibitor proteins to serine proteases and abolishes their inhibitory effects in in vitro tumor cell invasion." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, vol. 42, March 2001 (2001-03), pages 709-710, XP008007202 92nd Annual Meeting of the American Association for Cancer Research;New Orleans, LA, USA; March 24-28, 2001, March, 2001 ISSN: 0197-016X**

Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

Description

TECHNICAL FIELD

[0001] This invention relates to bacterial infections.

BACKGROUND

[0002] Sepsis is a systemic response to infection, e.g., a bacterial infection. It is commonly caused by endotoxins from Gram negative bacteria or exotoxins from Gram positive bacteria (which can trigger endotoxin-like responses). The systemic response can lead to septic shock, which is is characterized by a precipitous drop in blood pressure, cardiovascular collapse, and/or multiple organ failure. The mortality rate among patients diagnosed with septic shock can be as high as 35-45%. Rapidly and reliably detecting sepsis has been difficult using conventional diagnostic tools

SUMMARY

[0003] The invention provides a method of diagnosing sepsis in a mammal. The methods are also useful as a prognostic tool in predicting the outcome of those diagnosed with sepsis and those undergoing therapy for sepsis.

[0004] A method for diagnosis or prognosis sepsis in a mammal is carried out by contacting a bodily fluid, e.g., blood, plasma, or serum, from the mammal with a monoclonal antibody which binds to the light polypeptide chain moiety of an inter-alpha trypsin inhibitor (ITI) glycoprotein complex under conditions sufficient to form an ITI-antibody complex, detecting the complex, and determining an ITI level wherein a decrease of at least 10% in the level of ITI compared to normal level indicates a diagnosis of sepsis. The mammal is preferably a human patient; however, the assays are also applicable to veterinary use, e.g., to diagnose or prognose sepsis in animals such as dogs, cats, horses, cows and the like. Preferably, the antibody binds to an epitope of human ITI light chain. ITI is used as a marker for diagnosis or prognosis of sepsis in infants such as neonates as well as adult mammals.

[0005] A decrease in the level of ITI in a test sample compared to a normal level of ITI indicates a diagnosis of sepsis. A level of ITI that is at least 10%, more preferably at least 25%, more preferably at least 35% and most preferably at least 50% lower than the a normal level of ITI indicates a diagnosis of sepsis. For example, a normal level of ITI is approximately 1.2 mg/ml of ITI in plasma, and a level of 0.6 mg/ml ITI (or lower) is diagnostic of sepsis. For prognostic purposes, a level of ITI that is at least 10%, more preferably at least 25%, more preferably at least 35% and most preferably at least 50% lower than a diagnostic level of ITI indicates a high risk of mortality (i.e., a poor prognosis). For example, a diagnostic level of ITI is 0.6 mg/ml in plasma, and a level of 0.3 mg/ml ITI (or lower) is predictive of a poor outcome, e.g., septic shock and/or death.

[0006] The ITI-ligand used in the method is one that forms a detectable complex with ITI in a bodily fluid. For example, the ligand is an antibody or ITI-binding antibody fragment, e.g., a Fab or $(Fab)_2$ fragment; an engineered single chain Fv molecule; or a chimeric molecule such as an antibody which contains the binding specificity of one antibody, e.g., of murine origin, and the remaining portions of another antibody, e.g., of human origin. Preferably, the antibody is a monoclonal antibody (mAb) such as mAb 69.31. Alternatively, the ligand is a synthetic or proteolytically-generated peptide that binds to an ITI light chain. For detection purposes, the ligand, e.g., ITI-specific antibody, is directly or indirectly labelled using, e.g., a colorimetric or radioisotopic marker. The amount of an immune complex (which contains ITI antigen bound to ITI-specific antibody) is quantitated to determine the level of ITI in the fluid, and the level of ITI in the fluid is compared to a normal control level of ITI (e.g., a previously determined baseline value or the level of ITI from a subject known to be healthy). The claimed assays accurately and reliably diagnose sepsis rapidly (in less time than results are obtained from a conventional bacteremia or sepsis workup).

[0007] Prognosis of sepsis is also determined by measuring ITI over time. For example, the method includes the steps of (a) contacting samples of a bodily fluid from said mammal taken over time with a monoclonal antibody which binds to the light polypeptide chain moiety of an inter-alpha trypsin inhibitor (ITI) glycoprotein complex under conditions sufficient to form an ITI-antibody complex; (b) quantitating the amount of complex to determine the level of ITI in said samples of fluid; and (c) comparing the level of ITI in said samples of fluid over time, wherein decreasing levels of ITI over time indicates an adverse prognosis. Changes in the severity of the sepsis are monitored by comparing changes in the level of ITI in bodily fluids of the patient over time. Such temporal data is used to determine a course of treatment for the patient.

[0008] Reagents, e.g., an ITI-specific antibody such as mAb 69.31, for carrying out the diagnostic or prognostic assay may be packaged together as a kit. A kit for diagnosis or prognosis of sepsis in a mammal contains a ligand, e.g., an antibody or antibody fragment, which binds to an ITI light chain and a means of detecting a complex containing the ligand bound to an ITI light chain. The means of detection is preferably a colorimetric marker or radioisotopic marker. For example, the antibody is immobilized on a solid phase and packaged together with other reagents suitable for

detecting ITI-ligand complexes. Enzyme-conjugated reagents may be included in the kit. In some embodiments, the antibody or ITI ligand is immobilized on a solid phase such as an assay plate, an assay well, a nitrocellulose membrane, a bead, a dipstick, or a component of an elution column. The immobilized antibody acts as a capture antibody, and a secondary antibody is used to detect the immune complex (e.g., an ITI antigen bound to the mAb 69.31 antibody). The kit may optionally contain a purified ITI polypeptide or purified ITI complex as a control. The polypeptide or complex is purified from natural sources or recombinantly produced. The kit may also contain a second antibody or other detectable marker as described above. For example, the second antibody or marker is labelled, e.g., using a radioisotope, fluorochrome, or other means of detection.

[0009] Assays to detect and/or measure human ITI levels in biological samples using antibodies to the heavy chain of ITI are known - see Balduyck *et al*, J. Lab. Clin. Med., 135, 188-198 (2000).

[0010] Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

[0011]

FIG. 1 is a Fig. 1 is a line graph showing the specificity of binding of mAb 69.31 using a a competitive ELISA assay.

Figs. 2A-B are line graphs showing the range of ITI levels in plasma of human patients. Fig. 2A shows plasma ITI levels in healthy patients compared to patients with sepsis, and Fig. 2B shows plasma ITI levels in survivors compared to non-survivors of sepsis.

Fig. 3 is a line graph showing the range of ITI levels in plasma of human patients in an expanded population of patients. The levels detected in survivors compared to non-survivors of sepsis confirm the data shown in Fig. 2B.

Figs. 4A-B are bar graphs showing the correlation between plasma ITI level and mortality The data plotted in Fig. 4A shows that patients with low plasma ITI levels had a higher mortality rate compared to those with high plasma ITI levels. Fig. 4B shows the results of a followup study of patient ITI levels over time.

Fig. 5 is a line graph showing the effect of ITI on the mortality rate after CLP and caecal excision.

DETAILED DESCRIPTION

[0012] Rapid and reliable detection of sepsis is critical to timely intervention to prevent septic shock and death. The methods described herein are used to detect ITI in bodily fluids of individuals suspected of having or at risk of developing sepsis. Such individuals include those with an infection in the genitourinary tract, the liver or biliary (liver secretion) tract, the gastrointestinal tract, and the lungs. Hospitalized individuals are also at risk of developing sepsis. For example, nocosomial infections which may originate at the site of an intravenous line, surgical wound, surgical drain, or sites of skin breakdown, e.g., a skin ulcer or bedsore, can lead to sepsis. Elderly patients and immunocompromised patients are particularly at risk.

[0013] Risk factors associated with sepsis include recent bacterial pneumonia, meningitis, a urinary tract infection that does not respond to antibiotics, osteomyelitis, bacterial peritonitis, a recent dental procedure, a recent endoscopy procedure, a recent cardiovascular procedure, an indwelling urinary catheter, a recent major surgery, cellulites, or a recent therapy with antibiotics.

[0014] ITI is a diagnostic and prognostic marker for sepsis

[0015] Human ITI is a complex glycoprotein normally found in plasma at a relatively high concentration (ca. 0.8 mg/ml). It consists of one light and two heavy polypeptide chains covalently linked by a glycoaminoglycan bond. The light chain, called bikunin, inhibits various serine proteases involved in coagulation and inflammatory networks such as elastase, plasmin, and cathepsin G. Upon activation by serine proteases, bikunin is cleaved from the ITI complex and rapidly excreted by the kidneys. Using a specific monoclonal antibody against human ITI, e.g., mAb 69.31, a competitive ELISA assay was developed. The assay is used to quantitatively measure levels of human ITI in bodily fluids. In contrast to the assay described herein, previous assays for the detection of ITI used antibodies against the heavy polypeptide chains, as described, for example, in Albani *et al*., J. Lab. Clin. Med., vol. 130(3):334-347 (1997).

[0016] Blood samples from 25 septic patients were collected and plasma was separated for determining ITI levels. The results indicate that plasma levels of ITI of sepsis patients were decreased by 20-90% relative to plasma levels of ITI in healthy volunteers. The magnitude of the decrease in plasma ITI levels correlated with the mortality of the patients. Patients with severely decreased levels of ITI (plasma concentration <0.3 mg/ml) demonstrated a higher mortality rate. The mean $\pm$ SD ITI level was significantly lower in non-survivors (0.35 ± 0.20 mg/ml) compared to survivors (0.58 ± 0.13 mg/ml). These differences persisted for up to 7 days after onset of a severe sepsis ($p<0.05$).

[0017] The assays described herein are used to diagnose sepsis and as a prognostic marker to identify subpopulation of septic patients who might need more aggressive treatment to reduce the mortality risk associated with sepsis.

Neonatal Sepsis

[0018] Diagnosis of adult or neonatal sepsis is typically carried out using standard diagnostic tests for bacteremia, e.g., by drawing neonatal blood, performing a bacterial cell culture and blood cell count. Human neonatal sepsis may be caused by various pathogens, including Group B Streptococci, E. coli, Listeria, and viruses.

[0019] There are several disadvantages associated with a conventional neonatal sepsis workup. Often the blood volume obtained from an infant is suboptimal for conventional tests (5-10 times less than the blood volume drawn from an adult) and the workup can take up to 3 days. If the mother has been undergoing antibiotic treatment, the baby's bacterial culture can be negative, even if bacteria are present.

[0020] To detect sepsis in a human infant patient, a blood sample is obtained from the infant using conventional methods. For neonates, the size of the blood sample drawn is typically 200 µl. Larger sample are drawn according to standard medical practice, taking into account such factors as the size and age of the infant. A blood sample size less than or greater than 200 µl can be used. When the mother is known to have clinical risk factors for neonatal sepsis, a blood sample from the infant is obtained for testing shortly after birth. Maternal risk factors include maternal fever, fetal tachycardia, rupture of the amniotic membrane more than 12 hours before delivery, foul smelling amniotic fluid, uterine tenderness, increased white blood cell count, and meconium stained amniotic fluid.

[0021] If a maternal risk factor is not known to be present, an infant blood sample is obtained as soon as the infant displays signs of infection. Signs of infant infection are known in the art and include evidence of respiratory distress, apnea, petechiae (microbruises under the skin), irregular heart beat, rapid breathing, tachypnea, bulging anterior fontenelle, low blood sugar, and low core temperature.

[0022] The methods described herein are rapid (i.e., results can be obtained in a day, often in hours) and can be carried out on a small amount of patient plasma (less than 4-5 Tl of serum or plasma for multiple tests).

Production of ITI-specific antibodies

[0023] A panel of mAbs was generated against two different human plasma-derived Factor VII preparations using a standard subtractive immunization protocol. The mice were first immunized with a non-pasteurized preparation (containing ITI), treated with cyclophosphamide, and then injected with a pasteurized preparation. Following sacrifice of the mice, hybridomas were generated and screened using known methods. One of the antibodies (mAb 69.31) was found to bind to a complex glycoprotein which contains several polypeptide chains. Affinity chromatographic purification of the antigen complex with immobilized mAb 69.31 isolated polypeptide antigens of 250 kDa and 125 kDa in size. The bands (on SDS-PAGE) were identified as ITI by N-terminal amino acid sequence analysis;

AVLPQEEEGGGGGQ (SEQ ID NO:1); SLPGEXEEMMEEVD (SEQ ID NO:2);

SBLPWRRRGGGGGQL (SEQ ID NO:3); SLPGEXEEMMEEVDQ (SEQ ID NO:4).

Affinity-purified ITI inhibited serine proteases, such as trypsin, Plasmin, and elastase.

[0024] ITI found in the serum or plasma of human subjects contains one light chain and two heavy polypeptide chains covalently linked by a chondroitin sulfate chain. The light chain (with an apparent molecular mass of 30 kDa) inhibits several serine proteases such as trypsin, human leukocyte elastase (HLE), plasmin and cathepsin G, which proteases are involved in inflammation, shock, tumor invasion, and formation of metastases. Naturally-occurring and recombinant ITI light chains are purified with ITI mAb such as 69.31. Purified ITI light chains are useful to therapeutically inhibit serine proteases.

Deposit

[0025] A hybridoma cell line RI 69.31 which produces mAb 69.31 was deposited with the American Type Culture Collection (ATCC) under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure on December 16,1999, and bears the Patent Deposit Designation PTA-1066.

Binding specificity of ITI-specific antibodies

[0026] MAb 69.31 was reactive with the light chain of ITI. ITI was purified from human serum by using mAb 69.31 and treated with 0, 1, 5, and 10 ghy aluronidase to cleave the heavy and light chains of ITI. The samples were separated

on 12.5% SDS-PAGE and transferred onto nitrocellulose membrane for analysis by Western blot. Without hyaluronidase, two major bands (250 kDa and 125 kDa) were detected with mAb 69.31. With increasing amounts of hyaluronidase, an additional lower molecular weight band (approximately 25-30 kDa) was detected by the antibody. This lower band represented the light chain of ITI. The light and heavy chains of ITI are linked by glycoaminoglycan. Hyaluronidase cleaved the glycoaminoglycan (chondroitin sulfate) chain linking the heavy chain and light chains of the ITI complex, releasing the two chains. These results indicate that mAb 69.31 binds to an epitope located in the light chain of ITI.

[0027] The addition of mAb 69.31 to ITI blocked the serine protease inhibitory activity of ITI (as demonstrated by the observed reduction in ITI's inhibition of trypsin activity in the presence of the antibody). These data indicate that the epitope of mAb 69.31 is located in or proximal to the active site of the ITI molecule, i.e., when the 69.31 antibody binds to an ITI light chain molecule, the molecule cannot bind to its substrate. Alternatively, antibody binding alters the 3-dimensional confirmation of ITI, thereby decreasing its serine protease inhibitory activity. Other monoclonal antibodies with the binding specificity of mAb 69.31 are produced and identified using methods known in the art, e.g., competitive binding assays. For example, the active site of ITI light chain contains residues 20-32, residues 241-242, or residues 297-298 of SEQ ID NO:5 (Table 1).

Table 1: Amino acid sequence of human ITI light chain

MRSLGALLLL LSACLAVSAG PVPTPPDNIQ VQENFNISRI YGKWYNLAIG STCPWLKKIM 61
DRMTVSTLVL GEGATEAEIS MTSTRWRKGV CEETSGAYEK TDTDGKFLYH KSKWNITMES 121
YVVHTNYDEY AIFLTKKFSR HHGPTITAKL YGRAPQLRET LLQDFRVVAQ GVGIPEDSIF 181
TMADRGECVP GEQEPEPILI PRVRRAVLPQ EEEGSGGGQL VTEVTKKEDS CQLGYSAGPC 241
MGMTSRYFYN GTSMACETFQ YGGCMGNGNN FVTEKECLQT CRTVAACNLP IVRGPCRAFI 301
QLWAFDAVKG KCVLFPYGGC QGNGNKFYSE KECREYCGVP GDGDEELLRF SN
(SEQ ID NO:5; residues 1-19 = signal peptide)

Diagnostic/Prognostic Assays and Kits

[0028] The data described herein demonstrated that significantly lower levels of ITI were detected in plasma or serum of patients with sepsis in contrast to those without sepsis. While Western blot assays offer high specificity in detecting ITI, a quantitative assay such as ELISA is preferred to quantify the levels of ITI in bodily fluids. A normal value range of ITI levels in normal individuals (e.g., healthy volunteers) was determined as a baseline.

[0029] Collection of normal baseline data is carried out using conventional analytical techniques and well-known methods of statistical analysis. A "normal level" of ITI means the mean level of ITI in a given bodily fluid for a population of non-septic individuals. A normal range is a mean level among a population of non-septic individuals, plus or minus 10% of the mean, or plus or minus two standard deviations from the mean. Normal ranges are preferably age-matched, i.e., the normal range of ITI levels for a neonate is the mean level of non-septic neonates, and the normal range for an adult is the mean level for non-septic adults.

[0030] A standard competitive ELISA format using affinity purified human ITI and Mab 69.31 is used to quantify patient ITI levels. Alternatively, a sandwich ELISA using a capture antibody (Mab 69.31) and a second enzyme-labeled rabbit polyclonal antibody against ITI as a detection antibody is used.

[0031] Methods of detecting level of ITI in bodily fluids include contacting a component of a bodily fluid with an ITI-specific antibody bound to solid matrix, e.g., microtiter plate, bead, dipstick. For example, the solid matrix is dipped into a patient-derived sample of a bodily fluid, washed, and the solid matrix contacted with a reagent to detect the presence of immune complexes present on the solid matrix.

[0032] Proteins in a test sample are immobilized on (bound to) a solid matrix. Methods and means for covalently or noncovalently binding proteins to solid matrices are known in the art. The nature of the solid surface may vary depending upon the assay format. For assays carried out in microtiter wells, the solid surface is the wall of the well or cup. For assays using beads, the solid surface is the surface of the bead. In assays using a dipstick (i.e., a solid body made from a porous or fibrous material such as fabric or paper) the surface is the surface of the material from which the dipstick is made. Examples of useful solid supports include nitrocellulose (e.g., in membrane or microtiter well form), polyvinyl chloride (e.g., in sheets or microtiter wells), polystyrene latex (e.g., in beads or microtiter plates, polyvinylidine fluoride (known as IMMULON™), diazotized paper, nylon membranes, activated beads, and Protein A beads. Microtiter plates may be activated (e.g., chemically treated or coated) to covalently bind proteins. The solid support containing the antibody is typically washed after contacting it with the test sample, and prior to detection of bound immune complexes.

[0033] A common feature of all of these assays is that an ITI-specific antibody is contacted with a sample of bodily fluid under conditions that permit ITI to bind to the antibody forming an immune complex containing the patient ITI bound to an ITI-specific antibody. Such conditions are typically physiologic temperature, pH, and ionic strength. The

incubation of the antibody with the test sample is followed by detection of immune complexes by a detectable label. For example, the label is enzymatic, fluorescent, chemiluminescent, radioactive, or a dye. Assays which amplify the signals from the immune complex are also known in the art, e.g., assays which utilize biotin and avidin.

[0034] An ITI-detection reagent, e.g., an antibody, is packaged in the form of a kit. The kit may contain in separate containers an antibody (either already bound to a solid matrix or packaged separately with reagents for binding them to the matrix), control formulations (positive and/or negative), and/or a detectable label. Instructions (e.g., written, tape, VCR, CD ROM, etc.) for carrying out the assay may be included in the kit. The assay may be in the form of a standard sandwich ELISA format known in the art.

[0035] For example, an ITI capture antibody, e.g., mAb 69.31, is immobilized on a solid matrix such as a porous strip to form at least one ITI detection site. The measurement or detection region of the porous strip may include a plurality of sites containing an immobilized antibody. A test strip may also contain sites for negative and/or positive controls. Alternatively, control sites are located on a separate strip from the test strip. Optionally, the different detection sites may contain different amounts of immobilized antibody, i.e., a higher amount in the first detection site and lesser amounts in subsequent sites. For example, if 20 nanograms of antibody captures the equivalent of 1 nmol/min/ml of ITI, then the first detection site of an assay device might contain 50 nanograms of the antibody while the subsequent sites contain 10, 20, 30, etc. nanograms of antibody. Upon the addition of test sample, the number of sites displaying a detectable signal provides a quantitative indication of the amount of ITI present in the sample. The detection sites may be configured in any suitably detectable shape and are typically in the shape of a bar spanning the width of a teststrip.

[0036] A multi-capture assay configuration is prepared such that if a threshold amount of ITI is not present in the test sample, then substantially all of the ITI will bind to the antibody in the first capture site and thus become immobilized at that site. If a greater than threshold amount of ITI is present in the test sample, the remaining ITI binds to subsequent detection zones of immobilized antibody along the length of the teststrip. The greater the amount of ITI in the test sample, the greater the number of capture sites that will display a detectable signal due to the presence of ITI.

[0037] DNA encoding ITI polypeptides is introduced into target cells of the patient by standard vectors and/or gene delivery systems. Suitable gene delivery systems include liposomes, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses, and adenoviruses, among others.

Example 1: Plasma levels of ITI as a marker for sepsis

[0038] Plasma levels of ITI in healthy individuals and septic patients were measured by a competitive ELISA using a monoclonal antibody specific which binds to human ITI (e.g., MAb 69.31). A standard curve of the immunoassay was established by using serially diluted of known amount of purified ITI (Fig. 1). The assay was used to reliably and accurately measure the levels of ITI in plasma in a range between 0.1 - 2.5 μg/mL. To determine the ITI level in a test plasma or serum sample, the sample was diluted 1:1000 to 1:2500 in PBS and the concentration of ITI calculated based on the standard curve.

[0039] Plasma levels of ITI were determined in healthy individuals, individuals known to have sepsis (as determined by conventional clinical parameters), survivors of sepsis, and non-survivors of sepsis. Using the competitive ELISA, plasma ITI levels from a group of healthy individuals (n=17) were compared to the levels of septic patients (n=52). The results (Fig. 2) indicated that the mean of ITI levels of healthy individuals was significantly higher compared to the mean of septic patient group (1.266±0.519 mg/ml compared to 0.498±0.165 mg/ml). The difference is statistically significant ($p=<0.0001$).

[0040] When 25 plasma samples from septic patients were further analyzed based on the outcome, the group of survivors demonstrated higher levels of ITI compared to the patients who did not survive sepsis (non-survivors). The mean ITI levels were 0.58±0.13 mg/ml compared to 0.35±0.20 mg/ml, respectively. The difference between the ITI levels of each group was statistically significant ($p=0.031$). The results indicate that a decreased level of ITI (compared to a normal, non-septic level) correlates with increased septic mortality. The results shown in Fig. 2 were further confirmed in a larger septic patient study involving 32 survivors and 20 non-survivors. The results (Fig. 3) were consistent with the data shown in Fig. 2.

Example 2: Plasma ITI levels are prognostic of mortality

[0041] The correlation between low ITI plasma levels and mortality rate is shown in Figs. 4A-B. Sixty per cent (6 out of 10) of septic patients who died demonstrated severely decreased levels of ITI in the plasma (<0.3 mg/ml). In contrast, elevated levels of ITI were detected in 19% (8 out of 42) of septic patients who died (ITI levels > 0.30 mg/ml). The difference was statistically significant at $p=0.016$.

[0042] When patients were followed over time, patients with low levels of ITI demonstrated a higher mortality rate than patients with high levels of ITI (Fig. 4B) These differences persisted for up to 7 days after onset of a severe sepsis

(p<0.05).

[0043] These data indicate that serum or plasma ITI levels are useful to determine the prognosis of patients with infection (e.g., individuals at risk of developing sepsis or those who are undergoing treatment for sepsis). For example, in addition to use as a diagnostic and prognostic tool for sepsis, the assays described herein are useful to monitor the progress of individuals undergoing a particular treatment regimen (for infection and/or sepsis) and provide a basis for altering a prescribed treatment protocol.

SEQUENCE LISTING

[0044]

<110> Rhode Island Hospital, a Lifespan Partner
Lim, Yow-Pin
Hixson, Douglas C

<120> Inter-Alpha-Trypsin inhibitor as a Marker for Sepsis

<130> 21486-037

<140> PCT/US01/03827
<141> 2001-02-06

<150> 09/514,450
<151> 2000-02-28

<160> 5

<170> PatentIn Ver. 2.0

<210> 1
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Inter-Alpha-Trypsin Inhibitor

<400> 1

```
Ala Val Leu Pro Gln Glu Glu Glu Gly Gly Gly Gly Gly Gln
  1               5                  10
```

<210> 2
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Inter-Alpha-Trypsin Inhibitor

<220>
<221> VARIANT
<222> (6)
<223> Wherein X is any amino acid.

<400> 2

Ser Leu Pro Gly Glu Xaa Glu Glu Met Met Glu Glu Val Asp
1 5 10

5

<210> 3
<211> 15
<212> PRT
<213> Artificial Sequence

10

<220>
<223> Inter-Alpha-Trypsin Inhibitor

<400> 3

15

Ser Asx Leu Pro Trp Arg Arg Arg Gly Gly Gly Gly Gly Gln Leu
1 5 10 15

<210> 4

20

<211> 15
<212> PRT
<213> Artificial Sequence

<220>

25

<223> Inter-Alpha-Trypsin Inhibitor

<220>
<221> VARIANT

30

<222> (6)
<223> Wherein X is any amino acid.
<400> 4

35

Ser Leu Pro Gly Glu Xaa Glu Glu Met Met Glu Glu Val Asp Gln
1 5 10 15

<210> 5
<211> 352

40

<212> PRT
<213> Homo sapiens
<220>
<221> SIGNAL
<222> (1)..(19)

45

<400> 5

Met Arg Ser Leu Gly Ala Leu Leu Leu Leu Leu Ser Ala Cys Leu Ala
1 5 10 15

50

Val Ser Ala Gly Pro Val Pro Thr Pro Pro Asp Asn Ile Gln Val Gln
20 25 30

55

Glu Asn Phe Asn Ile Ser Arg Ile Tyr Gly Lys Trp Tyr Asn Leu Ala
35 40 45

5

Ile Gly Ser Thr Cys Pro Trp Leu Lys Lys Ile Met Asp Arg Met Thr
50 55 60

Val Ser Thr Leu Val Leu Gly Glu Gly Ala Thr Glu Ala Glu Ile Ser
10
65 70 75 80

Met Thr Ser Thr Arg Trp Arg Lys Gly Val Cys Glu Glu Thr Ser Gly
85 90 95

15

Ala Tyr Glu Lys Thr Asp Thr Asp Gly Lys Phe Leu Tyr His Lys Ser
100 105 110

Lys Trp Asn Ile Thr Met Glu Ser Tyr Val Val His Thr Asn Tyr Asp
20
115 120 125

Glu Tyr Ala Ile Phe Leu Thr Lys Lys Phe Ser Arg His His Gly Pro
130 135 140

25

Thr Ile Thr Ala Lys Leu Tyr Gly Arg Ala Pro Gln Leu Arg Glu Thr
145 150 155 160

Leu Leu Gln Asp Phe Arg Val Val Ala Gln Gly Val Gly Ile Pro Glu
30
165 170 175

Asp Ser Ile Phe Thr Met Ala Asp Arg Gly Glu Cys Val Pro Gly Glu
180 185 190

35

Gln Glu Pro Glu Pro Ile Leu Ile Pro Arg Val Arg Arg Ala Val Leu
195 200 205

Pro Gln Glu Glu Glu Gly Ser Gly Gly Gly Gln Leu Val Thr Glu Val
40
210 215 220

Thr Lys Lys Glu Asp Ser Cys Gln Leu Gly Tyr Ser Ala Gly Pro Cys
225 230 235 240

45

Met Gly Met Thr Ser Arg Tyr Phe Tyr Asn Gly Thr Ser Met Ala Cys
245 250 255

Glu Thr Phe Gln Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val
50
260 265 270

Thr Glu Lys Glu Cys Leu Gln Thr Cys Arg Thr Val Ala Ala Cys Asn
275 280 285
55

```
Leu Pro Ile Val Arg Gly Pro Cys Arg Ala Phe Ile Gln Leu Trp Ala
    290                 295                 300

Phe Asp Ala Val Lys Gly Lys Cys Val Leu Phe Pro Tyr Gly Gly Cys
305                 310                 315                 320

Gln Gly Asn Gly Asn Lys Phe Tyr Ser Glu Lys Glu Cys Arg Glu Tyr
                325                 330                 335

Cys Gly Val Pro Gly Asp Gly Asp Glu Glu Leu Leu Arg Phe Ser Asn
            340                 345                 350
```

5

10

15

20

25

30

35

40

45

50

55

Claims

1. A method for diagnosis of sepsis in a mammal, comprising contacting a sample of a bodily fluid from said mammal with a monoclonal antibody which binds to the light polypeptide chain moiety of an inter-alpha trypsin inhibitor (ITI) glycoprotein complex under conditions sufficient to form an ITI-antibody complex, detecting the complex, and determining an ITI level wherein a decrease of at least 10% in the level of ITI compared to normal level indicates a diagnosis of sepsis.

2. The method of claim 1, wherein a level of 0.6 mg/ml or lower of ITI glycoprotein in said bodily fluid indicates a diagnosis of sepsis.

3. A method for prognosis of sepsis in a mammal comprising the steps of

   (a) contacting samples of a bodily fluid from said mammal taken over time with a monoclonal antibody which binds to the light polypeptide chain moiety of an inter-alpha trypsin inhibitor (ITI) glycoprotein complex under conditions sufficient to form an ITI-antibody complex,
   (b) quantitating the amount of complex to determining the level of ITI in said samples of fluid; and
   (c) comparing the level of ITI in said samples of fluid over time, wherein decreasing levels of ITI over time indicates an adverse prognosis.

4. The method of claim 3, comprising contacting a sample of bodily fluid from said mammal with a monoclonal antibody which binds to an ITI light chain polypeptide-containing inter-alpha trypsin inhibitor (ITI) glycoprotein with a molecular mass of 250 kDa and an ITI light chain polypeptide-containing ITI glycoprotein with a molecular mass of 125 kDa under conditions sufficient to form an immune complex and detecting the immune complex to determine an ITI level, wherein said bodily fluid is blood, serum, or plasma, and wherein an ITI level of 0.3 mg/ml or lower indicates a high risk of mortality.

5. The method of claim 3 or claim 4, wherein a level of 0.3 mg/ml or lower of said ITI glycoprotein in said bodily fluid indicates a prognosis of septic shock or death.

6. The method of any preceding claim, wherein said mammal is a human.

7. The method of any preceding claim, wherein said mammal is a human infant.

8. The method of any preceding claim, wherein said fluid is blood, plasma or serum.

9. The method of any preceding claim, wherein said monoclonal antibody binds to an epitope of human ITI light chain.

10. The method of any preceding claim, wherein said monoclonal antibody is 69.31 (ATCC Deposit No. PTA-1066).

11. The method of any preceding claim, wherein the monoclonal antibody binds to an ITI light chain polypeptide-containing ITI glycoprotein with a molecular mass of 250 kDa and an ITI light chain polypeptide-containing ITI glycoprotein with a molecular mass of 125 kDa.

12. The method of any preceding claim, wherein said level of ITI glycoprotein is at least 25% lower than said normal level.

13. The method of any preceding claim, wherein said level of ITI glycoprotein is at least 35% lower than said normal level.

14. The method of any preceding claim, wherein said level of ITI glycoprotein is at least 50% lower than said normal level.

15. A kit for diagnosis or prognosis of sepsis in a mammal, comprising a monoclonal antibody which binds to an inter-alpha trypsin inhibitor (ITI) light chain polypeptide and a means of detecting said monoclonal antibody bound to said ITI polypeptide.

16. A kit as claimed in claim 15, wherein said antibody is 69.31 (ATCC Deposit No. PTA-1066).

17. A kit as claimed in claim 15, wherein said antibody binds to an ITI light chain polypeptide-containing ITI glycoprotein with a molecular mass of 250 kDa and an ITI light chain polypeptide-containing ITI glycoprotein with a molecular mass of 125 kDa.

18. The kit of any one of claims 15 to 17, wherein said antibody is immobilized on a solid phase.

19. The kit of claim 18, wherein said solid phase is selected from the group consisting of an assay plate, an assay well, a nitrocellulose membrane, a bead, a dipstick, and a component of an elution column.

Patentansprüche

1. Methode zur Diagnose von Sepsis in einem Säuger, umfassend das Kontaktieren einer Probe einer Körperflüssigkeit von dem Säuger mit einem monoklonalen Antikörper, welcher an die leichte Polypeptidketten-Komponente eines Inter-alpha-Trypsin-Inhibitor-(ITI)-Glycoprotein-Komplexes unter hinreichenden Bedingungen bindet, um einen ITI-Antikörper-Komplex zu bilden, das Nachweisen des Komplexes und das Bestimmen einer ITI-Menge, wobei eine Abnahme von mindestens 10 % bei der Menge des ITI im Vergleich zur normalen Menge eine Sepsis-Diagnose anzeigt.

2. Methode nach Anspruch 1, wobei eine Menge von 0,6 mg/ml oder weniger des ITI-Glycoproteins in der Körperflüssigkeit eine Sepsis-Diagnose anzeigt.

3. Methode zum Prognostizieren von Sepsis in einem Säuger, umfassend die folgenden Schritte:

   (a) Kontaktieren von über die Zeit genommenen Proben einer Körperflüssigkeit von dem Säuger mit einem monoklonalen Antikörper, welcher an die leichte Polypeptidketten-Komponente eines Inter-alpha-Trypsin-Inhibitor-(ITI)-Glycoprotein-Komplexes unter hinreichenden Bedingungen bindet, um einen ITI-Antikörper-Komplex zu bilden,
   (b) Quantifizieren der Menge des Komplexes zur Bestimmung der Menge an ITI in den Flüssigkeitsproben; und
   (c) Vergleichen der Menge an ITI in den Flüssigkeitsproben im Zeitverlauf, wobei abnehmende Mengen an ITI im Zeitverlauf eine ungünstige Prognose anzeigen.

4. Methode nach Anspruch 3, umfassend das Kontaktieren einer Probe einer Körperflüssigkeit von dem Säuger mit einem monoklonalen Antikörper, welcher an ein ITI-Leichtketten-Polypeptid-enthaltendes Inter-alpha-Trypsin-Inhibitor-(ITI)-Glycoprotein mit einer Molekülmasse von 250 kDa und ein ITI-Leichtketten-Polypeptid-enthaltendes ITI-Glycoprotein mit einer Molekülmasse von 125 kDa unter hinreichenden Bedingungen bindet, um einen Immunkomplex zu bilden, und das Nachweisen des Immunkomplexes, um eine ITI-Menge zu bestimmen, wobei die Körperflüssigkeit Blut, Serum oder Plasma ist, und wobei eine ITI-Menge von 0,3 mg/ml oder weniger ein hohes Risiko von Mortalität anzeigt.

5. Methode nach Anspruch 3 oder Anspruch 4, wobei eine Menge von 0,3 mg/ml oder weniger des ITI-Glycoproteins in der Körperflüssigkeit eine Prognose von septischem Schock oder Tod anzeigt.

6. Methode nach einem der vorangehenden Ansprüche, wobei der Säuger ein Mensch ist.

7. Methode nach einem der vorangehenden Ansprüche, wobei der Säuger ein Menschenkind ist

8. Methode nach einem der vorangehenden Ansprüche, wobei die Flüssigkeit Blut, Plasma oder Serum ist.

9. Methode nach einem der vorangehenden Ansprüche, wobei der monoklonale Antikörper an ein Epitop der humanen ITI-Leichtkette bindet.

10. Methode nach einem der vorangehenden Ansprüche, wobei der monoklonale Antikörper 69.31 ist (ATCC-Hinterlegungsnr. PTA-1066).

11. Methode nach einem der vorangehenden Ansprüche, wobei der monoklonale Antikörper an ein ITI-Leichtketten-Polypeptid-enthaltendes Inter-alpha-TrypsinInhibitor-(ITI)-Glycoprotein mit einer Molekülmasse von 250 kDa und ein ITI-Leichtketten-Polypeptid-enthaltendes ITI-Glycoprotein mit einer Molekülmasse von 125 kDa bindet.

12. Methode nach einem der vorangehenden Ansprüche, wobei die Menge an ITI-Glycoprotein um mindestens 25 % unter der normalen Menge liegt.

13. Methode nach einem der vorangehenden Ansprüche, wobei die Menge an ITI-Glycoprotein um mindestens 35 % unter der normalen Menge liegt.

14. Methode nach einem der vorangehenden Ansprüche, wobei die Menge an ITI-Glycoprotein um mindestens 50 % unter der normalen Menge liegt.

15. Kit für die Diagnose oder Prognose von Sepsis in einem Säuger, umfassend einen monoklonalen Antikörper, welcher an eine Inter-alpha-Trypsin-Inhibitor-(ITI)-Leichtketten-Polypeptid bindet, und eine Einrichtung zum Nachweisen des an das ITI-Polypeptid gebundenen monoklonalen Antikörpers.

16. Kit nach Anspruch 15, wobei der Antikörper 69.31 ist (ATCC-Hinterlegungsnr. PTA-1066).

17. Kit nach Anspruch 15, wobei der Antikörper an eine ITI-Leichtketten-Polypeptid-enthaltendes ITI-Glycoprotein mit einer Molekülmasse von 250 kDs und ein ITI-Leichtketten-Polypeptid-enthaltendes ITI-Glycoprotein mit einer Molekülmasse von 125 kDa bindet.

18. Kit nach einem der Ansprüche 15 bis 17, wobei der Antikörper an einer festen Phase immobilisiert ist.

19. Kit nach Anspruch 18, wobei die feste Phase ausgewählt ist aus der Gruppe, bestehend aus einer Assay-Platte, einem Assay-Well, einer Nitrocellulosemembran, einem Kügelchen, einem Tauchstift und einer Komponente einer Elutions-Säule.

Revendications

1. Méthode de diagnostic d'un état septique chez un mammifère, comprenant la mise en contact d'un échantillon d'un fluide corporel dudit mammifère avec un anticorps monoclonal qui se lie au fragment chaîne polypeptidique légère d'un complexe glycoprotéinique qui est l'inhibiteur inter-alpha de la trypsine (ITI) dans des conditions suffisantes pour former un complexe ITI-anticorps, la détection du complexe, et la détermination d'un niveau d'ITI, une diminution d'au moins 10% du niveau d'ITI par rapport au niveau normal indiquant un diagnostic d'un état septique.

2. Méthode selon la revendication 1, dans laquelle un niveau de 0,6 mg/ml ou moins de glycoprotéine ITI dans ledit fluide corporel indique un diagnostic d'un état septique.

3. Méthode de pronostic d'un état septique chez un mammifère comprenant les étapes consistant

(a) à mettre en contact des échantillons d'un fluide corporel dudit mammifère prélevés au cours du temps, avec un anticorps monoclonal qui se lie au fragment chaîne polypeptidique légère d'un complexe glycoprotéinique qui est l'inhibiteur inter-alpha de la trypsine (ITI), dans des conditions suffisantes pour former un complexe ITI-anticorps,
(b) à quantifier la quantité du complexe afin de déterminer le niveau d'ITI dans lesdits échantillons de fluide ; et
(c) à comparer le niveau d'ITI dans lesdits échantillons de fluide dans le temps, des niveaux décroissants d'ITI dans le temps indiquant un pronostic défavorable.

4. Méthode de la revendication 3, comprenant la mise en contact d'un échantillon de fluide corporel dudit mammifère avec un anticorps monoclonal qui se lie à une glycoprotéine qui est l'inhibiteur inter-alpha de la trypsine (ITI) contenant une chaîne polypeptidique légère d'ITI, d'une masse moléculaire de 250 kDa et une glycoprotéine qui est l'ITI contenant une chaîne polypeptidique légère d'ITI, d'une masse moléculaire de 125 kDa, dans des conditions suffisantes pour former un complexe immun et la détection du complexe immun afin de déterminer un niveau d'ITI, dans laquelle ledit fluide corporel est du sang, du sérum, ou du plasma, et dans laquelle un niveau d'ITI de 0,3 mg/ml ou moins indique un risque élevé de mortalité.

5. Méthode de la revendication 3 ou de la revendication 4, dans laquelle un niveau de 0,3 mg/ml ou moins de ladite glycoprotéine ITI dans ledit fluide corporel indique un pronostic de choc septique ou de mort.

6. Méthode de l'une quelconque des revendications précédentes, dans laquelle ledit mammifère est un humain.

7. Méthode de l'une quelconque des revendications précédentes, dans laquelle ledit mammifère est un nourrisson humain.

8. Méthode de l'une quelconque des revendications précédentes, dans laquelle ledit fluide est le sang, le plasma ou le sérum.

9. Méthode de l'une quelconque des revendications précédentes, dans laquelle ledit anticorps monoclonal se lie à un épitope de la chaîne légère de l'ITI humain.

10. Méthode de l'une quelconque des revendications précédentes, dans laquelle ledit anticorps monoclonal est le 69.31 (No de Dépôt ATCC PTA-1066).

11. Méthode de l'une quelconque des revendications précédentes, dans laquelle l'anticorps monoclonal se lie à une glycoprotéine ITI contenant une chaîne polypeptidique légère d'ITI, d'une masse moléculaire de 250 kDa et à une glycoprotéine ITI contenant une chaîne polypeptidique légère d'ITI, d'une masse moléculaire de 125 kDa.

12. Méthode de l'une quelconque des revendications précédentes, dans laquelle ledit niveau de glycoprotéine ITI est au moins 25% inférieur audit niveau normal.

13. Méthode de l'une quelconque des revendications précédentes, dans laquelle ledit niveau de glycoprotéine ITI est au moins 35% inférieur audit niveau normal.

14. Méthode de l'une quelconque des revendications précédentes, dans laquelle ledit niveau de glycoprotéine ITI est au moins 50% inférieur audit niveau normal.

15. Kit de diagnostic ou de pronostic d'un état septique chez un mammifère, comprenant un anticorps monoclonal qui se lie à un polypeptide à chaîne légère d'un inhibiteur inter-alpha de la trypsine (ITI) et des moyens de détection dudit anticorps monoclonal lié audit polypeptide d'ITI.

16. Kit tel que revendiqué dans la revendication 15, dans lequel ledit anticorps est le 69.31 (No. De Dépôt ATCC PTA-1066).

17. Kit tel que revendiqué dans la revendication 15, dans lequel ledit anticorps se lie à une glycoprotéine ITI contenant un polypeptide à chaîne légère d'ITI, d'une masse moléculaire de 250 kDa et à une glycoprotéine ITI contenant un polypeptide à chaîne légère d'ITI, d'une masse moléculaire de 125 kDa.

18. Kit selon l'une quelconque des revendications 15 à 17, dans lequel ledit anticorps est immobilisé sur une phase

solide.

19. Kit de la revendication 18, dans lequel ladite phase solide est choisie dans le groupe constitué par une plaque d'essai, un puits d'essai, une membrane de nitrocellulose, une perle, une tige plongeante, et un composant d'une colonne d'élution.

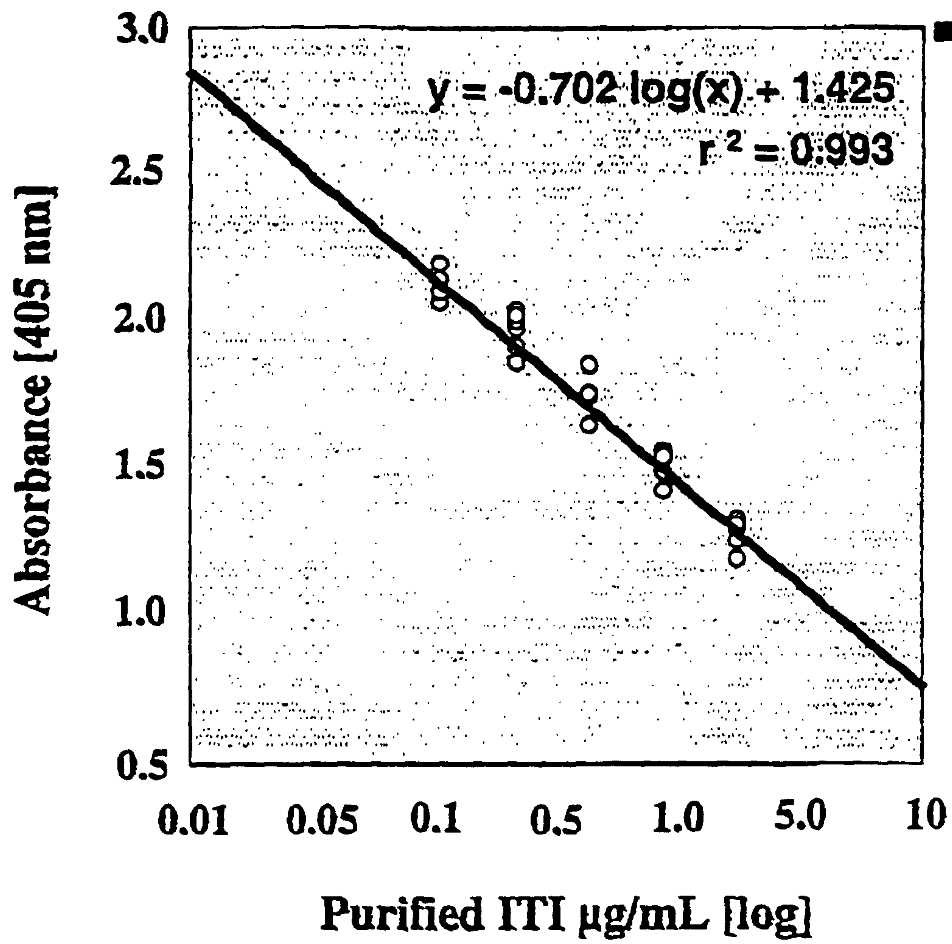


FIG. 1

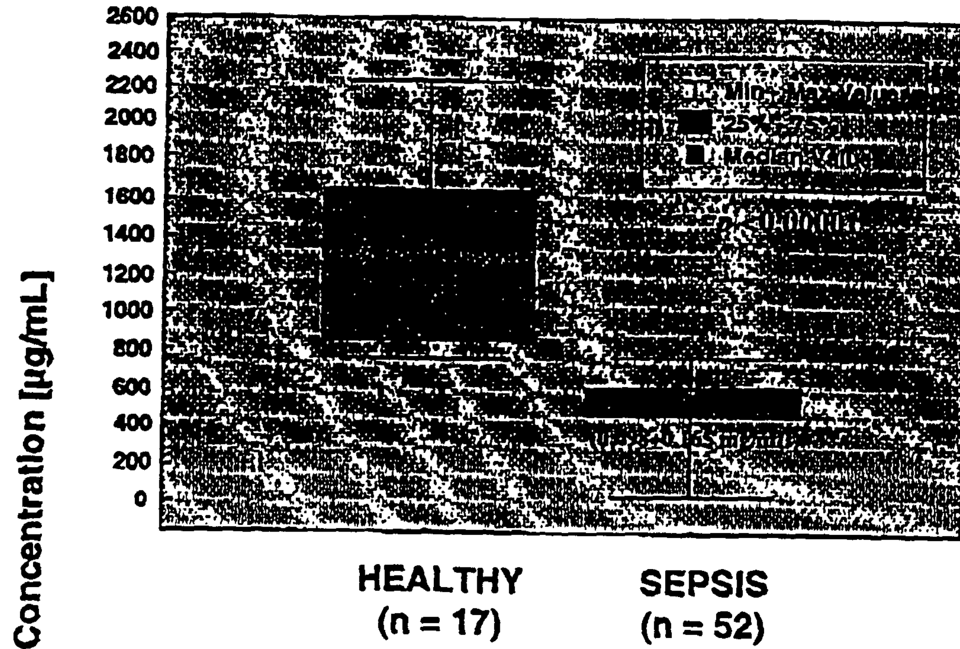


FIG. 2A

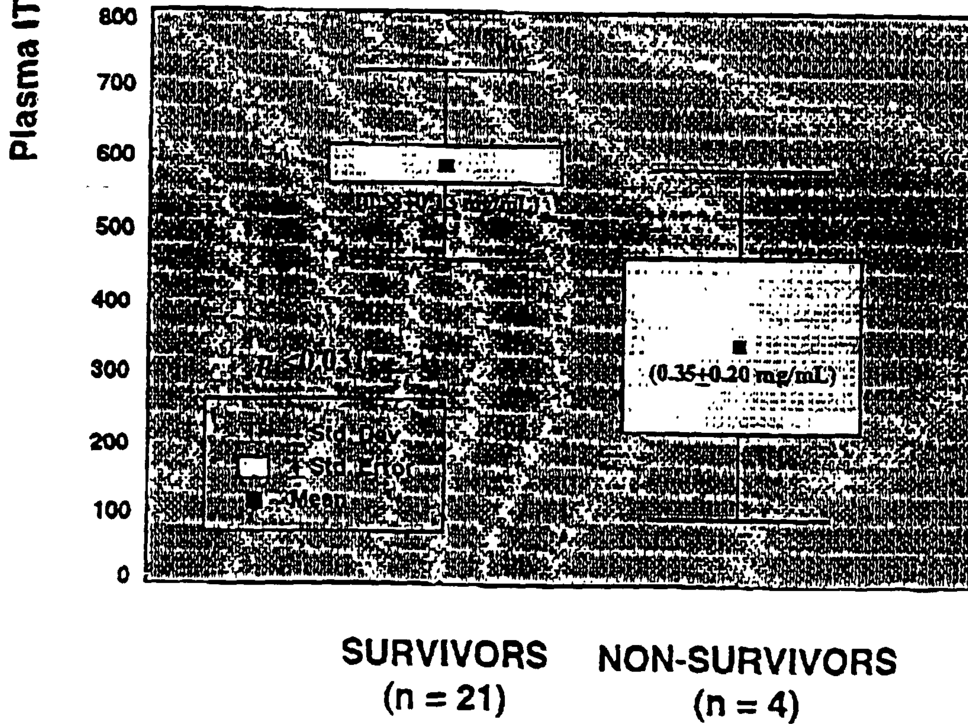


FIG. 2B

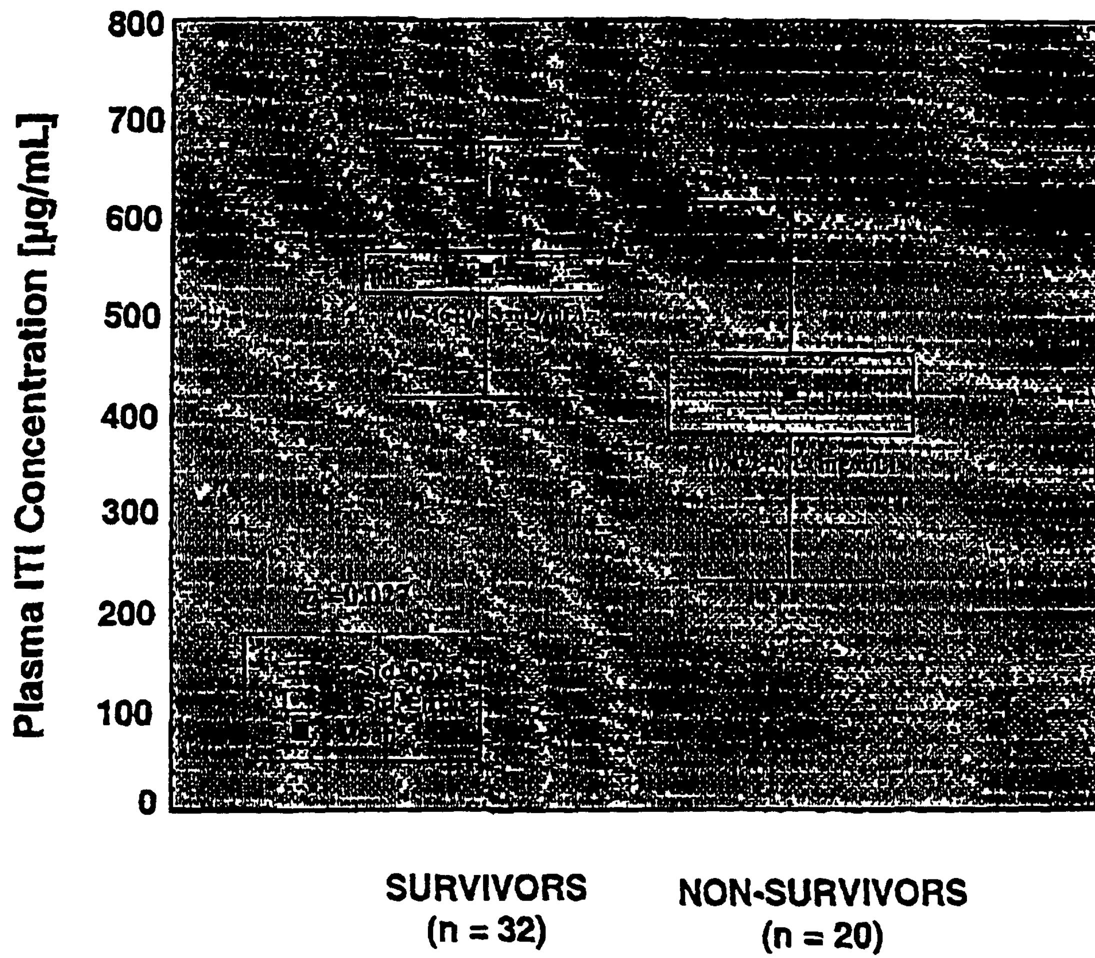


FIG. 3

FIG. 4A

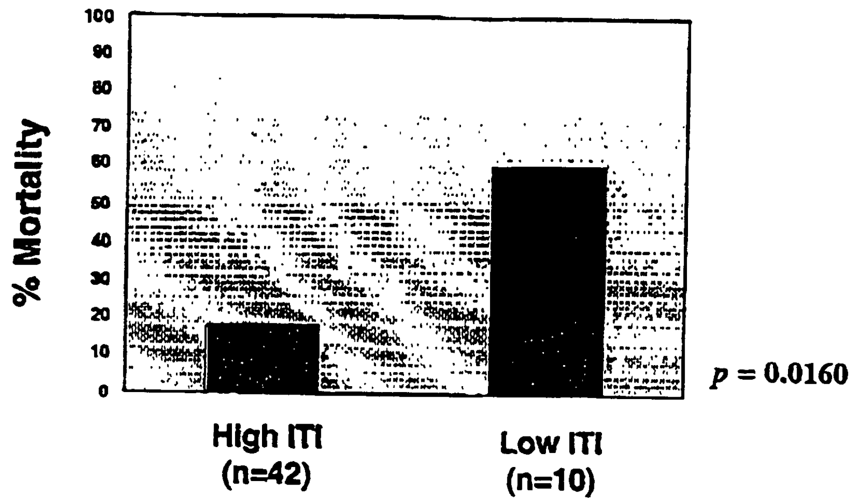
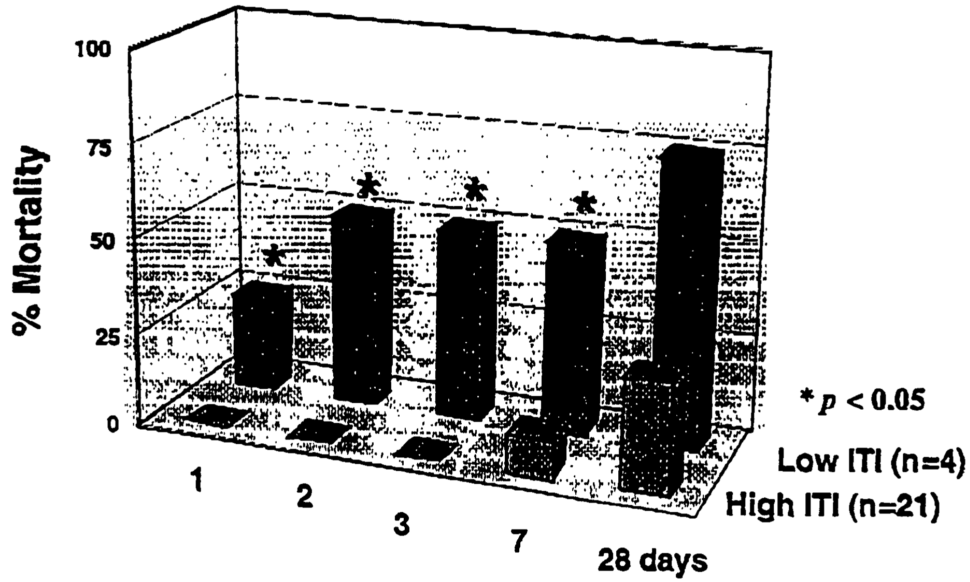


FIG. 4B



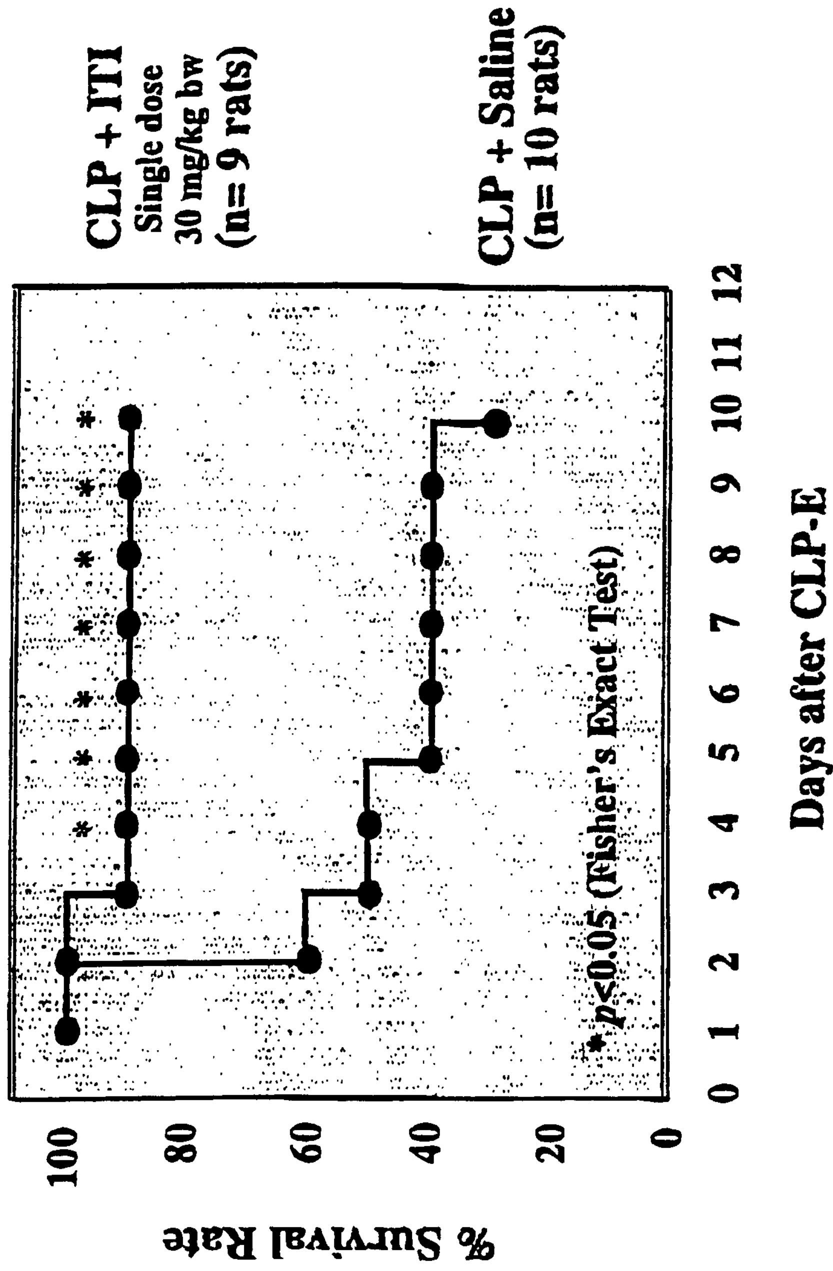


FIG. 5

patsnap

专利名称(译)	间α-胰蛋白酶作为败血症的标志物		
公开(公告)号	EP1295127B1	公开(公告)日	2005-11-09
申请号	EP2001934830	申请日	2001-02-06
申请(专利权)人(译)	罗德岛医院，的寿命合作伙伴		
当前申请(专利权)人(译)	罗德岛医院		
[标]发明人	LIM YOW PIN HIXSON DOUGLAS C		
发明人	LIM, YOW-PIN HIXSON, DOUGLAS, C.		
IPC分类号	G01N33/53 A61K38/55 A61P31/04 C07K16/38 C12Q1/37 G01N33/569 G01N33/573 G01N33/577 G01N33/68 A61K38/00		
CPC分类号	G01N33/573 C12Q1/37		
优先权	09/514450 2000-02-28 US		
其他公开文献	EP1295127A2		
外部链接	Espacenet		

摘要(译)

本发明提供了一种通过使来自哺乳动物的体液与在足以形成ITI-配体复合物并检测该复合物的条件下与α间胰蛋白酶抑制剂（ITI）多肽结合的配体接触来诊断哺乳动物败血症的方法。