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Analysis of the Association between Interleukin-10 Plasma Levels and the Incidence of Single and Multiple Organ Failure following Severe Multiple Trauma

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1 Introduction

1.1 Definition and Epidemiology of Multiple Trauma

ERTEL and TRENTZ defined the term “multiple trauma” as a serious, in various body regions simultaneously arisen, multiple bodily harm. At least one of these injuries, alone or in combination, must be life-threatening.[23] Clinical experience shows that, although those single injuries could be medically controllable and survivable, they overburden in combination the immunological and hemodynamical compensatory mechanisms of the injured organism.

In Germany, trauma is the leading cause of death in individuals up to 40 years of age[70] and 20328 persons died following an accident in the year 2000.[133] Multiple trauma is the fourth most common cause of death, after cancer, cardiovascular and respiratory disease.[133] In developed countries, multiple trauma is mostly caused by traffic accidents.[19, 89, 117] In the period of 1998-2000, 7752 people died annually from traffic accidents. The ratio of persisting disability to death is estimated to be 3. Young adult males form the majority of the multiple trauma population.[19, 89, 114] This means that victims are most often people who would be able to work and whose accident is a heavy burden on society and its social security systems.

1.2 Post-traumatic Complications

Normal homeostasis in human tissues requires precisely balanced interactions between cells and the network of secreted proteins known as the extracellular matrix. These co-operative interactions involve numerous cytokines acting through specific cell-surface receptors. Trauma initiates the acute phase reaction - a complex hormonal and cytokine response - and disturbs this sensitive balance. As a result of better intensive care unit (ICU) management, traumatic events that were previously lethal have been transformed into a chronic, possibly survivable state. This gave rise to a new spectrum of post-traumatic disorders that emerged a few decades ago.[6, 66, 75]

The liver is the most frequently injured intra-abdominal organ.[92, 109] Apart from direct injury to the liver, liver failure is a common complication in trauma patients that dramatically influences outcome and increases mortality.[41] Liver cells, both hepatocytes and Kupffer cells, are sensitive to oxygen deprivation and hypoperfusion.[31] Build-up of bacterial toxins and toxic metabolites within the liver interstitium is a potential hazard during periods of tissue hypoxia. This deterioration of cellular energy metabolism is proposed to be causative for the impairment of hepatic function. Hepatic dysfunction is aggravated by the inflammatory response probably through continuing derangement in hepatic sinusoidal microcirculation.[69] Shock induced microcirculatory failure may thus proceed to hepatic necrosis and contribute to the development of a multiple organ dysfunction syndrome (MODS).[128] Jaundice, coagulopathy, encephalopathy and altered mental status are the hallmarks of acute liver failure.[50, 60]

Severely injured patients may also develop renal dysfunction. This may lead to acute renal failure, a rare but serious complication.[78] Hemorrhagic shock is a common risk factor for the development of post-traumatic renal dysfunction. Other risk factors include the presence of hemoperitoneum, rhabdomyolysis, acute lung injury, a Glasgow Coma Scale (GCS)<10 and pre-existing pathological conditions such as diabetes and hypertension.[120, 127] Shock induced ischaemia/reperfusion (I/R) injury leads to nephrotoxic damage in the post-traumatic patient and is associated with high mortality and morbidity levels.[127] Two thirds of the cases of post-traumatic renal dysfunction develop relatively late i.e. 3 weeks after the initial traumatic event and are secondary to MODS.[78] Mortality rates vary from 20-30 % by oliguric renal failure to 70-90 % by nonoliguric renal failure.[115]

The acute respiratory distress syndrome (ARDS), first described three decades ago, is an important clinical problem in patients with multiple injuries.[2] ARDS may be regarded as an example of an excessive inflammatory response in the lung parenchyma with accumulation of both pro- and anti-inflammatory cytokines in the bronchoalveolar lavage fluid (BALF).[1] This ongoing inflammatory process is associated with severe injury to the epithelial and endothelial barriers[131] and results in the disruption of the alveolar-capillary barrier, severe hypoxemia, leakage of protein-rich fluid into the alveolar air spaces and, ultimately, pulmonary fibrosis.[123, 137] Major risk factors for the development of ARDS are sepsis, gastric

aspiration, massive blood transfusions and multiple trauma (particularly lung contusion and long bone fractures).[32, 94] The final outcome in patients with ARDS is related to the duration and the extent of the host's inflammatory response.[43] Mortality has remained between 50-70% and is mainly related to MODS rather than pulmonary dysfunction.[74, 94, 123]

One of the most feared and most serious complications following severe trauma is MODS. Multiple dysfunction of organs was first described by TILNEY et al. in 1973 as "distal organ failure".[119] The term Multiple Organ Failure (MOF) was introduced by BAUE in 1975.[6] In 1991, the American Society of Chest Physicians and the Society of Critical Care Medicine changed the term "Multiple Organ Failure" to "Multiple Organ Dysfunction Syndrome" to stress the possible reversibility and the dynamic nature of the syndrome.[10] Nowadays, MODS is described as a clinical syndrome in which the development of progressive and potentially reversible physiological dysfunction occurs in 2 or more organs or organ systems. It is characteristically induced by a variety of factors, including sepsis.[108]

The clinical manifestation of MODS varies and consists of a cumulative sequence of single organ failures. Often, the lung is the initial organ to fail, followed by dysfunction of the liver, heart, gut and kidneys.[97] MODS is the leading cause of morbidity and mortality in patients who initially survive multiple trauma.[6, 25, 33] Once MODS reaches an advanced stage, the prognosis is poor, reaching 50-70% mortality.[15]

The pathogenesis of MODS is a complex and interrelated mechanism that remains, despite intensive investigation, elusive. During the early eighties, several clinical and epidemiological studies led investigators to conclude that infection was the prime inciting event. FRY et al. maintained that "MODS is the most common fatal expression of uncontrolled infection".[33]

The last decade of trauma research has demonstrated that a variety of causes other than infection can lead to MODS. In addition, several new hypotheses to explain the initiation and progression of MODS have been proposed. Tissue hypoxia[87] and dysregulated apoptosis[66, 99] could cause endothelial and parenchymal cell injury associated with MODS. SCHOEMACHER et al. proposed the theory that occult oxygen debt and a decompensated state of shock encouraged the development of MODS.[112] Gut barrier failure was

considered to be an important factor in the genesis of MODS by FIDDIAN-GREEN.[27]

The hypothesis that a dysfunctional inflammatory response is the pivotal risk factor for MODS received the most attention. Severe trauma results in the activation of the host's immune defence mechanisms and is followed by a massive release of cytokines and other immune regulators.[64] Indeed, the post-traumatic immune response starts with a systemic and unspecific inflammatory response, referred to as systemic inflammatory response syndrome (SIRS).[10] SIRS is primarily driven by the release of pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF-α)[68, 93], Interleukin-1β (IL-1β)[116], Interleukin-6 (IL-6)[35, 68] and Interleukin-8 (IL-8)[51].

Within this inflammatory response, a fragile balance exists between the potential for tissue repair and the potential for tissue injury. Whereas a mild or moderate release of inflammatory mediators is a normal and beneficial reaction to injury stress, an uncontrolled inflammatory response harms the patient.[38] A variety of factors precipates an exaggerated and potentially auto-destructive systemic hyperinflammation. This process finally leads to generalised capillary damage, increased permeability, interstitial oedema and subsequently to organ dysfunction.[97] Almost all patients developing MODS manifested clinical evidence of an exaggerated SIRS response.[38,100]

An alternative model of the dysregulated inflammatory response that accompanies MODS suggests that the problem is a sustained anti-inflammatory state. It comes along with reduction of the specific, cell-mediated immune response.[24] This immunosuppression, termed the "compensatory anti-inflammatory response syndrome" (CARS), has repeatedly been demonstrated after trauma.[3, 21] CARS is characterised by suppressed T cell proliferation, deficient antigen presentation, macrophage paralysis and increased leukocyte apoptosis.[88] An imbalance between pro- and anti-inflammatory cytokines with a rise of anti-inflammatory cytokines such as Transforming growth factor-β (TGF-β), Interleukin-10 (IL-10) or Interleukin-2 (IL-2) is thought to be of pathogenic importance. Many of the components of CARS are presumed to be caused by the biological effects of IL-10.[9] BONE et al. suggested that MODS could also occur as a result of an inability to eradicate infection in the CARS state.[9]

The extent of the CARS response should depend on the intensity of SIRS i.e. the more serious a survived SIRS, the more serious CARS will develop. Thus, the outcome of the severely injured patient could depend on which pattern of immune response takes place: pro-inflammatory (SIRS), anti-inflammatory (CARS) or a balance between both.[11] The latter is named “mixed anti-inflammatory response syndrome” (MARS).[9]

1.3 Interleukin-10

1.3.1 Interleukin-10: Biochemical Structure and Function

Interleukin-10 is a pleiotrophic cytokine that regulates various functions of hematopoietic cells. This protein, 160 amino acid residues in length, is an acid sensitive, noncovalent homodimere of 2 interpenetrating polypeptide chains and has a molecular weight of 18.5 kDa.[77]

IL-10 is encoded by 5 exons on chromosome 1, spread over approximately 5.1 kb of the genome.[80] The promoter region has various polymorphic sites. Complementary DNA clones reveal that the IL-10 polypeptide shows extensive homology with an open reading frame in the Epstein-Bar virus, suggesting that the virus may have captured the IL-10 gene which allows the virus to interact with and suppress the host’s immune system.[46]

IL-10 was first described in 1989 as a product of type 2 T helper (Th) cells that could inhibit cytokine synthesis of type 1 Th cells (“cytokine synthesis inhibitory factor").[29] It is now known that IL-10 is produced by many other activated immune cell types[77], including B lymphocytes[80], mastcells[80], eosinophils[57], monocytes[14], macrophages[14, 134] and keratinocytes[20]. The IL-10 receptor, whose gene lies on chromosome 11, is expressed in a wide variety of cells, consistent with the response of many cells to IL-10.[80] Cell signalling after the engagement of IL-10 to its receptor mainly includes phosphorylation of Jak1 and Tyk2 and phosphorylation of STAT-3. [28, 132,88]

The principal routine function of IL-10 appears to be the limitation and eventual termination of cell-mediated immune responses. IL-10 exerts potent immunosuppressive activities by inhibiting the specific cell mediated immune

response both directly and indirectly.[57] The indirect inhibition is primarily linked to its downregulatory effect on the surface expression of class II MHC (major histocompatibility complex) molecules on a variety of antigen-presenting cells (APC's)[14], including dendritic cells, macrophages and Langerhans cells. IL-10 acts on co-stimulatory pathways as well by downregulating the surface expression of ICAM I, CD80 and CD86.[125] Reduced expression of these molecules significantly affects the T-cell activating capacity of APC's. IL-10 can also directly affect T cell function. IL-10 reduces IL-2, IFNγ and interleukin-5 (IL-5) production by T helper cells.[29, 71] Additionally, IL-10 may also contribute to the induction and maintenance of T-cell anergy, by downregulating ligand-receptor co-stimulatory interactions between APC's and T-cells.[105]

The bacterial endotoxin lipopolysaccharide (LPS) is a potent inducer of IL-10 secretion by macrophages. Treatment with IL-10 protects mice against lethal doses of LPS.[36, 121] This protective function is due to the cytokine synthesis-inhibiting properties of IL-10 in various cell types. IL-10 inhibits the release of TNF-α, IL-1α, IL-1β, IL-6, and IL-8 by monocytes and macrophages[14, 30, 36], polymorphonuclear neutrophiles and eosinophils.[77] IL-10 does not only inhibit production of these pro-inflammatory cytokines, but also augments expression of their natural antagonists such as interleukin-1 receptor antagonist (IL-1RA) and soluble TNF receptors (sTNFRs).[22, 65]

In addition, IL-10 also inhibits the generation of free oxygen radicals and downregulates the induction of nitric oxide synthase in macrophages, thereby diminishing their microbicidal activity against extracellular pathogens.[8, 57] Activation, proliferation and cytokine production by natural killer cells is inhibited by IL-10.[106] Also, the production of chemokines and of "granulocytes/macrophages-colony-stimulating factors" by activated monocytes and macrophages is inhibited.[7, 76]

In contrast to its powerful immunosuppressive properties, IL-10 can enhance immune activity as well. IL-10 stimulates the proliferation, activation and antibody production of B-lymphocytes, and increases proliferation, chemotaxis and cytolytic activity of CD8+ T cells.[40]

Since IL-10 is a potent anti-inflammatory cytokine, its clinical use has been studied in a large variety of inflammatory and infectious disorders. Alterations in the production of IL-10 have been linked to numerous disease states. IL-10

stimulation of immunoglobulin production by B-cells may play a deleterious role in the pathogenesis of systemic lupus erythematosus (SLE) and disease activity correlates with IL-10 titers in the sera of SLE patients.[45] In patients suffering from psoriasis, induction of IL-10 receptor helps to reduce local inflammation.[72] Systemic administration of IL-10 ameliorates collagen-induced arthritis in mice.[129] High IL-10 production as found in patients with rheumatoid arthritis may be protective against progression of joint destruction.[124] Therefore, IL-10 could possibly be an attractive candidate for therapeutic use and several clinical trials with IL-10 are in progress.

1.3.2 Interleukin-10 and Trauma

The main causes of acute mortality in multiple injured patients are devastating organ injuries (mostly central nervous system injuries) and massive blood loss. If the patient does not die at the site of the accident, the two major problems that follow are direct organ damage and sufficient blood loss to cause hemorrhagic shock resulting in tissue hypoxia.[44] This is reflected in parameters that are indicative of oxygen debt such as blood pressure, mean arterial pressure (MAP), pH and others.[113]

Patients who survive these acute trauma sequelae, are threatened by the reaction of the organism to the trauma. It has been well documented that major trauma results in a rapid increased secretion of pro-inflammatory cytokines such as IL-1, IL-6[104] and TNF-α, known as SIRS.[35, 36, 68, 93, 116] In response, there is a compensatory rise in anti-inflammatory cytokines such as TGF-β[58] and IL-10, thus initiating CARS.[9]

Only when the balance between these two forces is lost, these mediators may become harmful to the organism.[9 ,100] An exaggerated CARS response can result in massive impairment of immunologic reactivity and a paralysation of cell-mediated immunity.

IL-10 is part of this post-traumatic cytokine-network activity. IL-10's cytokine synthesis inhibitory activity influences the patient's immunological condition after trauma and suppresses the patient's immune response to infections. IL-10 could possibly play an important role in the development of post-traumatic complications.

2 Objective and Questions

The main objective of this study was to investigate whether IL-10 plasma levels were associated with outcome after severe trauma and in how far IL-10 could be seen as a marker or mediator of pathophysiological processes. So the following questions were addressed:

1) To what extent was IL-10 measurable in this patient cohort at various time-points?

2) Which pre- and initial clinical parameters influenced IL-10 plasma concentrations significantly?

3) Were IL-10 plasma concentrations associated with outcome after severe multiple trauma?

4) Which time-point of IL-10 determination showed the highest discriminative value for the development of post-traumatic organ failure in a receiver operating characteristic (ROC) analysis?

5) Which pre- and initial clinical parameters influenced the development of MODS significantly?

6) Which factors could be considered as statistically independent predictors for the development of MODS in a multivariate analysis?

3 Materials and Methods

3.1 Study Design

The study was performed in a prospective, single center, non-interventional design at the Unfallkrankenhaus Berlin, a level I trauma center in Berlin, Germany.

The study included consecutive patients, fulfilling the criteria for multiple trauma, according to the definition of multiple trauma by ERTEL and TRENTZ.[23] These criteria were an initial Injury Severity Score (ISS) >15, combined with at least one life-threatening injury and at least one additional severe injury in another body region. Exclusion criteria were: under 16 years of age; a constitutional or acquired immunodeficiency; major organ failure causing trauma, pregnancy and lack of or withdrawal of informed consent.

In the UKB, an expert team of trauma surgeons and anaesthetists assisted by neurosurgeons and other surgical specialists directed the treatment of patients. Diagnosis consisted of digital radiology and included an initial helical computed tomography of head and trunk in all patients.[81] All therapeutic interventions were performed according to general medical standards. No modifications of the treatment regimen were made in study participants.

The responsible ethics committee (Freie Universität Berlin) approved of the study. Informed consent was obtained from the patients or next of kin.

3.2 Assessment Instrument

Patients were assessed online on a 24-hour (h) basis. All data were documented independently of routine patient charts. Documentation was made using bivalent charts for both data inquiry and data recording. These charts assessed different aspects of the patient and their course by predefined items.

Clinical and laboratory parameters were assessed at the following time-points: 30 minutes after admission to the emergency department; at the beginning of the emergency operation; 6h, 12h and 18h after admission to the ICU. Documentation was monitored on a daily basis from day 2 on.

Time-point abbreviations were used in all tables: Thirty minutes denotes within 30 minutes of admission to the emergency department; OP denotes at the beginning of the emergency operation; 6h,12h,18h denote 6,12,18 hours after admission to the ICU.

3.3 Definition of Pre-traumatic Illness

To register pre-existing morbidity, patients were systematically questioned about their pre-admission health status. When such an anamnesis was not possible, the information was obtained from the responsible general practitioner or next of kin. Definitions of pre-traumatic disease were consistent with the international World Health Organisation standards.

3.4 Definition of Clinical Outcome Measures

The primary outcome measure was MODS, occurring in the first 30 days after trauma. Secondary outcome measures were liver failure, renal dysfunction and ARDS. Precise definitions of the outcome measures had been made before the beginning of the study according to the widely used multiple organ dysfunction score by MARSHALL (see Table 1).[67]

- MODS (Status: equal or more than 3 points in at least 2 organ systems, acquired according to the multiple organ dysfunction score by MARSHALL).
- Liver failure (Status: total serum bilirubin >60 μmol/L)
- Renal dysfunction (Status: total plasma creatinine >100 μmol/L)
- ARDS (Status: $P_aO_2/F_iO_2 <150$)

ORGANSYSTEM	PARAMETER	O POINTS	1 POINT	2 POINTS	3 POINTS	4 POINTS
LIVER	Serum-Bilirubin (µmol/L)	≤20	21-60	61-120	121-240	>240
KINDNEY	Serum-Creatinine (µmol/L)	≤100	101-200	201-350	351-500	>500
LUNG	PaO_2/ FiO_2	>300	226-300	151-225	76-150	≤75
CARDIOVASCULAR SYSTEM	Pressure-adjusted heart rate (PAR)	≤10.0	10.1-15.0	15.1-20.0	20.1-30.0	>30.0
HEMATOLOGICAL SYSTEM	Thrombocytes (10^3/µL)	>120	81-120	51-80	21-50	≤20
NEUROLOGICAL SYSTEM	GCS	15	13-14	10-12	7-9	≤6

Table 1: MARSHALL's Multiple Organ Dysfunction Score

In accordance with international agreement, single and multiple organ dysfunction can only be stated when the primary resuscitation period is surpassed.[101, 102] Consequently, only patients who were still alive on day 2 were included into the analysis of morbidity.

Cardiovascular function is calculated with this formula:

$$PAR = \frac{HR * MAP}{CVP}$$

HR denotes heart rate and CVP stands for central venous pressure. Patients could score a maximum of 20 points i.e. a maximal 4 points per organ system (liver, kidney, lung, cardiovascular system and hematological system). No points were attributed to the category “neurological system” since patients in our study cohort were almost always deeply sedated. The worst scores were integrated in the calculations on a daily basis.

3.5 Determination of Laboratory and Clinical Parameters and Values

3.5.1 Base-line Laboratory Values

The laboratory parameters were measured according to standard methods at the laboratory of the UKB (Berliner Betrieb für zentrale gesundheitliche Aufgaben). Blood-gas values (pH, pO_2) were determined directly at the emergency department or at the ICU. Given below is an alphabetic list of the various parameters with the required instruments, the method of determination and the range values.

3.5.1.1 Creatinine

Creatinine determination from serum was made following Jaffé's kinetic method without deproteinising using the Roche Hitachi 912 (Hoffmann-LaRoche, Basle, Switzerland). The Jaffé reaction is based on the observation that at an alkaline pH, creatinine reacts with picrate to form a red-orange adduct. The creatinine-picrate colour reaction results in a change in absorption, which is measured photometricly at a wavelength of 570 nm. The reference wavelength is 505 nm. All analyses were performed with a commercial analytic system (SYS 1 Boehringer Mannheim, Germany).

Normal ranges: Men under 30 years of age: $<$1.3 mg/dL or $<$115 µmol/L

Men over 30 years of age: $<$1.4 mg/dL or $<$124 µmol/L

Women: $<$1.1 mg/dL or $<$97 µmol/L

3.5.1.2 Hematocrit

Hematocrit was determined from Ethylenediamine tetraacetic acid (EDTA)- blood, using the SE-9000 from the Sysmex company (Kobe, Japan). Hematocrit is the proportion, by volume, of the blood that consists of red blood cells. The hematocrit calculation results from the erythrocyte count per mean corpuscular volume.

Normal ranges: men: 40-54%; women: 35-47%

3.5.1.3 Hemoglobin

Hemoglobin was determined from EDTA-blood according to the sodiumlaurylsulphate-hemoglobin method and using the SE 9000 from the Sysmex company (Kobe, Japan). Erythrocytes were haemolysed after sample dilution. Hemoglobin was transformed into SLS-hemoglobin by the reagent sulfolyser. In a spectrophotometric measurement at a wavelength of 555 nm, the not-absorbed quantity of light was in opposite proportion to the hemoglobin concentration.

The light signal that was not absorbed was transformed into an electrical signal by means of a photodetector. Each measurement assessed values from the light-absorption before the sample was induced and sample values that were subsequently subtracted from one another. This result then provided the hemoglobin concentration.

Normal ranges: men: 8.60-11.1 mmol/L; women: 7.40-9.90 mmol/L

3.5.1.4 Leukocytes

Leukocyte concentration was determined from EDTA-blood according to the impedance principle using a SE-9000 analysator (Kobe, Japan). The sample was diluted in a buffered electrolyte solution and was allowed to pan through the orifice of an aperture tube between two electrodes. After incubation of 13 seconds, the erythrocytes are hemolysed and the thrombocytes are shrivelled. Interruption of the current by the non-conducting blood cells alters the electric charge and a pulse is produced. The amplitude of each pulse is in proportion to the volume of the cell and the cell count is determined from the total number of pulses obtained from a measured volume of blood.

Normal ranges: 3.80-9.80 x $10^3/\mu L$

3.5.1.5 Partial Oxygen Pressure (pO_2)

The pO_2 value was determined from arterial full blood using the IL Synthesis 35 of the Instrumentation Laboratory company (Barcelona, Spain). Determination was based on CLARCK's electrodes-principle. The oxygen electrode is polarised by a constant electric voltage of 0.6V and thereby generates current, which is proportional to the partial pressure of oxygen that encounters the electrode surface. This current is the product of the oxygen reduction, which takes place at the cathode of the electrode and is expressed like this:

$$O_2 + 2\ H_2O + 4\ e^- \rightarrow 4\ OH^-$$

Each oxygen molecule binds four electrons. The electrode measured this electron-current.

Normal ranges: 71-104 mmHg or 9.5-13.9 kPa

3.5.1.6 pH

The pH was determined from arterial blood by blood gas analysis using the IL Synthesis 35 of the Instrumentation Laboratory company (Barcelona, Spain). The pH is defined as the negative decadical logarithm of the hydrogenous concentration: $pH = -\log [H^+]$.

The principle of the potentiometry is as follows: if a pH difference exists between two solutions, which are separated from another by a special composition H^+-sensitive glass membrane, a potential is produced across this membrane. This potential difference develops between the reference electrode and the measuring electrode. One solution is by definition held at a constant pH, therefore any change in potential is due to a change in pH of the second solution. In reference to this difference, the hydrogen-ions concentration of the second solution was calculated.

Normal ranges: 7.35-7.45

3.5.1.7 Potassium

Potassium determination was made from serum according to the potassium-selective electrode method and using the Roche Hitachi 912 (Hoffmann-LaRoche, Basle, Switzerland). The principle of this method: a measuring- and a reference-electrode, connected with each other over a leading bridge, measure the potential of a solution. The adjustment of the measuring electrode was chosen in such a way that it responded proportionally to the ion activity of potassium. The reference electrode held a constant voltage. The top of the measuring electrode was made of valinomycinmembrane that is highly selective for potassium (e.g. potassium compared with sodium 5000:1). When brought into contact with a potassium solution, the potassium ions pass through the membrane and alter the potential. The difference between the new surface potential of the measuring electrode and the constant potential of the reference electrode provided the change in voltage. This change is logarithmically proportional to the potassium activity. The calculation of the potassium concentration was dependent on an activity-coefficient.
Normal ranges: 3.5-5.0 mmol/L

3.5.1.8 Sodium

Sodium levels were determined from serum using the Roche Hitachi 912 (Hoffmann-LaRoche, Basle, Switzerland) following the sodium-selective electrode method. The principle of this method is the same as with potassium. The measuring electrode was made of lithium-aliminiumsalicate and had selectivity for sodium to potassium of 300:1.
Normal ranges: 135-145 mmol/L

3.5.1.9 Total Bilirubin

Total bilirubin determination from serum was made with the aid of the full-automatic sample-analysator Roche Hitachi 912 (Hoffmann-LaRoche, Basle, Switzerland), using the DPD-method. In an acidic environment (0.1 mol/L

hydrochloric acid), bilirubin, brought together with 2.5-dichlor-benzoldiazoniumacid, forms azobilirubin. The absorbency was measured quantitatively by 540 to 560 nm. To determine the total bilirubin level, the detergent Triton X-100 released indirect bilirubin. The reaction-mixture of the empty value samples only contained 0.1 mol/L HCl, so no dyeing reaction took place.
Normal ranges: $<$18.8 μmol/L

3.5.1.10 Thrombocytes

The thrombocyte concentration was determined from EDTA-blood following the resistance-measurement principle and using SE-9000 analysator from the Sysmex company (Kobe, Japan). The method of determination is explained in 1.5.1.9.
Normal ranges: 140-440 x 10^3 /μL

3.5.2 Clinical Parameters

3.5.2.1 Circulation Parameters

The parameters heart rate, arterial blood pressure and central venous pressure were determined at the given sampling points in accordance with international standards. From the second day on, the respectively worst values of the day were applied from the intensive medicine documentation. Systolic blood pressure was measured either with a full automatic blood pressure cuff or intra-arterial with the aid of a catheter. MAP was determined according to the following relationship:

MAP = diastolic blood pressure + one third of the difference
between the systolic and diastolic blood pressure

The CVP was measured with a pressure probe (TRUWAVE PX-600F Red, Edwards Lifescience, Irvine, USA). This pressure probe was positioned together with the central venous catheter at the entrance of the right atrium. The SC 9000 electronic transducer (Siemens, Munich, Germany) transformed the measured pressure into an electrical signal.

3.5.2.2 Respiratory Parameters

The respiratory parameters were determined on the defined time-points. From the second day on, the respectively worst score of the day was taken as basis for the calculations. FiO_2 is the oxygen content of the gases inspired by the patient expressed as a fraction. The positive end expiratory pressure (PEEP) was determined manometricly in the expiratory branch of the SC 9000 respirator (Siemens, Munich, Germany).

3.5.2.3 Temperature

Body temperature was measured by means of a trans-urethral probe (Type Modell Curity Thermistor YSI 400, Kendall, Massachusetts, USA) according to the resistance measurement principle using the Siemens SC 9000 (Siemens, Munich, Germany).

3.5.3 Scoring Systems

Several internationally valid scoring systems were used to classify the patients. The Abbreviated Injury Scale (AIS) and the Injury Severity Score (ISS) assess the anatomical impact of the injury. The Acute Physiology and Chronic Health Evaluation Score II (APACHE II) assesses the initial physiological traumatic stress.

3.5.3.1 Score-based Assessment of Anatomical Severity of Injury: Abbreviated Injury Scale and Injury Severity Score

In 1971, the "Association for the Advancement of Automotive Medicine" developed a classification system of traumatic injuries: the Abbreviated Injury Scale.[12] Revisions were made in 1990 and 1998. Each injury is allocated to one of nine body regions and is assigned an AIS score (Table 2). These scores go from one (minor injury) to six (unsurvivable injury).

BODY REGIONS IN THE AIS
Injuries of the head
Injuries of the face
Injuries of the neck
Injuries of the thorax
Injuries of the abdomen
Injuries of the spine
Injuries of the upper extremity
Injuries of the lower extremity
Unspecified injuries

Table 2: Classification of Injuries in the AIS

In 1974, BAKER et al. introduced the Injury Severity Score, a system for grading the severity of an injury.[5] This frequently used score allows an estimation of mortality of severely injured patients. The ISS classifies injuries by body region on a six point ordinal score. The six body regions are head and neck, face, thorax, abdomen, extremities including pelvis, and external injuries. Injuries of the spine are categorised with the corresponding regions.

Body regions in the ISS are not identical to those defined in the AIS. To calculate the ISS, only the highest AIS score in each body region is used. The three most severely injured body regions have their score squared and added together to produce the ISS score. (ISS= $AIS1^2 + AIS2^2 + AIS3^2$). The ISS score takes values from 0 to 75. If an injury is given an AIS score of 6, the ISS score is automatically assigned to 75.

3.5.3.2 Score based Assessment of Physiological Severity of Injury: The Acute Physiology and Chronic Health Evaluation Score II

KNAUS et al. published the APACHE II-score in 1985 (Table 3). This internationally used score quantifies the severity of the illness in intensive care patients.[53] The score takes values for 12 different physiological parameters plus ratings of age and chronic illness (Biopsy proven liver cirrhosis, cardiac insufficiency class IV according to the New York Heart Association (NYHA), severe chronic obstructive respiratory disease, chronic renal insufficiency obligatory of dialysis and immunosuppression). All chronic illnesses get five points. The valuation of age has four categories: younger than 44: 1 point; between 45 and 54: 2 points; between 65 and 74: 3 points; older than 75: 4 points.

PARA-METER	4 POINTS	3 POINTS	2 POINTS	1 POINT	0 POINTS	1 POINT	2 POINTS	3 POINTS	4 POINTS
TEMPERATURE °C	≥41	39-40.9		38.5-38.9	36-38.4	34-35.9	32-33.9	30-31.9	≤ 29.9
MAP mm Hg	≥160	130-159	110-129		70-109		50-69		≤49
HEART RATE /min	≥180	140-179	110-139		70-109		55-69	40-54	≤39
BREATHING RATE /MIN	≥50	35-49		25-34	12-24	10-11	6-9		≤5
$AaDO_2$ ($F_iO_2 \geq 0.5$) P_AO_2 ($F_iO_2 < 0.5$) mm Hg	≥500	350-499	200-349		<200 >70	61-70		55-60	<55
ARTERIAL pH	≥7.7	7.6-7.69		7.5-7.59	7.33-7.49		7.25-7.32	7.15-7.24	<7.15
SERUM-SODIUM mmol/L	≥180	160-179	155-159	150-154	130-149		120-129	111-119	≤110
SERUM-POTASSIUM mmol/L	≥7	6-6.9		5.5-5.9	3.5-5.4	3-3.4	2.5-2.9		<2.5
SERUM-CREATININE mg/dL	≥3.5	2-3.4	1.5-1.9		0.6-1.4		<0.6		
HEMATO-CRIT %	≥60		50-59.9	46-49.9	30-45.9		20-29.9		<20
LEUKOCYTES 1000/mm^3	≥40		20-39.9	15.0-19.9	3-14.9		1-2,9		<1
GLASGOW COMA SCALE	The difference of 15 minus GCS-score is entered								

Table 3: APACHE II

3.6 Sample Handling

One mL of full blood was drawn initially at the emergency department, prior to any needed transfusions and was frozen at –80°C for later analysis.

Withdrawal of EDTA plasma was scheduled at the following time points: 30 minutes after arriving at the emergency department; at the beginning of the first operation; 6h, 12h and 18h after admission to the ICU; on day 2 and on day 5. The samples were immediately placed on ice, transferred to the laboratory and immediately spun at 2500 rpm at 4°C. The plasma samples were instantly shock-frozen at -80°C until the analyses was performed.

3.7 Determination of IL-10 Levels

Serum IL-10 levels were measured using an enzyme linked immunosorbent assay system (ELISA) (DPC Biermann GmbH, Bad Nauheim, Germany).

3.7.1 Principle of the Procedure

The test kit was a solid phase enzyme immunometric assay in the microplate format, designed for the quantitative measurement of human IL-10. The microplate was coated with a first monoclonal antibody specific for IL-10.

Calibrators and patient samples were pipetted into the antibody-coated microplate. During two hours of incubation, present antigens in the sample bound the antibodies on the inner surface of the wells. Non-reactive sample components were removed by a washing step.

Afterwards, a ligand-labelled antibody, directed against another epitope of the IL-10 molecule, was added. During a two-hour incubation, a sandwich complex consisting of the two antibodies and the IL-10 was formed. After a washing step, a horseradish peroxidase-labelled anti-ligand reacted with the sandwich complex during a 30-minute incubation. Unreacted material was then removed by a washing step.

A chromogenic substrate, TMB (3.3'.5.5'-Tertra-Methyl-Benzidine) was added to all wells. During 30 minutes of incubation, the substrate was converted by the fixed enzyme to a blue end product. Enzyme reaction was stopped by dispensing of hydrochloric acid as stop solution (change from blue to yellow). The colour intensity was in direct proportion to the concentration of the IL-10 present in the sample. The optical density of the colour solution was measured with a microplate reader at 450 nm.

3.7.2 Immunoassay Procedure

Six tubes were labelled [G (500 pg/mL), F (250 pg/mL), D (62.5 pg/mL), C (31.3 pg/mL), and B (15.6 pg/mL)] and 0.5 mL of the Calibrator/Sample Diluent was pipetted into all tubes. Half a mL of the reconstituted IL-10 Master Calibrator was pipetted into tube G and mixed thoroughly. Then the 0.5 mL from tube G was transferred to tube F and again mixed thoroughly. This process was repeated successively to complete the 2-fold dilution series. The reconstituted IL-10 Calibrator served as the highest Calibrator H (1000 pg/mL). IL-10 Calibrator/Sample Diluent was used as the zero Calibrator A (0 pg/mL).

For determination of IL-10, 100 µL of calibrators and patient samples was pipetted into the wells and 50 µL of the IL-10 Calibrator/Sample diluent was added to every well. Subsequently, the plate was covered and rotated for 2 hours at room temperature (18-28°C) on a plate mixer (350-400 rpm). Afterwards, the content of the wells was discarded and washed 4 times with 300 µL buffered wash solution.

100 µL of ligand-labelled anti-IL-10 antibody was pipetted into each well. Again, the plate was covered and rotated for 2 hours at room temperature (18-28°C) on a plate mixer (350-400 rpm). The content of all wells was once more discarded and washed 4 times with 300 µL buffered wash solution. As much wash solution as possible was removed by beating the microplate carefully.

100 µL of IL-10 enzyme-labelled anti-ligand was pipetted into each well. The plate was covered and rotated for 30 minutes at room temperature on a plate mixer. The content of all wells was discarded and again washed 4 times with 300 µL buffered wash solution.

Then, 100 µL of TMB substrate solution was added to every well and incubated for 30 minutes at room temperature in the dark. Thereafter, 50 µL of acid stop solution was added to every well. Optical density could then be read at 450 nm, though bi-chromatic measurement with a reference at 600-690 nm was recommended. The developed colour was stable for at least 15 minutes. Optical densities were read during this time.

3.8 Statistical Analysis

If not otherwise mentioned, data are given as Median [Interquartile Range (IQR)]. Comparison for differences between the medians of two groups was performed with the MANN-WHITNEY U test for continuous variables or by the Chi-Square test or FISHER's exact test in case of proportions. FISHER's exact test was used when the number of cases was less than five. The risk factors were calculated according to the below given formula in a univariate analysis as Odds ratios (OR). The OR values were calculated for various cut-off points. A cut-off point is defined as the IL-10 plasma concentration where the sensitivity and specificity values are as equal as possible.

	EVENTFUL OUTCOME	**UNEVENTFUL OUTCOME**
IL-10 LEVELS HIGHER THAN X PG/ML	A	B
IL-10 LEVELS LOWER THAN X PG/ML	C	D

A = Number of patients with IL-10 plasma levels higher than x pg/mL and with an eventful outcome

B = Number of patients with IL-10 plasma levels higher than x pg/mL and with an uneventful outcome

C = Number of patients with IL-10 plasma levels lower than x pg/mL and with an eventful outcome

D = Number of patients with IL-10 plasma levels lower than x pg/mL and with an uneventful outcome

$$\text{Odds Ratio} = \frac{A * D}{B * C}$$

Sensitivity and specificity were calculated (using the abbreviations from the 2 by 2 table above) as follows:

$$\text{Sensitivity} = \frac{A}{A + C}$$

$$\text{Specificity} = \frac{D}{D + B}$$

Correlation analyses were done using SPEARMANN's rang correlation coefficient (r_s). According to general statistical usage, we defined the interpretations of the correlations according to Table 4.

R_S VALUE	INTERPRETATION
Under 0,2	Very low correlation
Under 0,5	Low correlation
Under 0,7	Median correlation
Under 0,9	High correlation
Over 0,9	Very High correlation

Table 4: Interpretations of SPEARMANN's Correlation Coefficient

In order to determine the association between the IL-10 plasma levels and outcome parameters, a ROC analysis was performed. The ROC-curve is constructed by plotting the true-positive fraction (sensitivity) against the false-negative fraction (1-specificity). The area under the curve (AUC) ranges from 0.50 for a non-informative test, to 1.0, indicating perfect discrimination of a test. The test has to be done under a hypothesis whether higher or lower values of the parameter represent a more positive result.

Parameters with a P value <0.1 in the univariate analysis were entered into a multivariate analysis with logistic regression (using forward approach analysis and according to the likelihood ratio criterium). The HOSMER-LEMESHOW-Goodness-of Fit Test was performed to compare the observed and the predicted outcome of the patient cohort. A parameter that was allowed into the final model and had a P<0.05, could be considered as an independent factor associated with the development of MODS, over and above the other parameters that were included in the equation.[96]

All tests of statistical significance were two-sided. A P value of less than 0.05 was considered indicative of statistical significance. Statistical analysis was performed using the SPSS for Windows software version 11.5 (SPSS, Munich, Germany).

4 Results

4.1 Study Population

4.1.1 Patient Characteristics

Between 01 May 1999 and 30 June 2002, 137 patients with severe multiple trauma were admitted to the emergency department of the UKB. Of those 137 patients, 19 (13.9%) died within 24 hours, leaving 118 patients who were enrolled in this study. The base-line characteristics of this patient cohort are shown in Table 5. Continuous variables are presented as median (IQR).

CHARACTERISTICS	ALL PATIENTS (N=118)
MEDIAN AGE (IQR) (YEARS)	30 (19-47)
SEX – NO. OF PATIENTS (%)	
Female	29 (24.6 %)
Male	89 (75.4%)
CAUSE OF INJURY – NO. OF PATIENTS (%)	
Traffic Accident	102 (86.4%)
Attempted Suicide	8 (6.8%)
Other	8 (6.8%)
ANATOMICAL INJURY SEVERITY	
Median ISS (IQR)	34 (27-34)
Severe Head Injury	77 (65.3%)
Severe Thorax Injury	102 (86.4%)
Severe Abdominal Injury	48 (40.7%)
Severe Extremity Injury	67 (56.8%)
PHYSIOLOGICAL INJURY SEVERITY	
Median APACHE II	20 (16-24)
Median Arterial pH	7.34 (7.25-7.39)
Median Hemoglobin (mmol/L)	6.14 (5.09-7.10)
Median Shock Index	0.80 (0.68-0.98)
Median Systolic Blood Pressure (mmHg)	120 (100-140)
Median MAP (mmHg)	86 (77-94)
Median Leukocytes (10^3/µL)	10.2 (84-14.0)
Median Red Blood Cell Transfusion in the first 24 h (mL)	3035 (990-4620)
Median Temperature (°C)	35.5 (34.5-36.1)

Table 5: Base-line Characteristics

The majority of patients was young [median (IQR) age 30 (19-47) years] and male (75.4%). Traffic accidents were the dominating cause of injury in the study population (86.4%), mainly involving motor vehicles. The general co-morbidity before trauma was low. Four patients (3.9%) suffered from cardiac insufficiency (≥ NYHA 1) and chronic obstructive lung disease. Three patients disease had been diagnosed with chronic liver (2.5%) and five patients with neurological disorders (4.2%). Arterial hypertension was the most common pre-traumatic disorder with 19 patients (16.1%) affected. None of the patients in the study population suffered from pre-traumatic renal dysfunction or cancer.

The median Injury Severity Score was 34 (IQR: 27-34, mean: 36.2; standard deviation: 12.1). The site of the major life-threatening injuries is given, but it is important to note that some patients had severe injuries at more than one site. The median APACHE II score amounted to 20 (IQR: 16-24, mean: 20.1; standard deviation: 4.8). The average number of operations per patient amounted to 3 (2-6). Patients had to stay at the ICU for a period of 14 (7-23) days on the average.

4.1.2 Outcome Measures in the Study Population

Table 6 reveals how many patients died within 24 hours and how many patients actually had an eventful outcome i.e. how many patients developed post-traumatic complications. The outcome measures in this study were MODS, liver failure, renal dysfunction and ARDS and were determined according to MARSHALL's MODS score (Table 1).

OUTCOME MEASURE	NO. OF PATIENTS	DAYS UNTIL OUTCOME
	n (%)	Median (IQR) days
Death after 24 h	14 (11.9)	5.1 (2.6-10.1)
MODS	26 (22.0)	3 (2-5.3)
Hepatic Failure	26 (22.0)	7 (4-9)
Renal Dysfunction	39 (33.1)	2 (2-2)
ARDS	21 (17.8)	4 (2-7.5)

Table 6: Outcome Measures in the Study Population

4.2 Description of IL-10 Plasma Levels

Table 7 shows the median (IQR) IL-10 plasma concentrations for all 118 patients at the various time-points. The results are presented in pg/mL.

N=118	30 MIN	OP	6H	12H	18H	DAY 2	DAY 5
IL-10 LEVELS	148 (78-439)	102 (43-259)	88 (50-244)	90 (43-159)	52 (37-83)	34 (20-53)	25 (14-39)

Table 7: IL-10 Plasma Concentrations at Single Time-points: Median (IQR) in pg/mL

IL-10 plasma concentrations were measurable throughout the whole observation period. The highest concentrations were measured 30 minutes after arrival at the emergency department and decreased gradually afterwards.

Table 8 shows the correlation of the IL-10 plasma levels to each other. Correlations are expressed with the correlation coefficient of SPEARMANN (r_s).
P values were computed using the MANN-WHITNEY U test. Significant associations are marked in grey.

		OP	6H	12H	18H	DAY 2	DAY 5
30 MIN	**r_s**	**0.51**	**0.27**	**0.43**	**0.27**	**0.19**	**0.09**
	P value	<0.0001	0.03	<0.0001	0.02	0.13	0.52
OP	**r_s**		**0.53**	**0.53**	**0.34**	**0.27**	**0.38**
	P value		<0.0001	<0.0001	0.002	0.02	0.003
6H	**r_s**			**0.62**	**0.46**	**0.37**	**0.23**
	P value			<0.0001	<0.0001	0.001	0.08
12H	**r_s**				**0.61**	**0.35**	**0.31**
	P value				<0.0001	0.001	0.02
18H	**r_s**					**0.43**	**0.35**
	P value					<0.0001	0.007
DAY 2	**r_s**						**0.42**
	P value						<0.0001

Table 8: Correlation of IL-10 Plasma Concentrations to Each Other

The general tendency was that IL-10 plasma levels at a certain time-point significantly correlated with the levels of adjacent time-points. The strength of the correlation diminished in course of time.

4.3 Correlation of Pre- and Initial Clinical Parameters with IL-10 Plasma Levels.

Table 9 and Table 10 show the correlation of pre- and initial clinical parameters with IL-10 plasma levels. All clinical values were determined within two hours after arrival in the emergency department. Severe injury means equal or more than 3 points according to the AIS.

The correlations are expressed with the correlation coefficient of SPEARMANN (r_s). The interpretations were made according to Table 4. P values were computed using the MANN-WHITNEY U test. Significant associations are marked in grey.

In Table 9, the correlation of the pre- and initial parameters with early IL-10 plasma levels is given. Early denotes the time span from arrival at the emergency department until 12 hours after admission to the ICU.

In Table 10, the correlation of initial parameters with late IL-10 plasma levels is shown. Late denotes the time span between 18 hours after admission to the ICU and hospital day 5.

PARAMETER	30 MIN IL-10 PLASMA LEVELS		OP IL-10 PLASMA LEVELS		IL-10 PLASMA LEVELS AT 6H		IL-10 PLASMA LEVELS AT 12H	
	R_S	P	R_S	P	R_S	P	R_S	P
AGE (YRS)	-0.07	0.51	0.05	0.64	-0.003	0.98	-0.01	0.97
FEMALE PATIENTS	0.02	0.84	0.10	0.34	0.14	0.18	0.06	0.52
ANATOMICAL INJURY SEVERITY								
ISS	0.07	0.52	0.10	0.36	0.09	0.36	0.15	0.14
Severe Head Injury	-0.06	0.62	-0.01	0.96	0.05	0.64	0.02	0.85
Severe Thorax Injury	0.20	0.07	0.19	0.07	0.23	0.02	0.26	0.01
Severe Abdominal Injury	0.01	0.91	-0.23	0.03	-0.30	0.003	-0.23	0.02
Severe Extremity Injury	0.009	0.93	0.11	0.32	-0.91	0.38	-0.167	0.11
PHYSIOLOGICAL INJURY SEVERITY								
APACHE II	0.26	0.02	0.29	0.006	0.23	0.02	0.38	<0.0001
Arterial pH	-0.26	0.02	-0.34	0.001	-0.33	0.001	-0.26	0.01
Hemoglobin (mmol/L)	-0.15	0.17	-0.24	0.03	-0.40	<0.0001	-0.42	<0.0001
Shock Index	0.22	0.05	0.32	0.002	0.41	<0.0001	0.45	<0.0001
Systolic Blood Pressure (mmHg)	-0.15	0.17	-0.34	0.001	-0.30	0.004	-0.43	<0.0001
MAP (mmHg)	-0.10	0.35	-0.25	0.02	-0.41	<0.0001	-0.46	<0.0001
Leukocytes (10^3/L)	-0.04	0.75	-0.07	0.52	-0.21	0.04	-0.19	0.06
Red Blood Cell Transfusion (mL)	0.21	0.05	0.35	0.001	0.54	<0.0001	0.54	<0.0001
Temperature (°C)	-0.28	0.04	-0.09	0.48	-0.26	0.04	-0.32	0.02

Table 9: Correlation of Pre- and Initial Clinical Parameters with Early IL-10 Plasma Concentrations

PARAMETER	IL-10 PLASMA LEVELS AT 18H		IL-10 PLASMA LEVELS ON DAY 2		IL-10 PLASMA LEVELS ON DAY 5	
	R_S	P	R_S	P	R_S	P
AGE (YRS)	-0.02	0.88	0.13	0.19	0.14	0.26
FEMALE PATIENTS	0.02	0.83	0.03	0.75	-0.03	0.79
ANATOMICAL INJURY SEVERITY						
ISS	0.09	0.39	0.03	0.76	0.12	0.32
Severe Head Injury	0.05	0.61	0.11	0.26	-0.02	0.87
Severe Thorax Injury	0.20	0.04	0.32	0.002	0.08	0.49
Severe Abdominal Injury	-0.14	0.18	-0.19	0.06	-0.14	0.23
Severe Extremity Injury	-0.13	0.22	0.05	0.64	-0.05	0.64
PHYSIOLOGICAL INJURY SEVERITY						
APACHE II	0.26	0.01	0.11	0.31	0.30	0.009
Arterial pH	-0.35	0.001	-0.09	0.39	-0.11	0.56
Hemoglobin (mmol/L)	-0.33	0.001	-0.15	0.13	-0.26	0.02
Shock Index	0.34	0.001	0.17	0.08	0.14	0.25
Systolic Blood Pressure (mmHg)	-0.29	0.004	-0.10	0.32	-0.25	0.04
MAP (mmHg)	-0.25	0.02	-0.17	0.11	-0.24	0.04
Leukocytes (10^3/L)	-0.14	0.17	-0.13	0.19	-0.24	0.05
Red Blood Cell Transfusion (mL)	0.31	0.003	0.29	0.004	0.22	0.06
Temperature (°C)	-0.12	0.39	0.05	0.71	-0.19	0.20

Table 10: Correlation of Pre- and Initial Clinical Parameters with Late IL-10 Plasma Concentrations

Age, sex and ISS score did not correlate significantly with IL-10 plasma levels. Also there was no correlation with the site of injury, except for a low negative correlation of abdominal injury with IL-10 concentration at time-points OP, 6h and 12h respectively. Severe thorax injuries positively correlated with IL-10 plasma concentration from time-point 6h on until and including day 2.

A positive correlation between IL-10 plasma levels and the APACHE II score could be ascertained throughout the whole observation period, except on day 2. There was a striking correlation between parameters indicative of oxygen debt and plasma IL-10 levels. Transfusion requirements and the shock index were significantly, positively correlated with IL-10 levels from the emergency operation on until and including day 2 and time-point 18h respectively. A low arterial pH value was attended with high IL-10 concentrations from time-point 30 min on until and including time-point 18h. Also, IL-10 concentrations were significantly lower in patients with a high systolic blood pressure and a high MAP from the time of the emergency operation on until and including 18 hours after admission to the ICU. Hemoglobin showed a negative correlation with IL-10 concentration from the OP time-point until and including 18 hours after admission to the ICU and on day 5. Markers of inflammation and infection, such as leukocyte concentration and body temperature showed a low, negative correlation with IL-10 plasma levels only at few early time-points.

4.4 IL-10 Plasma Concentration and Single Organ Failure

4.4.1 IL-10 Plasma Levels and Liver Failure

4.4.1.1 IL-10 Plasma Levels and Liver Failure: MANN-WHITNEY U Test

Table 11 shows the association between IL-10 plasma levels and liver failure at single time-points. P values were computed using the MANN-WHITNEY U test. Significant associations are marked in grey. Continuous variables are presented as median (IQR) in pg/mL.

Outcome is presented in a bimodal way i.e. per outcome measure, the patients are divided into a group with and a group without liver failure.

TIMEPOINT OF IL-10 MEASUREMENT	LIVER FAILURE		
	YES N=26	NO N=92	P
30 MIN	294 (81-685)	181 (77-346)	0.30
OP	292 (91-513)	92 (41-158)	0.004
6H	241 (86-431)	75 (47-168)	0.005
12H	118 (90-167)	80 (35-142)	0.03
18H	78 (55-138)	48 (32-71)	0.002
DAY 2	44 (30-60)	29 (19-50)	0.03
DAY 5	34 (16-51)	24 (13-35)	0.08

Table 11: IL-10 Plasma Concentrations and Liver Failure at Single Time-points

From the time of the emergency operation on, patients who developed liver failure showed significantly elevated IL-10 plasma levels over patients who did not until and including day 2. Median IL-10 plasma levels decreased gradually throughout the whole observation period in both groups.

4.4.1.2 IL-10 Plasma Levels and Liver Failure: ROC Analysis

The relationship between IL-10 plasma levels and liver failure in a ROC analysis is shown in Table 12. AUC denotes area under the curve and 95% CI stands for 95% confidence interval. Significant results are marked in grey. Also presented in Table 12 are the cut-off points i.e. the IL-10 plasma concentration corresponding with the highest accuracy (minimal false negative and false positive results).

TIMEPOINT OF IL-10 MEASUREMENT	AUC (95% CI)	CUT-OFF PG/ML	SENSITIVITY (95% CI)	SPECIFICITY (95% CI)
30 MIN	**0.58** (0.41-0.75)	>379	50.0 (26.1-73.9)	78.5 (66.5-87.7)
OP	**0.72** (0.58-0.87)	>157	72.2 (46.5- 90.2)	75.7 (64.0- 85.2)
6H	**0.70** (0.57-0.83)	>209	61.9 (38.5- 81.8)	84.9 (74.6- 92.2)
12H	**0.66** (0.54-0.78)	>87	80.0 (56.3- 94.1)	57.3 (45.4- 68.7)
18H	**0.73** (0.61-0.85)	>52	83.3 (58.6- 96.2)	61.3 (49.4- 72.4)
DAY 2	**0.65** (0.53-0.77)	>30	81.8 (59.7- 94.7)	53.4 (41.4- 65.2)
DAY 5	**0.63** (0.49-0.78)	>32	57.9 (33.5- 79.7)	71.7 (57.7- 83.2)

Table 12: IL-10 and Liver Failure: Receiver Operating Characteristic Analysis

The AUC value was significant from the time of the emergency operation on and remained significant throughout the whole observation period.

In Table 13, the OR value for the cut-off point at time-point OP was calculated. Time-point 18h provided a better –with very little distinction- AUC value. It was however more meaningful to calculate OR at a time-point as early as possible in light of predicative validity. 95% CI stands for 95% confidence interval.

CUT-OFF (PG/ML)	OR	LOWER 95% CI	HIGHER 95% CI	P (CHI SQUARE)
>157	8.1	2.5	26.0	<0.0001

Table 13: Odds Ratio for the Cut-off Point at Time-point OP

4.4.2 IL-10 Plasma Levels and Renal Dysfunction

4.4.2.1 IL-10 Plasma Levels and Renal Dysfunction: MANN-WHITNEY U Test

Table 14 shows the association between IL-10 plasma levels and renal dysfunction at single time-points. P values were computed using the MANN-WHITNEY U test. Significant associations are marked in grey. Continuous variables are presented as Median (IQR) in pg/mL.

Outcome is presented in a bimodal way i.e. per outcome measure, the patients are divided into 2 groups, one with and one without renal dysfunction.

TIMEPOINT OF IL-10 MEASUREMENT	**RENAL DYSFUNCTION**		
	YES N=39	**NO N=79**	**P**
30 MIN	204 (84-527)	168 (76-413)	0.60
OP	159 (85-352)	79 (38-186)	0.005
6H	165 (75-324)	72 (45-162)	0.008
12H	144 (90-286)	66 (32-109)	<0.0001
18H	60 (43-191)	49 (32-72)	0.03
DAY 2	42 (29-110)	29 (18-44)	0.006
DAY 5	28 (18-52)	23 (12-37)	0.12

Table 14: IL-10 Plasma Concentrations and Renal Dysfunction at Single Time-points

A significant elevation in IL-10 plasma levels in patients who developed renal dysfunction over patients who did not could be observed from time-point OP on until and including day 2.

4.4.2.2 IL-10 Plasma Levels and Renal Dysfunction: ROC Analysis

Table 15 shows the relationship between IL-10 plasma levels and renal dysfunction according to a ROC analysis. AUC denotes area under the curve and 95% CI stands for 95% confidence interval. Significant results are marked in grey.

Also presented in Table 15 are the cut-off points i.e. the IL-10 plasma concentration corresponding with the highest accuracy (minimal false negative and false positive results).

TIMEPOINT OF IL-10 MEASUREMENT	AUC (95% CI)	CUT-OFF PG/ML	SENSITIVITY (95% CI)	SPECIFICITY (95% CI)
30 MIN	**0.54** (0.41-0.67)	>35	100.0 (86.7-100.0)	17.5 (8.8-29.9)
OP	**0.69** (0.57-0.81)	>113	69.2 (46.2- 85.6)	66.1 (53.0- 77.7)
6H	**0.66** (0.55-0.77)	>74	80.0 (61.4- 92.2)	53.1 (40.2- 65.7)
12H	**0.78** (0.68-0.88)	>85	83.3 (65.3- 94.3)	63.1 (50.2- 74.7)
18H	**0.64** (0.52-0.76)	>199	24.1 (10.3- 43.5)	100.0 (94.3- 100.0)
DAY 2	**0.67** (0.55-0.79)	>53	46.9 (29.1- 65.2)	87.3 (76.5- 94.3)
DAY 5	**0.61** (0.47-0.74)	>20	75.9 (56.5- 89.7)	48.8 (33.3- 64.5)

Table 15: IL-10 and Renal Dysfunction: Receiver Operating Characteristic Analysis

The AUC value was significant from the time of the emergency operation on and remained significant until and including day 2.

Table 16 shows the odds ratio value, calculated for the cut-off point at time-point 12h (highest discriminative measurement). 95% CI stands for 95% confidence interval.

CUT-OFF (PG/ML)	OR	LOWER 95% CI	HIGHER 95% CI	P (CHI SQUARE)
>85	8.0	2.7	23.6	<0.0001

Table 16: Odds Ratio for the Cut-off Point at Time-point 12h

4.4.3 IL-10 Plasma Levels and ARDS

4.4.3.1 IL-10 Plasma Levels and ARDS: MANN-WHITNEY U Test

Table 17 shows the association between IL-10 plasma levels and ARDS at single time-points. P values were computed using the MANN-WHITNEY U test. Significant associations are marked in grey. Continuous variables are presented as Median (IQR) in pg/mL.

Outcome is presented in a bimodal way i.e. per outcome measure, the patients are divided into 2 groups, one with and one without ARDS.

TIMEPOINT OF IL-10 MEASUREMENT	ARDS		
	YES N=21	NO N=97	P
30 MIN	504 (168-806)	156 (75-346)	0.02
OP	95 (67-335)	105 (42-250)	0.41
6H	125 (64-268)	86 (49-232)	0.38
12H	127 (68-357)	84 (39-146)	0.06
18H	53 (37-97)	51 (36-80)	0.97
DAY 2	40 (29-64)	30 (19-49)	0.09
DAY 5	24 (13-41)	25 (14-39)	0.85

Table 17: IL-10 Plasma Concentrations and ARDS at Single Time-points

A significant difference in IL-10 plasma concentrations between patients who developed ARDS and patients who did not could only be observed at time-point 30 minutes.

4.4.3.2 IL-10 Plasma Levels and ARDS: ROC Analysis

Table 18 shows the relationship between IL-10 plasma levels and ARDS according to a ROC analysis. AUC denotes area under the curve and 95% CI stands for 95% confidence interval. Significant results are marked in grey.

Also presented in Table 18 are the cut-off points i.e. the IL-10 plasma concentration corresponding with the highest accuracy (minimal false negative and false positive results).

TIMEPOINT OF IL-10 MEASUREMENT	AUC (95% CI)	CUT-OFF PG/ML	SENSITIVITY (95% CI)	SPECIFICITY (95% CI)
30 MIN	**0.69** (0.54-0.85)	>611	46.7 (21.3-73.4)	91.2 (81.8- 96.7)
OP	**0.57** (0.41-0.73)	>53	84.6 (54.5- 97.6)	36.0 (25.2- 47.9)
6H	**0.57** (0.42-0.71)	>124	56.2 (29.9- 80.2)	64.1 (52.4- 74.7)
12H	**0.66** (0.50-0.82)	>121	61.5 (31.6- 86.0)	69.5 (58.4- 79.2)
18H	**0.50** (0.34-0.65)	>27	93.3 (68.0- 98.9)	21.8 (13.2- 32.6)
DAY 2	**0.62** (0.49-0.76)	>30	75.0 (50.9- 91.2)	50.7 (38.9- 62.4)
DAY 5	**0.49** (0.33-0.64)	>54	94.1 (71.2-99.0)	18.2 (9.1- 30.9)

Table 18: IL-10 and ARDS: Receiver Operating Characteristic Analysis

A statistically significant AUC value could only be established at time-point 30 minutes. A value of 0.69 indicates that this association is mild to moderate.

Table 19 shows the OR value, calculated for the cut-off point at time-point 30 minutes. 95% CI stands for 95% confidence interval. The OR value is not significant.

CUT-OFF (PG/ML)	OR	LOWER 95% CI	HIGHER 95% CI	P (CHI SQUARE)
>611	9.0	2.4	33.7	0.001

Table 19: Odds Ratio for the Cut-off Point at Time-point 30 Minutes

4.5 IL-10 Plasma Levels and Multiple Organ Dysfunction Syndrome

4.5.1 IL-10 Plasma Levels and MODS: MANN-WHITNEY U Test

Table 20 shows the association between MODS and IL-10 plasma levels at single time-points. Continuous variables are presented as median (IQR) in pg/mL. P values were computed using the MANN-WHITNEY U test. Significant associations are marked in grey.

Outcome is presented in a bimodal way i.e. per outcome measure, the patients are divided into 2 groups, one with and one without MODS.

TIMEPOINT OF IL-10 MEASUREMENT	MODS		
	YES N=26	NO N=92	P
30 MIN	195 (76-806)	183 (80-371)	0.29
OP	323 (111-541)	93 (41-190)	0.004
6H	236 (155-536)	72 (45-131)	<0.0001
12H	127 (87-440)	80 (35-135)	0.004
18H	78 (46-257)	49 (32-71)	0.005
DAY 2	52 (30-117)	29 (18-44)	0.001
DAY 5	33 (17-53)	24 (13-36)	0.07

Table 20: IL-10 Plasma Concentrations and MODS at Single Time-points

From the time of the emergency operation on, the IL-10 plasma concentrations in patients who developed MODS were significantly elevated over the IL-10 concentrations in patients who recovered without MODS and this difference remained significant until and including day 2. The difference between both groups was most impressive 6 hours after admission to the ICU with median IL-10 plasma levels of 236 (155-536) pg/mL in the +MODS group.

4.5.2 IL-10 Plasma Levels and MODS: ROC Analysis

Table 21 shows the relationship between IL-10 plasma levels and MODS according to a ROC analysis. AUC denotes area under the curve and 95% CI stands for 95% confidence interval. Significant results are marked in grey.

Also presented in Table 21 are the cut-off points i.e. the IL-10 plasma concentration corresponding with the highest accuracy (minimal false negative and false positive results).

TIMEPOINT OF IL-10 MEASUREMENT	AUC (95% CI)	CUT-OFF PG/ML	SENSITIVITY (95% CI)	SPECIFICITY (95% CI)
30 MIN	**0.58** (0.41-0.74)	>611	36.8 (16.4- 61.6)	90.6 (80.7-96.5)
OP	**0.75** (0.62-0.81)	>278	61.5 (31.6- 86.0)	84.0 (73.7- 91.4)
6H	**0.78** (0.64-0.86)	>124	80.8 (60.6- 93.4)	69.2 (58.7- 78.5)
12H	**0.71** (0.58-0.84)	>79	85.7 (63.6- 96.8)	47.9 (36.1- 60.0)
18H	**0.70** (0.57-0.84)	>78	52.4 (29.8- 74.3)	81.9 (71.1- 90.0)
DAY 2	**0.72** (0.61-0.84)	>52	54.2 (32.8- 74.4)	81.4 (70.3- 89.7)
DAY 5	**0.64** (0.50-0.78)	>28	60.0 (36.1- 80.8)	67.3 (52.9- 79.7)

Table 21: IL-10 and MODS: Receiver Operating Characteristic Analysis

The AUC value was significant from the time of the emergency operation on and remained significant until and including day 2. The time-point with the highest discriminative value could be observed at 6h (AUC=0.78). The AUC value gradually declined towards day 5.

Table 22 shows the odds ratio value, calculated for the cut-off point at time-point 6h (greatest AUC value). 95% CI stands for 95% confidence interval.

CUT-OFF (PG/ML)	OR	LOWER 95% CI	HIGHER 95% CI	P (CHI SQUARE)
>124	9.6	3.0	31.3	<0.0001

Table 22: Odds Ratio for the Cut-off Point at Time-point 6h

4.5.3 Clinical Parameters and MODS: Univariate and Multivariate Analysis

Table 23 shows the results of an univariate and a multivariate analysis of pre- and initial clinical parameters with the development of MODS. In this study, liver failure, renal dysfunction and ARDS were defined as secondary outcome measures. Therefore, these outcome measures were not included in the analyses in Table 23. The relevance of IL-10 plasma levels in the multivariate analysis was tested with 2 concentrations: the cut-off IL-10 plasma concentration with the highest AUC value in the ROC analysis (Table 21) and the median IL-10 plasma concentration (Table 7). The IL-10 plasma concentrations were ascertained as significant parameters in both multivariate analyses.

All metric parameters were dichotomised around their median value and tested in a univariate analysis for significant associations. Furthermore, the cut-off IL-10 plasma concentration with the highest AUC value in the ROC analysis of the association between IL-10 plasma levels and MODS (Table 21) was included. The univariate analysis was performed using two by two tables and Chi-Square test. Parameters with a P value <0.1 in the univariate analysis were entered into a multiple logistic regression analysis (using forward approach analysis and the likelihood ratio criterium). Three parameters were revealed as significant: IL-10 plasma levels at 6h >124 pg/mL; severe head injury and an arterial pH value <7.34. Based on these 3 parameters, it was possible to predict 81.3% of the patients correctly.

A HOSMER-LEMESHOW-Goodness-of-Fit Test was performed to compare the observed and the predicted outcome of the patient cohort. This test led to the following results: 2.1 (df:6). The P value amounted to 0.985, suggesting non-significant difference between the observed and the predicted patient groups.

A parameter that was allowed into the final model and had a P<0.05, could be considered as an independent factor associated with the development of MODS, over and above the other parameters that were included in the equation.[96] 95% CI stands for 95% Confidence Interval. Significant results (P<0.05) are marked in grey. All clinical values were determined within 2 hours after arrival at

the emergency room. Severe injury means equal or more than 3 points according to the AIS. One package of red blood cell concentrates contained 330 mL.

PARAMETER	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS	
	ODDS RATIO (95% CI)	P	ODDS RATIO (95% CI)	P
IL-10 AT 6H >124 PG/ML	9.6 (3.0-31.3)	<0.0001*	11.6 (3.1-42.9)	<0.0001
AGE >30	2.2 (0.9-5.6)	0.076*		0.071
MALE PATIENTS	2.1 (0.6-6.5)	0.218		
ANATOMICAL INJURY SEVERITY				
ISS >34	1.5 (0.6-3.6)	0.349		
Severe Head Injury	0.3 (0.1-0.7)	0.005*	0.2 (0.1-0.7)	0.009
Severe Thorax Injury	1.3 (0.4-3.5)	0.733		
Severe Abdominal Injury	3.7 (1.5-9.3)	0.004*		0.065
Severe Extremity Injury	0.7 (0.3-1.7)	0.429		
PHYSIOLOGICAL INJURY SEVERITY				
APACHE II >20	4.5 (1.6-12.3)	0.002*		0.104
Shock Index >0.8	3.2 (1.3-8.1)	0.012*		0.137
Arterial pH <7.34	3.4 (1.3-8.8)	0.015*	4.4 (1.3-15.5)	0.020
>9 Packages of Erythrocyte Concentrates in the 1st 24h	4.2 (1.5-12.1)	0.005*		0.277
Constant term			0.2	0.146
* Entered into the multivariate analysis				

Table 23: Univariate and Multivariate Analysis of Pre- and Initial Clinical Parameters and IL-10 Plasma Concentration >124 pg/mL with the Development of MODS

The univariate analysis revealed that age and sex were not significantly related with the development of MODS. Parameters indicative of anatomical injury severity did not show a significant association with the development of MODS, except for severe injury localised at the head or abdomen. The APACHE II score was associated with the development of MODS. Parameters indicative of oxygen debt (pH, transfusion requirements and shock index) were all significantly associated with the development of MODS.

All significant parameters mentioned above were entered into the multivariate analysis. Dichotomised IL-10 plasma concentrations at time-point 6h (>124 pg/mL), severe head injury and a dichotomised arterial pH value (<7.34) turned out to be the only 3 significant predictors for the development of MODS. Of these 3 parameters, IL-10 plasma levels >124 pg/mL had the highest odds ratio value of 11.6.

In the second multivariate analysis, the dichotomised median IL-10 plasma concentration (>88 pg/mL) at 6h (Table 7) was entered. All other median values remained identical to those in Table 23. This analysis also provided the parameters IL-10 plasma concentration, severe head injury and a pH value <7.34 as independent predictors of the development of MODS. The significant results are given in Table 24. A HOSMER-LEMESHOW-Goodness-of-Fit Test was performed with following results: 2.1 (df:6) and a P value of 0.909.

PARAMETER	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS	
	ODDS RATIO (95% CI)	P	ODDS RATIO (95% CI)	P
IL-10 AT 6H >88 PG/ML	9.0 (2.4-32.9)	<0.0001*	12.4 (2.9-52.1)	0.001
Severe Head Injury	0.3 (0.1-0.7)	0.005*	0.2 (0.1-0.6)	0.006
Arterial pH <7.34	3.4 (1.3-8.8)	0.015*	4.8 (1.4-16.5)	0.012
Constant term			0.2	0.163

Table 24: Uni- and Multivariate Analysis of Median IL-10 Plamsa Concentrations, Severe Head Injury and pH <7.34 with the Development of MODS

4.6 Summary of Results

A total of 118 patients were enrolled. The patients were mostly young with a median (IQR) age of 30 (19-47) years and median (IQR) ISS value of 34 (27-34). The majority of patients was male (75.4%) and had a low rate of co-morbid disease.

Twenty-six patients developed MODS, 26 patients developed liver failure and 21 patients suffered from ARDS. Renal dysfunction was the most common post-traumatic complication with 39 patients affected.

IL-10 plasma levels were markedly elevated following severe trauma. This elevation occurred quickly. IL-10 reached peak levels within 30 minutes after arrival at the emergency department with a gradual decrease afterwards.

Several initial clinical parameters correlated with IL-10 plasma levels. Parameters indicative of oxygen debt significantly correlated with IL-10 plasma levels. This correlation mainly began at the initial period (30 minutes and OP) until 18h after admission to the ICU. Red blood cell transfusion, APACHE II score and the shock index all positively correlated with IL-10 concentration. Negative correlations were found between IL-10 plasma levels and parameters pH, hemoglobin, systolic blood pressure and MAP. Age, sex and ISS score did not influence the IL-10 plasma levels.

IL-10 plasma levels were associated with outcome i.e. the IL-10 concentration was significantly higher in patients with an eventful outcome compared to patients who recovered without complications.

From the time of the emergency operation on, patients who developed liver failure showed significantly elevated IL-10 plasma concentrations over patients without liver failure until and including day 2. An AUC value of 0.72 was observed at time-point OP. The cut-off point then was 157 pg/mL and the OR value amounted to 8.1.

Significant higher IL-10 plasma concentrations in patients developing renal dysfunction could be observed from the time of the emergency operation until and including day 2. The highest AUC value was observed 12 hours after admission to the ICU with a cut-off value of 85 pg/mL and an OR value of 8.0.

There was significant difference between patients who developed ARDS and patients who did not only at time-point 30 minutes. The AUC values at that

time-point amounted to 0.69, which indicates that this association was moderate. The OR value amounted to 9.0.

From the time of the emergency operation on, IL-10 concentrations in patients who developed MODS were significantly elevated over IL-10 concentration in patients who recovered without MODS and this difference remained significant until and including day 2. The difference between both groups was most impressive 6 hours after admission to the ICU. The results of a ROC analysis show that 6h was also the time-point with the highest discriminative value (AUC=0.78). The cut-off points showed a general decreasing tendency in course of time. The cut-off point at 6h was 124 pg/mL and the OR value then amounted to 9.6.

In a univariate analysis, initial clinical parameters indicative of oxygen debt (arterial pH, transfusion requirements, shock index) showed a significant correlation with the development of MODS. Age, sex and ISS also did not correlate with MODS.

Multivariate analysis indicated that IL-10 plasma levels >124 pg/mL at time-point 6h, severe head injury and an initial arterial pH value <7.34 were simultaneously significantly associated with the development of MODS in severely injured patients.

5 Discussion

5.1 Patient Characteristics and MODS-Score

The relevance of IL-10 for the development of MODS, liver failure, renal dysfunction and ARDS was studied in this prospective single center study. The homogeneous cohort of 118 severely injured patients who were enrolled in this study show characteristics that are very frequently observed among multiple injured patients.[89, 114] Patients were mostly young men with a median age of 30 years and with a background of low prevalence of co-morbid disease. Traffic accidents –predominantly involving motor vehicles- were the dominating cause of injury. In this aspect, our patient cohort is different from cohorts in studies made in the USA. Penetrating trauma, mostly caused by gunshot wounds, is an increasing cause of death and disability in the USA and is even the leading cause of injury death in California.[52, 79] The median (IQR) ISS in our study population was 34 (27-34), which is high in comparison with other studies concerning multiple injured patients.[55, 85, 110]

The incidence of post-traumatic organ failure has remained unchanged between the early 70's and this present day.[82] This observation is remarkable, given intensive research into pathophysiological mechanisms, new concepts of therapy and better ICU facilities. An essential problem lies with the accurate description of organ failure. Investigations targeting the impact of single and multiple organ dysfunction or failure have been hampered by a lack of consensus about the definitions to use for specific organ dysfunction. Organ failure is not an all-or-nothing phenomenon, but has a gradual beginning and can vary from mildly altered function to total organ failure. Various degrees of organ failure can occur during the course of the disease. As a consequence, many different organ dysfunction scoring systems have been developed and there is no general agreement on which organs to assess and which parameters to use.[126]

The clinical investigation of MODS has suffered because of this imprecise definition and research data have to be interpreted according to the score that was used. We decided to diagnose outcome measures according to MARSHALL's MODS-score, which is internationally one of the most used scores to determine MODS.[67]

In this study, blood samples of severely injured patients were systematically obtained and tested on the appearance of IL-10. Furthermore, initial clinical parameters of the patients were related to IL-10 levels and the development of post-traumatic organ failure.

5.2 Measurement of IL-10 Plasma Levels in the Study Population

IL-10 plasma levels were measurable in all 118 patients throughout the whole observation period. Cytokines are not stored as preformed molecules in cells but must be synthesised after appropriate stimulation of the cell surface receptor. In healthy individuals, such an activating stimulus is not present and IL-10 is nearly undetectable in the blood stream. Healthy human volunteers have serum IL-10 levels ranging from 0.1-1 picomolar.[34]

In our study, the highest median (IQR) plasma levels of IL-10 were observed within 30 minutes after admission at the emergency department [148 (78-439) pg/mL] and remained high on day of admission with slowly decreasing levels thereafter. Other studies have also demonstrated that multiple trauma is associated with elevated levels of circulating IL-10. SHERRY et al. found detectable IL-10 levels in 40 of 66 severely injured patients and release of IL-10 occurred within 72 hours after admission.[110] In a study made by NEIDHARDT et al., 417 patients with multiple trauma showed elevated IL-10 concentration in comparison with healthy volunteers (n=137). The release of IL-10 occurred within 60 minutes and reached peak levels of 100.2±11.9 pg/mL at 4 hours after injury.[85] An immediate, transient release of IL-10 was also discovered in the blood serum and in the cerebrospinal fluid of patients suffering from severe traumatic brain injury.[13, 111] An increase in circulating serum IL-10 was detected after severe burn injury.[135] In contrast, MILLER-GRAZIANO et al. reported no increased production of IL-10 after injury.[73]

It should be pointed out that the highest median IL-10 plasma concentration measured in this study is substantially higher than levels measured in the above mentioned studies. A possible explanation is that our patient cohort consisted mainly of very severely injured patients with high ISS values.

NEIDHARDT et al. have demonstrated that the level of increase in IL-10 plasma levels was related to the severity of injury as reflected in the ISS score.[85]

The cellular source of the rapid elevated IL-10 levels after trauma has not been clarified in detail. Several cell lineages, localised both to the site of the injury and in the peripheral circulation can produce IL-10. In mouse burn models, both CD4+ T cells[63] and CD8+ T cells[54] have been identified as the major source of IL-10 production after injury. In humans, major injury induces an increased production of IL-10 from polymorhonuclear neutrophil granulocytes[55] and CD4+ T cells.[63]

5.3 IL-10 Plasma Levels and Pre- and Initial Clinical Parameters

We subsequently addressed the question which pre- and initial clinical parameters influence IL-10 plasma levels, a matter that never was systematically examined.

In a mouse model, it has recently been demonstrated that the IL-10 release following trauma shows age and gender related changes.[48] In our patient cohort, such a difference could not be established. Neither did we find a convincing correlation between IL-10 plasma levels and anatomical parameters such as ISS and site of injury. This is in contrast with findings from other studies that reported a post-traumatic enhanced release of IL-10 dependent on the severity of injury.[37, 85] Parameters of physiological injury severity did markedly influence IL-10 plasma levels. A low pH and hemoglobin value, high transfusion requirements, a high shock index value, admission hypotension and a low MAP value were all significantly correlated with high IL-10 plasma levels. Other studies have ascertained a relationship between IL-10 plasma levels and parameters that are indicative of oxygen debt, however not specifically in trauma patients. Indeed, hemorrhage elevates plasma levels of IL-10 in rats[95] and serum levels of IL-10 in humans are elevated after intra-cerebral hemorrhage.[18] A correlation between high IL-10 levels and a low MAP has also been established following cardiopulmonary bypass.[42]

5.4 IL-10 Plasma Levels and Outcome

This study concentrated on the association between IL-10 plasma levels and outcome. Our primary outcome measure was the occurrence of MODS; secondary outcome measures were liver failure, renal dysfunction and ARDS.

It was established that IL-10 levels differed significantly with regard to the outcome of patients. Patients developing MODS had significantly elevated IL-10 levels in comparison with patients who did not. This difference showed a time-dependent character with a rapid significant difference from the time of the emergency operation on until and including day 2. The difference between both groups was most impressive 6 hours after admission to the ICU with high median (IQR) IL-10 plasma levels of 236 (155-536) pg/mL in the +MODS group. This observation has been confirmed by other studies. NEIDHARDT et al. discovered that those increased IL-10 plasma levels significantly correlated with mortality and the development of post-traumatic complications such as sepsis and MODS.[85]

IL-10 release in patients who later developed liver failure differed from those who did not from the time of emergency operation on. The difference then stayed significant until and including day 2. An association between IL-10 concentration and liver failure has been established, however not in association with trauma. IL-10 synthesised during the course of liver inflammation and fibrosis may modulate Kupffer cell actions, and influence subsequent progression of fibrosis.[118] Increased levels of IL-10 have also been associated with poor outcome after liver transplantation.[56]

Patients who develop renal dysfunction also showed significantly elevated plasma levels of IL-10 for the first time during the emergency operation. The difference was no longer significant on day 5. In hemodialysis patients, the IL-10 genotype determines IL-10 production which down-regulates uraemia- and dialysis-induced chronic inflammation and helps to preserve immune defence functions.[39] Leukocytes may play an essential role in the parenchymal injury after I/R injury as well as in the regeneration process. Especially polymorphonuclear cells, which are an important source of IL-10 release[55] are considered as key mediators in this damaging process.[136]

The correlation between ARDS and IL-10 plasma levels is not strong. A significant difference could only be observed at time-point 30 minutes. It is,

however, widely recognised that ARDS results from the interplay of complex inflammatory responses.[1] Several studies were able to detect substantially elevated levels of anti-inflammatory mediators such as IL-10 in the BALF of patients suffering from ARDS.[1, 91] Moreover, low concentrations of IL-10 and IL-1RA in the BALF of patients with early ARDS may contribute to poor outcome.[16, 61] In this study, only a moderate, initial correlation could be established. This is probably due to the systemic measurement and indicates that results from a IL-10 measurement in the blood compartment as in this study can not be transferred to a local measurement without further ado. Also, IL-10 concentration in the lung generally amounts to much lower values.[1, 91]

Thus, with an exception for ARDS, it can be concluded that the post-traumatic IL-10 plasma concentration is a good marker of post-traumatic events.

5.5 Pre- and Initial Clinical Parameters and the Development of MODS

In a uni- and multivariate analysis, it was examined which pre- and initial clinical parameters were associated with the development of MODS.

There was no significant association between age older than the median age of 30 years and an ISS score higher than the median value of 34 and the development of MODS. This is consistent with results from studies by NAST-KOLB et al. and others.[59, 83] However, SAUAIA et al.[101, 103] and ROUMEN et al.[100] have demonstrated that age and ISS were early predictors of post-injury MODS.[101] A possible explanation for this discrepancy could be that ISS can predict outcome, only if a wide range of injury severity is accounted for in the patient population.[83] This was not the case in our cohort of trauma victims who all tended towards high median ISS scores (IQR= 27-34). Since our patient cohort consisted mostly of young patients (IQR= 19-47), the fact that age did not correlate with outcome could be due to similar reasons.

The site of injury did not significantly influence outcome measures, with two exceptions. Severe abdominal injury was associated with the development of MODS but only in the univariate analysis. In contrast, NAST-KOLB et al. found no difference between multiple injured patients with and without abdominal injury with

respect to the development of complications.[84] Head injury turned out to be independently associated with the development of MODS. This has never before been described, but it is known that post-traumatic mortality is mainly determined by severe head injury.[82, 107, 122] To our opinion, severe head injury was negatively related to the development of MODS, because many patients had died before they could develop MODS.

The main point of interest lies within hemorrhagic parameters (pH, RBC transfusion, shock index, systolic blood pressure and MAP). In the univariate analysis, a significant correlation between all these parameters and MODS could be established. These observations are in accordance with results from other studies. HECKBERT et al. could establish an association of hypotension with the occurrence of organ failure in trauma patients.[44] Lots of studies have also consistently shown that an initial high requirement for RBC transfusion is associated with MODS.[25, 83, 101] SAUAIA et al. established that more than 6 units of red blood cells transfused in the first 12 hours after trauma could predict MODS.[101] The fact that transfusions have immunosuppressive properties, has been implicated to play a role in this process.[86, 101] LAW et al., however, found no differences between patients with and without organ failure with respect to transfusion requirements.[59] Although all initial hemorrhagic parameters were associated with MODS, they did not improve on arterial pH for predicting MODS. An arterial pH value <7.34 was an independent predictor of the development of MODS in the multi-variate analysis. This is in accordance with other study results that showed that acidosis was a risk factor, independently related to MODS.[17, 98] In contrary, FALCONE et al. found that pH value was a good and independent predictor of the quantity of transfusion requirements, but had no value in predicting outcome after trauma.[26]

Other studies established an association between elevated IL-10 plasma levels and post-traumatic complications[85, 110 9] , but this is the first study to identify the IL-10 concentration as an independent factor associated with the development of MODS in a multivariate analysis.

5.6 Pathophysiological Role of Plasma IL-10 Elevation after Trauma

Increased IL-10 levels are an independent predictor of the development of MODS. A central question that still needs to be answered is what pathophysiological role should be attributed to this post-traumatic IL-10 elevation.

GORIS et al. held a generalized, autodestructive inflammation accountable for the development of MODS.[38] The post-traumatic rise in IL-10 is then supposed to counteract the exaggerated inflammatory response that follows severe trauma.[3, 21] Increased IL-10 plasma levels should therefore logically prevent patients from developing single and multiple organ failure. Lots of studies did adjudge protective and regenerative qualities to IL-10 and it was shown that IL-10 attenuates the development of zymosan-induced MODS in mice.[47] IL-10 counteracts the effects of pro-inflammatory cytokines[49] and thereby operates to minimise TNF-α induced damage.[130] IL-10 has inductive properties on the secretion of IL-1RA and sTNRFs following severe injury.[22, 65]

In contrast to the hypothesis from GORIS et al., other studies have established an association between elevated IL-10 plasma levels and the development of post-traumatic complications.[85, 110 9] IL-10 increases the host's susceptibility to infections[63] and in that way could bring on the trigger for the development of MODS and other complications. LYONS et al. demonstrated that blockage of IL-10 improves outcome in a mouse burn model.[62] A similar finding has been reported by O'SULLIVAN et al. that major injury leads to predominance of the type 2 Th cells and trauma victims with detectable IL-10 in the first 72h are more likely to develop sepsis during hospitalisation.[90]

In addition, the results of this study do not favour the theory based on a secondary rise in IL-10 as a counter-acting, beneficial mediator. The immunosuppressive properties of IL-10 seem to be injurious to the patient and patients with high IL-10 plasma levels have a greater risk to suffer from MODS. Also, in our patient cohort, IL-10 plasma levels increased rapidly, with peak levels 30 minutes after arrival at the emergency department. Afterwards, IL-10 levels did not reflect the course of the disease but had a continuous sinking tendency. This indicates that the instant and ongoing release of IL-10 appears to be induced by mechanisms that are active already in the very first hours after trauma. IL-10 could be seen as an early reactant reflecting initial pathophysiological disturbances, set

off by direct tissue injury, blood loss with consecutive ischemia and reperfusion-phenomena, impairment of gut barrier function and others.

It is known that severe post-traumatic hemorrhage, represented among others in a low arterial pH value, induces profound depression of cell-mediated immunity.[86, 101] IL-10 is thought to contribute directly to the decreased capacity to ward off infectious challenges seen following trauma.[4] In this study, evidence was found of a significant correlation between initial parameters of oxygen debt and IL-10 plasma levels and this relationship has also been ascertained by other studies.[18, 42, 95] To our mind, the post-traumatic increase in IL-10 plasma levels could be indicative of oxygen debt, either caused by massive blood loss or by direct lung damage.

In conclusion, this study adjudges a predominant role to the initial, injury-related pathophysiological disturbances, such as severe blood loss resulting in hemorrhagic shock, rather than to an exaggerated immunological answer in causing or priming the patient for MODS.

5.7 Predictive Value of post-traumatic IL-10 Levels

It was established that the IL-10 plasma levels differ between patient groups with an eventful and with an uneventful outcome and that IL-10 is an independent predictor of MODS. Obviously, there are still some open questions related to the pathophysiological role of IL-10 in this process, but this study did not aspire to answer them. The priority of this study was to adjudge a practical diagnostic value to these observations. The ideal way to do this would be to define one fixed limiting value of IL-10 concentration, which would differentiate between patients who will probably develop complications and patients who will probably not. This could enable physicians to take preventive measures and therapeutic interventions sooner, thereby diminishing the high mortality rates that accompany post-traumatic complications.

Oddly enough, this was never done before and a limiting value was never described. Various studies reported about the association and predictive value of IL-10 with respect to post-traumatic complications,[85, 110] but they never tried to

quantify this relationship with numbers. Therefore, a ROC analysis, which determined the AUC value and the cut-off point for all four outcome measures at every single time-point, was performed. The general tendency is that the cut-off points do not remain constant but vary for the same limiting values at every single time-point. One time-independent limiting value of IL-10 concentration can not be pinned down. This is due to the continuous drop in IL-10 plasma. IL-10 plasma levels that can initially be considered as low and relatively innocent, can be considered as high and indicative of the development of post-traumatic complications at a later time-point. The cut-off point with the highest AUC was taken as the limiting value.

For MODS, time-point 6h shows the highest discriminative value (AUC=0.78) with a cut-off point of 124 pg/mL. The OR value for 124 pg/mL amounted to 9.6. The odds of developing MODS in patients with IL-10 plasma concentrations over 124 pg/mL 6 hours after arrival at the ICU are 9.6 times greater than in patients with IL-10 plasma concentrations under 124 pg/mL.

In the case of liver failure, time-point OP shows an AUC value of 0.72; the cut-off point lies at 157 pg/mL and the OR value amounts to 8.1. The odds of developing liver failure in patients with IL-10 plasma concentrations over 157 pg/mL 6 hours after arrival at the ICU are 8.1 times greater than in patients with IL-10 plasma concentrations under 157 pg/mL.

For the prediction of renal dysfunction, time-point 12h provided the best discriminatory value (AUC=0.78) with a cut-off point of 85 pg/mL. The OR value for that concentration amounted to 8.0. Again it can be concluded: the odds of developing renal dysfunction are 8.0 times greater in patients with IL-10 plasma concentrations over 85 pg/mL than in patients with lower IL-10 plasma concentrations at that time-point.

For ARDS, the maximum AUC value amounted to the relatively low value of 0.69 at time-point 30 minutes, with a cut-off point of 611 pg/mL and a significant odds ratio value of 9.0. However, a significant association between the IL-10 plasma concentration and the development of ARDS could only be observed at one single time-point (30minutes). That does not allow us to make any specific conclusion or to draw any diagnostic consequences. It therefore seems to be of little or no use to apply the systemic IL-10 plasma levels in this study as a predictive marker for the possible development of ARDS.

Conclusively, IL-10 plasma levels can be used as a marker for monitoring severely injured patients and to identify cases with guarded prognosis. The optimal time-point of measurement depends on the prediction that is wished for. Following practical conclusions can be drawn: if a prediction about the possible development of MODS is required, it is best to determine the IL-10 plasma levels 6 hours after the patient's arrival at the ICU. The IL-10 plasma concentration during the emergency operation provides the most information with respect to the development of liver failure. If the focus of interest aims at finding out which patients are more endangered to suffer from renal dysfunction, a later measurement, preferably 12 hours after arrival at the ICU, is required.

6 Summary

Severe trauma results in alterations in immune functions, which are correlated with a dysbalanced cytokine synthesis. This imbalance endangers severely injured patients for post-traumatic complications such as MODS, liver failure, renal dysfunction and ARDS. IL-10, a powerful immunosuppressive cytokine that markedly inhibits lymphocyte and phagocytic functions, plays a central role in the immune response after severe trauma.

The relevance of IL-10 for single and multiple organ failure was studied in a prospective, single center study at a level I trauma center. Blood was systematically obtained from a total of 118 severely injured [median (IQR) ISS=34 (27-34)], young [median (IQR) age=30 (19-47) years] patients with a low rate of co-morbid disease. IL-10 plasma levels were measured over a 5-day period by ELISA.

Injured patients showed elevated IL-10 levels throughout the whole observation period of 5 days. IL-10 plasma levels rose rapidly after trauma and gradually declined towards day 5. Patients who developed complications demonstrated significantly elevated IL-10 levels compared with patients with an uneventful post-traumatic course. The odds of developing MODS were 9.6 times greater in patients with IL-10 plasma levels higher than 124 pg/mL 6 hours after arrival at the ICU versus patients who had IL-10 plasma concentrations lower than 124 pg/mL at that time-point.

Multivariate analysis showed that IL-10 plasma levels >124 pg/mL at time-point 6h, severe head injury and an arterial pH <7.34 were simultaneously significant predictors of the development of MODS in severely injured patients. IL-10 plasma concentrations were significantly correlated with hemorrhagic parameters. The dynamic with rapid increase and gradual decline in IL-10 plasma levels indicated that IL-10 is a marker of the initial damage to the organism caused by trauma, rather than a marker of somatic dysregulations. Evidence suggested that IL-10 is an early reactant reflecting oxygen debt.

7 Abbreviations

AIS	Abbreviated Injury Scale
APACHE II	Acute Physiology and Chronic Health Evaluation Score II
APC	Antigen-presenting Cell
ARDS	Acute Respiratory Distress Syndrome
AUC	Area under the Curve
BALF	Bronchoalveolar Lavage Fluid
C	Celsius
CARS	Compensatory Anti-inflammatory Response Syndrome
CI	Confidence Interval
CVP	Central Venous Pressure
df	Degrees of Freedom
dL	Decilitre
DPD	2.5-dichlorophenyldiazonium
e.g.	Exampli Gratia
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay System
et.al.	Et Alii
FiO_2	Fraction of Inspired Oxygen
GCS	Glasgow Coma Scale
h	Hours
HR	Heart Rate
i.e.	Id est
I/R	Ischemia / Reperfusion
ICU	Intensive Care Unit
IL	Interleukin
IL-1 RA	Interleukin-1 Receptor Antagonist
IQR	Interquartile Range
ISS	Injury Severity Score
Jak	Janus Kinase
KPa	Kilo Pascal
L	Litre
LPS	Lipopolysaccharide

MAP	Mean Arterial Pressure
mg	Milligram
Min	Minutes
mL	Millilitre
mmHg	Millimetres of Mercury
MODS	Multiple Organ Dysfunction Syndrome
MOF	Multiple Organ Failure
nm	Nanometre
No	number
NYHA	New York Heart Association
OR	Odds Ratio
PAR	Pressure-adjusted Heart Rate
PEEP	Positive End Expiratory Pressure
pg	Picogram
PGE_2	Prostaglandin E_2
pO_2	Partial Oxygen Pressure
resp	respectively
ROC	Receiver Operating Characteristic
rpm	Revolutions per Minute
r_s	Correlation Coefficient of Spearman
SIRS	Systemic Inflammatory Response Syndrome
STAT	Signal Transducers and Activators of Transcription
sTNFRs	Soluble TNF Receptors
TGF	Transforming Growth Factor
Th	T helper
TMB	3.3'.5.5'-Tetra-Methyl-Benzidine
TNF	Tumour Necrosis Factor
Tyk	Tyrosine Kinase
UKB	Unfallkrankenhaus Berlin
yrs	Years
µL	Microlitre

8 References

1. Armstrong L, Millar A: *Relative production of tumour necrosis factor alpha and interleukin 10 in adult respiratory distress syndrome*. Thorax 1997; 52: 442-446.

2. Ashbaugh D, Bigelow D, Petty T, Levine B: *Acute respiratory distress syndrome in adults*. Lancet 1967; 2: 319-323.

3. Ayala A, Ertel W, Chaudry I: *Trauma-induced suppression of antigen presentation and expression of major histocompatibility class II antigen complex in leukocytes*. Shock 1996; 5: 79-90.

4. Ayala A, Lehman D, Herdon C, Chaudry I: *Mechanism of enhanced susceptibility to sepsis following hemorrhage. Interleukin-10 suppression of T-cell response is mediated by eicosanoid-induced interleukin-4 release*. Archives of Surgery 1994; 129: 1172-1178.

5. Baker S, O'Neill B, Haddon W, Long W: *The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care*. Journal of Trauma 1974; 14: 187-196.

6. Baue A: *Multiple, progressive, or sequential systems failure. A syndrome of the 1970s.* Archives of Surgery 1975; 110: 779-781.

7. Berkmann N, John M, Roesems G, Jose P, Barnes P, Chung K: *Inhibition of macrophage inflammatory protein 1-alpha expression by IL-10. Differential sensitivities in human blood monocytes and alveolar macrophages*. Journal of Immunology 1995; 155: 4412-4418.

8. Bogdan C, Vodovotz Y, Nathan C: *Macrophage deactivation by IL-10*. Journal of Experimental Medicine 1991; 147: 1549-1555.

9. Bone R: *Immunologic dissonance: a continuing evolution in our understanding of the systematic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS)*. Annals of Internal Medicine 1996; 125: 680-687.

10. Bone R, Balk R, Cerra F, Dellinger R, Fein A, Knaus W, Schein R, Sibbald W: *Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis*. Chest 1992; 101: 1644-1655.

11. Bone R, Grodzin C, Balk R: *Sepsis: a new hypothesis for pathogenesis of the disease process*. Chest 1997; 112: 235-243.

12. Committee, safety omaoa. Rating the severity of tissue damage:1. The abbreviated injury scale. JAMA. Vol. 215, 1971:277-280.

13. Csuka E, Morganti-Kossmann M, Lenzlinger P, Joller H, Trentz O, Kossmann T: *IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-alfa, TGF-beta1 and blood-brain barrier function*. Journal of Neuroimmunology 1999; 101: 211-221.

14. de Waal Malefyt R, Abrams J, Bennett B, Figdor C, de Vries J: *Interleukin-10 inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes*. Journal of Experimental Medicine 1991; 174: 1209-1220.

15. Deitch E: *Multiple organ failure - pathophysiology and potential future therapy*. Annals of Surgery 1992; 216: 117-134.

16. Donnelly S, Strieter R, Reid P, Kunkel S, Burdick M, Armstrong I, Mackenzie A, Haslett C: *The association between mortality rates and decreased concentrations of interleukin-10 and interleukin-1 receptor antagonist in the lung fluid of patients with the adult respiratory distress syndrome*. Annals of Internal Medicine 1996; 125: 191-196.

17. Dunham C, Damiano A, Wiles C, Cushing B: *Post-traumatic multiple organ dysfunction syndrome-- infection is an uncommon antecedent risk factor*. Injury 1995; 26: 373-378.

18. Dziedzic T, Bartus S, Klimkowicz A, Motyl M, Slowik A, Szczudlik A: *Intracerebral hemorrhage triggers interleukin-6 and interleukin-10 release in blood.* Stroke 2002; 33: 2334-2335.

19. Ekkernkamp A, Brand J, Wernet E, Muhr G, Rehn J: *What modifies the outcome of abdominal trauma? An analysis of 558 patients*. Der Unfallchirurg 1992; 95: 380-386.

20. Enk A, Katz M: *Identification and induction of keratinocyte-derived IL-10*. Journal of Immunology 1992; 149: 92-95.

21. Ertel W, Faist E: *Immunologic monotoring after severe trauma*. Unfallchirurg 1993; 96: 200-212.

22. Ertel W, Keel M, Bonaccio M, Steckholzer U, Gallati H, Kenny J, Trentz O: *Release of anti-inflammatory mediators after mechanical trauma correlates with severity of injury and clinical outcome.* The Journal of Trauma: Injury, Infection and Critical Care 1995; 39: 879-887.

23. Ertel W, Trentz O: *Polytrauma und Multiorgan-Dysfunktionssyndrome (MODS). Definition - Pathophysiology - Therapie*. Zentralblatt für Chirurgie 1994; 119: 159-167.

24. Faist E: *The mechanisms of host defence dysfunction following shock and trauma*. Current Topics of microbiology and immunology 1996; 216: 259-274.

25. Faist E, Baue A, Dittmer H, Heberer G: *Multiple organ failure in polytrauma patients*. The Journal of Trauma 1983; 23: 775-785.

26. Falcone R, Santanello S, Schulz M, Monk J, Satiani B, Carey L: *Correlation of metabolic acidosis with outcomen following injury and its value as a scoring tool*. World Journal of Surgery 1993; 17: 575-579.

27. Fiddian-Green R: *Associations between intramucosal acidosis in the gut and organ failure*. Critical Care Medicine 1993; 21: 103-107.

28. Finbloom D, Winestock K: *IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1 and STAT3 complexes in human T cells and monocytes*. Journal of Immunology 1995; 155: 1079-1090.

29. Fiorentino D, Bond M, Mosmann T: *Two types of mouse T helper cells. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones*. Journal of Experimental Medicine 1989; 170: 2081-2095.

30. Fiorentino D, Zlotnik A, Mosmann T, Howard M, O'Garra A: *IL-10 inhibits cytokine production by activated macrophages*. The Journal of Immunology 1991; 147: 3815-3822.

31. Flint L: *Liver Failure*. Surgical Clinics of North America 1982; 62: 157-165.

32. Fowler A, Hamman R, Good J, Benson K, Baird M, Eberle D, Petty T, Hyers T: *Adult respiratory distress syndrome: risk with common predispositions*. Annals of Internal Medicine 1983; 98: 593-597.

33. Fry D, Pearlstein L, Fulton R, Polk H: *Multiple system organ failure: the role of uncontrolled infection*. Archives of Surgery 1980; 115: 136-140.

34. Fuchs A, Granowitz E, Shapiro L: *Clinical, hematologic, and immunologic effects of interleukin-10 in humans*. Journal of Clinical Immunology 1996; 16: 291-303.

35. Gebhard F, Pfetsch H, Steinbach G, Strecker W, Kinzel L, Bruchner U: *Is interleukin 6 an early marker of injury severity following major trauma in humans?* Archives of Surgery 2000; 135: 291-295.

36. Gerhard C, Bruyns C, Marchant A, Abramowicz D, Vandenabeele P, Delvaux A, Fiers W, Goldman M, Velu T: *Interleukin-10 reduces the release of tumour necrosis factor and prevents lethality in experimental endotoxemia*. Journal of Experimental Medicine 1993; 177: 547.

37. Giannoudis P, Smith R, Perry S, Windsor A, Dickson R, Bellamy M: *Immediate IL-10 expression following major orthopaedic trauma: relationship to anti-inflammatory response and subsequent development of sepsis*. Intensive Care Medicine 2000; 26: 1076-1081.

38. Goris R, te Boekhorst T, Nuytinck J, Gimbere J: *Multiple organ failure: generalized autodestructive inflammation?* Archives of Surgery 1985; 120: 1109-1115.

39. Grindt M, Sester U, Sester M, Deman E, Ulrich C, Kaul H, Köhler H: *The interleukin-10 promotor genotype determines clinical immune function in hemodialysis patients*. Kidney International 2001; 60: 2385-2391.

40. Groux H, Bigler M, de Vries J, Roncarolo M: *Inhibitory and stimulatory effects of IL-10 on human CD8+ T cells*. Journal of Immunology 1998; 160: 3188-3193.

41. Harbrecht B, Zenati M, Doyle H, McMichael J, Townsend R, Clancy K, Peitzman A: *Hepatic dysfunction increases length of stay and risk of death after injury*. Journal of Trauma 2002; 53: 517-523.

42. Harig F, Cesnjevar R, Mahmoud F, von der Ende J: *Perioperative factors influencing interleukin-10 release under cardiopulmonary bypass*. Thoracal and Cardiovascular Surgery 1999; 47: 361-368.

43. Headley A, Tolley E, Meduri G: *Infections and the inflammatory response in acute respiratory distress syndrome*. Chest 1997; 111: 1306-1321.

44. Heckbert S, Vedder N, Hoffman W, Winn R, Hudson L, Jurkovich G, Copass M, Harlan J, Rice C, Maier R: *Outcome after hemorrhagic shock in trauma patients*. The Journal of Trauma: Injury, Infection and Critical Care 1998; 45: 545-549.

45. Houssian F, Lefebrre C, Van den Berghe M, Lambert M, Devogelaer J, Renauld C: *Serum IL-10 titers in systemic lupus erythematosus reflect disease activity*. Lupus 1995; 4: 393-395.

46. Hsu D, de Waal Malefyt R, Fiorentino D, Dang M, Vieira P, de Vries J, Spits H, Mosmann T, Moore K: *Expression of IL-10 activity by Epstein-Barr virus protein BRCFI*. Science 1990; 250: 830-832.

47. Jansen M, Hendriks T, de Man B, van der Meer J, Goris R: *Interleukin-10 mitigates the development of the zymosan-induced multiple organ dysfunction syndrome in mice*. Cytokine 1999; 99: 713-721.

48. Kahlke V, Angele M, Ayala A, Schwacha M, Cioffo W, Blande K, Chaudry I: *Immune dysfunction following trauma-haemorrhage: influence of gender and age*. Cytokine 2000; 12: 69-77.

49. Karakozis S, Hinds M, Cook J, Kim D, Provido H, Kirkpatrick J: *The effects of interleukin-10 in hemorrhagic shock*. Journal of Surgical Research 2000; 90: 109-112.

50. Kelly D: *Managing liver failure*. Postgraduate Medical Journal 2002; 78: 660-667.

51. Khabar K, elBarbary M, Khouquer F, Deval E, al-Garin S, al-Halees Z: *Circulating endotoxin and cytokines after cardiopulmonary bypass : differential correlation with duration of bypass and systemic inflammatory response/multiple organ dysfunction syndromes*. Clinical Immunology and Immunopathology 1997; 85: 97-103.

52. Kim A, Trent R: *Firearm-related injury surveillance in California*. American Journal of Preventive Medicine 1998; 15: 31-37.

53. Knaus W, Draper E, Wagner D, Zimmerman J: *APACHE II: a severity of disease classification system*. Critical Care Medicine 1985; 13: 818-829.

54. Kobayashi M, Herndon D, Pollard R, Suzuki F: *CD4+ contra-suppressor T cells improve the resistance of thermally injured mice infected with HSV*. Journal of Leukocyte Biology 1995; 58: 159-167.

55. Köller M, Clasbrummel B, Kollig E, Hahn M, Muhr G: *Major injury increased production of interleukin-10 in human granulocyte fractions*. Langenbeck's Archive of Surgery 1998; 383: 460-465.

56. Kubala L, Ciz M, Vondrazek J, Cizova H, Cerny J, Nemec P, Studenik P, Duskova M, Lojek A: *Peri-and post-operative course of cytokines and the metabolic activity of neutrophils in human liver transplantation*. Cytokine 2001; 16: 97-101.

57. Lalani I, Bohl K, Razzaque A: *Interleukin-10: biology, role in inflammation and autoimmunity*. Annals of Allergy, Asthma, and Immunology 1997; 79: 469-484.

58. Laun R, Schröder O, Schoppnies M, Roher H, Ekkernkamp A, Schulte K: *Transforming growth factor beta1 and major trauma: time-dependent association with hepatic and renal insufficiency*. Shock 2003; 19: 16-23.

59. Law M, Cryer H, Abraham E: *Elevated levels of soluble ICAM-1 correlate with the development of multiple organ failure in severely injured trauma patients*. Journal of Trauma 1994; 37: 100-.

60. Lee W: *Acute liver failure*. Journal of Trauma 1994; 17: 3-9.

61. Lo C, Fu M, Cryer G: *Interleukin-10 inhibits alveolar macrophage production of inflammatory mediators involved in adult respiratory distress syndrome*. Journal of Surgical Research 1998; 79: 179-184.

62. Lyons A, Goebel A, Mannick J, Lederer J: *Protective effects of early interleukin-10 antagonism on injury-induced immune dysfunction*. Archives of Surgery 1999; 134: 1317-1324.

63. Lyons A, Kelly J, Rodrick M, Mannick J, Lederer J: *Major injury induces increased production of interleukin-10 by cells of the immune system with a negative impact on resistance to infection*. Annals of Surgery 1997; 226: 450-460.

64. Majetschak M, Obertacke U, Waydhas C, Nast-Kolb D, Ulrich Schade F: *Mediatorenmodulation bei Sepsis und Multiorganversagen*. Unfallchirurg 2000; 103: 903-907.

65. Marie C, Pitton C, Fitting C, Cavaillon J: *IL-10 and IL-4 synergise with TNF-alfa to induce IL-1ra production*. Cytokine 1996; 8: 147-151.

66. Marschall J: *Inflammation, coagulopathy, and the pathogenesis of multiple organ dysfunction syndrome*. Critical Care Medicine 2001; 29: 99-106.

67. Marshall J, Cook D, Christou N, Bernhard G, Sprung C, Sibbald W: *Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome*. Critical Care Medicine 1995; 23: 1638-1652.

68. Martin C, Boisson C, Haccoun M, Thomachot L, Mege J: *Patterns of cytokine evolution (tumor necrosis factor-alpha and interleukin-6) after septic shock, hemorrhagic shock and severe trauma.* Critical Care Medicine 1997; 25: 1813-1819.

69. Masai T, Sawa Y, Ohtake S, Nishida T, Nishimura M, Fukishima M, Yamaguchi N, Matsuda H: *Hepatic dysfunction after left ventricular, mechanical assistance in patients with end-stage heart failure: role of inflammatory response and hepatic microcirculation*. Annals of Thoracal Surgery 2002; 73: 549-555.

70. Matthes G, Seifert J, Ostermann P, Wurfel S, Ekkernkamp A, Wick M: *Early death of the severely injured patient- A retrospective analysis*. Zentralblatt für Chirurgie 2001; 126: 995-999.

71. McBride J, Jung T, deVries J, Aversa G: *IL-10 alters DC function via modulation of cell surface molecules resulting in impaired T-cell responses*. Cellular Immunology 2002; 215: 162-172.

72. Michel G, Gaillis A, Jatzebska-Deussen B: *1,25(OH)2, vitamin D3 and calcipotriol induce IL-10 receptor gene expression in human epidermal cells*. Inflammation Research 1997; 46: 32-34.

73. Miller-Graziano C, De A, Kodys K: *Altered IL-10 levels in trauma patients' monocytes and T lymfocytes*. Journal of Clinical Immunology 1995; 15: 93-104.

74. Montgomery A, Stager M, Carrico C, Hudson L: *Causes of mortality in patients with the adult respiratory distress syndrome*. American Review of Respiratory Disease 1985; 132: 485-489.

75. Moore F, Sauaia A, Moore E, Haenel J, Burch J, Lezotte D: *Postinjury multiple organ failure: a bimodal phenomenon*. The Journal of Trauma: Injury, Infection and Critical Care 1996; 40: 501-512.

76. Moore K, de Waal Malefyt R, Coffman R, O'Garra A: *Interleukin-10 and the interleukin-10 receptor*. Annual Review of immunology 2001; 19: 683-765.

77. Moore K, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann T: *Interleukin-10*. Annual Review of immunology 1993; 11: 165-190.

78. Morris J, Mucha P, Ross S, Moore B, Hoyt D, Gentilello L, Landercasper J, Feliciano D, Shackford S: *Acute posttraumatic renal failure: a multicenter perspective*. Journal of Trauma 1991; 31: 1584-1589.

79. Morrissey T, Byrd C, Deitch E: *The incidence of recurrent penetrating trauma in an urban trauma center*. Journal of Trauma 1991; 31: 1536-1538.

80. Mosmann T: *Properties and functions of IL-10*. Advanced Immunology 1994; 56: 1-26.

81. Mutze S, Madeja C, Paris S, Ostermann P, Ekkernkamp A: *Helical CT examination of multiple trauma patients in a digitized radiology department*. Emergency Radiology 1999; 6: 77-80.

82. Nast-Kolb D, Aufmkolk M, Ruchholtz S, Obertacke U, Waydhas C: *Multiple organ failure still a major cause of morbidity but not mortality in blunt multiple trauma*. Journal of Trauma 2001; 51: 835-841.

83. Nast-Kolb D, Waydhas C, Gippner-Steppert C, Schneider I, Trupka A, Ruchholtz S, Zettl R, Schweiberer L, Jochum M: *Indicators of the posttraumatic inflammatory response correlate with organ failure in patients with multiple injuries*. The Journal of Trauma: Injury, Infection and Critical Care 1997; 42: 446-455.

84. Nast-Kolb D, Waydhas C, Kastl S, Duswald K, Schweiberer L: *The role of an abdominal injury in follow-up of polytrauma patients*. Der Chirurg 1993; 64: 552-559.

85. Neidhardt R, Keel M, Steckholzer U, Safret A, Ungethuem U, Trentz O, Ertel W: *Relationship of Interleukin-10 plasma levels to severity of injury and clinical outcome in injured patients*. The Journal of Trauma: Injury, Infection and Critical Care 1997; 42: 863-871.

86. Nichols R, Smith J, Klein D, Trunkey D, Cooper R, Adinolfi M, Mills J: *Risk of infection after penetrating abdominal trauma*. New England Journal of Medicine 1984; 311: 1065-1070.

87. Nuytinck J, Goris R, Weerts J: *Acute generalized microvascular injury by activated complement and hypoxia: the basis of acute respiratory distress syndrome and multiple organ failure?* British Journal of Experimental Pathology 1986; 67: 537-548.

88. Oberholzer A, Oberholzer C, Moldawer L: *Interleukin-10: a complex role in the pathogenesis of sepsis syndromes and its potential as anti-inflammatory drug*. Critical Care Medicine 2002; 30: 58-63.

89. Odero W, Garner P, Zwi A: *Road traffic injuries in developing countries: a comprehensive review of epidemiological studies*. Tropical Medicine and International Health 1997; 2.

90. O'Sullivan S, Lederer J, Horgan A, Chin D, Mannick J, Rodrick M: *Major injury leads to predominance of the T helper-2 lymphocyte phenotype and diminished interleukin-12 production associated with decreased resistance to infection*. Annals of Surgery 1995; 222: 482-492.

91. Park W, Goodman R, Steinberg G, Ruzinski J, Radella F, Park D, Pugin J, Skerrett S, Hudson L, Martin T: *Cytokine balance in the lungs of patients with acute respiratory distress syndrome*. American Journal of Respiratory and Critical Care Medicine 2001; 164: 1896-1903.

92. Parks R, Chrysos E, Diamond T: *Management of liver trauma*. British Journal of Surgery 1999; 86: 1121-1135.

93. Pellegrini J, Puyana J, Lapchak P, Kodys K, Miller-Graziano C: *A membrane TNF-alpha/TNFR ratio correlates to MODS scores and mortality.* Shock 1996; 6: 389-396.

94. Petty T: *Indicators of risk, course and prognosis in adult respiratory distress syndrome (ARDS)*. American Review of Respiratory Disease 1985; 132: 471.

95. Phelan H, Stahls P, Hunt J, Bagby G, Molina P: *Impact of alcohol intoxication on hemodynamic, metabolic and cytokine responses to hemorrhagic shock*. Journal of Trauma 2002; 52: 675-682.

96. Ranjit J, Rajasinghe H, Chen J, Weinberg A, Sinha P, Itescu S, Lietz K, Mancini D, Oz M, Smith C, Rose E, Edwards N: *Impact of current management practices on early and late death in more than 500 consecutive cardiac transplant recipients*. Annals of Surgery 2000; 232: 302-311.

97. Regel G, Grotz M, Weltner T, Sturm J, Tscherne H: *Pattern of organ failure following severe trauma*. World Journal of Surgery 1996; 20: 422-429.

98. Robertson R, Eidt J, Bitzer L, Wallace B, Collins T, Parks-Miller C, Cone J: *Severe acidosis alone does not predict mortality in the trauma patient*. American Journal of Surgery 1995; 170: 691-694.

99. Rose S, Marzi I: *Pathophysiology des Polytraumas*. Zentralblatt für Chirurgie 1996; 121: 896-913.

100. Roumen R, Redl H, Schlag G, Zilow G, Sandtner W, Koller W, Hendriks T, Goris R: *Inflammatory mediators in relation to the development of multiple organ failure in patients after severe blunt trauma*. Critical Care Medicine 1995; 23: 474-480.

101. Sauaia A, Moore F, Moore E, Haenel J, Read R, Lezotte D: *Early predictors of postinjury multiple organ failure*. Archives of Surgery 1994; 129: 39-45.

102. Sauaia A, Moore F, Moore E, Norris J, Lezotte D, Hamman R: *Multiple organ failure can be predicted as early as 12 hours after injury*. Journal of Trauma 1998; 45: 291-301.

103. Sauiaia A, Moore F, Moore E, Lezotte D: *Early risk factors for postinjury multiple organ failure*. World Journal of Surgery 1996; 20: 394-400.

104. Schröder O, Schulte K, Ostermann P, Roher H, Ekkernkamp A, Laun R: *Heat shock protein 70 genotypes HSPA1B and HSPA1L influence cytokine concentrations and interfere with outcome after major injury*. Critical Care Medicine 2003; 31: 73-79.

105. Schwartz R: *Models of T cell anergy: is there a common molecular mechanism?* Journal of Experimental Medicine 1996: 1-8.

106. Schwarz M, Hamilton L, Tardelli L, Narula S, Sullivan L: *Stimulatilion of cytolytic activity by interleukin-10*. Journal of Immunotherapy 1994; 16: 95-104.

107. Shackford S, Mackersie R, Davis J, Wolf P, Hoyt D: *Epidemiology and pathology of traumatic deaths occurring at a level I trauma center in a regionalized system: the importance of secondary brain injury*. Journal of Trauma 1989; 29: 1392-1397.

108. Sharma S, Eschun G: *Multisystem organ failure of sepsis*. eMedicin Journal 2002; 3.

109. Sheldon G, Rutledger R: *Hepatic trauma*. Advanced Surgery 1989; 22: 179-194.

110. Sherry R, Cué J, Goddard J, Parramore J, DiPiro J: *Interleukin-10 is associated with the development of sepsis in trauma patients*. The Journal of Trauma: Injury, Infection and Critical Care 1996; 40: 613-617.

111. Shimonkevitz R, Bar-or D, Harris L, Dole K, McLaughlin L, Yuki R: *Transient monocyte release of interleukin-10 in response to traumatic brain injury*. SHOCK 1999; 12: 10-16.

112. Shoemacher W, Appel P, Keam H: *Tissue oxygen debt as a determinant of lethal and nonlethal postoperative organ failure*. Critical Care Medicine 1988; 16: 1117-1120.

113. Sims C, Seigne P, Menconi M, Monura J, Barlow C, Pettit J, Puyana J: *Skelatal muscle acidosis correlates with the severity of blood volume loss during shock and resuscitation*. The Journal of Trauma 2001; 51: 1137-1145.

114. Sluis van der C, Geertzen J, Werkman H, Duis ten H: *Epidemiological data on severely injured patients: a retrospective study of the period 1985-1989*. Nederlands Tijdschrift voor Geneeskunde 1994; 138: 2285-2289.

115. Stene J: *Renal failure in the trauma patient*. Overview of Trauma Anesthesia and Critical Care 1990; 6: 111-119.

116. Stith R, Templer L: *Peripheral endocrine and metabolic responses to centrally administered interleukin-1*. Neuroendocrinology 1994; 60: 215-224.

117. Street J, Winter D, Buckley S, Nicholson P, Twomney A: *Trauma on rural roads - the role of a peripheral hospital*. Injury 1999; 30: 337-340.

118. Thompson K, Maltby J, Fallowfield J, McAulay M, Millward-Sadler H, Sheron N: *Interleukin-10 expression and function in experimental murine liver inflammation and fibrosis*. Hepatology 1998; 28: 1597-1606.

119. Tilney N, Bailey G, Morgan A: *Sequential system failure after rupture of abdomic aortic aneurysms. An unsolved problem in postoperative care.* Annals of Surgery 1973; 178: 117-122.

120. Tran D, Cuesta M: *Acute renal failure in patients with severe civilian trauma*. Nephrology Dialysis Transplantation 1994; 9: 121-125.

121. van der Poll T, Jansen P, Montegut W, Braxton C, Calvano S, Stackpole S, Smith S, Swanson S, Hack C, Lowry S, Moldawer L: *Effects of IL-10 on systemic inflammatory responses during sublethal primate endotoxemia*. The Journal of Immunology 1997; 158: 1971-1975.

122. van der Sluis C, Geertzen J, Werkman H, ten Duis H: *Epidemiological data on severely injured patients; a retrospective study of the period 1985-1989*. Nederlands Tijdschrift voor Geneeskunde 1994; 138: 2285-2289.

123. Varon J, Wenker C: *The acute respiratory distress syndrome: myths and controversies*. The Internet Journal of Emergency and Intensive Care Medicine 1997; 1: 1-11.

124. Verhoef C, van Roon J, Vianen M, Bylsma J, Lafeber F: *Interleukin-10, not interleukin-4 or interferon-gamma production, correlates with progression of joint destruction in rheumatoid arthritis*. Journal of Rheumatology 2001; 28: 1960-1966.

125. Villegas E, Wille U, Craig L, Linsley P, Rennick D, Peach R, Hunter C: *Blockade of costimulation prevents infection-induced immunopathology in interleukin-10-deficient mice*. Infection and Immunology 2000; 68: 2837-2844.

126. Vincent J, Ferreira F, Moreno R: *Scoring systems for assessing organ dysfunction and survival*. Critical Care Clinics 2000; 16: 353-365.

127. Vivino G, Antonelli M, Moro M, Cottini F, Conti G, Bufi M, Cannata F, Gasparetto A: *Risk factors for acute renal failure in trauma patients*. Intensive Care Medicine 1998; 24: 808-814.

128. Vollmar B, Lang G, Post S, Menger M, Messner K: *Die Mikrozirkulation der Leber im hämorrhagischen Schock der Ratte und ihre Bedeutung für Energiestoffwechsel und Funktion*. Zentralblatt für Chirurgie 1993; 118: 218-225.

129. Walmsley M, Katsikis P, Abney E, Parry S, Williams R, Maini R, Feldmann M: *IL-10 inhibition of the progression of established collagen-induced arthritis*. Arthritis and Rheumatism 1996; 39: 495-503.

130. Wang P, Wu P, Siegel M, Egan R, Billah M: *IL-10 inhibits transcription of cytokine genes in human peripheral blood mononuclear cells*. The Journal of Immunology 1994; 153: 811-816.

131. Ware L, Matthay M: *The acute respiratory distress syndrome*. New England Journal of Medicine 2000; 342: 1334-1349.

132. Weber-Nordt R, Riley R, Greenlund A, Moore K, Darnell J, Schreiber R: *Stat3 recruitement by two distinct ligand-induced tyrosine phosphorylated docking sides in the IL-10 receptor intracellular domaine*. Journal of Biology and Chemistry 1996; 271: 27954-27961.

133. Wick M, Ekkernkamp A, Muhr G: *Epidemiologie des Polytraumas*. Chirurg 1997; 68: 1053-1058.

134. Williams K, Dooley N, Ulvestadt E, Becher B, Antel J: *IL-10 production by adult derived microglial cells*. Neurochemistry International 1996; 29: 55-64.

135. Yeh F, Lin W, Shen H: *Changes in circulating levels of anti-inflammatory interleukin-10 in burned patients*. BURNS 2000; 26: 454-459.

136. Ysebaert D, DeGreef K, Vercauteren S, Ghielli M, Verpooten G, Eyskens E, DeBroe M: *Identification and kinetics of leukocytes after severe ischemia/reperfusion renal injury*. Nephrology Dialysis Transplantation 2000; 15: 1562-1574.

137. Zapol W, Trelstad R, Coffey J, Tsai I, Salvador R: *Pulmonary fibrosis in severe acute respiratory failure*. American Review of Respiratory Disease 1979; 119.

9 Contents of Tables

Eidesstattliche Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbständig verfasst und keine anderen als die angegebenen Hilfsmittel benutzt habe.

Die Dissertation ist bisher keiner anderen Fakultät vorgelegt worden.

Ich erkläre, dass ich bisher kein Promotionsverfahren erfolglos beendet habe und dass eine Aberkennung eines bereits erworbenen Doktorgrades nicht vorliegt.

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