Eur J Med Res (2000) 5: 283-294

IMMUNOLOGICAL SURROGATE PARAMETERS IN A PROGNOSTIC MODEL FOR MULTI-ORGAN FAILURE AND DEATH*

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Abstract:
Objective: To assess the ability of clinical or biochemical parameters to predict outcome (survival or non-survival; severe or moderate/no complication) using multiple regression analyses.
Design: Prospective, descriptive cohort study with no interventions
Setting: 12 surgical intensive care units of university hospitals and large community hospitals; four medical school research laboratories in eight European countries
Patients: 128 surgical patients with major intra-abdominal surgery admitted for at least two days to an intensive care unit
Main Outcome Measures: Prediction of complications or survival based on analysis of clinical (Multiple Organ Dysfunction Score, Multi-Organ-Failure Score, Acute Physiology and Chronic Health Evaluation II scores) and immunological (plasma levels of endotoxin, endotoxin neutralizing capacity, IL-6, IL-8, cell associated IL-8, Fc-receptor polymorphism, soluble CD-14) parameters, with comparison of predicted and actual outcomes.
Results: APACHE II, MODS score, MOF score, platelets, IL-6, IL-8, ENC, cell ass. IL-8 were significantly different between survivors and non-survivors and patients with/without severe complications by univariate analysis. By multivariate analysis only MOF, MODS score, IL-6, platelets, comorbidity predicted complications with a sensitivity of 82% and a specificity of 87%. Multivariate analysis demonstrated that only APACHE II score, plasma IL-8 and complications predicted death (sensitivity 84%; specificity 90%).
Conclusion: Immunological surrogate parameters may predict complications and death of surgical ICU patients. The use of several parameters may add to increase sensitivity and specificity in a prognostic model.

Key words: Sepsis; multi-organ failure; mortality; immunological surrogate parameters; prognosis

INTRODUCTION

Despite improvement in intensive care, mortality among septic surgical patients remains high. It is well recognized that systemic inflammation is the condition for sepsis and that this inflammatory response is the result of a cascade of cell-derived mediators. Endotoxin, TNF-α, IL-1, IL-6 have been recognized as major mediators in sepsis [1, 2]. Animal experiments suggested it may be beneficial to block TNF-α in an attempt to reduce mortality [3]. Large clinical studies designed to investigate the blocking effect of anti-TNF-α antibodies in septic patients or to neutralize endotoxin were terminated without a reduction in the mortality rate [4, 5]. However, these studies revealed the need of a better stratification system and surrogate parameters based on scores and immune mediators related to cellular and organ function. Specific genetic disposition, cytokine patterns and treatment with antibiotics may influence the rate of complications and outcome of surgical intensive care patients. Several studies have investigated the prognostic capacity of cytokines in sepsis and peritonitis [6, 7, 8]. However, most studies were performed in one center with a limited amount of patients or used ELISA systems which may not be applicable in clinical situations. As a results from several cytokine studies it be-

* This work was presented at the SIS 1999 meeting in Seattle, Washington, USA

came obvious that the determination of plasma cytokines alone may have serious limitations. It may be the determination of the cellular response to sepsis which correlates better with outcome. There is a dissociation between plasma and monocyte associated cytokines during sepsis which may be responsible for the failure of plasma cytokine determination to identify patients at risk. IL-8 has been found in elevated concentrations in sepsis and inflammation and is involved in leukocyte-endothelial interactions and neutrophil migration leading to organ failure and shock [9, 10, 11, 12, 13]. IL-8 titers may be related more to cellular function (PMN) in sepsis and the cellular dysfunction (hypo- or hyperactivation) may influence the development of multi organ failure [14, 15]. Fc-receptors which provide a critical link between specific humoral responses and the cellular branch of the immune system and are important in immunoglobulin mediated phagocytosis were recently identified to express a genetic polymorphism [16]. Patients which were homozygous for Fcγ-R IIa H/H131 demonstrated a higher phagocytosis rate than patients homozygous for Fcγ-RR IIa R/R131 [17]. sCD-14 which can be recognized in serum, urine and other body fluids is increased in sepsis, polytrauma and severe burns [18]. sCD-14 seems to have prognostic function in diseases where monocytes and macrophages are involved [19].

Classification of patients with sepsis or SIRS according to scores based on overall health, age, acute physiology parameters, and extent of organ system function has been reported [20, 21, 22].

There are only few studies with a small number of patients who investigated the effect of immune surrogate parameters and scores on outcome prediction [23,24].

We have recognized that standard sepsis treatment, e.g., antibiotic administration, may influence the balance of the immune system. Antibiotics induced in vitro the release of free endotoxin in different quantities depending on the type of antibiotics (Penicillin Binding Protein 2-specific (PBP 2) antibiotics, e.g., imipenem , or Penicillin Binding Protein 3-specific (PBP 3) antibiotics, e.g., cephalosporins) (PBP 2-specific or PBP 3-specific antibiotics) [25]. In a recent clinical monocenter study the in vitro results have been confirmed in vivo [26].

The objective of this European multi-center-trial was to test the following hypotheses:

1. Surgical intensive care patients with Fc-R II HH receptor status may be associated with decreased rate of complications and lethal outcome,
2. Endotoxin, Endotoxin neutralizing capacity, plasma IL-6 and IL-8, sCD-14, and cell-associated IL-8 may correlate with severe complications and outcome,
3. Antibiotic treatment may induce endotoxin release resulting in different IL-6 plasma levels which are associated with outcome,
4. Multivariate analysis of disease-associated variables and scores will improve prediction for outcome in patients with sepsis

Patients and Methods

Patients and Experimental Design

Surgical patients admitted for major elective surgery, e.g., esophagectomy, gastrectomy, pancreatectomy, colectomy, or for emergency surgery, e.g., acute pancreatitis, intra-abdominal trauma, requiring intensive care therapy for two days or more, were studied in eleven European university or large community departments of surgery. Patients requiring therapy which may interfere with the immune system, e.g., corticosteroids, cyclosporin, were excluded from the study. The study design has been already described elsewhere [27]. The study was approved by the appropriate institutional review board according to the guidelines in each center. Between March 1996 and July 1997 128 patients were enrolled. The study was monitored by on-site visits of the coordinator or an assistant. The blood samples were obtained on admission to the ICU, the day of the operation, 120 and 180 minutes after antibiotic administration on admission to the ICU, and on days three, seven and nine after admission to the ICU. Blood samples were obtained in vacuum endotoxin-free tubes (Chromogenix, Essen, Germany) and centrifuged at 600 g for twenty minutes within thirty minutes after collection of blood. Samples were kept at 4° to 10° C during centrifugation and thereafter. Samples were then stored at -80° C until further processing.

Biological Assessments

Plasma IL-6 and IL-8 were determined by a solid phase, two-site chemiluminiscent enzyme immunometric assay (Immulite®, DPC, Bad Nauheim, Germany). This test allows single determination of cytokines within approximately 70 minutes (plasma IL-6: normal 0-11.3 pg/ml; sensitivity 1 pg/ml; range 2 - 2000 pg/ml; plasma IL-8: normal 0-70 pg/ml; sensitivity: 6.2 pg/ml; range 20 - 10.000 pg/ml). Cell-associated IL-8 was determined at the Institut Pasteur according to a recently published method by Cavaillon et al. (normal 65 - 210 pg/ml; minimal detection limit 60 pg/ml) [28]. Soluble CD-14 was determined by a commercial sandwich enzyme immuno assay (IBL, Gesellschaft für Immunchemie und Immunbiologie; Hamburg, Germany) (normal 1.4 - 4.5 (g/ml; sensitivity < 1.0 ng/ml). Fc-receptor analysis was performed after DNA-isolation in whole blood (Puregene Isolationkit, Biozym, Hess. Oldendorf, Germany) according to a method published by van den Herik-Oudijk et al. (H/H: high responder; H/R: intermediate responder; RR: low responder) [29]. For measurement of endotoxin and endotoxin neutralizing capacity (ENC) we used the turbidimetric, kinetic LAL-test with internal standardization as described by Urbaschek et al. [30]. Endotoxin standard (NP-3 (KSE) endotoxin standard, Salmonella abortus equi, 100 ng/ml) and lysate (Pyrospektro, Limulus amoebocyte lysate (LAL), Cape Cod) were provided by Pyroquant Diagnostik (Walldorf, Germany). Each sample was spiked by a known concentration of endotoxin. The kinetic reaction was read continuously in an ELISA plate reader (Molecular Devices, MWG Biotech, Ebersberg, Germany) and the endotoxin

concentration was calculated by a special software program. The sensitivity of the endotoxin test was 0.5 pg/ml. The cut-off point for endotoxin in this study was 0.5 pg/ml which is also the sensitivity of the endotoxin test we have used. The absolute amount of endotoxin measured at one single time point does not necessarily correlate to the clinical signs of sepsis; small amounts of endotoxin above 0.5 pg/ml, however, may suffice for the establishment of an acute phase reaction. It was the purpose of the study to investigate the effect endotoxin-positivity may have on immunological and clinical parameters. Endotoxin determination at one time point alone does not help to differentiate healthy volunteers from septic patients. It was therefore recommended by Urbaschek et al. to have several time points for measurement of endotoxin for analysis. Unheated samples were tested in a similar way for ENC. In the absence of adequate references it is difficult to assess the sensitivity and specificity of ENC measurements. ENC is an index describing the activity of human plasma to neutralize endotoxin added to the plasma in known quantities; it is influenced by plasma proteins and other factors known to neutralize endotoxin. Heating denatures the proteins, interfering with ENC measurements. Endotoxin Neutralizing Capacity is not expressed in pg/ml according to Urbaschek et al. but as index. The plasma is not heated and therefor the proteins which normally neutralize or bind endotoxin are preserved. Endotoxin is then added in known concentrations, then the ELISA reader analyzes the turbidimetric reaction and compares the capacity of the plasma to neutralize endotoxin. The analysis of this turbidimetric reaction is evaluated by a special software program, which was supported by the Fraunhofer Gesellschaft, and indicates the ability to neutralize endotoxin as an index ranging from 0 to 5000, where 5000 is the best result to neutralize endotoxin.

Clinical Assessments

The following items were recorded in all patients: diagnosis, operation, complications, APACHE II score, Multi-Organ-Failure score [31] (Goris), Multi-Organ-Dysfunction (MODS) score [32], type of antibiotics used, laboratory data (e.g., leukocytes, thrombocytes), and outcome. Aside of the multi-organ failures scores, definitions of the surgical site infections of the Center of Disease Control (CDC) (Atlanta, USA) and a complication key (not significant for the clinical course, moderate, severe) were used for registration of complications. Complications were classified as 0 = not significant for clinical outcome, 1 = moderate, 2 = severe for the respiratory, cardiovascular and renal system, bleeding, other nosocomial infections, GI tract complication, and wound healing. The surgical site infections (SSI) were classified according to the guidelines of the Center of Disease Control, Atlanta, USA in 1. Incisional surgical site infections a. superficial incisional, b. deep incisional and 2. Organ/space surgical site infection, nosocomial pneumonia, nosocomial urinary tract infection, blood stream infections, clinical sepsis. Furthermore we have used the Surgical Infection Society of North America definition of intra-abdominal infections [33].

Data Analysis

Descriptive statistics covered frequency for qualitative data and mean-standard deviation for quantitative data. Univariate comparisons between independent groups were performed by chi-square test using cut-off points for the immunological parameters, e.g., IL-6 $\geq$ 1000 pg/ml. For multivariate analysis stepwise logistic regression was used (BMDP LR, default values). Differences were considered significant when $p < 0.05$.

Results

In total 128 of 156 patients were eligible for statistical analysis. The following centers have contributed patients: Pisa (n = 7, 1 died), Rome (n = 9, 2 died), Santiago de Compostela (n = 4, 2 died), Barcelona (n = 8, none died), Foch/Paris (n=4, 1 died), Manchester (n = 10, 3 died), Oslo (n = 10, 1 died), Freiburg (n = 21, none died), Würzburg (n = 23, 10 died), Graz (n = 1, none died), Erlangen (n = 31, none died), Poissy/Paris (n = 0). 28 patients could not be enrolled due to missing data or samples. 87 of the 128 patients were male and 41 female. The mean APACHE II score on admission to the intensive care unit was 9.6 (7.2 SD; range 0-41). 20 patients (%) died and 108 patients survived. There were 116 elective operations and 12 emergency operations. Most patients were treated for a malignant disease of the gastro-intestinal tract (61%). Material tested for bacteriology were blood cultures, intravenous line tips, urine, intra-abdominal smears, wound smears, and tracheal aspiration, or sputum. The bacteriological test results show gram-positive cocci (13%), gram-negative rods (7%) and polymicrobial infection (9%). The patients received antibiotic prophylaxis perioperatively, mostly cephalosporins. While in ICU 94 patients received antibiotic treatment: cephalosporin with or without metronidazole (n = 37), imipenem (n = 11), quinolones or other antibiotics (n = 46).

Univariate analysis showed no difference with regard to the prediction of complication or death for age, gender, GI-tract resection, leukocytes, soluble CD-14, endotoxin, and Fc-receptor polymorphism. Admission status, malignant disease, treatment with catecholamines, continuous ventilatory support or comorbidity was associated with a higher risk for complications and death. APACHE II score ($\geq$ 10), MOF-score ($\geq$ 5), MODS-score ($\geq$ 3), plasma IL-6 ($\geq$ 1000 pg/ml), plasma IL-8 ($\geq$ 70 pg/ml), cell associated IL-8 ($\geq$ 1000 pg/ml) and endotoxin neutralizing capacity ($\leq$ 100) were significantly associated with complications or death by univariate analysis. (Table 1 and 2).

By multivariate analysis the parameters MOF-score, MODS-score, plasma IL-6, platelet count and comorbidity predicted complications with a sensitivity of 82% and a specificity of 87%. (Table 3)

APACHE II score, plasma IL-8 and the presence of a severe complication predicted death according to the multivariate analysis with a sensitivity of 84% and a specificity of 90%. (Table 4)

There was no preference for any antibiotic regimen. The patients treated with imipenem had already signs of

Table 1. Univariate analysis of acute physiology parameters, scores and immune parameters for complication: n.s. not significant; IL-6, IL-8, cell associated IL-8 measured in pg/ml; endotoxin measured in pg/ml; sCD-14 soluble CD-14 measured in µg/ml; HH high responder; RR low responder; ENC Endotoxin Neutralizing Capacity (Index).

Variable	No/moderate N (%)	Severe N (%)	Total N	p-value
Age				n.s.
< 50 years	21 (81)	5 (19)	26	
≥ 50 years	70 (69)	32 (31)	102	
Gender				n.s.
Male	60 (69)	27 (31)	87	
Female	31 (76)	10 (24)	41	
Malignancy				0.001
Yes	64 (82)	14 (18)	78	
No	27 (54)	23 (46)	50	
GI-tract resection				n.s
Yes	64 (75)	21 (25)	85	
No	27 (63)	16 (37)	43	
Comorbidity				0.001
Yes	23 (50)	23 (50)	46	
No	68 (83)	14 (17)	82	
APACHE II				0.001
≥ 10	23 (44)	29 (56)	52	
< 10	68 (90)	8 (11)	76	
Continuous ventilatory support				0.001
Yes	12 (34)	23 (66)	35	
No	79 (85)	14 (15)	93	
Catecholamines				0.001
Yes	9 (32)	19 (68)	28	
No	82 (82)	18 (18)	100	
Leukocytes				n.s.
≥ 10.000	49 (72)	19 (28)	68	
< 10.000	38 (69)	17 (31)	55	
Thrombocytes				0.001
≥ 100.000	85 (79)	23 (21)	108	
< 100.000	4 (22)	14 (78)	18	
Goris Score				0.001
≥ 5	3 (14)	19 (86)	22	
< 5	88 (83)	18 (17)	106	
Marshall Score				0.001
≥ 3	12 (31)	27 (69)	39	
< 3	78 (90)	9 (10)	87	
Plasma IL-6				0.001
≥ 1000	16 (49)	17 (52)	33	
< 1000	72 (81)	17 (19)	89	
Plasma IL-8				0.001
> 70	18 (47)	20 (53)	38	
≤ 70	69 (83)	14 (17)	83	
Cell assoc. IL-8				0.001
≤ 1000	43 (90)	5 (10)	48	
> 1000	44 (62)	27 (38)	71	
sCD-14				n.s.
> 4.5	52 (66)	27 (34)	79	
≤ 4.5	18 (69)	8 (31)	26	
Endotoxin				n.s.
≥ 0.5	70 (70)	30 (30)	100	
< 0.5	17 (81)	4 (19)	21	
ENC				0.04
≤ 100	47 (66)	24 (34)	71	
> 100	41 (84)	8 (16)	49	
Fcγ-RIIa				n.s.
HH	21 (72)	8 (28)	29	
RR	12 (63)	7 (37)	19	
Admission status				0.001
Emergency	3 (25)	9 (75)	12	
Elective	88 (76)	28 (24)	116	

Table 2. Univariate analysis of acute physiology parameters, scores and immune parameters for death: n.s. not significant; IL-6, IL-8, cell associated IL-8 measured in pg/ml; endotoxin measured in pg/ml; sCD-14 soluble CD-14 measured in µg/ml; HH high responder; RR low responder; ENC Endotoxin Neutralizing Capacity (Index).

Variable	Non-Survivor N (%)	Survivor N (%)	Total N	p-value
Age				n.s.
< 50 years	1 (4)	25 (96)	26	
≥ 50 years	19 (19)	83 (81)	102	
Gender				n.s.
Male	12 (14)	75 (86)	87	
Female	8 (20)	33 (81)	41	
Malignancy				0.001
Yes	5 (6)	73 (94)	78	
No	15 (30)	35 (70)	50	
GI-tract resection				n.s.
Yes	10 (12)	75 (88)	85	
No	10 (23)	33 (77)	43	
Comorbidity				0.005
Yes	13 (28)	33 (72)	46	
No	7 (9)	75 (92)	82	
APACHE II				0.001
≥ 10	18 (35)	34 (65)	52	
< 10	2 (3)	74 (97)	76	
Continuous ventilatory support				0.001
Yes	12 (34)	23 (66)	35	
No	8 (9)	85 (91)	93	
Catecholamines				0.005
Yes	10 (36)	18 (64)	28	
No	10 (10)	90 (90)	100	
Leukocytes				n.s.
≥ 10.000	12 (18)	56 (82)	68	
< 10.000	8 (15)	47 (86)	55	
Thrombocytes				0.005
≥ 100.000	12 (11)	96 (89)	108	
< 100.000	8 (44)	10 (56)	18	
Goris Score				0.001
≥ 5	12 (55)	10 (46)	22	
< 5	8 (8)	98 (93)	106	
Marshall Score				0.001
≥ 3	14 (36)	25 (64)	39	
< 3	5 (6)	82 (94)	87	
Plasma IL-6				0.01
≥ 1000	10 (30)	23 (70)	33	
< 1000	9 (10)	80 (90)	89	
Plasma IL-8				0.001
> 70	13 (34)	25 (66)	38	
≤ 70	6 (7)	77 (93)	83	
Cell assoc. IL-8				0.04
≤ 1000	3 (6)	45 (94)	48	
> 1000	14 (20)	57 (80)	71	
SCD-14				n.s.
> 4.5	15 (19)	64 (81)	79	
≤ 4.5	4 (15)	22 (85)	26	
Endotoxin				n.s.
≥ 0.5	18 (18)	82 (82)	100	
< 0.5	1 (5)	20 (95)	21	
ENC				0.04
≤ 100	15 (21)	56 (79)	71	
> 100	3 (6)	46 (94)	49	
FcγRIIa				n.s.
HH	6(21)	23 (79)	29	
RR	4 (21)	15 (79)	19	
Admission status				0.005
Emergency	6 (50)	6 (50)	12	
Elective	14 (12)	102 (88)	116	
Complications				0.001
Moderate/no complication	1 (1)	90 (99)	91	
Severe complication	19 (51)	18 (49)	37	

Table 3. Multivariate analysis of scores and immune parameters for complication: IL-6 measured in pg/ml.

Variable	Cut-off level	Coefficient (β)	β/SE	Relative risk Exp (β)	p-value	Sensitivity	Specificity
						82*	87*
Goris	5	2.432	2.91	11.4	0.001		
Marshall	3	1.419	2.11	4.13	0.001		
Plasma IL-6	1000	1.560	2.46	4.76	0.008		
Thrombo-cytes	< 100.000	2.047	2.229	7.74	0.027		
Comorbidity	Yes	1.083	1.67	2.95	0.097		
Constant		-3.458	-5.59	0.03	-		

Cut-off point = 0.3

Table 4. Multivariate analysis of scores and immune parameters for death: IL-8 measured in pg/ml.

Variable	Cut-off level	Coefficient (β)	β/SE	Relative risk Exp (β)	p-value	Sensitivity	Specificity
						84*	90*
APACHE II	10	1.986	2.19	7.28	0.022		
Plasma IL-8	> 70	1.162	1.60	3.20	0.104		
Complication	Severe	3.712	3.40	40.9	0.001		
Constant		-5.816	-4.50	0.003	-		

Cut-off point = 0.3

Table 5. MOF/MODS scores and plasma IL-6 levels after antibiotic administration.

Antibiotics	MOF-score (median)	MODS-score (median)	IL-6 pg/ml (median) on admission	N	IL-6 pg/ml (median) 120 minutes after antibiotic administration	N	IL-6 pg/ml (median) 180 minutes after antibiotic administration	N
Imipenem	5	5	275	11	134	8	162	8
Cephalosporin/ cephalosporin + metronidazole	0	1	724	9	588	9	515.5	9
p-value	0.03	0.09	0.04		0.07		0.08	

organ failure whereas patients treated with cephalosporins did not. The MOF-score was significantly increased ($p < 0.05$) compared to patients treated with cephalosporins; however, the IL-6 plasma levels of the imipenem patients were significantly lower than in patients with cephalosporin/ cephalosporin plus metronidazole treatment. (Table 5). Only 22% (n = 2) of patients treated with imipenem were endotoxin positive on admission to the ICU and 11% (n = 1) at 120 minutes after administration of antibiotics (Total n = 9). Although 64% (n = 7: total n = 11) of patients treated with cephalosporins were endotoxin positive on admission to ICU and 50% (n =4 ; total n = 8) 120 minutes after antibiotics administration, this difference did not reach statistical difference ($p = 0.09$, $p = 0.13$ respectively; 0.5 pg/ml was considered to be endotoxin-positive). There was no difference in the rate of complication and death in the two patient groups. Despite increased multi-organ failure scores at the beginning of imipenem treatment, a strong sign for serious sepsis, there was no difference in outcome to other patients and IL-6 levels which are a signal of acute phase response decreased substantially during treatment with imipenem.

Discussion

Sepsis and peritonitis remain an imminent danger for surgical patients admitted to an intensive care unit. Ten percent of patients in the ICU suffer from sepsis, 6% from severe sepsis and 2-3% from septic shock. SIRS occurs in 40 – 70% of all patients admitted to the ICU. The mortality of SIRS ranges from 6-7% and in septic shock more than 50%. Abdominal sepsis exhibits the highest mortality rate (72%) [34, 35]. Scores, cytokines, and other inflammatory mediators were analyzed for their potential ability to predict outcome and complications in surgical patients. However, in most studies the number of patients enrolled is rather small and seldom

several centers were included in such a study. Most of the immunological parameters seem to be still in the experimental phase and the clinical significance remains to be elucidated. The objective of this study was to study the clinical significance of several immune parameters (Fc-RIIa receptor polymorphism, endotoxin, endotoxin neutralizing capacity, plasma IL-6, plasma IL-8, cell-associated IL-8, sCD-14) on the day of admission to the surgical intensive care unit (the day of operation, in general), days three, seven and nine after admission with regard to complications and outcome. For stratification of the patients APACHE II score was evaluated. The development of multi-organ-failure in surgical intensive care patients was monitored using the MOF-score (Goris) and the MODS score (Marshall). To the best of our knowledge this is the first time that the significance of cytokines and scores for clinical outcome and complications has been investigated as primary objective in a multicenter study in Europe in surgical intensive care patients and did not result as a by-product from other study objectives, e.g. assurance of balance among treatment groups in pharmaceutical trials and/or single institution study with a limited number of genetically similar patients.

FC-Receptor Polymorphism

There is evidence that genetic polymorphism, e.g., Fc-receptor polymorphism, may be an important component of the predisposition to develop an infection [36]. Receptors for the Fc domain of IgG (Fcγ R) provide a critical link between specific humoral responses and the cellular branch of the immune system. By the interaction of this receptor with immunoglobulin a variety of biological responses are triggered, e.g. phagocytosis, release of inflammatory mediators and enhancement of antigen presentation [16]. The capacity of polymorphonuclear leukocytes homozygous for Fcγ IIa - H/H131 for IgG2 opsonized bacteria is significantly higher than phagocytosis by PMN homozygous for Fcγ RIIa - R/R131, independent of the Fcγ RIIb - NA1/NA2 (CD16) allelic polymorphism. The clinical significance of this polymorphism has been tested in 48 children with recurrent bacterial respiratory tract infections [17]. However, the significance of Fc-receptor polymorphism was not yet studied in surgical patients admitted to an intensive care unit. In this multi-center study most patients (34.4%) demonstrated an intermediate type (Fcγ RIIa-H/R131). 29 patients (22.7%) were homozygous for Fcγ RIIa-H/H131 and 19 patients (14.8%) homozygous for Fcγ RIIa-R/R131. This is in agreement with the normal values of Fc frequency in normal caucasians: HH 29%, HR 48%, RR 23% (P. Capel, personal communication). With regard to complications or outcome we did not find any difference in the surgical ICU patients. The role of antibacterial IgG2 which may be crucial in the immune defense against Staph. Aureus and Haemophilus influenzae type B, in opsonization and phagocytosis may be limited in patients homozygous for Fcγ RIIa H/R [37]. However, no pathogens were isolated in 72% of the study population and Gram-positive cocci, e.g. Staphylococcus aureus, were found in 16 patients (12.5%) only. The pathogens were isolated in blood, intra-abdominal smears, tracheal aspiration, urine. Surgical patients in intensive care units are associated with polymicrobial infections; the isolation of a single pathogen in septic surgical patients is rather uncommon. This may limit the conclusion for genetic Fc-receptor polymorphism in this study. The increased incidence and resistance of Gram-positive pathogens in intensive care units warrants further clarification of the role of Fc-receptor polymorphism.

Endotoxin and Endotoxin Neutralizing Capacity

Endotoxin has been considered a major trigger for the inflammatory response in sepsis and peritonitis. However, there are reports that endotoxin may not be necessary to trigger the inflammatory response [38]. It is known to us that the Limulus Amebocyte Lysate assay has its limitations. Endotoxin may be neutralized by plasma proteins, lipids and other compounds which may interfere with the endotoxin assays [30]. However, the kinetic chromogenic LAL assay with an internal standard used in this study has demonstrated that it may produce reliable results in surgical patients [39]. In many patients with gram-negative sepsis endotoxin may not be detected by the LAL assay [40]; however, we observed positive endotoxin plasma levels in 83% of our patients. Endotoxin plasma levels were not different in survivors and non-survivors, and thus may not be relevant for the prediction of outcome. However, there is evidence that not the absolute endotoxin plasma level may affect the outcome but the immune response of the patient to even minute endotoxin levels [30, 39]. The determination of endotoxin neutralizing capacity (ENC) may be a better reflection of this host response. In this study a decreased ENC titer (< 100) was associated with more complications ($p < 0.05$) and an increased death rate ($p < 0.05$). This is in agreement with findings in 92 postsurgical patients where the determination of ENC made the differentiation possible between patients with and without complications [41]. Other studies have successfully demonstrated that the neutralization of LPS by polymyxin B, lysozyme, immunoglobulins may be detected by an ENC assay [42]. There are only few reports available on endotoxin neutralizing capacity evaluation and certainly the detection of the responsible protein would help to establish this test for larger clinical use.

Plasma IL-6 and IL-8

There were several reports on the prognostic potential of IL-6 [6, 7, 8, 9] and IL-8 [11, 12] in surgical or critical care patients. IL-8, a proinflammatory cytokine, potent chemoattractant factor and activator of neutrophils is produced by many cell types after stimulation by IL-1, TNF or endotoxin. In septic patients high amounts of circulating IL-8 concentrations correlate with fatal outcome, whereas only low plasma concentrations of IL-8 are present in patients with non-septic multiple organ failure [14]. Thus the determination of IL-8 may help to discriminate between septic and non-septic multi-organ failure.

Although it has been demonstrated by in-vitro and

animal experiments that both cytokines, IL-6 and IL-8, are involved in the inflammatory response and outcome the clinical significance remains to be established. Comparable recognition of cytokines in biological samples was not possible in many commercial ELISA kits [43]. The determination of cytokines by ELISA did not meet the expectations of clinicians until recently a method for single sample and fast bed side cytokine analysis (Immulite®) became available which was used for IL-6 and IL-8 cytokine determination. In this study plasma IL-6 (cut-off level: 1000 pg/ml) was associated with significantly more complications ($p < 0.001$) and more deaths ($p < 0.01$). This is in agreement with the results of a study in patients with trauma and hemorrhagic shock where IL-6 concentrations were significantly elevated in patients with ARDS/MOF and IL-6 showed a good correlation with the daily MOF score during the whole 2 week observation period [44]. Plasma IL-8 (cut-off level: >70 pg/ml) correlated with more complications ($p < 0.001$) and increased incidence of death ($p < 0.001$). This in contrast to results reported by Fröhlich et al. who did not find significant differences in the intra-abdominal or plasma IL-8 levels between survivors and non-survivors [45]. Cavaillon has recently stated that plasma cytokine determination may only be the tip of the iceberg [46]. The determination of cell-associated cytokines showed superior results in critical care patients with regard to complications [47]. IL-8 may discriminate between septic and non-septic origin of the multiple organ failure [14], which may have significant clinical relevance in ICU patients. In this study, patients with increased cell-associated IL-8 (cut-off level: > 1000 pg/ml) showed significantly more complications ($p < 0.001$) and were associated with lethal outcome ($p < 0.04$). Increased cell-associated IL-8 and plasma IL-6 levels correlated to the presence of pathogens in ICU patients. Whether cytokine cut-off levels may be used for the diagnosis of severe bacterial infection in intensive care units may be an objective for further studies. However, this multi center study confirms an earlier report by Cavaillon that increased levels of cell-associated IL-8 correlates to MOF and complications [47].

Soluble CD-14

CD14, a glycopeptid (53 kDa) attached to the membrane of monocytes via phosphatidylinositol, is a specific marker for monocytes and functions as receptor for endotoxin [48]. It is highly concentrated on the surface of peripheral monocytes, macrophages and activated granulocytes (mCD14) [49]. Soluble CD 14 levels were increased in sepsis, polytrauma and severe burns [18]. sCD 14 seem to have a prognostic function in diseases where monocytes, macrophages and B-cells are involved. However, Ertel et al. have reported that binding of the LPS-LPS binding protein complex to the CD14 receptor may not play a pivotal role in sepsis [50]. This is in agreement with the results of this study. Soluble CD14 levels increased only marginally from 6.7 ± 3.1 (µg/ml; mean/SD) on admission to 8.1 ± 3.7 (µg/ml; mean/SD) on day 3 after admission to the SICU. There was no difference detectable in patients with complications and / or death when compared to patients without complications or surviving patients. The sCD-14 levels may be influenced by the amount of LPS in the plasma or anti-inflammatory cytokine release. LPS at low dose may increase CD14, at high doses decrease CD14 [51]. Cytokines, e.g., IL-4, were successful in downregulating the spontaneous sCD-14 release [19]. Recent findings demonstrated that sCD-14 may not even block the effect of LPS which may indicate that the clinical role of this compound remains to be established [52].

Scores

APACHE II score has been evaluated in several clinical sepsis studies for its predictive capacity of outcome [53, 54]. It has been suggested that neither APACHE II score nor clinical judgement were reliable when obtained within 24 hours of admission [55]. Unfortunately the inflammatory response in the first 24 hours may be decisive for outcome. The determination of multi-organ-failure or multiple organ dysfunction may be a reliable tool for the prediction of outcome in intensive care units [21, 22]. The combination of several scores and/or mediators may improve the reliability to predict outcome. It has been demonstrated that an increased mTNF/TNFR ratio correlates with high specificity to develop organ failure [56]. Casey et al. found that a "Lipopolysaccharide-cytokine score" based on endotoxin and cytokine concentrations, was associated with mortality [57]. In this study APACHE II score (cut-off level: 10) was associated with complications ($p < 0.001$) and death ($p < 0.001$). The multi-organ-failure score (Goris) correlated with complications ($p < 0.001$) and death ($p < 0.001$) (cut-off level: 5). The MODS score (Marshall) correlated with complication ($p < 0.001$) and death ($p < 0.001$) (cut-off level: 3). However, sensitivity and specificity of each score alone were rather different. The sensitivity to detect complications by the Goris score was rather low (51%) when compared to APACHE II score (78%) or endotoxin neutralizing capacity (72%). The sensitivity and specificity was improved when we used Goris (cut-off level: 5), Marshall (cut-off level: 3), plasma IL-6 (cut-off level: >1000 pg/ml), platelets (cut-off level: <100000) and comorbid disease: sensitivity 82% and specificity 87% (multivariate analysis). APACHE II cut-off level: (10), plasma IL-8 (cut-off level: >70 pg/ml) and severe complications could predict death with a sensitivity of 84% and a specificity of 90%. It has been suggested that organ dysfunction may better predict mortality than the acute physiology parameters measured by the APACHE score [58]. Marshall et al. developed a multiple organ dysfunction score as a reliable descriptor of a complex clinical outcome. ICU mortality was approximately 25% at 9 – 12 points [22]. However, with regard to the existing studies it makes sense to use both, APACHE score and MODS/MOF score, in surgical ICU patients; APACHE II scoring may be best for the grading of the severity of disease on admission to intensive care while MODS/MOF score is best for monitoring the degree of organ dysfunction and the intensity of supportive treatment [59]. The results of this study are in agreement with a recent report by Ohmann et al. who demonstrated that APACHE II score or Goris score alone were not of

particular use for therapeutic decision making in peritonitis [60]. In contrast to this study, Calvano et al. reported that MODS scores of 18 patients admitted to an intensive care unit with signs of sepsis were predictive for outcome, while plasma interleukin-6 was not. Compared to this study there is not only a difference in the patient population - entry criteria were sepsis syndrome - compared to this study; the authors agree that the study population was rather small. Nevertheless, this type of study may help to identify the clinical significance of cellular biological parameters [23].

Antibiotic Induced Endotoxin Release

There is increasing evidence that standard intensive care therapy may influence endotoxin and mediator release in patients with systemic inflammatory response syndrome [61]. Cell wall-active antibiotics differ in their mechanism of action in disrupting microbial growth and in their relative ability to induce the release of biologically active endotoxin both in vitro and in vivo [62]. The clinical significance of endotoxin release is subject of an open dispute. Endotoxin, although an important trigger, may not be the only factor to induce cytokine release [63]. Few clinical data are available on the antibiotic induced endotoxin release. In a posthoc analysis from a prospective randomized study designed to evaluate the efficacy of interferon gamma in preventing infection and death in trauma patients, it was demonstrated that antibiotics which were associated with greater release of endotoxin and production of TNF were also associated with a greater mortality in septic trauma patients [64]. In this study patients with a significant elevated MOF-score ($p < 0.05$) were treated with imipenem compared to patients treated with cephalosporins. IL-6 plasma levels were, however, significantly decreased in the patient group treated by imipenem ($p < 0.05$). These decreased IL-6 plasma levels were also observed 120 minutes and 180 minutes after antibiotic administration (Table 5). Due to the small numbers of patients in each group the imipenem treated patients did not have significantly less positive endotoxin test results ($p = 0.09$). Although there was a difference in the severity of organ failure we did not observe a difference in outcome.

It is evident that due to the enrollment criteria - the treating physician had the choice to use cephalosporins, imipenem or other antibiotics - the number of patients with imipenem treatment was rather low. The results of this study add evidence that the mechanism of antibiotic action may have a substantial effect in surgical ICU patients. The true effect on clinical parameters, e.g., cardiovascular system, may only be detected by careful monitoring. It should also be noted that in most clinical studies comparing antibiotics there is no difference in clinical and bacteriological outcome [65].

Acknowledgement: The authors like to thank Catherine Fitting, Institut Pasteur, Paris for the assistance in tests and the organization of the study. Dr. Renate Urbaschek, University Heidelberg has supported the endotoxin and ENC determination. Dr. Rosella DiStefano, Prof. A. Torres, Barcelona; Dr. S. Dalban-Sillas, Hopital Foch, Paris; Dr. J. Lagunilla, Santiago de Compostela; Dr. A. Fingerhut and J.Ch. Etienne, Hopital Poissy, Paris, Prof. A. Aasen and Mrs. C. Okkenhaug, Oslo, Norway, were active in the patient enrollment and data acquisition. Dr. Q. Yang, Theoretical Surgery, University of Düsseldorf, was especially helpful in the thorough statistical evaluation.

The following companies have generously supported the study: Merck Inc. USA (Medical School Grant), Novartis, Germany; Biotest Pharma, Germany; Eli Lilly, Germany; DPC, Germany; Smith Kline Beecham, Germany; Pfizer, Germany; Rhone-Poulenc-Rorer, Ger-many; Essex Pharma, Germany.

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Received: March 14, 2000 / Accepted: May 16, 2000

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