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Correlations between APACHE II Score and Plasma Levels of Cytokines in Postsurgical Patients

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This study was designed to assess whether plasma cytokines correlated with the clinical status of postsurgical patients. Plasma levels of cytokines in 72 patients admitted to the intensive care unit were measured using an Enzyme Linked Immuno Solubent Assay. The clinical status was evaluated with the Acute Physiology Age and Chronic Health Evaluation (APACHE) II scoring system. There were significant correlations between the APACHE II score and the plasma levels of Tumor Necrosis Factor (TNF) α and interleukin (IL) -1 β. But IL-8 and IL-6 did not correlate with the APACHE II score. Three patients died within postoperative 20 days. The plasma levels of TNF α, IL-1 β, IL-8 and IL-6 were significantly higher in nonsurvivors than in survivors. There was no significant difference in the APACHE II score and the plasma levels of IL-1 β, IL-8 and IL-6 between the survivors with Systemic Inflammatory Response Syndrome (SIRS) and those without SIRS. The survivors without SIRS had a higher concentration of TNF α than those with SIRS.

The results indicate that TNF α and IL-1 β correlate well with the severity of illness in the postsurgical patients, whereas IL-8 and IL-6 does not.

Key words : proinflammatory cytokines, critical illness, acute physiology age and chronic health evaluation (APACHE) II score, systemic inflammatory response syndrome (SIRS)

Introduction

There are increasing experimental and clinical evidences that a number of cytokines plays a pivotal role in the response to injury and infection and in the development of organ damage in critically ill patients. Sepsis is the most important cause of mortality in the intensive care units (ICU). At present, sepsis is understood to be the inflammatory response of the host to infection, rather than a direct effect of microbial aggression. From the clinical standpoint, this inflammatory response is known as systemic inflammatory response syndrome (SIRS). SIRS can result either from an infection i.e., sepsis or from a noninfectious insult, e.g., trauma, pancreatitis, or burns. Pathophysiologically, SIRS is characterized by the activation of several groups of cells (monocytes/macrophages, polymorphonuclears and endothelial cells) and by the release of inflammatory mediators, such as cytokines and others. TNF α seems to play a major role in SIRS secondary to infection, burns, trauma, hemorrhagic shock and pancreatitis[1]. Although patients undergoing major surgery are also known to suffer from a postsurgical systemic inflammatory response, the nature of which remains to be fully elucidated.

The Acute Physiology Age and Chronic Health Evaluation II (APACHE II) has been introduced as the scoring system to classify a severity of disease state[2]. The APACHE II score has been shown to correlate with the risk of death in the medical and surgical ICU and to predict the outcome of patients.

The present study was designed to investigate the role of cytokines in disease states of postsurgical patients. For this purpose, we measured plasma levels of TNF α, IL-1 β, IL-6 and IL-8 within 24 hours after the ICU admission, and compared these with the overall outcome and the APACHE II score.

Materials and Methods

Patients

Ethical Committee approval and informed patient consent were obtained. The subjects were seventy-two patients admitted to the general intensive care unit of Nagasaki University Hospital and five healthy controls. The admissions included surgical patients having a wide range of diagnosis. Survival was defined as discharged from the intensive care unit. For all patients, the usual clinical and biologic parameters, temperature and leukocytes were measured. The severity of illness was scored according to the APACHE II scoring system by an independent evaluator within 24 hours after admission. At the same time, a blood sample was drawn. Ethylenediaminetetraacetic acid was used as anticoagulant in the collection

vessels. Plasma was separated by centrifugation, immediately frozen and stored in divided aliquots at −40℃ until measurement of cytokines.

SIRS is defined by two or more of the following conditions : a) a temperature of >38℃ or <36℃ ; b) an increased heart rate of >90 beats/min ; c) tachypnea, as manifested by a respiratory rate of >20 breaths/min or hyperventilation, as indicated by a $PaCO_2$ of <32 torr (<4.3 kPa) ; and d) an altered WBC count of $>12{,}000$ cells/mm^3, or <4000 cells/mm^3, or the presence of $>10\%$ immature neutrophils. This definition was applied to the 72 patients, out of whom 51 patients (71%) were identified as SIRS.

Cytokine Assays

TNF α, IL-1 β, IL-8 and IL-6 were measured by ELISA system. A commercially available kits (Amersham International plc, England) were used. These assays employed the quantitative immunometric, 'sandwich' enzyme immunoassay technique. All assays were done in duplicate. The sensitivities of these cytokine assays were as follows : 7.5 pg/ml for TNF α, 0.3 pg/ml for IL-1 β, 18.1 pg/ml for IL-8, and 4.4 pg/ml for IL-6 respectively.

Statistical analyse

Data management and calculations were performed on Stat View-J 4.5 (Hulinks, Abacus Concepts, Inc., Berkeley, CA). Comparisons of biologic parameters or clinical scores between two groups of patients were made using two-tailed unpaired Student's test ; if the standard deviations of the groups being tested were significantly different (F-test), the Welch's test was used. When appropriate, non-parametric test (Mann-Whitney U test) was used (specific tests are indicated in results). Between-group evaluation of each variable was conducted using one-way analysis of variance. When significant differences were found, Scheffe's corrected paired t-test was employed as post-hoc testing. The relationships between biologic or clinical parameters were tested by linear regression using standard formulas. All data were presented as mean ± standard error (SE). The level of statistical significance was set at $p<0.05$.

Table 1. Demographic Data of the Study Population

Diagnosis	N	Age	Sex(F/M)	Death	SIRS
Aortic anurysm	13	68±3	4/9	2 (≦12hr)	6
Abdominal	9			1	
Thoracic	3			1	
Ischemic heart disease	9	60±3	1/8	0	7
Valvular disease	17	53±2*, #	8/9	0	15
Cancer	20	67±3	8/12	0	12
Lung	4				
Esophageal	2				
Gastric	4				
Rectal	6				
others	4				
Arterioscleosis obliterans	5	62±3	1/4	0	4
Others	8	47±6*, #	2/6	1 (after 3weeks)	9
Total	72		24/48	3	51

SIRS = Systemic Inflammatory Response Syndrome
Values are mean ± SE
* : different from aortic aneurysm, # : differnt from cancer, $p<0.05$ Scheffe's t-test

Table 2. Plasma Cytokine Levels and APACHE II scores in Healthy Controls and Patients

	Age	APACHE II score	Plasma level (pg/ml)			
			TNF α	IL-1 β	IL-8	IL-6
Control (n=5)	31±2		0.2±0.1	2.1±0.3	0.9±0.4	1.8±0.3
patient (n=72)	60±2*	10.0±0.8	6.9±1.0*	4.3±0.8*	448.3±218.1*	182.2±19.6*
*$p<0.05$ different from control						
Aortic aneurysm (n=11)		9.3±0.9	9.7±2.6	2.9±0.2	143.6±66.6	231.0±43.5
Ischemic heart disease (n=9)		9.7±1.7	4.1±1.5	2.5±0.3	49.6±17.3	100.0±36.8
Valvular disease (n=17)		6.9±0.6	2.6±0.9	2.3±0.2¶	41.4±15.6	180.0±53.6
Cancer (n=20)		10.3±0.7	7.9±1.6	3.7±0.2	66.0±11.9	161.3±31.6
Arterioscleosis obliterans (n=5)		8.4±0.5	2.4±1.2	2.6±0.4	30.0±12.1	139.6±57.8
Others (n=7)		9.7±1.0	7.3±3.1	2.8±0.2	70.3±29.9	214.4±82.0
¶$p<0.05$ different from cancer, Scheffe's t-test						

Table 3. Correlations between cytokines and usual clinical parameters in survivors

Diagnosis	N	R	P Value
TNF α vs WBC	62	0.304	0.016
TNF α vs CRP	63	0.263	0.037
TNF α vs ALB	63	0.329	0.009
TNF α vs CPK	62	0.390	0.002
TNF α vs CKMB	31	0.493	0.005
TNF α vs FDP	63	0.347	0.005
IL-1 β vs CRP	69	0.347	0.004
IL-1 β vs ALB	69	0.302	0.012
IL-1 β vs TP	69	0.266	0.027
IL-8 vs WBC	66	0.278	0.024
IL-8 vs CPK	67	0.844	<0.0001
IL-8 vs CKMB	30	0.771	<0.0001
IL-6 vs CPK	62	0.336	0.008
IL-6 vs FDP	66	0.242	0.05
IL-6 vs PLT	63	0.295	0.012

Results

Demographic data for 72 patients are presented in Table 1. Admissions after cardiovascular surgery accounted for 61% of the patients studied. APACHE II scores ranged between 4 to 47 with a mean of 10.0. The patients had higher plasma levels of TNF α, IL-1 β, IL-8 and IL-6 than the healthy controls. Plasma IL-1 β level was higher in patients with cancer than with valvular disease (Table 2). Thirty-eight percent had a detectable level of plasma TNF α (range ; nondetectable to 35.5 pg/ml). Seventy-six percent of the patients had a detectable level of plasma IL-1 β (range ; nondetectable to 38.7 pg/ml). Thirty-eight percent of the patients had a detectable level of plasma IL-8 (range ; nondetectalbe to 10460.7 pg/ml), and all of the patients (100%) had a detectable level of plasma IL-6 (range ; 27.8 to 570 pg/ml). Correlations between cytokines and clinical data in survivors are presented in Table 3. TNF α and IL-8 levels correlated inversely with leukocyte counts. IL-6 levels correlated inversely with platelet counts.

As shown in Figure 1, there were significant correlations between the APACHE II score and the plasma levels of TNF α and IL-1 β. But, there was no correlation between the APACHE II score and the plasma level of IL-8 or IL-6. A correlation was noticed between plasma IL-8 and TNF α, and between plasma IL-8 and IL-6 ($r = 0.612$; $p < 0.0001$, $r = 0.426$; p-0.0006, respectively). Further correlation analysis between these cytokines did not reveal any significant correlation.

Three of the 72 patients died (4%). All nonsurvivors met the criteria for SIRS. Patients who died had higher cytokine levels than patients who survived (Fig. 2).

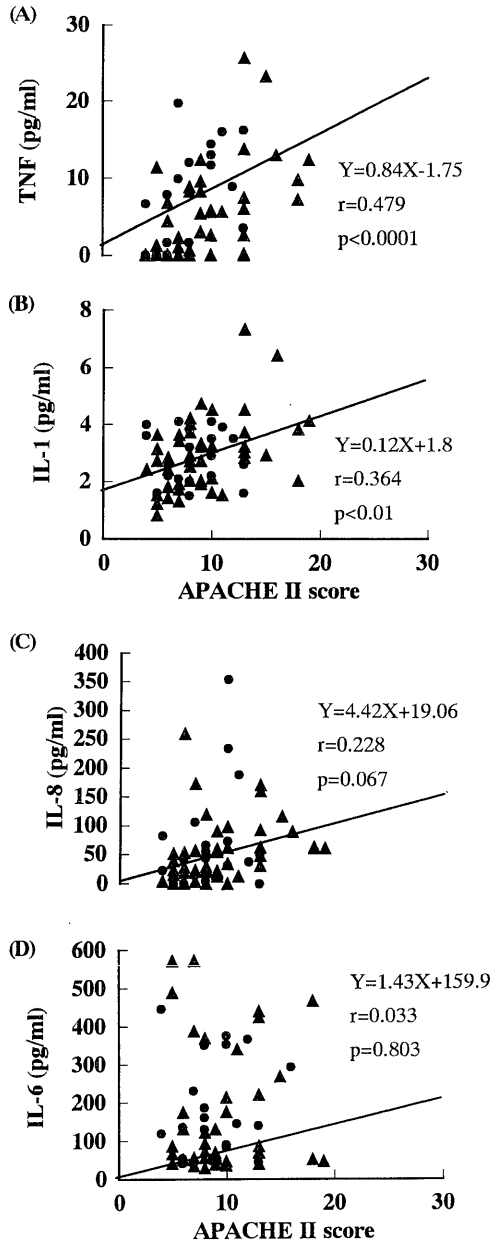

Figure. 1 Correlations between APACHE II score and the plasma levels of TNF α (A), IL-1 β (B), IL-8 (C) and IL-6 (D) in survivors. Circle reveals survivors without SIRS and triangle reveals survivors with SIRS.

No significant differences were detected in the APACHE II scores between survivors with or without SIRS. There were no significant differences in the plasma levels of IL-1 β, IL-8 and IL-6 between survivors with or without SIRS. However, the mean plasma level of TNF α was higher in survivors without SIRS than with SIRS (Fig. 3).

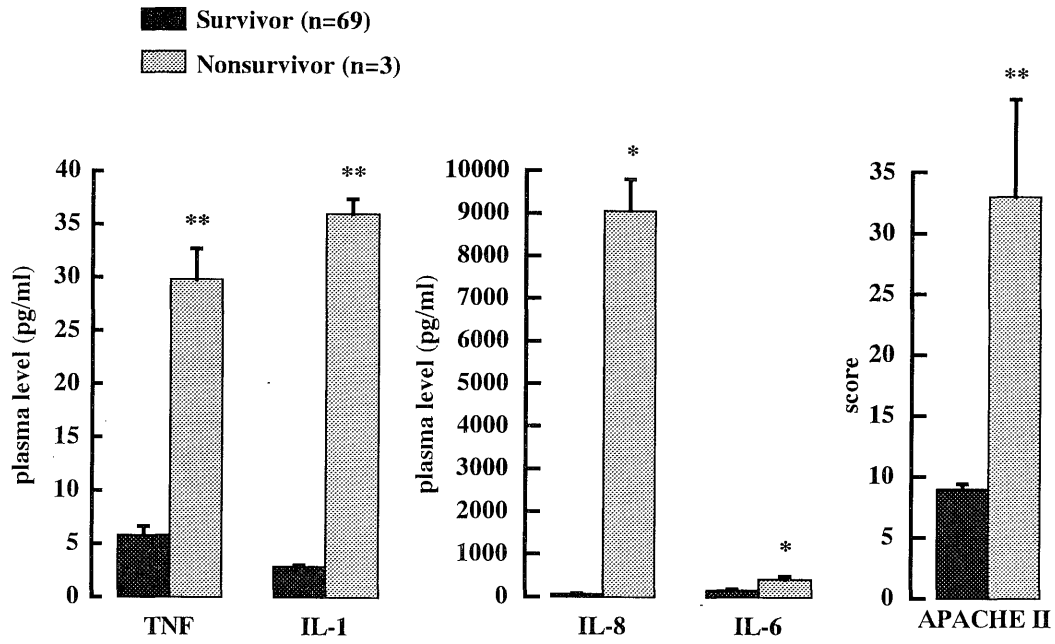

Figure. 2 Plasma Cytokines and APACHE II score in survivors and nonsurvivors.
*p<0.01, **p<0.0001, different from survivors

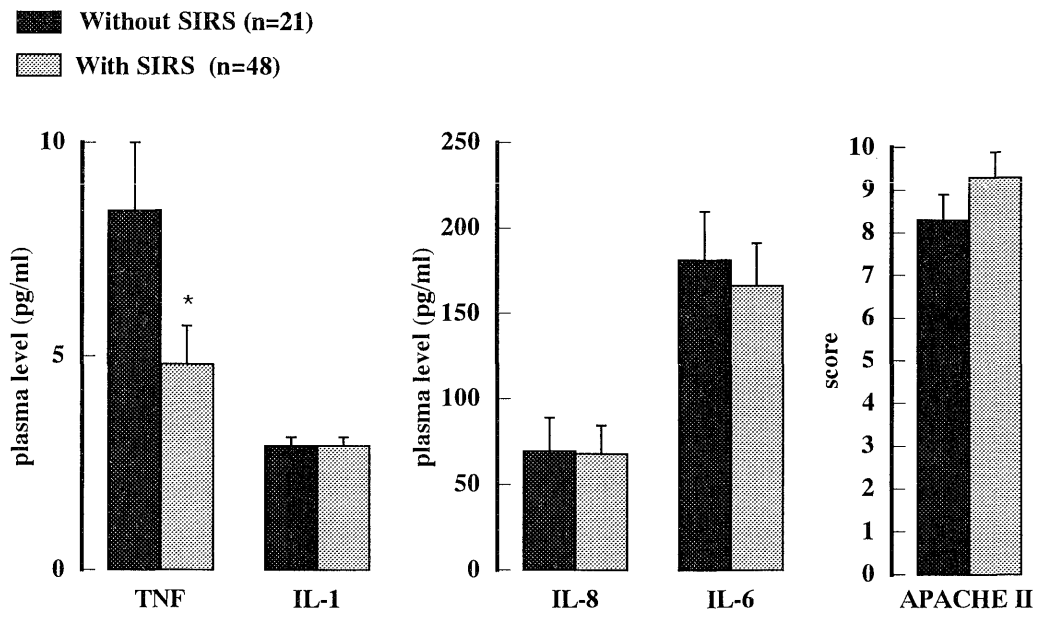

Figure. 3 Plasma cytokines and APACHE II scores in survivors without SIRS and with SIRS
*p<0.05, different from survivors without SIRS, Mann-Whitney U test

Discussion

The APACHE II classification system is the revised version of a prototype system, APACHE[3]. Although the maximum possible APACHE II score is 71, no patient has exceeded 55 in experience to date[2]. An increasing score was closely correlated with the subsequent risk of hospital death for intensive care admissions. This relationship was also found for many common disease. This scoring index can be used to evaluate the use of hospital resources and compare the efficacy of intensive care in different hospitals or over time.

Cytokines are essential mediators of cellular interactions and metabolic processes in many physiologic and pathophysiologic conditions. Experimental evidence suggests that at least four cytokines i. e., TNF α, IL-1 β, IL-6, and IL-8, participate in the host systemic inflammatory response in critical illness. In the present study, there were correlations between the APACHE II score and the plasma levels of TNF α and IL-1 β. But no significant correlations were found between the APACHE II score and the plasma levels of IL-6 or IL-8. It has been reported that the level of plasma IL-6 elevates soon after major elective surgery[4),5)] and surgical trauma alone is sufficient for IL-6 release, whereas endotoxemia might be an additional stimulus. Alternatively, the differences in IL-6 response may reflect the magnitude of the surgical trauma. Therefore the determination of IL-6, in contrast to TNF α, may not be helpful in predicting the presence of sepsis in patients with tissue trauma[6)]. Increased levels of IL-6 do not necessarily indicate an adverse role in sepsis, as reported recently regarding the use of monoclonal anti-IL-6 antibodies in sepsis[7)]. There is no doubt that in the postsurgical patients, IL-6 is massively produced and released in the circulation.

IL-8 is likely to prove to be important in the host defense to bacterial pathogens, as it is a powerful neutrophil chemoattractant involved in neutrophil trafficking across endothelial cells. The potential importance of IL-8 in the pathogenesis of inflammatory disease has been suggested by findings of increased synthesis by mononuclear cells in rheumatoid joints[8)], in idiopathic pulmonary fibrosis[9)] and in adult respiratory distress syndrome[10)]. The mechanism of IL-8 induction, however, remains speculative.

Most of patients (87%) had the plasma IL-1 β at a level similar to normal controls. The present data demonstrate that patients who subsequently die eventually exhibit much higher levels of TNF α, IL-1 β, IL-8 and IL-6 in the early postsurgical phase than those who survives. Any patient who showed marked concomitant increaces in the cytokines, as IL-1 β >34 pg/ml, TNF α >26 pg/ml, IL-8 > 7900 pg/ml and IL-6 > 260 pg/ml, did not survive. There seems to be marked synergy between these cytokines. The in vitro and in vivo biological effects of TNF α strongly resemble those of IL-1 β. As a matter of fact, TNF α can act as an inducer of IL-1 β, and IL-1 β and TNF α show synergy in a number of systems : lethality, Schwartzman-like reaction and leukocyte emigration. It was reported that circulating levels of TNF α and IL-1 β did not increase significantly in response to surgical stress[6),11-12)].

The interrelationship of infection, sepsis, and SIRS was characterized by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee. Diagnostic criteria for SIRS and related disorders were developed in a 1991 consensus conference[13)]. The role of TNF α in the development and maintenance of SIRS has been a topic of much discussion. New anti-TNF α therapies appear to attenuate the injurious actions of TNF α[14)]. As reported that the involvement of IL-6 in the pathophysiology of sepsis and septic shock may be questioned in view of the relationship between plasma levels of IL-6 and the severity of the disease[14)], involvement of IL-8 may also be questioned. When compared in survivors, there were no significant differences in the APACHE II scores or cytokines except for TNF α between patients with SIRS and without SIRS. TNF α showed a slight elevation in the patients without SIRS compared to those with SIRS. This finding suggests that TNF α might have favorable effects during the course of convalescence. While increased TNF α may be deleterious to the organism, there is also evidence that TNF α does have a protective effect in some bacterial infections[15)]. Furthermore, several cytokines not only are synergistic but also counterregulate each other[16-17)]. It is considered possible that the effects of cytokines are most likely dependent on their modulation and magnitude of secretion. Although the regulated release of TNF α may exert favorable effects, the uncontrolled production of TNF α may lead to organ dysfunction and death. The complexity of mechanisms involved in systemic inflammatory response make a general rule so difficult to establish. Indeed patient's response is highly individualized and it is not possible to know which moment of this dynamic process is being analyzed. In the present study, we measured plasma cytokines once on admission to examine the correlation between the severity scores and cytokines. It was reported that the persistence of cytokines in the plasma rather than peak levels of cytokines predicted a poor outcome in patients[18)]. The recent identification and characterization of endogenously derived circulating proteins with the capacity to inhibit proinflammatory cytokine activity presents additional questions referable to in vivo cytokine regulation[19-21)]. Thus further study would be needed to clarify the time course of change in plasma cytokines and to know whether circulating soluble receptors could have interfered the assays used to measure TNF α, IL-1 β, IL-8 and IL-6.

In conclusion,the plasma levels of TNF α and IL-1 β correlated to the severity of illness assessed by APACHE II score, but the plasma levels of IL-6 and IL-8 did not correlate to the severity of illness. The plasma level of IL-6 was elevated in all 72 patients. The results suggest that TNF α and IL-1 β, are involved in the pathophysiology of critical illness and that IL-6 and IL-8 seems to be an alarm signal leading to the recruitment of the host defense.

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