CHARLES UNIVERSITY IN PRAGUE

1st FACULTY OF MEDICINE

4th Department of Medicine

Oxidative stress, paraoxonase-1 activity and lipids in critically ill patients

Summary of Doctoral Thesis

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Prague 2009

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Opponents:

Summary of Doctoral Thesis sent:

Defense of the Thesis:

Place of Defence:

Study program: Biochemistry and pathobiochemistry

Chairman of the Biochemistry and Pathobiochemistry Commission:
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The thesis was elaborated within the postgraduate doctoral study in Biomedicine at the 4th Department of Internal Medicine, 1st Faculty of Medicine, Charles University in Prague.

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1 INTRODUCTION

Sepsis is an important cause of mortality and morbidity in critically ill patients, especially when associated with shock and/or multiple organ dysfunction syndrome (MODS)(5). Sepsis in combination with signs of organ dysfunction is called severe sepsis. Sepsis can be difficult to distinguish from other non-infectious conditions in critically ill patients admitted with clinical signs of acute inflammation. This issue is of paramount importance given that therapies and outcomes greatly differ between patients with and those without sepsis. Thus, there is an unmet need for clinical or laboratory tools distinguishing between the various forms of sepsis and systemic inflammatory response syndrome *(*SIRS). To date, no single clinical or biological indicator of sepsis has gained unanimous acceptance (102).

1.1 Multiple organ dysfunction syndrome in critically ill patients

Intensive care medicine is dealing with patients who usually require support for hemodynamic instability, airway and respiratory problems, acute renal failure or failure of other vital organs The condition usually results from infection, injury (accident, surgery), hypoperfusion and hypermetabolism. In severe cases, inflammation triggers a common pathway leading uniformly to organ dysfunction and failure(69). Frequently the cumulative effects of single organ dysfunctions lead to multiple organ dysfunction syndrom (MODS). A nonspecific condition that can be caused by inflammation, infection, ischemia, trauma or a combination of several non-infectious insults is called SIRS. Sepsis is the systemic response to infection and is the most common cause of MODS. In contrast to SIRS, it is very important to recognize and diagnose sepsis promptly, thus effective therapies for infection in critically ill patients could be started as soon as possible (30, 31). For definitions of diagnosic categories see Table 1-1.

1.2 Acute-phase response

In the face of acute inflammation, tissue damage, and/or commonly infection the human body undergoes a number of biochemical and physiological changes called the acute-phase response. Both innate and adaptive components of inflammatory response are controlled by mediators which are mostly produced or percepted by immune system itself. A key component of the acute-phase response is altered hepatic synthesis of a wide array of proteins (acute-phase reactants) involved in haemostasis, lipid metabolism, and the immune system (44). Among the potentially useful sepsis markers interleukin (IL)-6, IL-8, and procalcitonin (PCT) have been proposed to be the most promising candidates(21).

In bacterial sepsis, the inflammatory response is initiated by the bacterial products and toxins acting as exogenous mediators of inflammation. It has been shown that the cell wall fragments of both gram-negative and gram-positive bacteria can release tumor necrosis factor-α (TNF-α) and interferon-γ _(INF-γ), induce nitric oxide synthase (iNOS) in macrophages (60) *via* the activation of tyrosine kinases and nuclear factor kappa B (NF-kB)(61), and cause sepsis/septic shock and multiple organ failure(119).

Table 1-1 *Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Konference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Adapted from(15).*

Diagnostic category	Definition
Infection	microbial phenomenon characterized by an inflammatory response to the presence of microorganisms or the invasion of normally sterile host tissue by those organisms.
Bacteriemia	the presence of viable bacteria in the blood.
Systemic inflammatory response syndrome (SIRS)	the systemic inflammatory response to a variety of severe clinical insults. The response is manifested by two or more ofthe following conditions: **(1)** temperature >38°C or <36°C; **(2)** heart rate >90 beats per minute; **(3)** respiratory rate >20 breaths per minute or $PaCO_2$, <32 mm Hg; and **(4)** white blood cell count >12,000/mm^3, or <4,000/ mm^3, or >10% immature (band) forms
Sepsis	the systemic response to infection, manifested by two or more of the following conditions as a result of infection: **(1)** temperature >38°C or <36°C; **(2)** heart rate >90 beats per minute; **(3)** respiratory rate >20 breaths per minute or $PaCO_2$, <32 mm Hg; and **(4)** white blood cell count >12,000/mm^3, or <4,000/ mm^3, or >10% immature (band) forms
Severe sepsis	sepsis associated with organ dysfunction, hypoperfusion, or hypotension. Hypoperfusion and perfusion abnormalities may include, but are not limited to lactic acidosis, oliguria, or an acute alteration in mental status.
Septic shock	**s**epsis-induced with hypotension despite adequate fluid resuscitation along with the presence of perfusion abnormalities that may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status. Patients who are receiving inotropic or vasopressor agents may not be hypotensive at the time that perfusion abnormalities are measured.
Sepsis-induced hypotension	a systolic blood pressure <90 mm Hg or a reduction of 40 mm Hg from baseline in the absence of other causes for hypotension.
Multiple organ dysfunction syndrome	presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention.

1.3 Role of oxidative stress in critically ill patients

Activation of immune cells results in the production of reactive oxygen and nitrogen species (RONS) which potentiate the inflammatory response. The outcome of SIRS depends on its intensity, duration and on the balance between the pro and anti-inflammatory signals, and between pro- and antioxidant components. Oxidative stress results from either the excess in oxidant production, or the depletion of antioxidant defense.

These oxygen-driven reactions, however, bear the potential of forming intermediate species able to damage cells and tissues. These include leakage of electrons from cellular electron transfer chains, and as byproducts of membrane lipid metabolism (48, 100) A redox imbalance can enhance inflammation through the activation of stress kinases (c-jun N-terminal kinase, mitogen-activated protein kinase, p38) and redox-sensitive transcription factors such as nuclear factor kappa B (NF-κB) and activating protein-1, thereby modulating the expression of pro- and anti-inflammatory mediators (41, 95, 104). The tripeptide glutathione (L-g-glutamyl-L-

cysteinylglycine; GSH) together with its oxidized form glutathione disulfide (GSSG) comprises quantitatively the most important intracellular redox couple(32). In such way altering the redox state of the cell may contribute to the ongoing inflammatory cytokine production and progression of systemic inflammation, leading to organ injury (17).

Antioxidant substances can be divided into enzymatic and nonenzymatic groups. The enzymatic antioxidants include superoxide dismutase, which catalyzes the conversion of O_2^- to H_2O_2 and H_2O; catalase (CAT), which then converts H_2O_2 to H_2O and O_2; and glutathione peroxidase, which reduces H_2O_2 to H_2O by oxidizing glutathione (GSH). Re-reduction of the oxidized form of glutathione (glutathione disulfide) is then catalyzed by glutathione reductase.

The nonenzymatic antioxidants include the lipid-soluble vitamins (vitamin E, and vitamin A or β-carotene) and the water-soluble vitamins (vitamin C and glutathione). Vitamin E has been described as the major chain-breaking antioxidant in humans (88) Vitamin E can also directly quench oxygen reactive species (ROS). Vitamin A is a term encompassing a collection of retinols obtained in the diet primarily from dairy products, eggs, liver, and fortified cereals Vitamin C (ascorbic acid), obtained primarily from citrus fruits, functions as a water-soluble antioxidant capable of broadly scavenging ROS(23). Finally, GSH, which is synthesized intracellularly from cysteine, glycine, and glutamate, is capable of either directly scavenging ROS, or doing so via glutathione peroxidase enzymatically. In addition, GSH is crucial to the maintenance of enzymes and other cellular components in a reduced state. In all types of critical illness, such as sepsis, trauma, burn injury, acute pancreatitis, liver injury, severe diabetes, acute respiratory distress syndrome, AIDS and kidney failure, the occurrence of increased oxidative stress or a reduced antioxidative status has been studied. These studies have demonstrated mixed results; however, on the whole they support the presence of increased systemic oxidative stress and the depletion of antioxidant defenses during critical illness(17). As a result, several investigators evaluated the usefulness of antioxidant therapy for these patients. Their results were summarized in meta-analysis of antioxidant supplementation in critically ill patients (53).

The gold standard method for detecting oxygen radicals is electron spin resonance. Due to the exceptional instability of oxygen radicals, their low concentration and the relatively complicated measurement process, this method is not applicable in clinical settings. Methods based on the detection of products formed during macromolecule oxidation, the determination of the concentration or activity of components of the antioxidative defense system and several functionally oriented tests are more clinically relevant. Lipid peroxidation is investigated by a wide number of available methods. In clinical studies two methods have been more often used: the determination of malondialdehyde (MDA) (49) and the detection of F2-isoprostanes (79). Alternatively, an attempt can be made at determining the antioxidant defense potential of plasma or blood cells, mainly red blood cells. Furthermore, determination of the glutathione system, including reduced and oxidized glutathione (105) , or of glutathione peroxidase (GPX) activity is possible (106) Since ascertaining oxidative stress is rather complicated, it appears attractive to combine the determination of a number of different parameters, including oxidation related parameters (48). Several investigations show a close association of single or multiple parameters, such as total antioxidative capacity, lipid peroxidation, vitamins C and E, the activation of NF-κB and respiratory burst with the patient's outcome(3).

1.4 Plasma lipids and lipoproteins and paraoxonase 1 activity

Changes in lipoproteins during sepsis and recovery were first studied thoroughly by Alvarez. He has found that sepsis caused the decrease in concentrations of total cholesterol, high-density

lipoprotein cholesterol (HDL-C) , and apolipoproteins (apo) A and B in serum, whereas triacylglycerols (TAG) were increased (4).

Lipids have long been exclusively considered as nutrients providing calories, essential fatty acids (FA), and fat-soluble vitamins. More recently, their role as major biologic regulators, specifically in influencing the structure and function of cell membranes, membrane receptor activities, eicosanoid metabolism, cytokine production and interactions, and gene expression, has been increasingly recognized (121). Consequently, it has been widely accepted that lipids play an important role in pharmaconutritional regulation of inflammatory/ immune response, thereby influencing patient outcomes (52). Furthermore, the apolipoprotein composition of high-density lipoprotein (HDL) is changed during inflammation. Serum amyloid A (SAA), one of the major acute phase proteins, becomes associated with HDL and displaces apolipoprotein A-1 from particle (25, 71, 109). During severe sepsis, HDL is shifted to acute phase HDL, which is enriched in SAA and depleted of cholesterol and apo A-1(113) Enrichment with SAA enhances the catabolism of HDLs(18). The rapid decline of HDL during the acute-phase response may markedly reduce lipopolysaccharide (LPS)-neutralizing capacity (120).

The concept that HDLs may act as anti-inflammatory agents originated from large epidemiologic studies demonstrating a negative association between ischemic heart disease (viewed as a chronic inflammatory process) prevalence and circulating levels of HDLs(63, 76). Subsequently significant anti-inflammatory effects of HDLs have been demonstrated both *in vitro* and *in vivo*, probably as a result of (1) LPS binding and neutralization; (2) inhibition of adhesion molecule expression; (3) stimulation of endothelial nitric oxide synthase production; and (4) protection of low density lipoprotein (LDL) against peroxidative damage (120).

Paraoxonase (PON) [aryldialkylphosphatase (EC 3.1.8.1)] is a serum arylesterase. with ability to hydrolyze paraoxon, the active substance of insecticide parathion. There are three members of the PON gene family: PON1, PON2, and PON3. Enzymes PON1 and PON3 are mostly expressed in the liver and are carried in plasma bound to HDL(96). Paraoxonase 1 (PON1) is a HDL-associated lactonase and was shown to possess antioxidative properties (8, 81). These properties are probably attributed to the enzyme's capability to protect LDL (7, 80) as well as HDL (7) from oxidation and to decrease macrophage oxidative status (101).

To date, PON1 has been studied clinically in relation to health issues involving oxidative stress predominantly of noninfectious causes, including cardiovascular disease (22, 73), diabetes mellitus (70), chronic renal failure (33), inflammatory bowel disease (14), and elective surgery (66). PON1 activity has also been measured in patients with chronic infection caused by human immunodeficiency virus (90) or Helicobacter pylori (6). PON1 activity decreased in all of these disease states. As for sepsis, a drop in PON1 activity has been observed in an animal model following LPS application (38).

1.5 Fatty acid composition of plasma and erythrocyte lipids

The fatty acid (FA) composition of lipids is changing all over the life due to different specific situations e.g. starvation, aging, gravidity, changes in the diet, various diseases, etc. On the other hand these changes in FA composition and their metabolism affect reactivity of organism to diverse stimulations. FA profile is composed by saturated and unsaturated FA mostly esterified in a simple or complex lipids. As for unsaturated FA, the position of the first double bond from the methyl-end (ω position) of the hydrocarbon chain of FA is indicated as n-7, n-9, n-6 or n-3. Series of n-6 and n-3 denote polyunsaturated fatty acids (PUFA). Mammals are able to synthesise saturated FA (SFA) and monounsaturated FA (MUFA) of the n-7 and n-9 series only because they lack Δ12 and Δ15 desaturases (present in plants) for insertion of the

double bond at the n-6 or the n-3 position of PUFA. Thus mammalian cells must obtain essential n-6 (linoleic acid, 18:2n-6) and essential n-3 (α-linolenic acid, 18:3n-3) PUFA from the diet (29).

PUFA in membrane phospholipids contribute to the fluidity of the membrane and thus play a role in regulating the activity of membrane proteins. Membrane phospholipids are also the source of lipid signaling molecules such as diacylglycerols, phosphatidic acid, inositol-1,4,5-triphosphate, ceramides and arachidonic acid (AA) that are responsible for transfer of cellular signals across the hydrophobic membrane bilayer into different cellular compartments in order to promote appropriate responses. Moreover, 20-carbon PUFA and notably AA are substrates for the synthesis of eicosanoids e.g. prostaglandins, thromboxans, leucotriens, lipoxins and hydroxyeicosatetraenoic acids, etc(108). AA is usually the principal substrate for the synthesis of eicosanoids. This is why the cell membranes contain large amounts of AA compared with other eicosanoid precursors (e.g. eicosapentaenoic acid (EPA)). When diet is supplemented by fish oil, EPA is incorporated into cell membrane phospholipids partly at the expense of AA. Moreover, EPA inhibits the oxidation of AA by cyclooxygenase(83) and suppresses synthesis of prostaglandin E_2, thromboxan TXA_2 and leucotriene LTB_4. This way, n-3 PUFA can reduce platelet aggregation, blood clothing and modulate inflammatory cytokine production and so influence the immune function (20).

Number of studies shows substantial alterations in fatty acid metabolism in critically ill patients. Fatty acid oxidation rates, free fatty acid turnover, and lipolysis are elevated suggesting that the enhanced mobilization and oxidation of fat is one of the fundamental responses to stress (10, 117, 118). In addition, plasma levels of PUFA are reduced while SFA and MUFA are increased in burn injury, in patients with septicemia and established adult respiratory distress syndrome (ARDS), suggesting an essential fatty acid deficiency followed by increased oxidative stress (65, 92, 93).

2 SCOPE OF THE THESES

The aims of the study were:

1/ To compare septic and non-septic inflammatory process in critically ill patients with respect to inflammatory mediators, lipid profile and redox status.

2/ To follow up the changes of parameters in course of sepsis

3/ To assess the impact of sepsis severity on this particular combination of markers

3 SUBJECTS AND METHODS

3.1 Subjects

This was a prospective case control study in a medical adult intensive care unit (ICU) of the 4th Department of Internal Medicine of the First Faculty of Medicine and General Teaching Hospital in Prague. Thirty septic patients (SP), 15 age, sex and APACHE II score (Acute Physiology and Chronic Health Evaluation) matched non-septic critically ill patients with SIRS (NSP) and 30 age and sex matched healthy controls (HC) were included into the study. Healthy subjects were defined as individuals' without clinical and laboratory signs of sepsis, inflammation or known major disease. Septic patients had to fulfil the criteria of sepsis according to the Society of Critical Care Medicine/American College of Chest Physicians (SCCM/ACCP) definitions(15) together with the following inclusion criteria: APACHE II score >10(64) and C-reactive protein (CRP) in serum >20 mg/l. Exclusion criteria for all patients in the study were: antioxidant therapy, chronic dialysis, history of diabetes, generalized tumours, immunosupressive therapy and chemotheraphy. The group of non-septic critically ill patients had as additional exclusion criterion the presence of infection according to CDC criteria (54) and/or the presence of sepsis according to SCCM/ACCP (15). Routine patient eligibility screening was performed on every consecutive patient admitted in ICU between January 2006 and June 2008. All physiologic and laboratory data necessary for APACHE II(64) and sequential organ failure assessment (SOFA) score(40, 115) were collected prospectively.Both SP and NSP patients were classified as surgical (operative) or medical (non-operative) according to the major diagnosis. Approval of the study protocol by the locally appointed ethical committee was obtained, as was informed consent from all subjects.

3.2 Methods

3.2.1 Collection of blood samples

Fasting blood samples from septic patients were taken three times: during the first twenty-four hours after admission of patient(S1), 7 days after the first sampling (S2) and one week after the recovery (S3). All three samples were available in 19 cases, 8 patients died because of sepsis, one patient obtained pharmacological dose of zinc and selenium after first sampling and was excluded from follow up and last 2 patients were lost because they have never fully recovered from sepsis prior to transfer to another health-care facility. Blood samples from HC were obtained once and from NSP during the first twenty-four hours after admission. Blood samples were processed immediately after collection. For plasma, K_2EDTA (ethylendiamintetraacetic acid) was used as anticoagulant. Serum was prepared, following coagulation in vacutainer tubes, by centrifugation at 2500 x g at 4°C for 10 min. The samples were stored at -80°C until assay. Erythrocytes, separated from plasma, were washed three times with saline and separated by centrifugation at 2500 x g for 5 min.

3.2.2 Serum protein and lipid markers

Blood samples were obtained after overnight fast. Concentrations of CRP, PCT, cytokines (IL-6, IL-10 and TNF-α) were measured in serum. Concentration of CRP was measured immunoturbidimetric method using K-ASSAY CRP kit (Kamiya Biomedical Company, USA) on analyzer Hitachi Modular (Japan). Concentration of PCT was measured with immunoluminometric assay using BRAHMS PCT LIA-Kit (Brahms Diagnostica GmbH;

catalogue number 54.1, Berlin, Deutschland). All cytokines were analyzed using Flurokine MAP kits (R&D Systems, USA) and Luminex®100 analyzer. Serum amyloid A concentration was analysed by a solid phase sandwich ELISA kit (Invitrogen Corporation, USA). The concentration of albumin was assessed by colorimetric method using bromcresol green as chromogen (34).
Total cholesterol was analyzed in serum using a commercially purchased enzymatic-colorimetric test (CHOD-PAP) (Biola-test cholesterol 2500 kit; ; Pliva-Lachema, Czech Republic). Concentrations of cholesterol in HDL (HDL-C) was performed in serum samples using a BIO-LA-TEST HDL-Cholesterol kit (Pliva-Lachema) on a Cobas Mira analyzer (Roche, Switzerland). Oxidized-LDL measurement was performed by Oxidized LDL ELISA kit (Mercodia, Sweden). Oxidized LDL Competitive ELISA is based on the specific murine monoclonal antibody 4E6. Apo A-1 and apo B concentrations were measured in serum using rate nefelometric method kit on a Immage analyzer by Beckman Coulter (USA). Non-esterified fatty acids were determined spectrophotometrically at 550nm using acyl-coenzyme A synthetase and acyl-coenzyme A oxidase metod(72), kit by Randox laboratories UK, on Cobas Mira Plus analyzer, Roche (Switzerland).

3.2.3 Fatty acid composition of plasma and erythrocyte lipids

Plasma lipids were extracted according to modified method of Folch. Plasma (1 ml) was dissolved in 21 ml of a chloroform-methanol mixture (2:1) and shaken in a pear-shaped flask(42)**.** The serum protein precipitate was removed by filtration: 10 ml of chloroform-methanol-water mixture (3:48:47 v/v/v) was added and after a vigorous shaking, the lower lipid layer was separated and dried at 40 °C under a stream of nitrogen. Individual plasma lipids, i.e. total phospholipids (PL), triacylglycerols (TG) and cholesteryl esters (CE) were separated by one-dimensional thin-layer chromatography (0.5 mm Silica Gel H, Merck, Germany) using the solvent mixture hexane-ether-acetic acid (70:30:1 v/v/v), detected by 2,7-dichlorofluorescein (0.005% in methanol), scraped out and stored in a nitrogen atmosphere at -20 °C. On the next day, FA methyl esters were prepared and separated by gas chromatography (111). Erythrocyte lipids were extracted according to the method by Rose and Oklander(99). The same procedure for total erythrocyte phospholipids (EPL) separation and their fatty acid analysis as in plasma PL was performed.

3.2.4 Enzyme activities

Paraoxonaxe-1: The arylesterase activity of PON1 was measured according to the method as previously described by Eckerson et al. using phenylacetate as a substrate in tubes containing 945 µL of 20mM Tris-HCl (pH 8.0) with 1 mM $CaCl_2$ and 50 µL of serum(36). These tubes were incubated at 25 °C for 5 min. The reaction was started by 50 µL of 100 mM phenylacetate. The rate of phenol generation was monitored spectrophotometrically at 270 nm. Arylesterase activity of PON1 was calculated using the molar extinction coefficient of the produced phenol 1310 $M^{-1}cm^{-1}$ and expressed as U/ml serum.

Glutathione peroxidase 1 and glutathione reductase**:** Washed erythrocytes (50 µl) were lysed by adding deionised distilled water (200 µl). Haemolysate (20 µl) was then diluted with TRIS-HCl (580µl) for GPX1 and with phosphate buffer (580µl) for glutathione reductase (GR) activity. The activity of GPX1 was measured by the modified method of Paglia and Valentine using tert-butyl hydroperoxide as a substrate(89). The activity of GR was measured according to the method as previously described by Goldberg et al(47).

Catalase: Washed erythrocytes (200 µl) were added to deionise distilled water (1000 µl). Haemolysate (10 µl) was then diluted with phosphate buffer (4990 µl). The activity was determined by the modified method of Aebi(1).

CuZn Superoxide dismutase (SOD): Washed erythrocytes (350 µl) were lysed by adding deionised distilled water (1000 µl). Haemolysate (1000 µl) was mixed with ice-cold ethanol-chloroform mixture (800 µl; 5:3 v/v) and immediately shaken on Vortex for 20s. After 1.5 min the mixture was shaken 20 s again and after another 1.5 min was shaken last time for 20 s. The mixture was centrifuged at 12 000 rpm for 15 min. Supernatant (50 µl) was then diluted with phosphate buffer (450 µl). The activity was determined according to the modified method as previously described by Štípek(110).

3.2.5 Non-enzymatic antioxidants and cofactors

Vitamin A and E levels were analyzed using high-performance liquid chromatography, Radanal (Czech republic) . Folic acid was measured by electrochemiluminescence Immunoassay (ECLIA), kit by Roche Diagnostics, (Switzerland). Copper and zinc were measured by atomic absorption spectrometry (AAS) on Varian SpectrAA 220 FS. Selenium was assayed by atomic absorption spectrometry with electro thermal atomisation (ETAAS) on Varian SpectrAA 220 FS. Iron was measured by photometric method using ferrozine on Modular SWA analyzer, kit Roche Diagnostics, Switzerland. For measurement of ferritin levels was used chemiluminescence immunoasssay (CLIA) on ADVIA Centaur analyzer, kit Siemens, USA. Transferin was determined using immunoturbidimetric method on Modular SWA analyzer, kit Roche Diagnostics, Switzerland. Ceruloplasmin was assayed by nefelometry on Immage analyzer, kit Beckman Coulter, USA. Uric acid was determined by an enzymatic colorimetric method using uricase- peroxidase system on Modular SWA analyzer, kit Roche Diagnostics (Switzerland).
Urea was analyzed by UV enzymatic method on Modular SWA analyzer, kit Roche Diagnostics (Switzerland). Bilirubin concentration was measured by the 2,5-dichlorophenyldiazonium (DPD) method on Modular SWA analyzer, kit Roche Diagnostics (Switzerland). Concentrations of conjugated diene (CD)-LDL were determined spectrophotometrically. LDL was isolated by precipitation with buffered heparin. The assay for LDL oxidation products (LDL-BDC) is based on determination of baseline levels of conjugated dienes (BDC) in lipids extracted from LDL(2). Calculated Total Peroxyl Radical Trapping (cTRAP) was calculated according to formula(100):
cTRAP(µmol/l) = ((albumin (µmol/l)) 0.63 + (uric acid(µmol/l)) 1.02 + (bilirubin(µmol/l)) 1.50).

3.2.6 Statistical analysis

Data are expressed as mean ± standard deviation (SD) for parametric and as median and interquartile range (25^{th}-75^{th} percentiles) for non-parametric variables. Normality of data distribution was tested with Shapiro-Wilks W test. Differences between compared groups (septic group, non-septic and healthy controls) were tested with one-way ANOVA with Scheffé and Newman-Keuls *post tests* comparisons. For nonparametric analysis Kruskal-Wallis ANOVA was used. Friedman ANOVA was used for dependent analysis. The Spearman´s correlation coefficient was used for correlation analysis. All statistical analyses were performed using version 8.0 of StatSoft software Statistica (2007, CZ) and $P < 0.05$ was considered to be statistically significant.

4 RESULTS

4.1 Sepsis and systemic inflammation in critically ill patients

This part of the study presents the comparison of two different groups of critically ill patients characterised by SIRS of septic (SP) and non-septic etiology (NSP). Both patient groups were also compared with age matched healthy controls (HC). A total of 30 consecutive septic patients admitted to the ICU who met the all study inclusion and exclusion criteria were included in the study. Fifteen age, gender and APACHE II matched consecutive non-septic ICU patients with SIRS and thirty age and gender matched healthy controls were also included, see Table 4-1.

4.1.1 Acute-phase response markers, paraoxonase 1 activity and HDL

The comparison of acute phase response markers among SP, HC and NSP groups is demonstrated in Table 4-2. The considerable increase in concentrations of CRP, PCT, IL-6, IL-10 and TNF-α in serum of SP compared to HC was recorded. As for as NSP, the concentration of CRP, IL-6 and IL-10 also exceeded values in HC but in significantly smaller extent than in SP group. PCT and TNF-α in NSP did not differ from HC group. Figure 4-1 shows that the activity of PON 1 was lower in SP as well as in NSP groups regarding HC. The decrease in PON 1, HDL-C and apo A-1 concentrations was closely followed by the counter increase of SSA in both groups of patients. There was no difference in PON 1 activity between SP and NSP. However, the concentration of SSA was higher and of HDL-C and Apo A-1 lower in SP as compared to NSP.

Table 4-1 *Demographic and clinical parameters.*

Parameters/Groups	Septic patients	Healthy controls	Non-septic patients
Number	30	30	15
Gender (M/F)	12/18	12/18	9/6
AGE (years)	74 (61-79)	74 (58-81)	70 (57-79)
APACHE II	19.5 (14.0-28.0)	-	17.0 (13.0-20.0)
Diagnosis (medical/surgical)	20/10	-	8/7
Source of sepsis (lungs/others)	20/10	-	-
ICU hospitalization (days)	15.5 (9.0-48.0)	-	6.0 (4.0-7.0)
Hospitalization (days)	18.5 (15.0-58.0)	-	11.0 (7.0-18.0)
APV (number/percent)	17 (56.7%)	-	6 (40%)
CRRT (number/percent)	5 (16.7 %)	-	2 (13.3%)
Mortality (Number/percent)	8 (26.7%)	0 (0%)	2 (13.3%)

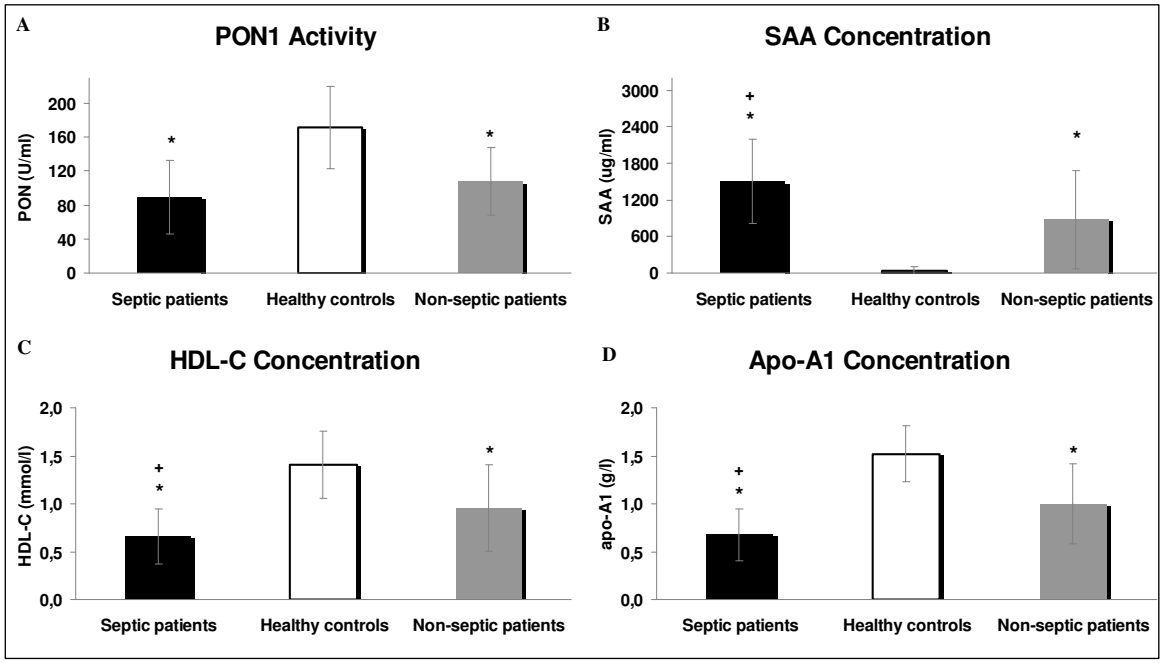
Abbreviations used: APACHE II: Acute Physiologic and Chronic Health Evaluation, ICU: Intensive Care Unit, APV: Artificial Pulmonary Ventilation, CRRT: Continuous Renal Replacement Therapy; data presented as median and interquartile range (25^{th}-75^{th} percentile).

Table 4-2 *Acute-phase response markers*

Parameters/Groups	Septic patiens (n = 30)	Healthy controls (n = 30)	Non-septic patients (n = 15)
CRP (mg/l)	127.5 (62.0-310.0)[a,b]	2.15 (2.0-5.8)	84.8 (4.8-130.6)[a]
PCT (mg/l)	3.15 (1.18-11.56)[a,b]	0.38 (0.26-0.78)	0.28 (0.14-0.73)
IL-6 (pg/ml)	127.1 (51.0-283.9)[a]	1.72 (1.2-2.9)	21.45 (10.9-48.0)[a]
IL-10 (pg/ml)	9.01 (4.68-14.48)[a]	0.54 (0.00-1.03)	5.16 (1.76-6.98)[a]
TNF-α (pg/ml)	20.2 (11.85-39.23)[a]	6.54 (5.35-7.72)	11.89 (6.82-14.47)

Abbreviations used: CRP: C-reactive protein, PCT: procalcitonin, IL: interleukin, TNF-α: tumor necrosis factor α; data presented as median and interquartile range (25^{th}-75^{th} percentile); [a]... septic patients and non-septic patients vs. healthy controls; [b]... septic patients vs. non-septic patients; $p < 0.05$

Figure 4-1 *PON1 activity and associated parameters. PON1: enzyme paraoxonase1 – arylesterase activity; SAA: serum amyloid; HDL-C: high density lipoprotein cholesterol, Apo A-1: apolipoprotein A-1; data presented as mean ± S.D., [*]… septic patients and non-septic patients vs. healthy controls; [+]... septic patients vs. non-septic patients; $p < 0.05$*

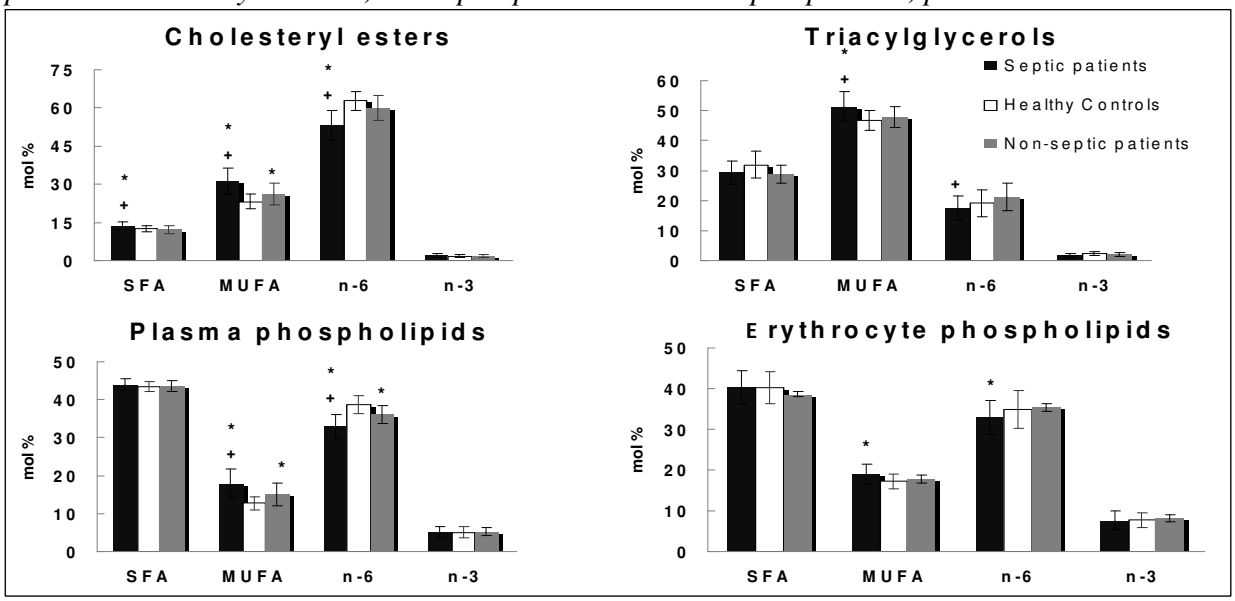


4.1.2 Fatty acid composition of plasma and erythrocyte lipids

Figure 4-2 demonstrates the comparison of saturated FA (SFA), monounsaturated FA (MUFA), n-6 and n-3 polyunsaturated FA (PUFA) proportions in plasma CE, TAG, PL and erythrocyte PL of SP, NSP and HC groups. The proportion of MUFA in septic patients was increased in plasma CE, TG and PL as compared to NSP and HC groups. This rise was compensated by the fall in n-6 PUFA for CE and PPL. As for plasma TAG and erythrocyte PL (EPL), the shift in the MUFA and n-6 PUFA proportions in SP was less expressed than in CE

and PPL. Regarding NSP versus HC, some trends were similar to those in SP but much less significant.

Figure 4-2 *Proportion of fatty acid classes in individual lipids. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, n-6: n-6 polyunsaturated fatty acids, n-3: n-3 polyunsaturated fatty acids; data presented as mean ± S.D., *… septic patients and non-septic patients vs. healthy controls, +… septic patients vs. non-septic patients, p < 0.05*



4.1.3 Markers of oxidative stress and antioxidative enzyme activities

The concentration of LDL associated oxidized LDL (ox-LDL) and CD, as markers of lipid peroxidation, were raised in both SP and NSP groups as compared with HC. However there was no difference between both patient groups (Figure 4-3).
Figure 4-4 shows activities of antioxidative enzymes. Whereas activities of CuZnSOD, CAT and GR in plasma of SP were higher and GPX1 lower regarding to HC, activities in NSP were similar those in HC. We found very the good positive correlation of CuZnSOD with all markers of inflamation and the negative with TRAP, CAT negatively correlated with CRP, IL-6, ox-LDL, CD, and positively with TARP while GR corelated positively with IL-6 and negatively with TRAP.

Figure 4-3 *Markers of lipid peroxidation. Ox-LDL: oxidized low density lipoproteins, LDL-C: low density lipoprotein cholesterol, CD: conjugated dienes; data presented as mean ± S.D., *… septic patients and non-septic patients vs. healthy controls, ** p < 0.01, * p < 0.05*

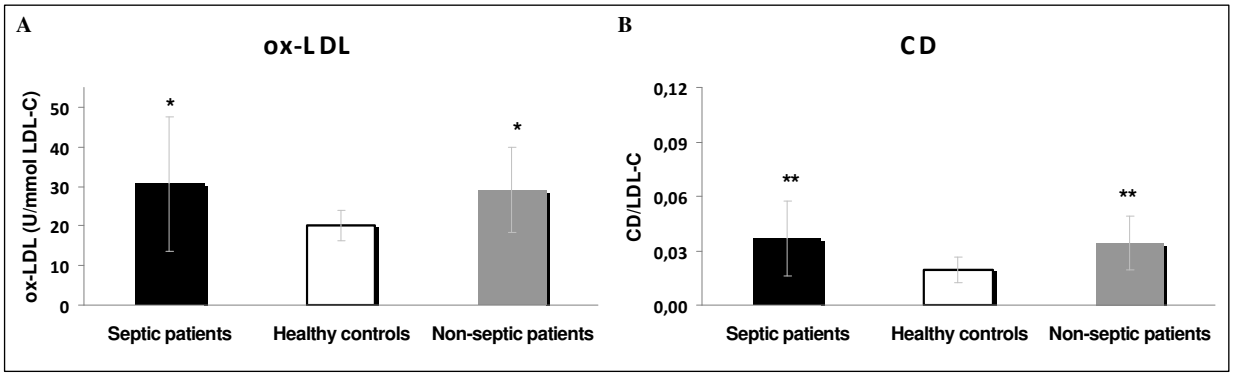
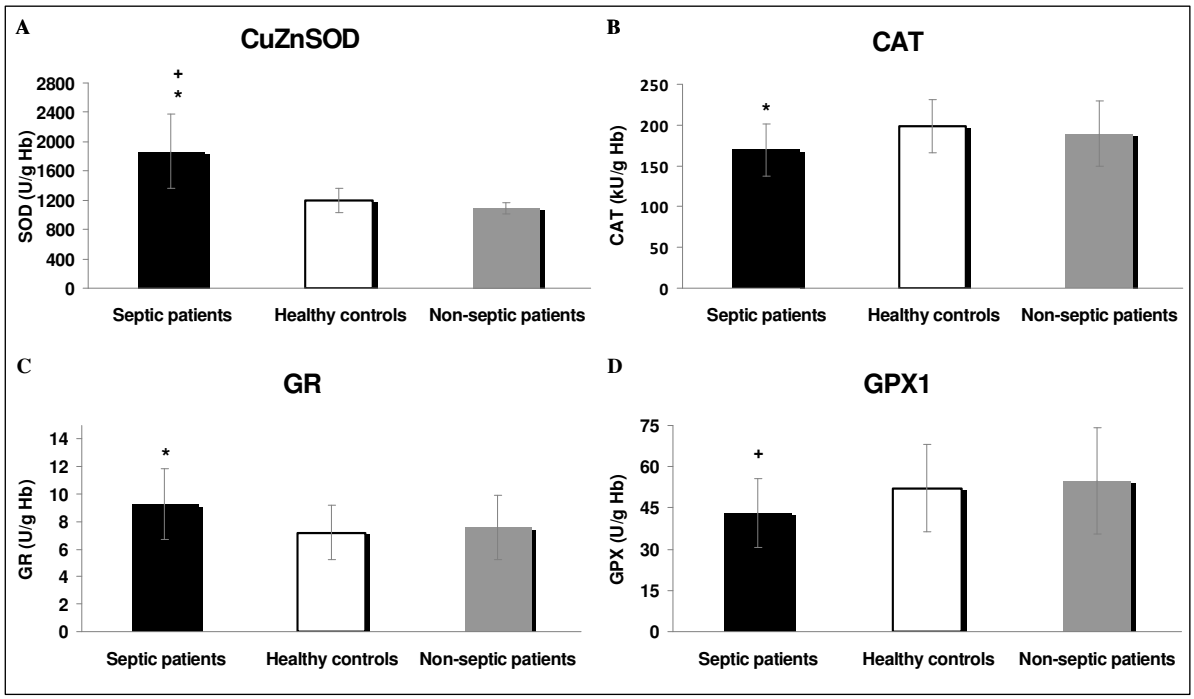


Figure 4-4 *Antioxidative enzyme activities. CuZnSOD: superoxide dismutase, CAT: catalase, GPX1: glutathione peroxidase1, GR: glutathione reductase; data presented as mean ± S.D., *… septic patients and non-septic patients vs. healthy controls, +… septic patients vs. non-septic patients, p < 0.05*



4.2 Critically ill patients in the course of sepsis

In the study twenty two septic patiens (SP) fully recovered from sepsis and 19 of them were available for three samplings: SP enrolled within 24 hours after the onset of sepsis (S1), SP 7 days after S1 (S2) and SP one week after the clinical and laboratory cessation of sepsis symptoms (S3).

4.2.1 Acute-phase response markers, paraoxonase 1 activity and plasma lipids

The follow up of acute phase response markers in course S1,S2 and S3 samplings is demonstrated in Figure 4-5. The increase in concentrations of CRP (not shown), IL-6, IL-10 and TNF-α in serum of SP in S1 compared to HC persisted in lesser extent until S3 in CRP, IL-6,IL-10 and TNF- α. The only exception was PCT that has already decreased in S2 and S3 to the levels of HC. The decrease in the PON 1 activity and apo A-1, HDL-C concentrations respectively persisted in all three samplings regarding HC. However, S3 value was significantly higher than S1 or S2 one (Figure 4-6). The concentration of SAA was the highest in S1 and gradually approaching HC. SAA in S3 sampling did not already differ from HC.

Figure 4-5 *Parameters of inflammation in course of sepsis. PCT: procalcitonin, TNF-α: tumor necrosis factor-α, IL-6: interleukin-6, IL-10: interleukin-10; data presented as mean ± S.D., *…S1/S2/S3 vs. healthy controls (HC); [+] S1/S2 vs. S3; [b] S1 vs. S2; ***…p< 0,001, **…p<0,01, *…p<0,05*

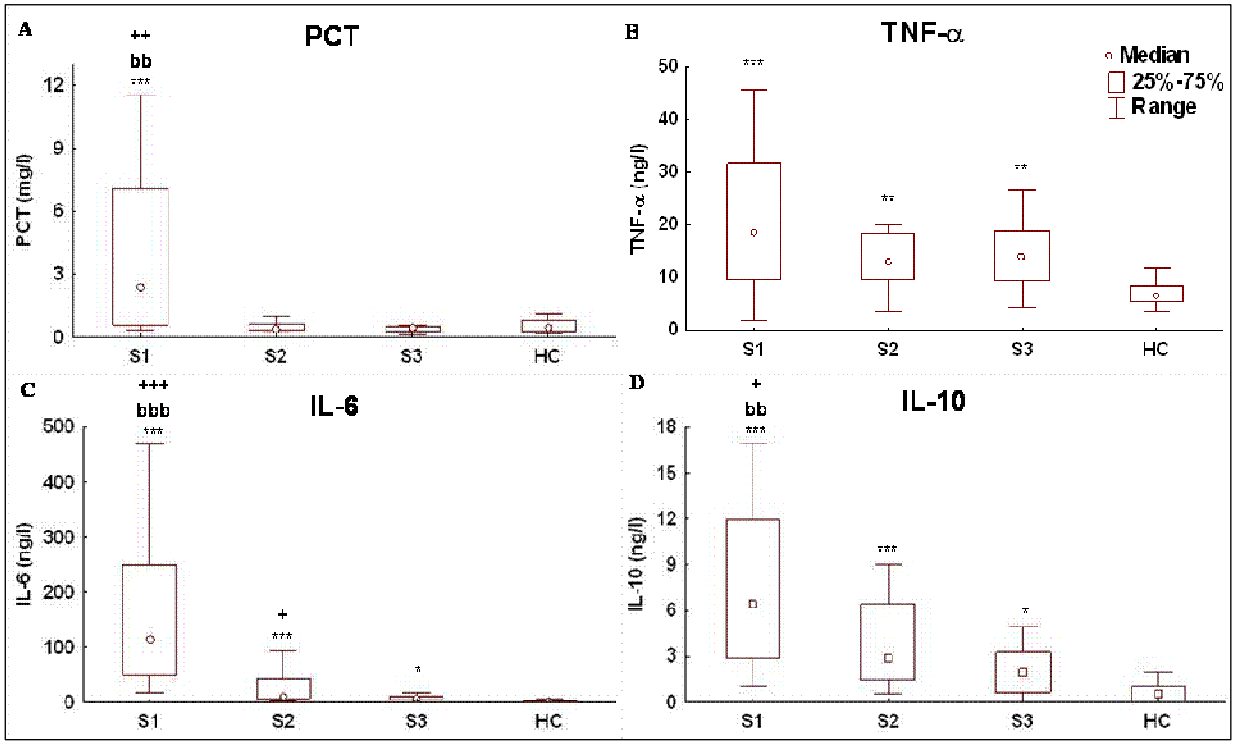
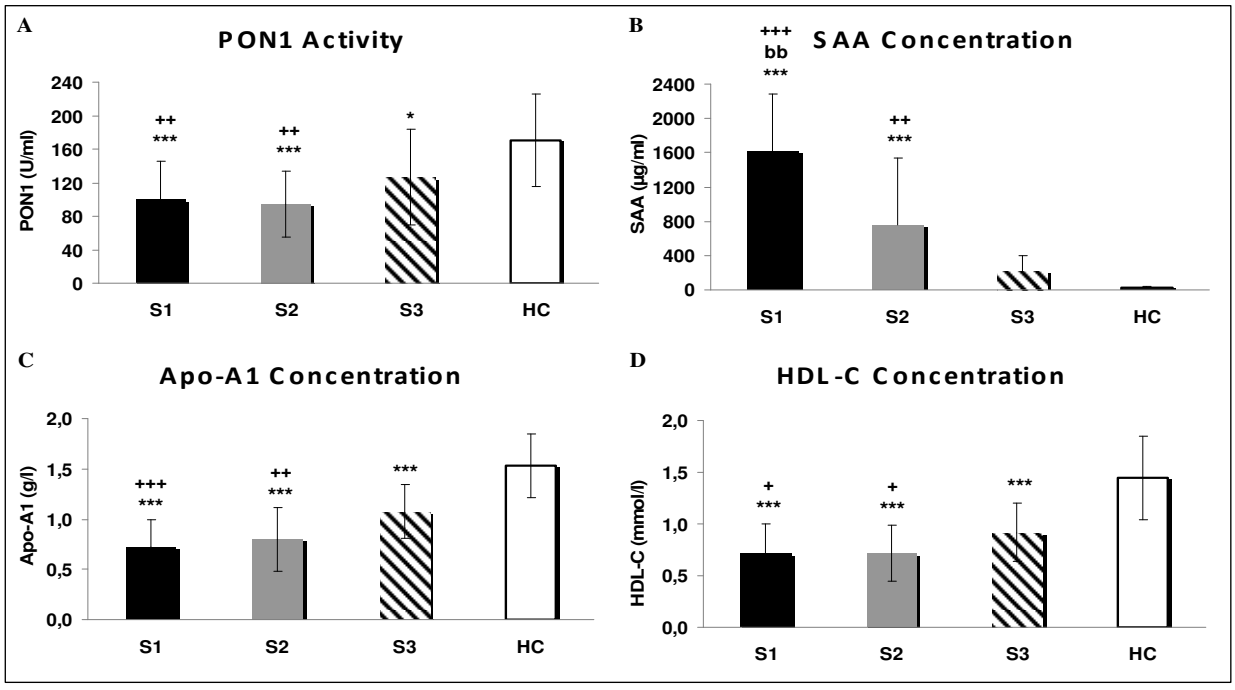


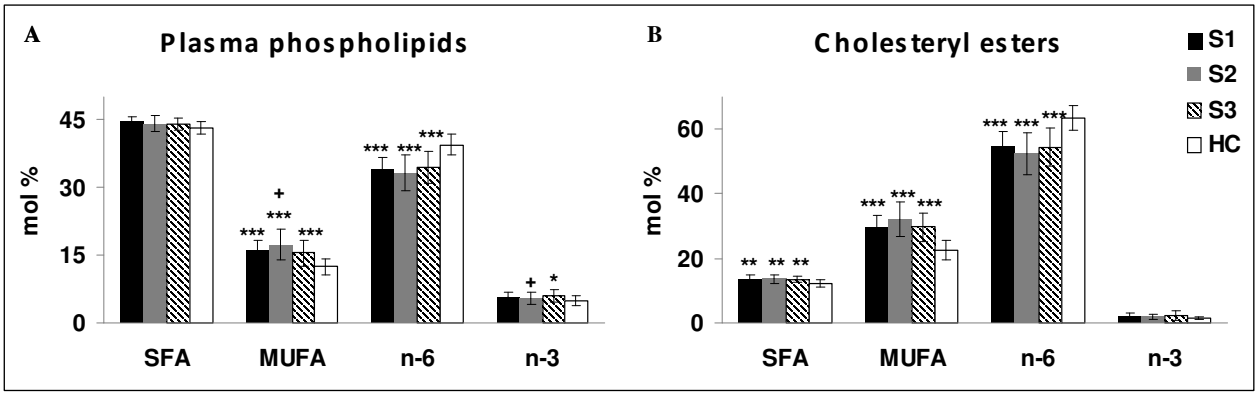
Figure 4-6 *PON 1 activity and associated parameters in course of sepsis. PON1: enzyme paraoxonase1 – arylesterase activity, SAA: serum amyloid, Apo A-1: apolipoprotein A-1, HDL-C: high density lipoprotein cholesterol; data presented as mean ± S.D., *…S1/S2/S3 vs. healthy controls (HC); [+] S1/S2 vs. S3; [b] S1 vs. S2; ***…p<0,001, **…p<0,01, *…p<0,05*



4.2.2 Fatty acid composition of plasma lipids

Figure 4-8 demonstrates the proportion of fatty acid classes in CE and PPL during the course of sepsis. There was the increase in MUFA proportion in all samplings and n-3 PUFA in S3 that was followed by the decrease of n-6 PUFA regarding to HC in PPL. As for as CE, the higher proportion of SFA and MUFA and lower n-6 PUFA in all samplings as compared to HC was found.

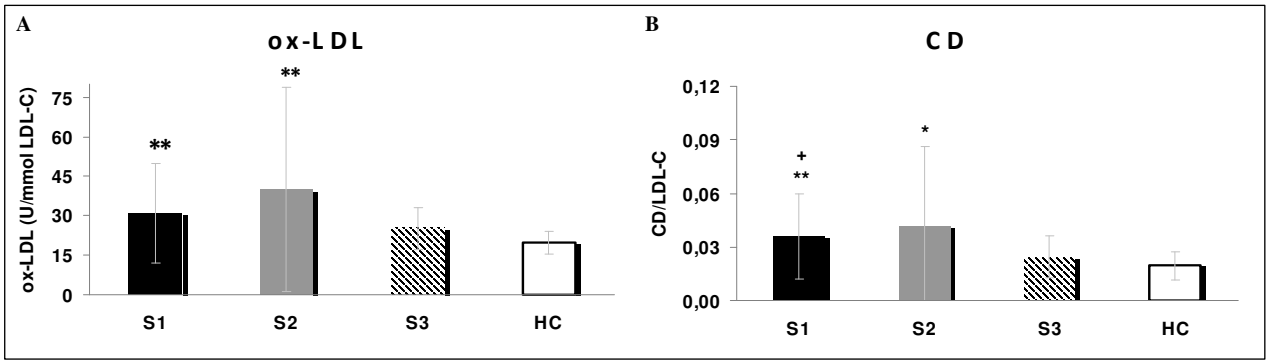
Figure 4-8 *Proportion of fatty acid classes in cholesteryl esters and plasma phospholipids during the course of sepsis. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, n-6: n-6 polyunsaturated fatty acids, n-3: n-3 polyunsaturated fatty acids; data presented as mean ± S.D., *…S1/S2/S3 vs. healthy controls (HC), + S1/S2 vs. S3, ***…p<0,001, **…p<0,01, *…p<0,05*



4.2.3 Markers of oxidative stress

Figure 4-9 shows the level of lipid peroxidation products during the course of sepsis. Ox-LDL as well as CD were higher in S1 and S2 samplings in comparison with HC. In S3, the level of both markers already reached HC values.

Figure 4-9 *Parameters of lipid peroxidation during the course of sepsis. Ox-LDL: oxidized low density lipoproteins, CD: conjugated dienes, LDL-C: low density lipoprotein cholesterol; data presented as mean ± S.D., *…S1/S2/S3 vs. HC, + S1/S2 vs. S3, **…p<0,01, *…p<0,05*



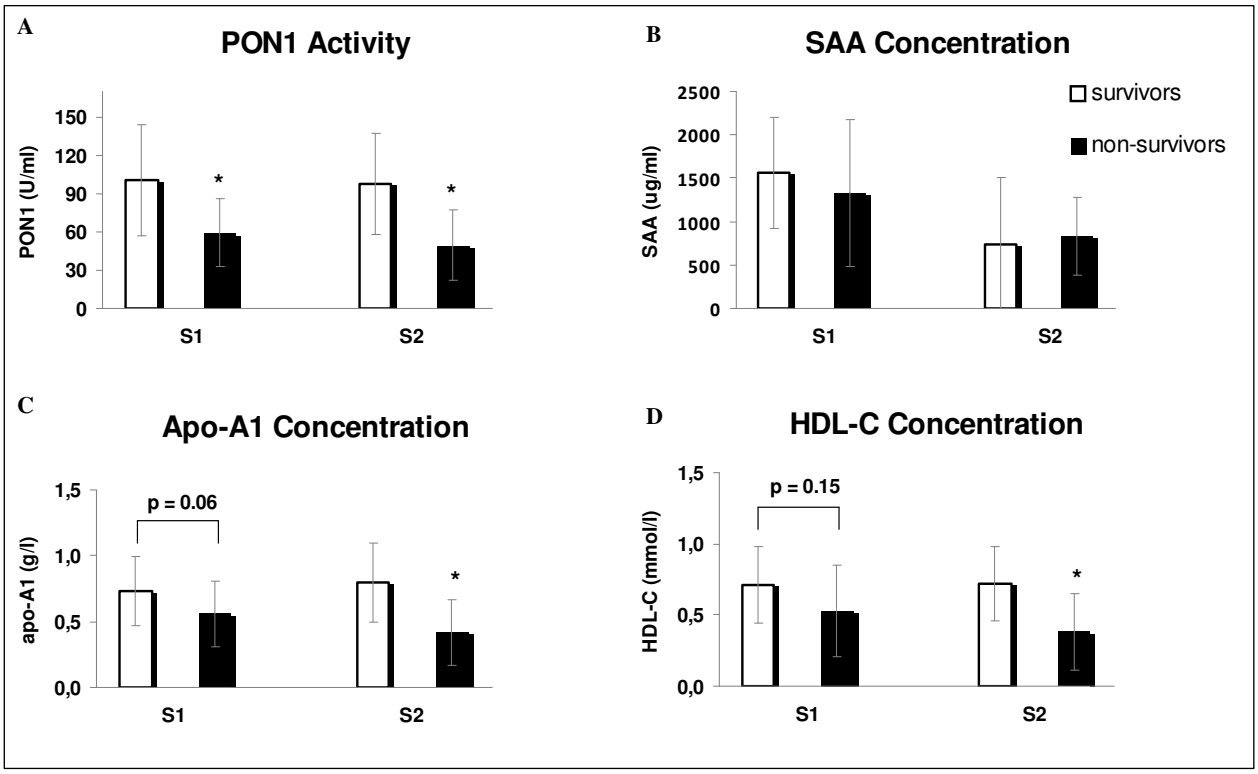
4.3 Severity of sepsis

This part of study compares septic patients who survived till recovery (survivors 1st sampling(S1), n=22 and survivors 2nd sampling(S2), n=21) with those who died after S1 or S2 and never recovered of sepsis (non-survivors S1, n=8, and non-survivors S2, n=4). S1 was done within 24 hours after the onset of sepsis; S2 was done 7 days after S2.

4.3.1 Paraoxonase 1 activity and plasma lipids

Figure 4-12 depicts the comparison of PON1 activity and associated parameters between survivors and non-survivors in S1 and S2 samplings. We observed lower PON1 activity and apo A-1, HDL-C concentrations in non-survivors compared to survivors S1 and S2 samplings. There was no difference between both groups of patients in SAA concentration.

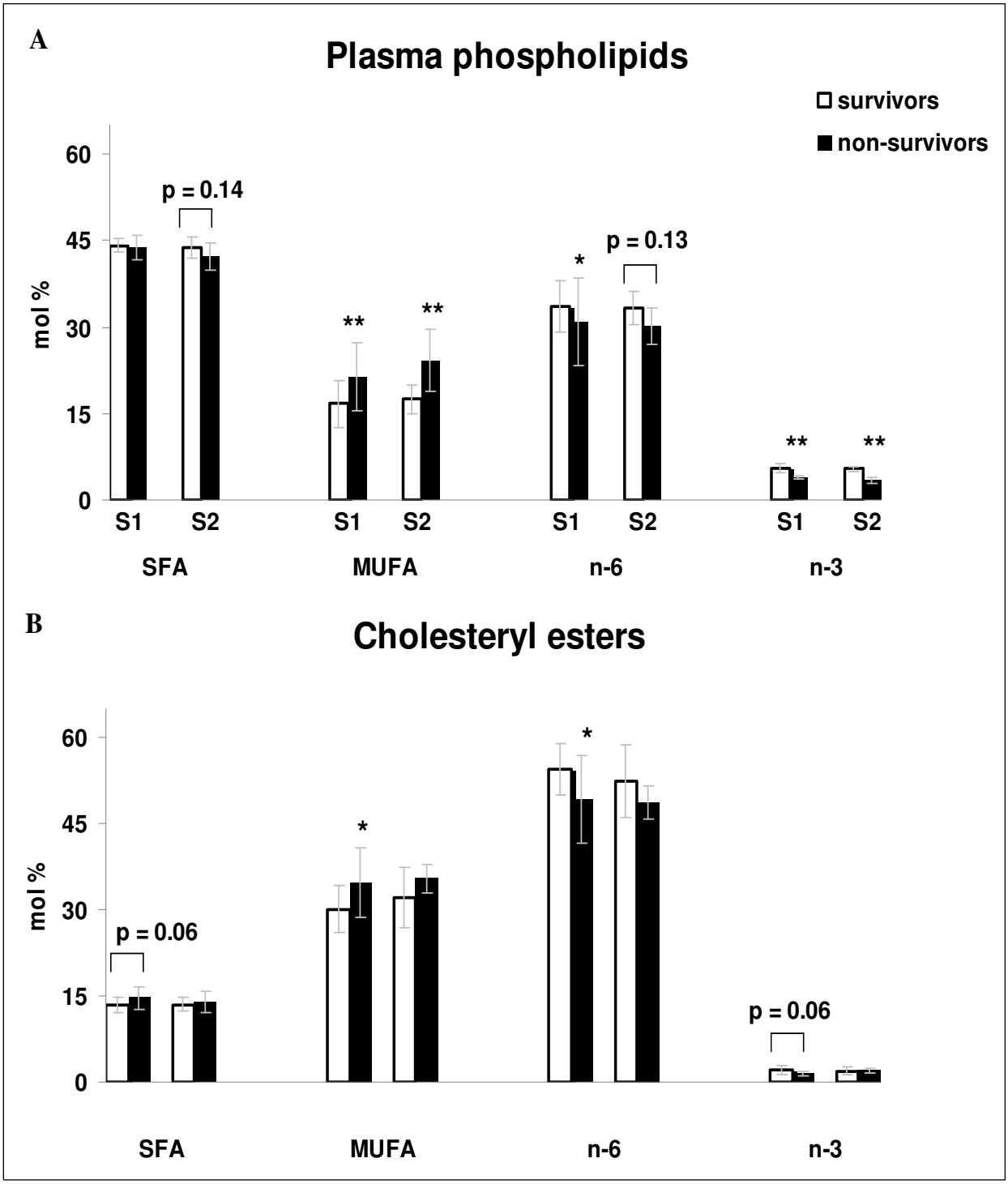
Figure 4-12 *PON1 activity and associated parameters – survivors/non-survivors. PON1: enzyme paraoxonase1 – arylesterase activity, SAA: serum amyloid, Apo A-1: apolipoprotein A-1, HDL-C: high density lipoprotein; data presented as mean ± S.D., *… non-survivors vs. survivors, p< 0,05*



4.3.2 Fatty acid composition of plasma lipids

Figure 4-14 illustrates the proportion of fatty acid classes in cholesteryl esters and plasma phospholipids in survivors and non-survivors. The proportion of MUFA was higher and both n-6 and n-3 PUFA lower in non-survivors then compared to survivors in plasma phospholipids. In cholesteryl esters, higher MUFA and lower n-6 PUFA proportion in non-survivors compared to survivors was found in S1 sampling only.

Figure 4-14 *Proportion of fatty acid classes in cholesteryl esters and plasma phospholipids – survivors/non-survivors. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, n-6: n-6 polyunsaturated fatty acids, n-3: n-3 polyunsaturated fatty acids; data presented as mean ± S.D., *… non-survivors vs. survivors, **…p<0,05, *…p<0,05*



5 DISCUSSION

This study compared critically ill patients with SIRS of septic and non-septic origin. Patients were selected according to 1992 consensus criteria. Those were criticized especially in SIRS category as being overly sensitive and unspecific. In 2001, the group of sepsis experts suggested modification of the definition of sepsis and severe sepsis at the International Sepsis Definitions Conference (69). However this definition has not been completed yet(102). Therefore the current concepts of SIRS, sepsis, severe sepsis, and septic shock remain useful to clinicians and researchers(69). All subjects involved in the study were well age and gender matched, and both patient groups were also matched successfully using APACHE II score. However other clinical parameters of NSP group such as mortality, length of ICU and hospital stay were clearly less severe probably due to the higher proportion of surgical and coronary care patients. This confirms that these scoring systems have limitations mainly due the fact that they primarily focus on the physiologic abnormalities(64). Nevertheless in large observational study, severe sepsis and severe non-septic SIRS have had similar mortality rate(35). Septic patients were also followed up in course of sepsis. Initial SOFA score gave evidence that most of the patients were in severe sepsis/septic shock diagnostic category. The score improved after one-week period. The severity of illness and mortality rate in our study were similar with data reported for patients with severe sepsis(43).

We have shown enhanced concentrations of acute-phase response markers (CRP, PCT, TNF-α, IL-6 and IL-10) in SP group regarding HC. This increase was also significant in NSP except of PCT that did not differ from HC. Markers like PCT or CRP respond to both infection and inflammation and hence reflect both microbiological findings and the host response. Nevertheless, our study confirms a number of studies that point out the superiority of PCT as a marker for diagnosis of sepsis and/or infection compared to CRP. According to results of the metaanalysis of Uzzan et al., PCT has higher specificity for sepsis than CRP in surgical patients, it means that the risk for a positive PCT test in infected patients was about 16-fold higher than in non-infected SIRS ones(112). PCT is highly specific for sepsis(11) that supports our results. From this point of view, stratification of SP and NSP subjects in our study was quite successful. On the other hand, there are studies showing elevated PCT in non-septic SIRS patients(97) and immediately after Surgery (74) or trauma(77), without obvious infection. It means that PCT is "indirect" marker of infection and its sensitivity and specificity for diagnosis of infection may vary in different patient groups and indications. We have also checked changes in acute-phase response markers during the course of the sepsis. PCT was the only marker which was increased just at the onset of sepsis and later on fell down to HC values while other markers were still increased above HC levels when sepsis has started to recede (S2). Dandona et al. injected endotoxin to healthy volunteers and ascertained that serum PCT become detectable at 4 hrs, maintaining a plateau till 24 hrs and following by an increase of pro-inflammatory cytokines e.g. TNF-α and IL-6(28). The comparison of surviving and non-surviving septic patients confirms that PCT might be a good predictor of sepsis prognosis. In our septic patients the level of PCT was substantially lower in survivors than in non-survivors at the onset of sepsis and completely diminished one week later while the high PCT level still persisted in non-survivors. In accordance with our results, decreasing levels of PCT were associated with a higher probability of survival and PCT levels were correlated with the severity of disease. Moreover, PCT was found to be an earlier marker of sepsis than CRP(24). Another study on critically ill children determined the values of PCT and CRP in diagnosing septic patients in correlation with the severity of disease. They concluded that PCT is a better diagnostic marker of sepsis in critically ill children than CRP(98). Both markers, i.e. the CRP and especially PCT, may become a helpful clinical tool to stratify patients with SIRS according to disease severity.

The pro-inflammatory cytokines such as tumor necrosis factor TNF-α, IL-6 and IL-8 proved to be of diagnostic and prognostic value in disease states followed by systemic inflammation/sepsis, and are frequently used for the monitoring of septic patients(26, 84, 85). A variety of activated immune cells can produce anti-inflammatory IL-10 but activated macrophages are suggested to be the major source of this cytokine. IL-10 appears to be a potent regulator of immune function because it can inhibit the production of other cytokines, including TNF-α, IL-1, IL-6 and INF-γ(78). Pro-inflammatory TNF-α, IL-6 and anti-inflammatory IL-10 cytokines were increased in both groups of patients under study and the elevation of both cytokines still persisted after recovery. Similarly to our findings, the multicentre cohort study of subjects with community-acquired pneumonia reported that the circulating cytokine response to pneumonia continues for more than a week after presentation. Individuals with high circulating levels of both pro-inflammatory (TNF-α, IL-6) and anti-inflammatory (IL-10) cytokines had a markedly increased risk of severe sepsis and death(59).

Our study showed that the activity of PON 1 was lower in SP and NSP groups compared to HC. The decrease in PON1, HDL-C and apo A-1 concentrations was closely followed by the counter increase of SSA in both groups of patients. There was no difference in PON1 activity between SP and NSP. However, the concentration of SSA was higher and of HDL-C and Apo A-1 lower in SP as compared to NSP. There were negative correlations between PON1 activity and other positive acute-phase reactants in the whole group of subjects under study. Decreased PON1 activity, low level of circulating HDL and modest increase in CRP have drawn considerable interest, and especially through this combination's relation to atherosclerosis that exemplifies a low-grade chronic inflammatory process(22). Mackness et al. supposed there to be a link between atherosclerosis development and combination of higher CRP level with low PON1 activity(70). A similar pattern characterized by very low HDL and high CRP concentrations has been found in sepsis representing a high-grade acute inflammatory state caused by infection(4). In patients with severe sepsis, an early rapid decrease in HDL lipoproteins and increase in CRP concentration has been observed. There was significant correlation between increased CRP and serum amyloid A, both positive acute phase reactants(113). Therefore, our finding of decreased PON1 activity in both SP and NSP is consistent with the aforementioned parallels.

Changes in lipid and protein composition of HDL caused by inflammation influence PON1 activity and function(58). During the acute-phase response, HDL is loosing apoprotein A-1, esterified cholesterol and most of the HDL-associated enzymes (including PON1). PON1 is replaced mainly by serum amyloid A with the concomitant loss of HDL antioxidative properties(114). A recent study suggests that HDL-associated PON1 promotes an apoprotein-dependent lactonase activity(45) that protects lipoproteins, probably via degradation of proinflammatory oxidized fatty acids(116). The decrease in PON1 activity during sepsis has been demonstrated in the serum of Syrian hamsters, a model of Gram-negative bacterial infection, within 24 h following LPS treatment. A marked decrease in the liver PON1 mRNA as early as 4 h after a single LPS treatment also has been observed. Moreover, independent TNF-α and IL-1 cytokines administration without LPS treatment moderately decreased serum PON1 activity and PON1 mRNA levels in the liver, indicating a partial direct effect of these cytokines on PON1 expression(38). PON1 that decreases during the inflammatory response regardless of the ethiology should be classified among the negative acute phase proteins.

The results of this study confirm that oxidant activity and antioxidant capacity are altered in SIRS patients. Surprisingly, we did not observe any difference in increased level of plasma lipid peroxidation products (Ox-LDL, CD) between SP and NSP. The elevation of both markers has already been found in S1, persisted at least one week and returned to the normal levels after recovery. Similarly, another study presented that the ox-LDL concentration increased during the

first week in patients with severe sepsis(12), however, the level of CD was not changed in the human septic shock(86). Endotoxin administration caused a rise in plasma levels of CD in the chronic porcine model of burn and sepsis(9). When we compared ox-LDL and CD in sepsis survivors and non-survivors, they did not differ at the onset of sepsis (S1) but later on in S2, non-survivors had profound elevation of both peroxidation markers in comparison to survivors. This may be a result of RONS overproduction that is not effectively balanced in context of MODS.

Erythrocyte antioxidative enzyme activities, their cofactors and vitamins were more influenced in SP than in NSP. In erythrocytes of NSP, none of the antioxidative enzymes activities changed compared to HC. In septic patients, we observed lower erythrocyte activities of CAT till S2 and GPX 1 till S3 and higher activity of ZnCuSOD in S1. As for sepsis survivors and non-survivors, we have not found any difference in individual components of antioxidant capacity (not shown) except GPX1 activity in S2. Accordingly in other study on non-septic patients, superoxide dismutase activity was not changed in erythrocytes of ischemic stroke patients with high level of lipid peroxidation products(57). In another study of ARDS patients, no alterations in the activities of CAT, SOD, GPX in erythrocytes were also detected(75). On the other hand, septic patients had increased circulating levels of catalase(67) and manganese superoxide dismutase and those were predictive of the development of ARDS. In addition, their serum had an increased antioxidant capacity, as reflected in an increased ability to scavenge hydrogen peroxide(68). Cowley et al. showed decreased plasma antioxidant potential at the onset of severe sepsis with normalization over time in survivors while non-survivors failed to achieve normal level(27).

Our observations of the lower concentration of total cholesterol and of both fractions HDL-C, LDL-C, Apo A-1 and Apo B compared to HC in SP and NSP is in line with the observation of Hardardóttir et al(51) and Sammalkorpi et al(103). They observed that the mass concentrations of both LDL and HDL were reduced during acute infections due to the lowering of their cholesterol, phospholipid, and protein contents. However there is a difference between rodents and primates in metabolism of cholesterol under conditions of inflammation. Whereas an increase in serum total cholesterol levels and hepatic cholesterol synthesis in rodents under inflammation occurs, there is either no change, or decrease in concentration of serum cholesterol in humans and non-human primates. The mechanism underlying this species difference is not known yet(62). As for as the comparison of concentration of HDL-C and Apo A-1 in patients and HC, the level was significantly lower in both patient groups and lower in SP than in NSP subjects. Similarly Cabana et al. observed a marked decrease in serum levels of HDL and apo A-1 during infection and inflammation(19). Besides these quantitative changes during inflammation, the apoprotein composition of HDL was changed in both septic and non-septic patient groups. SAA as one of the major acute-phase response proteins becomes associated with HDL and displaces apo A-1(71). In our study the displacement was greater in SP than in NSP group. Thus, during severe sepsis composition of HDL lipoproteins is shifted to acute phase HDL particles enriched by SAA and depleted of cholesterol and apo A-1(113).

Both patient groups did not differ in the concentration of plasma triglacylglycerols (TAG) and nonesterified fatty acids compared to HC group. Our finding does not correspond to other studies that have observed increased serum TAG levels in patients with gram-negative or gram-positive bacterial and viral infections(46, 50, 103). This non-compliance may be caused by the fact that the hypertriglyceridemic effect induced by inflammation is rapid process, occurring within 2 h after administration, sustaining for at least 24h(37, 39) and it is possible that hypertriglyceridemia as a very sensitive and rapid physiological part of the host response to infection already subsided at the time of S1 collection.

Another interesting observation of our study is the decrease of n-6 PUFA proportion (linoleic acid 18:2n-6) that was compensated by the proportional increase of MUFA in plasma CE, TAG

and PL first of all in SP and also in lesser extent in NSP. Increased production of free radicals presented in our study as an increased level of CD is in good negative correlation with PUFA level and positive correlation with MUFA level in plasma lipids. The similar shifts in FA composition in comparison with HC were observed also in erythrocyte phospholipids of SP and NSP patients. In accord, Pratt et al. determined reduced proportions of 20:4n-6, n-6 and n-3 PUFA and higher levels of MUFA and SFA in plasma and in red blood cells in burn patients early after injury(93). The lower level of 20:4n-6 and n-3 PUFA in plasma lipids of burn patients suggest increased use of these fatty acids for wound healing and immune function following burn injury. Also patients with ARDS had decreased plasma concentrations of total linoleic acid compensated by an increase in oleic and palmitoleic acids. As plasma linoleic acid concentrations decreased, there was usually an increase in concentrations of plasma 4-hydroxy-2-nonenal, one of its specific peroxidation products, suggestive of severe oxidative stress during ARDS leading to molecular damage to lipids(94). Das et al. demonstrated the relation between the increase in free radical generation and the decrease in the levels of n-6 and n-3 PUFA in plasma phospholipids in patiens with pneumonia, septicemia, and collagen vascular diseases such as rheumatoid arthritis and systemic lupus erythematosus was observed(29). It was also shown that both n-3 and n-6 PUFA are able suppress human T-cell proliferation, and inhibit the secretion of both TNF-α and IL-2 by lymphocytes in vitro, suggesting the importance of PUFA in the regulation of inflammation process(92). But there is still wider array of disorders and diseases that are able to elicit relevant PUFA deficiency either in plasma or blood cells. All these states either acute inflammatory states mentioned above or chronic in nature e.g. smoking, coronary heart disease, diabetes, cancer, Crohn´s disease, atopic eczema-psoriaris, Cystic fibrosis and multiple sclerosis, etc.(13, 16, 55, 56, 82, 87, 91, 107) are connected with the formation of free radicals in response to oxidative stress. Plasma resp. erythrocyte fatty acid status is a reflection of dietary fat intake. It also explores PUFA metabolism and gives information about the incorporation of these FA into cellular membranes. Erythrocyte and plasma FA analysis can detect PUFA insufficiencies and imbalances or metabolic abnormalities due to either lipid peroxidation during a disease state or by improper dietary FA intake. The decrease of PUFA in plasma or erythrocytes can be caused either by loss of their lipoperoxidation or by their increased usage as substrate for eicosanoid production or at site of anabolic pathways due to suppressed activities of delta desaturases and elongases. Specific PUFA supplementation can be helpful in the prevention and the control of both acute and chronic diseases.

6 CONCLUSIONS

This study compared critically ill patients with SIRS of septic and non-septic etiology. Septic patients were also followed up in the course of sepsis. The laboratory parameters were assessed according to clinical outcome.

1. Acute-phase response markers (PCT, CRP, TNF-α, IL-6 and IL-10) were enhanced in SP while concentrations of CRP, IL-6 and IL-10 in NSP were increased only in lesser extent. PCT was the only specific marker of sepsis with relatively fast normalization.

2. The decrease in PON 1 activity was found in both SIRS SP and NSP to the similar extent. The decrease in PON 1 activity was positively correlated with HDL-C, apo A-1 concentration and negatively correlated with PCT, CRP, TNF-α, IL-6 and IL-10 as mediators of SIRS and with ox-LDL as marker of oxidative stress..

3. The plasma levels of ox-LDL and CD was enhanced in both groups of patients. Sepsis non-survivors had profound elevation of both lipid peroxidation markers compared to survivors suggesting the presence of more severe oxidative stress.in subjects of fatal outcome.

4. Erythrocyte CuZnSOD and GR activities were enhanced while those of CAT and GPX1 decreased in SP but not in NSP. In the course of sepsis, CuZnSOD was increased in S1 only, CAT was suppressed in S1 and S2 and the decline in GPX1 activity persisted till the recovery.

5. The proportion of n-6 PUFA (mainly LA) was decreased in plasma CE, TAG, PL and in erythrocyte PL in SP as compared to NSP and HC groups. This fall was compensated by the rise in MUFA. The decrease in n-6 PUFA proportion in plasma CE and PL was in negative correlation with all acute-phase response markers and parameters of oxidative stress. Whereas sepsis survivors lost n-6 PUFA only, the deprivation of both n-6 and n-3 PUFA was observed in non-survivors.most probably due to the intensified oxidative stress

6. According to our results, the discriminating markers of SIRS SP vs NSP are mainly PCT, SAA, HDL-C, apo A-1, all antioxidative enzymes and n-6 PUFA. The discriminating markers between non-survivors and survivors are mainly PCT, IL6, IL10, PON1, n-3 PUFA in both S1and S2 while Apo A-1, HDL-C, LDL-C, total cholesterol, n-3 PUFA, ox-LDL, CD and GPX1 in S2 only.

In conclusion, our results support the position of PCT as relatively specific sepsis marker that may also be of good prognostic value. We documented markers of increased oxidative stress in both septic and non-sepstic SIRS. We propose PON1 activity as universal negative inflammatory marker with relatively late recovery. As for plasma lipid levels in acute inflammation, we have shown that the decrease of cholesterol persists long after septic period and through recovery. Our finding of PUFA deficit, especially severe in sepsis non-suvivors, necessitates careful management of lipid supplementation with respect to individual fatty acid classes.

7 ABBREVIATIONS

AA	arachidonic acid
APACHE	acute physiology and chronic health evaluation
Apo	apolipoprotein
ARDS	acute respiratory distress syndrome
CAT	catalase
CE	cholesteryl esters
CD	conjugated dienes
CRP	C-reactive protein
cTRAP	calculated total peroxyl radical trapping
EPA	eicosapentaenoic acid
FA	fatty acids
GPx	glutathione peroxidase
GR	glutathione reductase
HC	healthy controls
HDL-C	high-density lipoprotein cholesterol
HDL	high-density lipoprotein
ICU	intensive care unit
IL	interleukin
INF-γ	interferon-γ
LPS	lipopolysaccharide
LDL	low density lipoprotein
MDA	malondialdehyde
MODS	multiple organ dysfunction syndrom
MUFA	monounsaturated fatty acids
NF-κB	nuclear factor kappa B
NSP	non-septic patients
ox-LDL	oxidized LDL
PCT	procalcitonin
PL	phospholipids
PON1	paraoxonase-1
PUFA	polyunsaturated fatty acids
RONS	reactive oxygen and nitrogen species
ROS	reactive oxygen species
SAA	serum amyloid A
SFA	saturated FA
SIRS	systemic inflammatory response syndrome
SOD	superoxide dismutase
SOFA	sequential organ failure assessment
SP	septic patients
TAG	triglacylglycerols
TNF-α	tumor necrosis factor-α

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