Significance of increased lipid peroxidation in critically ill patients

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Abstract

OBJECTIVES: Increased oxidative stress and low-T_3 syndrome may develop in critically ill patients. The study aimed at evaluating the level of lipid peroxidation (LPO) in critically ill patients and at estimating the relationships among LPO level, the death rate, the rate of low-T_3 syndrome and patient's clinical status.

METHODS: Lipid peroxidation (LPO) level was studied in seventy (70) adult, critically ill patients and 48 healthy volunteers. Critically ill patients were classified into survivors and non-survivors, or those with and without the low-T_3 syndrome (normal-T_3).

RESULTS: LPO level was four times higher in critically ill patients than in healthy volunteers. Among non-survivors, LPO level was higher in patients with the low-T_3 syndrome than in patients without this syndrome, and among survivors, the tendency was opposite. Additionally, the extent, to which LPO level increased, depended on the kind of the disease. The degree of LPO variability was higher in survivors than in non-survivors. LPO level was lower in patients with higher number of therapeutic interventions.

CONCLUSION: A tremendous increase in oxidative damage to lipids in critically ill patients strongly depends on the kind of pathological process and, under certain conditions, higher LPO levels could be due to more favourable outcome.

ORIGINAL ARTICLE

Abbreviations

T_3	- triiodothyronine
T_4	- thyroxine
TSH	- thyrotropin
ROS	- reactive oxygen species
LPO	- lipid peroxidation
ICU	- Intensive Care Unit
FT_3	- free triiodothyronine
FT_4	- free thyroxine
TISS-28	- Therapeutic Intervention Scoring System
MDA+4-HDA	- malondialdehyde + 4-hydroxyalkenals
ANOVA	- one-way analysis of variance
SD	- standard deviation
SEM	- standard error of the mean
COPD	- chronic obstructive pulmonary disease

INTRODUCTION

Metabolic deregulation, endocrine dysfunction included, occurs in critically ill patients in response to both acute and chronic critical diseases (Nylen *et al.*, 2006; Vanhorebeek and Van den Berghe, 2006), and the mechanisms of this deregulation have not been completely explained till now. Among endocrine dysfunction, changes in thyroid hormone – triiodothyronine (T_3) and thyroxine (T_4) – concentrations are most frequent and they are manifested by the, so-called, low-T_3 syndrome (euthyroid sick syndrome), which – under serious conditions – may progress into low-T_3 and low-T_4 syndrome (Peeters *et al.,* 2006). The typical changes of thyroid hormone concentrations in the course of acute critical disorder or at the beginning of chronic critical diseases rely on decreased (below normal ranges) T_3 concentration (low-T_3 syndrome). In turn, in patients with chronic critical diseases, T_4 concentration also decreases (low-T_3 and low-T_4 syndrome). The low-T_3 (and, possibly, low-T_4) syndrome is associated with poor prognosis, independent of what kind of disease or external factor contributed to critical stage, and that is why it is treated as a very useful diagnostic and prognostic marker (Peeters *et al.,* 2006). Whereas thyrotropin (TSH) concentration remains usually in normal ranges, at least, at first stages of critical illnesses, it drops down below normal ranges at advanced steps of the disease. In patients, who are successfully treated for critical disease, thyroid hormone concentrations return to normal ranges, which is now associated with better prognosis.

It is worth mentioning that the low-T_3 syndrome is a strong prognostic predictor of death, for example, in patients with heart disease (Iervasi *et al.,* 2003).

Enhanced oxidative damage to macromolecules is expected to occur, due to the catabolic state, accompanying critical illnesses. It is to be recalled that reactive oxygen species (ROS) and free radicals are formed in living organisms – in certain amounts – under physiological conditions. An overproduction of ROS and of free radicals results in oxidative stress and may lead to several diseases. On the other hand, organ or tissue disturbances may secondarily cause increased ROS and free radical formation, leading, in consequence, to increased damage to macromolecules (Valko *et al.*, 2007). In turn, the products of oxidative damage to macromolecules may further enhance oxidative stress and damage all the components in the organism, deteriorating the antioxidant defence in such conditions as critical illnesses. Indeed, there is an imbalance of the cellular redox status in critically ill patients, relying on increased formation of ROS and on the decreased antioxidative capacity; this contributes to the pathogenesis of multiple organ dysfunction in critically ill patients (Crimi *et al.,* 2006). Additionally, the occurrence of enhanced oxidative stress is, in these patients, associated with poor prognosis (Roth *et al.,* 2004).

The level of lipid peroxidation (LPO) is one of the most frequently measured parameters of oxidative stress. The measurement of LPO products (the index of oxidative damage to lipids) in blood serum appeared to be a reliable marker of oxidative stress, both in humans [in newborns with sepsis, i.e. with overt critical disease (Gitto *et al.,* 2001), in adult patients with chronic fatigue syndrome (Maes *et al.*, 2006), in adult patients with growth hormone deficiency (Kokoszko *et al.*, 2006), or in smoking mothers and newborns (Argüelles *et al.*, 2006)], and in animal models [e.g. in response to potential carcinogens (Karbownik *et al.*, 2000a; 2000b), or other prooxidative conditions, such as thyroid dysfunction (Mogulkoc *et al.*, 2005; Sewerynek *et al.*, 2006; Wiktorska *et al.*, 2005)]. Increased LPO levels in critically ill patients have already been observed in previous studies (Cighetti *et al.*, 2005; Motoyama *et al.*, 2003), however, their specific meaning with respect to either the low-T_3 syndrome development or the prognosis in critically ill patients have not yet been unambiguously defined. Because both the changes in thyroid hormone concentrations, due to the low-T_3 syndrome and LPO are dynamic processes, depending mostly on health/disease status, we decided to observe during hospitalization period, if there was any direct or indirect relationship between them, and to what extent they determined death/survival.

The study aimed at evaluating the level of LPO products in blood serum, collected from critically ill patients and at estimating the relationships between LPO level and the death rate and/or the rate of the low-T_3 syndrome.

MATERIALS AND METHODS

Patients

The procedures, used in the study, were approved by the Local Ethics Committee of the Polish Mother's Memorial Hospital – Research Institute, and fully informed, written consent was obtained, either from the patients themselves or from their closest family members.

Seventy (70) adult, critically ill patients, either with acute or chronic diseases, hospitalised at the Intensive Care Unit (ICU) of the Regional Hospital in Opoczno (Poland) between November 2004 and September 2005, and 48 healthy volunteers (controls) were enrolled into the study (Table 1). The exclusion criteria constituted

biochemical evidence of overt hyper- or hypothyroidism on admission.

Patients with critical illnesses and healthy volunteers (controls) were well matched at baseline, in terms of age and sex (no statistically significant differences between the groups were found) (Table 1).

Critically ill patients did not constitute a diagnostically uniform group. The final single and the final diagnosis, being one of several other diagnoses, are presented with relation to death/survival and the low-T_3 syndrome in Table 2 (Table 2A and Table 2B).

Sampling and biochemical analysis

In case of the critically ill patients, peripheral blood was collected for the measurement of LPO and hormone [free triiodothyronine (FT_3), free thyroxine (FT_4), and TSH] concentrations on admission to ICU; thereafter, blood LPO level was measured every day within the hospitalisation period and hormone concentrations were occasionally evaluated – in each patient more than once and, usually, 3–5 times within the hospitalisation period. In case of the controls, single measurements of LPO level and of hormone concentrations were performed.

All the critically ill patients were classified into patients, who survived (survivors), and those, who died within the hospitalisation period (in-hospital deaths; non-survivors). Additionally, all the critically ill patients were classified into patients without low-T_3 syndrome (normal-T_3) and patients, suffering from low-T_3 syndrome (low-T_3), the latter included both low-T_3 syndrome and low-T_3 and low-T_4 syndrome. From the practical point of view, the following groups were also considered in Discussion: the least severely ill patients, i.e. those without the low-T_3 syndrome, who did survive; the most severely ill patients, i.e. those with the low-T_3 syndrome, who did not survive; and patients being "in-between", i.e. non-survivors without the low-T_3 syndrome, as well as survivors with the low-T_3 syndrome.

Patients were conventionally assigned to the group with the low-T_3 syndrome on the basis of free thyroid hormone concentrations, regardless, whether their values indicated the low-T_3 syndrome once or more frequently, and reverse-T_3 concentration was not evaluated in the study. It should be noted that the cut-off point of the analysed period of time was the last hospitalisation day at the ICU, due to either patient's death or discharge from hospital (or transfer from the ICU to another ward).

The severity of illness was clinically evaluated every day, using the simplified Therapeutic Intervention Scoring System (TISS-28), in which, higher scores indicate higher number of therapeutic interventions (Miranda *et al.*, 1996); the scores <25 were regarded in the present study as "1", the scores between 25 and 35 – as "2", and scores > 35 – as "3". TISS-28 scale is the only system, used in intensive care units in Poland which is refunded by the National Healthcare Fund.

Hormone assay

After rapid centrifugation of blood samples, FT_3, FT_4 and TSH concentrations were measured by immunoenzymatic assay, using the Immulite 2000 System (DPC Cirrus Inc., Randolph, NJ, USA). Normal ranges for FT_3 concentration were 1.5–4.1 pg/ml, for FT_4 concentration – 0.8–1.9 ng/dl, and for TSH concentration – 0.27–4.2 μIU/ml.

LPO assay

After collection, blood was centrifuged (3000 × g, 10 min, 4 °C) in order to obtain serum, and stored at –80 °C until assay. The concentrations of malondialdehyde + 4-hydroxyalkenals (MDA+4-HDA), as the index of LPO, were measured in blood serum, using an LPO-586 kit, purchased from Calbiochem (La Jolla, CA). Serum (200 μl) was mixed with 650 μl of a methanol:acetonitrile (1:3, v/v) solution, containing a chromogenic reagent, N-methyl-2-phenylindole, and vortexed. After

Table 1. Clinical characteristics of healthy subjects (controls) and the studied critically ill patients. The latter group was classified to survivors and non-survivors or to normal-T_3 and low-T_3; ♠significant versus survivors, ♦significant versus normal-T_3. Statistical evaluation was done by Student's *t* test (for age and days of hospitalisation) or by the ratio comparison test.

Characteristic	Controls n=48	All patients n=70	Survivors n=32	Non-survivors n=38	Normal-T_3 n=36	Low-T_3 n=34
Male sex – no (%)	28 (58.3%)	43 (61.4%) p=0.37	17 (53.1%)	26 (68.4%) p=0.10	20 (55.6%)	23 (67.6%) p=0.15
Age – yr (mean ± SEM)	54.88 ± 2.04	59.87 ± 2.05 p=0.10	55.00 ± 3.36	63.97 ± 2.34♠ p=0.03	56.06 ± 2.90	63.91 ± 2.77♦ p<0.05
Hospitalisation period – days (mean ± SEM)	----	20.94 ± 2.23	18.63 ± 2.05	23.00 ± 3.79 p=0.33	19.53 ± 2.80	22.53 ± 3.56 0.50
Low-T_3 syndrome – no (%)	----	34 (48.6%)	6 (18.8%)	28 (73.7%)♠ p<0.0001	----	----
In-hospital deaths – no (%)	----	38 (54.3%)	----	----	10 (27.8%)	28 (82.4%)♦ p<0.0001

adding 150 µl of methanesulfonic acid (15.4 M), incubation was carried out at 45 °C for 40 min. The reaction between MDA+4-HDA and N-methyl-2-phenylindole yields a chromophore, which is spectrophotometrically measured at the absorbance of 586 nm, using a solution of 4-hydroxynonenal (10 mM) as the standard. The level of LPO was expressed as the amount of MDA+4-HDA (nmol) per 1 ml of serum.

The parameters, considered in statistical evaluation, were defined, as follows:

LPO_{first} - LPO level, evaluated on admission
LPO_{LFT3} - LPO level, found at the time of the lowest FT_3 ($FT_{3\,L}$) concentration
LPO_{LFT4} - LPO level, found at the time of the lowest FT_4 ($FT_{4\,L}$) concentration
LPO_{max} - the highest LPO level
LPO_{mean} - the mean LPO level
$FT_{3\,first}$ - FT_3 concentration, evaluated on admission
$FT_{3\,L}$ - the lowest FT_3 concentration
$FT_{3\,LFT4}$ - FT_3 concentration, found at the time of the lowest FT_4 ($FT_{4\,L}$) concentration
$FT_{4\,first}$ - FT_4 concentration, evaluated on admission
$FT_{4\,L}$ - the lowest FT_4 concentration
$FT_{4\,LFT3}$ - FT_4 concentration, found at the time of the $FT_{3\,L}$ concentration
TSH_{first} - TSH concentration, evaluated on admission
TSH_{LFT3} - TSH concentration, found at the time of the $FT_{3\,L}$ concentration
TSH_{LFT4} - TSH concentration, found at the time of the $FT_{4\,L}$ concentration.

Statistical analysis

The data were statistically analysed, using Student's unpaired t-test or the one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls' test – for continuous variables, or the Ratio Comparison Test – for the frequency of events. Univariate logistic regression analysis was used to determine which continuous variable might have predicted death/survival; in order to adjust for several risk factors, multivariate logistic regression analysis was performed with all the variables, found to be significant at the univariate analysis, entering in a single step. The χ^2 test of independence was used to determine, which dichotomised variable might have predicted death/survival. The mean standard deviation (SD) and the mean standard error of the mean (SEM) of LPO in particular patients were compared among chosen groups to evaluate the degree of variability/stability of LPO level within the hospitalisation period. The results are presented as means ± SEM. For the evaluation of correlation among particular parameters, Pearson's cor-

Table 2A. Single final diagnosis [presented in no (%)], being the direct death cause or the most important in case of patient discharge, found in all the studied critically ill patients, classified to survivors and non-survivors, or classified to normal-T_3 and low-T_3; p – level of statistical significance ($p<0.05$) marked by "♠", when significant versus survivors, or marked by "♦", when significant versus normal-T_3. Statistical evaluation was done by the ratio comparison test.

Diagnosis(es)	All patients n=70	Survivors n=32	Non-survivors n=38	p-value	Normal-T_3 n=36	Low-T_3 n=34	p-value
Circulatory and/or respiratory failure	8 (11.4%)	0 (0.0%)	8 (21.0%)♠	0.004	1 (2.8%)	5 (14.7%)♦	0.04
Circulatory arrest	9 (12.8%)	3 (9.4%)	6 (15.8%)	0.19	5 (13.85%)	4 (11.9%)	0.40
Peritonitis (with abdominal surgery)	9 (12.8%)	2 (6.3%)	7 (18.4%)	0.07	1 (2.8%)	8 (23.5%)♦	0.006
Multiple trauma	7 (10.0%)	6 (18.8%)	1 (2.6%)♠	0.02	3 (8.3%)	4 (11.9%)	0.29
COPDa	2 (2.9%)	2 (6.3%)	0 (0.0%)	0.07	2 (5.6%)	1 (2.9%)	0.27
Cerebral stroke	8 (11.4%)	2 (6.3%)	6 (15.8%)	0.10	5 (13.85)	3 (8.8%)	0.26
Pulmonary oedema	5 (7.1%)	3 (9.4%)	2 (5.4%)	0.26	3 (8.3%)	2 (5.9%)	0.37
Myocardial infarction	2 (2.9%)	1 (3.0%)	1 (2.6%)	0.50	2 (5.6%)	0 (0.0%)	0.08
Pneumonia	4 (5.7%)	3 (9.4%)	1 (2.6%)	0.14	3 (8.3%)	1 (2.9%)	0.20
Status epilepticus	3 (4.3%)	3 (9.4%)	0 (0.0%)♠	0.03	3 (8.3%)	1 (2.9%)	0.20
Gastrointestinal bleeding	2 (2.9%)	1 (3.0%)	1 (2.6%)	0.50	1 (2.8%)	1 (2.9%)	0.50
Acute pancreatitis	3 (4.3%)	3 (9.4%)	0 (0.0%)♠	0.03	3 (8.3%)	0 (0.0%)	0.05
Hepatic cirrhosis	2 (2.9%)	0 (0.0%)	2 (5.4%)	0.10	1 (2.8%)	1 (2.9%)	0.50
Major surgery	2 (2.9%)	1 (3.0%)	1 (2.6%)	0.50	0 (0.0%)	2 (5.9%)	0.07
Poisoning	2 (2.9%)	2 (6.3%)	0 (0.0%)	0.07	2 (5.6%)	0 (0.0%)	0.08
Encephalitis	1 (1.4%)	0 (0.0%)	1 (2.6%)	0.16	1 (2.8%)	0 (0.0%)	0.16
Liver failure	1 (1.4%)	0 (0.0%)	1 (2.6%)	0.16	0 (0.0%)	1 (2.9%)	0.15

[a]COPD – chronic obstructive pulmonary disease

relation coefficient was used. For the evaluation of the relationship between everyday LPO level and everyday TISS-28 score, assigned either as TISS-28 "2" or TISS-28 "3", Spearman's rank correlation coefficient was used. Statistical significance was determined at the level of $p<0.05$.

RESULTS

No statistical differences were found in sex distribution, either between the survivors and the non-survivors or between patients with normal-T_3 and those with low-T_3. The following statistical differences in age distribution were found: the non-survivors were older than the patients who survived, and patients with low-T_3 were older than those with normal-T_3. Low-T_3 syndrome was much more frequently found in the non-survivors than in the survivors; consequently, the death rate was much higher in the group of patients with low-T_3 than in the patients with normal-T_3 (Table 1).

Some differences were found in the frequencies of particular diagnoses related to death/survival rate as well as to development of the low-T_3 syndrome.

In case of single final diagnosis, being the direct death cause or the most important one at patient's discharge, circulatory and/or respiratory failure were recorded more frequently in non-survivors than in survivors, as well as more frequently in patients with low-T_3 than in patients with normal-T_3. Peritonitis occurred more frequently in patients with low-T_3 than with normal-T_3. In turn, multiple trauma, status epilepticus and acute pancreatitis occurred more frequently in survivors than in non-survivors (Table 2A).

When the final diagnosis, being one of several other diagnoses, circulatory and/or respiratory failure, and

Table 2B. Final diagnosis [presented in no (%)], being one of several other diagnoses, found in all the studied critically ill patients, classified to survivors and non-survivors, or classified to normal-T_3 and low-T_3; p – level of statistical significance ($p<0.05$) marked by "♠", when significant versus survivors, or marked by "♦", when significant versus normal-T_3. Statistical evaluation was done by the ratio comparison test.

Diagnosis(es)	All patients n=70	Survivors n=32	Non-survivors n=38	p-value	Normal-T_3 n=36	Low-T_3 n=34	p-value
Circulatory and/or respiratory failure	49 (70.0%)	11 (34.4%)	38 (100.0%)♠	<0.001	19 (52.8%)	30 (88.2%)♦	0.001
Circulatory arrest	36 (51.4%)	6 (18.8%)	30 (78.9%)♠	<0.001	13 (36.1%)	23 (67.6%)♦	0.005
Peritonitis (with abdominal surgery)	15 (21.4%)	7 (21.9%)	8 (21.1%)	0.46	4 (11.1%)	11 (32.4%)♦	0.02
Renal failure	15 (21.4%)	3 (9.4%)	12 (31.6%)♠	0.01	7 (19.4%)	8 (23.5%)	0.31
Arterial hypertension	14 (20.0%)	6 (18.8%)	8 (21.1%)	0.42	9 (25.0%)	5 (14.7%)	0.15
Multiple trauma	13 (18.6%)	7 (21.9%)	6 (15.8%)	0.26	5 (13.9%)	8 (23.5%)	0.14
COPDa	13 (18.6%)	5 (15.6%)	8 (21.1%)	0.30	4 (11.1%)	9 (26.5%)	0.05
Ischaemic heart disease	12 (17.1%)	9 (28.1%)	3 (7.9%)♠	0.02	10 (27.8%)	2 (5.9%)♦	0.01
Cerebral stroke	12 (17.1%)	3 (9.4%)	9 (23.7%)	0.05	6 (16.7%)	6 (17.6%)	0.46
Pulmonary oedema	8 (11.4%)	4 (12.5%)	4 (10.5%)	0.40	4 (11.1%)	4 (11.8%)	0.45
Myocardial infarction	7 (10.0%)	5 (15.6%)	2 (5.3%)	0.07	6 (16.7%)	1 (2.9%)♦	0.03
History of cancer	7 (10.0%)	3 (9.4%)	4 (10.5%)	0.40	2 (5.6%)	5 (14.7%0	0.11
Diabetes mellitus	6 (8.6%)	1 (3.1%)	5 (13.2%)	0.07	2 (5.6%)	4 (11.8%)	0.20
Pneumonia	5 (7.1%)	2 (6.3%)	3 (7.9%)	0.37	4 (11.1%)	1 (2.9%)	0.10
Status epilepticus	5 (7.1%)	4 (12.5%)	1 (2.6%)	0.06	4 (11.1%)	1 (2.9%)	0.10
Gastrointestinal bleeding	4 (5.7%)	2 (6.3%)	2 (5.3%)	0.43	1 (2.8%)	3 (8.8%)	0.15
Acute pancreatitis	3 (4.3%)	3 (9.4%)	0 (0.0%)♠	0.03	3 (8.3%)	0 (0.0%)	0.05
Hepatic cirrhosis	3 (4.3%)	1 (3.1%)	2 (5.3%)	0.34	2 (5.6%)	1 (2.9%)	0.27
Major surgery	2 (2.9%)	1 (3.1%)	1 (2.6%)	0.50	0 (0.0%)	2 (5.9%)	0.07
Poisoning	2 (2.9%)	2 (6.3%)	0 (0.0%)	0.07	2 (5.6%)	0 (0.0%)	0.76
Brain oedema	1 (1.4%)	0 (0.0%)	1 (2.6%)	0.16	0 (0.0%)	1 (2.9%)	0.15
Brain contusion	1 (1.4%)	0 (0.0%)	1 (2.6%)	0.16	0 (0.0%)	1 (2.9%)	0.15
Encephalitis	1 (1.4%)	0 (0.0%)	1 (2.6%)	0.16	1 (2.8%)	0 (0.0%)	0.16
Liver failure	1 (1.4%)	0 (0.0%)	1 (2.6%)	0.16	0 (0.0%)	1 (2.9%)	0.15

[a]COPD – chronic obstructive pulmonary disease

circulatory arrest were recorded more frequently in non-survivors than in survivors, as well as more frequently in patients with low-T_3 than in patients with normal-T_3. Peritonitis occurred more frequently in patients with low-T_3 than in patients with normal-T_3. Renal failure occurred more frequently in non-survivors than in survivors. Ischemic heart disease was recorded more frequently in survivors than in non-survivors, as well as more frequently in patients with normal-T_3 than in patients with low-T_3. Myocardial infarction occurred more frequently in patients with normal-T_3 than in patients with low-T_3. In turn, acute pancreatitis occurred more frequently in survivors than in non-survivors (Table 2B).

LPO level was approximately four times as high in blood serum, collected from critically ill patients, as that in healthy volunteers, independent of which LPO value, measured in critically ill patients, was taken into consideration. When LPO levels were considered separately in the survivors and in the non-survivors, they were, in both

Table 3. Mean (± SEM) values of lipid peroxidation and hormone concentrations in controls and in all the critically ill patients, classified to survivors and non-survivors or to normal-T_3 and low-T_3; *significant versus controls, ♠significant versus survivors, ♦significant versus normal-T_3. Statistical evaluation was done by unpaired Student's *t* test or by ANOVA followed by Student-Newman-Keuls' test.

	Controls n=48	All patients (%) n=70	Survivors n=32	Non-survivors n=38	Normal-T_3 n=36	Low-T_3 n=34
LPO_{first} (nmol/ml)	5.07 ± 0.39	19.77 ± 2.07* p<0.0001	18.29 ± 3.36* p=0.00014	21.02 ± 2.57* p=0.00012* p=0.38	17.17 ± 3.13* p=0.00022	22.53 ± 2.64* p=0.00011* p=0.08
LPO_{LFT3}		21.45 ± 2.29* p<0.0001	24.16 ± 4.19* p=0.00011	19.17 ± 2.34* p=0.00016* p=0.15	21.98 ± 3.85* p=0.00012	20.89 ± 2.47* p=0.00011* p=0.75
LPO_{LFT4}		19.63 ± 2.01* p<0.0001	21.48 ± 3.56* p=0.00011	18.07 ± 2.18* p=0.00013* p=0.25	19.14 ± 3.29* p=0.00011	20.15 ± 2.27* p=0.00012* p=0.74
LPO_{max}		30.81 ± 3.05* p<0.0001	33.73 ± 5.23* p=0.00011	28.34 ± 3.52* p=0.00011* p=0.24	31.45 ± 4.90* p=0.00011	30.13 ± 3.62* p=0.0001* p=0.77
LPO_{mean}		21.75 ± 2.20* p<0.0001	23.61 ± 3.87* p=0.00011	20.17 ± 2.44 p=0.00011* p=0.30	21.87 ± 3.61* p=0.00012	21.62 ± 2.50* p=0.00011* p=0.94
$FT_{3\ first}$ (pg/ml)	2.71 ± 0.12	2.60 ± 0.14 p=0.60	2.98 ± 0.19 p=0.25	2.28 ± 0.20♠ p=0.08 p=0.01♠	3.27 ± 0.17* p=0.009	1.89 ± 0.16*♦ p=0.00029* p=0.00011♦
$FT_{3\ L}$		2.05 ± 0.13* p=0.00077	2.55 ± 0.20 p=0.47	1.64 ± 0.14*♠ p=0.00012* p=0.00015♠	2.84 ± 0.15 p=0.45	1.22 ± 0.09*♦ p=0.0001* p=0.00011♦
$FT_{3\ LFT4}$		2.12 ± 0.13* p=0.003	2.75 ± 0.20 p=0.85	1.60 ± 0.13*♠ p=0.00010* p=0.00011♠	3.00 ± 0.15 p=0.08	1.19 ± 0.05*♦ p=0.0001* p=0.00011♦
$FT_{4\ first}$ (ng/dl)	1.75 ± 0.13	1.28 ± 0.06* p=0.00031	1.39 ± 0.09* p=0.02	1.20 ± 0.07* p=0.001* p=0.20	1.39 ± 0.08* p=0.02	1.17 ± 0.08* p=0.00074* p=0.15
$FT_{4\ L}$		1.04 ± 0.06* p<0.0001	1.28 ± 0.07* p=0.002	0.84 ± 0.07*♠ p=0.00011* p=0.003♠	1.28 ± 0.05* p=0.002	0.79 ± 0.08*♦ p=0.00011* p=0.001♦
$FT_{4\ LFT3}$		1.08 ± 0.06* p<0.0001	1.33 ± 0.08* p=0.006	0.88 ± 0.07*♠ p=0.00011* p=0.003♠	1.33 ± 0.06* p=0.005	0.82 ± 0.08*♦ p=0.00011* p=0.00088♦
TSH_{first} (μIU/ml)	1.28 ± 0.12	1.51 ± 0.26 p=0.49	1.94 ± 0.50 p=0.10	1.14 ± 0.21 p=0.74 p=0.12	1.97 ± 0.46 p=0.08	1.02 ± 0.21♦ p=0.51 p=0.046♦
TSH_{LFT3}		1.00 ± 0.14 p=0.17	1.22 ± 0.28 p=0.82	0.82 ± 0.10 p=0.15 p=0.10	1.25 ± 0.25 p=0.90	0.74 ± 0.11♦ p=0.07 p=0.04♦
TSH_{LFT4}		1.10 ± 0.16 p=0.45	1.38 ± 0.30 p=0.70	0.87 ± 0.17 p=0.14 p=0.14	1.34 ± 0.26 p=0.82	0.86 ± 0.18 p=0.12 p=0.18

Table 3A. Mean (± SEM) values of lipid peroxidation in critically ill patients with different diagnoses or other pathologies, classified to survivors and non-survivors or to normal-T_3 and low-T_3; ♠significant versus survivors, ♦significant versus normal-T_3. Statistical evaluation was done by unpaired Student's *t* test.

	Survivors	Non-survivors	Normal-T_3	Low-T_3
Circulatory and/or respiratory failure (n=49)	(n=11)	(n=38)	(n=19)	(n=30)
LPO_{first} (nmol/ml)	27.06 ± 7.56	21.02 ± 2.57 p=0.34	19.58 ± 5.06	24.15 ± 2.81 p=0.40
LPO_{LFT3}	31.69 ± 7.74	19.18 ± 2.34♠ p=0.04	21.26 ± 5.27	22.45 ± 2.65 p=0.82
LPO_{LFT4}	28.14 ± 6.84	18.07 ± 2.18 p=0.07	18.86 ± 4.63	21.26 ± 2.47 p=0.62
LPO_{max}	42.82 ± 9.56	28.34 ± 3.52 p=0.09	30.60 ± 6.79	32.22 ± 3.93 p=0.83
LPO_{mean}	31.20 ± 7.39	20.17 ± 2.44 p=0.07	21.59 ± 5.20	23.32 ± 2.66 p=0.75
Circulatory arrest (n=36)	(n=6)	(n=30)	(n=13)	(n=23)
LPO_{first} (nmol/ml)	16.99 ± 4.22	21.04 ± 3.03 p=0.57	13.06 ± 3.21	24.50 ± 3.41♦ p=0.03
LPO_{LFT3}	30.67 ± 6.91	18.77 ± 2.68 p=0.09	17.67 ± 4.55	22.50 ± 3.13 p=0.37
LPO_{LFT4}	25.72 ± 6.58	17.59 ± 2.43 p=0.20	15.46 ± 3.91	20.92 ± 2.86 p=0.27
LPO_{max}	41.81 ± 10.37	28.11 ± 4.08 p=0.19	26.33 ± 7.01	32.68 ± 4.59 p=0.44
LPO_{mean}	27.45 ± 6.67	19.73 ± 2.76 p=0.27	17.14 ± 4.60	23.21 ± 3.04 p=0.26
Multiple trauma (n=13)	(n=9)	(n=4)	(n=5)	(n=8)
LPO_{first} (nmol/ml)	10.59 ± 2.52	33.81 ± 5.86♠ p=0.001	8.53 ± 3.49	23.49 ± 5.05 p=0.06
LPO_{LFT3}	9.08 ± 2.22	33.64 ± 5.87♠ 0.0005	8.53 ± 3.49	21.70 ± 5.45 p=0.11
LPO_{LFT4}	10.24 ± 2.48	33.64 ± 5.87♠ 0.001	8.53 ± 3.49	23.01 ± 5.15 p=0.07
LPO_{max}	22.38 ± 7.89	51.72 ± 15.83 p=0.09	27.64 ± 14.24	33.76 ± 10.10 p=0.73
LPO_{mean}	15.21 ± 5.84	35.03 ± 8.86 p=0.09	19.13 ± 10.44	22.67 ± 6.36 p=0.76
COPD[a] (n=13)	(n=5)	(n=8)	(n=4)	(n=9)
LPO_{first} (nmol/ml)	22.76 ± 10.62	18.68 ± 5.47 p=0.71	24.04 ± 13.61	18.56 ± 4.82 p=0.64
LPO_{LFT3}	41.86 ± 12.69	15.51 ± 3.67♠ p=0.03	42.19 ± 16.38	18.29 ± 4.27 p=0.08
LPO_{LFT4}	38.39 ± 10.29	14.65 ± 3.81♠ p=0.03	37.85 ± 13.26	17.52 ± 4.42 p=0.09
LPO_{max}	41.90 ± 12.66	28.21 ± 7.98 p=0.35	42.24 ± 16.33	29.58 ± 7.17 p=0.42
LPO_{mean}	30.93 ± 10.27	19.88 ± 5.15 p=0.31	31.39 ± 13.25	20.90 ± 4.66 p=0.36
Admission hyperglycemia (n=27)	(n=10)	(n=17)	(n=13)	(n=14)
LPO_{first} (nmol/ml)	26.58 ± 6.76	17.89 ± 2.95 p=0.19	22.58 ± 5.90	19.74 ± 2.83 p=0.66
LPO_{LFT3}	37.47 ± 6.58	19.79 ± 3.67♠ p=0.02	29.35 ± 6.36	23.55 ± 4.06 p=0.44
LPO_{LFT4}	32.45 ± 5.45	17.73 ± 3.09♠ p=0.02	24.90 ± 5.37	21.59 ± 3.38 p=0.60
LPO_{max}	53.89 ± 9.27	23.98 ± 4.52♠ p=0.003	42.27 ± 9.18	28.36 ± 4.95 p=0.19
LPO_{mean}	37.69 ± 6.98	18.61 ± 3.37♠ p=0.01	30.18 ± 6.63	21.50 ± 3.63 p=0.25

[a]COPD – chronic obstructive pulmonary disease

groups, significantly higher than LPO levels, measured in the controls, however, without statistical differences, found between the survivors and the non-survivors in the entire study group of critically ill patients. In turn, when LPO level was considered separately in patients with normal-T_3 and low-T_3, both groups demonstrated significantly higher values than the controls, however, without statistical differences, found between normal-T_3 and low-T_3 in the entire study group of critically ill patients (Table 3).

However, when the comparison of LPO values was done with relation to different pathological processes (final diagnoses) (each from Table 2B) or with relation to such parameters as acidosis, anemia, hyperglycemia, and hypokalemia, the following statistically significant differences were found. In patients with circulatory and/or respiratory failure, as well as in patients with chronic obstructive pulmonary disease (COPD), LPO level was higher in survivors than in non-survivors. In contrast, in patients with multiple trauma, LPO level was higher in non-survivors than in survivors, and in patients with circulatory arrest, LPO level was higher in low-T_3 than in normal-T_3. In patients with admission hyperglycemia, LPO level was higher in survivors than in non-survivors (Table 3A).

Then, LPO levels were compared between particular subgroups. In the group of critically ill patients who did not survive, LPO level was twice higher in patients with the low-T_3 syndrome, comparing to those with normal-T_3, with statistical significance for each LPO value. Concerning the differences in LPO level, found between other subgroups, they did not reach statistical significance. However, some tendencies were noted – the least severely ill patients, i.e. survivors without the low-T_3 syndrome had LPO levels as high as those, observed in the most severely ill patients, i.e. non-survivors with the low-T_3 syndrome (no statistical differences were found between those subgroups); at the same time, however, patients being "in-between", i.e. non-survivors without the low-T_3 syndrome, as well as survivors with the low-T_3 syndrome, had lower LPO level (without statistical differences between these subgroups) (Table 4).

Concerning thyroid hormone concentrations between either particular groups or particular subgroups, the expected results were obtained. FT_3 concentration, as well as that of FT_4, was significantly lower in critically ill patients than in healthy subjects (Table 3). FT_3 and FT_4 concentrations were lower in the non-survivors than in the survivors, when the entire study group of critically ill patients was considered (Table 3). Both FT_3 and FT_4 concentrations were significantly lower in patients with low-T_3 syndrome than in patients with normal-T_3 in the entire studied group of critically ill patients (Table 3) and in the non-survivors (Table 4). In the survivors, only FT_3 concentration was lower in patients with low-T_3 than in patients with normal-T_3, and such differences did not concern FT_4 concentration (Table 4). Additionally, non-survivors with low-T_3 syndrome had lower FT_4 concentration than survivors with low-T_3 syndrome (Table 4). TSH concentration was lower in patients with low-FT_3 syndrome than in patients with normal-T_3, when the entire study group was considered (Table 3).

For the entire study group of critically ill patients, as well as for patients with normal-T_3 and patients with low-T_3 syndrome, several parameters, such as LPO level, hormone concentrations, glucose, creatinine, urea and bilirubin concentrations were submitted to a univariate and a multivariate logistic regression model. The purpose of the model was to determine which of those continuous variables might predict survival/death.

No death predictive value for LPO was documented at logistic regression analysis in the entire group of critically ill patients (Table 5). However, when the test was performed with relation to different pathological processes (final diagnoses) or such parameters as acidosis, anemia, hyperglycemia and hypokalemia, negative death predictive value for LPO was found in patients with hyperglycemia on admission (Table 5A).

For the entire study group of critically ill patients, FT_3 and FT_4 concentrations constituted independent negative death risk factors. At multivariate analysis, both determinants lost their predictive value. For the patients with normal-T_3 or for the patients with low-T_3 syndrome, none of the analysed variables were found to have predictive significance (data not shown) (Table 5).

Among routinely measured biochemical parameters, urea and creatinine concentrations had positive, death predictive values for the entire study group of critically ill patients (Table 5), as well as for patients with normal-T_3 (OR=1.02, 95%CI=1.00–1.04, p=0.02; and OR=1.65, 95%CI=1.02–2.66, p=0.04, respectively), but not for the patients with the low-T_3 syndrome (data not shown). No predictive value was found either for glucose or for bilirubin concentrations at univariate regression analysis (Table 5).

Among dichotomised variables in the entire group of critically ill patients, death predictive value was accounted to acidosis, diabetes mellitus, renal failure (which preserved its positive predictive values in normal-T_3 patients), cardiac arrest and circulatory-respiratory failure (the two latter preserved its positive predictive values in both normal-T_3 patients and low-T_3 patients); additionally, predictive value positive of death was found in the death predictive value low-T_3 syndrome for myocardial infarction (Table 6).

Correlations were evaluated between LPO levels and other measured parameters. Positive correlations were found between LPO level and patient's age in healthy subjects, in the entire study group of critically ill patients, as well as in the patients with the low-T_3 syndrome and in the non-survivors. Negative correlation was found between the lowest FT_3 concentration and LPO level in patients with normal-T_3 (Table 7).

The degree of variability/stability of LPO level within the hospitalisation period was compared between the survivors and the non-survivors, as well as between patients

Table 4. Mean (± SEM) values of lipid peroxidation and hormone concentrations in survivors (n=32) and non-survivors (n=38), classified to normal-T_3 and low-T_3.

	Survivors n=32		Non-survivors n=38	
	Normal-T_3 n=26	Low-T_3 n=6	Normal-T_3 n=10	Low-T_3 n=28
LPO_{first} (nmol/ml)	19.70 ± 4.05	12.19 ± 3.10	10.59 ± 3.48	24.75 ± 2.99♦ p=0.01
LPO_{LFT3}	26.34 ± 4.93	14.69 ± 5.51	10.67 ± 3.44	22.22 ± 2.74♦ p=0.03
LPO_{LFT4}	22.65 ± 4.20	16.43 ± 5.41	10.01 ± 3.32	20.95 ± 2.52♦ p=0.03
LPO_{max}	37.20 ± 6.16	18.71 ± 4.94	16.50 ± 5.20	32.58 ± 4.16♦ p=0.04
LPO_{mean}	26.16 ± 4.55	12.55 ± 3.87	10.70 ± 3.66	23.56 ± 2.81♦ p=0.02
$FT_{3\ first}$ (pg/ml)	3.28 ± 0.18	1.68 ± 0.22♦ p=0.00031	3.26 ± 0.40	1.94 ± 0.19♦ p=0.002
$FT_{3\ L}$	2.87 ± 0.20	1.15 ± 0.10♦ p=0.00032	2.77 ± 0.19	1.23 ± 0.10♦ p<0.0001
$FT_{3\ LFT4}$	3.09 ± 0.19	1.26 ± 0.21♦ p<0.0001	2.78 ± 0.19	1.18 ± 0.05♦ p<0.0001
$FT_{4\ first}$ (ng/dl)	1.38 ± 0.09	1.44 ± 0.28	1.43 ± 0.17	1.11 ± 0.07 p=0.05
$FT_{4\ L}$	1.30 ± 0.06	1.24 ± 0.30	1.26 ± 0.11	0.69 ± 0.06♦♠ p<0.0001 p=0.008
$FT_{4\ LFT3}$	1.35 ± 0.07	1.25 ± 0.30	1.28 ± 0.09	0.73 ± 0.07♦♠ p=0.00014 p=0.01
TSH_{first} (μIU/ml)	2.23 ± 0.61	0.69 ± 0.22	1.29 ± 0.44	1.09 ± 0.25 p=0.68
TSH_{LFT3}	1.33 ± 0.34	0.74 ± 0.34	1.03 ± 0.24	0.74 ± 0.11 p=0.24
TSH_{LFT4}	1.52 ± 0.35	0.79 ± 0.33	0.86 ± 0.22	0.87 ± 0.21 p=0.97

♦significant versus normal-T_3 (within the same main group – survivors or non-survivors; ♠significant versus survivors with low-T_3). Statistical evaluation was done by Student's *t* test.

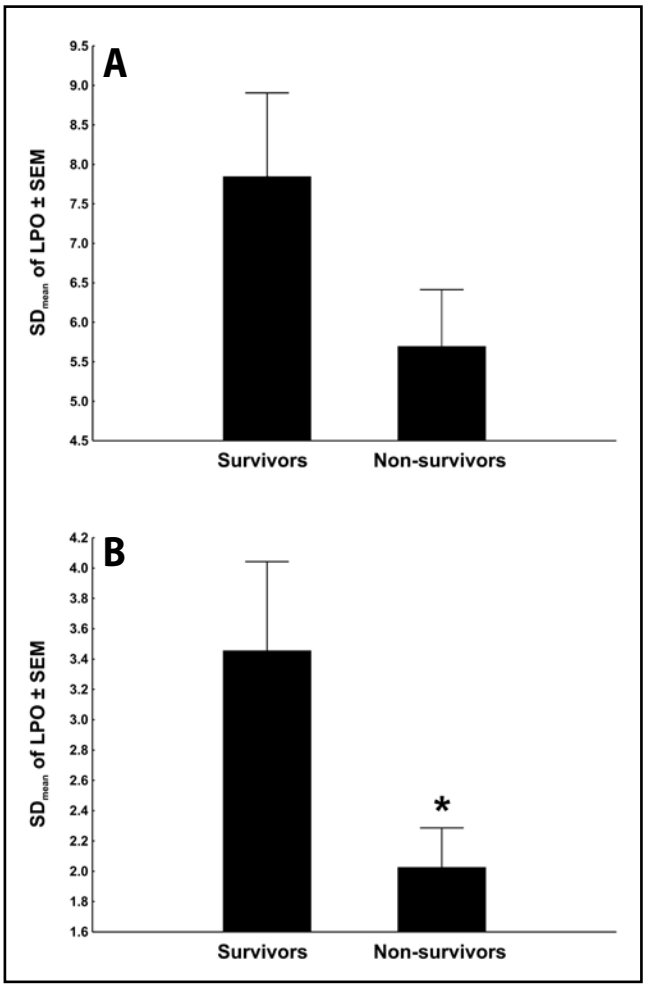

Figure 1. Variability of lipid peroxidation level, expressed as the mean SD (SD_{mean}) of LPO (Figure 1A) or as the mean SEM (SEM_{mean}) of LPO (Figure 1B), within the hospitalisation period in all the critically ill patients, classified to survivors and non-survivors. *significant versus survivors, p<0.05. Statistical evaluation was done by unpaired Student's t test.

with normal-T_3 and those with the low-T_3 syndrome. LPO variability was higher in the survivors than in the non-survivors, with statistical significance obtained, when the comparison employed the mean SEM (Figures 1A–1B). LPO variability was something higher in patients with normal-T_3 than in those with low-T_3 syndrome, but the differences for neither the mean SD nor the mean SEM were statistically significant (data not shown).

The TISS-28 score was always, at least, 25 or more in all studied critically ill patients, within the whole hospitalisation period. Thus, each patient was assigned either TISS-28 "2", or TISS-28 "3", and none of them was assigned TISS-28 "1", on any particular day of the hospitalisation period. Higher TISS-28 scores (assigned TISS-28 "3") were found much more frequently in the non-survivors than in the survivors but the difference between the patients with low-T_3 and the patients with normal-T_3 was less clear and statistically significant only in one case of the highest LPO level (Table 8).

For the entire study group of critically ill patients, LPO level was unexpectedly lower in the patients, classified as TISS-28 "3" than in those, classified as TISS-28 "2". Similar differences were found in patients with normal-T_3, in the survivors and in the survivors with normal-T_3. Conversely, when the groups of more severely ill patients (non-survivors or with low-T_3 syndrome) were considered, LPO level did not differ between the groups with lower or higher TISS-28 scores (Table 9). Lower LPO

Table 5. Univariate and multivariate logistic regression analysis of the univariate death determinant (variables), such as lipid peroxidation and hormone concentrations, performed in all the critically ill patients (n=70); OR, odds ratio; CI, confidence interval.

VARIABLE	UNIVARIATE REGRESSION		MULTIVARIATE REGRESSION	
	OR	95% CI	OR	95% CI
LPO_{first} (nmol/ml)	1.01 p=0.51	0.98–1.04	----	----
LPO_{LFT3}	0.99 p=0.29	0.96–1.01	----	----
LPO_{LFT4}	0.99 p=0.40	0.96–1.02	----	----
LPO_{max}	0.99 p=0.38	0.97–1.01	----	----
LPO_{mean}	0.99 p=0.44	0.96–1.02	----	----
$FT_{3\ first}$ (pg/ml)	0.60* p=0.02	0.37–0.92	1.35 p=0.46	0.60–3.04
$FT_{3\ L}$	0.40* p=0.002	0.23–0.71	1.65 p=0.61	0.23–11.69
$FT_{3\ LFT4}$	0.30* p=0.00025	0.16–0.56	0.24 p=0.18	0.03–1.97
$FT_{4\ first}$ (ng/dl)	0.40 p=0.10	0.13–1.21	----	----
$FT_{4\ L}$	0.07* p=0.00071	0.1–0.30	0.20 p=0.60	0.0004–100.60
$FT_{4\ LFT3}$	0.07* p=0.00037	0.02–0.32	0.92 p=0.98	0.002–433.13
TSH_{first} (μIU/ml)	0.81 p=0.16	0.61–1.09	----	----
TSH_{LFT3}	0.73 p=0.18	0.50–1.16	----	----
TSH_{LFT4}	0.75 p=0.14	0.50–1.10	----	----
glucose[a] (mmol/l)	1.01 p=0.10	0.99–1.02	----	----
urea[a] (mmol/l)	1.02* p=0.01	1.00–1.04	1.03 p=0.07	0.99–1.06
creatininea (mmol/l)	1.66* p=0.045	1.01–2.74	0.79 p=0.56	0.36–1.76
bilirubina (mmol/l)	1.33 p=0.31	0.75–2.35	----	----

[a] evaluated only on admission; *p<0.05.

Table 5A. Univariate and multivariate logistic regression analysis of lipid peroxidation, as the univariate death determinant (variable), performed in the critically ill patients with admission hyperglycemia (n=27); OR, odds ratio; CI, confidence interval.

VARIABLE	UNIVARIATE REGRESSION		MULTIVARIATE REGRESSION	
	OR	95%CI	OR	95%CI
LPO_{first} (nmol/ml)	0.97 p=0.22	0.91–1.02	----	----
LPO_{LFT3}	0.94* p=0.04	0.89–0.99	1.07 p=0.43	0.89–1.30
LPO_{LFT4}	0.93* p=0.04	0.88–0.99	0.90 p=0.28	0.74–1.09
LPO_{max}	0.95* p=0.02	0.91–0.99	0.76 p=0.06	0.57–1.01
LPO_{mean}	0.94* p=0.04	0.89–0.99	1.38 p=0.09	0.95–2.00

*p<0.05.

levels in the patients with higher TISS-28 scores vs. those with lower TISS-28 scores, as determined by the use of Student's *t* test, were confirmed in univariate regression analysis (data not shown).

Negative relationship (evaluated by Spearman's rank correlation coefficient) was found between the everyday LPO level and the everyday TISS-28 score, either for the entire study group of critically ill patients or for non-survivors or for normal-T_3 or for low-T_3 (Table 10).

DISCUSSION

Products of oxidative damage to macromolecules, including LPO products, present in blood, are formed not only in the vascular compartment (Szasz *et al*., 2007) but they may also derive from different tissues and organs (from all the subcellular compartments), the latter probably provide the predominant amount of LPO products in blood. Thus, LPO products, present in blood of critically ill patients, are expected to represent oxidative damage to lipids, being constituents of different cellular membranes (cytoplasmic, microsomal, mitochondrial, nuclear membranes, etc.) of all tissues and organs.

The present study demonstrates some evidence for increased oxidative damage to lipids in patients, suffering from critical illnesses. This part of our results is in concordance with numerous earlier studies, performed in critically ill patients or using animal models, in which different indices of oxidative damage to macromolecules were examined (Barichello *et al*., 2006; Cighetti *et al*., 2005; Crimi *et al*., 2006; Idris *et al*., 2005; Mishra *et al*., 2005; Motoyama *et al*., 2003; Rokyta *et al*., 2003).

However, certain discrepancy exists between previous results of other authors and our observations. In opposite to the earlier findings, showing higher oxidative damage to lipids in non-survivors, comparing to critically ill patients, who did survive (Alonso de Vega *et al.,* 2002; Mishra *et al*., 2005), LPO blood levels in the survivors and in the non-survivors did not differ in our study at all. Moreover, the lack of death predictive value for LPO, found in the present study, does not confirm the earlier assumption that the increased level of oxidative damage to lipids in critically ill patients could be treated as a prognostic marker of poor prognosis (Mishra *et al*., 2005). It should be stressed, however, that the above discussion refers to the entire group of critically ill patients, without taking

Table 6. χ^2 test of independence analysis of the dichotomized death determinant (variables), performed in all the critically ill patients (n=70), in patients with normal-T_3, and in patients with low-T_3 syndrome.

Dichotomized variable	All patients (n=70)		Normal-T_3 (n=36)		Low-T_3 (n=34)	
	χ^2		χ^2		χ^2	
circulatory-respiratory failure (n=49)	35.63*	p<0.0001	12.39*	p<0.001	21.16*	p<0.001
cardiac arrest (n=36)	24.41*	p<0.001	11.56*	p<0.001	8.25*	p=0.004
anemia (n=30)	0.50	p=0.48	0.85	p=0.36	0.001	p=0.98
hypokalemia (n=27)	1.81	p=0.18	0.18	p=0.67	0.33	p=0.59
admission hyperglycemia (n=27) (> 6.1 mmol/l)	1.33	p=0.25	1.16	p=0.28	0.19	p=0.67
acidosis (n=21)	4.82*	p=0.03	2.37	p=0.12	3.16	p=0.08
renal failure (n=15)	7.28*	p=0.006	8.31*	p=0.004	0.73	p=0.40
arterial hypertension (n=14)	0.86	p=0.35	1.43	p=0.23	1.86	p=0.17
ischemic heart disease (n=12)	3.04	p=0.08	0.01	p=0.90	1.12	p=0.29
cancer (n=7)	0.04	p=0.84	0.81	p=0.37	0.01	p=0.91
myocardial infarction (n=7)	1.55	p=0.21	0.39	p=0.53	4.31*	p=0.04
diabetes mellitus (n=6)	4.07*	p=0.04	1.71	p=0.19	1.42	p=0.23

*p<0.05.

Table 7 (7A-7E). Pearson's correlation coefficients between lipid peroxidation and age, or hormone concentrations in healthy subjects (n=48) (Table 7A), in all the critically ill patients (n=70) (Table 7B), in non-survivors (n=38) (Table 7C), in patients with normal T_3 (n=36) (Table 7D), and in patients with low-T_3 (n=34) (Table 7E).

Table 7A	**LPO** (nmol/ml)
age (yr)	0.34*

Table 7B	LPO_{first} (nmol/ml)	LPO_{LFT3}	LPO_{LFT4}	LPO_{max}	LPO_{mean}
age (yr)	0.27*	0.16	0.19	0.14	0.17

Table 7C	LPO_{first} (nmol/ml)	LPO_{LFT3}	LPO_{LFT4}	LPO_{max}	LPO_{mean}
age (yr)	0.35*	0.14	0.19	0.18	0.25

Table 7D	LPO_{first} (nmol/ml)	LPO_{LFT3}	LPO_{LFT4}	LPO_{max}	LPO_{mean}
$FT_{3\ first}$ (pg/ml)	-0.07	-0.10	-0.07	-0.15	-0.15
$FT_{3\ L}$	-0.25	-0.28	-0.27	-0.37*	-0.34*
$FT_{3\ LFT4}$	-0.22	-0.23	-0.27	-0.26	-0.26

Table 7E	LPO_{first} (nmol/ml)	LPO_{LFT3}	LPO_{LFT4}	LPO_{max}	LPO_{mean}
age (yr)	0.42*	0.33	0.33	0.36*	0.43*

*p<0.05.

Table 8. The frequency of higher TISS-28 scores (>35, assigned as TISS-28 "3") on the first day, at the time of the lowest FT_3 concentration ($FT_{3\,L}$), at the time of the lowest FT_4 concentration ($FT_{4\,L}$) or at the time of the highest lipid peroxidatio level (LPO_{max}), evaluated in survivors and in non-survivors, as well as patients with normal-T_3 and low-T_3 syndrome.

	Survivors n=32	Non-survivors n=38	Normal-T_3 n=36	Low-T_3 n=34
TISS-28 "3" on the first day – no (%)	19 (59.4%)	35 (92.1%)♠ p=0.002	25 (69.4%)	29 (85.3%) p=0.12
TISS-28 "3" by the $FT_{3\,L}$ – no (%)	14 (43.8%)	29 (76.3%)♠ p=0.008	20 (55.6%)	23 (67.6%) p=0.31
TISS-28 "3" by the $FT_{4\,L}$ – no (%)	17 (53.1%)	30 (78.9%)♠ p=0.02	23 (63.9%)	24 (70.6%) p=0.53
TISS-28 "3" by the LPO_{max} – no (%)	11 (34.4%)	34 (89.5%)♠ p<0.0001	17 (47.2%)	28 (82.4%)♦ p=0.03

♠significant versus survivors, ♦significant versus normal-T_3. Statistical evaluation was done by the ratio comparison test.

into consideration particular pathological processes. Further statistical analyses revealed that the differences in LPO levels depended on the kind of pathological process. In case of some diagnoses, such as circulatory and/or respiratory failure or chronic obstructive pulmonary disease, LPO level was much higher in survivors than in non-survivors, but in patients with multiple trauma, which could be treated as a more acute state than the two pathologies mentioned before, the result was opposite. In turn, circulatory arrest, which is obviously an acute state, was associated with higher LPO level in patients with low-T_3 syndrome. Thus, on the basis of the present results, we can conclude that, although LPO level may – to some extent – determine death/survival, it depends strongly on the kind of critical disease.

Another novel observation relates to the comparison of LPO level in particular subgroups. This analysis did reveal differences in LPO level, being distinctly dependent on both outcome measures (in terms of survival/death and low-T_3 syndrome development), but only when those two criteria were considered together. Although statistical differences were found in LPO level only between patients with low-T_3 syndrome and without this syndrome, a certain tendency is easily visible. The least severely ill patients, i.e. survivors without low-T_3 syndrome had as high LPO levels as the most severely ill patients, i.e. non-survivors with low-T_3 syndrome. At the same time, however, patients being "in-between", i.e. non-survivors without low-T_3 syndrome, as well as survivors with low-T_3 syndrome, had lower LPO level.

The explanation of this absolutely novel finding could be as follows. In the least severely ill patients, high LPO levels suggest that oxidative processes, occurring at higher level, causing stronger damage to macromolecules and – probably – inducing stronger defence mechanisms, result in better outcome. Conversely, in the most severely ill patients, higher levels of LPO products did not produce any favourable outcome, what could be due to the absolute disruption (inefficiency) of any protective mechanisms, antioxidative mechanisms included. Indeed, total or subtotal dysfunction of many organs at cellular and subcellular levels is typical for critical stages of different diseases. This is in agreement with the observation, discussed above, showing that higher oxidative damage could be associated with higher death rate in patients with acute disorders but, in some chronic disorders, the dependence may be opposite.

The higher variability of LPO among patients who survived, that was observed in the present study, further confirms the above assumption that red-ox processes could be more dynamic and intensive in patients with better clinical status, better prognosis, and – consequently – better outcome.

The significance of intensive red-ox processes, producing higher oxidative damage, in the better final outcome of critically ill patients is further supported by the negative relationship between LPO level and TISS-28 scores. Among less severely ill patients (survivors or without low-T_3 syndrome), lower LPO level was found in case of higher TISS-28 scores, requiring more therapeutic interventions (thus constituting more severe clinical stage). Such a difference between LPO level in higher and lower TISS-28 scores was not found in more severely ill patients (non-survivors or with low-T_3 syndrome). Weaker damage to lipids in patients with higher TISS-28 scores was also confirmed in the present study by negative correlation between LPO level and adjusted TISS-28 score, both measured daily in each patient during the entire hospitalisation period. These observations suggest again that, under more serious conditions (more advanced disease), oxidative processes may occur less intensively, producing weaker damage to macromolecules but – at the same time – inducing weaker defence mechanisms, consequently leading to unfavourable outcome. However, the latter assumption related to protective mechanisms requires clinical evidence.

In order to make the discussion on our, rather unexpected, results complete, not entirely clear results of other studies should be mentioned, related to oxidative stress in critically ill patients. For example, the serum total antioxidant status was found to be higher in the non-survivors than in critically ill patients who survived (MacKinnon *et al.*, 1999). A decreased level of nitric oxide, being a free radical was established in patients with acute respiratory distress syndrome (Kumar *et al.*, 2000). Furthermore, increased formation of reactive nitrogen intermediates in saliva of critically ill patients may enhance endogenous bactericidal defence effects, resulting in better outcome (Bjorne *et al.*, 2007).

Table 9. Mean (± SEM) values of lipid peroxidation level in all the critically ill patients, classified to patients with lower TISS-28 scores (TISS-28 "2") and with higher TISS-28 scores (TISS-28 "3")

	LPO (nmol/ml)	TISS-28 "2" (mean ± SEM)	n	TISS-28 "3" (mean ± SEM)	n	p-value
All the critically ill patients (n=70)	LPO_{first}	25.88 ± 5.74	16	17.96 ± 2.04	54	0.11
	LPO_{LFT3}	31.96 ± 4.34	27	14.86 ± 2.03*	43	0.00016
	LPO_{LFT4}	28.90 ± 4.19	23	15.09 ± 1.87*	47	0.00086
	LPO_{max}	34.00 ± 5.60	25	29.12 ± 3.61	45	0.45
Normal-T_3 (n=36)	LPO_{first}	27.61 ± 7.95	11	12.57 ± 2.47*	25	0.02
	LPO_{LFT3}	38.44 ± 6.25	16	8.83 ± 1.99*	20	<0.0001
	LPO_{LFT4}	32.36 ± 6.51	13	11.66 ± 2.61*	23	0.001
	LPO_{max}	35.91 ± 7.03	19	26.46 ± 6.78	17	0.34
Low-T_3 (n=34)	LPO_{first}	22.08 ± 6.57	5	22.61 ± 2.92	29	0.94
	LPO_{LFT3}	22.55 ± 4.48	11	20.10 ± 3.02	23	0.65
	LPO_{LFT4}	24.41 ± 4.63	10	18.38 ± 2.56	24	0.23
	LPO_{max}	27.97 ± 7.33	6	30.74 ± 4.15	28	0.78
Survivors (n=32)	LPO_{first}	26.14 ± 6.76	13	12.91 ± 2.83	19	0.05
	LPO_{LFT3}	36.87 ± 5.79	18	7.82 ± 1.64*	14	0.00016
	LPO_{LFT4}	31.98 ± 5.73	15	12.22 ± 3.05*	17	0.004
	LPO_{max}	35.64 ± 6.36	21	30.10 ± 9.49	11	0.62
Non-survivors (n=38)	LPO_{first}	24.75 ± 11.47	3	20.70 ± 2.67	35	0.68
	LPO_{LFT3}	22.16 ± 4.82	9	18.26 ± 2.70	29	0.49
	LPO_{LFT4}	23.13 ± 5.35	8	16.72 ± 2.36	30	0.24
	LPO_{max}	25.44 ± 11.14	4	28.81 ± 3.76	34	0.77
Survivors with normal-T_3 (n=26)	LPO_{first}	30.05 ± 8.37	10	13.22 ± 3.30*	16	0.04
	LPO_{LFT3}	40.79 ± 6.19	15	6.64 ± 1.86*	11	0.00012
	LPO_{LFT4}	34.79 ± 6.57	12	12.24 ± 3.70*	14	0.005
	LPO_{max}	37.54 ± 7.23	18	36.44 ± 12.45	8	0.94
Survivors with low-T_3 (n=6)	LPO_{first}	13.10 ± 5.32	3	11.28 ± 4.34	3	0.80
	LPO_{LFT3}	17.25 ± 11.79	3	12.12 ± 2.56	3	0.70
	LPO_{LFT4}	20.73 ± 11.02	3	12.12 ± 2.56	3	0.49
	LPO_{max}	24.24 ± 9.22	3	13.18 ± 2.51	3	0.31
Non-survivors with normal-T_3 (n=10)	LPO_{first}	3.14	1	11.43 ± 3.78	9	0.51
	LPO_{LFT3}	3.14	1	11.51 ± 3.73	9	0.50
	LPO_{LFT4}	3.14	1	10.77 ± 3.62	9	0.52
	LPO_{max}	6.64	1	17.59 ± 5.69	9	0.56
Non-survivors with low-T_3 (n=28)	LPO_{first}	35.56 ± 6.67	2	23.92 ± 3.14	26	0.32
	LPO_{LFT3}	24.53 ± 4.75	8	21.29 ± 3.38	20	0.60
	LPO_{LFT4}	25.99 ± 5.22	7	19.27 ± 2.89	21	0.27
	LPO_{max}	31.70 ± 13.02	3	32.85 ± 4.47	25	0.93

*significant versus TISS-28 "2". Statistical evaluation was done by unpaired Student's *t* test.

Table 10. The relationship between lipid peroxidation level and the everyday TISS-28 score.

	n	R
All the critically ill patients	623	–0.18*
Survived	240	–0.07
Not survived	383	–0.20*
Normal-T_3	346	–0.17*
Low-T_3	277	–0.18*

n – the sum of hospitalisation days of all the patients belonging to particular groups; R – Spearman's rank correlation coefficient; * $p<0.05$.

When discussing increased oxidative damage to lipids in critically ill patients, it should be mentioned that not well balanced food consumption, which is frequently typical for critical disorders, contributes to increased oxidative stress (Staruchova *et al.*, 2006).

Just as expected, thyroid hormone concentrations appeared to be valuable in estimating patient's clinical status and in predicting death. First, low-T_3 syndrome was found much more frequently in the non-survivors than in the survivors, and FT_3 and FT_4 concentrations were lower in the non-survivors than in the survivors. Second, FT_3 and FT_4 concentrations constituted independent negative death risk factors. These observations are consistent with numerous previous results (Peeters *et al.,* 2006; Vanhorebeek and Van den Berghe, 2006).

It is worth stressing that our expected results on the significance of low thyroid hormone concentration, as markers for poor prognosis, were found in the same critically ill patients, high LPO levels were noted with relation to good prognosis and good final outcome.

Although both LPO levels and free thyroid hormone concentrations are of value in estimating the current clinical status, only a poor relationship was found between those two parameters in the present study. The negative correlation, which was found between FT_3 concentration and LPO level in patients with normal-T_3, could only support the above suggestion that increased oxidative damage to lipids may be associated with more dynamic red-ox reactions, leading – under certain conditions – to better outcome.

The observed positive death predictive values of urea and creatinine concentrations are in agreement with another finding of the present study, showing death predictive value for renal failure. Other parameters or diagnoses, for which death predictive value was found in the present study, constitute: acidosis, diabetes mellitus, cardiac arrest, circulatory-respiratory failure and, additionally, myocardial infarction, the latter observed only in patients with low-T_3 syndrome. These findings are in agreement with well-known mortality risk factors at ICU.

The positive correlation, found between LPO level and patient's age (both healthy subjects and critically ill patients), is in agreement with very well known increased oxidative damage to macromolecules, occurring with age in all living organisms (Martin and Grotewiel, 2006), however it has been not confirmed that this phenomenon results from increased ROS production (Stritesky Larssen and Lyberg, 2006).

The increased oxidative damage to lipids, found in critically patients in the present study, supports the ideas to examine potential protective effects of antioxidants in such patients (Berger, 2005; Heyland *et al.*, 2006; Pontes-Arruda *et al.*, 2006).

As normoglycemia control has recently been proposed as one of the most important determinants in prevention of organ failure and death in critically ill patients (Langouche *et al.*, 2005; Turina et *al.*, 2006; Van den Berghe *et al.*, 2003), the issue requires some discussion. No predictive values were found in the present study, either for glucose concentration or for hyperglycemia, as evaluated on admission. Only the diagnosis of diabetes mellitus was found to be a positive death determinant in the studied population of all critically ill patients. Thus, our results do not confirm the significance of hyperglycemia in either oxidative damage or death determination. But this is probably due to the fact that neither daily glucose nor insulin infusion control was monitored in the present study. Instead, however, higher LPO levels were associated with lower death rates and LPO level appeared to be a negative death predicting factor in patients with hyperglycemia on admission. This finding confirms again that, under some conditions, high oxidative damage to macromolecules may result in better outcome.

In conclusion, a tremendous increase in oxidative damage to lipids in critically ill patients strongly depends on the kind of pathological process and, under certain conditions, higher LPO levels could be due to more dynamic red-ox reactions (and, possibly, to more intensive defence mechanisms), resulting in more favourable outcome.

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