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Routine clinical laboratory tests correspond to increased serum levels of 3-hydroxy fatty acids, markers of endotoxins, in cardiosurgery patients

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Abstract

Introduction: Endotoxemia developing during cardiosurgery as elevated endotoxin concentrations in patient's serum may prevail over 24 h after operation. A major reason is thought to be increased gut permeability resulting in endotoxin and bacterial leakage. In this study we aimed to measure endotoxin levels on samples obtained during and after cardiovascular procedures and compare them with clinical observations and laboratory test results.
Materials and Methods: 3-Hydroxy fatty acids (3-OH FAs) of 10–18 carbon chain length, chemical markers of endotoxin (lipopolysaccharide), were determined in patient sera by gas chromatography-mass spectrometry-based analysis. Results were compared with routine laboratory tests: blood morphology, urine, ALT, AST, bilirubin, kidney parameters, clotting parameters, and gasometry.
Results: Of a total of 16 patients, 5 patients (group I) showed increased serum 3-OH FA levels and 11 patients (group II) did not show any change in 3-OH FA levels 24 h after operation. All group I patients revealed leukocytosis, two developed post-operative anemia. Significantly different changes were observed: the initial, pre-operative 3-OH FA levels were similar for both groups, while group I patients showed increased levels of all the studied 3-OH FAs during the operation ($p \leq 0.05$), and 3-OH C14 and 3-OH C16 remained elevated 24 h after the operation.
Conclusions: Cardiosurgery may strongly promote gut endotoxin translocation to the blood in some patients. Prolonged leukocytosis, deep anemia, and increased liver dysfunction markers may indicate the need for observation of possible endotoxemia development. It is recommended to monitor the endotoxin level and/or endotoxemia markers in cardiosurgery patients.

Key words: cardiosurgery, endotoxin, chemical markers, 3-hydroxy fatty acids.

Abbreviations: 3-OH FAs – 3-hydroxy fatty acids, LPS – lipopolysaccharide, CABG – coronary artery bypass grafting, GLC-MS – gas-liquid chromatography-mass spectrometry, ALT – alanine aminotransferase, AST – aspartate aminotransferase, CPK – creatinine kinase, CK-MB – heart isoenzyme of the creatinine kinase, ACT – activating clotting time.

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INTRODUCTION

Cardiovascular surgery may lead to increased intestinal epithelial permeability due to, for example, cellular hypoxia, mesenteric prolonged ischemia, oxidant stress, cytokines, bacterial toxins, endotoxemia, and total parenteral nutrition [6, 7, 17]. Among these factors, gut ischemia is considered to be the most important, since hypoperfusion of the splanchnic bed is not tolerated for longer periods. The epithelial cells of villi are very sensitive for hypoxia, and prolonged ischemia can lead to necrosis of epithelial cells and, finally, necrosis of the tips of mucosal villi. This may result in dysfunction of the gut barrier [8] leading to "gut leakage", including the translocation of bacteria and bacterial products, such as endotoxin (lipopolysaccharide – LPS), from the intestinal lumen to mesenteric lymph nodes, blood, and inner organs [1, 2, 14].

The splanchnic blood flow rate of cardiosurgery patients varies when using a lung-heart machine. One reason is the prolonged hypotension that begins with connecting the patient to the lung-heart machine after cross-clamping the aorta [9], when the mean arterial pressure may decrease to 50–60 mm Hg. Moreover, the administration of catecholamines, acting on α-adrenergic receptors, also decreases the splanchnic flow rate. Another reason is the loss of blood during surgery and the use of heparin during and after operation. Hypothermia (body temperature is kept at 28°C) constricts vessels and decreases visceral blood flow. After disconnection from the lung-heart machine, the cardiac output and, consequently, blood flow through viscera increase. This is considered a reason for reperfusion syndrome, which also affects the blood-gut barrier and causes bacterial translocation [3].

Endotoxemia as a result of increased gut permeability (particularly through intercellular tight junctions) has been observed during coronary artery bypass grafting (CABG) and post-operative periods. Levels of endotoxin may remain elevated even more than 24 h after surgery and may strongly influence patient status [3, 4]. Despite these findings, endotoxin determination, in connection with cardiosurgery as well as surgery in general, does not belong to routine clinical laboratory practice.

We previously used 3-hydroxy fatty acids (3-OH FAs) for monitoring endotoxin levels in the blood and sera of rats [15]. 3-OH FAs, structural constituents of the lipid A part of LPS, are chemical markers of endotoxin and are quantified by an analytic method [13]. In the present studies this approach was applied to determine endotoxin in the sera of patients before, during, and after surgery. The aim of the study was to relate poor outcome of patients revealed by high levels of endotoxin markers to clinical observations and routine laboratory tests performed on patients in accordance with the procedure so that we could find indications for the convalescent process after the operation.

MATERIALS AND METHODS

Patients

Sixteen patients of a total of 28 undergoing elective CABG and/or artificial valve implantation at the Cardiosurgery Clinical Hospital in Poznań, Poland, were enrolled. The project was approved by the Ethical Committee at the Medical Academy of Poznań. Patients with a history of inflammatory bowel disease or who had been using either steroids or non-steroidal anti-inflammatory drugs (other than a cardiological dose of aspirin) within one month prior to surgery were excluded from the study. Blood and urine samples were cultured and patients with positive test results for bacteria were also excluded from the study.

Pre-operative period

The patients had abstained from aspirin one week before operation. They received their last meal (liquid) at noon the day before operation, and then they could have either water or tea. In the evening an enema was done and each patient was shaved clean. Patients received intravenous injections of antibiotics (amoxicillin/clavulonic acid; Amoksiklav, Lek, Slovenia, 3×1.2 g) on the day of operation and for 3 days thereafter.

Anesthesia and surgery

Intramuscular injection of morphine (0.1 mg/kg) was given one hour before operation. Anesthetic induction consisted of fentanyl, droperidol, propofol (2 mg/kg), and pancuronium (0.08 mg/kg). Anesthesia was maintained by a mixture of oxygen with air (2.2 l/min) and isofluran (0.5–0.75 MAC) and intravenous infusion of propofol (2–4 mg/kg/h). Flaccidity was maintained by infusion of pancuronium (0.02 mg/kg). Ventilation was controlled by an Aestiva 3000 anesthetic system (Datex Ohmeda, USA). The extracorporeal circuit consisted of a roller pump with a Jostra model HL membrane oxygenator (Jostra AG, Hirrlingen, Germany), and non-pulsatile flow was maintained at 2.4 l/min/m^2 body surface area. The mean blood pressure during the extracorporeal circuit was maintained between 50–60 mm Hg measured from the radial catheter.

Two central and two peripheral veins were cannulated. Blood pressure, central venous pressure, ECG, O_2 saturation (Sa O_2), body temperature, hourly diuresis, ionogram, and hematocrit were measured during the cardiovascular operation. The operation was performed in general hypothermia (27–29°C) and local heart ice-cooling. The heart was arrested with St. Thomas' Hospital cardioplegic solution administered directly to the aortic bulb (10 ml/kg) and after each bypass suturing (5 ml/kg). Heparin was given intravenously before cannulation of the aorta (2–3 mg/kg). Effective anticoagulation was monitored by activating clotting time (ACT). The heparin dose was repeated when ACT was shorter than 400 s. Heparin action was neutralized by the administration of prothamine sulfate at a dose of 2 mg per each 1 mg of heparin. During the operation the patients were given 3 units of erythrocytic mass (1 unit – 300 ml); H2 blockers (2×20 mg, famotidine, for 48 h, Quamatel, Richter Gedeon, Hungary) were also administered intravenously.

Generally, two types of cardiovascular operation with use of the extracorporeal circulation were performed: CABG or implantation of an artificial valve. However, since some of the patients with valve disease also had significant coronary constrictions, they needed both artificial valve implantation and CABG.

Post-operative period

After the operation, heparin was given subcutaneously for 2–3 days until the patients could move nor-

mally. Intravenous administration of antibiotic and of H2 blockers (orally, 1×20 mg famogast, Polpharma, Poland) was continued for 3–5 days.

General information

Information about the patients' age and history of diseases that could either influence the splanchnic blood flow (e.g. hypertension and diabetes) or correlate with intense atherosclerosis of splanchnic circulation (e.g. ischemic apoplexy and obliterative atherosclerosis) was collected; cigarette and alcohol abuse were documented. The hemodynamic status of the patients (ejection fraction) was diagnosed by echocardiography and, in some cases, by ventriculography. Time of aorta closure (time of aortic cross-clamp) and lung-heart machine action (duration of extracorporeal circulation) were recorded. All complications during and after the operation (e.g. bleeding, post-operative psychosis, low output syndrome, arrhythmia, and pulmonary problems) were documented. Times and doses of catecholamines given after operation were recorded.

The type of cardiovascular operation (CABG, artificial valve implantation, or both) was documented. In patients with CABG, the number of grafts along with the number of significant coronary constrictions in coronarography was recorded: 4 patients of group I had CABG and one had implantation of an artificial aortic valve. Six patients of group II had CABG, 3 had of the artificial valve implantation (two aortic and one mitral), and two had both artificial mitral valve implantation and CABG.

Laboratory tests

Laboratory tests were performed before (one day), during (when the aorta was closed) and after the operation (every 12 h for 2 days and every 24 h the following 3 days), and included an assay for blood morphology with blood smear and determination of metabolic parameters of the liver (ALT, AST and bilirubin) and kidney (creatinine and urea). The concentrations of glucose and electrolytes, acid-base balance parameters, clotting parameters (ACT), creatinine kinase CPK and CK-MB activities as well as a gasometry assay were recorded. A one hundred percent increase in leukocyte number and parameters of liver and kidney metabolic dysfunctions compared with the levels before operation were considered as important changes.

Blood collection

One-ml blood samples were taken from the radial artery in the operating room 3 times: before anesthesia, immediately after restoration of heart function (after disconnecting the lung-heart machine), and 24 h after operation. The blood clot was centrifuged and the serum collected and frozen at –20°C until analyzed.

Sample preparation and analysis

Samples of 200 μl of serum were methanolyzed and the resulting methyl esters of fatty acids were processed to obtain trimethylsilyl volatile derivatives suitable for gas-liquid chromatography-mass spectrometry (GLC-MS) analysis as described previously [16]. Briefly, the first step provided a mixture of hydrolyzed monomers of fatty acids and other lipids, which was enriched by the internal standard (deuterized 3-OH C14:0, methyl ester). This was extracted with heptane and the hydroxy fatty acids were isolated from the part containing fatty acids methyl esters by solid-phase extraction. The last step involved trimethylsilyl derivatisation and analysis on a Saturn 2000 GLC-MS ion trap instrument (Varian Analytical Instruments, Walnut Creek, CA, USA) operating in electron impact mode using MSMS as described previously [13].

Statistical analysis

The Mann-Whitney non-parametric test was used for the statistical analyses. p-Values ($p<0.005$) indicated significance.

RESULTS

The initial, pre-operative 3-OH FAs levels were similar in all patients (Fig. 1A). However, in the course of surgery, elevated levels were seen in 5 of the patients, who later developed prolonged leukocytosis (Fig. 1B,C). According to these data, two patient groups were created: group I patients (n=5) with clearly elevated 3-OH FAs (over 60 pmol/ml of serum) and group II patients (n=11) without such increase. The levels of all 3-OH fatty acids rose during surgery in the group I patients ($p\leq0.05$), and those of longer chain length, *viz.* 3-OH C14 and 3-OH C16, remained high at least 24 h after operation (Table 1).

Table 2 presents the characteristics of the patients. All group I patients revealed increased leukocytosis (over 100% compared with pre-operative values) that remained at least 72 h after surgery (pre-operative leukocytosis was normal for all the patients in both groups: 7.46±2.3 G/l in group I and 7.92±1.92 G/l in group II); two of these patients revealed post-operative anemia that decreased progressively with blood supplementation. Leukocytosis was observed also in 3 of the group II patients, but in one patient it lasted for 24 h after surgery and in the other two it was connected with post-operative anemia (defined as a 30% drop in hemoglobin level compared with the pre-operative level). Pre-operative concentrations of the metabolic liver parameters ALT and bilirubin were within normal values for all the patients: ALT 31±8 IU/l in group I and 33±7 IU/l in group II, and bilirubin 12.4±3.8 μmol/l and 11.65±3.87 μmol/l, respectively. In post-operative tests, elevated levels (over 100% compared with pre-operative

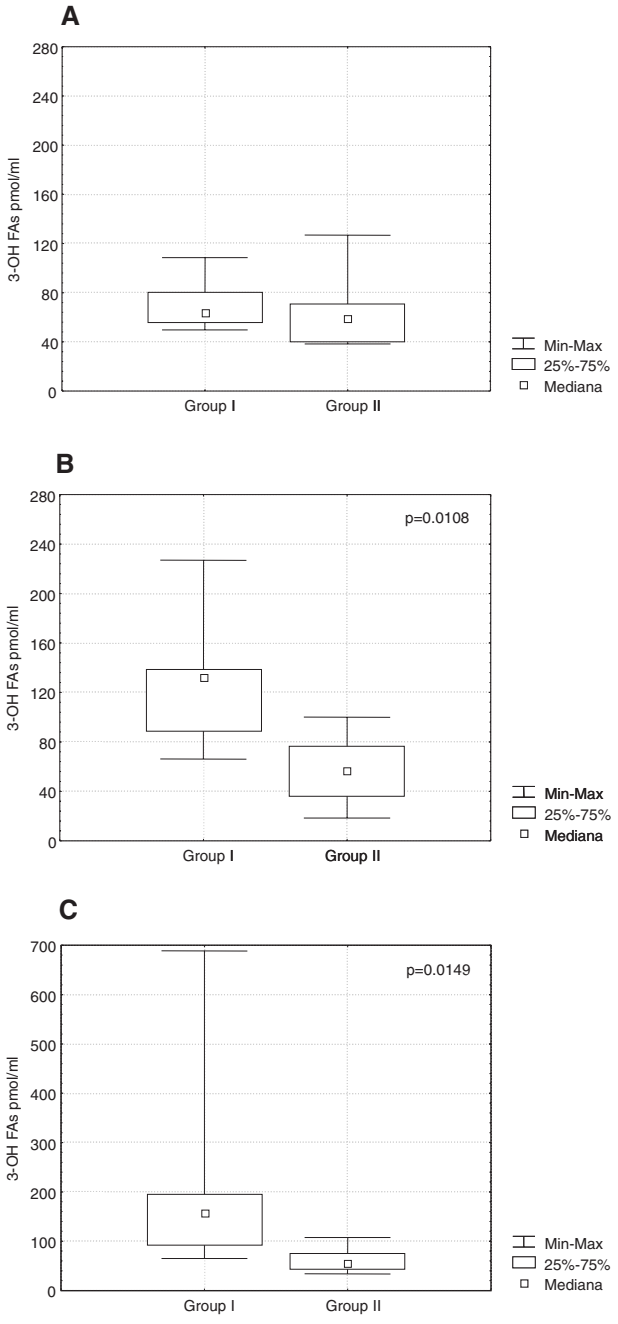

Fig. 1. Changes in the level of 3-OH FAs (average of four acids), markers of endotoxin, in serum collected from patients in the course of cardiosurgery: **A** – at 24 h before operation, **B** – during the operation (after cross-clamping), **C** – at 24 h after operation.

Table 1. Levels of 3-OH FAs (pmol/ml serum) of patients before, during, and 24 h after cardiosurgery

	Group I		Group II	
3-OH C10				
before	41.7	(23.9–85.6)	37.0	(6.2–101.9)
during*	68.4	(33.3–159.1)	31.5	(6.7–122.3)
after	38.0	(28.5–1207.0)	27.3	(4.2–79.5)
3-OH C12				
before	41.3	(16.9–83.0)	25.2	(9.5–100.3)
during*	100.0	(42.6–252.9)	35.2	(10.8–86.9)
after	40.9	(23.1–812.9)	42.4	(5.7–125.5)
3-OH C14				
before	65.1	(54.6–139.6)	50.4	(0.0–169.8)
during*	100.3	(63.3–341.0)	39.1	(21.9–132.5)
after*	176.3	(93.1–497.2)	48.5	(3.5–130.2)
3-OH C16				
before	104.7	(91.7–125.5)	103.9	(44.6–165.1)
during*	138.7	(125.3–267.9)	89.5	(34.2–162.0)
after*	149.5	(114.1–236.7)	99.8	(62.9–132.0)
Mean 3-OH FAs				
before	63.8	(49.7–108.4)	58.8	(38.3–126.8)
during*	132.5	(66.1–226.9)	57.0	(18.4–100.0)
after*	157.3	(64.7–688.5)	55.5	(33.7–107.3)

Values are shown as medians (minimal-maximal values);
* p≤0.05.

Table 2. Characteristics of patients (n=16) undergoing CABG and/or valve implantation

	Group I (n = 5)	Group II (n = 11)
Patients age (mean)	62	58
Sex	4 females/1 male	3 females/8 males
Body mass index	26±2	31±5
Leukocytosis	5 (72 h)	3 (24–48 h)
Anemia	2	2
Increase of ALT and bilirubin	3	1
Post-operative complications (excluding hemorrhage)	1 (psychosis and respiratory problems)	0

levels) were recorded in 3 patients of group I (two of them with leukocytosis) and in only one group II patient. Patients did not show signs of kidney disturbance (mean creatinine level in group I was 90.2± ±13.7 μmol/l and in group II 86.5±15.8 μmol/l); the other parameters tested did not reveal any changes in the pre- and post-operative periods.

Except for leukocytosis, no other correlation was found between increased levels of 3-OH FAs and anamnesis data such as hypertension, diabetes, ischemic apoplexy, obliterate atherosclerosis, nicotinism, and alcohol abuse. The hemodynamic state of patients before the operation did not influence 3-OH FA concentration. The ejection fraction of the group I patients was 54.75±6.49% and of the group II patients 56.5±9.7%. The duration of extracorporeal circulation in both groups was comparable (87.4±11.3 and 91.0±32.2 min, respectively). No significant changes in the duration of aortic cross-clamp between both groups of patients (48.6±13.8 and 56.5±28.1 min, respectively) were recorded. There was no relation between the type of cardiovascular operation (CABG or/and valve implantation) and 3-OH FA increase. The elevated level of 3-OH FAs did not correlate with the ratio of the number of grafts to the number of significant coronary

constrictions; the ratio was higher in group I (0.87) than in group II (0.78). The type, dose, and time of catecholamine infusion did not correlate with 3-OH FA level at 24 h after operation. In group I, "soft" catecholamines such as dopamine were used in the first 24 h after operation in 4 patients, and additional "hard" catecholamines (norepinephrine) in small doses for 48 h were given to one patient of this group. In 8 patients of group II, "soft" catecholamines in the first 24 h after operation, and "hard" catecholamines were used in one patient because of low cardiac output syndrome. In two patients of this group, catecholamines were not administered.

Some complications of the revascularisation appeared such as transitory psychosis and respiratory problems, cardiac arrhythmia, bleeding, and low cardiac output syndrome. One patient of group I had transitory psychosis and respiratory problems (hypoventilation) after implantation of the artificial aortic valve. In anamnesis he had cerebral stroke. Because of transient hypotension, "soft" catecholamines and norepinephrine in small doses were administered during the first 24 h. Two patients of group II had paroxysmal atrial fibrillation: one in the first 24 h after CABG and the other on the second day after implantation of an artificial mitral valve (without CABG). This second patient also had low cardiac output syndrome and intraaortic counterpulsation was used with infusion of "soft" and "hard" catecholamines for 24 h.

One patient of group II had ventricular arrhythmia in the first 24 h after implantation of an artificial aortic valve. Bleeding after operation was recorded in two patients of group I (with mild loss of blood) and in two patients of group II (both had anemia). In one case only mild loss of blood and in the second a loss of 1800 ml of blood in the first 24 h after the operation was recorded.

DISCUSSION

Previous observations in patients subjected to heart revascularisation have revealed significant increases in endotoxin concentration in serum during the operation after closure of the aorta, reaching a maximum after completing the aortic clamp [8]. Jansen et al. [4] and Rocke et al. [11] found that LPS levels decreased a few hours after operation. In another study, however, the endotoxin level remained elevated at 24 h after the operation [3]. These discrepancies may be due to difficulties in and different procedures of measuring endotoxin levels.

Determination of endotoxin is not currently performed in routine laboratory examinations for patients undergoing cardiovascular operations, although translocation of endotoxin from the gastrointestinal tract to the blood is a known phenomenon in cardiovascular by-pass operations [4]. Treatment of patients with short-term antibiotics before cardiosurgery may lead to an increase in the endotoxin level in blood due to bacterial disruption, leading further to release of cell wall fragments.

Earlier studies [15, 16] have shown that the distribution of 3-OH FAs in mammalian tissues and in blood can be detected by GLC-MS ion trap instrumentation. An experimental model of germ-free rats indicated a dual origin of these compounds in the blood and soft tissues: because of the unique structure of LPS it is believed that 3-OH FAs are chemical markers of Gram-negative bacteria. On the other hand, in the ubiquitous β-oxidation process of fatty acid metabolism in an organism, β-hydroxy fatty acids appear as intermediates, causing a pathological problem in individuals suffering an inborn lack of β-oxidation enzymes [5]. We hypothesize that the 3-OH FAs reflect the endotoxin levels in blood, since the anamnesis data did not reveal any metabolic disorders in our patients. In addition, the initial 3-OH FA levels were similar in all of them. The results presented above remain in agreement with previous findings [11]. Similar to the observations done on a rat model, a distinct correlation with liver impairment and circulating LPS management was noted in group I of patients in the present study. Increases in the parameters of liver dysfunction (bilirubin, ALT) can be correlated to the increased 3-OH FAs.

In the present study we used a chemical analytical method for determining enodotoxin markers in serum. Increased levels of these markers were recorded in 5 of 16 patients at 24 h after cardiosurgery. This increase was independent of the type of cardiovascular operation (CABG and/or valve operation), but was related to the occurrence of leukocytosis that persisted for a long time, as was found in all patients with increased endotoxin markers (group I). The increase found in leukocyte number may be due to an increased blood concentration of tumor necrosis factor α induced by the endotoxemia and/or an increase in C3a concentration due to the endotoxin activation of the complement cascade through both the classic and alternative pathways. Leukocytosis has also previously been connected with bacterial gut translocation to the blood [10]. Post-operative bleeding has been reported to increase endotoxin translocation from the gastrointestinal tract in patients with hemorrhagic shock [12]. In our study, increased concentrations of the endotoxin markers were found in two patients with anemia and secondary leukocytosis, but leukocytosis was also found in two group II patients with loss of blood. However, leukocytosis in the patients of group I lasted much longer despite the compensation of hematological parameters. We noted no fever in the patients with increased LPS concentrations, in accord with another study done on a similar group of patients [3]. We found no correlation between 3-OH FA level changes and anamnesis data and hemodynamic state, which is in agreement with previous studies [3]. The lack of hemodynamic splanchnic blood flow disturbances in our patients may be explained by adequate adaptation reserves of the blood flow and, possibly, the net effect of hypothermia, which protects against ischemic gut injury [3].

During surgery, blood may be enriched by cellular debris and proteins derived from the disintegration of

tissues. This may lead to insufficient neutralization of endotoxins by Browicz-Kupfer cells, which may explain the prolonged increased level of endotoxin markers in the group I patients. Indeed, the elevated liver metabolic factors suggest severe liver dysfunction, although the cause may also be due to ischemic injury during the cardiovascular operation [11].

Cardiosurgery may strongly promote the translocation of gut endotoxin to blood in some patients. According to our findings, prolonged leukocytosis and an increase in markers for deep anemia and liver dysfunction may indicate the need for further observation of the possible development of endotoxemia in these patients. Therefore it is recommended to monitor the endotoxin level and/or endotoxemia markers in the cardiosurgery clinic.

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