Universitätsklinikum Ulm

Klinik für Anästhesiologie

Leiter: Prof. Dr. med. Dr. h. c. Michael Georgieff

Sektion Anästhesiologische Pathophysiologie und Verfahrensentwicklung

Leiter: Prof. Dr. med. Dr. h. c. Peter Radermacher

A new long-term porcine model of fecal peritonitis-induced septic shock hemodynamics, gas exchange, metabolism, and organ function

Dissertation

zur Erlangung des Medizinischen Doktorgrades der Medizinischen Fakultät

der Universität Ulm

vorgelegt von

Nguyen Duy Cuong

aus Thai Binh, Vietnam

2008

Amtierender Dekan**: Prof.Dr. Klaus-Michael Debatin**

1. Berichterstatter**: Prof.Dr.med.Dr.h.c. Peter Radermacher**
2. Berichterstatter**: PD.Dr. Hubert Schelzig**

Tag der Promotion: 18.07.2008

Content

Abbreviations

A	Artery
ALF	Acute liver failure
ALI	Acute lung injury
ALT	Alanin aminotransferase
ARDS	Acute respiratory distress syndrome
ARF	Acute renal failure
AST	Aspartat aminotransferase
bpm	Beat per minute
BPU	Blood perfusion unit
CBIG	Blood clearance of ICG
CLP	Cecal ligation and perforation
CO	Cardiac output
CO_2	Carbon dioxide
CVP	Central venous pressure
DO_2 liv	Liver oxygen delivery
ECG	Electro cardiograph
EVLW	Extra vascular lung water
f	Female
FiO_2	Fraction of inspired oxygen
GEDV	General end diastolic volume
GOE	Gut oxygen extraction
ha	Hepatic artery
Hb	Hemoglobin
HCO_3^-	Bicarbonate
HR	Heart rate
hv	Hepatic vein
I.D	Internal diameter
I/E	Inspiratory and expiratory ratio

IAP	Intra abdominal pressure
ICG	Indocyanine green
ICU	Intensive care unit
IL1	Interlerkine 1
ITBV	Intra thorax blood volume
IV	Intravenous
lac	Lactate
LDF	Laser Doppler flowmetry
LV	Left ventricular
m	Male
MAP	Mean arterial pressure
MDS	Myocardial depressant substance
Mea	Measure point
min	minute
mmHg	Millimeter mercury
MOD	Multiple organ dysfunction
MODS	Multiple organ dysfunction syndrome
MPAP	Mean pulmonary arterial pressure
NO	Nitric oxide
NOs	Nitric oxide synthesis
O_2	Oxygen
OER	Oxygen extraction ratio
$PaCO_2$	Carbon dioxide partial pressure
PaO_2	Oxygen partial pressure
PAOP	Pulmonary arterial occlusion pressure
PBV	Pulmonary blood volume
PCO_2	Ileal intramucosal
PCO_2 gap	Ileal mucosal-arterial gap
PDRig	Plasma disappearance rate
PEEP	Positive end expiratory pressure
pO_2	Tissue oxygenation

pv	Portal vein
pyr	Pyruvate
Qha	Flow hepatic artery
Qliv	Flow liver
Qpv	Flow portal vein
Qs	Shunt flow
Qt	Total flow
RQ	Respiratory quotient
SaO_2	Oxygen saturation
SV	Stroke volume
SVR	Systemic ventricular resistant
TBV	Total blood volume
TNF	Tumor necrosis factor
U/kg/h	Unit per kilogram per hour
VCO_2	Carbon dioxide elimination
VO_2	Oxygen consumption
VO_2 liv	Liver oxygen consumption
Vt	Tidal volume

1. Introduction

1.1. Definition and Epidemiology of sepsis

Sepsis is the systemic inflammatory responses to the infection. The symptoms include 2 or more of the following conditions:

+ Rectal temperature greater than 38^0C or less than 36^0C.

+ Tachycardia: heart rate greater than 90 beat per minute (bpm).

+ Tachypnea: respirator rate greater than 20 breaths per minute or $PaCO_2$ less than 32 mmHg

+ White blood cell count greater than 12,000/μL, or less than 4000/μL, or 10% immature (band) forms, and including at least 1 of the following manifestations of inadequate organ function or organ perfusion:

+ Alteration in mental state

+ Hypoxemia: PaO_2 < 72 mmHg at FiO_2 21%

+ Elevated plasma lactate level

+ Oliguria: urine output < 30 ml or < 0.5 ml/kg for at least 1 hour

Severe sepsis is sepsis associated with organ dysfunction, hypoperfusion, or hypotension. Hypoperfusion and perfusion abnormalities may include lactic acidosis, oliguria, or acute alteration in mental status. Hypotension (systolic blood pressure < 90 mmHg or reduce > 40 mmHg from baseline) may develop despite adequate fluid.

Septic shock is severe sepsis induced hypotension despite adequate fluid resuscitation.

Multiple organ dysfunction syndrome (MODS) is manifestations of altered organ function in the patient being acutely ill and in whom homeostasis cannot be maintained without intervention.

The incidence of sepsis is increasing. Martin and co-workers have analyzed the occurrence of sepsis from 1979 to 2000 in the United States found the number of sepsis increased from 82.7 cases per 100,000 populations to 240.4 cases per 100,000 populations over 22 years and annually increasing ratio is 8.7 percent (76). Radermacher 1998, sepsis is one of the main causes of death in adult, mortality rate of sepsis varies from 6 to 7 percent, particularly it is highest when septic shock and multiple organ dysfunction (MOD) developed, reaching to 72 percent (107).

1.2 Effects of septic shock on hemodynamics

1.2.1 Hypotension and vasodilatation

Septic shock is manifested by persistent arterial hypotension and vasodilatation despite adequately resuscitated volume and elevated cardiac output (CO). Arterial hypotension results from redistribution of intravascular fluid volume, which is caused by the decreased arterial vascular tone, the decreased returning venous blood from the dilated vein, and released the myocardial depressant substances (MDS) (e.g. tumor necrosis factor-TNF) (25, 59). In septic shock, nitric oxide (NO) is major contributor for vasodilatation status, it changes cell wall transport mechanism and intracellular factors, leading to reducing intracellular calcium, increasing vasodilatation and resisting to vasopessor agents. Furthermore, in sepsis a secretion of vasopressin may impair, therefore it encourages the vasodilatation. In septic shock not all vessels are dilated, in some vital organs, such as the heart and the brain, blood flow is remained relatively better than the non-vital organs such as gut, kidneys. This state is called maldistribution of blood flow.

In initial stage of septic shock, cardiac output (CO) is increased to maintain the blood pressure, this phase is termed “warm shock”, and then cardiac output is decreased associating with vasodilatation causing persistent hypotension and shock (18, 25, 59).

1.2.2 Microcirculation

Sepsis is a disorder of the microcirculation and conversely. The microcirculation is the main injured target organ of septic shock. The microcirculation dysfunction caused by sepsis occurs in all three microcirculation elements: arterioles, capillaries, and venules. The arterioles respond poorly to vasoconstrictors and vasodilators. A number of the perfused capillaries are reduced, hence oxygen diffusing to mitochondria is impacted. In some tissues (e.g., mesentery), venules have an inflammatory response defined by neutrophil infiltration and protein leakage (66, 125, 52).

Factors caused insufficient oxygen extraction and death cells include a vascular dysregulation, a loss of barrier function (capillary leak syndrome), an impaired endothelial cell function of microcirculation caused by intrinsic and extrinsic capillary compression, an increasing clotting in capillary lumen, a rigid blood cell, and an activating leukocyte together with inflammatory (18, 66).

1.2.3 Hepato-Splanchnic organs

The Hepato-Splanchnic organs are considered to play a key role in the septic progression causing multiple organ failure. The liver perfusion is supplied 75-80% blood flow from the portal vein and 20-25% blood flow from hepatic artery. Splanchnic organ perfusion frequently depends on cardiac output. When sepsis developed, decreasing cardiac output (in the late stage of septic shock) and disorder of microcirculation lead to lack of oxygen in the liver cells, and therefore the metabolism in the liver activates on an anerobic condition releasing numerous metabolism mediators, such as cytokine (e.g., tumor

necrosis factor-TNF). And the injury of the liver capillaries is favourable condition for penetrating of bacteria and bacterial production into blood circulation. In sepsis, the barrier function of the gut may be injured, accordingly, endotoxin and bacteria can enter in the systemic circulation and spread septic response (3, 60).

1.3 Effects of septic shock on gas exchange

The most important role of gas exchange is extracting oxygen (O_2) from alveoli and then delivering oxygen to the cells, and carrying away metabolic product carbon dioxide (CO_2) from tissue to the lung and then diffusing into alveoli. Many factors effect on the gas exchange process, such as pneumonia which reduces passing oxygen and carbon dioxide through alveoli-capillaries membrane. Consequently, that causes increasing carbon dioxide, decreasing oxygen in the blood, and developing respiratory failure. On the other hand, when the gas exchange process is disordered, it badly impacts on existing diseases. For example, developing gas exchange failure during sepsis, the metabolic process activates on the hypoxia condition causing increase metabolic mediators and oxygen deficiency persisting on tissues, which are the main cause of organ failure and death (18, 49).

Blood gas analysis in clinic is needed for the critical ill patients. Although arterial blood gas values can not inform completely to diagnose diseases, for instance they can not discriminate between lung and heart diseases, but analytic values of blood gas are very important to evaluate disorders in respiratory system, cardiac circulation, and especially in metabolic function. Detecting acid-base balance disorder in critical ill patients, especially in severe sepsis patients must base on pH of blood, partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), and bicarbonate HCO_3^-. In addition, PaO_2/FiO_2 ratio calculated by using blood gas analysis values and fraction of inspired

oxygen (FiO_2) is the most important indicator to diagnose injuries of the lung, such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), which are the severe complications relating to poor outcome of patients undergoing septic shock (18, 49).

1.4 Effects of septic shock on metabolism

The patients experienced with severe sepsis or septic shock, metabolic deficiencies occur frequently. The most common finding of metabolic deficiencies is glucose metabolic disorders. In tissue hypoxia condition, glucose metabolism causes an increasing blood lactate concentration resulted from pyruvate cannot enter the krebs cycle. Hence, high blood lactate concentration expresses the state of tissue hypoxia. Several groups studied on animal have reported that an acute lung injury produced a huge lactate production, it is higher than whole basal endogenous lactate production of the body. In addition, the lactate clearance plays an important role in increasing blood lactate concentration. Lactate is metabolized mainly by the liver (50%) and kidney (20%). A liver malfunctions, and reducing blood flow in liver and kidney cause decreasing lactate clearance and increasing blood lactate level. Hyperlactatemia disturbs acid-base balance, induces lactic acidosis, and impacts severely on patient survival (18, 118).

In addition, nitric oxide (NO) metabolic disorder plays an important role in progression of septic shock. In severe sepsis and septic shock, cytokines are increasingly released and lead to the over production of nitric oxide (NO) resulted from L-arginine converting to L-citruline through an enzyme group nitric oxide synthesis (NOs). Nitric oxide relaxes the smooth muscle in the walls of arterioles causing arterial vasodilatation, a common manifestation persisting on septic shock, and mediates tissue injury (18).

1.5 Effects of septic shock on organ function

The severe and common complication of sepsis and septic shock is multiple organ dysfunction (MOD), that is commonest cause of mortality in intensive care unit (ICU). Mortality rate varies from 30-70% correlating with the number of organ dysfunction (107). Multiple organ dysfunction syndrome is an altered organ function in acutely ill patients, in those homeostasis cannot be maintained without intervention. Organ dysfunction is usually developed in two or more organ systems.

1.5.1 Cardiovascular dysfunction

In the initial period of septic and sepsis shock (the hyperdynamic phase of sepsis and septic shock) cardiac output (CO) usually elevates to maintain blood pressure and metabolic demand, but the rise of cardiac output is often limited by some factors, such as hypovolemia, a low cardiac preload, a myocardial depression caused by myocardial depressant substances, a reduced coronary blood flow, an increase of releasing various cytokines (e.g. TNF) and nitric oxide (NO), and reduced beta-receptor. Hence, in the hypodynamic phase of sepsis and septic shock the blood pressure is decreased. The alteration of central venous pressure (CVP) and pulmonary artery occlusion pressure (PAOP) are also an evidence to evaluate central circulation dysfunction. In addition, a disorder of microcirculation involved in cardiovascular dysfunction critically contributes to the septic shock. The inflammation and mediators stimulated in sepsis and septic shock compromise the microcirculation manifested by a dysregulation, a loss of barrier function of capillary, an impaired function of endothelial cells, an increasing clotting, a rigidity of red blood cells, and leukocyte activations. Eventually, the microcirculation dysfunction which together with inflammatory mediators lead to death cell and multiple organ dysfunction (25, 66).

1.5.2 Pulmonary dysfunction

Although not strongly associated with the mortality rate as cardiovascular, renal, or hepatic dysfunction, the pulmonary dysfunction is the most common finding of organ dysfunction during the onset of sepsis, as many as 20% - 40% acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) cases are developed from severe sepsis (5) . In sepsis and septic shock, the pulmonary endothelial vascula are injured leading to the disturbed capillary blood flow and the increased microvascular permeability causing edema in interstitial cells and alveoli, which together with the injured alveolar capillary membrane resulted from neutrophil entrapment in the pulmonary microcirculation cause ALI or ARDS. Both ALI and ARDS are acute onset and manifested by the bilateral infiltration on chest x-ray, the pulmonary artery wedge pressure ≤ 18 mmHg and no clinical evidence of the increased left atrial pressure. While ALI is defined with the PaO_2/FiO_2 ratio between 200 and 300 mmHg, ARDS is defined more severe with PaO_2/FiO_2 ratio < 200 mmHg and in the prognosis, ARDS is more severe than ALI (5, 103).

1.5.3 Hepatic failure

Acute liver failure (ALF) is the lethal clinical syndrome usually observed in clinical practice. The mortality rate in patients undergoing ALF is high, ranging from 30 – 80% depending on the aetiology. Critical disturbances in a circulatory control, a blood pressure and a blood flow in cerebrum, a susceptibility to renal failure, a pervasive sepsis, and metabolic disorders such as hypoglycemia are the major causes of high mortality rate related to ALF. In sepsis and septic shock, the marked disturbance in microcirculation including arteriole-venous shunting and impaired end-organ perfusion cause tissue hypoperfusion, tissue hypoxia, and lactic acidosis, which consequently cause ALF. The

manifestations of ALF are clinical symptoms (e.g. hepatic encephalopathy and cerebral edema) and evidences of changes in blood analysis result. Some of the important changes in blood analysis involve the decreases of platelet amount, coagulation factors (e.g. factor V, VII, XIII, fibrinogen), and prothrombin-T, which manifest the hemorrhage syndrome. The increases of endotoxemia, bacteraemia, and cytokines (e.g. IL1, TNF) manifest the infection and the immunity disorder. And the decreases of almost al serum electrolytes such as hyponatremia, hypokalemia, hypocalcemia, hypomagnesemia are the common manifestations of ALF. In addition, the histological analysis of the liver structure is an important contribution to diagnostic of ALF (103).

1.5.4 Renal failure

Acute renal failure (ARF) is the one of the most common complications of sepsis, appropriating 23% – 51% proportional to severe sepsis and septic shock. The mortality rate of ARF associated with sepsis is high, approximately 70-80%. In sepsis and septic shock, the major mechanism induced ARF is hypotension causing decreasing renal blood flow and reducing glomerular filtration, therefore the renal clearance is impaired. Additionally, the activation of the neurohumoral axis (e.g. the rennin-angiotensin system and the sympathetic nervous system) to maintain blood pressure also effects on the renal artery, causing the arterial vasoconstriction, especially pre-glomerular arterial vasoconstriction, which more badly disturbs the renal function. Furthermore, the renal inflammation, especially the injury of glomerular and tubular kidney is also the main cause of ARF. The hall marks for diagnosing ARF are the elevation of serum creatinine, with the creatinine serum concentration is higher 3.0 times than baseline creatinine or creatinine concentration > 355 μmol/l (with a rise of > 44 μmol/l), and urine output < 0.3

ml/kg for 24 hours. In addition, the histological analysis of the kidney structure is an important contribution to diagnostic of ARF (60).

1.6. Objective

We use a long-term porcine fecal peritonitis-induced septic shock as a new model to investigate any changes of the pigs subjected to infection and developed septic shock. The main findings were the disorders of hemodynamics, gas exchange, metabolism, and organ function.

2. **Material and methods**

2.1 Anaesthesia and surgery

The study was approved by the protection of animal representative of the University of Ulm as well as by governmental presidium Türbingen, Baden-Württenberg for the care of animal subjects.

The pigs (body weight 45-50 kg) were fasted in research animal centre of Ulm University for 18 hours prior to the experiment, with unrestrained access to water. On an experiment, the pig was used premedication with Atropin (0.05 mg/kg Atropinsulfat®, B.Braun, Melsungen, Germany) and Azaperon (4mg/kg Strenil, Janssen-Cilag, Germany) and then was transported to "Tier-OP" of department "Anästhegiologische Pathophysiologie und Verfahrensentwicklung" in Parkstrasse 11, 89073 Ulm. For anesthesia a canunla was induced in an auricular vein and the pig was respired with the oxygen (10 liters/min). Then anesthesia was commenced with Atropin (0.5 mg), Ketaminhydrochlorid (2 mg/kg Ketavet®, Pharmacia, Erlangen, Germany), and Propofol (2mg/kg Propofol®, B.Braun, Melsungen, Germany). Fingerclip pulse-oxymeter (Datex Capnomac Ultima® UTL-S-3310, Datex Instrumentarium Corp, Helsinki Finland) was attached to the pig's tail to monitor O_2-saturation. The orotracheal intubation with I.D 8.5 tube was performed and mechanical ventilation (Servo 900C®, Siemens, Sweden) was started according to protective ventilation ARDS Network strategy (Volume Control Ventilation set with Tidal Volume (Vt) 8 ml/kg; Peak Airway Pressure < 40 cmH_2O; Positive End Expiratory Pressure (PEEP) 10 cmH_2O; Inspiratory and Expiratory ratio (I/E ratio) 1:1.5; The respiratory rate was titrated to maintain $PaCO_2$ 34-45 mmHg; If PaO_2/FiO_2 < 300 mmHg changed I/E ratio to 1:1 and PEEP to 12 cmH_2O; If PaO_2/FiO_2 < 200 mmHg changed PEEP to15 cmH_2O. Anesthesia was maintained by continuous

infusion of Pentobarbital (4.5 mg/kg Narcoren®, Merial, Hallbergmoos, Germany) and muscle relaxation obtained with Alcuroniumchlorid (0.28 mg/kg Alloferin®, Valeant, Germany). Fluid management during experiment consisted of Ringer solution (Ringerlösung Fresenius, Fresenius Kabi, Bad Homburg, Germany) 10 ml/kg/h and after operation reduced to 7.5 ml/kg/h, and Hydroxyethyl starch (Vitafusal® 6%, Serumweg Bernburg AG, Bernburg, Germany) was infused 15 ml/kg/h after first measure point. If pulmonary artery occlusion pressure (PAOP) was over 18 mmHg reduced fluid to 10 ml/kg/h. For antithromboses, Heparin (Heparin-Natrium, B.Braun, Melsungen, Germany) was added 16 U/kg/h (0.04 ml/kg/h).

After washing, shaving and in sterilized condition, a Pulsion catheter 5Fr was introduced in femoral artery for blood sampling, continuous arterial pressure, and cardiac output (CO) monitoring. A thermistor-equipped fiberoptic catheter 4Fr was also introduced in the other femoral artery for thermal-dye double indicator dilution (indocyanine green-ICG) measurements to estimate the liver and lung function. Two jugular veins and carotid arteries were also isolated. A three lumen catheter was introduced in the right jugular vein, it was used for fluid management, intravenous anesthesia infusion and bringing in a 7Fr hepatic vein catheter under the control of ultrasonography, as well as a central venous catheter. Via left side jugular vein, a Swan-Ganz catheter 7.5Fr was introduced in the pulmonary artery. Through the left carotid artery a combined conductance/Millar-tip catheter 6Fr was introduced into the left ventricle. In the right carotid artery a multiparameter sensor Paratrend was placed. After midline laparotomy a Doppler calibrated flow probes were set around the common hepatic artery and portal vein to permit continuous blood flow measurement, and in the portal vein a pediatric Two-Lumen central venous catheter 4Fr was also introduced for blood sampling and portal

vein pressure monitoring. After ileostomy, a double-lumen ileostomy was constructed to introduce a tonometer for intramucosal PCO_2 measurement. For monitoring of mucosal and serosal microcirculation, combined Laser Doppler Flowmetry (OxyFlo XP) probe and tissue oxygenation pO_2 E-Series probe were placed in the gut wall. In addition, a microdialysis probe (CMA/20) was introduced in the gut wall as well as in the subcutaneous tissue. Two ascitic drains were introduced through abdominal wall into Douglas space and above the liver. Laparotomy in below abdomen was also operated to allow placing an epicystic catheter to monitor amount of urine output and measure intra-abdominal pressure (IAP). The incision was closed with sutures. Two Laser Doppler flowmeter (Transonic® Flowprobes) were placed on the skin in the neck and the abdomen. After the surgical preparation an 8-hour phase of recovery was allowed.

For the induction of peritonitis a standardized feces solution was prepared prior to the experiment. For this purpose autologous feces (1 g/kg) was collected and then suspended in 500 ml Ringer lactate (Ringer-Lactat-Lösung, Frisenius Kabi, Bad Homburg, Germany) and cultivated at 37 ^{0}C for 12 hours. After measure point 1, 100 ml solutions was put into each ascitic drains. Two drains were clamped for 12 hours and then opened.

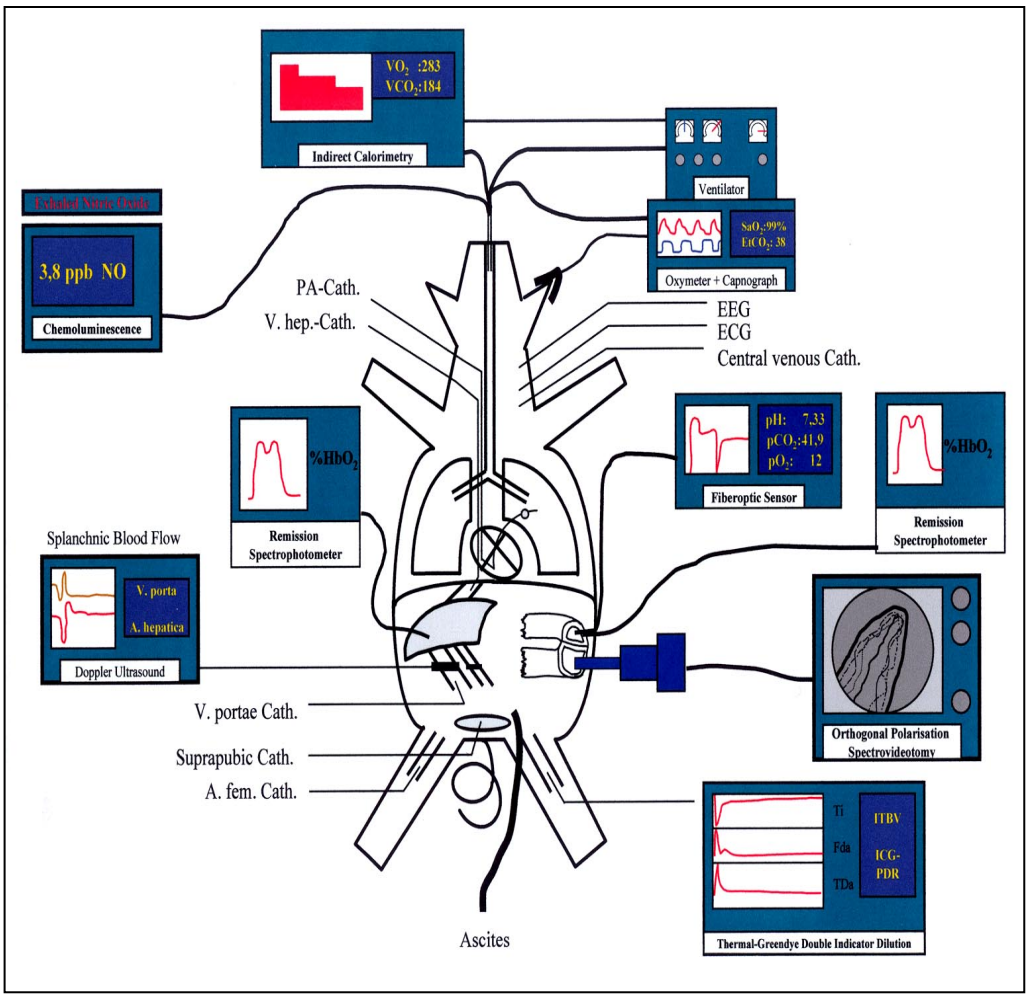

Figure 1: *Introduction of experimental instruments and monitor.*

(Adapted from: WestnerK.F)

PA-Cath: Pulmonary catheter; V.hep-Cath: Hepatic vein catheter; V.porta Cath: Portal vein catheter; A.fem.Cath: Femoral arterial catheter; EEG: Electroencephalogram; ECG: Electrocardiogram; Central venous Cath: Central venous catheter

2.2. Experimental design

The purpose of this project is to have the results as much extrapolatable to the critically ill as possible. Thus, the intention is to perform the study in an animal model designed to meet the criteria of human septic shock model as defined by several authors (Fink 1990,

Deitch 1998, Traber 1999). For that reason, the whole project was proceeded on a 40-hours, hyperdynamic, fecal peritonitis induced septic shock model.

Large animal model is well suited for septic shock research for several reasons (Dodds 1982): 1) it closely mimics human sepsis (hyperdynamic circulation with low systemic vascular resistance, persistent hypermetabolism, acute respiratory failure requiring mechanical ventilation, and multiple organ dysfunction), 2) unlike other species, it presents with optimum anatomy (location and configuration of intrathoracic and abdominal organs), metabolic as well as physiological and pathophysiological homology with human, 3) the model permits an uncomplicated access to the splanchnic structures and the use of such methods for the estimation of regional hemodynamic parameters, O_2 transport and metabolic activity as are utilized in human research, and 4) the results are faithfully easy to extrapolate into intensive medicine thanks to the about-mentioned analogy in physiological and metabolic parameters.

15 pigs were randomly assigned 2 groups:

- Sham operated group (5 pigs): this group serves as control group for monitoring, comparing the changes with peritonitis group. The pigs in this group were not subjected to sepsis, and were ventilated with the least FiO_2 compatible with an arterial hemoglobin O_2 saturation (SaO_2 > 88%, according to the protocol of the ARDS Network 2000). The only therapy provided was the fluid substitution with hydroxyethylstarch (15 ml/kg/h, reduce to 10 ml/kg/h if pulmonary artery occlusion pressure (PAOP) > 18 mmHg).

- Peritonitis Group (10 pigs): the pigs in this group were subjected to peritonitis to detect the changes of hemodynamics, gas exchange, metabolism, and organ function during sepsis. Surgical instrumentation, ventilation, as well as volume resuscitation did not differ from the “control group”. Furthermore a standardized feces solution was put into an

abdominal pig cavity to induce peritonitis, and continuous infusion of noradrenaline was added as soon as if need. When volume resuscitation failed to maintain arterial pressure at pre-peritonitis levels, starting noradrenaline with 0.05 μg/kg/min and an increments of 0.05 μg/kg/min every 5 minutes, but noradrenaline infusion rate was not further increased if heart rate reaches a maximum of 160 bpm to avoid tachycardia-induced myocardial ischemia.

2.3. Research protocol

There were 4 measurement points (Mea) at 17h30 (Mea1-base line), and in next day at 6h30 (Mea2 – after 12 hours peritonitis introduced), 12h30 (Mea3 – after 18 hours peritonitis introduced) and 17h30 (Mea4 – after 24 hours peritonitis introduced). After operation a recoverable phase was extended for about 4 hours and then first measurement point (Mea1) was started. All measurements and samples taking were baseline prior to introduction of septic shock and then Mea 2, 3, 4 at 12, 18 and 24 hours after sepsis induced respectively.

Each measurement point, all the below listed measurements and sample taking was performed:

Heart rate, arterial and pulmonary artery pressure as well as central venous pressure (CVP) were shown on monitor.

Pulmonary artery occlusion pressure (PAOP) was measured when catheter's distal balloon was inflated.

Cardiac output (CO) was measured with 4 x 10 ml NaCl 0.9% cooled below 10^0C.

Intra-abdominal pressure (IAP) was measured by an indirect measurement technique based on the intra-vesical pressure

Measure blood flow in the common hepatic artery and portal vein

Measure the portal vein and hepatic vein pressure.

Analyze ileum intramucosal CO_2.

Analyze ileum mucosal microcirculation

Analyze CO_2 production and O_2 consumption.

Blood gas analysis of arterial, hepatic, portal, and mixed-venous.

Measure lactate and pyruvate concentration of portal and hepatic veins and artery

Collect expired gas.

Arterial serum levels of bilirubine, alanine aminotranferase, aspartate aminotranferase, amylase, lipase, creatinine kinase, troponine and creatinine (plus urine creatinine).

Take liver and kidney specimens for histological analysis (end of the experiment).

After all data were retrieved the experimental animals were sacrificed by an injection of kaliumchlorid (20 ml 1M-Kaliumchlorid-Lösung, Frisenius Kabi, Germany) under deep pentobarbital anaesthesia.

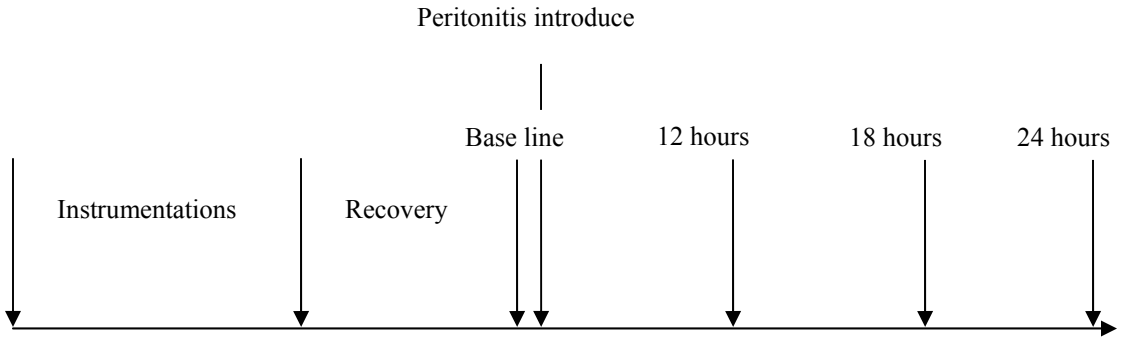

Figure 2 *Outline of timetable experiment*

2.4. Measure parameter

2.4.1 Hemodynamic system

Heart rate as well as electro cardio graph (ECG), arterial pressure (diastole, systole and mean artery pressure (MAP)), central venous pressure (CVP) were continuously monitored by monitor (Hewlett Packard Model 66 S, Boeblingen, Germany).

2.4.2 Pulmonary artery occlusion pressure

The balloon flotation pulmonary catheter was used for measurement of pulmonary artery occlusion pressure (PAOP). The catheter was connected with the electronic pressure transducer (Hewlett Packard Model 66 S, Boeblingen, Germany) and when catheter's distal balloon was inflated the catheter rapidly, distally migrated into pulmonary artery by the force of pulmonary blood flow against the inflated balloon until it was impacted by the pulmonary, in there an internal diameter was the same or less than that of the balloon and PAOP was measured at the tip of catheter. PAOP was used for estimating pulmonary venous pressure and left ventricular (LV) preload.

2.4.3 Cardiac output

Cardiac output (CO) was measured by transpulmonary arterial thermodilution using PiCCO device (Pulsion Medical Systems, Munich, Germany) and Cardiac Output Computer (Baxter, Germany) with 4x10 ml cold NaCl 0.9% (below 10^0C) and CO value was average of four time measurement values.

2.4.4 Calculated vascular resistances

Resistance is the impediment to blood flow in a vessel, but it cannot be directly measured. Instead, vascular resistance must be calculated from measurements of blood flow and pressure difference in the vessel. Mean arterial pressure (MAP), central venous

pressure (CVP) and cardiac output (CO) were measured and then systemic vascular resistance (SVR) was calculated by the equation, as follows:

$$SVR = \frac{MAP - CVP}{CO} \times 79.99$$

2.4.5 Intra-abdominal pressure

Intra-abdominal pressure (IAP) was measured by an indirect measurement technique the intra-vesical pressure. Two stopcocks were bound together, one way was connected with a vescical catheter and other way with urine bag. 50 ml syringe was attached with second stopcock and first stopcock was connected to a pressure transducer (Hewlett-Packard Monitor) via rigid pressure tubing. IAP was measured for 10 times, with 2 times x 25 ml and 3 times x 50 ml urine was instilled in the bladder and then 3 times x 50 ml and 2 times x 25 ml urine is taken out of the bladder. Each time, after opening the stopcock to the pressure transducer, the mean IAP was measured from the monitor and IAP value was an average of 10 time measurements.

2.4.6 Blood gas analysis

Analyzing blood gas for pulmonary gas exchange from arterial and mixed-venous blood gas, as well as inspiratory and mixed-exspiratory gases to calculate the PaO_2/FiO_2 ratio, and acid-base balance, hemoglobin concentration and oxygen saturation. 2 ml blood drawn from arterial, pulmonary artery catheter, hepatic and portal veins catheter were analyzed in blood gas analyzer Stat Profile Ultra (Nova, Waltham, USA) and CO-Oxymeter 682 (IL Company, Lexington, USA).

2.4.7 Noradrenaline dose

Noradrenaline was used to maintain mean arterial pressure at pre-peritonitis levels. It was started with 0.05µg/kg/min (Arterenol, Aventis, Frankfurt Germany) and increased in increments of 0.05µg/kg/min every 5 minutes until the target blood pressure was achieved. Noradrenaline infusion rate was not further increased if heart rate reaches a maximum 160 bpm to avoid tachycardia-induced myocardial ischemia. Noradrenaline was weighed before and after use as well as infusion period was recorded.

2.4.8 Indocyanine green clearance

Indocyanine green (ICG-Pulsion®, Medical Systems AG, Munich Germany) 0.3 mg/kg and solution was kept cold below 10^0C, and injected via central venous. Transpulmonary ICG concentration curves were analyzed automatically by using a computer system (COLD Z-021 Pulsion Medical Systems, Munich Germany). Computer expressed the results of CO (Cardiac Output), GEDV (General end diastolic volume in ml), ITBV (Intrathoracic Blood Volume in ml), TBV (Total Blood Volume in ml), PBV (Pulmonary Blood Volume in ml), EVLW (Extra Vascular Lung Water in ml), CBIG (Blood Clearance of ICG in ml/min), and PDRig (Plasma disappearance rate in %/min).

2.4.9 Blood flow in the common hepatic artery and portal vein

Laser Doppler Flowmetry (LDF) is a noninvasive, continuous measurement of microcirculatory blood flow. Doppler flow probes were placed around the common hepatic artery and portal vein. Blood flow of the hepatic artery and portal vein was continuously measured by Transonic Systems (Inc, Ithaca New York, United States).

2.4.10 Ileum intramucosal CO_2

Determination of the ileum intramucosal CO_2 has been introduced for the early detection of impaired splanchnic perfusion. After ileostomy a balloon-tipped tonometry tube for

gaseous sample was placed. The balloon was pumped with 15 ml air for about 1 hour before measurement. 10 ml gaseous sample was analyzed by Radiometer (ABL™ System 600, Copenhagen, Denmark). Simultaneously, arterial CO_2 was measured to define the difference between mucosal partial carbon dioxide tension and arterial partial carbon dioxide tension, PCO_2 gap.

2.4.11 Monitoring of ileum mucosal microcirculation and dissolved partial pressure of oxygen

The OXY LAB ^{LDF} (Microvascular Perfusion Monitor) is an instrument capable of monitoring red blood cell (erythrocyte) perfusion in the microcirculation of a tissue. The Oxy Flo XP probe was placed on the gut wall with the suture and connected with Microvascular Perfusion Monitor (OXY LAB ^{LDF}, Oxford Optronix Ltd, Oxford, OX4 4GA, UK). On the monitor displayed the perfusion measurement and the backscatter was measured.

The OXY LAB pO_2 (Tissue Oxygenation Monitor) is an instrument capable of continuous, quantitative measurement of dissolved partial pressure of oxygen (pO_2). The pO_2 E-Series Probe was punctured in the gut wall with the guide of intravenous catheter (BD Venflon™, Becton Dickinson, Helsingborg, Sweden) and then was connected with Tissue Oxygenation Monitor (OXY LAB pO_2, Oxford Optronix Ltd, Oxford, OX4 4GA, U.K). On the monitor displayed temperature and the pO_2 value.

2.4.12 Indirect calorimetry

Indirect calorymetry is used for continuous measurement of CO_2 production and O_2 consumption. The expired gas from the lung was connected with metabolic monitor (Detatrac, Datex-Ohmeda, Duisburg, Germany). Then CO_2 volume and O_2 volume were measured and Respiratory Quotient (RQ) was calculated.

2.4.13 Endogenous glucose production

Use ^{13}C-labelled glucose, stable isotope method to assess the turnover rates of glucose, through this method giving the dynamic view of carbohydrate metabolism. As soon as after introducing arterial catheter, blood for glucose was drawn and expired gas was collected and then ^{13}C-labelled glucose infusion was started with 15 ml/min and second ^{13}C-labelled glucose syringe was infused 1.5 ml/min, when the first syringe was empty. The ^{13}C-labelled glucose syringes were weighed before and after use as well as infusion period was recorded. Expired gas was collected to measure $^{13}CO_2$ in HeliFAN-System (HeliFAN, Fischer Analyser Instrumente, Leipzig, Germany) and blood sample was drawn to assess $^{13}C_6$-Glucose in photometer (Spectronic Genesys 2, Milton Roy, USA)

2.4.14 Blood analysis

After blood samples were drawn in each measurements point, blood serum levels of bilirubine, alanine aminotransferase, aspartate aminotransferase, amylase, lipase, creatinine kinase, troponine and creatinine were analysed by photometer (Spectronic Genesys 2, Milton Roy, USA).

2.5 Statistics

Data were expressed as median, quantities and range. Main criteria were tested for normal distribution using the Kolmogorov-Smirnov test. Time dependent changes within each group were tested with a Friedman analysis of variance on ranks and the Dunn's test for multiple comparisons. Differences between groups were analyzed by the Mann-Whitney rank sum test for unpaired samples. In all cases, $p < 0.05$ was regarded as signification.

3. Results

3.1. General collection data

In this study, 15 German land pigs with both genders were used. 5 pigs for the control group with the average body weight 42.6 kg (42-50 kg; m : f = 2 : 3), and 10 pigs for the peritonitis group with the average body weight is 51.6 kg (46-58.5 kg; m : f = 7 : 3).

3.2. Haemodynamic

3.2.1 Hydroxyethylstarch

Amount of hydroxyethylstarch, milliliter per kilogram body weight per hour in peritonitis group significantly increased in Mea2, Mea3, and Mea4 when compared with those of Mea1 ($p < 0.05$), whereas this amount of control group was no significant difference between measure points.

Results of hydroxyethylstarch amount are shown in figures 3.

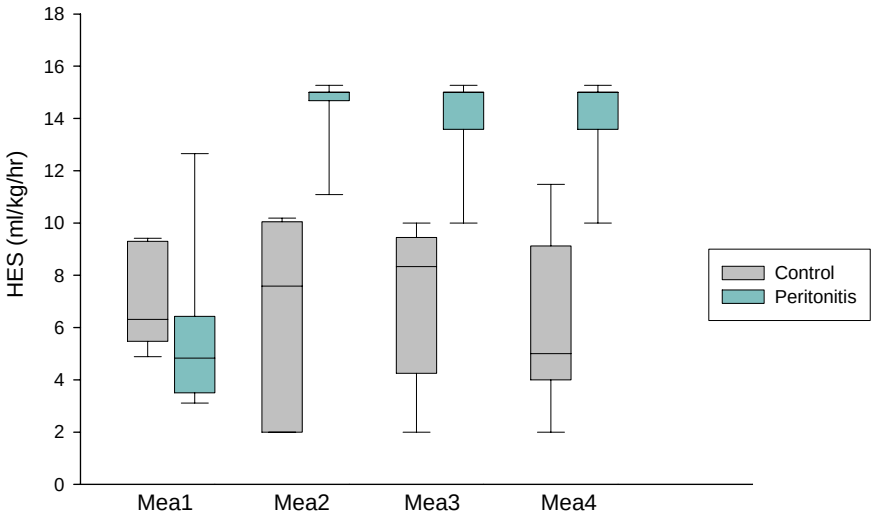

Figure 3 Hydroxyethylstarch (HES, milliliter per kilogram per hour) . Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.

Mea: measure point

.2.2 Urine amount

Calculated urine amount, milliliter per kilogram body weight per hour in control group was not different from Mea2 to the end of experiment (Mea4) when compared with Mea1. But this amount significantly increased in peritonitis group when compared the urine amount of Mea2, Mea3, and Mea4 with Mea1 ($p < 0.05$).

Results of urine amount are shown in figures 4.

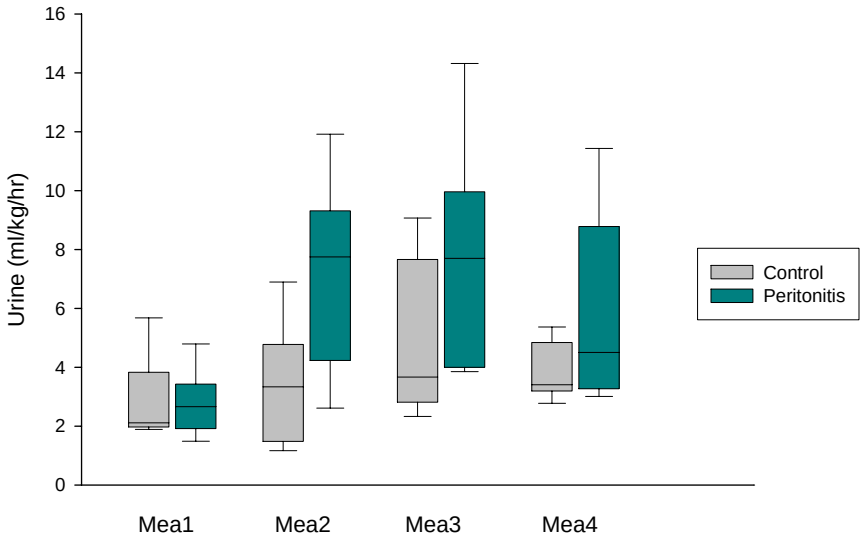

Figure 4 Urine (milliliter per kilogram per hour) . Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.2.3 Protein artery

In control group, there was no difference of protein artery (Protein A) of Mea2, Mea3 and Mea4 compared with that of Mea1. In the other group, protein A of Mea2, Mea3 and Mea4 significantly decreased when compared with that of Mea1 ($p < 0.05$).

Results of protein A are shown in figure 5.

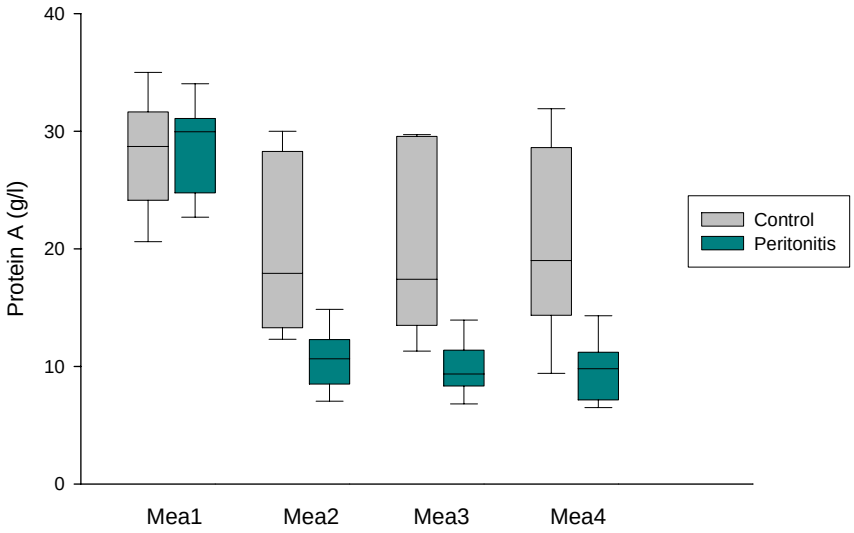

Figure 5 Protein in artery (gram per liter). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.2.4 Hemoglobin

Hemoglobin (Hb) in peritonitis group significantly increased when compared Hb of Mea2, Mea3, and Mea4 with that of Mea1 ($p<0.05$). In control group Hb was no difference between measure points.

Results of hemoglobin are shown in figure 6.

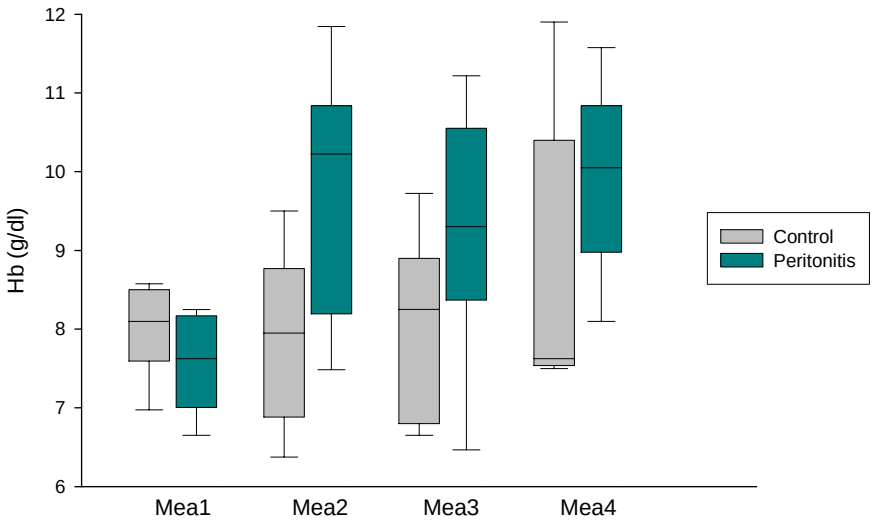

Figure 6 Hemoglobin (Hb, gram per deciliter) . Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.2.5 Heart rate and mean arterial pressure

In both groups, heart rate (HR) significantly increased, but in the peritonitis group, heart rate increased earlier (from Mea2) than that of control group from Mea3 ($p<0.05$).

There were no differences of mean arterial pressure (MAP) in both groups between Mea2, Mea3, and Mea4 with Mea1.

Results of heart rate and mean arterial pressure are shown in figure 7 and 8.

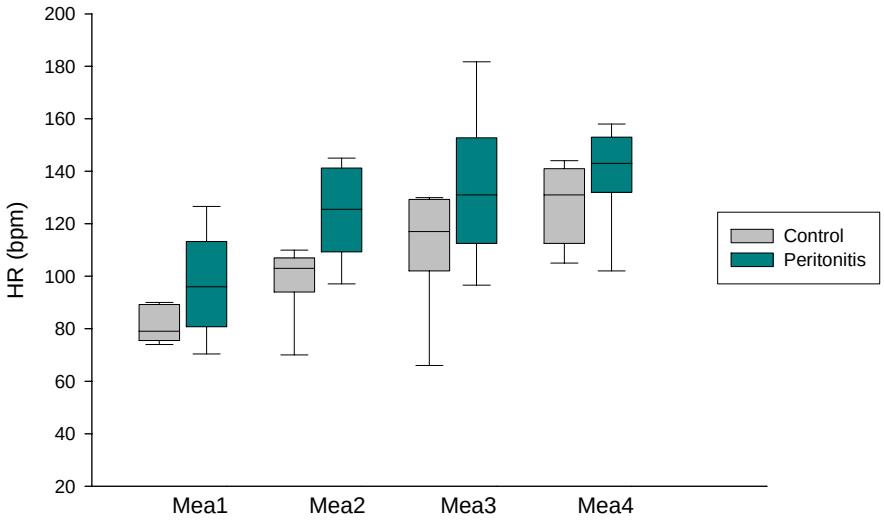

Figure 7 Heart rate (HR, beat per minute). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

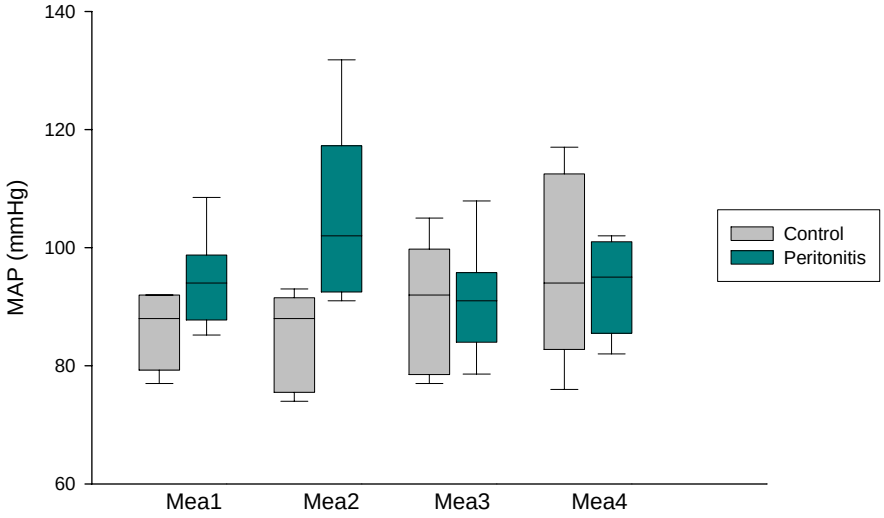

Figure 8 Mean arterial pressure (MAP, millimeter mercury). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians. Mea: measure point

3.2.6 Mean pulmonary arterial pressure

In peritonitis group, mean pulmonary arterial pressure (MPAP) in Mea2, Mea3, and Mea4 significantly increased when compared with that of Mea1 ($p<0.05$). Whereas, in control group there was no differences of MPAP in Mea2, Mea3, and Mea4 compared with those of Mea1.

Results of mean pulmonary arterial pressure are shown in figure 9.

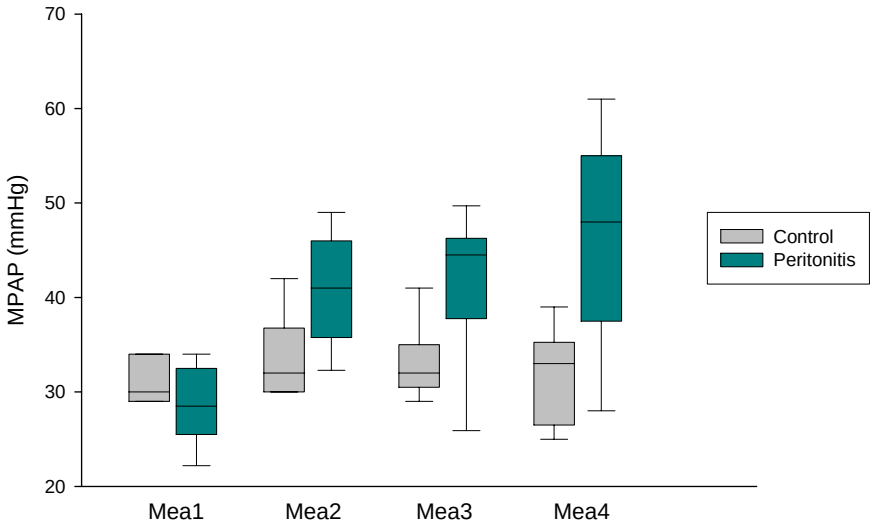

Figure 9 Mean pulmonary arterial pressure (MPAP, millimeter mercury). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.

Mea: measure point

3.2.7 Central venous pressure

In the control group, central venous pressure (CVP) was not significantly different when compared the CVP value of Mea2, Mea3 and Mea4 with CVP value in base line (Mea1). But in the peritonitis group, CVP significantly increased from Mea3 ($p<0.05$) to the end of experiment, and in Mea2 it was not different with base line (Mea1).

Results of central venous pressure are shown in figure 10.

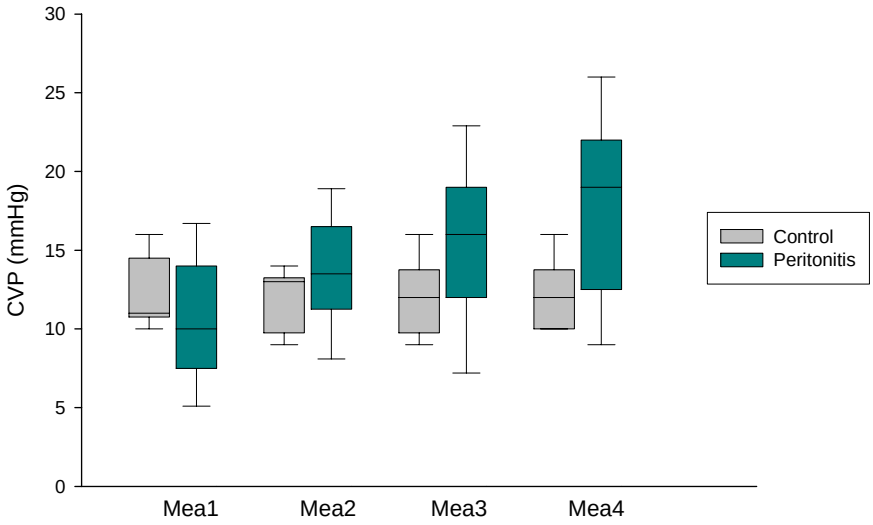

Figure 10 Central venous pressure (CVP, millimeter mercury). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.

Mea: measure point

3.2.8 Pulmonary arterial occlusion pressure

In peritonitis group pulmonary arterial occlusion pressure (PAOP) in Mea2, Mea3, and Mea4 significantly increased when compared with that of Mea1 ($p<0.05$). Whereas, in control group there was no difference of PAOP in Mea2, Mea3, and Mea4 compared with that of Mea1.

Results of pulmonary arterial occlusion pressure are shown in figure 11.

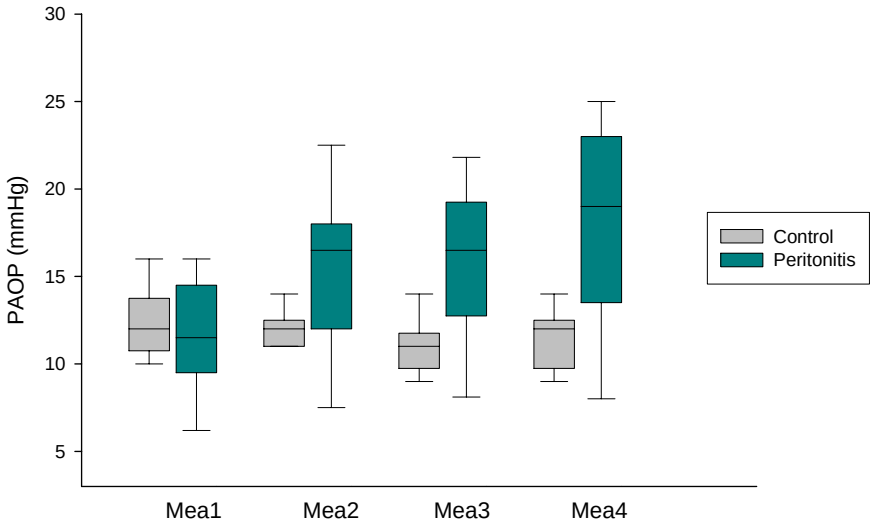

Figure 11 Pulmonary arterial occlusion pressure (PAOP, millimeter mercury). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.

Mea: measure point

3.2.9 Cardiac output index

Cardiac output index (CO index) in this study significantly increased after pigs were subjected to infection, in Mea2, Mea2, and Mea4 CO index was higher than that of Mea1 ($p<0.05$). Whereas in control group, CO index remained unchanged .

Results of cardiac output index are shown in figure 12.

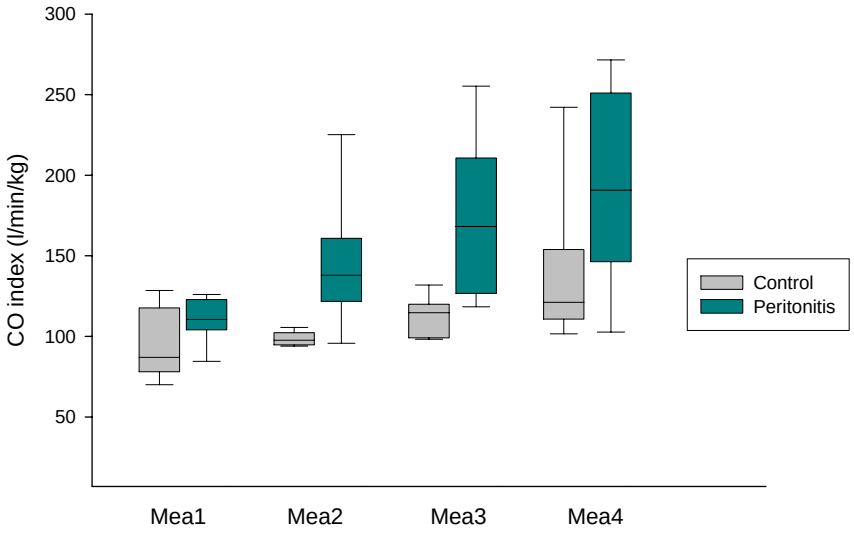

Figure 12 Cardiac output index (CO index, litter per minute per kilogram body weight). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.

Mea: measure point

3.2.10 Systemic venous resistant

In peritonitis group, systemic venous resistant (SVR) significantly increased from Mea3 to the end of experiment (Mea4), and SVR of Mea1 was not different with base line. SVR of the pigs in control group remained unchanged ($p < 0.05$).

Results of systemic venous resistant are shown in figure 13.

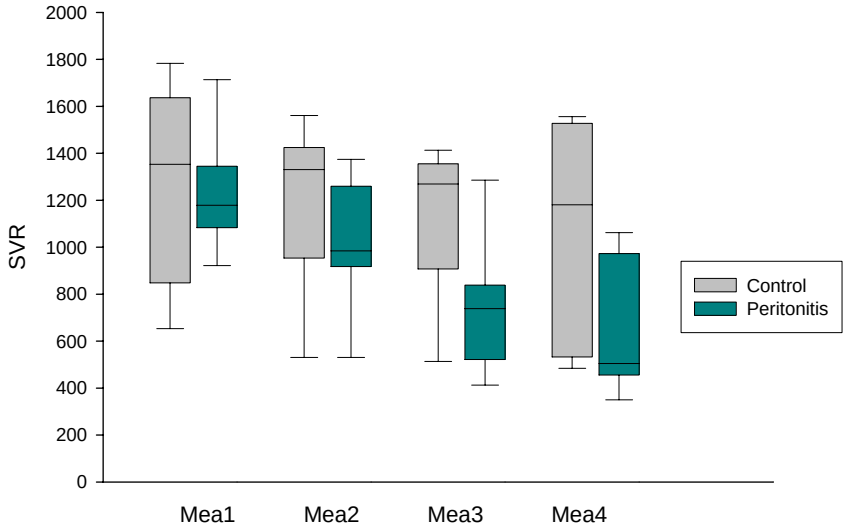

Figure 13 Systemic venous resistant (SVR). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.2.11 Stroke volume index

There was not significantly different of stroke volume index (SV index) in both groups when compared SV index of Mea2, Mea3, and Mea4 with that of Mea1 ($p < 0.05$).

Results of stroke volume index are shown in figure 14.

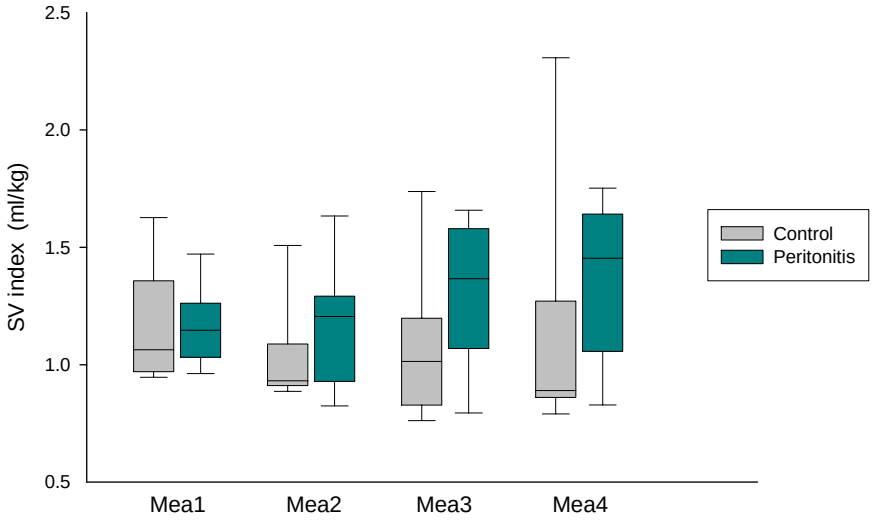

Figure 14 Stroke volume index (SV index, millimeter per kilo body weight). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.

Mea: measure point

3.2.12 Intra thorax blood volume index

Intra thorax blood volume index (ITBV index) of control group was not different between Mea2, Mea3, and Mea4 with Mea1 and in the peritonitis group, ITBV index increased in the late of experiment (Mea4) ($p < 0.05$).

Results of intra thorax blood volume index are shown in figure 15.

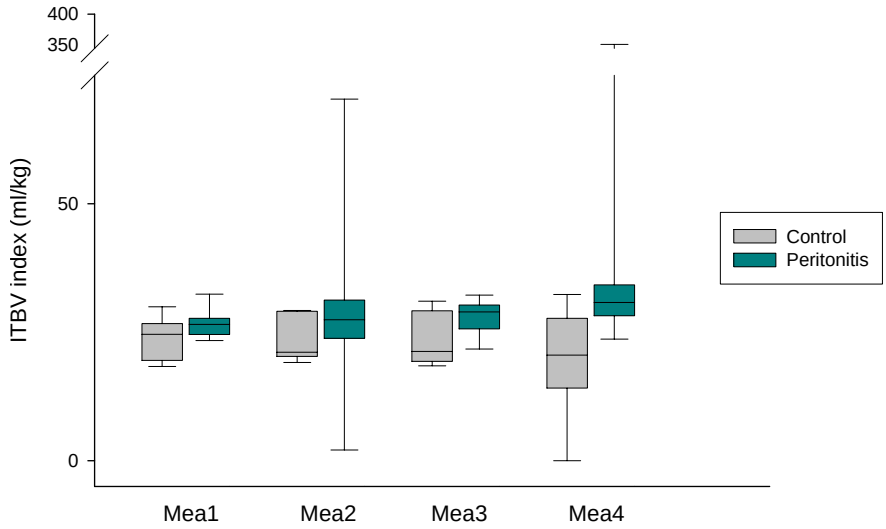

Figure 15 Intra thorax blood volume index (ITBV index, millimeter per kilo body weight). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.2.13 Extravacular lung water

Extravascular lung water (EVLW) of control group was not different between Mea2, Mea3, and Mea4 with Mea1 and in the peritonitis group EVLW significantly increased from Mea2 to the end of experiment ($p < 0.05$).

Results of extravascular lung water are shown in figure 16.

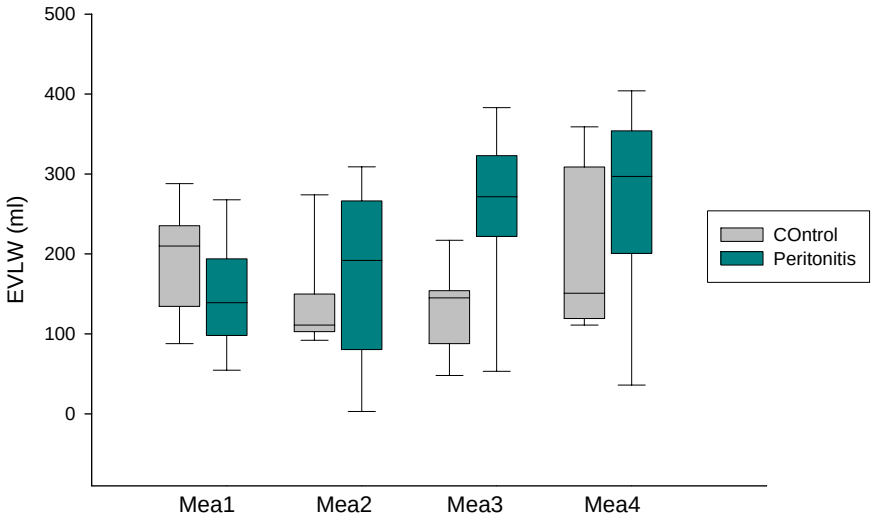

Figure 16 Extravascular lung water (EVLW, milliliter) . Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians. Mea: measure point

3.3. Gas exchange

3.3.1 Oxygen partial pressure

There was a significant decrease of oxygen partial pressure (PaO_2) in Mea2, Mea3, and Mea4 when compared with that of Mea1 in peritonitis group ($p<0.05$). In control group PaO_2 remained unchanged.

Results of oxygen partial pressure are shown in figure 17.

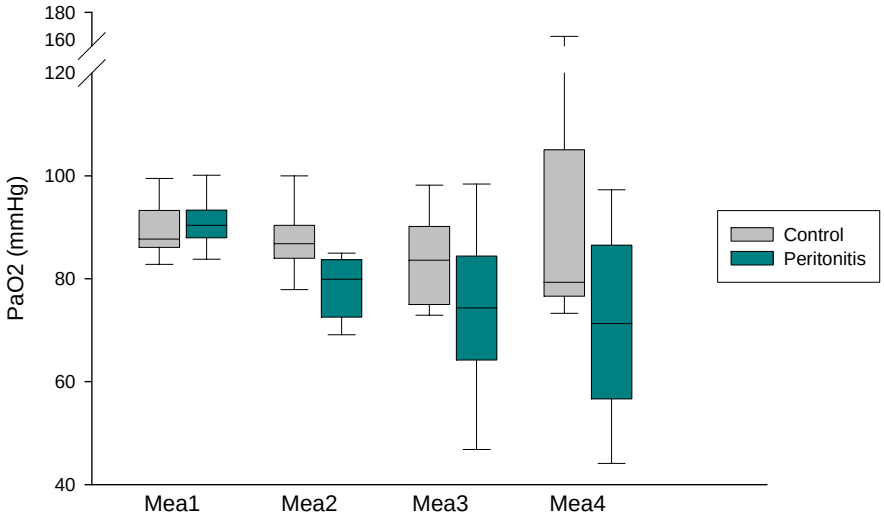

Figure 17 Oxygen partial pressure (PaO_2, millimeter mercury) . Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.

Mea: measure point

3.3.2 Arterial partial pressure and fraction of inspired oxygen ratio

In control group, arterial partial pressure and fraction of inspired oxygen ratio (PaO_2/FiO_2 ratio - horowitz) was not different from Mea2, Mea3, and Mea4 compared with Mea1, but in peritonitis group, horowitz of Mea2, Mea3, and Mea4 significantly decreased when compared with that of Mea1 ($p < 0.05$).

Results of horowitz are shown in figure 18.

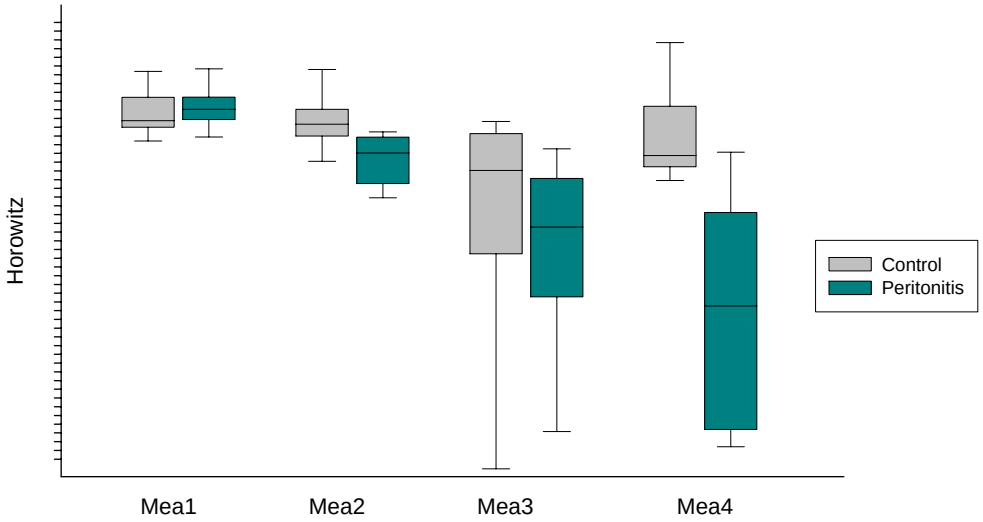

Figure 1

Figure 18 Arterial partial pressure and fraction of inspired oxygen ratio – PaO2/FiO2 (Horowitz) . Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.

Mea: measure point

3.3.3 Oxygen saturation

There was a significant decrease of oxygen saturation (SaO_2) in Mea2, Mea3, and Mea4 when compared with that of Mea1 in peritonitis group ($p<0.05$). In control group there was no difference of SaO_2 between measure points versus with baseline.

Results of oxygen saturation are shown in figure 19.

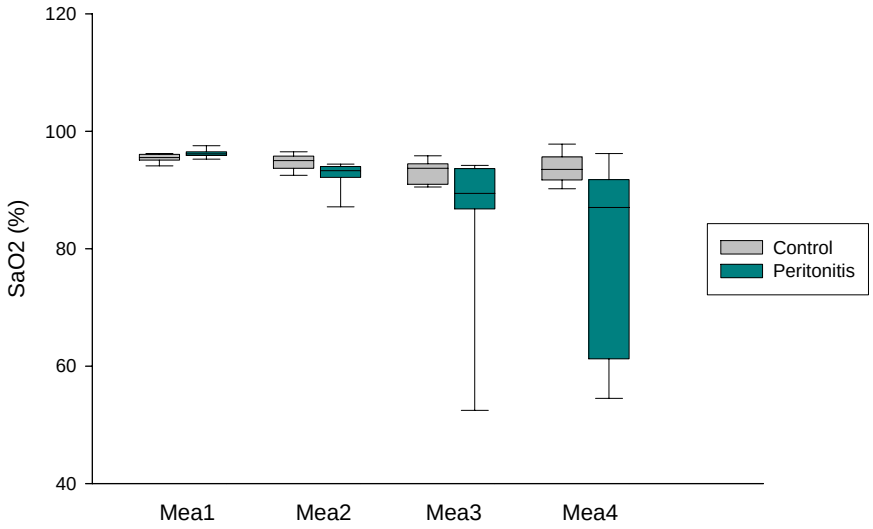

Figure 19 Oxygen saturation (SaO_2, percentage) . Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.3.4 Carbon dioxide partial pressure

There was no difference of carbon dioxide partial pressure ($PaCO_2$) in both control and peritonitis groups when compared $PaCO_2$ of Mea2, Mea3, and Mea4 with that of Mea1 ($p < 0.05$).

Results of carbon dioxide partial pressure are shown in figure 20.

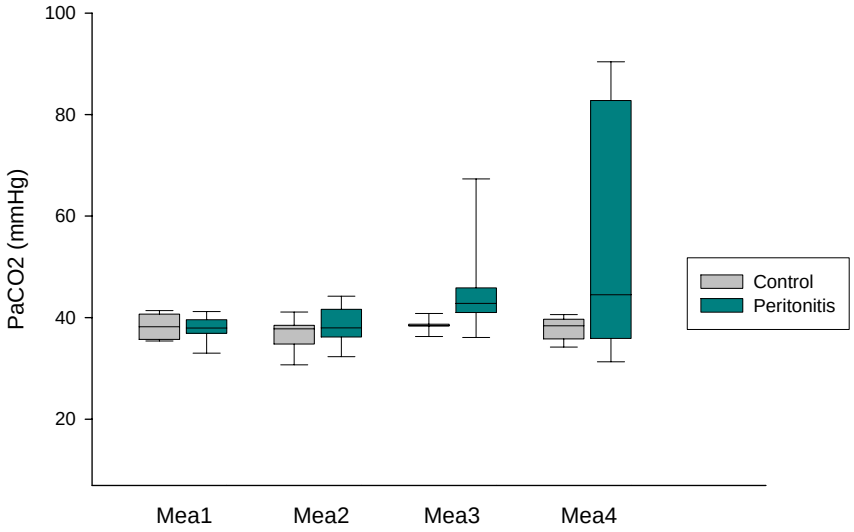

Figure 20 Carbon dioxide partial pressure ($PaCO_2$, millimeter mercury) . Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.

Mea: measure point

3.3.5 Shunt flow and total flow ratio

Ratio of shunt flow and total flow (Qs/Qt) significantly increased in peritonitis group when compared Qs/Qt ratio of Mea2, Mea3, and Mea4 with that of Mea1 ($p<0.05$), whereas in control group, this ratio was remained unchanged.

Results of shunt flow and total flow ratio are shown in figure 21.

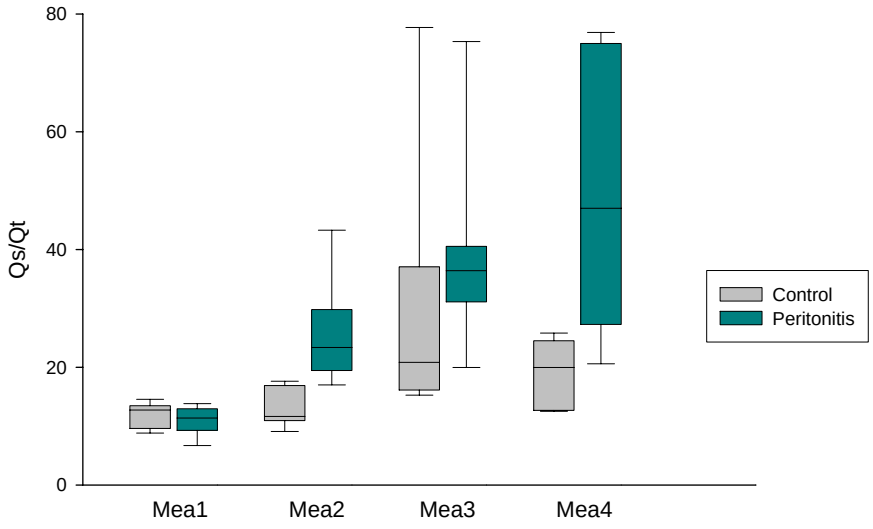

Figure 21 Shunt flow and total flow ratio (Qs/Qt). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.3.6 Blood pH of artery

In peritonitis group, pH of artery significantly decreased from Mea2 to the end of experiment (Mea4) when compared with that of Mea1 ($p<0.05$). In control group, pH artery remained unchanged.

Results of blood pH of artery are shown in figure 22.

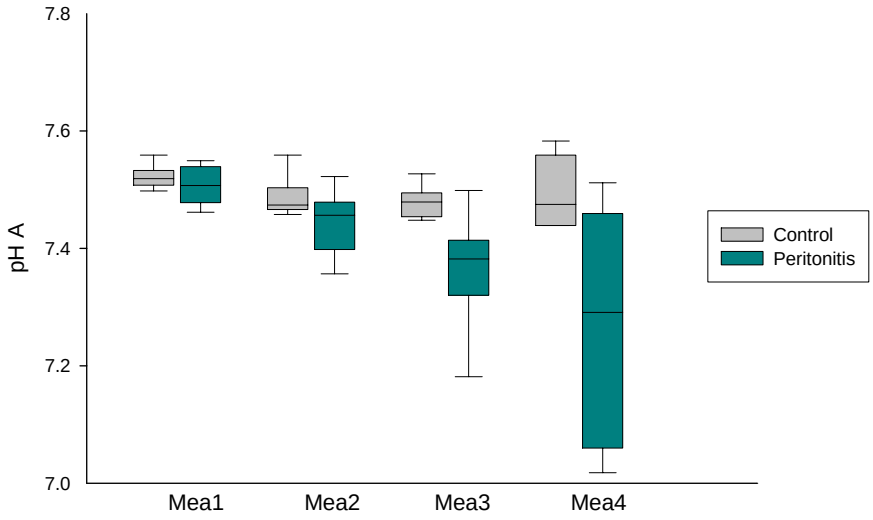

Figure 22 pH of arterial blood (pH A). MEA1 (base line) after recovery phase. Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.4 Metabolism

3.4.1 Carbon dioxide elimination index

Carbon dioxide elimination index (VCO_2 index) increased only in Mea4 of control group, and in other measure points of both groups, VCO_2 index remained unchanged ($p < 0.05$). Results of carbon dioxide elimination index are shown in figure 23.

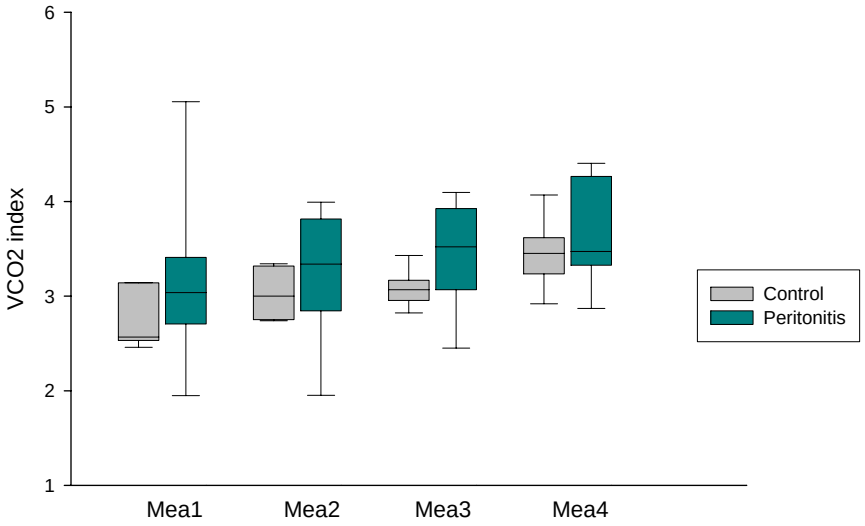

Figure 23 Carbon dioxide elimination index (VCO_2 index). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians. Mea: measure point

3.4.2 Global oxygen consumption index

In both groups, global oxygen consumption index (VO_2 global index) significantly increased from Mea4 and in other measure points VO_2 global index remained unchanged ($p < 0.05$).

Results of global oxygen consumption index are shown in figure 24.

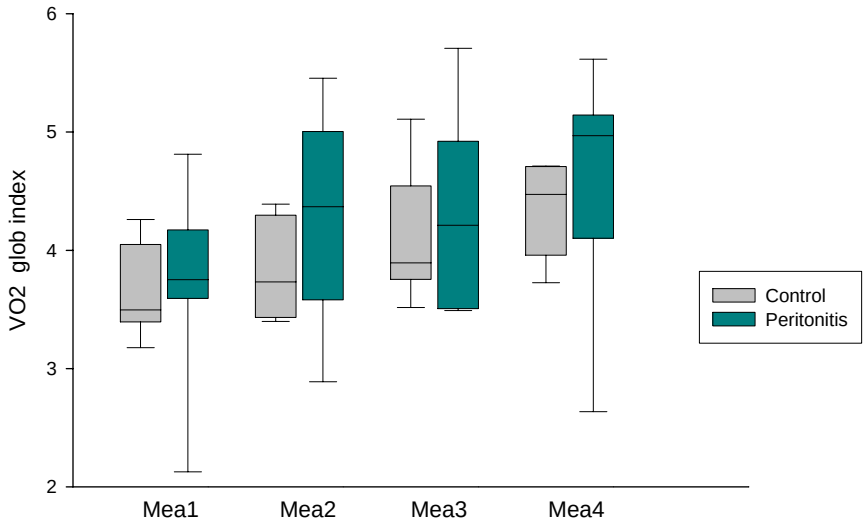

Figure 24 Global oxygen consumption index (VO_2 glob index). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.

Mea: measure point

3.4.3 Arterial lactate concentration

In peritonitis group, lactate concentration in arterial blood (Lac A) of Mea2, Mea3, and Mea4 significantly increased compared with Mea1 ($p<0.05$). Whereas in control group, Lac A of Mea2 and Mea3 significantly reduced, and in Mea4 Lac A was not different when compared with that of Mea1 ($p<0.05$).

Results of arterial lactate concentration are shown in figure 25.

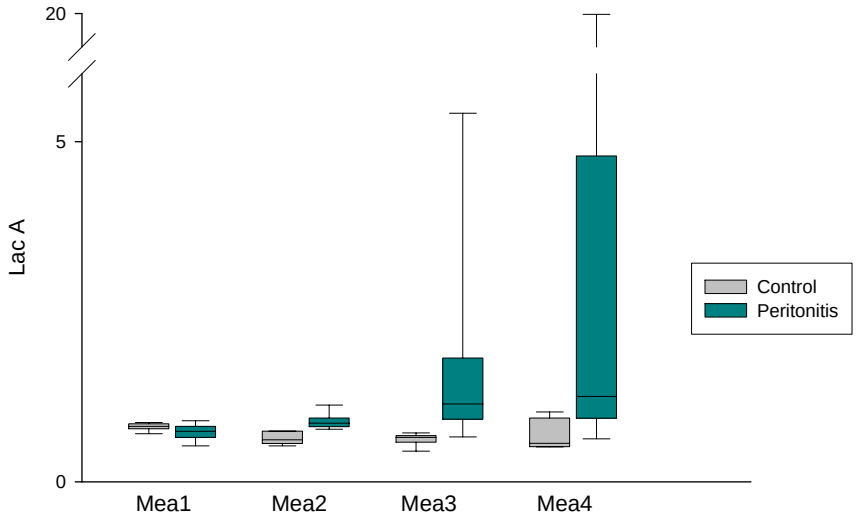

Figure 25 Lactate artery concentration (Lac A). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.4.4 Lactate and pyruvate ratio of artery.

In peritonitis groups, lactate and pyruvate ratio of artery (Lac/Pyr A) significantly increased from Mea2 to the end of experiments (Mea4) when compared with that of Mea1 ($p < 0.05$). Whereas, in control group, Lac/Pyr A was not different between Mea2, Mea3, and Mea4 when compared with that of Mea1 ($p < 0.05$)

Results of lactate and pyruvate ratio of artery are shown in figure 26.

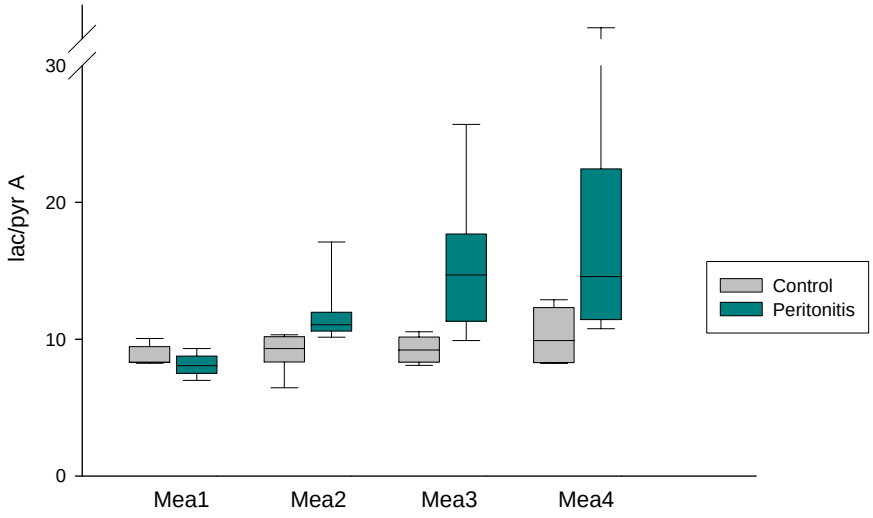

Figure 26 Arterial lactate and pyruvate ratio (lac/pyr A). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians. Mea: measure point

3.5 Regional organs: heamodynamics, metabolism and function

3.5.1 Intra abdominal pressure

In peritonitis group intra abdominal pressure (IAP) in Mea2, Mea3, and Mea4 significantly increased when compared with that of Mea1 ($p<0.05$). Whereas in control group, IAP remained unchanged ($p < 0.05$) .

Results of intra abdominal pressure are shown in figure 27.

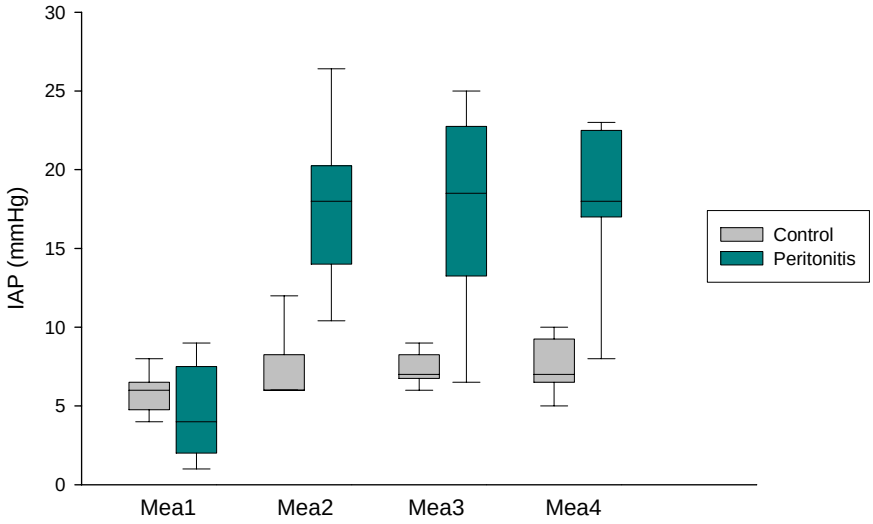

Figure 27 Intra abdominal pressure (IAP, millimeter mercury) . Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.

Mea: measure point

3.5.2 Flow portal vein and hepatic artery index

In peritonitis group, flow portal vein index (Qpv index) significantly increased in whole experiment Mea2, Mea3, and Mea4 when compared with that of Mea1 ($p<0.05$), and in control group, only Qpv index of Mea4 increased, whereas Qpv index of Mea2 and Mea3 remained unchanged.

Flow hepatic artery index (Qha index) was not significantly different in both groups ($p < 0.05$).

Results of flow portal vein and hepatic artery index are shown in figures 28 and 29.

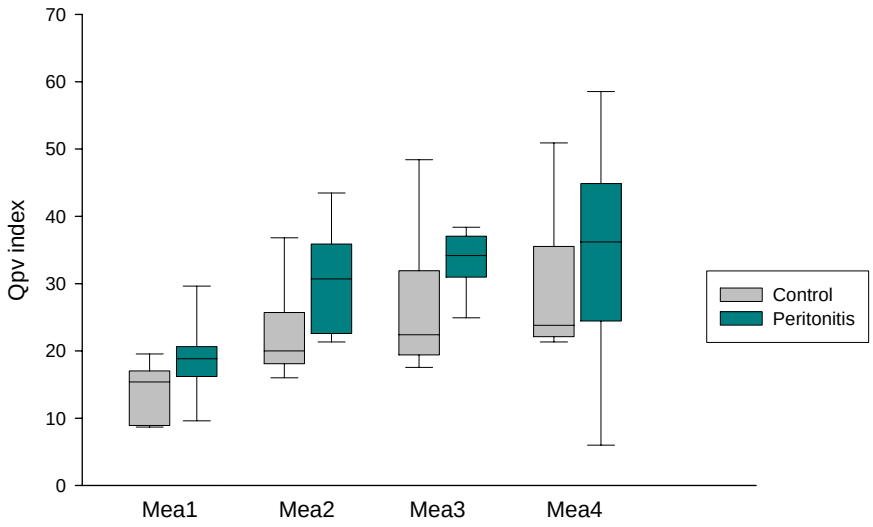

Figure 28 Flow portal vein index (Qpv index). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

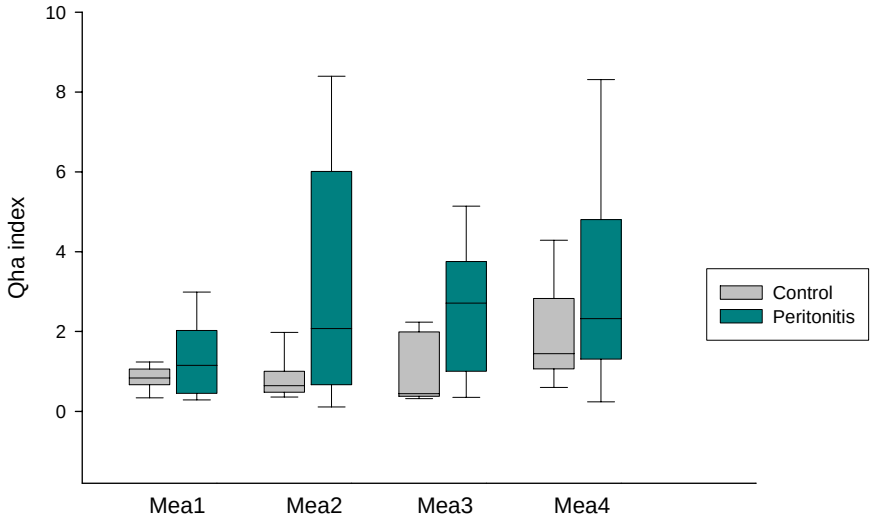

Figure 29 Flow hepatic artery index (Qha index). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.5.3 Flow liver index

Flow liver index (Qliv index) in peritonitis group early and persistently increased from Mea2 to the end of experiment (Mea4) when compared with that of Mea1 ($p<0.05$). Whereas in control group, Qliv index increased from Mea4 ($p < 0.05$) .

Results of flow liver index are shown in figure 30.

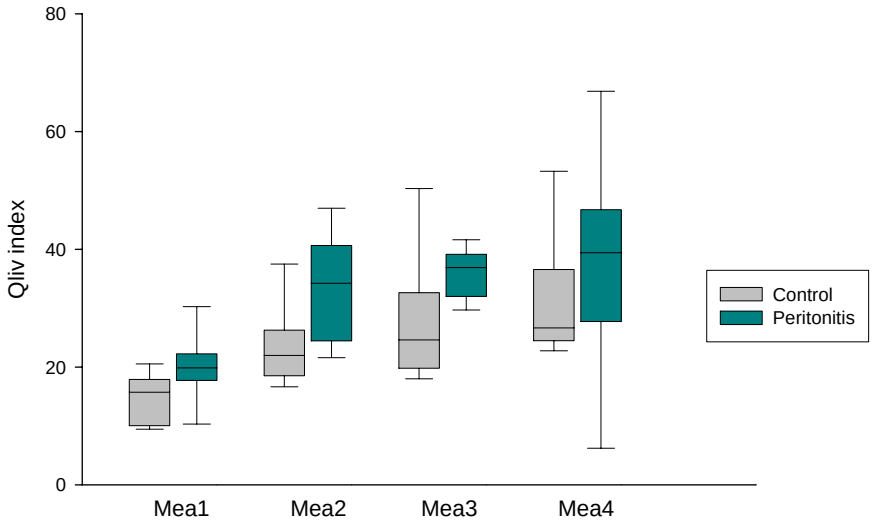

Figure 30 Flow liver index (Qliv index). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.5.4 Flow portal vein, liver and cardiac output ratio

In both groups, there were no significant differences of flow portal vein and cardiac output ratio (Qpv/CO), and flow liver and cardiac output ratio (Qliv/CO) in Mea2, Mea3, and Mea4 when compared with those of Mea1 ($p < 0.05$).

Results of flow portal vein and cardiac output ratio, and flow liver and cardiac output ratio are shown in figures 31 and 32.

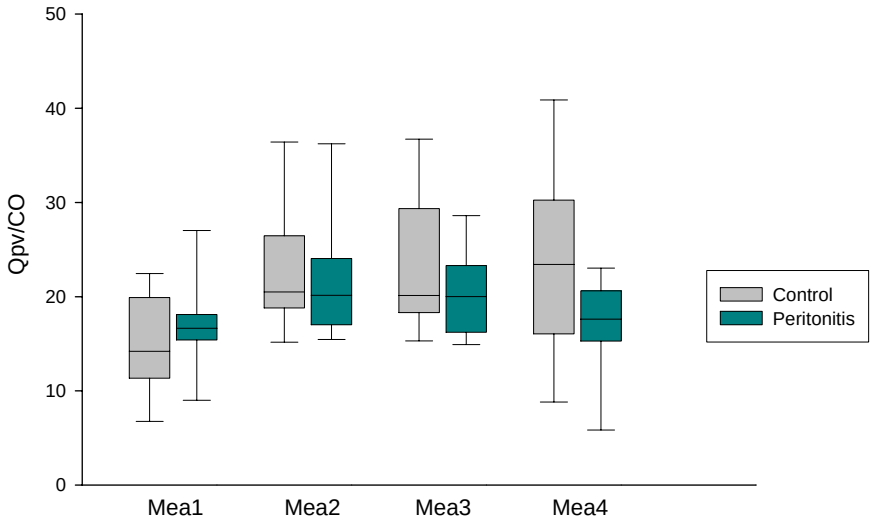

Figure 31 Ratio of flow portal vein to cardiac output (Qpv/CO). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.

Mea: measure point

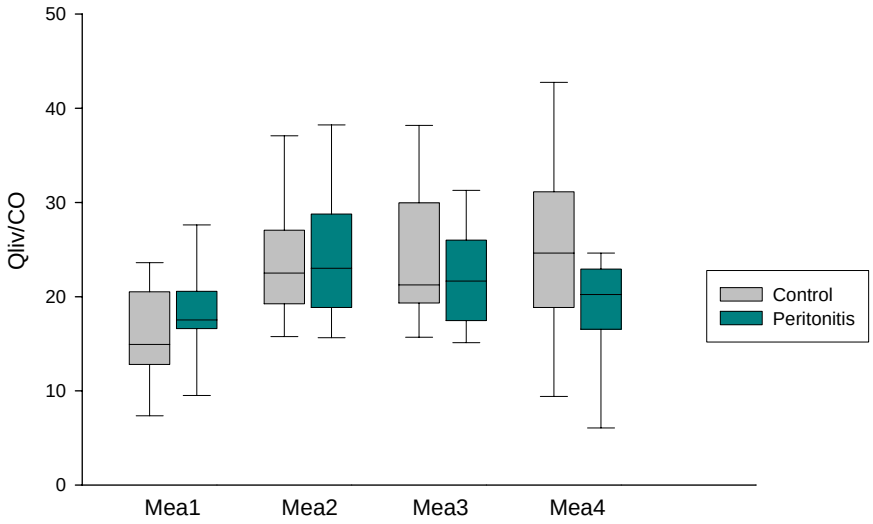

Figure 32 Ratio of flow liver to cardiac output (Qliv/CO). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians. Mea: measure point

3.5.5 Liver oxygen delivery index

Liver oxygen delivery index (DO_2 liv index) of Mea2, Mea3, and Mea4 significantly increased when compared with that of Mea1 in peritonitis group ($p<0.05$). In control group, DO_2 liv index remained unchanged.

Results of liver oxygen delivery index are shown in figure 33.

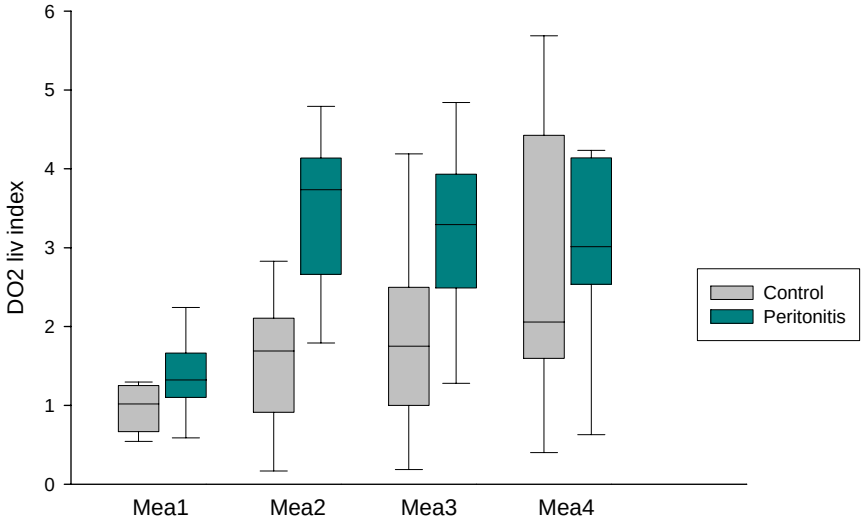

Figure 33 Liver oxygen delivery index (DO_2 liv index). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians. Mea: measure point

3.5.6 Gut oxygen extraction

In peritonitis group, gut oxygen extraction (GOE), significantly decreased from Mea2 to the end of experiment (Mea4) when compared with that of Mea1 ($p<0.05$). Whereas, in control group GOE remained unchanged .

Results of gut oxygen extraction are shown in figures 34.

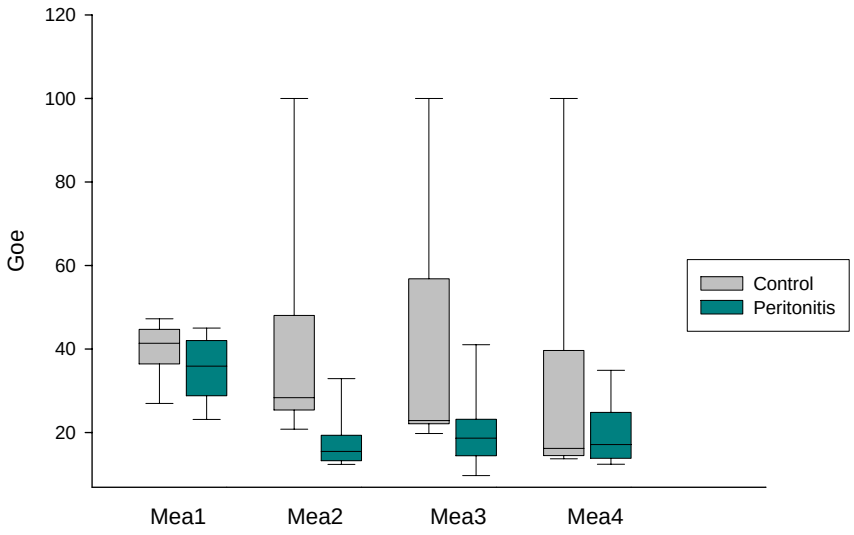

Figure 34 Gut oxygen extraction (GOE). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.5.7 Liver oxygen consumption index

There was not significantly different of liver oxygen consumption index (VO_2 liv index) in both control and peritonitis groups when compared VO_2 liv index of Mea2, Mea3, and Mea4 with that of Mea1.

Results of liver oxygen consumption index are shown in figure 35.

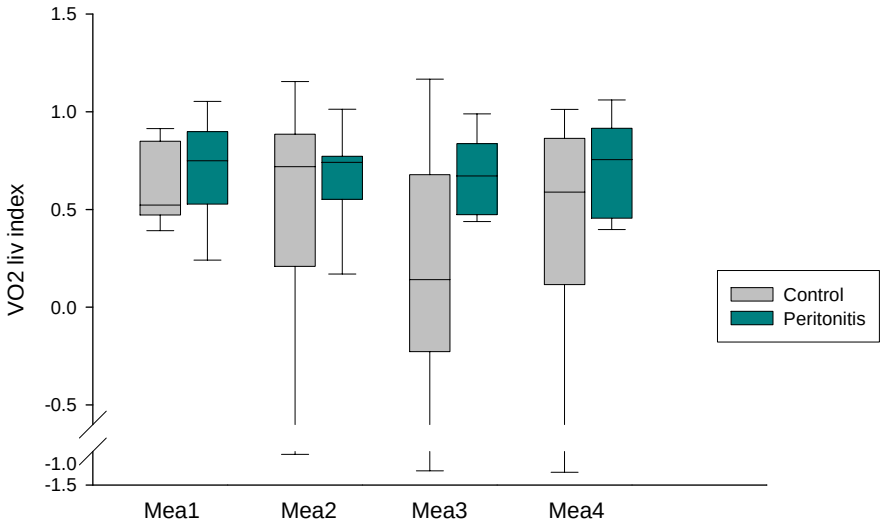

Figure 35 Liver oxygen consumption index (VO_2 liv index). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.

Mea: measure point

3.5.8 Blood pH of portal vein

In peritonitis group, pH of portal vein (pH pv) significantly decreased from Mea2 to the end of experiment (Mea4) when compared with that of Mea1 ($p<0.05$). In control group, pH pv remained unchanged.

Results of blood pH of portal vein are shown in figure 36.

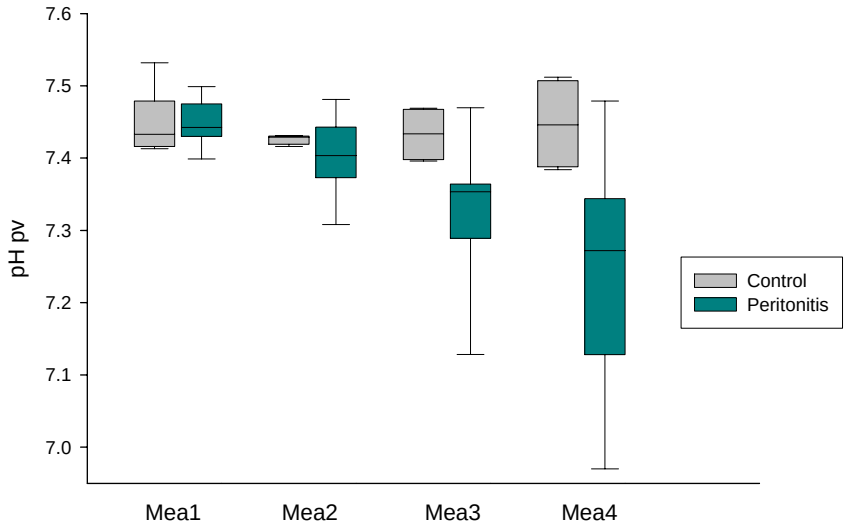

Figure 36 pH of portal vein blood (pH pv). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.5.9 Blood pH hepatic vein

In peritonitis group, pH of hepatic vein (pH hv) significantly decreased from Mea2 to the end of experiment (Mea4) when compared with that of Mea1 ($p<0.05$). In control group, pH hv remained unchanged.

Results of blood pH of hepatic vein are shown in figure 37.

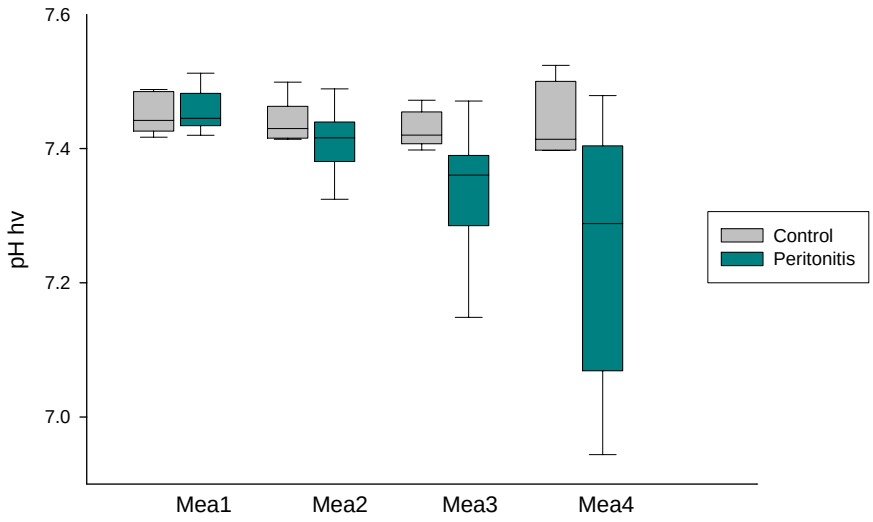

Figure 37 pH of hepatic vein blood (pH hv). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.5.10 Ileal mucosal arterial delta

In peritonitis group, only ileal mucosal arterial delta (PCO_2 gap) in Mea2 significantly increased, and in other measure points of peritonitis group and in whole control group PCO_2 gap was not different when compared with that of Mea1 ($p < 0.05$).

Results of ileal mucosal arterial delta are shown in figure 38.

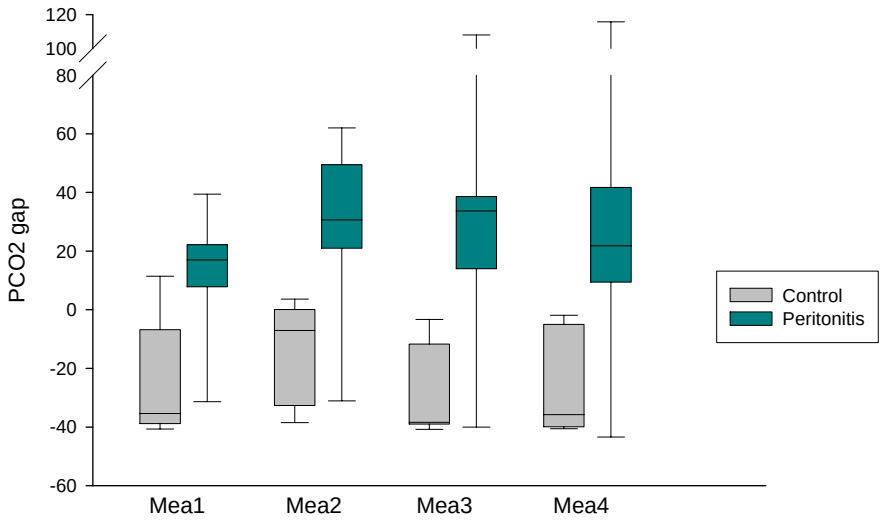

Figure 38 Ileal mucosal arterial delta (PCO_2 gap). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians. Mea: measure point

3.5.11 Lactate and pyruvate ratio of portal vein and hepatic vein.

In peritonitis groups, lactate and pyruvate ratio of portal vein (lac/pyr pv) and hepatic vein (lac/pyr hv) significantly increased from Mea2 to the end of experiments (Mea4) when compared with those of Mea1. Whereas, in control groups, lac/pyr pv and lac/pyr hv remained unchanged ($p < 0.05$).

Results of lactate pyruvate ratio of portal vein and hepatic vein are shown in figure 39 and 40.

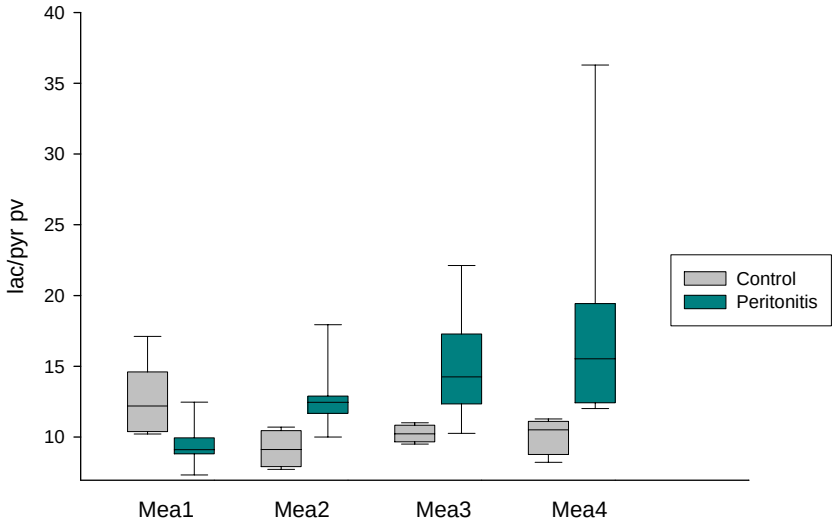

Figure 39 Portal vein lactate pyruvate ratio (lac/pyr pv). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians. Mea: measure point

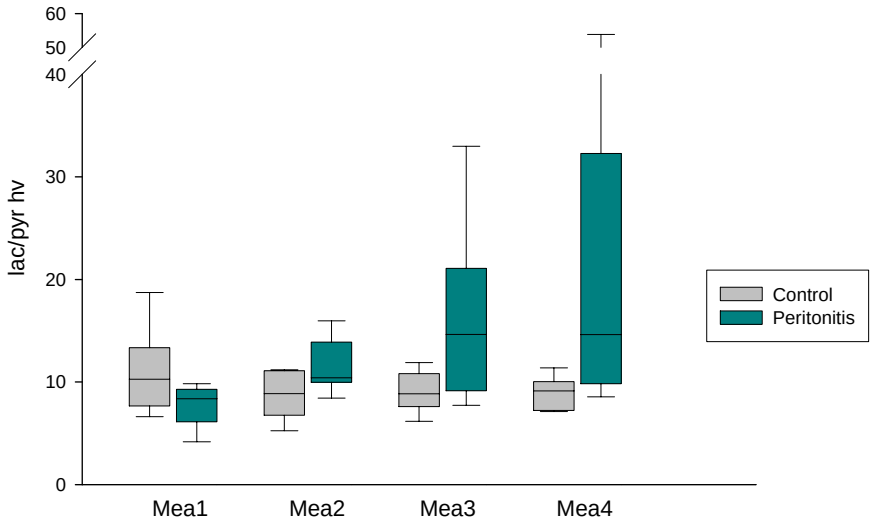

Figure 40 Hepatic vein lactate pyruvate ratio (lac/pyr hv). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians. Mea: measure point

3.5.12 Indocyanine green plasma disappearance rate

Indocyanine green plasma disappearance rate (PDRig) in both groups was not significantly different between Mea2, Mea3, and Mea4 when compared with that of Mea1.

Results of indocyanine green plasma disappearance rate are shown in figure 41.

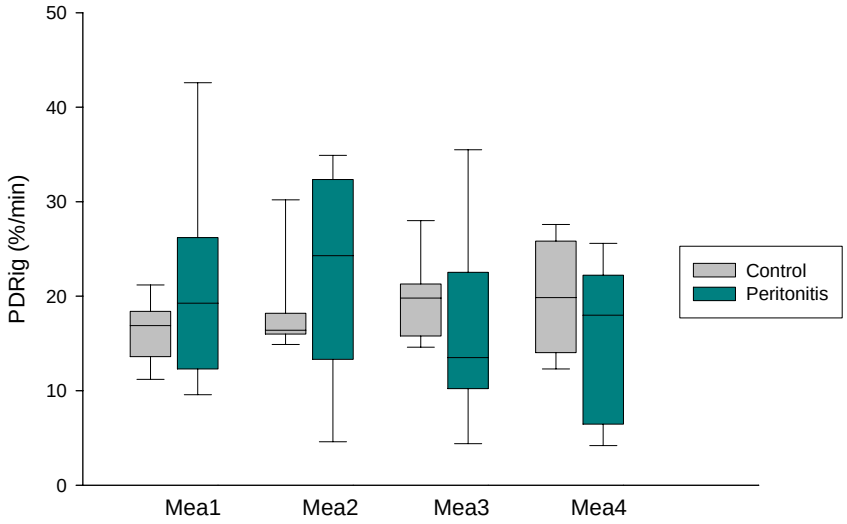

Figure 41 Indocyanine green plasma disappearance rate (PDRig, percentage per minute). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.5.13 Lactate balance liver

Lactate balance liver (lac bal liv) was not significantly different in both groups between Mea2, Mea3, and Mea4 when compared with that of Mea1.

Results of lactate balance liver are shown in figure 42.

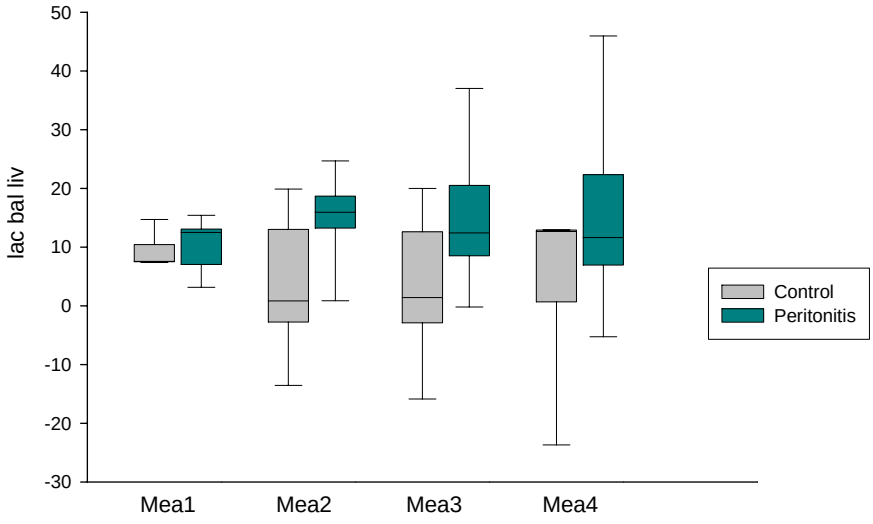

Figure 42 Lactate balance liver (lac bal liv). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.5.14 Aspartat aminotransferase and alanin aminotransferase of hepatic vein blood

Aspartat aminotransferase (AST) of hepatic vein blood per gram protein significantly increased in both groups, and AST in peritonitis group was higher than AST in control group at the same measure points ($p < 0.05$).

Alanin aminotransferase (ALT) of hepatic vein blood per gram protein in peritonitis group significantly increased from Mea2 to the end of experiment (Mea4) compared with Mea1, whereas in control group ALT remained unchanged ($p < 0.05$).

Results of aspartat aminotransferase and alanin aminotransferase of hepatic vein blood per gram protein are shown in figure 43 and 44.

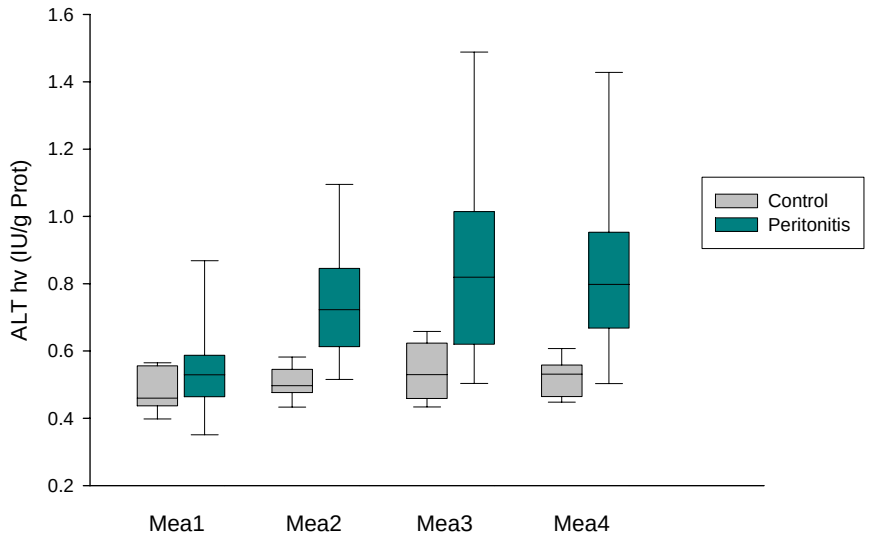

Figure 43 Alanin aminotransferase of hepatic vein blood per gram protein (ALT hv, unit per gram protein). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

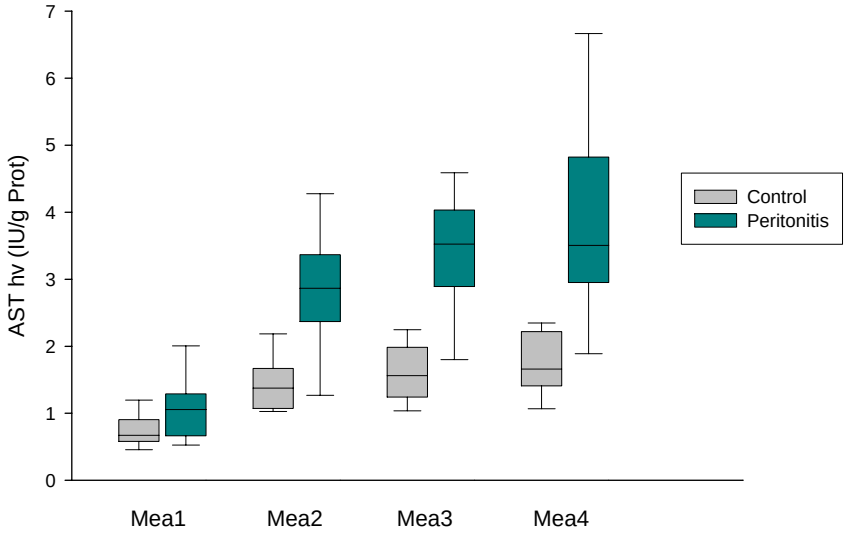

Figure 44 Asprartat aminotransferase of hepatic vein blood per gram protein (AST hv, unit per gram protein). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.5.15 Bilirubin

In peritonitis group, bilirubin of hepatic vein blood per gram protein (bilirubin hv) of Mea2, Mea3, and Mea4 significantly increased when compared with that of Mea1. In control group bilirubin hv remained unchanged ($p < 0.05$).

Results of bilirubin of hepatic vein blood per gram protein are shown in figure 45.

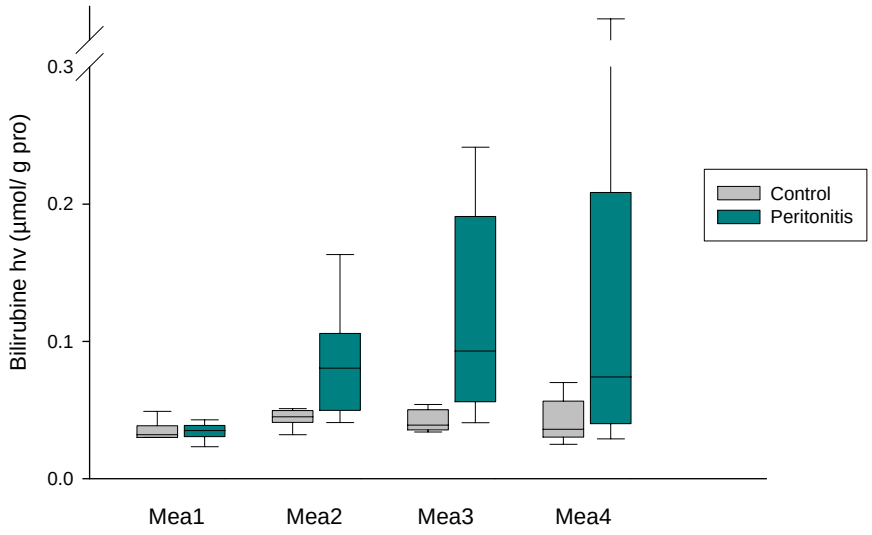

Figure 45 Bilirubin of hepatic vein blood per gram protein (Bilirubine hv, micromole per gram protein). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

3.5.16 Creatinine

In peritonitis group, creatinine per gram protein of Mea2, Mea3, and Mea4 significantly increased when compared with that of Mea1. Whereas in control group creatinine remained unchanged ($p < 0.05$).

Results of creatinine per gram protein are shown in figure 46.

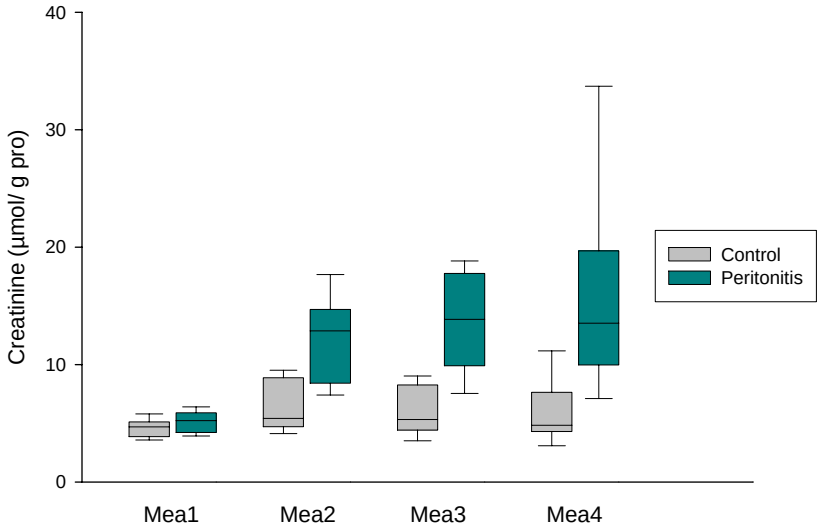

Figure 46 Creatinine per gram protein (Creatinine, micromole per gram proteine). Mea1 (base line) after recovery phase. Mea2, Mea3, and Mea4 after Mea1: 12, 18 and 24 hours respectively. Data are medians.
Mea: measure point

4. Discussion

4.1. Method

Laboratory sepsis experiments were designed to mimic the sepsis in human. There are three experimental sepsis model usually used: i, Bolus intravascular (IV) bacterial or endotoxin models; ii, Endotoxicosis models: models that use small (sublethal) dose of lipopolysaccharide or provide aggressive resuscitation of intravascular volume or utilize a continuous of lipopolysaccharide, and models use lipopolysaccharide injecting intraperitoneally; iii, and peritonitis models: cecal ligation and perforation (CLP), and fecal peritonitis (28, 34). Each sepsis models have advantages and limitations. Bolus intravascular bacterial or endotoxin models, although many experiment have been intravenously introduced bolus or continuously short term (1-4h) of large doses of bacteria (10^9 – 10^{10}/kg), these models are not match with preclinical models (28, 101). The seeds of bacteria in the IV bolus models are over a short period of time, that not correlate with sepsis human, the seeds of bacteria are overtime. In sepsis model using massive IV bolus of bacteria or endotoxin generally produce a rapid and critical decrease in cardiovascular function and cardiac output, hence, the hemodynamics response observed in IV bolus model is hypodynamic response rather than hyperdynamic response observed in sepsis patients (28). The survival time of animals in IV bolus models are generally short, thus the time for progression of disease is limited. Other limitation of IV bolus model is response of cytokine, in IV bolus models cytokine response is in short time and greater in magnitude than cytokine response observed in sepsis patients (28).

Endotoxicosis models are very useful to mimic human sepsis. Endotoxin (as well as other bacterial cell wall products) induces the septic responses such as hemodynamic, hematology, metabolic changes that are similar to those observed in human volunteers, who were injected of small dose (4 ng/kg) of endotoxine (35). There is only a limitation of this model that is a number of bacterial species induced sepsis. In endotoxicosis models, even by using two or three species, these models fail to correctly mimic with the human sepsis, which is characterized by a confrontation with the hundreds of different bacterial species and adjuvant factors from native feces induced peritonitis (35). This is taken into account in another class of multibacterial models of sepsis, namely fecal sepsis (35).

The two described and most commonly used peritonitis models are the cecal ligation and perforation (CLP), and the fecal peritonitis model, in which bacteria suspended in a fibrin clot is implanted into the peritoneal cavity. In our sepsis model, we introduced fecal autologous feces (1g/kg body weigh) suspended in Ringer's solution directly into peritoneal cavity. These models have been used successfully to mimic preclinical sepsis in both small and large animal species. In these models, animal confront with its individual flora, this type matches the situation in human sepsis (35). In our experiment, fecal peritonitis-induced septic shock model we found that microbiology included many species bacteria, both negative and positive gram bacteria.

Kazarian et al, in 1994 (39) contrasted two types of intraperitoneal soilage, autologous fecal inoculum versus pure culture E.coli. Changes were identified early (1-4h) and late (24-72h) in both groups. The E.coli group was manifested by early hypotension, decreased cardiac output, increased systemic and pulmonary vascular resistance, together with leucopenia, hypoglycemia, and high lactic acid. In addition, in the survived animals, physiology returned rapidly to baseline. In the autologous fecal inoculum, hypotension manifestation was associated with increased cardiac output and reduced systemic vascular resistance. These changes persisted for days and associated with leukosytosis (39). Other important difference is that endotoxin was quicker eliminated in the E.coli group but persisted longer in blood in the autologous fecal inoculum group. A major advantage of peritonitis models is the development an initial hyperdynamic cardiovascular response, characterized by an increased cardiac output and decreased systemic vascular resistance that is similar with what observed in human sepsis (28). Other advantage of this model is the magnitude of the serum cytokine response, it persisted over time similarly to that observed in human sepsis. Furthermore, animals in peritonitis model become worse over time and reach to critical condition, septic shock (cardiovascular collapse), and death in several days (28). Therefore, this model is reasonable choice for testing preclinical goals: improving survival in patients with severe sepsis and septic shock (35).

Pig has been chosen as an animal model of human sepsis in our experiments because of the remarkable similarities in anatomy, physiology and pathophysiology of circulation, respiration, digestion and metabolism to humans as underlined by several publication (28,

20, 31, 34, 35). Out of the remarkable pathophysiological response to sepsis between pigs and rodents, pigs can also sufficiently provide blood samples for different tests (31, 100).

There are some limitations of the fecal peritonitis model. In clinical practice, neonates and elderly are the common patients subjected to sepsis. In neonates, the coagulation factor concentration, especially the vitamin K and prothrombin levels are very low. This changes probably promote either hemorrhage or thrombosis process in response to inflammation (3). In elderly human, diabetes, hypertension and atherosclerosis are common conditions and function of the immune system in elderly human decrease. Thus, some of the results gained from healthy animal studies may give different results observed in elderly patients, especially studying the effects of drugs (3). In addition, there are major differences between experimental animals and clinical practice that is the ways managed sepsis in patients and animals. Most of septic patients are ventilated, resuscitated with fluids, and given vasopressor to maintain blood pressure. Usually, in animal sepsis models, these procedures are rarely employed, that's why some experimental results are not matches with those observed in patients. For example, in experimental animals without ventilation, drug improves lung function, but in patients this advantage might be negated because of mechanical intervention (3). To minimize those differences, in our sepsis model, we placed all animals on a lung-protective ventilation protocol similar to the ARDS Network protocol. In addition, the Ringer's solution, hydroxyethyl starch and continuous intravenous norepinephrine were adjusted.

4.2. Hemodynamic

4.2.1 Systemic circulation

Sepsis-induced hemodynamic disturbances characterized by an elevated cardiac output (CO) and decreased systemic venous resistance (SVR) (27). Some early studies (31, 82) reported that CO reduced in septic shock, but it likely that all of these patients had an inadequate blood volume resuscitated. Since the central venous catheter and pulmonary catheter were used to measure central venous pressure (CVP) and pulmonary artery occlusion pressure (PAOP) respectively, volume resuscitation status and left ventricular pressure were sufficiently evaluated, hence it is realized that the decrease of CO in sepsis is uncommon (111). Cardiac output is the blood amount pumped out of the heart per

minus to maintain oxygen demand of the body, and cardiac output index is CO value divided to body size (l/min/m^2) to account for the changes in CO that occurs in patients with different size. In the present study, HR in both groups was increased, whereas SV was not changed, but CO and CO index were significantly increased in the peritonitis group and in control group these values were not changed. Our finding was consistent with many our previous studies on long-term hyperdynamic porcine endotoxemia, on hyperdynamic murine septic shock (2, 9, 51, 106, 125), and studies in other research center studied in clinical septic shock (1, 42, 70) found that CO and CO index were significantly increased after sepsis induced and hyperdynamic shock occurred.

In addition, systemic vascular resistance (SVR) is very important parameter reflecting the afterload and the vascular tone influencing blood pressure and organ perfusion. Although septic shock is usually manifested by vasodilatation, but not all vessel are dilated. Some vessel, especially the vessel supplied blood for vital organs, such as cerebral artery, remain vasoconstricted. Otherwise, vasodilated vessels normally occur in sub-vital organs, such as gut, kidney, and periphery (18). This situation leads to maldistribution of blood flow caused decreased blood pressure and an inadequate tissue perfusion (18, 111). SVR is calculated by mean arterial pressure, central venous pressure, and cardiac output parameters as the following equation

$$SVR = (MAP - CVP) \times 80/CO$$

Where SVR is systemic vascular resistance; MAP is mean arterial pressure, CVP is central venous pressure; and CO is cardiac output. In our study, we found that CVP and MAP were not different in both groups in all measure points versus with baseline. Stable CVP value indicated that volume resuscitation with Hydroxyethyl starch following to protocol was adequate, therefore preload was comparable between the two experimental groups during the experiment. Accordingly to protocol, MAP in the present study was maintained by vasopressor therapy. Noradrenaline was used to maintain blood pressure equally with baseline pressure. Noradrenaline effects on α-adrenergic agonist increasing the MAP due to an increase in peripheral vascular resistance, and noradrenaline also effects on β-adrenergic increasing splanchnic blood flow (3, 18). This result was matched

with results of our previous studies (2, 9, 51, 106, 125) and investigators in other research center (1, 42, 70). They studied in endotoxemia induced hyperdynamic shock model in porcine and murine, and in clinical shock patients respectively, and found that SVR was significantly decreased within group versus with baseline when shock induced.

4.2.2 Regional circulation

Multiple organ dysfunction syndrome (MODS) still remain the most frequent cause of death in intensive care (3, 68). The organs of the hepato-splanchnic system are believed to be the significant contributors to the development of this syndrome during septic shock (3, 27, 68). The inflammatory cells and mediators induced by sepsis effect badly in the microcirculation causing vascular dysregulation, loss of barrier function (capillary leak), impaired endothelial cell function, and increasing disturbances of blood such as increased clotting, red blood cell rigidity, and leukocyte activation (3, 51). The most important hepato-splanchnis organs related to the development of septic shock include liver, gut, and kidneys.

The liver has three fundamental roles including vascular function, metabolic achievements, and secretory and excretory function. In this study, we focus on vascular function and metabolic achievement (mention later). The supplied blood of liver is unique and special that includes blood from the common hepatic artery (Qha) and blood from the portal vein (Qpv). The portal vein supplies 75-80% and the hepatic artery supplies 20-% of liver blood flow. It implies that liver blood flow (Qliv) is the sum of Qha and Qpv (3). In the present study, Laser Doppler Flowmetry (LDF) was used to continuously measure microcirculatory blood flow. We found that although the Qha was not affected in either group, but Qpv and Qliv index (the quotient of liver flow and body weigh (kg) to account for the changes of liver flow that occur in patients with different size) were significantly increased in the peritonitis animals in all measure points versus with Qpv and Qliv index in baseline. This findings were consistent with our previous studies on hyperdynamic porcine endotoxemia (6, 40, 51), and study in other research center on porcine fecal peritonitis (59). The hepato-splanchnic blood flow account for 30% of the cardiac output (3), therefore elevated CO may be leading to increased hepato-splanchnic blood flow. In our study, although CO increased in peritonitis group but the ratios between Qha, Qpv, and Qliv with CO (Qha/CO; Qpv/CO; Qliv/CO respectively)

were not different. Our finding was matched with finding of Santak et al. in 1998 (106) studied in endotoxin-induced shock in pigs found that although CO increased by 50-80% in endotoxin group, Qliv was only augmented 30-40%.

The gut is perfused by the coeliac branch and mesenteric arteries, and is drained via the portal system. The gut takes the important part of spreading sepsis and developing septic shock (41). Decreased perfusion and impaired oxygenation of the intestinal mucosa can lead to disruption of gastrointestinal mucosal barrier creating favorable condition for penetrating of bacteria and bacterial production into blood circulation. Subsequently, which promote inflammatory response, increase oxygen demand, and supply inadequate oxygen (3, 41, 104). In the present study, the Laser Doppler microvascular perfusion (OXY LAB ^{LDF}) monitoring red blood cell (erythrocyte) perfusion in the microcirculation of a tissue was used to monitor perfusion of the gut, expressing in blood perfusion unit (BPU). We found that BPU of the gut did not change during the experiment in both groups. In addition to evaluating microvascular perfusion, the tonometer was used for measuring ileal mucosal PCO_2, simultaneously an arterial blood sample was obtained for arterial PCO_2 measurement, and the ileal mucosal-arterial PCO_2 gap was calculated (12, 19, 44). In our study, we did not observe any alteration of PCO_2 gap in both groups. Our finding was consistent with the results from other research groups (6, 10). These investigators studied hyperdynamic porcine endotoxemia and found not any differences of PCO_2 gap. On the contrary, Asfar et al, in 2005 (40), and Hauser et al, in 2005 (9) found that PCO_2 gap was increased during porcine endotoxemia. The reason for the difference between our present finding and results of other investigators (9, 40) may be that, the PCO_2 gap mirrors both variations in microvascular flow and in carbon dioxide metabolism, thus there is no apparent correlation between PCO_2 gap and global or regional haemodynamic measurements in sepsis patients (3, 44).

4.3. Gas exchange

The most important role of gas exchange is extracting oxygen from alveoli and then delivering to the cells, and carrying away metabolic product, carbon dioxide from tissue to the lung and then diffusing into alveoli. Sepsis and septic shock cause hypotension, maldistribution of blood perfusion, and the inability using oxygen of cells. Therefore, monitoring and evaluating specific indicators of delivering and consuming oxygen, and

extracting oxygen is warranted in critically ill patients because continued tissue hypoxia may occur despite macrohemodynamic stability (18). Total pulmonary blood flow (Qt) includes capillary and noncapillary pulmonary blood flow. In capillary, blood in the pulmonary blood flow is oxygenated, and in noncapillary, blood in the pulmonary blood flow (Qs (shunt)) is considered the wasted blood because it is not oxygenated. Ratio Qs/Qt expresses the amount of wasted blood and total pulmonary blood flow, and when disorders of the lung occur, this ratio is increased (18). In present study, this ratio was significantly increased in septic animals among measure points compared to baseline. Our finding was consistent with the study on the endotoxemic pigs (125). Investigators found that Qs/Qt ratio was significantly increased during sepsis in the septic pig model.

In addition, several global indicators of tissue oxygenation are measured or calculated to provide insight into overall oxygenation status. Oxygen delivery (DO_2) is the amount of oxygen delivered to the tissue each minute. Evaluating the factors affected DO_2 (SaO_2, Hb, and CO) provides important information for guiding therapy for tissue oxygenation disorders (18). Haemoglobin (Hb) is not only important in the carriage of oxygen (O_2) to the tissues but also in the transport of carbon dioxide (CO_2) to the lung and in buffering hydrogen ions. Its buffering effect is strongest in the tissues. In the present study, although adequately resuscitated, which is ensured by stable intrathorax blood volume index (ITBV index), Hb in the pigs of peritonitis group was significantly increased whereas in control group Hb was not different. Our finding was contrast with other investigators (9, 40) they found that Hb was reduced in the pigs induced endotoxemia. The reasons for this difference may depend on volume resuscitation and model induced sepsis, the fecal peritonitis induced septic shock and hyperdynamic porcine endotoxemia. Contrary to increasing of Hb, in our study oxygen saturation (SaO_2), an indicator of the percentage of hemoglobin saturated with oxygen was decreased, 87-93.3% versus normal range of Sao_2 95-100%. Finally, in the present study, the increase of Hb and CO (mentioned above) rather than the decrease of SaO_2 causing DO_2 index of liver and gut in sepsis animals significantly increased. Our finding was consistent with many other investigators (9, 40, 59, 111) they found that DO_2 was increased, whereas many other studies (6, 76, 77, 113, 116) found that DO_2 was not different. The difference between our result and other investigator finding (6, 78, 79, 115, 118) may be explained that

almost all these investigators mentioned DO_2 of liver, only Matejovic et al (79), in 2004 extra measured mesenteric oxygen delivery, and they studied in different sepsis model, hyperdynamic porcine endotoxemia.

In addition to DO_2, oxygen consumption (VO_2) is important indicator representing oxidative activity. In the present study, we observed not any changes of VO_2 of liver (VO_2 liv). This result was consistent with other studies (6, 8, 9, 14, 125) these investigators studied on hyperdynamic porcine endotoxemia and hyperdynamic porcine fecal peritonitis and found that VO_2 liv remained unchanged.

Furthermore, oxygen extraction ratio (OER) is more sensitive indicator for evaluating the adequacy of the balance between DO_2 and VO_2. Normal range of OER is approximately 0. (i.e. % of delivery oxygen is consumed). In normal condition, when DO_2 varies causing OER changes proportionally, which results in the stable VO_2, thus in this condition the VO_2 is independent of DO_2. However, when DO_2 is below a critical level, although OER increases but not enough to maintain adequate tissues oxygen consumption and consequently anaerobic metabolism occur. In this situation, the VO_2 becomes dependent on the amount of DO_2 (18). In our study, we found that gut oxygen extraction ratio (GOE ratio) decreased in animals in peritonitis group, whereas in control group we did not observe any difference of GOE ratio. Our result was different to other investigations (76, 116), they studied hyperdynamic porcine endotoxemia and found that GOE ratio was not changed after the pigs subjected to sepsis. However, our finding was consistent with many other investigators (6, 8, 9, 10, 14, 87, 125), they studied on hyperdynamic porcine endotoxemia and hyperdynamic porcine fecal peritonitis, and observed that GOE ratio was significantly decreased.

4.4. Metabolism and organ function

In the human body, bicarbonate is the most important buffer system because carbon dioxide (CO_2) is removed by the lung and bicarbonate regenerated by the kidney. It is very important parameter, together with pH blood to detect acid-base balance disorder. In the blood, pH range is very narrow 7.35 to 7.45, or $[H^+]$ = 35-45 nmol/l, indicating acidosis when pH lower than 7.35 and alkalosis when pH higher than 7.45. In the present study, we found that pH blood was significantly decreased in peritonitis group after 12, 18, 24 hours versus with baseline and after 24 hour pH was lower than minimum value of

normal range of pH blood (7.35) in all blood samples of artery (A), hepatic vein (hv), and portal vein (pv). The pH blood in fourth measure of A, hv, pv was 7.29, 7.21, 7.27 respectively, whereas pH was not different in all blood samples in control group. In addition, bicarbonate was decreased in the pigs in both groups. Our findings were consistent with many investigators studied in pigs, dogs, sheep, and mice designed sepsis model (9, 10, 14, 40) found that pH decreased in all blood samples of A, hv, and pv and bicarbonate decreased in arterial blood sample of sepsis animals. The decrease of pH blood parallel with the changes of bicarbonate blood samples of A, hv, and pv demonstrated that this metabolic acidosis state occurred not only in metabolic system but also in the splanchnic organs. This metabolic acidosis disorder may result from an excess of acid and/or reduced buffering capacity (120). Reduce of buffering capacity represented a low concentration of bicarbonate may result from losing of large amount of bicarbonate by the small bowel and the kidneys. Excess acid may occur due to increased production of organ acids, lactic or pyruvic acid. In the glucolysis process, pyruvate is formed and then it may enter four different pathways: conversion to lactate, transamination to alanine, oxidation (Krebs cycle), and gluconeogenesis. Although hyperlactatemia and lactate and pyruvate ratio (lac/pyr ratio) as useful parameters attributed to hypoxia phenomenon is still controversial, but the increase of lactate and lac/pyr ratio is consequence of anaerobic cellular metabolism was admitted (120). In our study, arterial lactate and lactate/pyruvate ratio of A, hv, and pv were significantly increased in sepsis pigs. Our results were consistent with other investigators (9, 40, 121) they studied porcine hyperdynamic endotoxemia model and found that lactate and lac/pyr ratio increased after pigs were infected.

Multiple organ dysfunction (MOD) still remains the most frequent cause of death in intensive care, especially in septic shock, and deterioration of splanchnic organs is considered a main cause in pathogenesis of MOD. Therefore monitoring and evaluating regional organ function is often crucial for guiding therapy in critically ill patients (3, 58, 94, 105, 106). The indocyanine green plasma disappearance rate (PDRig) is alternative measure for evaluating liver function. After injection into the circulation, indocyanine green (ICG) is nearly completely eliminated by the liver into the bile, and elimination of IGC depends on hepatic blood flow, hepatic cellular uptake and hepatic excretion (105).

In the present study, we did not observe any difference of PDRig in animal subjected and not subjected with sepsis. This result was corresponding with the finding of Vassilev et al, in 2004 (125), and Hauser et al, in 2005 (40). They found no difference of PDRig in the pigs in porcine endotoxemia model. Whereas Sakka et al (105), from 1996 to 2000, studied PDRig in critically ill patients and found that PDRig was significantly lower in nonsurvivors than in survivors. The difference between our finding and Sakka et al may be explained by the difference of study models, septic animals and critically ill patients. In addition to evaluating hepatic function, bilirubin, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are the useful parameters. Bilirubin is a brownish yellow substance found in bile, and produced when the liver breaks down old blood cells. The change of bilirubin is related to many factors, such as rapid destruction of red blood cells, poisoned some medicines (e.g indomethacin, phenytoin, diazepam…), injured gallbladder or cholecystitis, and especially liver cells damaged. AST and ALT are the enzymes in the cells. AST is normally found in red blood cells, heart, liver, muscle tissue, pancreas, and kidney, whereas ALT is found mainly in the liver, thus ALT has more unique quality than AST when liver cells damaged (40). In our study, AST per gram protein increased in the pigs of both groups. ALT per gram protein and bilirubin per gram protein increased when pigs subjected to sepsis, whereas in control group we did not observe any difference. Our results of ALT and bilirubin were consistent with the finding of other investigators (9, 40, 125), they studied hyperdynamic porcine endotoxemia model and found that ALT and bilirubin increased when pigs subjected to sepsis. Furthermore, these investigators observed that AST increased in the septic pigs, but they did not study in healthy pigs (not subjected with sepsis), so that there was lack of information to compare with our finding of increased AST in the uninfected animals.

Besides liver and gut, kidney is also the splanchnic organ playing important role in pathogenesis and complication of sepsis. The more serious sepsis is the more acute renal failure occur, the rate 19%, 23%, and 51% acute renal failure occurred correlate with moderate sepsis, severe sepsis, and septic shock respectively (108), and acute renal failure combined with sepsis associates 70 percent mortality (67, 108). The kidneys are the natural filtration system of the body. They remove from bloodstream all waste products like urea and toxins, along with excess fluids in the form of urine. Creatinine is

a chemical waste molecule produced from creatine, the major important molecule for energy production in muscles. Creatinine is transported through bloodstream to the kidneys, filtered out and disposed in the urine. Although it is a waste, creatinine has been found to be a fairly reliable indicator of kidney function. When kidneys are impaired the creatinine will rise and urine output usually decreases (62, 67). In the present study, we found that creatinine per gram protein increased after pigs subjected to sepsis, and did not observe any differences of creatinine in the pigs of control group. Whereas, instead of decreasing, urine amount per kilogram body weigh significantly increased in the septic animals, and in control group this amount was not different. Our finding of creatinine per gram protein was consistent with other investigators (9, 40), but our finding of urine amount per kilogram body weigh was contrast with them. These investigators studied in porcine hyperdynamic endotoxemia found that creatinine per gram protein was increased, and urine amount per kilogram body weigh was decreased when pigs subjected to sepsis. The difference of the increased urine amount per kilogram body weight in our experiment may be explained by the use of vasopressor drug, norepinephrine in the present study. Norepinephrine effects on α-adrenergic agonist, which increases mean arterial pressure due to an increase in peripheral vascular resistance, and also effects on β-adrenergic increasing splanchnic blood flow resulting raising urine amount (3, 11, 18, 58).

Sepsis syndrome is one of the most frequent causes of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), leading to increased lung permeability, enhanced polymorphonuclear neutrophil sequestration and respiratory failure (5, 85). Although several progresses have been made in supportive care, the mortality arising from ARDS is still high, ranging from 24 to 60 percent (77, 103). Oxygen partial pressure (PaO_2) by itself is insufficient information to define disorders of respiratory system, but when along with the fraction of inspired oxygen (FiO_2) to calculate PaO_2/FiO_2 ratio (Horowitz quotient), become most important criterion detecting disturbances in the lung, especially ALI and ARDS. Horowitz range normally is over 400, from 200-300 refer to ALI, and < 200 refer to ARDS (5, 17). In our experiment, Horowitz quotient was significantly reduced in the pigs subjected to infection, whereas in control group Horowitz quotient was normal. Low Horowitz quotient (205 in Mea4) expressed that ALI developed in the late phase of our experiment. Our finding was consistent with other investigators (10, 14,

40) who found that Horowitz indicator decreased in septic animals of the endotoxemia models. In addition to the evaluation of lung injury, extravascular lung water (EVLW) is an indicator of pulmonary dysfunction. The hallmark of sepsis is a raise of capillary permeability, manifesting in the lung with the altered alveolar-capillary barrier function and characterized by an increase of EVLW (21). In the present study, we found that EVLW was increased after 18 hours in the septic animals, whereas we observed not any differences of EVLW in the pigs of control group. Our finding was different with Vassilev et al, in 2004 (125). They observed not any differences of EVLW when the pigs subjected with sepsis. The difference between our result and Vassilev finding may be resulted from the different sepsis model. Our sepsis model was feacal peritonitis induced septic shock and their model was long term porcine endotoxemia.

5. Summary

The aim of the study was to investigate a new model of fecal peritonitis induced septic shock in pig. 15 ventilated, anesthetized pigs, average body weight 48.6 kg (42 – 58.5 kg) were randomly assigned to two groups. In the peritonitis group (n = 10), the pigs were instrumented then a standardized feces solution were inoculated in abdominal cavity to induce peritonitis. During the observation period of 24 hours, the pigs were resuscitated with hydroxyethylstarch (15 ml/kg/h), and noradrenaline as needed to maintain blood pressure equal to baseline. 5 pigs served as control pigs (control group). These pigs were not subjected to sepsis and were also observed for 24 hours.

When compared to control group, we found the following results in the pigs induced sepsis and septic shock:

Cardiac output (CO) and cardiac output index were significantly increased. In addition, systemic vascular resistant (SVR) was significantly decreased. In regional circulation: hepatic arterial blood flow (Qha) was not affected by sepsis, but portal vein blood flow (Qpv) and liver blood flow index (Qliv index) were significantly increased. In the gut, microvascular circulation evaluated by Laser Doppler Flowmetry (LDF) and the arterial-ileal mucosal PCO_2 gap was not changed.

The ratio of the total pulmonary blood flow and the pulmonary blood flow shunt (Qs/Qt) was increased. Oxygen delivery index (DO_2 index) of liver and gut was significantly increased, whereas oxygen consumption of liver (VO_2 liv) unchanged. Furthermore gut oxygen extraction ratio (GOE ratio) was significantly decreased

Metabolic acidosis occurred at systemic level and in splanchnic organs (e.g. gut and liver) manifested by the decreased pH and bicarbonate in blood samples of artery, hepatic vein, and portal vein. Cellular metabolism activated in anaerobic status manifested by the increase of lactate and lac/pyr ratio of artery, hepatic vein, and portal vein.

An injured liver was indicated by the increases of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin, but liver excretory function was unevident, because although bilirubin was increased but indocyanine green plasma disappearance rate (PDRig) remained unchanged. Lung function was impaired as shown by the reduced ratio of oxygen partial pressure and fraction of inspired oxygen

(PaO_2/FiO_2) (Horowitz quotient) and by the increase of extravascular lung water (EVLW). The present data of the increased urine amount per kilogram body weigh per hour along with the increased hemoglobin concentration implicated that noradrenaline caused the kidney blood flow increased.

In conclusion, our model of fecal peritonitis induced sepsis/septic shock was closely mimic with the clinical condition. Therefore, it may be a useful research approach for the clinical studies of possible therapies.

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ACKNOWLEGEMENT

I would like to sincerely thank Prof.Dr.med.Dr.h.c. Peter Radermacher for the design and excellent supervision of this project.

Special thank Prof. Dr. med. Enrico Calzia for his scientific guidance, and his critical reviews of the manuscripts.

I am indebted to Tanja Schulz, Andrea Söll, and Wolfgang Siegler for skilful assistance.

I am thankful to Mrs. Christa Klein for her engaged help in relation to my administrative issues.

I am also grateful to the Vietnam Ministry of Education and Training as well as the German Academic Exchange Service for the scholarship offered to me to study in the University of Ulm, Germany.

In addition, I thank my friends for their friendly help and their encouragement.

Finally, I want to thank my parent and especially thanks to my wife Tran Thi Minh Thu and my children Nguyen Duy Quang and Nguyen Thu Trang for their support and optimism.

Curriculum vitae

Full name: **Nguyen Duy Cuong**

Nationality: Vietnamese

Sex: Male

Date of birth: February 06, 1966

Place of birth: Thai Binh, Vietnam

Marital status: Married

Office: Department of Internal Medicine of Thai Binh Medical University

Office address: 373 LyBon Street Thai Binh City.

Tel: (036) 838.545 - Fax: (036) 847.509

Email: ndcuong66@yahoo.com

Academic degree: General doctor, September, 1990

Master of Science in Medicine, December 1996

Education

Name of University: Thai Binh Medical University

Place: Thai Binh, Vietnam

Subject: Medicine (full time 6 years)

Degree: General doctor

Period: Sep.1984 to Sep.1990

Name of University: Hanoi Medical University

Place: Hanoi, Vietnam

Subject: Intensive Care Medicine (full time 3 years)

Degree: Master of Intensive Care Medicine

Period: Dec.1993 to Dec.1996

Previous employment

Oct.2004 until now: Studying at Department of Anästhesiologische Pathophysiologie und Verfahrensentwicklung, Univesitätklinikum Ulm, Germany.

Jan.1997 – Sep.2004: Lecturer of Internal Medicine Department of Thai Binh Medical University

Dec.1993 – Dec.1996: Studied at Hanoi Medical University, Hanoi Vietnam, awarded the good degree of Master of Intensive Care Medicine

Oct.1990 - Nov.1993: Lecturer of Internal Medicine Department of Thai Binh Medical University

Sep.1984 – Sep.1990: Studied at Thai Binh Medical University, Thai Binh Vietnam, awarded the good degree of general doctor.