

**SHORT THESIS FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY (Ph.D.)**

**Cerebral blood flow examination in  
patients with sepsis associated encephalopathy**

**by Szilárd Szatmári M.D.**

Supervisor: Prof. Béla Fülesdi D.Sc.



**UNIVERSITY OF DEBRECEN  
DOCTORAL SCHOOL OF NEUROSCIENCES**

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# **Cerebral blood flow examination in patients with sepsis associated encephalopathy**

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The Examination takes place at Library of the Department of  
Anesthesiology and Intensive Care, University of Debrecen,  
11.00 a.m. 3 Dec, 2012

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The Ph.D. Defense takes place at the Lecture Hall of the  
1st Department of Medicine, Institute for Internal Medicine,  
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## 1. INTRODUCTION

Systemic sepsis and its severe complications (e.g. septic shock, multiorgan failure) are the main mortality causes in the intensive care unit. According to statistical data, the risk of sepsis related mortality is between 10 and 50%. Previous studies confirmed that mortality rates are significantly higher when sepsis is accompanied by disturbance of consciousness.

Sepsis-related encephalopathy is the most common type of encephalopathy in the clinical intensive care units, and it is presumably underdiagnosed. According to present knowledge sepsis-related encephalopathy is a severe organ manifestation of the systemic sepsis syndrome, not a primer disease, therefore it should be considered as an early organ complication of the syndrome. The precise definition and nomenclature of the sepsis-related encephalopathy is not yet clarified. Different authors refer to the same condition by using various expressions: septic encephalopathy, sepsis-induced encephalopathy, sepsis-induced brain dysfunction, sepsis-associated delirium or sepsis-associated metabolic encephalopathy. All these medical terms cover sepsis-related consciousness disturbance of different severity, as a leading symptom.

Based on the previously described statements, sepsis-associated encephalopathy is usually defined as a diffuse cerebral dysfunction induced by the systemic response to the infection without evidence of direct infectious involvement of the central nervous system. The term “sepsis-associated encephalopathy” (SAE) has become the most widely accepted expression in the international medical literature since the study of Wilson and Young was published in this topic. The expression “septic encephalopathy” is misleading, because most of the authors include direct infections of the central nervous system to this topic. The not yet completely revealed and still continuously investigated pathomechanism of this

encephalopathy form could be the reason for the not unified nomenclature.

It is hard to determine the prevalence of SAE by using data from the medical literature, because its definition is not clear yet. Depending on the executed study, the prevalence of SAE in patients with severe sepsis has a wide range from 9% to 71%.

Clinical symptoms are not typical, any grade of disturbance of consciousness is possible from confusion, agitation, somnolence up to stupor and coma. The presence of postoperative alteration of consciousness or its development in elderly patients make it complicated to get at the diagnosis of SAE, because it is often similar to the postoperative cognitive dysfunction, which is a frequent postoperative condition.

Therefore health-care staff often doesn't consider mild alteration of consciousness or alertness as a sign of sepsis syndrome. Vigilance fluctuations are common in moderate disturbance of consciousness, which makes SAE identification even more complicated. Moreover, sedation of a patient with severe sepsis is often indispensable, which limits not only the possibility to recognize consciousness disturbance, but also makes objective evaluation challenging.

Severe disturbance of consciousness cases are rather associated with delirium or coma. It has to be emphasized how important the sepsis-associated encephalopathy is, since it serves as an early warning sign for the upcoming septic state. Unfortunately clinical experiences show that septic patients frequently have multiorgan failure by the time they are admitted to an intensive care unit, thus they are already in a critical condition, and have worse life expectancies.

Therefore, it cannot be overemphasized that the identification of the symptoms of the early phase of sepsis is crucial for every clinical department.

The exact pathophysiological background of the sepsis-associated encephalopathy is still under investigation. It

is known, that this type of encephalopathy is most likely a multifactorially determined syndrome, where complicated and not fully clarified vascular, inflammatory, toxic and coagulation processes are also involved. In our study we aimed to investigate the vascular component of this process.

## **2. OBJECTIVES**

Taking into consideration, that the development of sepsis-associated encephalopathy presumably involves the alteration of the cerebral microvascular function, in our present study we aimed to find an answer for the following question:

Is cerebral vasomotor-reactivity impaired in the early and later course of sepsis?

## **3. PATIENTS AND METHODS**

This study can be divided for two parts according to topic and implementation. In the first part of this study patients with sepsis-associated encephalopathy were observed in the early phase of the septic process without signs of any other organ dysfunction. In the second part of the study patients with sepsis-related encephalopathy were also observed, however this time in the later course of sepsis with signs of multiorgan dysfunctions. The study was conducted between 2009-06-01 and 2011-06-01.

### ***3.1 Patient enrollment***

This prospective study was conducted in the 18-bed perioperative intensive care unit of the Department of Surgery at the University of Debrecen Medical and Health Science Centre. The study was approved by the local Medical Ethics Committee of the Debrecen University Medical and Health

Science Centre. The nearest relatives of the patients were informed about the nature of the study in detail, and they were asked for giving written, informed consent. In the early phase of sepsis 14, in the later course of sepsis 16 patients were enrolled in the study. All the patients fulfilled the criteria of clinical sepsis according to the guideline of the ACCP/SCCM Consensus Conference Committee.

In the first part of the study (patients in the early phase of septic process) fourteen patients (5 female, 9 male) with sepsis-associated encephalopathy were enrolled, ages were between 60-86 years, means of ages were  $72 \pm 9,7$  years. In 8 cases the source of sepsis in patients with SAE was peritonitis, in 5 cases it was pneumonia and in 1 case it was femoral abscess.

In the second part of the study (patients in the later course of septic process) sixteen severe septic patients (7 female, 9 male) were enrolled, ages were between 37-88 years, means of ages were  $70 \pm 14,1$  years. In 11 cases the source of sepsis in patients with severe sepsis was peritonitis, in 2 cases it was gangrene of lower limb, in 1 case it was mediastinitis caused by traumatic injury, in 1 case it was thoracic emphysema caused by esophageal perforation and in 1 case it was retroperitoneal abscess.

The enrollment and the transcranial Doppler tests of patients with sepsis-related encephalopathy in an early phase of sepsis were performed within 24 hours after diagnosing sepsis. Sepsis-related encephalopathy was defined as a combination of the following: patients had to meet the criteria of clinical sepsis and had to show disturbance of consciousness or alertness of any severity. Any other metabolic causes of consciousness disturbance were excluded (hypoxemia, hyper- or hypoglycemia, increased serum urea, creatinin or ammonia levels). Those with hemodynamic

unstability, in need of hemodynamic support or with signs of hypoperfusion of different organs were excluded. Patients were not under mechanical ventilation prior to or during the study.

The inclusion of severe septic patients with SAE and transcranial Doppler tests were performed within 24 hours after diagnosing severe sepsis. Severe sepsis was defined according to recent internationally accepted guidelines if hypoperfusion or dysfunction of at least 1 organ system was present in the patients. Only those with at least 2 organ manifestations related to the septic process beside the brain affection entered the present study. Of the 16 patients, 14 were under mechanical ventilation, all in assisted mode (continuous positive airway pressure or BiPAP mode (bi-level positive airway pressure) along with 5 to 10 cm H<sub>2</sub>O positive end-expiratory pressure as was necessary for proper oxygenation). The BiPAP frequencies were kept at 4 to 6 per minute in all cases to preserve patient's own respiratory effort. Controlled ventilatory mode was not administered in any of the patients. Sedative drugs were not administered during the transcranial Doppler tests or were stopped before. Neurologic assessments were performed as a part of the routine measures of the weaning protocol for mechanically ventilated patients at our department. Neurologic examination as well as acetazolamide tests were performed during this period.

A certified neurologist performed a detailed neurologic assessment of all the patients to exclude direct infectious involvement of the central nervous system (such as meningitis or encephalitis). Sedative drugs were not administered before the neurologic assessment. Consciousness disturbance was graded numerically according to the Richmond Agitation and Sedation Score (RASS) and the Ramsay scale.

### **3.2 Control group**

Transcranial Doppler measurements were performed (in the first part of the study on 20, in the second part of the study on 16) age- and sex-matched persons who were free of sepsis, diabetes mellitus, hypertension, and significant stenoses of the cerebral arteries, which could have influenced vasoreactivity tests. These subjects served as controls for the study.

### **3.3 Transcranial Doppler acetazolamid test**

#### **a) Acetazolamide**

Acetazolamide (Diamox, Lederle Pharmaceuticals, Carolina, Puerto Rico, USA) is the reversible inhibitor of carbonic anhydrase, which is located at the surface of the erythrocytes. The enzyme catalyses the following reaction:



It also induces a slight temporary hypercapnia lasting for approximately 20 minutes, which results in vasodilation of the cerebral arterioles, most probably through inducing nitric oxide synthesis.

#### **b) Transcranial Doppler ultrasonography measurements**

Transcranial Doppler measurements were performed in supine position using a Rimed Digilite Transcranial Doppler sonograph (Rimed Ltd, Ra'anana, Israel). A 2-MHz probe was used for insonation. Sample volume, gain, and power were kept constant during the investigation. Temporal window was used for insonation; probes were fixed by LMY-2 probe holder (Rimed Ltd, Ra'anana, Israel). The device enabled the assessment of the best available signal of the middle cerebral



artery (MCA) between the depths of 45 to 55 mm. Systolic, diastolic, and mean blood flow velocities were registered, and pulsatility indices were calculated by the device. After blood flow measurements were performed at resting state, 15 mg/kg BW acetazolamide was injected intravenously. Blood flow velocities were registered continuously until 20 minutes after injection of the vasodilatory drug.

Cerebrovascular reactivity (CVR) was defined as the percentage increase of the MCA mean blood flow velocity after administration of acetazolamide, calculated as follows:

$$CVR = (MCAV_{ACZ} - MCAV_{REST}) / MCAV_{REST}$$

where  $MCAV_{ACZ}$  is the MCA mean blood flow velocity measured at 5, 10, 15, and 20 minutes after acetazolamide, and  $MCAV_{REST}$  is the MCA mean blood flow velocity at rest.

In addition, the maximal percentage increase of the vasodilatory response (cerebrovascular reserve capacity – CRC), was also calculated using the following formula:

$$CRC = (MCAV_{ACZmax} - MCAV_{rest}) / MCAV_{rest}$$

where  $MCAV_{ACZmax}$  is the highest MCA mean blood flow velocity measured within 20 minutes after administration of acetazolamide.

Transcranial Doppler measurements can be performed at the level of the middle cerebral artery and cerebral arterioles cannot be directly assessed. When an arteriolar vasodilation occurs, the cerebrovascular resistance (CVR) of the corresponding arterial territory decreases, resulting in an increase of the cerebral blood flow velocity (CBFV) measured in the middle cerebral artery. Thus, cerebral arteriolar function cannot be directly measured. Only changes of the cerebrovascular resistance induced by acetazolamide can be indirectly assessed by measuring cerebral blood flow velocities in the middle-sized arteries of the corresponding territory.

### ***3.4 Blood gas analysis***

Blood gas analysis was performed on enrolled septic patients during the cerebral vasomotor test in order to follow the temporary hypercapnia induced by acetazolamide.

On these patients arterial catheter was used for arterial sampling for blood gas analysis. During the transcranial Doppler ultrasonography measurements arterial sampling for blood gas analysis was performed before administering the vasodilatory drug and at the 5th, 10th, 15th and 20th minutes after acetazolamide administration. These subjects were tested by point-of-care blood gas analyzer device (GEM Premier 3000, Instrumentation Laboratory, Bedford, Massachusetts, USA) and results were documented on worksheets.

In control persons, arterial sampling for blood gas analysis was only performed at resting state because we considered inserting a radial artery catheter or serial arterial blood gas analysis sampling unethical.

### ***3.5 Statistical analysis***

Means and SDs are shown for all values. Before analyzing the data, normality tests were performed. Repeated measure analysis of variance was used to detect differences in MCAV and CVR values after acetazolamide administration. When significant differences were detected, pairwise comparisons were performed between the groups using the Mann-Whitney U test.  $P < 0,05$  was accepted as statistically significant.

## 4. RESULTS

### *4.1 Results of patients with SAE in early phase of sepsis*

Fourteen patients with sepsis-associated encephalopathy and twenty control persons were enrolled. The distribution of the Ramsay scales were in the septic groups as follows: Ramsay 1 = 6 cases, Ramsay 3 = 4 cases, Ramsay 4 = 4 cases. There were six cases with RASS +1 and a further eight cases with RASS -1. Thus, in all cases either a sepsis-related delirious state or somnolence was present. Blood pressures and blood gas analysis parameters were comparable in the two groups at rest. In septic patients, pH slightly decreased, while pCO<sub>2</sub> and partial pressure of oxygen slightly increased during the acetazolamide test.

Related to the results of the transcranial Doppler measurements it has to be noted that pulsatility indices were higher at the resting state in patients with sepsis-related encephalopathy and this difference remained unchanged after administration of acetazolamide. Absolute blood flow velocities after the vasodilator drug were higher among control subjects than in septic patients.

In a further analysis we checked the timecourse of the vasomotor reaction to acetazolamide (cerebrovascular reaction – CVR). Patients with sepsis-associated encephalopathy reacted more slowly to the vasodilatory stimulus than control persons.

When assessing the maximal vasodilatory ability of the cerebral arterioles to acetazolamide (cerebrovascular reserve capacity – CRC) during 20 minutes of vasomotor testing, we realized that patients with sepsis-associated encephalopathy reacted to the drug to a lesser extent than control subjects.

## ***4.2 Results of patients with SAE in later phase of sepsis***

Sixteen severe septic patients and sixteen control persons were enrolled. The distribution of RASS scores in the severe septic group is as follows: RASS -1 = 5 cases; RASS -2 = 7 cases; and RASS -3 = 4 cases. According to neurologic clinical assessment, there were 7 cases of somnolency and 9 cases of stupor. Before starting the acetazolamide test, systolic blood pressures were slightly higher, whereas diastolic blood pressures were lower among severe septic patients. Mean arterial pressures did not differ among the groups. There were no significant differences between pretest pH values of severe septic and control persons. In contrast to this, arterial PaCO<sub>2</sub> was lower and PaO<sub>2</sub> was higher among severe septic patients before administration of acetazolamide.

Results of transcranial Doppler tests: Generally speaking, systolic, diastolic, and mean blood flow velocities measured within the MCA did not differ at rest or after acetazolamide administration between severe septic and control persons. It is worth noting that pulsatility indices were higher among severe septic patients already before acetazolamide stimulation, and this difference continued to exist throughout the 20 minutes of the vasodilatory test. It is also clear from the results that administration of acetazolamide led to an increase in cerebral blood flow (CBF) velocities and a decrease in pulsatility indices both in severe septic patients and in healthy controls, indicating vasodilation of the arterioles and a consequent increase in CBF.

Related to cerebrovascular reactivity values (percentual increase of MCA mean blood flow velocity after acetazolamide at different time points – CVR) measured in septic and control persons the percentual change of the MCAV after acetazolamide was similar in the 2 groups in the first 15 minutes of acetazolamide test, whereas it declined earlier in healthy subjects.

Cerebrovascular reserve capacity (the maximal percentage increase of the MCA mean blood flow velocity after acetazolamide – CRC) was similar in severe septic and healthy persons, indicating that the maximal vasodilatory capacity of the 2 groups is comparable

## **5. DISCUSSION**

It has been shown that, at the beginning of the septic process, a heterogenous disturbance of microcirculation occurs at the level of vessels less than 150  $\mu\text{m}$  in diameter. There are 2 main processes that may be responsible for this heterogenous peripheral circulation:

(a) capillary occlusion due to stiff leukocytes, red blood cells, fibrin clots, and endothelial cell swallowing and

(b) capillary leakage.

The combination of these procedures impairs perfusion of brain. In this phase of the septic process, nitric oxide (NO) synthase (NOS) enhances the production of NO, which penetrates into tissue cells and inhibits oxygen use resulting in augmentation of  $\text{O}_2$  diffusion distance. Thus, in this early perfusion-mediated phase of the septic process, a local endothelial overproduction of NO occurs. It has to be noted that according to previous observations, cerebral vasodilatory stimuli (such as hypotension, increased  $\text{PaCO}_2$ ) exert their action on the endothelial NOS resulting in production NO and, thus, vasodilation of the cerebral arterioles. A similar effect of acetazolamide on NOS has been recently demonstrated by Aamand and co-workers. It is conceivable that if a vasodilation due to the local endothelial overproduction of NO precedes cerebral vasoreactivity tests in the early phase of sepsis, external vasodilatory stimuli may not induce increase cerebral blood flow in the magnitude that is observed in normal brain circulation.

In the later course of sepsis, however, the dominant process is the inhibition of the mitochondrial respiration within the cells, resulting in hibernation-like state, enhanced apoptotic process, and necrosis. According to the concept of flow-metabolism coupling, the blood flow to the brain is adjusted to the metabolic needs of the brain parenchyma. Thus, if the tissue metabolism of the brain is reduced through the decreased mitochondrial respiration, cerebral perfusion also decreases. In such cases, however, reactivity of the cerebral arterioles (ie. the percentage increase of the cerebral blood flow to vasodilatory stimulus) may be normal.

Previous studies on cerebral vasoreactivity and pressure autoregulation in septic patients have yielded conflicting results. Matta and Stow found that cerebral vasoreactivity to CO<sub>2</sub> is not altered in sepsis, and similar results were also published by others. In another set of studies, it was shown that CO<sub>2</sub> reactivity is impaired in septic patients. Similar discrepancies were detected in cerebral autoregulation studies performed among septic patients; although Matta and Stow could not prove any autoregulatory disturbances in the brain circulation of septic patients, Smith and co-workers as well as Pfister and co-workers demonstrated disturbed autoregulatory responses in SAE. In septic shock, Taccone and co-workers reported on impaired cerebral autoregulatory response that seemed to be influenced by hypercapnia.

Although critically evaluating our results, we have to mention that systolic blood pressures were higher and that diastolic pressures were lower among septic patients, whereas mean arterial pressures were similar to those of control persons. The explanation for this difference is that in the septic group arterial pressures were normalized by norepinephrine in all severe septic patients. Despite this, diastolic blood pressures remained low indicating peripheral vasodilation of the capacitance-vessels. We believe that this did not influence our results because cerebral perfusion pressure is proportional

to mean arterial pressure, and the latter was comparable in septic and nonseptic patients. Another factor that might have influenced our results is lower arterial  $\text{PaCO}_2$  in septic patients compared with healthy persons. To explain this, we have to mention that 14 of the 16 patients were under mechanical ventilation. In the present study, however, we induced further increases in  $\text{PaCO}_2$  using the carbonic anhydrase inhibitor acetazolamide and assessed the percentage increase of cerebral blood flow velocities after vasodilatory stress. Thus, during these tests, baseline values were compared with stress test values, and differences were expressed as percent changes compared with resting values. We decided to use this method for analyzing our data because previous studies comparing transcranial Doppler and cerebral blood flow measurements proved that it is not the absolute blood flow velocities but rather the percent changes after acetazolamide tests that reflect cerebral blood flow alterations during vasoreactivity testing. As we mentioned before carbon dioxide increased and pulsatility indices decreased in the septic group after acetazolamide administration, indicating that the drug caused further increases in  $\text{PaCO}_2$  and a consequent vasodilation of the cerebral arterioles.

Based on results of our investigation we conclude, that:

1. In case of patients in the early phase of septic process without hemodynamic instability, or need of hemodynamic support the dilatational ability of cerebral resistance arterioles is impaired.
2. During the later progression of the septic process the vasoreactivity of cerebral arterioles get normalized.

3. Our study reinforces the hypothesis based on previous experimental results that in early phase of sepsis perfusion mediated changes take place whereas in more severe states energetic failure mediated changes take place and all of these are presumably valid for the brain.

Because of the timeliness of the topic and the controversies in medical literature we believe that it is crucial to perform further investigations of a bigger subject number to justify or disprove the shown concept. In any case these studies contribute to getting to know the pathomechanism of SAE better which is inevitable for the research of further possible therapies.



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List of publications related to the dissertation

1. Fülesdi, B., Szatmári, S., Antek, C., Fülepi, Z., Sárkány, P., Csiba, L., Molnár, C.: Cerebral vasoreactivity to acetazolamide is not impaired in patients with severe sepsis.  
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