AChE-activity in critically ill patients with suspected septic encephalopathy: a prospective, single-centre study

Benedikt Zujalovic (✉ b.zujalovic@gmx.de)  
Universitätsklinikum Ulm  https://orcid.org/0000-0002-7988-3947

Benjamin Mayer  
Universität Ulm

Sebastian Hafner  
Universitätsklinikum Ulm

Florian Balling  
Universitätsklinikum Ulm

Eberhard Barth  
Universitätsklinikum Ulm

Research article

Keywords: Septic associated encephalopathy, Cholinergic dysfunction, Acetylcholinesterase-activity, Delirium, Cognitive Dysfunction

DOI: https://doi.org/10.21203/rs.2.23128/v3

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

**Background** Up to 70% of septic patients develop a diffuse brain dysfunction accompanying with an increase in mortality, which is referred to as “septic associated encephalopathy”. Neuroinflammation as well as a disturbance of cholinergic transmission are assumed to be the causes of both delirium and septic associated encephalopathy. A possible change in cholinergic activity can be objectified by measuring the erythrocytic acetylcholinesterase activity. It has been shown, however, that the acetylcholinesterase activity, if only single measurements are carried out, is controversial in its significance. Therefore, we wanted to test the hypothesis whether a longitudinal analysis of acetylcholinesterase activity in critically ill patients can help to diagnose a suspected septic-associated encephalopathy and whether acetylcholinesterase activity differs in comparison to non-septic patients.

**Methods** In this prospective, observational, single-center study, 175 patients, admitted to the surgical Intensive Care Unit of the University hospital Ulm, Germany, were included.

45 patients were septic, 130 patients were non septic. All patients were examined daily for the presence of delirium using the CAM-ICU. Daily measurement of the acetylcholinesterase activity was performed in all patients. The acetylcholinesterase activity was analyzed over time using a linear regression model taking into account repeated measurements. By using a time adjusted model, the effect of further possible predictors of acetylcholinesterase activity was analyzed too. For nonparametric distributions quantitative data were compared using Wilcoxon matched-pairs test. For the analysis of the independent samples the Mann-Whitney test was performed.

**Results** In approximately 90% of the septic patients with suspected septic associated encephalopathy a statistically significant, time-dependent in- or decrease in acetylcholinesterase activity could be demonstrated over a period of at least 5 consecutive days.

**Conclusion** The longitudinal measurement of acetylcholinesterase activity over several consecutive days revealed a shift compared to baseline values exclusively in septic patients with supposed septic associated encephalopathy. Therefore, longitudinal measurement of acetylcholinesterase activity may help to diagnose septic associated encephalopathy in patients with sepsis and accompanying delirium symptoms.

**Trial registration** Retrospectively registered at German Clinical Trials Register, registration number DRKS 00020542, date of registration: January 27, 2020

**Background**

In accordance to the third international consensus definition for sepsis and shock (Sepsis-3) sepsis is defined as a syndrome of physiological, pathological and biochemical changes due to an infection (1,2). As a result of systemic inflammation up to 70% of septic patients develop a diffuse brain dysfunction called "septic associated encephalopathy (SAE)" (3,4). Patients with sepsis and associated cognitive
changes in the sense of delirious symptoms show increased mortality compared to septic patients without changes in cognition. In this context, the brain dysfunction appears to be an outcome relevant factor and therefore deserves special attention in the diagnosis and treatment of critically ill patients with suspected SAE (5,6). In addition, patients who have survived sepsis often complain of long-term cognitive impairment, which can manifest itself as memory and attention disorders. (7). From a clinical point of view, SAE is not a singular disease, but can be assigned to the generic term delirium. The delirium, in turn, can have different clinical manifestations. To differentiate SAE from other causes of delirium in critically ill intensive care patients, the underlying pathophysiological causes should be examined more closely. The occurrence of delirium symptoms in critically ill patients has multifactorial causes, including neuronal ageing, proinflammation, disorders of the blood-brain barrier, in particular changes in its permeability and neurotransmitter imbalance (8–11). Inflammation and disorders of cerebral metabolism appear to play an important role in the pathogenesis of both delirium and SAE. In detail, the release of pro-inflammatory mediators leads to an impairment of the integrity of the blood-brain barrier, to a microvascular endothelial dysfunction and an excessive activation of inflammatory cells, e.g. microglia cells of the central nerve system (CNS). In consequence, the impairment of the cerebral metabolism results in a step by step loss of neurons, especially of cholinergic neurons of the forebrain (6,15,16,17). Additionally, the depletion and the loss of cholinergic activity leads to attention deficits, cognitive impairment and memory disorders (13). The clinical manifestation is among other things the delirium of the critically ill intensive care patient. In addition to cognitive impairment, the disturbance of cholinergic activity has a direct influence on the immune response, which is mediated by the vagus nerve. As Tracey et al. described more than a decade ago the interaction between the CNS and the peripheral immune response, known as the cholinergic anti-inflammatory pathway, plays a pivotal role in the control of inflammation, and consequently in the pathophysiology of SAE (3,14–18). In experimental sepsis there is growing evidence that the use of indirect parasympathomimetics leads to an improved immune response by stimulating the cholinergic anti-inflammatory reflex (19–21). It is conceivable that a therapy of SAE using indirect CNS-permeating parasympathomimetic drugs could have beneficial effects to critically ill patients. In this context, AChE-activity plays a much more important role in patients with sepsis and accompanying cognitive impairment than has been assumed up to now (22–25). The binding of acetylcholine (Ach) to CNS-localized nicotinic acetylcholine receptors modulates the neuronal level of excitation as well as learning and memory competence (8). If disturbances occur within the cholinergic transmitter system these alterations can manifest themselves as typical delirium symptoms, as they also arise in the SAE (9,17). The determination of the erythrocytic acetylcholinesterase activity has been shown to be most suitable for this purpose. The erythrocytic AChE is characterized by its high affinity for the transmitter acetylcholine, its inhibition by high Ach-concentrations and its low affinity for non-cholinesters (26). The fact that the AChE-activity in cerebrospinal fluid is equivalent to the plasma AChE-activity suggests that the erythrocytic AChE-activity partially reflects the (central) cholinergic transmitter balance (27). The possibility of determining the AChE-activity within a few minutes by point-of-care diagnostic has proven to be useful since the suspicion of a disturbance in the central cholinergic transmission can be objectified. Alongside a potential benefit in diagnosis of a SAE the daily measurement of the AChE-activity can potentially provide an aid in monitoring a therapy with indirect,
central acting parasympathomimetics (28,29). Due to the increasing knowledge about the role of cholinergic transmission in sepsis, neuroinflammation and concomitant cognitive disorders, this study investigated whether AChE-activity is altered in septic patients with SAE/delirium, and its capability to reflect neuroinflammation and, as a secondary endpoint, to differentiate between supposed SAE and other causes of delirium in critically ill patients.

**Methods**

This prospective, observational, single-center study was conducted in the observation period from 03/2017 until 03/2018, after the positive ethics committee vote of the University of Ulm (Trial-Code No. 363/16). The data evaluation took place in the period from 05/2018 to 06/2019. The study was retrospectively registered at the German Clinical Trials Register (DRKS-ID: DRKS00020542). The study protocol conformed to the Declaration of Helsinki ethical guidelines. All patients or their legal designees signed written informed consent to take part in this study.

Inclusion criteria were as follows:

- Age $\geq$ 18 years
- need for intensive care treatment due to an emergency or elective surgery
- expected stay on the ICU for at least 24 hours
- $\geq$ 2 values of AChE-activity
- ability to understand and speak the German language.

Exclusion criteria were as follows:

- Age < 18 years
- $< 2$ consecutive measurement values of the AChE-activity
- missing informed consent

The following patient-related data were collected during the stay on the ICU:

- Age at enrollment
- gender
- ICU length of stay
- disease severity scores (SAPS II, TISS-28)
- primary reason for ICU admission
- several laboratory parameters (subsumed under the SOFA-Score, TISS-28, SAPS II)
- vital signs (heart rate, blood pressure, respiratory rate)

For characterization of the study population the relevant baseline data (demographic data, primary reason for ICU admission) were collected. The severity of illness was quantified using the Simplified
Acute Physiology Score (SAPS II), as well as the Therapeutic Intervention Scoring System (TISS-28). The TISS-28 records the daily condition of the patient by recording the therapeutic, diagnostic and nursing measures. Among other things, 23 different items such as the lungs, the cardiovascular system, frequency and extent of dressing changes, the kidney, the CNS, etc. are assigned to a certain point value. 5 of these items are additionally graded in their intensity (32). Ultimately, both scoring systems serve to make the severity of the illness of critically ill patients measurable and thus comparable within the framework of studies.

**Definition of sepsis**

Sepsis and septic shock respectively were diagnosed according to the third international consensus definitions for sepsis and septic shock (Sepsis-3) in 2016 (2). Patients were classified as septic if they met the criteria of the Sepsis-3 definition at admission or within 24 hours after admission to the intensive care unit (1,2). Beside the SOFA-Score inflammatory parameters (CRP, PCT, white blood cells) were collected.

**Definition of delirium and its differential diagnoses - cognitive dysfunction and septic associated encephalopathy**

**Definition of delirium**

Delirium is a common syndrome on ICU and will be divided into the hypoactive-, the hyperactive- and the mixed-type (30). In the present study, the delirium was diagnosed by using the Confusion assessment method for the Intensive Care Unit (CAM-ICU), German Version. It was performed by trained personal (nurses and physicians) at least once every eight hours, or more often if considered necessary. Delirium was primarily diagnosed in all patients with a positive CAM-ICU test result, regardless of the presence of sepsis.

**Definition of cognitive dysfunction**

For example, critically ill patients with a limited vigilance level (RASS < -3) cannot be evaluated with current delirium screening instruments. Those patients cannot comply with simple prompts despite an appropriate sedation break and a RASS value greater than minus 3. Typical requests include show one's teeth and tongue, squeezing one's hand e.g. These patients may also show up by an uncoordinated adaption to the respirator, agitation and the inability to reach a sufficient level of contact. The limitations mentioned for performing the CAM-ICU are often observed in critically ill patients with intracranial bleeding or neurocognitive disorders. For these patients the somewhat controversial term "cognitive dysfunction" has been chosen, which should be interpreted in a purely descriptive manner (29).

**Definition of septic associated encephalopathy**

Delirious conditions often occur in septic patients and can be the clinical manifestation of septic-associated encephalopathy. It is important to note that in particular structural changes of the brain due to
craniocerebral trauma or ischemia as well as adverse drug reactions must be excluded before an SAE can be diagnosed (31,32). Validated delirium screening tools like the CAM-ICU have proven to be suitable for diagnosing SAE (33). Aware that the CAM-ICU can support the suspected diagnosis of SAE, but cannot prove it, the following consideration should be taken into account: Septic patients in whom the CAM-ICU cannot be reliably performed, for example due to cerebral damage, should not be classified in the category "SAE". These patients are referred to as septic patients with cognitive dysfunction. Patients in the present study were suspected to have SAE under the following criteria: Diagnosed sepsis with concomitant delirious symptoms and positive CAM-ICU test result.

**AChE-activity measurements**

Since acetylcholine cannot be measured directly due to its rapid enzymatic degradation by acetylcholinesterase, it is necessary to define an appropriate surrogate parameter for the (central) cholinergic acetylcholine metabolism. The erythrocytic acetylcholinesterase activity (AChE-activity) has proven to be a suitable surrogate parameter in numerous studies (33). One EDTA-Blood sample (1 ml) was collected once daily over a period of maximum six days at 5:00 a.m. Several patients got an infusion of indirectly acting parasympathomimetics, usually at 6:00 a.m. (for intestinal stimulation, average half-life up to 80 minutes) which could suppress AChE-activity. The first blood sample was taken in the morning after admission on the ICU, labeled as “day 1”. Between 7:00 and 12:00 a.m. the erythrocyte AChE-activity was determined by using LISA-ChE (Dr. F. Koehler Chemie GmbH), a point-of-care testing device. The measurement of the AChE-activity is based on the modified Ellman method, a colorimetric method, improved by Worek et al (28). The literature based reference values of AChE-activity ranges from 26.7 U/gHb until 50.9 U/gHb (36,37). Due to a high inter- and intra-individual variability of AChE-activity, in clinical practice, a modified reference range from 30.0 to 50.0 U/gHb is more suitable (29). It is therefore useful to use the reference range of AChE-activity under clinical aspects as a rough guide. However, it should not be interpreted in a purely dogmatic way. Nevertheless, the majority of studies refer to these postulated reference ranges (26.7-50.9 U/gHb) when interpreting AChE-activity. However, it is often ignored that the underlying basis of these reference values are studies on healthy agricultural workers (34,35). Studies on the re-evaluation of reference values of AChE-activity in intensive care patients are still missing. In particular, inter- and intraindividual variability as well as time-dependent changes in AChE-activity must be considered when interpreting corresponding study results in critically ill patients.

The primary endpoint of this study was to investigate whether AChE-activity is altered in septic patients with suspected SAE compared to non-septic patients with and without delirium. The secondary endpoint was to investigate whether AChE-activity is capable of differentiating between SAE and other causes of delirium in critically ill patients.

**Sample size calculation and power analysis**

With reference to previously published study results on AChE-activity, one of the main considerations in determining the number of cases was that, if available, statistically significant differences between non-
deliriant and deliriant intensive care patients could be detected even in small case numbers. Further considerations for determining the sample size were based on the following facts:

The average number of intensive care patients in the interdisciplinary surgical intensive care unit, University hospital Ulm, is about 550 patients per year. The prevalence of sepsis in German intensive care units was about 12.4% (sepsis) and 11.0% (severe sepsis and septic shock) in the observation period. Based on these facts, the number of cases was planned with around 200 patients in GPower 3.1. A post-hoc power calculation was done using the study data of septic and non-septic patients. Specifically, a simulation-based approach has been used in order to assess the power associated to a longitudinal AChE-activity regression model including the time point of measurement, group status of the patient (septic vs. non-septic) as well as the corresponding interaction term. This analysis was conducted by means of the SIMR package in R (version 3.6.1), which revealed that a number of about 100 patients per group (septic and non-septic, i.e. n = 200 patients in total) would be required to be assessed longitudinally in order to reach a statistical power of 80%. Based on the currently available sample size of about 40 patients in the smaller (septic) subgroup, the simulation reveals an empirical power of about 60%. Due to the large difference in cohort size (45 septic patients vs. 130 non-septic patients) the overall empirical power thus ranges somewhere between 60-80%.

**Statistical analysis**

Data were collected in Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) and analyzed by using GraphPad PRISM, Version 5 for Windows and SAS Version 9.4.

AChE-activity was analyzed over the course of time by using a linear regression model accounting for repeated measures. The AChE-activity was defined as the dependent variable and the time of measurement (a maximum of six consecutive days) was defined as the continuous independent predictor of primary interest. By using a time adjusted model, the effect of further possible predictors of AChE-activity was analyzed.

Quantitative data were expressed as median, minimum and maximum and, for nonparametric distributions, were compared using Wilcoxon matched-pairs test. For the analysis of the independent samples, we used the Mann-Whitney test. All results reported shall be interpreted in an exploratory manner, since we did not adjust the p-values for multiple testing.

**Results**

During the observation period, 241 potentially eligible patients were admitted to the surgical intensive care unit at Ulm University Hospital, Germany. However, 66 patients did not meet inclusion criteria. In detail, for 38 patients the number of AChE-activity was less than 2 readings (length of stay on ICU < 24 hours: 10 patients deceased, 28 patients were relocated to the Intermediate Care Unit), 10 patients had an incomplete dataset, 18 patients had a missing written consent at the time of data evaluation. Finally, a total of 175 patients were included in the further analysis. As displayed in table 1 in both groups the
analysis of the patient-related data shows an imbalance in the gender distribution in favor of the male sex. Patients with diagnosed sepsis had a longer stay in the intensive care unit and higher disease severity scores compared to non-septic patients. A comparison of the delirium patients in both groups showed that the hypoactive course was more common in septic patients with suspected SAE.

Results of the non-septic patients:

130 patients were classified as non-septic with a median age of 64 years at enrollment. 89 of them were men, 41 women. The median length of stay on the ICU was 8 days. A total of 10 non-septic patients deceased during the observation period. 36 of the non-septic patients were CAM-ICU positive, i.e. they had the clinical symptoms of a delirium. In consideration of the delirium subtypes 12 of them exhibited a hypoactive-, 5 a hyperactive- and 19 a mixed-form. In 24 of the non-septic patients, the CAM-ICU could not be performed, for example, due to cerebral hemorrhage. Taking into account the definition of cognitive dysfunction mentioned above, these patients were assigned to this term. Figure 1 shows the course of AChE-activity in 130 non-septic patients. With the exception of a statistically significant increase in AChE-activity between day 1 and day 3 (Wilcoxon matched pairs test, p = 0.03), no relevant change in AChE-activity over the further observation period could be detected. In Fig. 2 and Fig. 3, the course of AChE-activity in 10 of 130 non-septic patients with a positive CAM-ICU test result was shown. Of these, five patients showed a non-significant increase and another five patients a non-significant decrease in AChE-activity of at least 10% compared to the baseline value at admission to the ICU. Of the remaining 26 non-septic patients with delirium symptoms (CAM-ICU positive) the changes in AChE-activity were also not significant, i.e. less than 10% change of the AChE-activity compared to the baseline value. Therefore, the corresponding box plots were not shown separately.

Results of the septic patients:

45 of 175 patients could be defined as septic according to the above definition. 40 of these patients showed delirious symptoms with corresponding positive CAM-ICU test results. Taking into account the above-mentioned definition of SAE, these patients were all assigned to the suspected diagnosis of SAE. One septic patient was not accessible for CAM-ICU testing due to an accompanying cerebral hemorrhage. Therefore, he was classified as a septic patient with cognitive dysfunction, taking into account the criteria mentioned above. Three patients were under permanent sedation until death and thus also inaccessible to testing by CAM-ICU. One septic patient was permanently without delirious symptoms during the intensive care stay. The median age in the septic group was 61 years. The gender distribution in the group of the septic patients showed 33 male and 12 female patients. The median length of stay in the ICU was 14 days for the septic subgroup. 22 of the septic patients deceased during the observation period. Compared to the non-septic patients all septic patients showed a change in AChE-activity, corresponding to a 10% increase or decrease from baseline values. In 15 of the 40 septic patients with suspected SAE (positive CAM-ICU) a statistically significant increase in AChE-activity from day 1 to day 6 could be demonstrated. (Wilcoxon matched pairs test, *** p < 0.0001, ** p < 0.01, * p < 0.05), (Fig. 4). In 30 of the 40 septic patients with suspected SAE (positive CAM-ICU) a statistically significant decrease in AChE-
activity from day 1 to day 5 could be demonstrated. (Wilcoxon matched pairs test, *** p < 0.0001, day 1 to day 6 p > 0.05), (Fig 5).

**Univariate analysis of septic patients**

The univariate analysis accounting for repeated measures of the AChE-activity exhibit that in septic patients the AChE-activity can also in- or decrease over time statistically significant, starting from admission to the ICU over a period of at least 5 days (AChE-activity decrease: p = 0.023, AChE-activity increase: p = 0.002). In contrast, there was neither a correlation between AChE-activity and age, sex, SAPS II, an increase or decrease of the AChE-activity, SOFA-Score nor the incidence of delirium/SAE or cognitive dysfunction in septic patients. In the non-septic patients neither a dependence of AChE-activity on time nor a correlation of AChE-activity with the parameters mentioned above could be demonstrated. Therefore, we refrained from displaying these results. The statistically significant p-score with respect to the TISS-28 score in septic patients with a decrease in AChE-activity (p = 0.041) is of no clinical significance, as the SAPS score does not show a statistically significant difference (Table 2). Due to the lack of significance in the univariate models (type 1 error level was set to α = 5%) we did not run a more complex multivariable regression model afterwards.

**Discussion**

In the present study it could be demonstrated that the AChE-activity in critically ill septic patients with postulated SAE changes statistically significantly for at least 5 consecutive days after admission to ICU in relation to their baseline value. In contrast, no change in AChE-activity was observed in the group of non-septic patients, regardless of delirium symptoms or cognitive impairment.

**Decrease of AChE activity**

The time-dependent decrease in AChE-activity in the majority of septic patients with suspected SAE in the present study is consistent with the findings of Bitzinger et al. A significant and time-dependent decrease in AChE-activity was reported in a rat model with cecal ligation puncture (CLP) induced sepsis (24). Interestingly, these authors showed that a decrease in AChE-activity could be detected much earlier than other conventional laboratory tests generally used to diagnose sepsis (e.g. development of lactic acidosis) (22,36,37). The assumption that the decrease in AChE-activity is an early indicator of sepsis could not be confirmed in the results of the present study. This can be explained by the fact that, in contrast to a CLP-model, the onset of sepsis in a heterogeneous patient cohort cannot be reduced to a defined point in time. Compared to non-septic patients with delirious symptoms, in patients with suspected SAE, inflammation seems to be one of the major factors causing a change in AChE-activity. In the course of sepsis, among other things, oxygen radicals are released which can cause neuronal damage (38). If damage to cholinergic neurons occurs, the transmitter acetylcholine decreases consecutively. Accordingly, there is a reduced activity of the surrogate parameter AChE-activity. Clinically, a deficiency of acetylcholine can manifest itself as delirium, or through attention and memory deficits. (39). Interestingly, comparable pathophysiological changes also were observed in the development of
Alzheimer's disease. Me´ndez-Garrido et al. were able to show that in patients with Alzheimer's disease, higher concentration of reactive oxygen species, e.g. H2O2, in central nervous system were detected (6). Oxidative stress decreases the AChE-activity and simultaneously increases the acetylcholine hydrolysis, which ultimately contributes to a central cholinergic deficiency (40).

Increase of AChE-activity

In the present study, a time-dependent increase in AChE-activity was demonstrated in about one third of the septic patients with suspected SAE. In a CLP-induced sepsis model, the survivors had a decrease in cholinergic neurons in the basal forebrain, a significant increase in AChE-activity and an increase in the expression of their coding gene in the hippocampus and cortex, probably caused by microglial activation (41). An increase in AChE-activity leads to an increased breakdown of acetylcholine and ultimately to a cholinergic deficit with the associated characteristic symptoms such as memory disorders, disorientation, hypo- or hyperactivity (8,9). Changes in cholinergic transmission in the hippocampus of septic patients appear to play a central role in the pathogenesis of septic-associated encephalopathy. Many of the symptoms associated with SAE, such as memory disorders, attention deficits and consciousness disorders, can be attributed to changes in this particular area of the brain. Zivkovic et al. “identified the hippocampus as the site of dysfunction and pathology in sepsis induced delirium” by MRI-imaging (21). Given the possible pathophysiological changes postulated in SAE, both an increase and a decrease in AChE-activity seem plausible. So far, however, there is a lack of evidence that can explain these different courses of AChE-activity in septic patients with associated septic associated encephalopathy. Some studies on delirium and cholinergic transmission in critically ill patients have addressed, among other things, the activity of the butyrylcholinesterase (peripheral cholinesterase activity, BChE-activity) (42–44). BChE-activity together with AChE-activity in the CNS is responsible for central cholinergic transmitter homeostasis. However, unlike AChE-activity, it is not a surrogate for central cholinergic transmission (45). BChE-activity correlates inversely with the C-reactive protein and could be a predictor for the outcome of various diseases (37,44). Overall, BChE-activity appears to reflect the inflammatory level, while AChE-activity may indicate a change in central cholinergic transmission.

Perspective for the clinical and therapeutic relevance of AChE-activity in patients with presumed SAE

Although no clinical study has yet been able to prove the efficacy of cholinesterase inhibitors for the treatment of delirium in critically ill patients, there is evidence in several experimental sepsis models that indirect parasympathomimetics can positively influence the anti-inflammatory immune response in the central nervous system (21,42,47,48). Assuming that a change in the cholinergic transmitter balance is partially responsible for the cognitive impairment in SAE. It is conceivable that the application of indirect parasympathomimetic drugs that cross the blood-brain barrier may be an interesting therapeutic approach. Up to now, several studies addressing the use of indirect parasympathomimetics and their possible positive effects on delirium have failed. Taking into account the mentioned considerations it is crucial to identify the cholinergic change as a possible cause of septic-associated encephalopathy. In the clinical context, SAE can only be classified as a diagnosis of exclusion under the syndrome complex
delirium. The "ex-juvantibus" treatment of patients with delirium with indirect parasympathomimetics, as it has been performed so far, showed poor response rates. The observed time-dependent change in AChE-activity in the present study could therefore be the rationale for (targeted) therapy with centrally acting cholinesterase inhibitors such as physostigmine in critically ill patients with suspected SAE. In summary, in septic patients with delirious symptoms, positive CAM-ICU test result and suspected SAE, there was a statistically significant increase or decrease in AChE-activity for at least 5 consecutive days compared to the initial measurement. In comparison, no statistically significant, time-dependent change in AChE-activity could be detected in non-septic patients, independent of delirium or cognitive dysfunction. Therefore, the time-dependent change in AChE-activity could serve as a diagnostic criterion in the clinical diagnosis of a suspected SAE. In contrast, a single measurement of the AChE-activity does not appear to be suitable to support the diagnosis of SAE.

There are some limitations of the present study. The study was conducted in an interdisciplinary surgical intensive care unit in predominantly surgical patients. Therefore, the present results cannot easily be transferred to other patient groups. The time of onset of sepsis could not be determined exactly, so that an increase or decrease in AChE-activity may reflect different phases of the course of sepsis. The time-dependent changes in AChE-activity could be influenced by other factors, such as the type and number of anticholinergic drugs. Furthermore, there is no established diagnostic tool that allows a reliable diagnosis of SAE. The CAM-ICU has been validated for the diagnosis of delirium, but it is not known whether the high sensitivity and specificity also applies to patients with suspected SAE. Further studies are needed to investigate the significance of AChE-activity in patients with suspected SAE.

**Conclusion**

From a clinical point of view, SAE remains an exclusion diagnosis. So far, SAE has been suspected in critically ill septic patients with concomitant delirious symptoms and positive CAM-ICU test results. However, the presumed underlying pathological condition is the change in cholinergic transmission which was ultimately not considered up to now. Repeated measurement of AChE-activity can support the suspected diagnosis of SAE. The present novel approach with daily longitudinal measurements of AChE-activity in critically ill septic patients with suspected SAE captures the intra- and interindividual course of this parameter. Furthermore, a time-dependent increase or decrease in AChE-activity in septic patients with suspected SAE could be the rationale for the targeted use of indirect CNS-targeted parasympathomimetic drugs.

**Abbreviations**

| Abbreviation | Description                  |
|--------------|------------------------------|
| Ach:         | acetylcholine                |
| AChE-activity:| Acetylcholinesterase-activity|
| BChE-activity:| Butyrylcholinesterase-activity|
Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of the University Ulm. Written informed consent was obtained from all patients, their next of skin, or another surrogate decision maker, as appropriate. If patients were unable to provide informed consent and the next of skin or a designated person was not available, the inclusion procedure for emergency situations was applied. Post hoc consent was obtained in these latter patients.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

**Funding**

Not applicable.

**Authors’ contributions**

BZ and EB conducted the study, interpreted data and drafted the manuscript.

BM revised the manuscript and did the statistical analysis. SH and FB revised the manuscript. All authors read and approved the final manuscript.

**Acknowledgements**

Not applicable.

**References**

1. Bracht H, Hafner S, Weiß M. Sepsis-Update: Definition und Epidemiologie. AINS - Anästhesiol · Intensivmed · Notfallmedizin · Schmerzther. 2019 Jan;54(01):10–20.
2. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016 Feb 23;315(8):801–10.
3. Andonegui G, Zelinski EL, Schubert CL, Knight D, Craig LA, Winston BW, et al. Targeting inflammatory monocytes in sepsis-associated encephalopathy and long-term cognitive impairment. JCI Insight. 2018 May 3;3(9).
4. Young GB. Encephalopathy of Infection and Systemic Inflammation: J Clin Neurophysiol. 2013 Oct;30(5):454–61.
5. Sprung CL, Peduzzi PN, Shatney CH, Schein RM, Wilson MF, Sheagren JN, et al. Impact of encephalopathy on mortality in the sepsis syndrome. The Veterans Administration Systemic Sepsis Cooperative Study Group. Crit Care Med. 1990 Aug;18(8):801–6.
6. Sonnville R, Verdonk F, Rauturier C, Klein IF, Wolff M, Annane D, et al. Understanding brain dysfunction in sepsis. Ann Intensive Care. 2013;3(1):15.
7. Gordon SM, Jackson JC, Ely EW, Burger C, Hopkins RO. Clinical identification of cognitive impairment in ICU survivors: insights for intensivists. Intensive Care Med. 2004 Nov;30(11):1997–2008.
8. Hshieh TT, Fong TG, Marcantonio ER, Inouye SK. Cholinergic Deficiency Hypothesis in Delirium: A Synthesis of Current Evidence. J Gerontol A Biol Sci Med Sci. 2008 Jul;63(7):764–72.
9. Steiner LA. Postoperative delirium. Part 1: pathophysiology and risk factors. Eur J Anaesthesiol. 2011 Sep;28(9):628–36.
10. Pandharipande P, Cotton BA, Shintani A, Thompson J, Pun BT, Morris JA, et al. Prevalence and risk factors for development of delirium in surgical and trauma intensive care unit patients. J Trauma. 2008 Jul;65(1):34–41.

11. Chaiwat O, Chanidnuan M, Pancharoen W, Vijitmala K, Danpornprasert P, Toadithep P, et al. Postoperative delirium in critically ill surgical patients: incidence, risk factors, and predictive scores. BMC Anesthesiol. 2019 Mar 20;19(1):39.

12. Field RH, Gossen A, Cunningham C. Prior Pathology in the Basal Forebrain Cholinergic System Predisposes to Inflammation-Induced Working Memory Deficits: Reconciling Inflammatory and Cholinergic Hypotheses of Delirium. J Neurosci. 2012 May 2;32(18):6288–94.

13. Ballinger EC, Ananth M, Talmage DA, Role LW. Basal Forebrain Cholinergic Circuits and Signaling in Cognition and Cognitive Decline. Neuron. 2016 Sep;91(6):1199–218.

14. Abou-Hatab K, O'Mahony M, Patel S, Carey D, Woodhouse K. Plasma esterase activities in young and old patients undergoing open inguinal hernia repair. Arch Gerontol Geriatr. 2000 Dec;31(3):193–8.

15. Tracey KJ. Physiology and immunology of the cholinergic anti-inflammatory pathway. J Clin Invest. 2007 Feb;117(2):289–96.

16. Tracey KJ. Reflex control of immunity. Nat Rev Immunol. 2009 Jun;9(6):418–28.

17. Trzepacz PT. Is there a final common neural pathway in delirium? Focus on acetylcholine and dopamine. Semin Clin Neuropsychiatry. 2000 Apr;5(2):132–48.

18. Semmler A, Frisch C, Debeir T, Ramanathan M, Okulla T, Klockgether T, et al. Long-term cognitive impairment, neuronal loss and reduced cortical cholinergic innervation after recovery from sepsis in a rodent model. Exp Neurol. 2007 Apr;204(2):733–40.

19. Weismüller K, Bauer M, Hofer S, Weigand M. Sepsis – Die Bedeutung der neuroendokrinen Achse in der Pathophysiologie der Sepsis. AINS - Anästhesiol · Intensivmed · Notfallmedizin · Schmerzther. 2010 Sep;45(09):574–9.

20. Hofer S, Eisenbach C, Lukic IK, Schneider L, Bode K, Brueckmann M, et al. Pharmacologic cholinesterase inhibition improves survival in experimental sepsis*: Crit Care Med. 2008 Feb;36(2):404–8.

21. Zivkovic AR, Sedlaczek O, von Haken R, Schmidt K, Brenner T, Weigand MA, et al. Muscarinic M1 receptors modulate endotoxemia-induced loss of synaptic plasticity. Acta Neuropathol Commun. 2015 Dec;3(1).

22. Bitzinger DI, Gruber M, Tümmler S, Malsy M, Seyfried T, Weber F, et al. In Vivo Effects of Neostigmine and Physostigmine on Neutrophil Functions and Evaluation of Acetylcholinesterase and Butyrylcholinesterase as Inflammatory Markers during Experimental Sepsis in Rats. Mediators Inflamm. 2019 Jan 20;2019:1–12.

23. van Gool WA, van de Beek D, Eikelenboom P. Systemic infection and delirium: when cytokines and acetylcholine collide. The Lancet. 2010 Feb;375(9716):773–5.

24. Zaghoul N, Addorisio ME, Silverman HA, Patel HL, Valdés-Ferrer SI, Ayasolla KR, et al. Forebrain Cholinergic Dysfunction and Systemic and Brain Inflammation in Murine Sepsis Survivors. Front
25. Pavlov VA, Wang H, Czura CJ, Friedman SG, Tracey KJ. The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. Mol Med Camb Mass. 2003 Aug;9(5–8):125–34.

26. Santarpia L, Grandone I, Contaldo F, Pasanisi F. Butyrylcholinesterase as a prognostic marker: a review of the literature. J Cachexia Sarcopenia Muscle. 2013 Mar;4(1):31–9.

27. Thomsen T, Kaden B, Fischer JP, Bickel U, Barz H, Gusztany G, et al. Inhibition of acetylcholinesterase activity in human brain tissue and erythrocytes by galanthamine, physostigmine and tacrine. Eur J Clin Chem Clin Biochem J Forum Eur Clin Chem Soc. 1991 Aug;29(8):487–92.

28. Worek F, Mast U, Kiderlen D, Diepold C, Eyer P. Improved determination of acetylcholinesterase activity in human whole blood. Clin Chim Acta. 1999 Oct;288(1–2):73–90.

29. Barth E, Bracht H, Georgieff M, Zujalovic B. AChE- und BChE-Aktivität als Entscheidungshilfe für die medikamentöse Therapie von Delir und kognitiver Dysfunktion bei Intensivpatienten. Barth E Bracht H Georgieff M Zujalovic B AChE- BChE-Akt Als Entscheid Für Medikam Ther Von Delir Kognitiver Dysfunktion Bei Intensiv. 2019 May 10;(5–2019):233–42.

30. Krewulak KD, Stelfox HT, Leigh JP, Ely EW, Fiest KM. Incidence and Prevalence of Delirium Subtypes in an Adult ICU: A Systematic Review and Meta-Analysis*. Crit Care Med. 2018 Dec;46(12):2029–35.

31. Terborg C. [Septic encephalopathy]. Med Klin Intensivmed Notfallmedizin. 2012 Nov;107(8):629–33.

32. Lacobone E, Baillly-Salin J, Polito A, Friedman D, Stevens RD, Sharshar T. Sepsis-associated encephalopathy and its differential diagnosis. Crit Care Med. 2009 Oct;37(SUPPL. 10).

33. Chaudhry N, Duggal AK. Sepsis Associated Encephalopathy. Adv Med. 2014;2014:1–16.

34. Brüning Th, Bünger J, Welge P, Schindler B, Göen Th, Koch HM, et al. Arbeitsmedizinische Leitlinie Arbeiten unter Einwirkung von organischen Phosphorverbindungen [Internet]. [cited 2019 Jul 25]. Available from: https://www.awmf.org/leitlinien/detail/ll/002-022.html

35. Rathish D, Senavirathna I, Jayasumana C, Agampodi S. Red blood cell acetylcholinesterase activity among healthy dwellers of an agrarian region in Sri Lanka: a descriptive cross-sectional study. Environ Health Prev Med. 2018 Jun 21;23(1):25.

36. Pierrakos C, Vincent J-L. Sepsis biomarkers: a review. Crit Care. 2010;14(1):R15.

37. Zivkovic AR, Schmidt K, Sigl A, Decker SO, Brenner T, Hofer S. Reduced Serum Butyrylcholinesterase Activity Indicates Severe Systemic Inflammation in Critically Ill Patients. Mediators Inflamm. 2015;2015:1–11.

38. Berg RMG, Møller K, Bailey DM. Neuro-oxidative-nitrosative stress in sepsis. J Cereb Blood Flow Metab. 2011 Jul;31(7):1532–44.

39. Maldonado JR. Delirium pathophysiology: An updated hypothesis of the etiology of acute brain failure. Int J Geriatr Psychiatry. 2018 Nov;33(11):1428–57.

40. Méndez-Garrido A, Hernández-Rodríguez M, Zamorano-Ulloa R, Correa-Basurto J, Mendieta-Wejebe JE, Ramírez-Rosales D, et al. In Vitro Effect of H2O2, Some Transition Metals and Hydroxyl Radical
Produced Via Fenton and Fenton-Like Reactions, on the Catalytic Activity of AChE and the Hydrolysis of ACh. Neurochem Res. 2014 Nov;39(11):2093–104.

41. Zaghloul N, Addoriso ME, Silverman HA, Patel HL, Valdés-Ferrer SI, Ayasolla KR, et al. Forebrain Cholinergic Dysfunction and Systemic and Brain Inflammation in Murine Sepsis Survivors. Front Immunol. 2017;8:1673.

42. Cerejeira J, Batista P, Nogueira V, Firmino H, Vaz-Serra A, Mukaeotva-Ladinska EB. Low preoperative plasma cholinesterase activity as a risk marker of postoperative delirium in elderly patients. Age Ageing. 2011 Sep 1;40(5):621–6.

43. Müller A, Olbert M, Heymann A, Zahn PK, Plaschke K, von Dossow V, et al. Relevance of peripheral cholinesterase activity on postoperative delirium in adult surgical patients (CESARO): A prospective observational cohort study. Eur J Anaesthesiol. 2019 Feb;36(2):114–22.

44. John M, Ely EW, Halfkann D, Schoen J, Sedemund-Adib B, Klotz S, et al. Acetylcholinesterase and butyrylcholinesterase in cardiosurgical patients with postoperative delirium. J Intensive Care. 2017 Dec;5(1):29.

45. Hajjawi OS. Acetylcholinesterase in Human Red Blood Cells. Eur J Sci Res. 2012;Vol.75(4):510–22.

46. Gamberini M, Bolliger D, Lurati Buse GA, Burkhart CS, Grapow M, Gagneux A, et al. Rivastigmine for the prevention of postoperative delirium in elderly patients undergoing elective cardiac surgery—a randomized controlled trial. Crit Care Med. 2009 May;37(5):1762–8.

47. Liptzin B, Laki A, Garb JL, Fingeroth R, Krushell R. Donepezil in the prevention and treatment of postsurgical delirium. Am J Geriatr Psychiatry Off J Am Assoc Geriatr Psychiatry. 2005 Dec;13(12):1100–6.

Tables
Table 1 Characteristics of patient population
### Variable Patients

\[ n = 175 \]

**non-septic patients**  
**septic patients**  

\[ n = 130 \]  
\[ n = 45 \]

| Age at enrollment |  |
|-------------------|---|
| Median (min., max.) | 64.0 (20.0 – 95.0) | 61.0 (30.0 – 91.0) |

| Gender, n (%) |  |
|---------------|---|
| Male | 89.0 (68.5) | 33 (73.3) |
| Female | 41.0 (31.5) | 12 (26.7) |

| ICU – LOS - days |  |
|-----------------|---|
| median (min., max.) | 8 (1.0 – 86.0) | 14.0 (1.0 – 87.0) |

| Overall mortality, n (%) |  |
|--------------------------|---|
| 10 (7.7) | 22 (48.9) |

| Disease severity scoring |  |
|--------------------------|---|
| SAPS II, |  |
| median (min., max.) | 26.8 (9.8 – 54.5) | 35.4 (7.0 – 58.1) |

| TISS-28, |  |
|----------|---|
| median (min., max.) | 10.3 (4.0 – 30.2) | 18.6 (5.0 – 29.9) |

| Delirium n (%) |  |
|----------------|---|
| hypoactive | 12 (33.3) | 35 (87.5) |
| hyperactive | 5 (13.9) | 1 (2.5) |
| mixed-form | 19 (52.8) | 4 (10.0) |

| cognitive dysfunction n (%) |  |
|-----------------------------|---|
| 24 (18.5) | 1 (2.2) |

Table 2 – univariate analysis of septic patient subgroups
| Sedation until decease n (%) | 3 (6.7) |
|-----------------------------|--------|
| Permanently CAM-ICU negative n (%) | 1 (2.2) |

**Primary reason for ICU admission, n (%)**

| Reason                        | n   | %    |
|-------------------------------|-----|------|
| Neurosurgery & brain haemorrhage | 40  | (30.8) |
| Abdominal surgery              | 28  | (21.5) |
| Trauma surgery                 | 20  | (15.4) |
| Cardiac surgery                | 15  | (11.5) |
| Vascular surgery               | 12  | (9.2)  |
| Thoracic surgery               | 11  | (8.5)  |
| Respiratory failure            | 4   | (3.1)  |
| Haematology-oncology           | 2   | (4.4)  |
| Urinary system                 | 1   | (2.2)  |

ICU: Intensive Care Unit, LOS: length of stay, SAPS II: Simplified Acute Physiology Score II, TISS-28: Therapeutic Intervention Scoring System 28, CAM-ICU: Confusion Assessment Method for the Intensive Care Unit, SAE: Septic Associated Encephalopathy,
AChE-activity - independent variable

|                      | AChE-activity decrease | AChE-activity increase |
|----------------------|------------------------|------------------------|
|                      | Estimate | SE  | p-value | Estimate | SE  | p-value |
| Time                 | -0.57     | 0.25 | 0.023   | 1.44     | 0.45 | 0.002   |
| Age                  | -0.04     | 0.09 | 0.674   | -0.07    | 0.13 | 0.574   |
| Sex (male vs female) | -0.37     | 2.82 | 0.896   | 1.31     | 4.71 | 0.782   |
| SAPS II              | 0.14      | 0.09 | 0.144   | 0.08     | 0.17 | 0.608   |
| TISS-28              | 0.42      | 0.20 | 0.041   | 0.14     | 0.22 | 0.522   |
| SOFA                 | -0.20     | 0.52 | 0.703   | -0.66    | 0.57 | 0.251   |
| Delir – DD SAE (yes vs no) | -6.52 | 3.67 | 0.078   | -1.40    | 4.65 | 0.764   |
| cognitive dysfunction (yes vs no) | 5.72 | 7.26 | 0.433   | n.e.     | n.e. | n.e.    |

All models are adjusted for time; SE=standard error of the estimate; n.e.= not estimable (since none of the patients in the AChE-activity increase subgroup showed cognitive dysfunction). SAPS II: Simplified Acute Physiology Score II, TISS-28: Therapeutic Intervention Scoring System 28, SOFA: Sequential Organ Failure Assessment, SAE: Septic associated encephalopathy.

Figures
Figure 1

Course of AChE-activity in 30 septic patients with a decrease of AChE-activity (Confusion assessment method for the intensive care unit positive, differential diagnosis septic associated encephalopathy (n = 27), n = 3 permanently sedated until decease, n = 1 CAM-ICU negative. Statistical significance was calculated using Wilcoxon matched-pairs test. *** p < 0.001. Number of patients per day: d 1: n = 30, d 2: n = 30, d 3: n = 28, d 4: n = 27, d 5: n = 26, d 6: n = 22
Figure 2

Course of AChE-activity over a period of 6 days in 15 septic patients with an increase of AChE-activity (Confusion assessment method for the intensive care unit positive, differential diagnosis septic associated encephalopathy). Statistical significance was calculated using Wilcoxon matched-pairs test.

*** p < 0.001, ** p < 0.01, * p < 0.05 Number of patients per day: d 1: n = 15, d 2: n = 15, d 3: n = 14, d 4: n= 12, d 5: n = 11, d 6: n = 10
Figure 3

Course of AChE-activity in non-septic, CAM-ICU positive patients over a 6-day observation period with a trend towards a decrease of the AChE-activity (no statistically significance difference over the course of time between day 1 until day 6 was observed – Wilcoxon matched-pairs test). Number of patients per day: d 1: n = 5, d 2: n = 5, d 3: n = 4, d 4: n = 4, d 5: n = 4, d 6: n = 2
Figure 4

Course of AChE-activity in non-septic, CAM-ICU positive patients over a 6-day observation period with a trend towards an increase of the AChE-activity (no statistically significance difference over the course of time between day 1 until day 6 was observed – Wilcoxon matched-pairs test). Number of patients per day: d 1: n = 5, d 2: n = 5, d 3: n = 5, d 4: n= 5, d 5: n = 5, d 6: n = 5
Figure 5

Course of AChE-activity in non-septic patients over a period of 6 days. Statistically significant difference between day 1 and day 3 (p = 0.03), calculated with Wilcoxon matched-pairs test. Number of patients per day: d 1: n = 130, d 2: n = 130, d 3: n = 99, d 4: n= 74, d 5: n = 69, d 6: n = 56