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Baseline acetylcholinesterase activity and serotonin plasma levels are not associated with delirium in critically ill patients

Atividade basal de acetilcolinesterase e níveis plasmáticos de serotonina não se associam ao delirium em pacientes gravemente enfermos

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ABSTRACT

Objective: The aim of this study was to investigate whether plasma serotonin levels or acetylcholinesterase activities determined upon intensive care unit admission could predict the occurrence of acute brain dysfunction in intensive care unit patients.

Methods: A prospective cohort study was conducted with a sample of 77 non-consecutive patients observed between May 2009 and September 2010. Delirium was determined using the Confusion Assessment Method for the Intensive Care Unit tool, and the acetylcholinesterase and serotonin measurements were determined from blood samples collected up to a maximum of 24 h after the admission of the patient to the intensive care unit.

Results: In the present study, 38 (49.6%) patients developed delirium during their intensive care unit stays. Neither serum acetylcholinesterase activity nor serotonin level was independently associated with delirium. No significant correlations of acetylcholinesterase activity or serotonin level with delirium/coma-free days were observed, but in the patients who developed delirium, there was a strong negative correlation between the acetylcholinesterase level and the number of delirium/coma-free days, indicating that higher acetylcholinesterase levels are associated with fewer days alive without delirium or coma. No associations were found between the biomarkers and mortality.

Conclusions: Neither serum acetylcholinesterase activity nor serotonin level was associated with delirium or acute brain dysfunction in critically ill patients. Sepsis did not modify these relationships.

Keywords: Acetylcholinesterase/drug effects; Serotonin/drug effects; Delirium; Sepsis; Intensive care units

INTRODUCTION

Delirium is a frequent complication among patients admitted to intensive care units (ICU) and is independently associated with increases in the durations of mechanical ventilation (MV) and ICU stay, long-term neuropsychological dysfunction, and mortality.[1-3]

Several different mechanisms have been proposed to explain the development of delirium in ICU patients. It has been suggested that drug toxicity, inflammation and acute stress responses[4-6] could affect several aspects of mental function.[7] Other potential mechanisms include reductions of cerebral blood flow and oxygen extraction by the brain, disruptions of the blood-brain barrier, impairments of astrocyte function, and neuronal degeneration.[8,9] The

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final common pathway that leads to delirium is probably the disruption of neurotransmission in the brain.

The cholinergic and serotoninergic pathways are the major neurotransmitter pathways that have been implicated in the development of delirium.[5,10] The activation of cholinergic receptors modulates cognition, arousal, learning, and memory, which are the major brain functions that are affected by delirium. The synthesis of acetylcholine is vulnerable to different stressors, such as impairments in energetic metabolism and inflammation.[11,12] Serotonin has a role in the arousal and sleep-wake cycles, and an increase in serotonin activity occurs in hepatic encephalopathy.[13,14] Additionally, imbalances in the levels of the precursors of serotonin synthesis occur in delirium patients, which suggests a role of serotonin in the genesis of delirium.[15,16] Pandharipande et al.[16] demonstrated that either very low or very high levels of plasma tryptophan, a serotonin precursor, are independent risk factors for the occurrence of delirium in mechanically ventilated ICU patients. Cholinesterase activity has been proposed to have a role in the development of delirium in postoperative patients,[17,18] but no definitive evidence suggests the roles of these neurotransmitters in the development of delirium in critically ill patients.

Therefore, we hypothesized that the plasma level of serotonin and acetylcholinesterase activity determined upon ICU admission would predict the occurrence of acute brain dysfunction in ICU patients. Additionally, because the metabolisms of both neurotransmitters can be altered by inflammation, we also hypothesized that these neurotransmitters would be differentially modulated in patients with sepsis-associated delirium.

METHODS

Study design, setting and patient selection

A prospective cohort study was conducted with a convenience sample of 77 patients between May 2009 and September 2010. Patients over the age of 18 years who were admitted for more than 24 hours to a 20-bed medical-surgical ICU at a university hospital in Brazil were included. Each included patient was only allowed to enter the study once. The patients' medical records were carefully reviewed for diagnoses such as previous central nervous system disease, depression, dementia, and schizophrenia. Patients who could not be assessed for delirium at any time during their ICU stay and patients who were admitted as a result of brain trauma, delirium or other severe neurological condition that precluded the evaluation of delirium (e.g., stroke and subarachnoid hemorrhage) were excluded. This study complied with the Declaration of Helsinki. The institutional review board of the *Hospital São José* approved this study (Ref. 49/2008), and the informed consent requirement was waived.

Data collection and definitions

The demographic variables and disease characteristics of all admitted patients were collected. Mental status was assessed daily from enrollment until ICU discharge or to a maximum of 28 days. Delirium was evaluated using the Confusion Assessment Method for the Intensive Care Unit (CAM-ICU)[19,20] twice per day during the ICU stays at 8:00 am and 2:00 pm. The ICU routinely utilized a daily sedation stop protocol in which sedation interruptions were performed daily at 7:00 am. Thus, the CAM-ICU was generally assessed after sedation had been lightened to the point of wakefulness. The patients were diagnosed with delirium if they met least one positive CAM-ICU screening criterion. The level of sedation was evaluated with the Richmond Agitation-Sedation Scale (RASS).[21] Coma was defined by a RASS score of -4 or -5 (i.e., responsive to physical stimuli only or unresponsive). Acute brain dysfunction was evaluated by estimating the number of delirium/coma-free days, which represented the number of days that the patient was alive without delirium or coma.[22] At enrollment, the patients were allocated to septic or non-septic groups according to internationally agreed upon criteria.[23] The patients who developed sepsis during their ICU stays but were not septic at admission were counted as non-septic.

Blood samples collection and storage

Blood samples were collected within a maximum of 24 hours after ICU admission. Blood was drawn into dry tubes, and the serum was stored at -80ºC until the determination of the serotonin level and acetylcholinesterase activity.

Serum acetylcholinesterase measurements

Acetylcholinesterase activity was assayed according to the method of Ellman et al.[24] The reaction mixture (2mL

final volume) contained 100mM K+-phosphate buffer (pH 7.5) and 1mM 5.5-dithiobisnitrobenzoic acid. This method is based on the formation of the yellow 5.5-dithio-bis-acid-nitrobenzoic anion, which is measured by the absorbance at 412nm during a 2 min incubation at 25ºC. The enzyme (40 - 50µg of protein) was preincubated for 2 min. The reaction was initiated by the addition of 0.8mM acetylthiocholine iodide (AcSCh). All samples were run in duplicate, and the enzyme activity is expressed in micromoles of AcSCh per hour per milligram of protein. The protein levels were measured using the method of Lowry et al.[25] with bovine serum albumin as the standard.

Serum serotonin measurements

The serum serotonin levels were measured using a commercial enzyme immunoassay kit (Immuno-Biological Laboratories, Inc. - IBL - Minneapolis, USA). The serotonin levels are expressed in nanograms per milliliter.

Statistical analysis

Continuous variables are described as the medians (interquartile ranges), and categorical data are described as frequencies and proportions. The Mann-Whitney U test was used to compare continuous variables. The chi square test was performed to compare categorical variables. Linear regression was used to study the associations of the acetylcholinesterase and serotonin levels with the delirium/coma-free days (DCFDs). Binary logistic regression was performed to examine the associations between the biomarkers and delirium (the dependent variable). The model was adjusted for sedative use, sepsis, Sequential Organ Failure Assessment (SOFA) score, the biomarker and the interaction between sepsis and the biomarker. Therefore, one model was generated for each biomarker. To identify the factors associated with mortality, univariate analysis was performed, and binary logistic regression was performed to examine the associations between the biomarkers and mortality (the dependent variable). The model was adjusted for physiological changes that might have confounded the relationships between the biomarkers and survival. Thus, the model incorporated disease severity, age, the need for MV, coma, the need for vasoactive drugs and the need for sedation. The need for vasoactive drugs was already incorporated in the SOFA score. Thus, to avoid double-counting, the need for vasoactive drugs was not used as a separate covariate in the model. Co-linearity was observed between the use of sedation and MV. Thus, a model was generated using the SOFA score (and not the Acute Physiology and Chronic Health Evaluation - APACHE score, which is a variable that is associated with disease severity), age, the need for MV, and the biomarkers. Therefore, one model was generated for each biomarker. The Hosmer-Lemeshow goodness-of-fit test was used to evaluate the agreement between the observed and expected mortalities. Statistical significance was defined by a p value < 0.05. All analyses were completed using version 17.0 of the Statistical Package for the Social Sciences software (SPSS, IBM Corporation, New York, USA).

RESULTS

A total of 77 patients were included in the present study. There were 39 (50.6%) non-delirium and 38 (49.6%) delirium patients. The demographic and clinical variables are shown in table 1.

Acetylcholinesterase activity and serotonin measurements and the occurrence of delirium

The serum acetylcholinesterase activities and serotonin levels were comparable in the delirium and non-delirium patients (Table 1). In the binary regression models, neither serum acetylcholinesterase activity nor serotonin level was independently associated with delirium. Additionally, there was no significant interaction between the biomarkers and sepsis (Table 2).

Correlations of acetylcholinesterase activity and serotonin level with acute brain dysfunction and mortality

There were no significant correlations of acetylcholinesterase activity or serotonin level with the number of DCFDs ($r = 0.003$, $p = 0.959$ and $r = 0.014$, $p = 0.317$, respectively). Among the patients who developed delirium, there was a strong negative correlation between the acetylcholinesterase enzyme (AChE) level and the number of DCFDs ($r = 0.838$, $p \leq 0.001$); patients with higher AChE levels spent fewer days alive without delirium or coma.

In the univariate analyses, sedation and MV use, age, SOFA score upon admission, the number of days in a coma, and the DCFDs were associated with mortality (Table 3). Thus, multivariate analysis was performed using age, SOFA score, the need for MV and the biomarkers. Only MV was found to be a risk factor for death in this sample (odds ratio - OR:

Table 1 - Demographic and baseline characteristics

Variables	Non-delirium N = 39 (50.6%)	Delirium N = 38 (49.4%)	All patients N = 77 (100%)	p value
Age (years)	55 (29)	57 (28)	56 (25)	0.362
Male	30 (76.9)	23 (60.5)	53 (68.8)	0.123
APACHE II	18.5 (11.5)	19.5 (14)	18 (11)	0.996
SOFA score on admission	7 (5.5)	8 (6)	7 (5.75)	0.749
Emergency surgery patients	8 (20.5)	3 (7.9)	11 (14.3)	
Elective surgery patients	5 (12.8)	9 (23.7)	14 (18.2)	0.182
Medical patients	26 (66.7)	26 (68.4)	52 (67.5)	
Mechanical ventilation (yes)	23 (67.6)	24 (63.2)	47 (65.3)	0.805
Sedation use	21 (55.3)	28 (73.7)	49 (64.5)	0.09
Mental status at enrollment				
Comatose	16 (41)	15 (39.5)	31 (40.3)	
Delirious	0	1 (2.6)	1 (1.3)	0.990
Normal	23 (59)	22 (57.9)	45 (58.4)	
Reasons for admission				
Cardiovascular	5 (13.2)	5 (13.2)	10 (13.2)	
Postoperative	8 (21.1)	5 (13.2)	13 (17.1)	
Neurological	9 (23.7)	7 (18.4)	16 (21.1)	
Trauma	7 (18.4)	4 (10.5)	11 (14.5)	
Respiratory distress (excluding sepsis)	5 (13.2)	8 (21.1)	13 (17.1)	0.625
Sepsis	2 (5.3)	1 (2.6)	3 (3.9)	
Shock (excluding sepsis)	1 (2.6)	2 (5.3)	3 (3.9)	
Digestive	1 (2.6)	3 (7.9)	4 (5.3)	
Renal/metabolic	0	1 (2.6)	1 (1.3)	
Other	0	2 (5.3)	2 (2.6)	
AChE activity (μmol AcSCh/h mg protein)	1.7 (1.2)	1.5 (1.6)	1.7 (1.3)	0.181
Serotonin (ng/mL)	3.1 (0.8)	2.9 (1.1)	3.0 (0.9)	0.845
Hospital length of stay (days)	19 (13.5)	18 (19)	18.5 (16)	0.466
Delirium (days)	0	1 (1)	-	-
Coma (days)	3 (12)	3 (6)	3 (6.75)	0.719
Delirium/coma-free days	25 (22)	22 (14)	23 (15.75)	0.543
Mortality within 28 days of enrollment	8 (20.5)	12 (31.6)	20 (26)	0.307

AChE - acetylcholinesterase enzyme; APACHE II - Acute Physiology and Chronic Health Evaluation II; SOFA - Sequential Organ Failure Assessment. The data are presented as the median (IQR) or as the number (percentage). * Indicates a significant difference (i.e., p value ≤ 0.05).

7.2, confidence interval 95% - CI95%: 1.03 - 50.9, p = 0.047).

DISCUSSION

In the present study, the plasma serotonin levels and acetylcholinesterase activities upon ICU admission were not associated with the occurrence of acute brain dysfunction or mortality. These findings oppose the hypothesis that neurotransmitter imbalance is a key factor in the pathogenesis of acute brain dysfunction.

The cholinergic system of the brain modulates attention, learning, memory, movement control, and other peripheral functions[26] and plays extensive roles in attention and consciousness. Acetylcholine focuses awareness by acting as a modulator of the signal-to-noise ratios of sensory and cognitive inputs. Irregularities in these brain functions can cause symptoms of both hypoactive and hyperactive delirium, including inattention, disorganized thinking, and perceptual disturbances.[11] Studies have demonstrated that higher serum AChE activity is

Table 2 - Binary regression analyses of the characteristics associated with the occurrence of delirium according to each biomarker

	OR (CI 95%)	p value
AChE	0.86 (0.48 - 1.5)	0.62
Sepsis	1.7 (0.26 - 12)	0.55
SOFA	0.98 (0.82 - 1.16)	0.83
Sedation	1.4 (0.35 - 5.5)	0.62
AChE* sepsis	0.84 (0.39 - 1.8)	0.66
Serotonin	2.9 (0.59 - 14)	0.18
Sepsis	57 (0.15 - 21)	0.18
SOFA	1.06 (0.88 - 1.2)	0.48
Sedation	1.03 (0.25 - 4.2)	0.96
Serotonin* sepsis	0.29 (0.046 - 1.8)	0.19

OR - odds ratio; CI - confidence interval; AChE - acetylcholinesterase enzyme; SOFA - Sequential Organ Failure Assessment. Hosmer and Lemeshow goodness-of-fit - AChE $\chi^2 = 7.05$, $p = 0.53$, serotonin $\chi^2 = 11$, $p = 0.15$. * Indicates interaction between biomarker and sepsis.

associated with delirium in the post-operative period in elderly patients.[12,27,28] In contrast, patients who develop postoperative delirium have also been described to exhibit lower AChE activity.[17,18] Therefore, there is no consensus regarding whether acetylcholine is involved in the development of delirium at all or whether its serum biomarkers can serve as surrogate markers of brain dysfunction and disease severity.[5,17] Despite the finding that the AChE activities upon admission did not predict acute brain dysfunction in the patients who were generally critically ill, we demonstrated that among the patients who developed delirium, the duration of the acute brain dysfunction was correlated with AChE activity. Thus, acetylcholine might play a role in the maintenance of brain dysfunction by interfering with the basic mechanisms of attention and memory. Additionally, cholinergic signaling protects striatal, hippocampal, and cortical neurons against the neurotoxicity induced by excitotoxic amino acids and other toxic insults. Despite these theoretical mechanisms, clinical trials have failed to demonstrate beneficial effects of cholinergic agonists in the treatment of delirium.[29,30]

The cholinergic pathway acts as a predictor of the individual variation in the systemic inflammatory response to infection; thus, by modulating systemic inflammation, the cholinergic system can indirectly affect brain function.[31] Lower plasma AChE activity has been associated with higher levels of proinflammatory markers during acute illness,[32,33] and the metabolism of acetylcholine can be altered by inflammation.[10,32,33] Additionally, similar to the peripheral cholinergic anti-inflammatory pathway, acetylcholine and nicotine[34] modulate lipopolysaccharide -induced tumor necrosis factor release from microglia through the activation of acetylcholine receptors. Thus, it is possible that the decrease in cholinergic neurons during systemic inflammation decreases the availability of an "anti-inflammatory" signal in the brain. Although it is plausible that sepsis could interfere with the relationship between acetylcholine and delirium, we were unable to demonstrate any interaction between sepsis, AChE activity and the occurrence of delirium.

Serotonin is considered to have a role in the development of delirium due to its relationships with thought, perception, arousal level, learning and memory.[13-15] However, the role of serotonin in ICU delirium remains unclear. In addition to the evidence of the down-regulation of serotonin synthesis, there are also suggestions that serotonin levels are elevated during delirium.[14-16] Serotoninergic syndrome presents with symptoms that are similar to those of delirium,[14-16] and serotonin levels can be altered by drugs that are used in the ICU.[14,35,36] Additionally, increases in serotonin levels have been related to cholinergic deficiencies in experimental models.[37] In mechanically ventilated patients, both extremely low and extremely high levels of tryptophan have been associated with increases in the risk of delirium. Nevertheless, it remains unclear whether the symptoms of delirium are associated with the production of the neurotoxic metabolites of tryptophan, fluctuations in serotonin and melatonin levels, or both of these processes.[16] In contrast to these previous findings, our results do not support a role for serotonin in the prediction of the development of delirium.

There are some limitations to our study. First, this study may have lacked the statistical power to detect some clinically important associations. Because of the relatively small sample size, only a limited number of covariates could be incorporated into the regression models, but with a sample size of 38 patients per group, it was possible to identify a difference of 35% between the groups. Thus, although the negative results reported here could have been due to secondary to beta errors, we believe that a difference of less than 35% does not have biological significance in the development of the disease. Second, we examined only the baseline acetylcholinesterase activities and serotonin levels of samples acquired upon ICU admission. Examinations of the temporal patterns of these measurements may yield additional information. Additionally, the use of delirium duration and delirium-free days as outcome measures may lead to bias in cases of prolonged periods of coma because assessments of delirium cannot be performed in comatose patients.[38] Finally, we measured acetylcholinesterase

Table 3 - Variables associated with mortality

	Univariate analysis			Multivariate analysis	
	Survivors N = 57 (74%)	**Non-survivors N = 20 (26%)**	**p value**	**OR (CI95%)**	**p value**
Age (years)	52 (30)	64 (29)	0.034*	0.96 (0.92 - 1.0)	0.058
Male	41 (71.9)	12 (60)	0.402	-	-
APACHE II	17.5 (13.5)	22.5 (16.75)	0.307	-	-
SOFA score on admission	7 (5.25)	9 (5.5)	0.042*	0.98 (0.01 - 1.19)	0.800
Emergency surgery patients	9 (15.8)	2 (10)			
Elective surgery patients	9 (15.8)	5 (25)		-	-
Medical patients	39 (68.4)	13 (65)	0.587		
Mechanical ventilation (yes)	30 (56.6)	17 (89.5)	0.011*	7.2 (1.03 - 50.9)	0.047*
Sedation use	32 (57.1)	18 (94.7)	0.002*	-	-
Mental status at enrollment					
Comatose	25 (43.9)	6 (30)			
Delirious	2 (3.5)	0	0.335	-	-
Normal	30 (52.6)	14 (70)			
Reasons for admission					
Cardiovascular	8 (14.3)	2 (10)			
Postoperative	9 (16.1)	4 (20)			
Neurological	11 (19.6)	5 (25)			
Trauma	10 (17.9)	1 (5)			
Respiratory distress (excluding sepsis)	10 (17.9)	3 (15)	0.198	-	-
Sepsis	1 (1.8)	2 (10)			
Shock (excluding sepsis)	2 (3.6)	1 (5)			
Digestive	4 (7.1)	0			
Renal/metabolic	1 (1.8)	0			
Other	0	2 (10)			
Hospital length of stay (days)	18.5 (19.5)	18 (13.5)	0.981	-	-
Delirium (days)	1 (1)	1 (1)	0.535	-	-
Coma (days)	2 (6)	5 (7.5)	0.003*	-	-
Sepsis (yes)	21 (51.2)	14 (73.7)	0.159	-	-
Delirium/coma-free days	25 (6.25)	4 (17.25)	≤ 0.001*		
AChE (μmol AcSCh/h mg protein)	1.77 (1.24)	1.25 (1.56)	0.155	1.14 (0.66 - 1.96)	0.648
Serotonin (ng/mL)	3.06 (0.86)	2.98 (1.08)	0.959	1.54 (0.77 - 3.07)	0.225

OR - odds ratio; CI - confidence interval; APACHE - Acute Physiology and Chronic Health Evaluation; SOFA - Sequential Organ Failure Assessment; AChE - acetylcholinesterase enzyme. Univariate analysis: the data are presented as the median (IQR) or number (percentage). Multivariate analysis: Hosmer and Lemeshow goodness-of-fit - $\chi^2 = 4.11$, $p = 0.767$. * indicates a significant difference (i.e., p value ≤ 0.05).

activities and serotonin levels in the serum and not the cerebrospinal fluid or brain tissue, which precludes a definitive confirmation of a brain-specific effect. However, it has been demonstrated that blood serotonin levels are a reliable indicator of brain serotonin levels.[39] Additionally, serum acetylcholinesterase activity has been found to be involved in cognitive recovery following ischemia in an animal model, and serum acetylcholinesterase activity has been used to evaluate cognitive recovery in vascular dementia, suggesting that serum acetylcholinesterase activity reflects acetylcholine levels in the brain.[40]

CONCLUSIONS

In conclusion, neither serum acetylcholinesterase activity nor serum serotonin level was associated with the occurrence of delirium or acute brain dysfunction in critically ill patients, and sepsis did not modify the relationships of these biomarkers with the occurrence of delirium.

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RESUMO

Objetivo: Investigar se os níveis plasmáticos de serotonina e atividade de acetilcolinesterase determinados por ocasião da admissão à unidade de terapia intensiva preveem a ocorrência de disfunção cerebral aguda em pacientes internados em unidade de terapia intensiva.

Métodos: Foi conduzido no período entre maio de 2009 e setembro de 2010 um estudo prospectivo de coorte em uma amostra com 77 pacientes não consecutivos. A ocorrência de *delirium* foi determinada utilizando a ferramenta *Confusion Assessment Method for the Intensive Care Unit*, tendo sido determinadas as avaliações de acetilcolinesterase e serotonina em amostras de sangue coletadas até um máximo de 24 horas após admissão do paciente à unidade de terapia intensiva.

Resultados: No presente estudo, 38 pacientes (49,6%) desenvolveram *delirium* durante sua permanência na unidade de terapia intensiva. Nem os níveis de atividade de acetilcolinesterase nem os de serotonina tiveram associação independente com *delirium*. Não se observaram correlações significantes entre atividade de acetilcolinesterase e níveis de serotonina com o número de dias livres de *delirium*/coma, porém, em pacientes que desenvolveram *delirium*, ocorreu uma forte correlação negativa entre níveis de acetilcolinesterase e número de dias livres de *delirium*/coma, demonstrando que níveis mais elevados de acetilcolinesterase se associaram com menos dias de vida sem *delirium* e coma. Nenhuma associação foi identificada entre os biomarcadores e mortalidade.

Conclusão: Nem a atividade de acetilcolinesterase nem os níveis séricos de serotonina se associaram com *delirium* ou disfunção cerebral aguda em pacientes gravemente enfermos. A ocorrência de sepse não modificou esse relacionamento.

Descritores: Acetilcolinesterase/efeito de drogas; Sertonina/efeito de drogas; Delírio; Sepse; Unidades de terapia intensiva

REFERENCES

1. Ely EW, Gautam S, Margolin R, Francis J, May L, Speroff T, et al. The impact of delirium in the intensive care unit on hospital length of stay. Intensive Care Med. 2001;27(12):1892-900.
2. Ely EW, Shintani A, Truman B, Speroff T, Gordon SM, Harrell FE Jr, et al. Delirium as a predictor of mortality in mechanically ventilated patients in the intensive care unit. JAMA. 2004;291(14):1753-62.
3. Ouimet S, Kavanagh BP, Gottfried SB, Skrobik Y. Incidence, risk factors and consequences of ICU delirium. Intensive Care Med. 2007;33(1):66-73.
4. Fong TG, Tulebaev SR, Inouye SK. Delirium in elderly adults: diagnosis, prevention and treatment. Nat Rev Neurol. 2009;5(4):210-20.
5. Gunther ML, Morandi A, Ely EW. Pathophysiology of delirium in the intensive care unit. Crit Care Clin. 2008;24(1):45-65, viii.
6. Maclullich AM, Ferguson KJ, Miller T, de Rooij SE, Cunningham C. Unravelling the pathophysiology of delirium: a focus on the role of aberrant stress responses. J Psychosom Res. 2008;65(3):229-38.
7. Dantzer R. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. Eur J Pharmacol. 2004;500(1-3):399-411.
8. 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;18(8):801-6.
9. Papadopoulos MC, Davies DC, Moss RF, Tighe D, Bennett ED. Pathophysiology of septic encephalopathy: a review. Crit Care Med. 2000;28(8):3019-24.
10. Cerejeira J, Firmino H, Vaz-Serra A, Mukaetova-Ladinska EB. The neuroinflammatory hypothesis of delirium. Acta Neuropathol. 2010;119(6):737-54.
11. 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;63(7):764-72.
12. Han L, McCusker J, Cole M, Abrahamowicz M, Primeau F, Elie M. Use of medications with anticholinergic effect predicts clinical severity of delirium symptoms in older medical inpatients. Arch Intern Med. 2001;161(8):1099-105.
13. Koponen HJ, Lepola U, Leinonen E. A long-term follow-up study of cerebrospinal fluid 5-hydroxyindoleacetic acid in delirium. Eur Arch Psychiatry Clin Neurosci. 1994;244(3):131-4.
14. White S. The neuropathogenesis of delirium. Rev Clin Gerontol. 2002;12(1):62-7.
15. van der Mast RC, Fekkes D, Moleman P, Pepplinkhuizen L. Is postoperative delirium related to reduced plasma tryptophan? Lancet. 1991;338(8771):851-2.
16. Pandharipande PP, Morandi A, Adams JR, Girard TD, Thompson JL, Shintani AK, et al. Plasma tryptophan and tyrosine levels are independent risk factors for delirium in critically ill patients. Intensive Care Med. 2009;35(11):1886-92.
17. Cerejeira J, Batista P, Nogueira V, Firmino H, Vaz-Serra A, Mukaetova-Ladinska EB. Low preoperative plasma cholinesterase activity as a risk marker of postoperative delirium in elderly patients. Age Ageing. 2011;40(5):621-6.
18. Cerejeira J, Nogueira V, Luís P, Vaz-Serra A, Mukaetova-Ladinska EB. The cholinergic system and inflammation: common pathways in delirium pathophysiology. J Am Geriatr Soc. 2012;60(4):669-75.
19. Ely EW, Margolin R, Francis J, May L, Truman B, Dittus R, et al. Evaluation of delirium in critically ill patients: validation of the Confusion Assessment Method for the Intensive Care Unit (CAM-ICU). Crit Care Med. 2001;29(7):1370-9.

20. Gusmao-Flores D, Salluh JI, Dal-Pizzol F, Ritter C, Tomasi CD, Lima MA, et al. The validity and reliability of the Portuguese versions of three tools used to diagnose delirium in critically ill patients. Clinics (Sao Paulo). 2011;66(11):1917-22.
21. Sessler CN, Gosnell M, Grap MJ, Brophy GM, O'Neal PV, Keane KA, et al. The Richmond Agitation-Sedation Scale: validity and reliability in adult intensive care patients. Am J Respir Crit Care Med. 2002;166(10):1338-44.
22. Pandharipande PP, Sanders RD, Girard TD, McGrane S, Thompson JL, Shintani AK, Herr DL, Maze M, Ely EW; MENDS investigators. Effect of dexmedetomidine versus lorazepam on outcome in patients with sepsis: an a priori-designed analysis of the MENDS randomized controlled trial. Crit Care. 2010;14(2):R38. Erratum in: Crit Care. 2011;15(1):402.
23. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G; International Sepsis Definitions Conference. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Intensive Care Med. 2003;29(4):530-8.
24. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88-95.
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75.
26. Pavlov VA, Parrish WR, Rosas-Ballina M, Ochani M, Puerta M, Ochani K, et al. Brain acetylcholinesterase activity controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. Brain Behav Immun. 2009;23(1):41-5.
27. Flacker JM, Lipsitz LA. Serum anticholinergic activity changes with acute illness in elderly medical patients. J Gerontol A Biol Sci Med Sci. 1999;54(1):M12-6.
28. Mussi C, Ferrari R, Ascari S, Salvioli G. Importance of serum anticholinergic activity in the assessment of elderly patients with delirium. J Geriatr Psychiatry Neurol. 1999;12(2):82-6.
29. Van Eijk MM, Roes KC, Honing ML, Kuiper MA, Karakus A, van der Jagt M, et al. Effect of rivastigmine as an adjunct to usual care with haloperidol on duration of delirium and mortality in critically ill patients: a multicentre, double-blind, placebo-controlled randomised trial. Lancet. 2010;376(9755):1829-37.
30. Marcantonio ER, Palihnich K, Appleton P, Davis RB. Pilot randomized trial of donepezil hydrochloride for delirium after hip fracture. J Am Geriatr Soc. 2011;59 Suppl 2:S282-8.
31. Ofek K, Krabbe KS, Evron T, Debecco M, Nielsen AR, Brunnsgaad H, et al. Cholinergic status modulations in human volunteers under acute inflammation. J Mol Med (Berl). 2007;85(11):1239-51.
32. Abou-Hatab K, Nixon LS, O'Mahony MS, Newsway V, Patel S, Shale DJ, et al. Plasma esterases in cystic fibrosis: the impact of a respiratory exacerbation and its treatment. Eur J Clin Pharmacol. 1999;54(12):937-41.
33. Hubbard RE, O'Mahony MS, Calver BL, Woodhouse KW. Plasma esterases and inflammation in ageing and frailty. Eur J Clin Pharmacol. 2008;64(9):895-900.
34. Thomsen MS, Mikkelsen JD. The α7 nicotinic acetylcholine receptor ligands methyllycaconitine, NS6740 and GTS-21 reduce lipopolysaccharide-induced TNF-α release from microglia. J Neuroimmunol. 2012;251(1-2):65-72.
35. Del Angel-Meza AR, Dávalos-Marín AJ, Ontiveros-Martinez LL, Ortiz GG, Beas-Zarate C, Chaparro-Huerta V, et al. Protective effects of tryptophan on neuro-inflammation in rats after administering lipopolysaccharide. Biomed Pharmacother. 2011;65(3):215-9.
36. Sanders RD, Hussell T, Maze M. Sedation & immunomodulation. Crit Care Clin. 2009;25(3):551-70, ix.
37. Hirano H, Day J, Fibiger HC. Serotonergic regulation of acetylcholine release in rat frontal cortex. J Neurochem. 1995;65(3):1139-45.
38. Adams Wilson JR, Morandi A, Girard TD, Thompson JL, Boomershine CS, Shintani AK, et al. The association of the kynurenine pathway of tryptophan metabolism with acute brain dysfunction during critical illness. Crit Care Med. 2012;40(3):835-41.
39. Collins CM, Kloek J, Elliott JM. Parallel changes in serotonin levels in brain and blood following acute administration of MDMA. J Psychopharmacol. 2013;27(1):109-12.
40. Xiao Y, Guan ZZ, Wu CX, Li Y, Kuang SX, Pei JJ. Correlations between cholinesterase activity and cognitive scores in post-ischemic rats and patients with vascular dementia. Cell Mol Neurobiol. 2012;32(3):399-407.