

The Cholinergic System and Inflammation: Common Pathways in Delirium Pathophysiology

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OBJECTIVES: To investigate whether delirium is associated with an unbalanced inflammatory response or a dysfunctional interaction between the cholinergic and immune systems.

DESIGN: Cohort observational study.

SETTING: General hospital orthopedic ward.

PARTICIPANTS: One hundred one individuals aged 60 and older with no previous cognitive impairment undergoing elective arthroplasty.

MEASUREMENTS: Incidence of postoperative delirium, plasma cholinesterase activity (acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE)) and inflammatory mediators (C-reactive protein (CRP), interleukin (IL)-1 beta, tumor necrosis factor alpha, IL-6, IL-8, IL-10) before and after surgery.

RESULTS: Thirty-seven participants developed postoperative delirium and had greater production of CRP and pro-inflammatory to anti-inflammatory ratio after surgery. In participants with delirium, but not in controls, preoperative levels of plasma cholinesterase activity correlated with Δ CRP (AChE: $\rho = 0.428$, $P = .008$ and BuChE: $\rho = 0.423$, $P = .009$), Δ IL-6 (AChE: $\rho = 0.339$, $P = .04$), and Δ P/A ratio (AChE: $\rho = 0.346$, $P = .04$).

CONCLUSION: Delirium was associated not only with an unbalanced inflammatory response, but also with a dysfunctional interaction between the cholinergic and immune systems. Comprehensive understanding of the relationship between the cholinergic and immune systems is crucial to developing new insights into delirium pathophysiology and novel therapeutic interventions. *J Am Geriatr Soc* 2012.

Key words: delirium; inflammation; cholinesterases; C-reactive protein; cytokines

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Delirium is a common neuropsychiatric syndrome characterized by sudden onset of impaired consciousness and inattention with subsequent cognitive and behavioral disturbance.¹ From a pathophysiological perspective, this syndrome can be best conceptualized as a dysfunction in the homeostatic response to a precipitating factor (e.g., medical or surgical condition) resulting in an acute failure of the central nervous system (CNS). The two dominant theories regarding delirium pathophysiology are cholinergic deficiency and aberrant stress response and neuroinflammation.²

Surgery (e.g., hip arthroplasty) is a controlled traumatic event that directly induces tissue ischemia and destruction amid a diversity of factors such as hypotension, hypoxia, pain, blood loss, anesthetics, and drugs. Globally, the effect of these factors on the body elicits a number of intricate physiological responses characterized by the production and release of proinflammatory cytokines into the bloodstream, activation of the inflammatory cascade, and recruitment of immune cells. Protective immunity is critically dependent on adequate balance between pro- and anti-inflammatory responses to allow proper repair of tissue damage and to combat overwhelming infection without compromising organ function. Thus, counterregulatory mechanisms are simultaneously activated to inhibit the systemic innate immune response. These consist of anti-inflammatory cytokines (e.g., interleukin (IL)-10), stress hormones, and signals originating from the CNS culminating in the release of acetylcholine by the vagus nerve, which interacts with nicotine acetylcholine receptors in immune cells.³ Disruption of these complex homeostatic mechanisms may be involved in delirium pathophysiology as increased secretion of proinflammatory cytokines into the bloodstream triggers an exaggerated neuroinflammatory reaction, affecting synaptic and neuronal function and coupled with cognitive and behavioral symptoms similar to delirium.²

The few clinical studies conducted so far exploring the inflammatory response in delirium have provided contradictory results. Although high levels of C-reactive protein

(CRP) have been associated with delirium in medical⁴⁻⁶ and surgical⁶ patients, other studies have not supported these findings.⁷⁻¹¹ Likewise, negative findings^{8-10,12-14} have followed positive associations between delirium and higher plasma levels of IL-6,⁴⁻⁶ IL-8,^{12,15} and IL-10.⁷ High serum cytokines have also been found in sickness behavior independently of delirium occurrence (e.g., IL-6 and TNF- α)¹⁶ or postoperatively in individuals with impaired cognition.¹⁷

Thus, the relationship between the absolute levels of serum inflammatory mediators and delirium is inconsistent, heterogeneous, and difficult to generalize, compromising their clinical utility as biomarkers for this clinical syndrome. This also confirms that we are far from understanding to what extent the presence of inflammatory mediators in the bloodstream contributes to the development of neuropsychiatric symptoms leading to delirium. Additionally, although the cholinergic antiinflammatory pathway has been well characterized in animal models¹⁸ and implicated in inflammatory and infectious disorders,³ there is a lack of studies analyzing the interconnection between the cholinergic system and the innate immune response in individuals with delirium. A recent study demonstrated that individuals who develop postoperative delirium have substantially lower plasma cholinesterase activity before surgery, suggesting that an altered cholinergic function in resting conditions is a risk marker of delirium.¹⁹

The current study built on these findings and sought to clarify whether delirium is associated with an unbalanced inflammatory response or a dysfunctional interaction between the cholinergic and immune systems. It specifically addressed whether individuals with delirium would have an imbalance of proinflammatory to antiinflammatory cytokine concentration before or after surgery and a dysfunction in baseline plasma cholinesterases activity influencing the magnitude of the innate immune response to surgery.

MATERIALS AND METHODS

Preoperative Assessment

The study included consecutive individuals aged 60 and older undergoing elective total hip arthroplasty and not fulfilling the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision*, (DSM-IV-TR) criteria for dementia or delirium at the time of their preoperative medical evaluation, which included collection of demographic and clinical data and an assessment using the Charlson Comorbidity Index, Barthel Index, Mini-Mental State Examination (MMSE), and 15-item Geriatric Depression Scale.

Medical and Surgical Procedures and Perioperative Factors

Nonsteroidal anti-inflammatory and antiplatelet drugs were discontinued for at least 1 week before surgery, and thromboprophylaxis (subcutaneous low-molecular-weight heparin) was administered to each participant daily during that week until the day before surgery, with prophylactic intravenous antibiotherapy being initiated on the day of

surgery. General anesthesia was induced with propofol, and after the administration of a muscular relaxant, patients were intubated and mechanically ventilated. Alternatively, regional anesthesia (administration of analgesic medication (e.g., levobupivacaine) into the subarachnoid space through a catheter) was used. All patients were monitored during surgery, with fluid loss being replaced using an intravenous crystalloid solution and, if necessary, transfusion of red blood cells or whole blood. A lateral surgical approach with trochanteric osteotomy was used for the arthroplasty.

Postoperative Assessment

After surgery, all participants were assessed for delirium using the Confusion Assessment Method (CAM) (performed by a trained psychiatrist) on 3 consecutive days between 6 and 8 p.m. The first assessment occurred on the evening of the surgery day and the second and third took place on postoperative days 2 and 3. The CAM was rated during a brief interview with the participant that also included the MMSE. Positive cases of delirium (according to the CAM criteria) were confirmed according to the DSM-IV-TR criteria. The final sample consisted of 101 participants, 37 of whom developed delirium during the study period. Participants with delirium did not differ from those without on any preoperative or perioperative variable, except that they were less likely to consume alcohol regularly (Table 1).

Measurements of Serum Cholinesterase Catalytic Activities

Venous blood samples were collected from each participant in the morning of the day before surgery and in the morning of the first postoperative day. Plasma was immediately separated by centrifugation and stored at -80°C until analysis. Plasma acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity was assayed by measuring the production of thiocholine from the hydrolysis of the respective specific substrates acetylthiocholine iodide and S-butyrylthiocholine iodide. The production of 5-thio-2-nitrobenzoate was monitored using a Cary 100 Spectrophotometer (Varian, Cary, NC) at 412 nm over time (every minute for 8 minutes). Enzyme activity is expressed as μmol of 5,5'-dithiobis-(2-nitrobenzoic acid) transformed per mL of plasma per minute.¹⁹

Measurement of Inflammatory Mediators

The concentrations of five cytokines (IL-8, IL-1 beta (β), IL-6, IL-10, tumor necrosis factor alpha (TNF- α)) and CRP were determined using the xMAP Bio-Plex Suspension Array System 200 with multiplex assay kits purchased from Bio-Rad (Hercules, CA) and Invitrogen (Carlsbad, CA). Standard curves were constructed for determination of each analyte concentration according to the manufacturers' instructions. The detection limits of the assays were 0.002 ng/mL for CRP, 0.8 pg/mL for IL-1 β , 3 pg/mL for TNF- α , 1.1 pg/mL for IL-6, 0.5 pg/mL for IL-8, and 0.9 pg/mL for IL-10. In accordance with standard practice, samples with undetectable cytokine levels were entered at half of the minimum detection level derived from the

Table 1. Characteristics of the Analyzed Sample

Characteristic	Total Sample (N = 101)	Delirium (n = 37)	No Delirium (n = 64)	P-Value
Male, n (%)	50 (49.5)	15 (40.5)	35 (54.7)	.22*
Age, mean \pm SD (range)	73 \pm 6.3 (60–89)	73.6 \pm 5.9 (64–89)	72.7 \pm 6.5 (60–87)	.46 [†]
Education, years, n (%)				
0	20 (19.8)	8 (21.6)	12 (18.7)	.85*
1–4	69 (68.3)	24 (64.9)	45 (70.3)	
≥ 5	12 (9.9)	5 (13.5)	7 (10.9)	
Smoking, n (%)				
No smoking	82 (81.2)	30 (81.1)	52 (81.3)	>.99*
Past or active smoking	19 (18.8)	7 (18.9)	12 (18.7)	
Alcohol, n (%)				
No active drinking	46 (45.5)	24 (64.9)	22 (34.4)	.004*
Active drinking	55 (54.5)	13 (35.1)	42 (65.6)	
Charlson Comorbidity Index, mean \pm SD (range)	0.5 \pm 0.7 (0–4)	0.7 \pm 0.9 (0–4)	0.5 \pm 0.6 (0–3)	.36 [‡]
Mini-Mental State Examination score, mean \pm SD (range)	26.7 \pm 2.8 (19–30)	26.4 \pm 2.8 (19–30)	26.8 \pm 2.8 (19–30)	.38 [‡]
Barthel Index, mean \pm SD (range)	91.0 \pm 12.6 (45–100)	88.5 \pm 14.3 (50–100)	92.4 \pm 11.3 (45–100)	.15 [‡]
Geriatric Depression Scale, mean \pm SD (range)	4.5 \pm 3.2 (0–12)	4.6 \pm 3 (0–12)	4.4 \pm 3.2 (0–12)	.55 [‡]
Number of different medications taken by the participant outside the hospital during the week before the surgery and administered in the hospital until the day before surgery, mean \pm SD (range)	3.4 \pm 2.4 (0–9)	4.0 \pm 2.8 (0–9)	3.0 \pm 2.1 (0–9)	.11 [‡]
Anticholinergic activity of administered medications in the week before surgery as determined by the Anti-cholinergic Drug Scale, ²⁰ mean \pm SD (range)	0.7 \pm 1.1 (0–5)	0.8 \pm 1.3 (0–5)	0.6 \pm 0.7 (0–3)	.7 [‡]
General anesthesia, n (%)	68 (67.3)	29 (78.4)	39 (60.9)	.08*
Perioperative transfusion, n (%)	31 (30.7)	15 (40.5)	16 (25)	.08*

* Chi-square test.

[†] Student *t*-test.[‡] Mann Whitney test.

SD = standard deviation.

standard curve. Because IL-10 could be measured in all samples, plasma levels of each cytokine were used to calculate the proinflammatory to anti-inflammatory ratio (P/A) according to the formula (IL-1 β + TNF- α + IL-6 + IL-8)/ IL-10.

Statistical Analysis

Data were analyzed using the SPSS, version 17.0.2 (PASW Statistics 17, SPSS, Inc., Chicago, IL). The significance of the difference between the pre- and postoperative levels of each biomarker was determined using the Student *t*-test for paired samples or the Wilcoxon test as a nonparametric alternative. Comparison between participants with and without delirium for pre and postoperative values was performed using the Student *t*-test for independent samples (normal distributions) or the Mann Whitney *U*-test (non-normal distributions). The Pearson chi-square test was used to analyze the relationship between categorical variables. The association between preoperative cholinesterase activity and the difference between the two determinations within participants (end-point – baseline levels, Δ) for each inflammatory marker was assessed using the Spearman correlation coefficient. A type I (α) error probability of 0.05 was considered for all inferential analysis.

RESULTS

Plasma Cholinesterase Activity

Although surgery induced an overall reduction in AChE and BuChE plasma activity in the control and delirium groups, participants who developed delirium had substantially lower levels of plasma esterase activity before surgery than controls (Table 2).

Table 2. Pre- and Postoperative Activity Levels of Plasma Cholinesterases

Plasma Cholinesterase	Preoperative		Postoperative		
	Mean \pm SD	P-Value*	Mean \pm SD	P-Value*	P-Value [†]
Acetylcholinesterase					
Delirium	2.42 \pm 0.57	.01	1.88 \pm 0.46	.09	<.001
No delirium	2.71 \pm 0.51		2.04 \pm 0.45		<.001
Butyrylcholinesterase					
Delirium	4.79 \pm 1.28	.04	3.24 \pm 0.74	.03	<.001
No delirium	5.33 \pm 1.21		3.61 \pm 0.84		<.001

* Student *t*-test for independent samples.[†] Student *t*-test for paired samples.

SD = standard deviation.

Plasma Levels of Inflammatory Mediators

Preoperative levels of CRP, IL-1 β , and TNF- α were below the detection level in 8%, 21%, and 27% of participants, respectively. All participants who developed delirium presented preoperatively with detectable levels of CRP (0.002 ng/mL), whereas this marker was measurable in 87.5% of the remaining participants ($P = .02$). In the postoperative sample, CRP, IL-1 β , TNF- α , and IL-6 were not measurable in 4%, 28%, 44%, and 3% of participants, respectively. These percentages were similar in participants with and without delirium. The proportion of participants with CRP of 5 ng/mL or greater (a cutoff value used in clinical practice) was similar between the groups with and without delirium before surgery (10.8% vs 7.8%, $P = .72$) and on the first postoperative day (78.4% vs 81.2%, $P = .80$).

No differences were observed between the two groups regarding plasma levels of each mediator pre- or postoperatively (Table 3). Participants with delirium had a significantly higher P/A ratio on the first postoperative day (Table 3).

Correlation Between Baseline Cholinesterase Activity and Magnitude of Inflammatory Reaction (Δ)

Plasma activity of AChE and BuChE before surgery correlated with Δ CRP in participants who developed delirium ($\rho = 0.428$, $P = .008$, and $\rho = 0.423$, $P = .009$; Figure 1A and B, respectively) but not in controls ($\rho = 0.057$, $P = .65$ and $\rho = 0.085$, $P = .50$). Similarly, AChE activity correlated with Δ IL-6 ($\rho = 0.339$, $P = .04$; Figure 1C) and with Δ P/A ratio ($\rho = 0.346$, $P = .04$, Figure 1D) in participants

with delirium but not in those who did not developed delirium ($\rho = 0.028$, $P = .82$ and $\rho = 0.207$, $P = .10$, respectively). No correlations were found between baseline plasma cholinesterase activity and within-individual difference in each inflammatory mediator (data not shown).

DISCUSSION

It has been proposed that central cholinergic dysfunction and a deregulated neuroinflammatory reaction in response to systemic inflammation may be involved in delirium pathophysiology.^{2,21} In addition, the CNS modulates the innate immune response through the cholinergic output of the vagus nerve.³ It has recently been reported that individuals with delirium had lower plasma levels of cholinesterase activity than controls even before surgery, suggesting that lower plasma levels of cholinesterase activity may precede and even contribute to the development of an acute confusional state.¹⁹

The current study demonstrated that, when people were challenged with surgical trauma, production of CRP was significantly higher and that a shift toward a proinflammatory response (as shown by a greater P/A ratio) was observed in patients with delirium. This suggests that an imbalance between proinflammatory and antiinflammatory factors, in favor of a proinflammatory response, might be involved in delirium. Conversely, in agreement with several studies⁷⁻¹⁴ none of the plasma levels of each inflammatory marker measured in this study were related to delirium.

Although these findings are discordant with previous reports of a possible association between inflammatory

Table 3. Plasma Levels of Inflammatory Markers Before and After Surgery and within (Pre- and Postoperative) and Between (Delirium vs No Delirium) Groups

Inflammatory Marker	Preoperative		Postoperative		<i>P</i> -Value [†]
	Median (IQR)	<i>P</i> -Value*	Median (IQR)	<i>P</i> -Value*	
C-reactive protein					
Delirium	0.38 (1.17)	.86	18.31 (11.82)	.67	<.001
No delirium	0.51 (0.91)		15.89 (15.31)		<.001
IL-1 β					
Delirium	0.40 (0.55)	.57	0.4 (0.39)	.86	.90
No delirium	0.46 (0.61)		0.4 (0.38)		.05
Tumor necrosis factor alpha					
Delirium	6.12 (9.28)	.54	1.5 (5.01)	.98	.50
No delirium	6.2 (9.96)		1.5 (8.3)		.22
IL-6					
Delirium	7.95 (8.59)	.65	117.39 (148.07)	.14	<.001
No delirium	8.27 (8.49)		93.18 (99.25)		<.001
IL-8					
Delirium	9.16 (7.02)	.56	18.83 (17.3)	.14	<.001
No delirium	8.6 (6.51)		16.59 (11.25)		<.001
IL-10					
Delirium	1.81 (1.64)	.10	3.17 (3.23)	.97	<.001
No delirium	2 (1.37)		3.18 (3.85)		<.001
Pro-/anti-inflammatory ratio					
Delirium	12.44 (9.61)	.18	38.06 (27.13)	.049	<.001
No delirium	11.14 (5.71)		28.31 (26)		<.001

* Mann Whitney test.

† Wilcoxon test.

IL = interleukin.

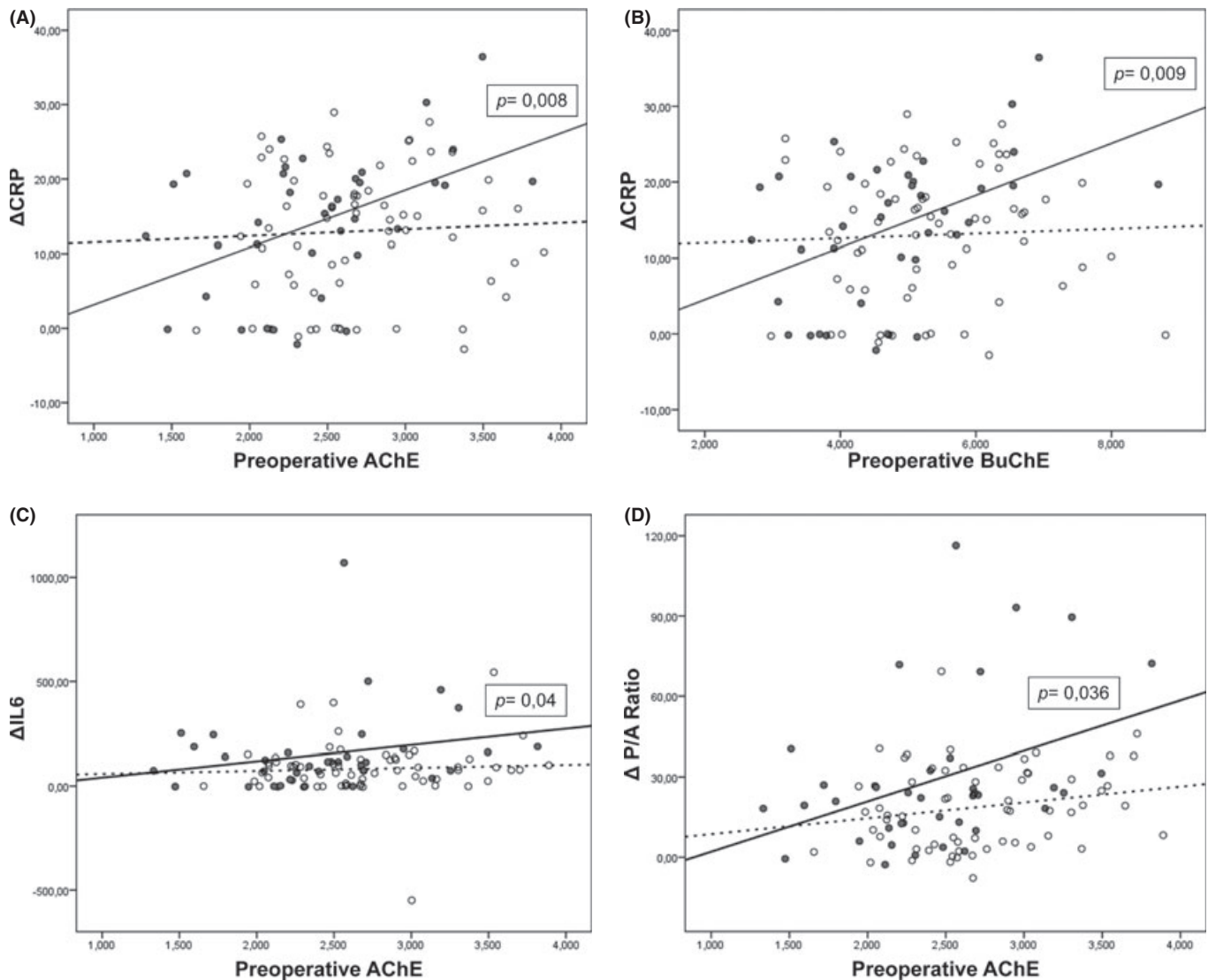


Figure 1. Scatter plots and Spearman correlation coefficients of significant association between preoperative plasma cholinesterase activity and inflammatory markers in participants who developed delirium (●—) and controls (○- -). Δ = end point minus baseline levels; P/A = proinflammatory/anti-inflammatory ratio.

markers and delirium^{4-7,12,15} it is likely that isolated levels of cytokines in the bloodstream do not accurately capture the complex homeostatic dysfunction occurring during a delirium episode. This is not surprising, considering that cytokines have pleiotropic and redundant functions within a complex network of various interactions between them.²² Consequently, the organism response to acute release of inflammatory mediators into the bloodstream depends more on the integrated actions of overlapping intracellular signaling pathways shared by different pathophysiological routes than on the level of a particular cytokine. P/A ratios might be a more valuable index of the association between systemic cytokine response and relevant clinical outcomes not only in coronary syndromes^{23,24} and sepsis,^{25,26} but also in delirium, as the current results suggest.

A dynamic and intricate cross-talk with the central nervous system and neuroendocrine systems tightly modulates immune system actions, including cytokine production and effects.²⁷ In particular, the inhibitory control of

the cholinergic anti-inflammatory pathway on the innate immune response is a recently described homeostatic mechanism maintaining proinflammatory responses within an adequate range.³ In response to systemic inflammation, the CNS stimulates vagus nerve outflow with the release of acetylcholine. Through inhibition of nuclear factor kappa B nuclear translocation and activation of janus kinase signal transducer and activator of transcription pathways, acetylcholine attenuates the production of TNF, IL-1 β , IL-6, and IL-18 immune cells expressing the nicotinic acetylcholine receptor subunit $\alpha 7$.¹⁸ Under resting conditions, the set point of this reflex arch contributes to establishing the magnitude of the innate inflammatory response to surgical trauma.³ Plasma cholinesterase activity, resulting from the conjoined action of peripheral AChE and BuChE, is responsible for the hydrolysis of acetylcholine, thus regulating peripheral cholinergic signaling.

A positive correlation was found between baseline plasma cholinesterase activity and high CRP, IL-6, and

P/A ratio after surgery in participants with delirium but not in controls. Thus, overactivity of the relationship between cholinergic and inflammatory systems emerges as a common feature in the participants with delirium, in whom exposure to surgery induced different degrees of inflammation according to baseline status of the peripheral cholinergic system (Figure 2). In contrast, the inflammatory response after surgery was independent of baseline cholinesterase activity in participants without delirium. It is plausible that a hyperactive cholinergic anti-inflammatory pathway may underlie the greater strength of correlation between plasma cholinesterase activity and the levels of inflammatory markers observed in individuals with delirium. The lack of correlation in the control group indicates a weaker relationship between the cholinergic and immune systems and the contribution of other factors, not addressed in this study, maintaining the inflammatory response tightly regulated. The detailed mechanisms by which the responsiveness of the cholinergic antiinflammatory pathway is regulated remain unclear. Recent evidence suggests that vagal reactivity and cholinesterase activity depend on the constitutive expression of acetylcholine receptors at the peripheral organs.²⁸

The results of the current study indicate that plasma cholinesterase activity can be a candidate biomarker of the inhibitory cholinergic tone at the periphery. When considering the group of participants with delirium, higher baseline cholinesterase activity was associated with heightened proinflammatory response, whereas participants with lower cholinesterase activity before surgery had a lower proinflammatory reaction. A definite conclusion cannot be reached about how AChE and BuChE activity levels correlate with peripheral levels of acetylcholine or with the responsiveness of vagal nerve to inflammation, but a dose-dependent positive correlation between cholinesterase activity and peripheral cytokine response to immune chal-

lenge, largely mediated by the vagus nerve, has been demonstrated.^{29,30} Also, the rate of acetylcholine degradation in peripheral organs critically determines the physiological effects of vagus nerve activity.^{31,32} It is therefore conceivable that lower plasma AChE and BuChE levels are associated with a greater supply of acetylcholine for ligation with cholinergic receptors in immune cells downregulating inflammation (Figure 2). This provides insight into the potential reasons for failure in previous attempts to modulate the cholinergic and inflammatory imbalance through administration of AChE inhibitors. Thus, in a recent randomized controlled trial, critically ill patients with delirium receiving a cholinesterase inhibitor (rivastigmine) had greater mortality than participants not treated with this drug.³³ Alternatively, recent experimental evidence shows that administration of AChE inhibitors suppresses systemic inflammation and enhances survival of animals exposed to lipopolysaccharides^{29,30} or infection.³⁴ These conflicting results demonstrate that any modulation of the cholinergic system should take into account the complex regulatory mechanisms determining the participant's baseline immune set point and the magnitude of responsiveness to inflammatory challenge.

An obvious strength of this study is the use of a prospective design in which several markers were simultaneously measured before and after a controlled traumatic event (hip arthroplasty) in a well-characterized sample of participants with no prior cognitive impairment, although this limits the generalization of the results to clinical samples more likely to benefit from interventions to prevent and treat delirium. The technology used in this study (multiplex arrays) has a number of advantages over traditional enzyme-linked immunosorbent assay (ELISA), offering the possibility of analyzing a panel of cytokines in a cost-effective fashion. Nevertheless, although some studies in delirium have used multiplex technology,^{8,12,14,15} experience with this method

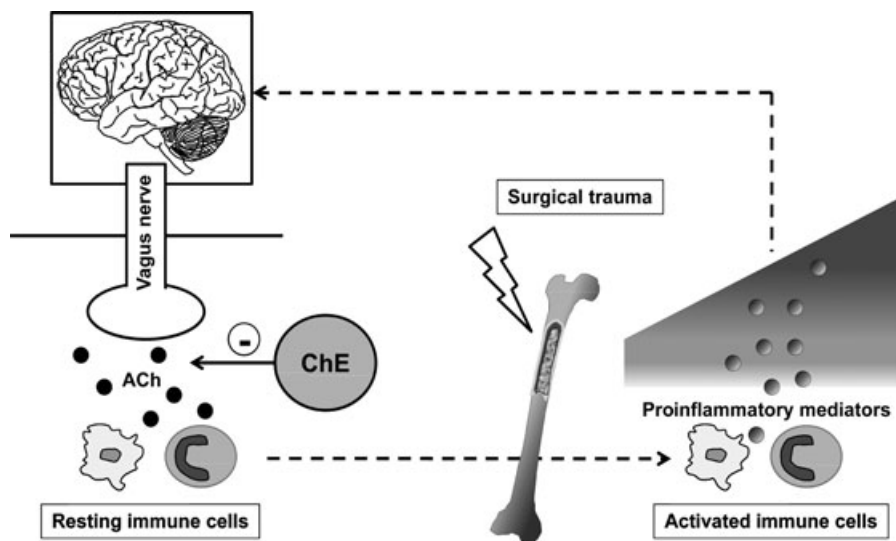


Figure 2. Relationship between the cholinergic and inflammatory systems in delirium. At the periphery, the cholinergic anti-inflammatory pathway modulates the immune system through the interaction of acetylcholine (ACh) with immune cells. In baseline conditions, acetylcholine availability depends on vagus nerve output and its degradation by cholinesterases (ChE). In patients with delirium, exposure to surgical trauma induced different degrees of inflammation according to the baseline status of the peripheral ChE activity, suggesting that delirium is associated with an overactive relation between cholinergic and inflammatory systems.

remains limited, and comparisons with prior studies using ELISA should be made cautiously because concordance between the two methods has not been definitively established.³⁵ Although the assay used in the current study provides a lower limit of detection of IL-1 β than previous studies, the levels of this cytokine, as in the case of TNF- α , were undetectable in a significant proportion of samples, and no changes were observed after surgery, confirming that these potent proinflammatory cytokines, which have brief half-lives in circulation, are unlikely to be useful markers of acute injury or illness.³⁶ Because the postoperative blood draw and the clinical assessment of delirium were not coincident in time, the observed changes in postoperative cytokine levels may not reflect the status of the immune system during the delirium episode.

In conclusion, delirium is associated with an inflammatory imbalance favoring the production of proinflammatory cytokines. Furthermore, changes in the cholinergic system, observed preoperatively, predicted the magnitude of the inflammatory response to surgical trauma. Thus, this study strengthens the view that a comprehensive understanding of the relationship between the cholinergic and immune systems is crucial to yielding new insights into delirium pathophysiology.

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Conflict of Interest: The authors declare that they have no competing conflict of interest in relation to the work described.

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