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Associations between Markers of Inflammation and Cholinergic Blockade and Delirium in Intensive Care Unit Patients: A Pilot Study

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Abstract The purpose of this study was to determine the associations of serum C-reactive protein (CRP) and plasma anticholinergic activity (PAA) with delirium in critically ill and injured patients. Prospective cohort study of 32 patients admitted a university-based intensive care unit. All patients were evaluated for delirium with the Confusion Assessment Method for the Intensive Care Unit, and blood was collected for measurement of serum CRP and PAA. These biomarkers and other factors, including patient demographics, intubation, and Acute Physiology and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) scores were compared between patients who developed delirium and those who did not. Furthermore, the levels of serum CRP and PAA were examined for correlation with each other. Intubated and mechanically ventilated patients were more likely to be delirious than non-intubated patients ($p < 0.001$). The APACHE II and SOFA scores were significantly higher in delirious patients than in non-delirious patients ($p = 0.007$ and $p = 0.04$, respectively). Serum CRP levels were significantly higher in intubated patients ($p < 0.02$) as were PAA levels ($p = 0.001$). A moderately strong correlation was found between serum CRP and PAA in the enrolled patients (Spearman's rho = 0.65, $p<0.0001$). Inflammation and cholinergic blockade, as measured by serum CRP and PAA levels, respectively, were associated with delirium in this study of critically ill patients, and serum CRP and PAA were correlated with each other.

Key words: delirium, inflammation, C-reactive protein (CRP), acetylcholine, Confusion Assessment Method for the Intensive Care Unit (CAM-ICU)

Introduction

The development of delirium in critically ill patients is associated with significant morbidity and mortality; in fact, this common complication is an independent predictor of mortality in mechanically ventilated patients in the intensive care unit (ICU).[1] Though many risk factors for delirium have been identified, including patient characteristics such as age and preexisting cognitive impairment as well as factors of the acute illness itself such as sepsis and hypoxemia,[2] the mechanisms leading to delirium in the ICU have not been fully elucidated. Such knowledge could substantially contribute to patient care in the ICU by directing interventions to prevent and treat delirium in this vulnerable population.

Inflammation is now known to play a central role in the pathogenesis of organ failure resulting from critical illness, and an association between delirium and serum C-reactive protein (CRP)-an inflammatory marker routinely used in Japan to evaluate the degree of inflammation or infection-has been found in several studies.[3][4] Serum CRP was increased in postoperative elderly patients and significantly and independently increased in the patients with impaired mental status.[4]

In addition to inflammation, several neurotransmitter derangements have been implicated in delirium. Three of the neurotransmitter systems purportedly involved in the pathophysiology of delirium are dopamine, g-aminobutyric acid (GABA) and acetylcholine,[5] the latter having been studied using a competitive binding radionuclide assay that determines serum or plasma anticholinergic activity (PAA), a measure of cholinergic blockade.[6] The reduced cholinergic neurotransmission in the brain is indirectly demonstrated by higher levels of PAA. Several non-ICU studies have found a strong association between PAA and the development of delirium.[7][8][9]

In this observational study, we sought to test the primary hypothesis that inflammation (as measured by CRP) is associated with delirium as well as the secondary hypotheses that cholinergic blockade (as measured by PAA) is associated with delirium and that inflammation is associated with cholinergic blockade.

Methods

Patients

The study population included both mechanically ventilated and non-ventilated adult ICU patients admitted from April 1, 2005 to July 31, 2007 to the Advanced Medical Emergency & Critical Care Center at Yamaguchi University Hospital. Critically ill and injured patients transported by ambulance are admitted to this Level 1 trauma center. The institutional review board approved this study, and informed consent was obtained from the patients or their families. Patients expected to stay in the ICU for more than 72 hours were eligible for the study. Exclusion criteria defined *a priori* included expected persistent disturbed consciousness (e.g., due to anoxic encephalopathy, brain stem hemorrhage, head trauma), age less than 19 years old, history of chronic dementia, psychosis, mental retardation, neuromuscular disease, and ongoing treatment with antipsychotics or morphine at the time of enrollment. Morphine treatment in our ICU typically indicates limitation or withdrawal of life support.

Data collection and study design

Information collected prospectively at the time of enrollment included patient demographics, clinical diagnoses, Acute Physiology and Chronic Health Evaluation (APACHE) II score,[10] and Sequential Organ Failure Assessment (SOFA) score.[11][12] Patients were followed throughout their ICU stay, and duration of mechanical ventilation, medication exposure, ICU length of stay, and ICU mortality were recorded.

An intensivist (RT) who had received training at Vanderbilt University Medical Center in the administration of the Confusion Assessment Method for the ICU (CAM-ICU)[13][14] and had translated the CAM-ICU in Japanese (see downloadable translation[15]) assessed sedation level via the Richmond Agitation Sedation Scale (RASS)[16][17] and diagnosed delirium using the CAM-ICU. The intensivist conducted delirium assessments among the non-intubated patients using the CAM-ICU

during the first 5 days of their ICU stay in accordance with methods used by previous investigators.[18] The evaluation was held daily between 8 am and 11 am. Because intubated patients were typically not assessable during the first 5 days due to coma (RASS scores of -4 or -5), assessments were conducted within 2 hours of extubation and continued for 5 consecutive days thereafter (Table 1). During the study period, the patients recognized as CAM-ICU positive were defined as delirious. No sedation protocol was followed at the time of the study. The sedative (initially midazolam or propofol) was chosen by the attending physician and was occasionally switched to dexmedetomidine within 24 hours of extubation.

Serum CRP was measured as part of routine clinical care by immunoturbidimetry within 24 hours after admission for non-intubated patients and within 24 hours of extubation for intubated patients. Blood samples were collected at the same time for PAA and stored at −80℃. SOFA scores were calculated at the time of blood collection. PAA was measured using the competitive binding radionuclide assay described by Tune and Coyle[6] at the Panapharm Laboratories Co., Ltd. (Uto, Kumamoto, Japan). PAA levels were quantified by the degree to which the subject's plasma inhibited binding of an acetylcholine analogue, tritiated quinuclidinyl benzilate ([^{3}H]-QNB), to rat forebrain membranes, and were expressed as picomole atropine equivalent/ml plasma (pmol/ml). All samples were run in the same batch and measured in duplicate. The minimum detectable quantity was 1.95 pmol/ml. When the mean [^{3}H]-QNB counts of 0 standard atropine were more than the maximum [^{3}H]-QNB counts of the patient sample or the minimum [^{3}H]-QNB counts of 0 standard atropine were more than the mean [^{3}H]-QNB counts of the patient sample, the PAA levels were expressed as 1.95 pmol/ml. In the other undetectable cases, they were expressed as 0 pmol/ml.

Statistical analysis

Data are represented as the mean ± standard deviation or median (interquartile range). Statistical analyses were conducted using SPSS software package ("Dr." SPSS II, Tokyo, Japan). We performed univariate analyses in which the data were compared between delirious and non-delirious patients using the Mann-Whitney U test for continuous variables and [chi]^2 test for categorical variables. Spearman's rank correlation was used to analyze the correlation between serum CRP levels and PAA levels. Significance was accepted at a value of $p < 0.05$.

Results

Patient characteristics

The baseline characteristics of the enrolled patients are presented in Table 2. Sixteen patients were intubated and mechanically ventilated, whereas 16 patients were non-

Table 1 Delirium assessment protocol

	24 to 2 hours before extubation (Day 0)	In 24 hours after extubation (Day 1)	Day 2	Day 3	Day 4	Day 5
Intubated patients	B, C, S	B, C, S	C	C	C	C
		In 24 hours after admission (Day 0)	Day 1	Day 2	Day 3	Day 4
Non-intubated patients		B, C, S	C	C	C	C

B, blood collected for serum C-reactive protein (CRP) and plasma anticholinergic activity (PAA); C, confusion assessment method for the ICU (CAM-ICU); S, sequential organ failure assessment (SOFA) score.

Table 2 Clinical characteristics of non-intubated or intubated patients

Variable	Non-intubated (n = 16)	Intubated (n = 16)
Age (yrs)	60 ± 14	57 ± 15
Gender, % male	100	75
Diagnosis		
Trauma	12	7
Infection	1	3
Cardiovascular	1	2
Other	2	4
APACHE II score	11 ± 7	17 ± 6
SOFA score	3 ± 2	5 ± 2
Use of sedatives & analgesics	1	15
Operation	0	10
Blood purification	1	5
Use of steroids	1	1
Use of catecholamine	0	3
Length of ventilator (days)	0	7 ± 5
Length of ICU stay (days)	10 ± 5, 8 (7-13)	19 ± 18, 13 (11-20)
Mortality in ICU	0	0
Serum CRP (mg/dl)	5.5 ± 7.1, 3.4 (0.3-7.8)	11.9 ± 6.6, 10.9 (6.4-17.1)
PAA (pmol/ml)	2.3 ± 1.5	5.9 ± 3.4

Values are mean ± SD or median (interquartile ranges). CRP, C-reactive protein; PAA, plasma anticholinergic activity.

Table 3 Clinical characteristics of patients with no delirium or delirium

Variable	No delirium (n = 19)	Delirium (n = 13)	*p* Value
Age (yrs)	59 ± 13	57 ± 16	0.94
Gender, % male	89	85	1.66
Intubated patients	3	13	<0.001
Use of sedatives & analgesics	3	13	<0.001
Operation	3	7	0.06
Blood purification	3	3	1.67
Use of steroids	1	1	1.69
Use of catecholamine	0	3	0.12
Infection	3	8	0.02
APACHE II score	11 ± 7	18 ± 6	0.007
SOFA score	3 ± 2	5 ± 2	0.04
Serum CRP (mg/dl)	6.7 ± 7.8 4.7 (0.4-10.3)	11.6 ± 6.2 11.0 (7.2-17.2)	0.02
PAA (pmol/ml)	2.9 ± 2.4 2.0 (2.0-4.0)	5.8 ± 3.5 5.8 (2.3-8.3)	0.005

Values are mean ± SD or median (interquartile ranges). CRP, C-reactive protein; PAA, plasma anticholinergic activity.

intubated. Trauma was the most common admission diagnosis. None of the patients died in the ICU. Serum CRP and PAA levels were measured twice before and after extubation. All patients who developed delirium did so on study day 0 or 1, and the blood samples on the day of diagnosis were utilized. For patients who did not develop delirium,

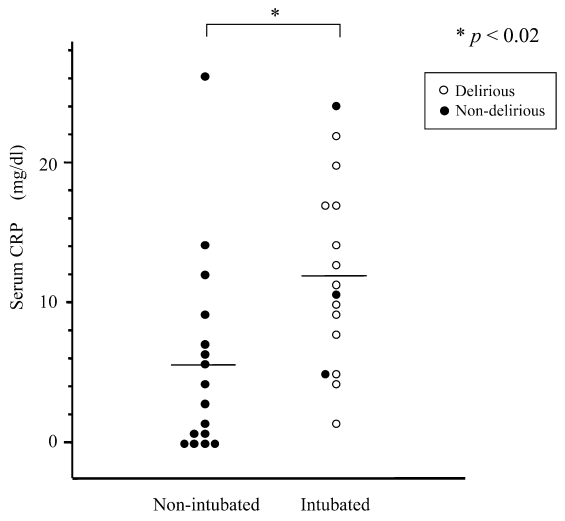

Fig. 1 Serum C-reactive protein levels in intubated and non-intubated patients. Serum CRP levels were significantly higher in intubated patients than in non-intubated patients [10.9 (6.4-17.1) vs. 3.4 (0.3-7.8) mg/dl, $p < 0.02$]. The horizontal line is a mean value.

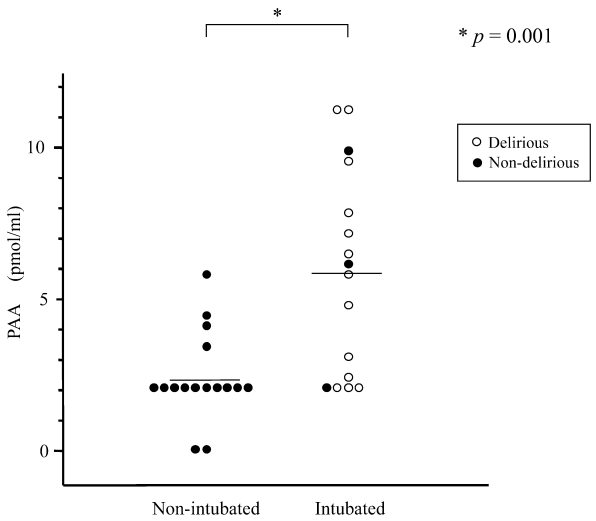

Fig. 2 Plasma anticholinergic activity levels in intubated and non-intubated patients. PAA levels were significantly higher in intubated patients than in non-intubated patients [5.9 (2.3-8.8) vs. 2.0 (2.0-2.6) pmol/ml, $p = 0.001$]. The horizontal line is a mean value.

blood samples from study day 0 were utilized. The SOFA scores were also calculated on the Day 0.

Thirteen of 16 intubated patients (81%) developed delirium, but none of the non-intubated patient developed delirium ($p < 0.001$, Table 3). All of the delirious patients received sedation, whereas only 3 intubated patients (16%) who did not become delirious received sedation ($p < 0.001$). Eleven patients had infections (sepsis in 4, pneumonia in 4, intraabdominal in 2, and soft tissue in 1), and delirious patients were significantly more likely to have had infection than non-delirious patients ($p = 0.02$). APACHE II and SOFA scores were significantly higher in delirious patients than in non-delirious patients ($p = 0.007$ and $p = 0.04$, respectively).

Inflammation and cholinergic blockade

Serum CRP levels were significantly higher in intubated patients than in non-intubated patients [10.9 (6.4-17.1) vs. 3.4 (0.3-7.8) mg/dl, $p < 0.02$, Fig. 1]. PAA levels were also significantly higher in intubated patients than in non-intubated patients [5.9 (2.3-8.8) vs. 2.0 (2.0-2.6) pmol/ml, $p = 0.001$, Fig. 2].

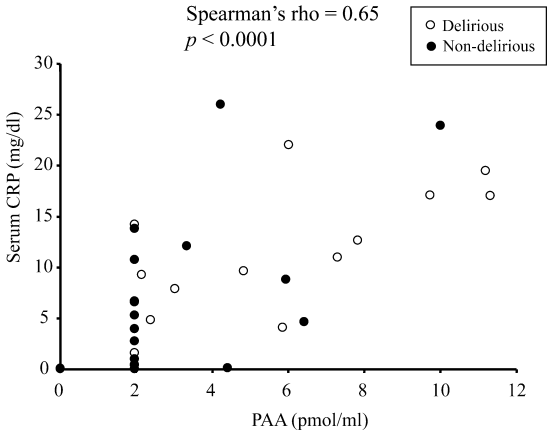

Fig. 3 The correlation between serum C-reactive protein and plasma anticholinergic activity levels in the patients (n = 32). PAA was significantly correlated with serum CRP (Spearman's rho = 0.65, $p<0.0001$).

A moderately strong correlation was found between serum CRP and PAA within the entire study population (n = 32, Spearman's rho = 0.65, $p < 0.0001$, Fig. 3) and within the subgroup of delirious patients (n = 13, Spear man's rho = 0.64, $p = 0.02$).

Discussion

In our study, serum C-reactive protein and plasma anticholinergic activity (PAA) were higher in patients with ICU delirium than in those without delirium, and a moderately strong correlation between these biological markers was noted. Thus, our data suggest that inflammation and cholinergic blockade may be part of the same or related pathophysiologic mechanisms leading to delirium rather than being independent pathways.

Several studies have demonstrated a strong association between PAA level and delirium in medical and surgical patients.[7)8)9)] Although this may result from exposure to anticholinergic medications, including sedatives, cholinergic blockade may be caused by the critical illness itself; acute illness may lead to the development of endogenous anticholinergic substances that can promote delirium.[19)] Accordingly, we investigated the relationship between PAA and inflammation during critical illness. Our data showed significantly higher levels of PAA and serum C RP—a summary measure of inflammation—in delirious patients and good correlation between PAA and CRP. Thereby, our data connect two central theories of delirium pathogenesis, systemic inflammation and acetylcholine imbalances,[5)] suggesting that one or more of the numerous molecules released as part of the inflammatory response during critical illness may have anticholinergic activity that alters neurotransmission in the brain, causing delirium.

Patients admitted to the ICU after traumatic injury, as were most patients in our study, are likely to have less comorbid illness than patients admitted to a medical and/or surgical ICU, which may explain the absence of delirium in the non-intubated patients we studied. The rate of delirium among the intubated patients, however, was similar to that reported in other studies examining mechanically ventilated patients, regardless of whether their critical illness was trau matic, medical, or surgical in nature.[1))13)18)20)21)] Not surprisingly, intubated patients had significantly higher APACHE II and SOFA scores than non-intubated patients, and length of ICU stay was longer for intubated patients.

There are several limitations to our study. Because we conducted this investigation as a pilot study, the sample size was small and we were limited in statistical power and could not perform multivariable regression to adjust for potential confounders according to the rule of thumb that a multivariable model must fit no more than m/10 parameters for it to be reliable on future similar patients, where m is the effective sample size (e.g., the number of patients with delirium in the current study).[22)] Mechanical ventilation and sedation are associated with high severity of illness on admission (i.e., APACHE II score) and multiple organ dysfunction (i.e., SOFA score), both of which may be associated with elevated serum CRP and PAA levels. We were not able to distinguish the independent effects of inflammation and cholinergic blockade on the brain from the effects of severity of illness, mechanical ventilation, or sedation. Pandharipande et al. have reported that lorazepam[23)] and midazolam,[24)] which is frequently used in Japan, are independent risk factors for daily transition to delirium. There are alternations in drug metabolism in critically ill patients and anticholinergic side effects involving these sedatives. Additionally, anticholinergic medications prescribed in the ICU were not investigated in this study, so we cannot determine whether cholinergic blockade was associated with exposure to such medications. Sedatives, antiarrhythmics, antihistamines, and other drugs have anticholinergic activity, and medications with few or no anticholinergic effects may be less likely to promote delirium in the ICU. This may be one reason that Pandharipande and colleagues recently found that mechanically ventilated patients sedated with dexmedetomidine-an α_2-adrenaergic receptor agonist-experienced more days without delirium or coma than those sedated with lorazepam.[25)] Short-term use of dexmedetomidine is allowed in Japan, and the effects of switching to it from conventional sedatives shortly before extubation are unknown. Finally, our results may be limited to less severely ill ICU patients; none of the patients enrolled in our study died in the ICU.

Conclusion

Inflammation and cholinergic blockade, as measured by serum CRP and PAA levels, respectively, were associated with delirium in this pilot study of critically ill patients, and serum CRP and PAA were correlated with each other. Future research is needed to determine whether these potential mechanisms of delirium in ICU patients are part of one pathophysiologic mechanism of brain dysfunction in this vulnerable population of patients.

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