BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-031212
Article Type:	Research
Date Submitted by the Author:	22-Apr-2019
Complete List of Authors:	Adam, Elisabeth; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt, Haas, Victoria; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt Lindau, Simone; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt Zacharowski, Kai; University Hospital Frankfurt, Clinic of Anesthesiology, Intensive Care Medicine and Pain Therapy Scheller, Bertram; Evangelisches Krankenhaus Düsseldorf
Keywords:	Postoperative delirium, Cardiac surgery < SURGERY, Cholinesterease, Acetylcholinesterase, Butyrylcholinesterase

Preoperatively decreased acetylcholinesterase is associated with delirium after cardiosurgery

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Short title: Cholinesterases and postoperative delir

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EA: wrote the manuscript, analyzed and interpreted the data

VH: conceived the study idea and collected data

SL: collected data, provided critical feedback and contributed to the final version of the manuscript

KZ: supervised the project and contributed to the final version of the manuscript

BS: conceived the study idea, analyzed the data and contributed to the final version of the manuscript

All authors read and approved the final version of the manuscript.

Author Disclosure Statement

The authors have reported no conflicts of interest.

Word count

2778

Data statement

Data will be made available via Dryad upon acceptance of the manuscript

Abstract

Objectives

Postoperative delirium (POD) is a common complication after elective cardiac surgery. Recent evidence indicates that a disruption in the normal activity of the cholinergic system may be associated with delirium.

Design

Prospective observational study

Setting

Single-center at a European academic hospital.

Primary and secondary outcome measures

In our study the enzyme activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were determined preoperatively as well as on the first and second postoperative day. The confusion assessment method for the intensive care unit (CAM-ICU) was used to screen patients for the presence of POD.

Results

A total of 114 patients were included in the study. POD was associated with a decrease in BChE activity on postoperative day one. In addition, patients who developed POD, had significantly lower preoperative AChE activity than patients without POD. Multivariate analysis identified a preoperatively decreased AChE activity, anticholinergic treatment, elevated EuroSCORE and age to be independently associated with the development of POD.

Conclusions

We conclude that a reduction in the acetylcholine hydrolyzing enzyme activity in patients undergoing cardiac surgery may correlate with the development of POD.

Strengths and limitations of this study

- One strength of this study results from the prospective nature
- Another strength is the data acquisition from a high-volume center
- A limitation is the exclusive inclusion of cardiac surgery patients. Whether these data can be extrapolated to other patient cohorts remains unclear and warrants further validation.
- It is known that delirium can fluctuate strongly and occur acutely during the course of the day. In this study, only one assessment was performed in the morning of the day of measurement. Thus, it is possible that not all patients who developed a delirium were detected with the applied screening method.

Keywords

Postoperative delirium

Cardiac surgery

Cholinesterase

Acetylcholinesterase

Butyrylcholinesterase

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Introduction

A delirium is a complex neuropsychiatric syndrome that is clinically characterized by sudden onset and fluctuating course and may influence disorders of consciousness as well as cognition in all its aspects. According to the criteria of the ICD-10 classification for Mental and Behavioural Disorders, a delirium is characterized as an etiologically unspecific cerebro-organic syndrome by the presence of disorders of consciousness and at least two of the following areas simultaneously: attention, perception, thinking, memory, psychomotor activity, emotionality or sleep-wake rhythm. The duration of delirium varies greatly and the severity ranges from mild to severe. [1]

The causes for delirium are multifactorial. Risk factors include dehydration, sleep deprivation, hypoxia, intoxication, anemia and hypoglycemia. In the general population, the incidence is below 0.4%, in hospitalized patients between 15-22%.[2 3] Particularly after surgical interventions, patients are at risk of developing postoperative delirium (POD). The incidence is described to be as high as 52%.[4] The consequences of a POD are very different and range from prolonged hospital stay, increased risk of wound infections, reduced quality of life, more frequent discharge into nursing homes to increased mortality in the first year after surgery.[5-8]

Higher age, longer duration of surgery as well as a reduced preoperative cognitive condition are frequently found in cardiac surgery patients and increase the risk for development of POD in this group of patients.[3] In the literature, the incidence of POD after cardiac surgery varies from 8 to 52%.[3 4 8 9] The duration of the POD in such patients varies widely, on average the POD lasts up to three days.[5 10] Patients with POD are at risk for developing chronic postoperative cognitive dysfunction (POCD) over time and for suffering from severe long-term cognitive deficits.[11]

There are different hypotheses about the molecular mechanisms involved in the development of delirium. The most common hypothesis for the development of POD are based on a central cholinergic deficit resulting from a deficit of Acetylcholine (ACh). Pathologies at the

presynapse, in the synaptic cleft or at the postsynaptic receptor may trigger a central cholinergic deficit. Acetylcholinesterase (AChE) is an enzyme which cleaves ACh in the synaptic cleft and terminates the transmission of a stimulus, a prerequisite for generating a new impulse. If the AChE is restricted in its function ACh remains in the synaptic cleft and blocks a new stimulus transmission.[12] However, several authors have found data challenging this hypothesis as they did not identify an association of preoperative serum anticholinergic activity with the development of POCD[13] or a therapeutic effect of rivastigmine for the prevention of POD.[14]

Butyrylcholinesterase (BChE) is an enzyme which splits choline compounds as well as other esters.[15] For a long time BChE was thought to have a less important function, but recent literature demonstrated that BChE may in part and with a significantly slower rate and affinity act as a substitute in the absence of AChE. A recently published study identified a significant decrease in the enzyme activity of AChE and BChE in patients with POD after hip surgery.[16] The impact of a choline esterase deficit in patients undergoing cardiac surgery remains unclear, however.

Due to the far-reaching consequences of a POD, it is of great importance to identify patients at risk for the development of such disorder. Our study investigated the extent to which changes in bed-side enzyme activity of cholinesterases correlates with the development of POD in cardiac surgery patients and to identify possible factors influencing the development of POD.

Material and methods

This manuscript includes data gained during a prospective observational study at the first author's institution. The institutional review board approved the conduct of the study prior to its initiation (428/12 of 19 December 2012).

Patients were included between February 2013 and February 2014. Over this period, 150 patients who received elective cardiac surgery at the authors' institution were screened for inclusion. The participating patients were informed about the study verbally and in writing. Only patients with written consent were included in the study.

Potential patients had to meet the following inclusion criteria: elective cardiac surgery with and without the use of a cardiopulmonary bypass (CPB) and age over 18 years. Exclusion from the study was based on: preoperatively existing delirium; preoperatively sedated patients with Richmond Agitation and Sedation Scale (RASS) < -2; no proficiency of the German or English language or missing patient consent.

After obtaining consent, patients were examined preoperatively and on the first and second postoperative day. Patients were examined for the presence of a POD using the confusion assessment method for the intensive care unit (CAM-ICU) clinical test[17]. In brief, the CAM-ICU assesses and scores clinical features associated with delirium. Further, blood samples were analyzed AChE and BChE activity as measured with the ChE Check mobile ® (Securetec Detektions-Systeme AG, Neubiberg, Germany). Depending on the results from CAM-ICU, a patient was assigned to either the postoperative delirium group (POD) or the no postoperative delirium (no POD) group. The patient was assigned to the PDO group if a delirium was diagnosed at least once as per the CAM-ICU. If a patient was either under too much sedation or the examiner was not able not make apply the CAM-ICU, the patient was not included for analysis.

Assessment of parameters

Both, BChE and AChE activity were assessed using the ChE Check Mobile® as per the manufacturer's instruction. Preoperatively, blood samples were drawn from the fingertip (10µL). Postoperatively, blood samples (1mL) were obtained via an arterial line. As two enzymes were determined in different measurements, two blood samples were taken at different times and analyzed independently.

Data collection

Basic demographic data, medication, hospitalization period, the length of stay on the intensive care unit, ventilation time as well as postoperative medication, transfusion, information about secondary diagnoses, weight, EuroSCORE, laboratory values as well as obtained scores were extracted from the patient data management system. The duration of anesthesia, intraoperative medication, aortic clamping time (APC) and the duration of CPB were extracted from the anesthesia and premedication protocols. The data and results were inserted and maintained in an Excel database.

Statistics

All data were tested for normality using the D'Agostino and Pearson omnibus normality test. Data comparisons of patient characteristics were made using Mann-Whitney U- or $\chi 2$-test, where applicable. To compare activities of cholinesterases between different days, a Wilcoxon signed rank test was used. Univariate analysis was performed using the $\chi 2$-test. Non binary-parameters were stratified by the median. Parameters with a p-value less than 0.1 were included for multivariable analysis, as carried out by binary logistic regression.

Length of ventilation was defined as the time of intubation until extubation; length of stay on the intensive care unit (ICU) was defined as the time from surgery to the discharge from the postoperative ICU; length of stay in the hospital was defined as the time from surgery to discharge from the primary care hospital. For survival analysis, groups were compared using a log rank test

and pointwise 95% confidence intervals (CI). A multivariable Cox's proportional hazards regression backward stepwise model (likelihood ratio) was performed to find independent predictors for outcome parameters.

Results with $p<0.05$ were considered to be statistically significant. All calculations/analyses were performed with SPSS (Version 25, Chicago, IL) or Graphpad Prism (Version 5.0, La Jolla, CA).

Results

Of the 150 patients screened for this study, 13 were excluded due to cancelled surgery and 23 were excluded due to an unavailability for assessment of delirium resulting from prolonged sedation thus leaving 114 patients available for analysis. Of the 114 patients included within our study, 31 patients (27.2%) developed a postoperative delirium (POD), while 83 patients (72.8%) did not show signs of a POD.

Baseline characteristics

No statistical differences were observed for sex, BMI, in-hospital death, preoperative incidence of alcohol abuse, the preoperative prescription of anticholinergic drugs or the performed procedure (Table 1). However, patients who went on to develop a POD had a significantly better EuroSCORE ($p=0.02$). Further, patients who developed POD were significantly older than patients without the development of POD ($p<0.001$).

Outcome dependent on the development of POD

Patients without the development of POD displayed a significantly shorter length of ventilation ($p=0.02$), shorter length of stay in the ICU ($p<0.001$) and shorter length of hospitalization ($p<0.001$) (Table 1). No differences were observed in regard to mortality, when comparing patients with or without the development of POD.

Assessment of cholinesterases

In the overall study population, the butyrlcholinesterase (BChE) decreased significantly over time, when comparing mean BChE activity on postoperative days one ($p<0.001$) and two ($p<0.001$) with the preoperative BChE activity (Figure 1A). Further, the mean acetylcholinesterase (AChE) activity increased over time, when comparing the AChE activity on postoperative day two with the preoperative AChE activity ($p<0.05$).

Significant differences were observed in regard to the activity of BChE on postoperative day one (p=0.03) (Figure 2B), when comparing patients from the POD and the no-POD groups. Further, patients with the development of POD displayed significantly lower levels of AChE activity preoperatively (p=0.003) and on postoperative days one (p=0.002) and two (p=0.007) (Figure 2 D-F).

Parameters associated with POD

To identify parameters associated with the development of POD in patients undergoing cardiac surgery, we performed a univariate analysis and identified age > 71 years, EuroSCORE ≥ 4, anticholinergic premedication and a preoperative AChE activity of < 44.3 U/g Hb (Table 2). To rule out potential confounding we performed a multivariate analysis and confirmed age > 71 years, EuroSCORE ≥4, preoperative anticholinergic medication and preoperatively AChE activity of < 44.3 U/g Hb as parameters independently associated with the development of POD.

Parameters associated with length of stay on the ICU

Survival analysis demonstrated, that patients with POD after cardiothoracic surgery displayed significantly longer LOS in the intensive care unit (Figure 3). To identify further parameters associated with prolonged stay in the ICU following cardiothoracic surgery, we performed various univariate analyses and identified EuroSCORE ≥ 4, preoperative anticholinergic medication, length of ventilation, transfusion of PRBCs, reduced AChE activity on postoperative day one, reduced postoperative BChE activity on postoperative day one and the development of POD as potentially associated (Table 3). To identify confounders, we performed a multivariate analysis and identified length of ventilation, reduced BChE activity on postoperative day one and the development of POD as independently associated with prolonged length of stay in the ICU.

Discussion

The purpose of this study was to analyze a potential correlation between AChE and BChE activities and the incidence of POD in cardiosurgical patients and to identify further possible predictors for the development of POD.

The incidence of POD in our study population is in line with the literature.[2 3] Our results show that a preoperative AChE activity was significantly lower in patients who went on to develop POD than in patients without the development of POD. Further, BChE activity was significantly lower in patients with POD on the first postoperative day. Our data revealed that the patients who developed a POD were significantly older than those who did not suffer from a POD. These patients were more frequently on anticholinergic medication. Further, the EuroSCORE was higher in such patients and they were longer ventilated. In addition, patients with POD stayed significantly longer in the intensive care unit and were discharged significantly later for follow-up treatment.

Patients who went on to develop POD showed lower preoperative AChE activity compared to patients without the development of POD. This finding is in agreement with the current hypothesis that a reduction in AChE activity is associated with POD. It is hypothesized that due to this deficit, cholinesterase cannot efficiently cleave the neurotransmitter ACh in the synaptic cleft. As a consequence, the stimulus transmission cannot be terminated, and ultimately a new stimulus transmission cannot be initiated.[18]

In a recently published study, Cerejeira et al. measured AChE and BChE activities pre- and postoperatively in patients who had undergone elective hip surgery and examined patients for the development of a POD using CAM-ICU.[16] They came to the conclusion that patients with POD after surgery showed reduced preoperative AChE activity. As in our results, preoperative BChE activity was decreased in patients with POD. Contrary to their findings however, our patient population groups with or without the development of POD did not differ significantly in preoperative BChE activity. This discrepancy might be attributed to different assays measuring enzyme activities. Most importantly, these findings need to be discussed in light of the 2017

publication by John et al.[19] This group did not find any differences regarding both AChE and BChE activity between patients with or without the development of POD. However, there are some considerable differences in the study design: no preoperative samples were collected in the study by John et al. Further, some samples were refrigerated before analysis, thereby potentially altering the measured enzyme activity. Zivkovic et el., however, have also identified a reduced BChE following surgery.[20] They suggested a cholinergic modulation of the inflammatory response that is independent of the POD. The stark contrast of our observed results needs to be addressed in further studies specifying the potential impact of cholinesterases in the development of POD, also in the context of inflammation.

In a study conducted in 2008, Hubbard et al. were able to show that a higher age was associated with deficits in the anticholinergic system.[21] They suspected that age was associated with changes in enzyme activity. The association between age and the development of POD observed for our patient population fits well with the literature that described such association before.[22]

In our cohort, patients with a history of anticholinergic medication suffered from a POD significantly more often than patients in the comparison group. This result supports the assumption that the anticholinergic predisposition has an influence on the development of the POD. It reduces the function of ACh and might also attribute to a cholinergic deficit. Anticholinergic medication is used when patients are regularly treated with antidepressants (e.g. amitriptyline, doxepin), anticonvulsants (e.g. gabapentin) or for Parkinson's disease (benserazide, L-DOPA). These drugs all have in common that they reduce ACh activity through direct and indirect anticholinergic action. In a study conducted in 2015, Naja et al. investigated geriatric patients with regard to the treatment with anticholinergic drugs before and during hospitalization and the incidence of delirium. They came to the conclusion that the anticholinergic burden was associated with the occurrence of delirium and that anticholinergic exposure correlated with the incidence of delirium and increased mortality.[23]

Patients with POD had a significantly longer anesthesia duration and were also operated on for longer periods of time. Long-lasting surgery is associated with many other risk factors such as hypoxemia, pain and disturbance of the sleep-wake rhythm.[22 24] The anesthesia itself interferes with various neuronal processes in the brain. It interacts with ion channels, such as the nicotinic acetylcholine receptors, neurotransmitters and second messengers, as well as metabolic processes.[25] The factors mentioned may have influenced the development of POD.

The effects of a POD are far-reaching. In our study, patients with POD not only stayed longer in the ICU, they also spent significantly more days in hospital postoperatively. These observations may be attributed to multiple factors such as delayed mobilization and physiotherapy.[26] Patients with POD require more intensive care from nurses and physicians, so that a transfer to the normal ward is only possible with delay and resulting in higher costs.[27] In a study published in 2004, Ely et al. showed that delirium is an independent predictor of significantly higher 6-month mortality and prolonged hospitalization in ventilated patients in the ICU.[28] Our patients did not show an increased in-hospital mortality in patients with POD while we, however, did not follow up patients for 6 months. Conclusions on associations between long-term mortality and cholinesterase activity may therefore not be drawn from the results of our study.

Strengths and limitations

Our study has several limitations that must be considered when evaluating the results. This study comprises exclusively cardiac surgery patients. Whether these data can be extrapolated to other patient cohorts remains unclear and warrants further validation.

It is known that delirium can fluctuate strongly and occur acutely during the course of the day.[29] In this study, only one measurement was performed in the morning of the day of measurement. Thus, it is possible that not all patients who developed a delirium were detected with the applied screening method.

The patient population was reduced from a total of 150 patients to 114, who were ultimately included for analysis. One reason for the exclusion of patients was excessive sedation at postoperative days one and two and thus an exclusion criterion for the CAM-ICU. Future studies should cover a longer observation period in order to be able to include such patients for analysis and to enable further conclusions to be drawn about the temporal development of POD.

Conclusions

We demonstrated that the development of POD after cardiac surgery correlates with postoperative decrease of BChE activity. In addition, patients who developed POD in the course of surgery showed significantly lower preoperative AChE activity as compared to patients without POD. We were able to identify a low preoperative AChE activity, an anticholinergic pre-medication, an increased EuroSCORE and a higher age as predictors for development. In addition, patients with POD differed from their peers by a longer postoperative ventilation time, an extended stay at the ICU and prolonged hospitalization.

Our data show that the cholinergic deficit hypothesis may be of importance for the development of POD. Anticholinergic medication may intervene in this pathophysiological system and may influence AChE and BChE activity resulting in neuroinflammation.

There are various studies investigating the risk factors for the occurrence of POD. Some correlations in the development of POD have been identified. However, the molecular basis of multifactorial POD has not yet been sufficiently understood. Nonetheless, this is necessary in order to develop preventive measures. Further studies are needed to investigate the exact pathomechanisms of risk factors for such disease.

Acknowledgements

None.

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Table and Figure legends

Table 1. Patient characteristics.

	No postoperative delirium (n=83)	Postoperative delirium (n=31)	
Age (y[IQR])	69 (58 – 74)	74 (71-78)	<0.001*
Female sex (n[%])	22 (26.5)	9 (29)	0.79
EuroSCORE (n[%])			0.02
1-5	59 (71.1)	13 (41.9)	
6-10	22 (26.5)	16 (30.2)	
11-15	2 (2.4)	2 (6.6)	
Body Mass Index (kg/m^2[SD])	27.6 (±4.8)	28 (4.8)	0.7*
Alcohol abuse (n[%])	2 (2.4)	0	1
Anticholinergic premedication (n[%])	8 (9.9)	10 (32.3)	0.003
Procedure (n[%])			0.3
ACVB	33 (39.8)	15 (48.4)	
AVR	24 (28.9)	6 (19.4)	
Combined Procedure	10 (12)	6 (19.4)	
TAVI	4 (4.9)	3 (9.7)	
MVR	6 (7.2)	1 (3.1)	
Other	6 (7.2)	0	
Length of ventilation (min[SD])	471 (±159)	1427 (±3565)	0.02*
Length of stay on ICU (h[SD])	20.1 (±20.1)	93.5 (±183)	<0.001*
Length of stay in hospital (d[SD])	13.1 (±5)	20.9 (13.9)	<0.001*
In-hospital death (n[%])	1 (1.2)	1 (3.2)	0.47*
Preop BchE activity (U/g Hb[SD])	2773 (±885)	2734 (±922)	0.83
PO day 1 BchE activity (U/g Hb[SD])	1966 (±588)	1674 (±730)	0.03
PO day 2 BchE activity (U/g Hb[SD])	1870 (±564)	1694 (±596)	0.16
Preop AchE activity (U/g Hb[SD])	45.4 (±5.7)	42.2 (±6.3)	0.003*
PO day 1AchE activity (U/g Hb[SD])	45.1 (±5.1)	41.8 (±5.5)	0.002*
PO day 2 AchE activity (U/g Hb[SD])	45.5 (±4.6)	42.7 (±5.8)	0.007*

Table 1. Patient characteristics. Data are given as means except for age which is presented as the median. Data comparisons were made with the *t*-test or the χ^2-test, where applicable. * denotes the use of a non-parametric test due to non-normal distribution of data. ICU = intensive care unit, CABG = coronary artery bypass grafting, AVR = aortic valve replacement, TAVI = transcatheter aortic valve replacement, MVR = mitral valve replacement, BChE = butyrylcholinesterase, AChE = acetylcholinesterase, PO = postoperative. IQR indicates interquartile range.

Table 2. Univariate and multivariate analysis of parameters associated with the development of postoperative delirium.

	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	*p* value	OR (95% CI)	p value
Age > 71 years	4.48 (1.74 – 11.54)	0.001	3.02 (1.06 – 8.62)	0.04
BMI > 27.5	1.31 (0.57 – 2.99)	0.67		
Male sex	1.13 (0.45 - 2.84)	0.82		
EURO-Score ≥ 4	5.43 (1.74 – 16.91)	0,002*	3.68 (1.04 – 12.99)	0.04
Known alcohol abuse	**	1.0*		
Anticholinergic premedication	6.02 (1.96 – 18.52)	0.001	5.09 (1.51 – 17.23)	0.009
Length of ventilation > 456 min	1.56 (0.68 -3.6)	0.29		
Transfusion of PRBC	2.26 (0.96 – 5.31)	0.06		0.28
Preop AchE activity of < 44.3 U/g Hb	2.74 (1.15 – 6.54)	0.02	3.1 (1.14 – 8.46)	0.03
Preop BchE activity of 2762 U/g Hb	1.31 (0.57 – 2.99)	0.53		

Table 2. Univariate and multivariate analysis of parameters associated with the development of postoperative delirium. Data comparisons were made with χ2-test for univariate analysis, binary logistic regression with stepwise exclusion was used for multivariable analysis. BMI = body mass index, PRBC = Packed red blood cells, BChE = butyrylcholinesterase, AChE = acetylcholinesterase. OR indicates odds ratio, CI indicates confidence interval. For multivariate analysis OR is only displayed in significant outcome parameters/where applicable.

Table 3. Univariate and multivariable analysis of parameters associated with length of stay in the ICU.

	Univariate Analysis		Multivariate Analysis		
	Median (95% CI)	*p* value	HR	(95% CI)	*p* value
Age		0.97			
Age > 71 years	0.75 (0.65 – 0.86)				
Age < 71 years	0.79 (0.56 -1.03)				
BMI		0.24			
BMI > 27.5	0.79 (0.68 – 0.91)				
BMI ≤ 27.5	0.71 (0.48 – 0.94)				
Sex		0.89			
Male	0.75 (0.55 – 0.95)				
Female	0.75 (0.64 – 0.86)				
EURO-Score		0.001			0.33
EURO-Score ≥ 4	0.79 (0.65 – 0.94)				
EURO-Score < 4	0.42 (0.11 – 0.72)				
Known alcohol abuse		0.76			
Present	0.75 (0.66 – 0.84)				
Absent	0.38 (-)*				
Anticholinergic premedication		0.05			0.39
Present	0.75 (0.59 – 0.91)				
Absent	0.75 (0.64 – 0.86)				
Length of ventilation		<0.001	2.77 (1.83 – 4.2)		<0.001
Length of ventilation > 456 min	1.04 (0.87 – 1.2)				
Length of ventilation < 456 min	0.33 (0.28 – 0.39)				
Transfusion of PRBC		0.04			0.98
Present	0.92 (0.76 – 1.07)				
Absent	0.5 (0.28 – 0.72)				
PO day 1 AchE activity		0.034			0.47
PO day 1 AchE activity of < 44.3 U/g Hb	0.79 (0.66 – 0.93)				
PO day 1 AchE activity of > 44.3 U/g Hb	0.71 (0.44 – 0.98)				
PO day 1 BchE activity		<0.001	1.84 (1.24 – 2.75)		0.003

PO day 1 BchE activity of < 2762 U/g Hb	1 (0.84 – 1.16)			
PO day BchE activity of > 2762 U/g Hb	0.5 (0.29 – 0.71)			
Delir		<0.001	1.79 (1.1 – 2.91)	0.019
Present	1.08 (0.48 – 1.69)			
Absent	0.71 (0.51 – 0.91)			

Table 3. Univariate and multivariable analysis of parameters associated with length of stay in the ICU. Data comparisons were made with Kaplan-Meier estimates for univariate analysis. Column median indicates median of parameter displayed. Cox-regression analysis with stepwise exclusion was used for multivariable analysis. BMI = Body mass index, EuroSCORE = European System for Cardiac Operative Risk Evaluation, PRBC = Packed red blood cells, BChE = butyrylcholinesterase, AChE = acetylcholinesterase, PO = postoperative. HR indicates hazard ratio, CI indicates confidence interval. For multivariate analysis HR is only displayed in significant outcome parameters/where applicable.

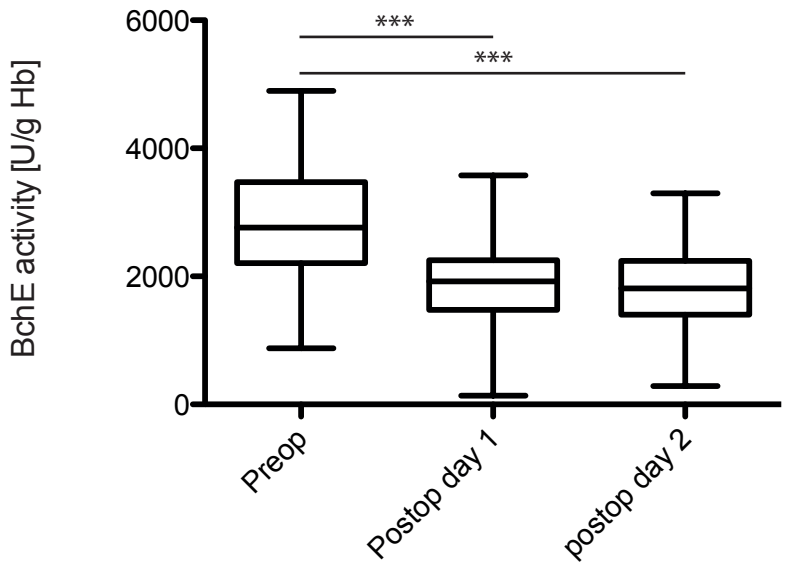
Figure 1. Activity of BChE and AChE in the overall patient population. Activity of A) butyrylcholinesterase (BChE) and B) acetylcholinesterase (AChE) were assessed preoperatively and on postoperative days one and two. *** indicates a p-value of <0.001; * indicates a p-value of <0.05.

Figure 2. Activity of BChE and AChE in patients without or with the development of POD. Activity of butyrylcholinesterase (BChE) was assessed A) preoperatively and on postoperative days B) one and C) two. Activity of acetylcholinesterase (AChE) were assessed D) preoperatively and on postoperative days E) one and F) two. * indicates a p-value of <0.05

Figure 3. Kaplan-Meier estimate. Time to discharge from ICU (logrank test $\chi^2 = 14.88$, $p < 0.001$)

Figure 1

A



B

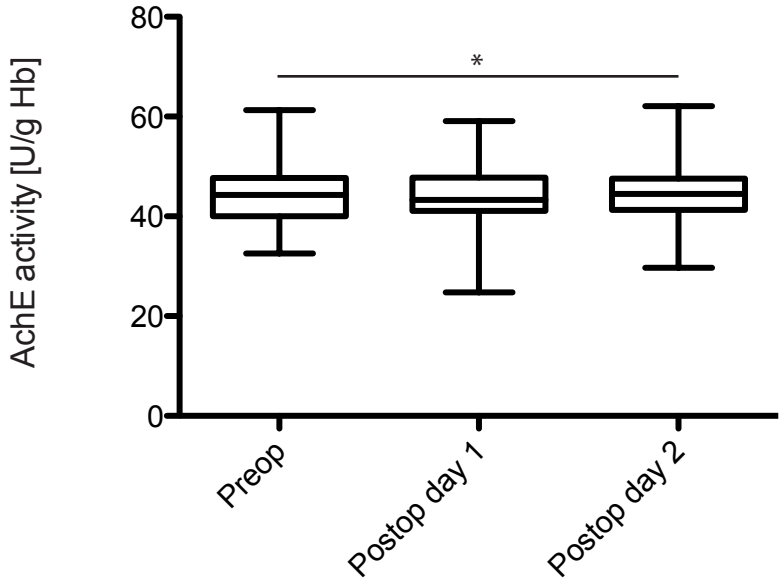
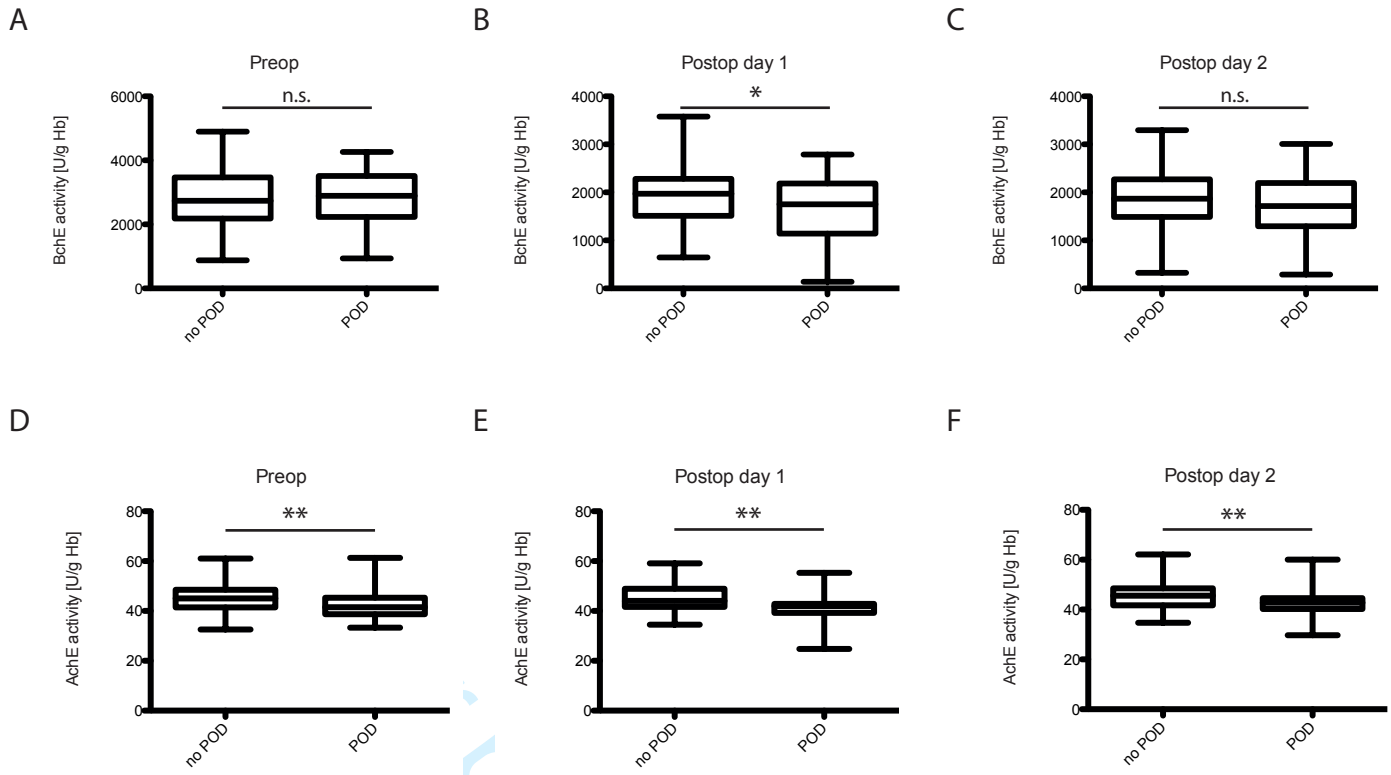
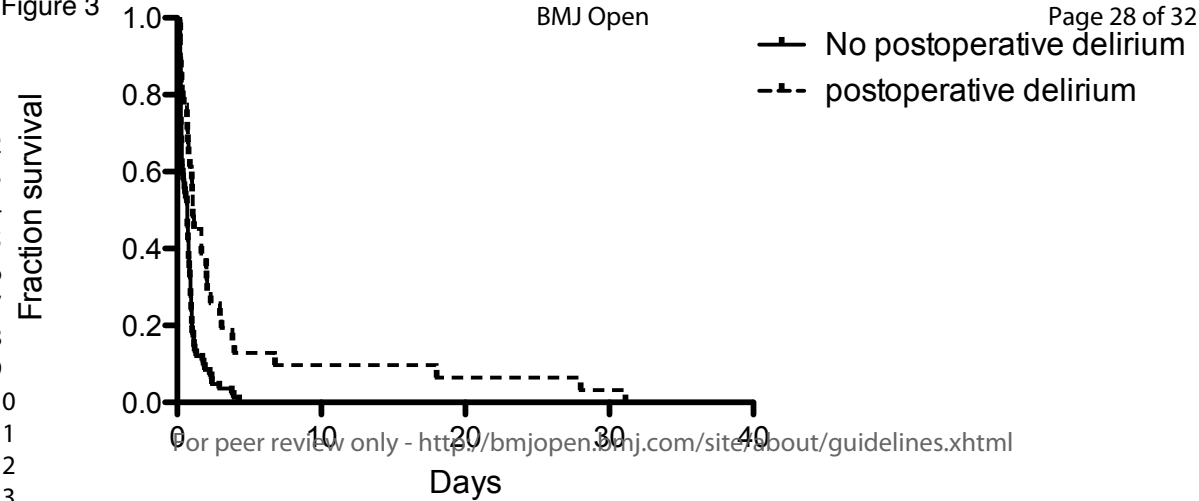


Figure 2





Reporting checklist for prediction model development and validation study.

Based on the TRIPOD guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the TRIPODreporting guidelines, and cite them as:

Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): The TRIPOD statement.

		Reporting Item	Page Number
Title			
	#1	Identify the study as developing and / or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract			
	#2	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	3
Introduction			
	#3a	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	6
	#3b	Specify the objectives, including whether the study describes the	7

		development or validation of the model or both.	
Methods			
Source of data	#4a	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	9
Source of data	#4b	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	8
Participants	#5a	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	8
Participants	#5b	Describe eligibility criteria for participants.	8
Participants	#5c	Give details of treatments received, if relevant	8
Outcome	#6a	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	9
Outcome	#6b	Report any actions to blind assessment of the outcome to be predicted.	9
Predictors	#7a	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured	9
Predictors	#7b	Report any actions to blind assessment of predictors for the outcome and other predictors.	9
Sample size	#8	Explain how the study size was arrived at.	8
Missing data	#9	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	9
Statistical analysis methods	#10a	If you are developing a prediction model describe how predictors were handled in the analyses.	9
Statistical analysis methods	#10b	If you are developing a prediction model, specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	9
Statistical analysis methods	#10c	If you are validating a prediction model, describe how the predictions were calculated.	9
Statistical analysis	#10d	Specify all measures used to assess model performance and, if relevant,	n/a

methods		to compare multiple models.	
Statistical analysis methods	#10e	If you are validating a prediction model, describe any model updating (e.g., recalibration) arising from the validation, if done	n/a
Risk groups	#11	Provide details on how risk groups were created, if done.	n/a
Development vs. validation	#12	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	9
Results			
Participants	#13a	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	11
Participants	#13b	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	11
Participants	#13c	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	11
Model development	#14a	If developing a model, specify the number of participants and outcome events in each analysis.	n/a
Model development	#14b	If developing a model, report the unadjusted association, if calculated between each candidate predictor and outcome.	12
Model specification	#15a	If developing a model, present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	n/a
Model specification	#15b	If developing a prediction model, explain how to the use it.	12
Model performance	#16	Report performance measures (with CIs) for the prediction model.	12
Model-updating	#17	If validating a model, report the results from any model updating, if done (i.e., model specification, model performance).	n/a
Discussion			
Limitations	#18	Discuss any limitations of the study (such as nonrepresentative sample,	15-16

		few events per predictor, missing data).	
Interpretation	#19a	For validation, discuss the results with reference to performance in the development data, and any other validation data	n/a
Interpretation	#19b	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	13
Implications	#20	Discuss the potential clinical use of the model and implications for future research	13-15
Other information			
Supplementary information	#21	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	n/a
Funding	#22	Give the source of funding and the role of the funders for the present study.	5

This checklist was completed on 22. April 2019 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

Cholinesterase alterations in delirium after cardiosurgery: a monocentric prospective study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-031212.R1
Article Type:	Original research
Date Submitted by the Author:	15-Jul-2019
Complete List of Authors:	Adam, Elisabeth; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt, Haas, Victoria; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt Lindau, Simone; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt Zacharowski, Kai; University Hospital Frankfurt, Clinic of Anesthesiology, Intensive Care Medicine and Pain Therapy Scheller, Bertram; Evangelisches Krankenhaus Düsseldorf
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Anaesthesia
Keywords:	Postoperative delirium, Cardiac surgery < SURGERY, Cholinesterease, Acetylcholinesterase, Butyrylcholinesterase

Cholinesterase alterations in delirium after cardiosurgery: a monocentric prospective study

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Short title: Cholinesterases and postoperative delirium

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Author contributions

EA: wrote the manuscript, analyzed and interpreted the data

VH: conceived the study idea and collected data

SL: collected data, provided critical feedback and contributed to the final version of the manuscript

KZ: supervised the project and contributed to the final version of the manuscript

BS: conceived the study idea, analyzed the data and contributed to the final version of the manuscript

All authors read and approved the final version of the manuscript.

Author Disclosure Statement

The authors have reported no conflicts of interest.

Word count

3416

Data statement

Deidentified participant data are available from the corresponding author upon reasonable request

Abstract

Objectives

Postoperative delirium (POD) is a common complication after elective cardiac surgery. Recent evidence indicates that a disruption in the normal activity of the cholinergic system may be associated with delirium.

Design

Prospective observational study

Setting

Single-center at a European academic hospital.

Primary and secondary outcome measures

In our study the enzyme activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were determined preoperatively as well as on the first and second postoperative day. The confusion assessment method for the intensive care unit (CAM-ICU) was used to screen patients for the presence of POD.

Results

A total of 114 patients were included in the study. POD was associated with a decrease in BChE activity on postoperative day one (p=0.03). In addition, patients who developed POD, had significantly lower preoperative AChE activity than patients without POD (p<0.01). Multivariate analysis identified a preoperatively decreased AChE activity (OR 3.1; 95%CI 1.14-8.46), anticholinergic treatment (OR 5.09; 95%CI 1.51-17.23), elevated EuroSCORE (OR 3.68; 95%CI 1.04-12.99) and age (OR 3.02; 95%CI 1.06-8.62) to be independently associated with the development of POD.

Conclusions

We conclude that a reduction in the acetylcholine hydrolyzing enzyme activity in patients undergoing cardiac surgery may correlate with the development of POD.

Strengths and limitations of this study

- One strength of this study results from the prospective nature
- Another strength is the data acquisition from a high-volume center
- A limitation is the exclusive inclusion of cardiac surgery patients. Whether these data can be extrapolated to other patient cohorts remains unclear and warrants further validation.
- It is known that delirium can fluctuate strongly and occur acutely during the course of the day. In this study, only one assessment was performed in the morning of the day of measurement. Thus, it is possible that not all patients who developed a delirium were detected with the applied screening method.

Keywords

Postoperative delirium

Cardiac surgery

Cholinesterase

Acetylcholinesterase

Butyrylcholinesterase

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Introduction

A delirium is a complex neuropsychiatric syndrome that is clinically characterized by sudden onset and fluctuating course and may influence disorders of consciousness as well as cognition in all its aspects. According to the criteria of the ICD-10 classification for Mental and Behavioural Disorders, a delirium is characterized as an etiologically unspecific cerebro-organic syndrome by the presence of disorders of consciousness and at least two of the following areas simultaneously: attention, perception, thinking, memory, psychomotor activity, emotionality or sleep-wake rhythm. The duration of delirium varies greatly and the severity ranges from mild to severe.[1]

The causes for delirium are multifactorial. Risk factors include dehydration, sleep deprivation, age, hypoxia, substance intoxication, anemia and hypoglycemia. In the general population, the incidence is below 0.4%, in hospitalized patients between 15-22%.[2 3] Particularly after surgical interventions, patients are at risk of developing postoperative delirium (POD). The incidence is described to be as high as 52%.[4] The consequences of a POD are very different and range from prolonged hospital stay, increased risk of wound infections, reduced quality of life, more frequent discharge into nursing homes to increased mortality in the first year after surgery.[5-8]

Higher age, longer duration of surgery as well as a reduced preoperative cognitive condition are frequently found in cardiac surgery patients and increase the risk for development of POD in this group of patients.[3] In the literature, the incidence of POD after cardiac surgery varies from 8 to 52%.[3 4 8 9] The duration of the POD in such patients varies widely, lasting three days on average.[5 10] Patients with POD are at risk for developing chronic postoperative cognitive dysfunction (POCD) over time and for suffering from severe long-term cognitive deficits.[11]

There are different hypotheses about the molecular mechanisms involved in the development of delirium.[12] The most common hypothesis for the development of POD is based on a central cholinergic deficit resulting from a deficit of Acetylcholine (ACh): Pathologies at the presynapse, in the synaptic cleft or at the postsynaptic receptor may trigger a central cholinergic

deficit. Acetylcholinesterase (AChE) is an enzyme which cleaves ACh in the synaptic cleft and terminates the transmission of a stimulus, a prerequisite for generating a new impulse. If the AChE is restricted in its function ACh remains in the synaptic cleft and blocks a new stimulus transmission.[13] However, several authors have found data challenging this hypothesis as they did not identify an association of preoperative serum anticholinergic activity with the development of POCD[14] or a therapeutic effect of rivastigmine for the prevention of POD.[15] Other hypotheses (e.g. brain injury, metabolic abnormalities) are based on localized or general brain energy deprivation critical to attentional processes such as the nucleus caudatus or frontal cholinergic pathways[16] or in case of systemic inflammation changes including pro-inflammatory cytokines and prostaglandins mediated by humoral and neural signaling pathways leading to symptoms of delirium.[17]

Butyrylcholinesterase (BChE) is an enzyme which splits choline compounds as well as other esters.[18] For a long time BChE was thought to have a less important function, but recent literature demonstrated that BChE may in part and with a significantly slower rate and affinity act as a substitute in the absence of AChE with a relevant role in the development of a cholinergic deficit.[19 20] A recently published study identified a significant decrease in the enzyme activity of AChE and BChE in patients with POD after hip surgery.[21] The impact of a choline esterase deficit in patients undergoing cardiac surgery remains unclear, however.

Due to the far-reaching consequences of a POD, it is of great importance to identify patients at risk for the development of such a disorder. Our study investigated the extent to which changes in bed-side enzyme activity of cholinesterases correlates with the development of POD in cardiac surgery patients and to identify possible factors influencing the development of POD.

Material and methods

This manuscript includes data gained during a prospective observational study at the University Hospital Frankfurt . The institutional review board approved the conduct of the study prior to its initiation (428/12 of 19 December 2012).

Patients were included between February 2013 and February 2014. Over this period, 150 patients who received elective cardiac surgery at the University Hospital Frankfurt were screened for inclusion. The participating patients were informed about the study verbally and in writing. Only patients with written consent were included in the study.

Potential patients had to meet the following inclusion criteria: elective cardiac surgery with and without the use of a cardiopulmonary bypass (CPB) and age over 18 years. Exclusion from the study was based on: preoperatively existing delirium; preoperatively sedated patients with Richmond Agitation and Sedation Scale (RASS) < -2; no proficiency of the German or English language or missing patient consent.

After obtaining consent, patients were examined preoperatively and on the first and second postoperative day. Patients were examined for the presence of a POD using the confusion assessment method for the intensive care unit (CAM-ICU) clinical test[22]. In brief, the CAM-ICU assesses and scores clinical features associated with delirium. Further, blood samples were analyzed for AChE and BChE activity as measured with the ChE Check mobile ® (Securetec Detektions-Systeme AG, Neubiberg, Germany). Depending on the results from CAM-ICU, a patient was assigned to either the postoperative delirium group (POD) or the no postoperative delirium (no POD) group. The patient was assigned to the PDO group if a delirium was diagnosed at least once as per the CAM-ICU. If a patient was either under too much sedation or the examiner was not able not make apply the CAM-ICU, the patient was not included for analysis.

Assessment of parameters

All included patients were scheduled for elective surgery and assessed directly before surgery at 7am to determine the presence of delirium. First, the RASS score was obtained, then blood samples were taken for the assessment of butyryl- and acetylcholinesterase activity. Both, BChE and AChE activity were assessed using the ChE Check Mobile® as per the manufacturer's instruction. Preoperatively, blood samples were drawn from the fingertip (10µL). Postoperatively, blood samples (1mL) were obtained via an arterial line. As two enzymes were determined in different measurements, two blood samples were taken at different times and analyzed independently. To provide consistency between assessments, measurement of AChE was always performed first, followed by assessment of BChE activity. Measurements were about 10 min apart. As animal data on the circadian changes of cholinesterase reveal an relevant increase during the sleep phase, we have hence taken samples at the same time as preoperatively (±1 hour) to ensure consistency of measurements.[23]

Data collection

Basic demographic data, medication, hospitalization period, the length of stay on the intensive care unit, ventilation time as well as postoperative medication, transfusion, information about secondary diagnoses, weight, EuroSCORE, laboratory values as well as obtained scores were extracted from the patient data management system. Medication was considered to be anticolinergic based on the study by Ancelin et al.[24] The duration of anesthesia, intraoperative medication, aortic clamping time (APC) and the duration of CPB were extracted from the anesthesia and premedication protocols. The data and results were inserted and maintained in an Excel database.

Statistics

All data were tested for normality using the D'Agostino and Pearson omnibus normality test. Data comparisons of patient characteristics were made using Mann-Whitney U- or $\chi 2$-test,

where applicable. To compare activities of cholinesterases between different days, a Wilcoxon signed rank test was used. Univariate analysis was performed using the χ2-test. Non binary-parameters were stratified by the median. Parameters with a p-value less than 0.1 were included for multivariable analysis, as carried out by binary logistic regression.

Length of ventilation was defined as the time of intubation until extubation; length of stay on the intensive care unit (ICU) was defined as the time from surgery to the discharge from the postoperative ICU; length of stay in the hospital was defined as the time from surgery to discharge from the primary care hospital. For survival analysis, groups were compared using a log rank test and pointwise 95% confidence intervals (CI). A multivariable Cox's proportional hazards regression backward stepwise model (likelihood ratio) was performed to find independent predictors for outcome parameters.

Results with $p<0.05$ were considered to be statistically significant. All calculations/analyses were performed with SPSS (Version 25, Chicago, IL) or Graphpad Prism (Version 5.0, La Jolla, CA).

Results

Of the 150 patients screened for this study, 13 were excluded due to cancelled surgery and 23 were excluded due to an unavailability for assessment of delirium resulting from prolonged sedation thus leaving 114 patients available for analysis. Of the 114 patients included within our study, 31 patients (27.2%) developed a postoperative delirium (POD), while 83 patients (72.8%) did not show signs of a POD.

Baseline characteristics

No statistical differences were observed for sex, BMI, in-hospital death, preoperative incidence of alcohol abuse, the preoperative prescription of anticholinergic drugs or the performed procedure (Table 1). Of note, none of the patients without previous history of anticholinergic medication received anticholinergic medication throughout the ICU stay. However, patients who went on to develop a POD had a significantly better EuroSCORE ($p=0.02$). Further, patients who developed POD were significantly older than patients without the development of POD ($p<0.01$).

Outcome dependent on the development of POD

Patients without the development of POD displayed a significantly shorter length of ventilation ($p=0.02$), shorter length of stay in the ICU ($p<0.01$) and shorter length of hospitalization ($p<0.01$) (Table 1). No differences were observed in regard to mortality, when comparing patients with or without the development of POD.

Assessment of cholinesterases

In the overall study population, the butyrylcholinesterase (BChE) decreased significantly over time, when comparing mean BChE activity on postoperative days one ($p<0.01$) and two ($p<0.01$) with the preoperative BChE activity (Figure 1A). Further, the mean acetylcholinesterase

(AChE) activity increased over time, when comparing the AChE activity on postoperative day two with the preoperative AChE activity ($p<0.05$) (Figure 1B).

No significant difference in pre- and postoperative BChE activity were observed (Figure 2A). Significant differences were observed in regard to the activity of BChE on postoperative day one ($p=0.03$) (Figure 2B), when comparing patients from the POD and the no-POD groups. Howewer, no significant difference in pre- and postoperative BChE activities was observed on postoperative day 2 (Figure 2C). Further, patients with the development of POD displayed significantly lower levels of AChE activity preoperatively ($p<0.01$) and on postoperative days one ($p<0.01$) and two ($p<0.01$) (Figure 2D-F).

Parameters associated with POD

To identify parameters associated with the development of POD in patients undergoing cardiac surgery, we performed a univariate analysis and identified age > 71 years, EuroSCORE ≥ 4, anticholinergic premedication and a preoperative AChE activity of < 44.3 U/g Hb (Table 2). To rule out potential confounding variables we performed a multivariate analysis and confirmed age > 71 years, EuroSCORE ≥4, preoperative anticholinergic medication and preoperatively AChE activity of < 44.3 U/g Hb as parameters independently associated with the development of POD.

Parameters associated with length of stay on the ICU

Survival analysis demonstrated, that patients with POD after cardiothoracic surgery displayed significantly longer LOS in the intensive care unit (Figure 3). To identify further parameters associated with prolonged stay in the ICU following cardiothoracic surgery, we performed various univariate analyses and identified EuroSCORE ≥ 4, preoperative anticholinergic medication, length of ventilation, transfusion of PRBCs, reduced AChE activity on postoperative day one, reduced postoperative BChE activity on postoperative day one and the development of POD as potentially associated (Table 3). To identify confounders, we performed

a multivariate analysis and identified length of ventilation, reduced BChE activity on postoperative day one and the development of POD as independently associated with prolonged length of stay in the ICU.

Discussion

The purpose of this study was to analyze a potential correlation between AChE and BChE activities and the incidence of POD in cardiosurgical patients and to identify further possible predictors for the development of POD.

The incidence of POD in our study population is in line with the literature.[2 3] Our results show that a preoperative AChE activity was significantly lower in patients who went on to develop POD than in patients without the development of POD. Further, BChE activity was significantly lower in patients with POD on the first postoperative day. Our data revealed that the patients who developed a POD were significantly older than those who did not suffer from a POD. These patients were more frequently on anticholinergic medication. Further, the EuroSCORE was higher in such patients and they were longer ventilated. In addition, patients with POD stayed significantly longer in the intensive care unit and were discharged significantly later for follow-up treatment.

Patients who went on to develop POD showed lower preoperative AChE activity compared to patients without the development of POD. This finding is in agreement with the current hypothesis that a reduction in AChE activity is associated with POD. It is hypothesized that due to this deficit, cholinesterase cannot efficiently cleave the neurotransmitter ACh in the synaptic cleft. As a consequence, the stimulus transmission cannot be terminated, and ultimately a new stimulus transmission cannot be initiated.[25]

In a recently published study, Cerejeira et al. measured AChE and BChE activities pre- and postoperatively in patients who had undergone elective hip surgery and examined patients for the development of a POD using CAM-ICU.[21] They came to the conclusion that patients with POD after surgery showed reduced preoperative AChE activity. As in our results, preoperative BChE activity was decreased in patients with POD. Contrary to their findings however, our patient population groups with or without the development of POD did not differ significantly in preoperative BChE activity. This discrepancy might be attributed to different assays measuring enzyme activities. Most importantly, these findings need to be discussed in light of the 2017

publication by John et al.[26] This group did not find any differences regarding both AChE and BChE activity between patients with or without the development of POD. However, there are some considerable differences in the study design: no preoperative samples were collected in the study by John et al. Further, some samples were refrigerated before analysis, thereby potentially altering the measured enzyme activity. Zivkovic et el., however, have also identified a reduced BChE activity following surgery.[27] They suggested a cholinergic modulation of the inflammatory response that is independent of POD. This finding of a postoperatively decreased BChE activity and a potential association with POD as observed within our study needs to be addressed in further studies specifying the potential impact of cholinesterases in the development of POD, also in the context of inflammation.

In a study conducted in 2008, Hubbard et al. were able to show that a higher age was associated with deficits in the anticholinergic system.(Hubbard, O'Mahony et al. 2008) Photometric determination of AChE revealed no significant difference for BChE activity between younger and older age, but a significantly lower activity of cholinesterases in the older people displaying a significant amount of frailty. They suspected that age was associated with changes in enzyme activity. While a deficit in cholinesterase activity may be observed in elderly patients, a significant correlation with age could not be demonstrated.[28-30] The association between age and the development of POD observed for our patient population fits well with the literature that described such association before.[31] In our cohort, patients with a history of anticholinergic medication suffered from a POD significantly more often than patients in the comparison group. This result supports the assumption that the anticholinergic predisposition has an influence on the development of the POD. It reduces the function of ACh and might also attribute to a cholinergic deficit. Anticholinergic medication is used when patients are regularly treated with antidepressants (e.g. amitriptyline, doxepin), anticonvulsants (e.g. gabapentin) or for Parkinson's disease (benserazide, L-DOPA). These drugs all have in common that they reduce ACh activity through direct and indirect anticholinergic action. In a study conducted in 2015, Naja et al. investigated

geriatric patients with regard to the treatment with anticholinergic drugs before and during hospitalization and the incidence of delirium. They came to the conclusion that the anticholinergic burden was associated with the occurrence of delirium and that anticholinergic exposure correlated with the incidence of delirium and increased mortality.[32]

Patients with POD had a significantly longer anesthesia duration and were also operated on for longer periods of time. Long-lasting surgery is associated with many other risk factors such as hypoxemia, pain and disturbance of the sleep-wake rhythm.[31 33] The anesthesia itself interferes with various neuronal processes in the brain. It interacts with ion channels, such as the nicotinic acetylcholine receptors, neurotransmitters and second messengers, as well as metabolic processes.[34] The factors mentioned may have influenced the development of POD.

The effects of a POD are far-reaching. In our study, patients with POD not only stayed longer in the ICU, they also spent significantly more days in hospital postoperatively. These observations may be attributed to multiple factors such as delayed mobilization and physiotherapy.[35] Patients with POD require more intensive care from nurses and physicians, so that a transfer to the normal ward is only possible with delay and resulting in higher costs.[36] In a study published in 2004, Ely et al. showed that delirium is an independent predictor of significantly higher 6-month mortality and prolonged hospitalization in ventilated patients in the ICU.[37] Our patients did not show an increased in-hospital mortality in patients with POD while we, however, did not follow up patients for 6 months. Conclusions on associations between long-term mortality and cholinesterase activity may therefore not be drawn from the results of our study.

To determine the diagnosis of delirium, the Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) was used, which is recommended by clinical guidelines.[38] While the CAM-ICU test is a tool for the diagnosis of delirium with the benefits of rapid assessment and no requirement for verbal communication with the patient, the CAM-ICU test does not provide information about motor subtypes of delirium.[39] We believe that future studies addressing this question are potentially of value to help understanding the pathology of this disease.

Strengths and limitations

Our study has several limitations that must be considered when evaluating the results. This study comprises exclusively cardiac surgery patients. Whether these data can be extrapolated to other patient cohorts remains unclear and warrants further validation. On a statistical note, we have not performed multiple comparison for the assessment of enzyme activities with a consecutive potential increase of the alpha error.

One limitation may be found in the lack of a consensus on a single classification system for anticholinergic medication. While several classification systems exist (as reviewed by Duran et al. [40]), the true effects of preoperative anticolinergic medication may differ depending on the classification system applied for analysis.

It is known that delirium can fluctuate strongly and occur acutely during the course of the day.[41] In this study, only one measurement was performed in the morning of the day of measurement. Thus, it is possible that not all patients who developed a delirium were detected with the applied screening method.

The patient population was reduced from a total of 150 patients to 114, who were ultimately included for analysis. One reason for the exclusion of patients was excessive sedation at postoperative days one and two and thus an exclusion criterion for the CAM-ICU. Future studies should cover a longer observation period in order to be able to include such patients for analysis and to enable further conclusions to be drawn about the temporal development of POD.

Conclusions

We demonstrated that the development of POD after cardiac surgery correlates with postoperative decrease of BChE activity. In addition, patients who developed POD in the course of surgery showed significantly lower preoperative AChE activity as compared to patients without POD. We were able to identify a low preoperative AChE activity, an anticholinergic pre-medication, an increased EuroSCORE and a higher age as predictors for development. In addition, patients with POD differed from their peers by a longer postoperative ventilation time, an extended stay at the ICU and prolonged hospitalization.

Our data show that the cholinergic deficit hypothesis may be of importance for the development of POD. Anticholinergic medication may intervene in this pathophysiological system and may influence AChE and BChE activity resulting in neuroinflammation.

There are various studies investigating the risk factors for the occurrence of POD. Some correlations in the development of POD have been identified. However, the molecular basis of multifactorial POD has not yet been sufficiently understood. Nonetheless, this is necessary in order to develop preventive measures. Further studies are needed to investigate the exact pathomechanisms of risk factors for such disease.

Acknowledgements

None.

Patient and public involvement

No patient involved.

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Table and Figure legends

Table 1. Patient characteristics.

	No postoperative delirium (n=83)	Postoperative delirium (n=31)	
Age (y[IQR])	69 (58 – 74)	74 (71-78)	<0.01*
Female sex (n[%])	22 (26.5)	9 (29)	0.79
EuroSCORE (n[%])			0.02
1-5	59 (71.1)	13 (41.9)	
6-10	22 (26.5)	16 (51.6)	
11-15	2 (2.4)	2 (6.5)	
Body Mass Index (kg/m^2[SD])	27.6 (±4.8)	28 (4.8)	0.7*
Alcohol abuse (n[%])	2 (2.4)	0	1
Anticholinergic premedication (n[%])	8 (9.9)	10 (32.3)	<0.01
Procedure (n[%])			0.3
ACVB	33 (39.8)	15 (48.4)	
AVR	24 (28.9)	6 (19.4)	
Combined Procedure	10 (12)	6 (19.4)	
TAVI	4 (4.9)	3 (9.7)	
MVR	6 (7.2)	1 (3.1)	
Other	6 (7.2)	0	
Length of ventilation (min[SD])	471 (±159)	1427 (±3565)	0.02*
Length of stay on ICU (h[SD])	20.1 (±20.1)	93.5 (±183)	<0.01*
Length of stay in hospital (d[SD])	13.1 (±5)	20.9 (13.9)	<0.01*
In-hospital death (n[%])	1 (1.2)	1 (3.2)	0.47*
Preop BchE activity (U/g Hb[median, SD])	2773 (2740±885)	2734 (2891±922)	0.83
PO day 1 BchE activity (U/g Hb [median, SD])	1966 (1971±588)	1674 (1752±730)	0.03
PO day 2 BchE activity (U/g Hb [median, SD])	1870 (1868±564)	1694 (1715±596)	0.16
Preop AchE activity (U/g Hb [median, SD])	45.4 (45±5.7)	42.2 (41.5±6.3)	<0.01*
PO day 1AchE activity (U/g Hb [median, SD])	45.1 (44.1±5.1)	41.8 (42±5.5)	<0.01*
PO day 2 AchE activity (U/g Hb [median, SD])	45.5 (45.6±4.6)	42.7 (42.8±5.8)	<0.01*

Table 1. Patient characteristics. Data are given as means except for age which is presented as the median and as indicated. Data comparisons were made with the *t*-test or the χ^2-test, where applicable. * denotes the use of a non-parametric test due to non-normal distribution of data. ICU = intensive care unit, CABG = coronary artery bypass grafting, AVR = aortic valve replacement, TAVI = transcatheter aortic valve replacement, MVR = mitral valve replacement, BChE =

butyrylcholinesterase, AChE = acetylcholinesterase, PO = postoperative. IQR indicates interquartile range.

Table 2. Univariate and multivariate analysis of parameters associated with the development of postoperative delirium.

	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	*p* value	OR (95% CI)	p value
Age > 71 years	4.48 (1.74 – 11.54)	<0.01	3.02 (1.06 – 8.62)	0.04
BMI > 27.5	1.31 (0.57 – 2.99)	0.67		
Male sex	1.13 (0.45 - 2.84)	0.82		
EURO-Score ≥ 4	5.43 (1.74 – 16.91)	<0.01*	3.68 (1.04 – 12.99)	0.04
Known alcohol abuse	**	1.0*		
Anticholinergic premedication	6.02 (1.96 – 18.52)	<0.01	5.09 (1.51 – 17.23)	<0.01
Length of ventilation > 456 min	1.56 (0.68 -3.6)	0.29		
Transfusion of PRBC	2.26 (0.96 – 5.31)	0.06		0.28
Preop AchE activity of < 44.3 U/g Hb	2.74 (1.15 – 6.54)	0.02	3.1 (1.14 – 8.46)	0.03
Preop BchE activity of 2762 U/g Hb	1.31 (0.57 – 2.99)	0.53		

Table 2. Univariate and multivariate analysis of parameters associated with the development of postoperative delirium. Data comparisons were made with χ2-test for univariate analysis, binary logistic regression with stepwise exclusion was used for multivariable analysis. BMI = body mass index, PRBC = Packed red blood cells, BChE = butyrylcholinesterase, AChE = acetylcholinesterase. OR indicates odds ratio, CI indicates confidence interval. For multivariate analysis OR is only displayed in significant outcome parameters/where applicable.

Table 3. Univariate and multivariable analysis of parameters associated with length of stay in the ICU.

	Univariate Analysis		Multivariate Analysis		
	Median (95% CI)	*p* value	HR	(95% CI)	*p* value
Age		0.97			
Age > 71 years	0.75 (0.65 – 0.86)				
Age < 71 years	0.79 (0.56 -1.03)				
BMI		0.24			
BMI > 27.5	0.79 (0.68 – 0.91)				
BMI ≤ 27.5	0.71 (0.48 – 0.94)				
Sex		0.89			
Male	0.75 (0.55 – 0.95)				
Female	0.75 (0.64 – 0.86)				
EURO-Score		<0.01			0.33
EURO-Score ≥ 4	0.79 (0.65 – 0.94)				
EURO-Score < 4	0.42 (0.11 – 0.72)				
Known alcohol abuse		0.76			
Present	0.75 (0.66 – 0.84)				
Absent	0.38 (-)*				
Anticholinergic premedication		0.05			0.39
Present	0.75 (0.59 – 0.91)				
Absent	0.75 (0.64 – 0.86)				
Length of ventilation		<0.01	2.77 (1.83 – 4.2)		<0.01
Length of ventilation > 456 min	1.04 (0.87 – 1.2)				
Length of ventilation < 456 min	0.33 (0.28 – 0.39)				
Transfusion of PRBC		0.04			0.98
Present	0.92 (0.76 – 1.07)				
Absent	0.5 (0.28 – 0.72)				
PO day 1 AchE activity		0.03			0.47
PO day 1 AchE activity of < 44.3 U/g Hb	0.79 (0.66 – 0.93)				
PO day 1 AchE activity of > 44.3 U/g Hb	0.71 (0.44 – 0.98)				
PO day 1 BchE activity		<0.01	1.84 (1.24 – 2.75)		<0.01

PO day 1 BchE activity of < 2762 U/g Hb	1 (0.84 – 1.16)			
PO day BchE activity of > 2762 U/g Hb	0.5 (0.29 – 0.71)			
Delirium		<0.01	1.79 (1.1 – 2.91)	0.02
Present	1.08 (0.48 – 1.69)			
Absent	0.71 (0.51 – 0.91)			

Table 3. Univariate and multivariable analysis of parameters associated with length of stay in the ICU. Data comparisons were made with Kaplan-Meier estimates for univariate analysis. Column median indicates median of parameter displayed. Cox-regression analysis with stepwise exclusion was used for multivariable analysis. BMI = Body mass index, EuroSCORE = European System for Cardiac Operative Risk Evaluation, PRBC = Packed red blood cells, BChE = butyrylcholinesterase, AChE = acetylcholinesterase, PO = postoperative. HR indicates hazard ratio, CI indicates confidence interval. For multivariate analysis HR is only displayed in significant outcome parameters/where applicable.

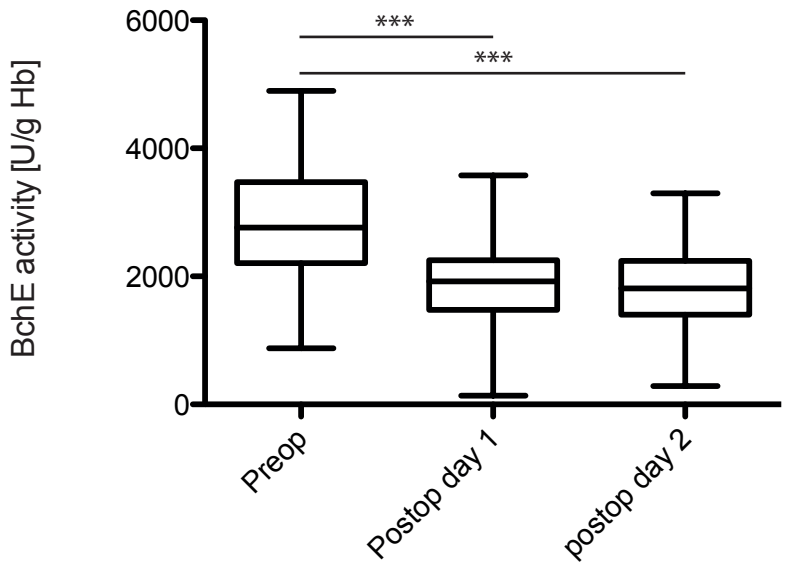
Figure 1. Activity of BChE and AChE in the overall patient population. Activity of A) butyrylcholinesterase (BChE) and B) acetylcholinesterase (AChE) were assessed preoperatively and on postoperative days one and two. *** indicates a p-value of <0.01; * indicates a p-value of <0.05.

Figure 2. Activity of BChE and AChE in patients without or with the development of POD. Activity of butyrylcholinesterase (BChE) was assessed A) preoperatively and on postoperative days B) one and C) two. Activity of acetylcholinesterase (AChE) were assessed D) preoperatively and on postoperative days E) one and F) two. * indicates a p-value of <0.05

Figure 3. Kaplan-Meier estimate. Time to discharge from ICU (logrank test $\chi^2 = 14.88$, $p < 0.01$)

Figure 1

A



B

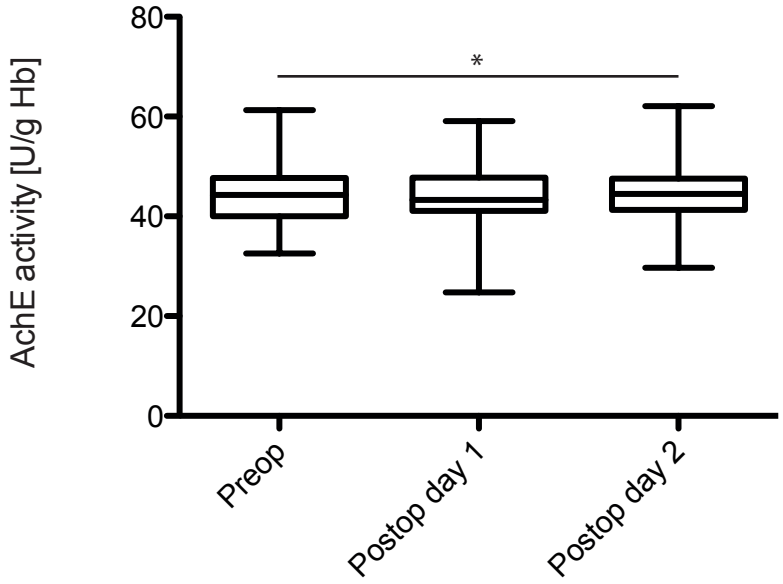
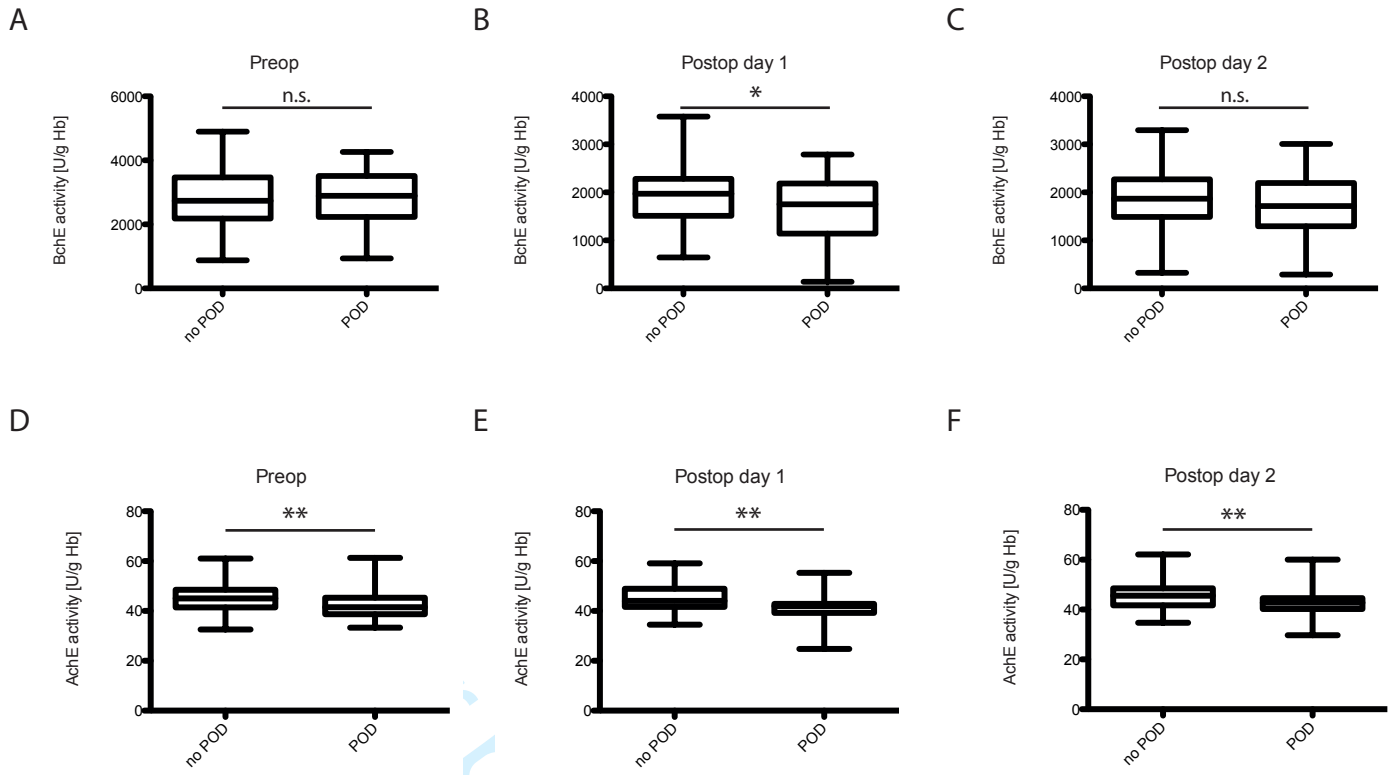
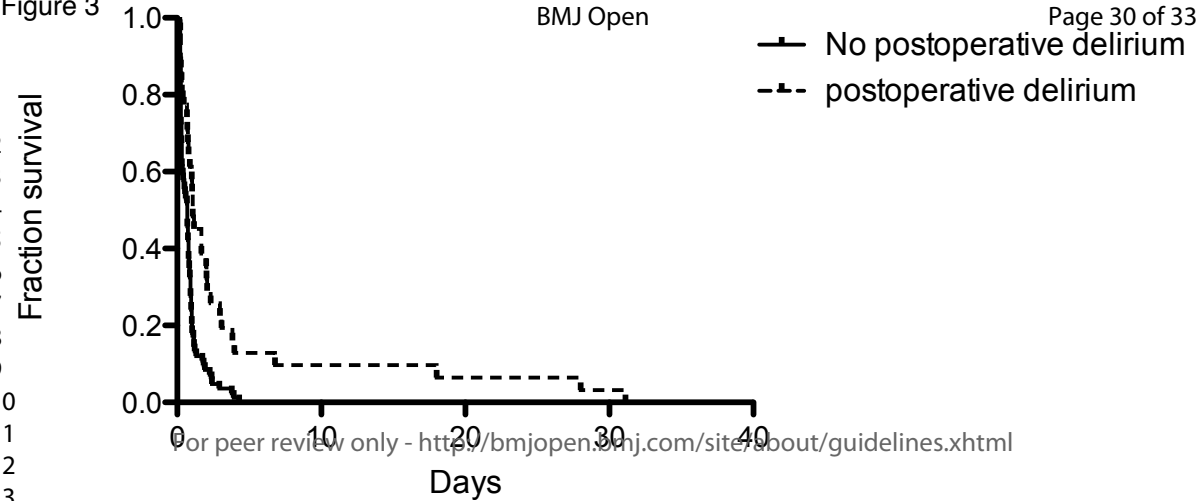


Figure 2





STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Section and Item	Item No.	Recommendation	Reported on Page No.
Title and Abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/Rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	8
Methods			
Study Design	4	Present key elements of study design early in the paper	9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	9
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	9
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9, 10

Section and Item	Item No.	Recommendation	Reported on Page No.
Data Sources/ Measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9, 10
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study Size	10	Explain how the study size was arrived at	n/a
Quantitative Variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11
Statistical Methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11, 12
		(*b*) Describe any methods used to examine subgroups and interactions	11, 12
		(*c*) Explain how missing data were addressed	n/a
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	n/a
		(*e*) Describe any sensitivity analyses	17
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12
		(b) Give reasons for non-participation at each stage	12
		(c) Consider use of a flow diagram	n/a
Descriptive Data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12
		(b) Indicate number of participants with missing data for each variable of interest	n/a
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	n/a
Outcome Data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	n/a
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	

Section and Item	Item No.	Recommendation	Reported on Page No.
Main Results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12, 13
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other Analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12, 13
Discussion			
Key Results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	6

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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BMJ Open

Cholinesterase alterations in delirium after cardiosurgery: a German monocentric prospective study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-031212.R2
Article Type:	Original research
Date Submitted by the Author:	22-Sep-2019
Complete List of Authors:	Adam, Elisabeth; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt, Haas, Victoria; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt Lindau, Simone; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt Zacharowski, Kai; University Hospital Frankfurt, Clinic of Anesthesiology, Intensive Care Medicine and Pain Therapy Scheller, Bertram; Evangelisches Krankenhaus Düsseldorf
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Anaesthesia
Keywords:	Postoperative delirium, Cardiac surgery < SURGERY, Cholinesterease, Acetylcholinesterase, Butyrylcholinesterase

Cholinesterase alterations in delirium after cardiosurgery: a German monocentric prospective study

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Short title: Cholinesterases and postoperative delirium

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Author contributions

EA: wrote the manuscript, analyzed and interpreted the data

VH: conceived the study idea and collected data

SL: collected data, provided critical feedback and contributed to the final version of the manuscript

KZ: supervised the project and contributed to the final version of the manuscript

BS: conceived the study idea, analyzed the data and contributed to the final version of the manuscript

All authors read and approved the final version of the manuscript.

Author Disclosure Statement

The authors have reported no conflicts of interest.

Word count

3416

Data statement

Deidentified participant data are available from the corresponding author upon reasonable request

Abstract

Objectives

Postoperative delirium (POD) is a common complication after elective cardiac surgery. Recent evidence indicates that a disruption in the normal activity of the cholinergic system may be associated with delirium.

Design

Prospective observational study

Setting

Single-center at a European academic hospital.

Primary and secondary outcome measures

In our study the enzyme activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were determined preoperatively as well as on the first and second postoperative day. The confusion assessment method for the intensive care unit (CAM-ICU) was used to screen patients for the presence of POD.

Results

A total of 114 patients were included in the study. POD was associated with a decrease in BChE activity on postoperative day one (p=0.03). In addition, patients who developed POD, had significantly lower preoperative AChE activity than patients without POD (p<0.01). Multivariate analysis identified a preoperatively decreased AChE activity (OR 3.1; 95%CI 1.14-8.46), anticholinergic treatment (OR 5.09; 95%CI 1.51-17.23), elevated EuroSCORE (OR 3.68; 95%CI 1.04-12.99) and age (OR 3.02; 95%CI 1.06-8.62) to be independently associated with the development of POD.

Conclusions

We conclude that a reduction in the acetylcholine hydrolyzing enzyme activity in patients undergoing cardiac surgery may correlate with the development of POD.

Strengths and limitations of this study

- One strength of this study results from the prospective nature
- Another strength is the data acquisition from a high-volume center
- A limitation is the inclusion limited to cardiac surgery patients as it remains unclear whether the results can be extrapolated to other patient cohorts.
- As the symptoms of a delirium may vary over time, there may be a possibility that not all patients with a delirium were detected, due to a single assessment of delirium per day.

Keywords

Postoperative delirium

Cardiac surgery

Cholinesterase

Acetylcholinesterase

Butyrylcholinesterase

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Introduction

A delirium is a complex neuropsychiatric syndrome that is clinically characterized by sudden onset and fluctuating course. Clinical symptoms according to the actual definition of the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-5[1]) may be disturbances in attention, awareness and cognition. Delirium is characterized as an etiologically unspecific cerebro-organic syndrome representing a decompensation of cerebral function. The duration of delirium varies greatly and the severity ranges from mild to serious conditions.

The causes for delirium are multifactorial. Risk factors include dehydration, sleep deprivation, age, hypoxia, substance intoxication, anemia and hypoglycemia In the general population, the incidence is below 0.4%, in hospitalized patients between 15-22%.[2 3] Particularly after surgical interventions, patients are at risk of developing postoperative delirium (POD). The incidence is described to be as high as 52%.[4] The consequences of a POD are very different and range from prolonged hospital stay, increased risk of wound infections, reduced quality of life, more frequent discharge into nursing homes to increased mortality in the first year after surgery.[5-8] . Recent literature suggests an association between frailty and the development of POD, while limitations are considerable due to notable methodological heterogeneity between the methods of studies on such associations.[9] Another risk factor for the development of POD may be a preoperative cognitive impairment, as observed in patients undergoing vascular surgery.[10]

Higher age, longer duration of surgery as well as a reduced preoperative cognitive condition are frequently found in cardiac surgery patients and increase the risk for development of POD in this group of patients.[3] In the literature, the incidence of POD after cardiac surgery varies from 8 to 52%.[3 4 8 11] The duration of the POD in such patients varies widely, lasting three days on average.[5 12] Patients with POD are at risk for developing chronic postoperative cognitive dysfunction (POCD) over time and for suffering from severe long-term cognitive deficits.[13]

There are different hypotheses about the molecular mechanisms involved in the development of delirium.[14] The most common hypothesis for the development of POD is based

on a central cholinergic deficit resulting from a deficit of Acetylcholine (ACh): Pathologies at the presynapse, in the synaptic cleft or at the postsynaptic receptor may trigger a central cholinergic deficit. Acetylcholinesterase (AChE) is an enzyme which cleaves ACh in the synaptic cleft and terminates the transmission of a stimulus, a prerequisite for generating a new impulse. If the AChE is restricted in its function ACh remains in the synaptic cleft and blocks a new stimulus transmission.[15] However, several authors have found data challenging this hypothesis as they did not identify an association of preoperative serum anticholinergic activity with the development of POCD[16] or a therapeutic effect of rivastigmine for the prevention of POD.[17] Other hypotheses (e.g. brain injury, metabolic abnormalities) are based on localized or general brain energy deprivation critical to attentional processes such as the caudate nucleus or frontal cholinergic pathways.[16] Systemic inflammation may cause alterations including pro-inflammatory cytokines and prostaglandins mediated by humoral and neural signaling pathways leading to symptoms of delirium.[17]

Butyrylcholinesterase (BChE) is an enzyme which splits choline compounds as well as other esters.[18] For a long time BChE was thought to have a less important function, but recent literature demonstrated that BChE may in part and with a significantly slower rate and affinity act as a substitute in the absence of AChE with a relevant role in the development of a cholinergic deficit.[19 20] A recently published study identified a significant decrease in the enzyme activity of AChE and BChE in patients with POD after hip surgery.[21] However, the impact of a choline esterase deficit in patients remains unclear.

Due to the far-reaching consequences of a POD, it is of great importance to identify patients at risk for the development of such a disorder. Our study investigated the extent to which changes in bed-side enzyme activity of cholinesterases correlates with the development of POD in cardiac surgery patients and to identify possible factors influencing the development of POD.

Material and methods

This manuscript includes data gained during a prospective observational study at the University Hospital Frankfurt. The institutional review board approved the conduct of the study prior to its initiation (428/12 of 19 December 2012).

Participants

Patients were included between February 2013 and February 2014. Over this period, 150 patients who received elective cardiac surgery at the University Hospital Frankfurt were screened for inclusion. The participating patients were informed about the study verbally and in writing. Only patients with written consent were included in the study.

Potential patients had to meet the following inclusion criteria: elective cardiac surgery with and without the use of a cardiopulmonary bypass (CPB) and age over 18 years. Exclusion from the study was based on: preoperatively existing delirium; preoperatively sedated patients with Richmond Agitation and Sedation Scale (RASS) < -2; no proficiency of the German or English language or missing patient consent.

Design

After obtaining consent, patients were examined preoperatively and on the first and second postoperative day. Patients were examined for the presence of a POD using the confusion assessment method for the intensive care unit (CAM-ICU) clinical test[22]. In brief, the CAM-ICU assesses and scores clinical features associated with delirium. Depending on the results from CAM-ICU, a patient was assigned to either the postoperative delirium group (POD) or the no postoperative delirium (no POD) group. The patient was assigned to the POD group if a delirium was diagnosed at least once as per the CAM-ICU. If a patient was either under too much sedation or the examiner was not able to apply the CAM-ICU, the patient was not included for analysis.

Assessment of parameters

All included patients were scheduled for elective surgery and assessed directly before surgery at 7am to determine the presence of delirium. First, the RASS score was obtained, then blood samples were taken for the assessment of butyryl- and acetylcholinesterase activity. Further, blood samples were analyzed for AChE and BChE activity as measured with the ChE Check mobile ® (Securetec Detektions-Systeme AG, Neubiberg, Germany). Both, BChE and AChE activity were assessed using the ChE Check Mobile® as per the manufacturer's instruction. Preoperatively, blood samples were drawn from the fingertip (10µL). Postoperatively, blood samples (1mL) were obtained via an arterial line. As two enzymes were determined in different measurements, two blood samples were taken at different times and analyzed independently. To provide consistency between assessments, measurement of AChE was always performed first, followed by assessment of BChE activity. Measurements were about 10 min apart. As animal data on the circadian changes of cholinesterase reveal an relevant increase during the sleep phase, we have hence taken samples at the same time preoperatively (±1 hour) to ensure consistency of measurements.[23]

The ChE-Check mobile device incorporates a variety of factors contributing to a more precise analysis of cholinesterase activity.[24] Working conditions and technical data for this device are published online.[25] Resulting from the incorporation of these measures, the results obtained from this device can be considered to be highly reproducible and reliable, when compared to a reference method for determining choline esterase activity.[26]

Data collection

Basic demographic data, medication, hospitalization period, the length of stay on the intensive care unit, ventilation time as well as postoperative medication, transfusion, information about secondary diagnoses, weight, EuroSCORE[27], laboratory values as well as obtained scores were extracted from the patient data management system. Medication was considered to be

anticholinergic based on the study by Ancelin et al.[28] The duration of anesthesia, intraoperative medication, aortic clamping time (APC) and the duration of CPB were extracted from the anesthesia and premedication protocols. The data and results were inserted and maintained in an Excel database.

Statistics

All data were tested for normality using the D'Agostino and Pearson omnibus normality test. Data comparisons of patient characteristics were made using Mann-Whitney U- or χ2-test, where applicable. To compare activities of cholinesterases between different days, a Wilcoxon signed rank test was used. Univariate analysis was performed using the χ2-test. Non binary-parameters were stratified by the median. Parameters with a p-value less than 0.1 were included for multivariate analysis, as carried out by binary logistic regression.

Length of ventilation was defined as the time of intubation until extubation; length of stay on the intensive care unit (ICU) was defined as the time from surgery to the discharge from the postoperative ICU; length of stay in the hospital was defined as the time from surgery to discharge from the primary care hospital. For survival analysis, groups were compared using a log rank test and pointwise 95% confidence intervals (CI). A multivariate Cox's proportional hazards regression backward stepwise model (likelihood ratio) was performed to find independent predictors for outcome parameters.

Results with $p<0.05$ were considered to be statistically significant. All calculations/analyses were performed with SPSS (Version 25, Chicago, IL) or Graphpad Prism (Version 5.0, La Jolla, CA).

Patient and public involvement

No patient involved.

Results

Of the 150 patients screened for this study, 13 were excluded due to cancelled surgery and 23 were excluded due to an unavailability for assessment of delirium resulting from prolonged sedation thus leaving 114 patients available for analysis. Of the 114 patients included within our study, 31 patients (27.2%) developed a postoperative delirium (POD), while 83 patients (72.8%) did not show signs of a POD.

Baseline characteristics

No statistical differences were observed for sex, BMI, in-hospital death, preoperative incidence of alcohol abuse, the preoperative prescription of anticholinergic drugs or the performed procedure (Table 1). Of note, none of the patients without previous history of anticholinergic medication received anticholinergic medication throughout the ICU stay. However, patients who went on to develop a POD had a significantly higher EuroSCORE ($p=0.02$). Further, patients who developed POD were significantly older than patients without the development of POD ($p<0.01$).

Outcome dependent on the development of POD

Patients without the development of POD displayed a significantly shorter length of ventilation ($p=0.02$), shorter length of stay in the ICU ($p<0.01$) and shorter length of hospitalization ($p<0.01$) (Table 1). No differences were observed in regard to mortality, when comparing patients with or without the development of POD.

Assessment of cholinesterases

In the overall study population, the butyrylcholinesterase (BChE) decreased significantly over time, when comparing mean BChE activity on postoperative days one ($p<0.01$) and two ($p<0.01$) with the preoperative BChE activity (Figure 1A). Further, the mean acetylcholinesterase

(AChE) activity increased over time, when comparing the AChE activity on postoperative day two with the preoperative AChE activity ($p<0.05$) (Figure 1B).

No significant preoperative difference in BChE activity was observed in patients with or without POD (Figure 2A). Significant differences were observed in regard to the activity of BChE on postoperative day one ($p=0.03$) (Figure 2B), when comparing patients from the POD and the no-POD groups. However, no significant difference in BChE activity was observed on postoperative day 2 (Figure 2C) between patients with or without POD. Further, patients with the development of POD displayed significantly lower levels of AChE activity preoperatively ($p<0.01$) and on postoperative days one ($p<0.01$) and two ($p<0.01$) (Figure 2D-F).

Parameters associated with POD

To identify parameters associated with the development of POD in patients undergoing cardiac surgery, we performed a univariate analysis and identified age > 71 years, EuroSCORE ≥ 4, anticholinergic premedication and a preoperative AChE activity of < 44.3 U/g Hb (Table 2). To rule out potential confounding variables we performed a multivariate analysis and confirmed age > 71 years, EuroSCORE ≥4, preoperative anticholinergic medication and preoperative AChE activity of < 44.3 U/g Hb as parameters independently associated with the development of POD.

Parameters associated with length of stay on the ICU

Survival analysis demonstrated that patients with POD after cardiothoracic surgery displayed significantly longer LOS in the intensive care unit (Figure 3). To identify further parameters associated with prolonged stay in the ICU following cardiothoracic surgery, we performed various univariate analyses and identified EuroSCORE ≥ 4, preoperative anticholinergic medication, length of ventilation, transfusion of PRBCs, reduced AChE activity on postoperative day one, reduced postoperative BChE activity on postoperative day one and the development of POD as potentially associated (Table 3). To identify confounders, we performed

a multivariate analysis and identified length of ventilation, reduced BChE activity on postoperative day one and the development of POD as independently associated with prolonged length of stay in the ICU.

Discussion

The purpose of this study was to analyze a potential correlation between AChE and BChE activities and the incidence of POD in cardiosurgical patients and to identify further possible predictors for the development of POD.

The incidence of POD in our study population is in line with the literature.[2 3] Our results show that a preoperative AChE activity was significantly lower in patients who went on to develop POD than in patients without the development of POD. Further, BChE activity was significantly lower in patients with POD on the first postoperative day. Our data revealed that the patients who developed a POD were significantly older than those who did not suffer from a POD. These patients were more frequently on anticholinergic medication. Further, the EuroSCORE was higher in such patients and they were longer ventilated. In addition, patients with POD stayed significantly longer in the intensive care unit and were discharged significantly later for follow-up treatment.

Patients who went on to develop POD showed lower preoperative AChE activity compared to patients without the development of POD. This finding is in agreement with the current hypothesis that a reduction in AChE activity is associated with POD. It is hypothesized that due to this deficit, cholinesterase cannot efficiently cleave the neurotransmitter ACh in the synaptic cleft. As a consequence, the stimulus transmission cannot be terminated, and ultimately a new stimulus transmission cannot be initiated.[29]

In a recently published study, Cerejeira et al. measured AChE and BChE activities pre- and postoperatively in patients who had undergone elective hip surgery and examined patients for the development of a POD using CAM-ICU.[21] They came to the conclusion that patients with POD after surgery showed reduced preoperative AChE activity. As in our results, preoperative BChE activity was decreased in patients with POD. Contrary to their findings however, in our patient population groups with or without the development of POD did not differ significantly in preoperative BChE activity. This discrepancy might be attributed to different assays measuring enzyme activities. Most importantly, these findings need to be discussed in light of the 2017

publication by John et al.[30] This group did not find any differences regarding both AChE and BChE activity between patients with or without the development of POD. However, there are some considerable differences in the study design: no preoperative samples were collected in the study by John et al. Further, some samples were refrigerated before analysis, thereby potentially altering the measured enzyme activity. Zivkovic et el., however, have also identified a reduced BChE activity following surgery.[31] They suggested a cholinergic modulation of the inflammatory response that is independent of POD. This finding of a postoperatively decreased BChE activity and a potential association with POD as observed within our study needs to be addressed in further studies specifying the potential impact of cholinesterases in the development of POD, also in the context of inflammation.

In a study conducted in 2008, Hubbard et al. were able to show that a higher age was associated with deficits in the anticholinergic system.[32] Photometric determination of AChE revealed no significant difference for BChE activity between younger and older age, but a significantly lower activity of cholinesterases in the older people displaying a significant amount of frailty. They suspected that age was associated with changes in enzyme activity. While a deficit in cholinesterase activity may be observed in elderly patients, a significant correlation with age could not be demonstrated.[33-35] The association between age and the development of POD observed for our patient population fits well with the literature that described such association before.[36] In our cohort, patients with a history of anticholinergic medication suffered from a POD significantly more often than patients in the comparison group. This result supports the assumption that the anticholinergic predisposition has an influence on the development of the POD. It reduces the function of ACh and might also attribute to a cholinergic deficit. Anticholinergic medication is used when patients are regularly treated with antidepressants (e.g. amitriptyline, doxepin), anticonvulsants (e.g. gabapentin) or for Parkinson's disease (benserazide, L-DOPA). These drugs all have in common that they reduce ACh activity through direct and indirect anticholinergic action. In a study conducted in 2015, Naja et al. investigated geriatric patients with regard to the treatment

with anticholinergic drugs before and during hospitalization and the incidence of delirium. They came to the conclusion that the anticholinergic burden was associated with the occurrence of delirium and that anticholinergic exposure correlated with the incidence of delirium and increased mortality.[37]

When interpreting effect sizes of the above-named potential risk factors for the development of POD, preoperative anticholinergic medication had the highest odds ratio for the development of such condition. Further, age was identified to display a high odds ratio with a potential association of POD. A comparable effect on the development of POD was identified for the preoperative EuroSCORE. However, such finding needs to be interpreted with caution, as age is one of the parameters utilized for the calculation of the EuroSCORE. A reduced preoperative AChE activity had the smallest effect (as per an odds ratio of 3.1) of all significant parameters on the development of POD. These findings both demonstrate the importance of a cholinergic deficit and of age as risk for the development of POD.

Patients with POD had a significantly longer duration of anesthesia and were also operated on for longer periods of time. Long-lasting surgery is associated with many other risk factors such as hypoxemia, pain and disturbance of the sleep-wake rhythm.[36 38] The anesthesia itself interferes with various neuronal processes in the brain. It interacts with ion channels, such as the nicotinic acetylcholine receptors, neurotransmitters and second messengers, as well as metabolic processes.[39] The factors mentioned may have influenced the development of POD.

The effects of a POD are far-reaching. In our study, patients with POD not only stayed longer in the ICU, they also spent significantly more days in hospital postoperatively. These observations may be attributed to multiple factors such as delayed mobilization and physiotherapy.[40] Patients with POD require more intensive care from nurses and physicians, so that a transfer to the normal ward is only possible with delay and resulting in higher costs.[41] In a study published in 2004, Ely et al. showed that delirium is an independent predictor of significantly higher 6-month mortality and prolonged hospitalization in ventilated patients in the ICU.[42] Our

patients did not show an increased in-hospital mortality in patients with POD while we, however, did not follow up patients for 6 months. Conclusions on associations between long-term mortality and cholinesterase activity may therefore not be drawn from the results of our study.

To determine the diagnosis of delirium, the Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) was used, which is recommended by clinical guidelines.[43] While the CAM-ICU test is a tool for the diagnosis of delirium with the benefits of rapid assessment and no requirement for verbal communication with the patient, the CAM-ICU test does not provide information about motor subtypes of delirium.[44] We believe that future studies addressing this question are potentially of value to help understanding the pathology of this disease.

Strengths and limitations

Our study has several limitations that must be considered when evaluating the results. This study comprises exclusively cardiac surgery patients. Whether these data can be extrapolated to other patient cohorts remains unclear and warrants further validation. On a statistical note, we have not performed multiple comparison for the assessment of enzyme activities with a consecutive potential increase of the alpha error.

While the literature proposes a myriad of risk factors for the development of POD, differences in the methodology based on different definitions of delirium, differences in assessment of both risk factors and delirium and others, do not allow for a definitive list of risk factors. In conclusion, confounding by potential risk factors not addressed within this study (e.g. frailty) may limit the application of the results found within this study.

One limitation may be found in the lack of a consensus on a single classification system for anticholinergic medication. While several classification systems exist (as reviewed by Duran et al. [45]), the true effects of preoperative anticholinergic medication may differ depending on the classification system applied for analysis.

It is known that delirium can fluctuate strongly and occur acutely during the course of the day.[46] In this study, only one measurement was performed in the morning of the day of measurement. Thus, it is possible that not all patients who developed a delirium were detected with the applied screening method. One limitation of our study might be the short duration of two days measurement, which might have led to patients with postoperative delirium not being diagnosed with delirium. Further, a substantial variation of results was observed within the study, potentially limiting the conclusions drawn from the results.

The patient population was reduced from a total of 150 patients to 114, who were ultimately included for analysis. One reason for the exclusion of patients was excessive sedation at postoperative days one and two and thus an exclusion criterion for the CAM-ICU. Future studies should cover a longer observation period in order to be able to include such patients for analysis and to enable further conclusions to be drawn about the temporal development of POD.

Conclusions

We demonstrated that the development of POD after cardiac surgery correlates with postoperative decrease of BChE activity. In addition, patients who developed POD in the course of surgery showed significantly lower preoperative AChE activity as compared to patients without POD. We were able to identify a low preoperative AChE activity, an anticholinergic pre-medication, an increased EuroSCORE and a higher age as predictors for development of POD. In addition, patients with POD differed from their peers by a longer postoperative ventilation time, an extended stay at the ICU and prolonged hospitalization.

Our data show that the cholinergic deficit hypothesis may be of importance for the development of POD. Anticholinergic medication may intervene in this pathophysiological system and may influence AChE and BChE activity resulting in neuroinflammation.

There are various studies investigating the risk factors for the occurrence of POD. Some correlations in the development of POD have been identified. However, the molecular basis of multifactorial POD has not yet been sufficiently understood. Nonetheless, this is necessary in order to develop preventive measures. Further studies are needed to investigate the exact pathomechanisms of risk factors for such disease.

Acknowledgements

None.

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Table and Figure legends

Table 1. Patient characteristics.

	No postoperative delirium (n=83)	Postoperative delirium (n=31)	
Age (y[IQR])	69 (58 – 74)	74 (71-78)	<0.01*
Female sex (n[%])	22 (26.5)	9 (29)	0.79
EuroSCORE (n[%])			0.02
1-5	59 (71.1)	13 (41.9)	
6-10	22 (26.5)	16 (51.6)	
11-15	2 (2.4)	2 (6.5)	
Body Mass Index (kg/m^2[SD])	27.6 (±4.8)	28 (4.8)	0.7*
Alcohol abuse (n[%])	2 (2.4)	0	1
Anticholinergic premedication (n[%])	8 (9.9)	10 (32.3)	<0.01
Procedure (n[%])			0.3
ACVB	33 (39.8)	15 (48.4)	
AVR	24 (28.9)	6 (19.4)	
Combined Procedure	10 (12)	6 (19.4)	
TAVI	4 (4.9)	3 (9.7)	
MVR	6 (7.2)	1 (3.1)	
Other	6 (7.2)	0	
Length of ventilation (min[SD])	471 (±159)	1427 (±3565)	0.02*
Length of stay on ICU (h[SD])	20.1 (±20.1)	93.5 (±183)	<0.01*
Length of stay in hospital (d[SD])	13.1 (±5)	20.9 (13.9)	<0.01*
In-hospital death (n[%])	1 (1.2)	1 (3.2)	0.47*
Preop BChE activity (U/g Hb[median, SD])	2773 (2740±885)	2734 (2891±922)	0.83
PO day 1 BChE activity (U/g Hb [median, SD])	1966 (1971±588)	1674 (1752±730)	0.03
PO day 2 BChE activity (U/g Hb [median, SD])	1870 (1868±564)	1694 (1715±596)	0.16
Preop AChE activity (U/g Hb [median, SD])	45.4 (45±5.7)	42.2 (41.5±6.3)	<0.01*
PO day 1AChE activity (U/g Hb [median, SD])	45.1 (44.1±5.1)	41.8 (42±5.5)	<0.01*
PO day 2 AChE activity (U/g Hb [median, SD])	45.5 (45.6±4.6)	42.7 (42.8±5.8)	<0.01*

Table 1. Patient characteristics. Data are given as means except for age which is presented as the median and as indicated. Data comparisons were made with the *t*-test or the χ^2-test, where applicable. * denotes the use of a non-parametric test due to non-normal distribution of data. ICU = intensive care unit, CABG = coronary artery bypass grafting, AVR = aortic valve replacement, TAVI = transcatheter aortic valve replacement, MVR = mitral valve replacement, BChE =

butyrylcholinesterase, AChE = acetylcholinesterase, PO = postoperative. IQR indicates interquartile range.

Table 2. Univariate and multivariate analysis of parameters associated with the development of postoperative delirium.

	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	*p* value	OR (95% CI)	p value
Age > 71 years	4.48 (1.74 – 11.54)	<0.01	3.02 (1.06 – 8.62)	0.04
BMI > 27.5	1.31 (0.57 – 2.99)	0.67		
Male sex	1.13 (0.45 - 2.84)	0.82		
EURO-Score ≥ 4	5.43 (1.74 – 16.91)	<0.01*	3.68 (1.04 – 12.99)	0.04
Known alcohol abuse	**	1.0*		
Anticholinergic premedication	6.02 (1.96 – 18.52)	<0.01	5.09 (1.51 – 17.23)	<0.01
Length of ventilation > 456 min	1.56 (0.68 -3.6)	0.29		
Transfusion of PRBC	2.26 (0.96 – 5.31)	0.06		0.28
Preop AchE activity of < 44.3 U/g Hb	2.74 (1.15 – 6.54)	0.02	3.1 (1.14 – 8.46)	0.03
Preop BchE activity of < 2762 U/g Hb	1.31 (0.57 – 2.99)	0.53		

Table 2. Univariate and multivariate analysis of parameters associated with the development of postoperative delirium. Data comparisons were made with χ2-test for univariate analysis, binary logistic regression with stepwise exclusion was used for multivariate analysis. BMI = body mass index, PRBC = Packed red blood cells, BChE = butyrylcholinesterase, AChE = acetylcholinesterase. OR indicates odds ratio, CI indicates confidence interval. For multivariate analysis OR is only displayed in significant outcome parameters/where applicable.

Table 3. Univariate and multivariate analysis of parameters associated with length of stay in the ICU.

	Univariate Analysis		Multivariate Analysis		
	Median (95% CI)	*p* value	HR	(95% CI)	*p* value
Age		0.97			
Age > 71 years	0.75 (0.65 – 0.86)				
Age < 71 years	0.79 (0.56 -1.03)				
BMI		0.24			
BMI > 27.5	0.79 (0.68 – 0.91)				
BMI ≤ 27.5	0.71 (0.48 – 0.94)				
Sex		0.89			
Male	0.75 (0.55 – 0.95)				
Female	0.75 (0.64 – 0.86)				
EURO-Score		<0.01			0.33
EURO-Score ≥ 4	0.79 (0.65 – 0.94)				
EURO-Score < 4	0.42 (0.11 – 0.72)				
Known alcohol abuse		0.76			
Present	0.75 (0.66 – 0.84)				
Absent	0.38 (-)*				
Anticholinergic premedication		0.05			0.39
Present	0.75 (0.59 – 0.91)				
Absent	0.75 (0.64 – 0.86)				
Length of ventilation		<0.01	2.77 (1.83 – 4.2)		<0.01
Length of ventilation > 456 min	1.04 (0.87 – 1.2)				
Length of ventilation < 456 min	0.33 (0.28 – 0.39)				
Transfusion of PRBC		0.04			0.98
Present	0.92 (0.76 – 1.07)				
Absent	0.5 (0.28 – 0.72)				
PO day 1 AchE activity		0.03			0.47
PO day 1 AchE activity of < 44.3 U/g Hb	0.79 (0.66 – 0.93)				
PO day 1 AchE activity of > 44.3 U/g Hb	0.71 (0.44 – 0.98)				
PO day 1 BchE activity		<0.01	1.84 (1.24 – 2.75)		<0.01

PO day 1 BchE activity of < 2762 U/g Hb	1 (0.84 – 1.16)			
PO day BchE activity of > 2762 U/g Hb	0.5 (0.29 – 0.71)			
Delirium		<0.01	1.79 (1.1 – 2.91)	0.02
Present	1.08 (0.48 – 1.69)			
Absent	0.71 (0.51 – 0.91)			

Table 3. Univariate and multivariate analysis of parameters associated with length of stay in the ICU. Data comparisons were made with Kaplan-Meier estimates for univariate analysis. Column median indicates median of parameter displayed. Cox-regression analysis with stepwise exclusion was used for multivariate analysis. BMI = Body mass index, EuroSCORE = European System for Cardiac Operative Risk Evaluation, PRBC = Packed red blood cells, BChE = butyrylcholinesterase, AChE = acetylcholinesterase, PO = postoperative. HR indicates hazard ratio, CI indicates confidence interval. For multivariate analysis HR is only displayed in significant outcome parameters/where applicable.

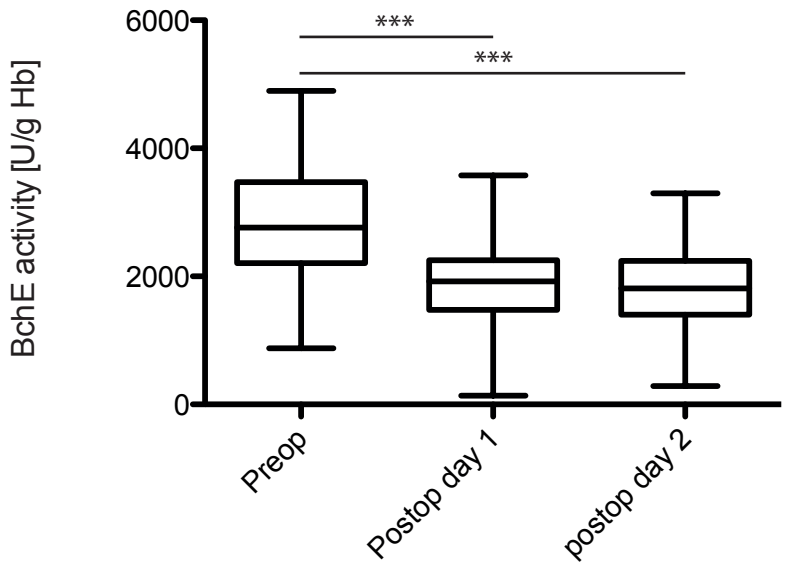
Figure 1. Activity of BChE and AChE in the overall patient population. Activity of A) butyrylcholinesterase (BChE) and B) acetylcholinesterase (AChE) were assessed preoperatively and on postoperative days one and two. *** indicates a p-value of <0.01; * indicates a p-value of <0.05.

Figure 2. Activity of BChE and AChE in patients without or with the development of POD. Activity of butyrylcholinesterase (BChE) was assessed A) preoperatively and on postoperative days B) one and C) two. Activity of acetylcholinesterase (AChE) were assessed D) preoperatively and on postoperative days E) one and F) two. * indicates a p-value of <0.05

Figure 3. Kaplan-Meier estimate. Time to discharge from ICU (logrank test $\chi^2 = 14.88$, $p < 0.01$)

Figure 1

A



B

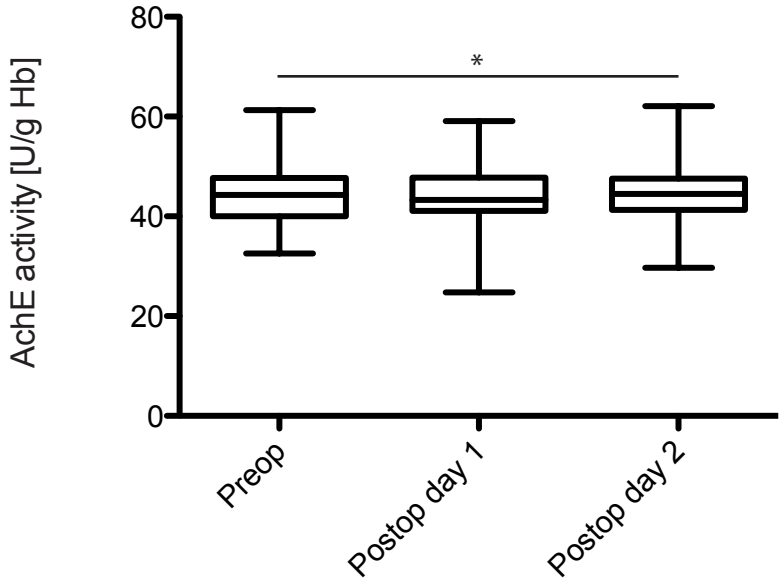
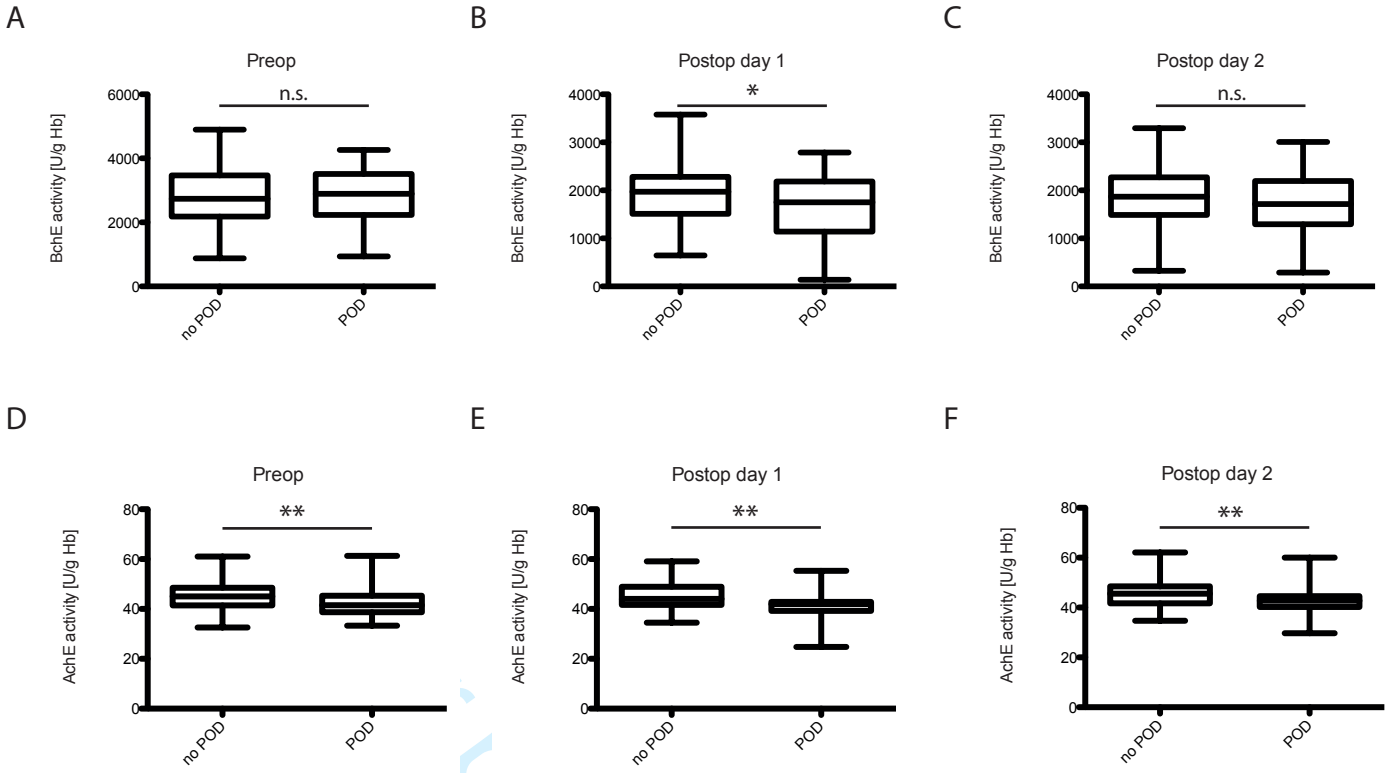
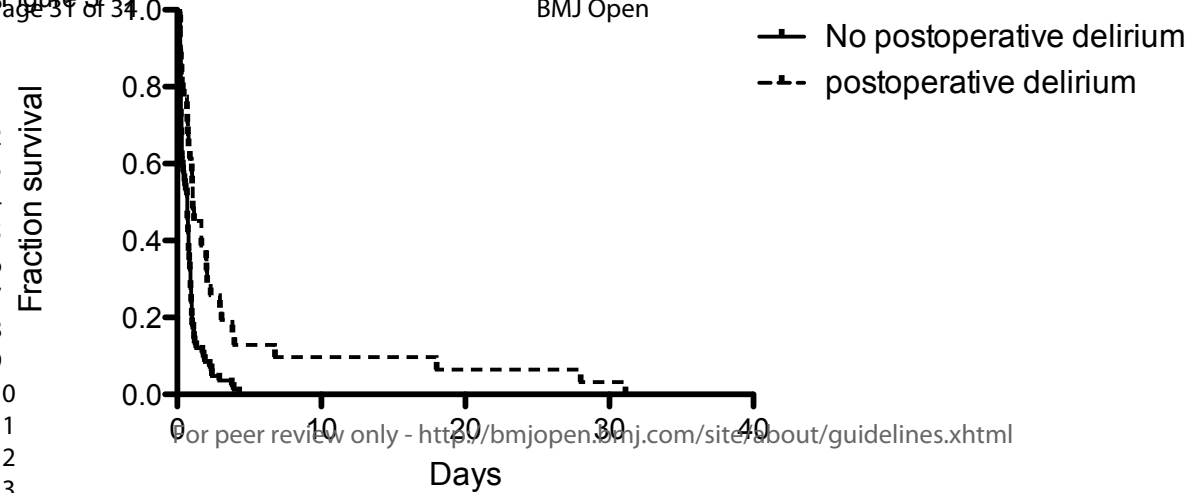


Figure 2





STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Section and Item	Item No.	Recommendation	Reported on Page No.
Title and Abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/Rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	8
Methods			
Study Design	4	Present key elements of study design early in the paper	9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	9
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	9
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9, 10

Section and Item	Item No.	Recommendation	Reported on Page No.
Data Sources/ Measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9, 10
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study Size	10	Explain how the study size was arrived at	n/a
Quantitative Variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11
Statistical Methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11, 12
		(*b*) Describe any methods used to examine subgroups and interactions	11, 12
		(*c*) Explain how missing data were addressed	n/a
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	n/a
		(*e*) Describe any sensitivity analyses	17
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12
		(b) Give reasons for non-participation at each stage	12
		(c) Consider use of a flow diagram	n/a
Descriptive Data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12
		(b) Indicate number of participants with missing data for each variable of interest	n/a
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	n/a
Outcome Data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	n/a
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	

Section and Item	Item No.	Recommendation	Reported on Page No.
Main Results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12, 13
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other Analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12, 13
Discussion			
Key Results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	6

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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Cholinesterase alterations in delirium after cardiosurgery: a German monocentric prospective study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-031212.R3
Article Type:	Original research
Date Submitted by the Author:	19-Nov-2019
Complete List of Authors:	Adam, Elisabeth; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt, Haas, Victoria; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt Lindau, Simone; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt Zacharowski, Kai; University Hospital Frankfurt, Clinic of Anesthesiology, Intensive Care Medicine and Pain Therapy Scheller, Bertram; Evangelisches Krankenhaus Düsseldorf
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Anaesthesia
Keywords:	Postoperative delirium, Cardiac surgery < SURGERY, Cholinesterease, Acetylcholinesterase, Butyrylcholinesterase

Cholinesterase alterations in delirium after cardiosurgery: a German monocentric prospective study

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Short title: Cholinesterases and postoperative delirium

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All authors read and approved the final version of the manuscript.

Author Disclosure Statement

The authors have reported no conflicts of interest.

Word count

3416

Data statement

Deidentified participant data are available from the corresponding author upon reasonable request

Abstract

Objectives

Postoperative delirium (POD) is a common complication after elective cardiac surgery. Recent evidence indicates that a disruption in the normal activity of the cholinergic system may be associated with delirium.

Design

Prospective observational study

Setting

Single-center at a European academic hospital.

Primary and secondary outcome measures

In our study the enzyme activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were determined preoperatively as well as on the first and second postoperative day. The confusion assessment method for the intensive care unit (CAM-ICU) was used to screen patients for the presence of POD.

Results

A total of 114 patients were included in the study. POD was associated with a decrease in BChE activity on postoperative day one (p=0.03). In addition, patients who developed POD, had significantly lower preoperative AChE activity than patients without POD (p<0.01). Multivariate analysis identified a preoperatively decreased AChE activity (OR 3.1; 95%CI 1.14-8.46), anticholinergic treatment (OR 5.09; 95%CI 1.51-17.23), elevated EuroSCORE (OR 3.68; 95%CI 1.04-12.99) and age (OR 3.02; 95%CI 1.06-8.62) to be independently associated with the development of POD.

Conclusions

We conclude that a reduction in the acetylcholine hydrolyzing enzyme activity in patients undergoing cardiac surgery may correlate with the development of POD.

Strengths and limitations of this study

- One strength of this study results from the prospective nature
- Another strength is the data acquisition from a high-volume center
- A limitation is the inclusion limited to cardiac surgery patients as it remains unclear whether the results can be extrapolated to other patient cohorts.
- As the symptoms of a delirium may vary over time, there may be a possibility that not all patients with a delirium were detected, due to a single assessment of delirium per day.

Keywords

Postoperative delirium

Cardiac surgery

Cholinesterase

Acetylcholinesterase

Butyrylcholinesterase

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Introduction

A delirium is a complex neuropsychiatric syndrome that is clinically characterized by sudden onset and fluctuating course. Clinical symptoms according to the actual definition of the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-5[1]) include disturbances in attention, awareness and another cognitive domain. Delirium is characterized as an etiologically unspecific cerebro-organic syndrome representing a decompensation of cerebral function.[2] The duration of delirium varies greatly and the severity ranges from mild to serious conditions.

The causes for delirium are multifactorial. Risk factors include dehydration, sleep deprivation, age, hypoxia, substance intoxication, anemia and hypoglycemia In the general population, the incidence is below 0.4%, in hospitalized patients between 15-22%.[3 4] Particularly after surgical interventions, patients are at risk of developing postoperative delirium (POD). The incidence is described to be as high as 52%.[5] The consequences of a POD are very different and range from prolonged hospital stay, increased risk of wound infections, reduced quality of life, more frequent discharge into nursing homes to increased mortality in the first year after surgery.[6-9] . Recent literature suggests an significant association between frailty and the development of POD with an OR of almost 10, while limitations are considerable due to notable methodological heterogeneity between the methods of studies on such associations.[10] Another risk factor for the development of POD may be a preoperative cognitive impairment, as observed in patients undergoing vascular surgery with a demonstrated OR of greater than 2.[11]

Higher age, longer duration of surgery as well as a reduced preoperative cognitive condition are frequently found in cardiac surgery patients and increase the risk for development of POD in this group of patients.[4] In the literature, the incidence of POD after cardiac surgery varies from 8 to 52%.[4 5 9 12] The duration of the POD in such patients varies widely, lasting three days on average.[6 13] Patients with POD are at risk for developing chronic postoperative cognitive dysfunction (POCD) over time and for suffering from severe long-term cognitive deficits.[14]

There are different hypotheses about the molecular mechanisms involved in the development of delirium.[15] The most common hypothesis for the development of POD is based on a central cholinergic deficit resulting from a deficit of Acetylcholine (ACh): Pathologies at the presynapse, in the synaptic cleft or at the postsynaptic receptor may trigger a central cholinergic deficit. Acetylcholinesterase (AChE) is an enzyme which cleaves ACh in the synaptic cleft and terminates the transmission of a stimulus, a prerequisite for generating a new impulse. If the AChE is restricted in its function ACh remains in the synaptic cleft and blocks a new stimulus transmission.[16] However, several authors have found data challenging this hypothesis as they did not identify an association of preoperative serum anticholinergic activity with the development of POCD[17] or a therapeutic effect of rivastigmine for the prevention of POD.[18] Other hypotheses (e.g. brain injury, metabolic abnormalities) are based on localized or general brain energy deprivation critical to attentional processes such as the caudate nucleus or frontal cholinergic pathways.[17] Systemic inflammation may cause alterations including pro-inflammatory cytokines and prostaglandins mediated by humoral and neural signaling pathways leading to symptoms of delirium.[18]

Butyrylcholinesterase (BChE) is an enzyme which splits choline compounds as well as other esters.[19] For a long time BChE was thought to have a less important function, but recent literature demonstrated that BChE may in part and with a significantly slower rate and affinity act as a substitute in the absence of AChE with a relevant role in the development of a cholinergic deficit.[20 21]

A recently published study identified a significant decrease in the enzyme activity of AChE and BChE in patients with POD after hip surgery.[22] However, the impact of a choline esterase deficit in patients remains unclear. Previously published manuscripts on the impact of cholinesterase activity on POD in surgical patients reach different conclusions. While postoperative measurement of AChE and BChE did not discern between patients with and without POD in a study published by John et al., Muller et al. found a potential relationship between

cholinesterase activity and the development of POD.[23] [24] While John et al. only studied postoperative cholinesterease activity, we sought to incorporate preoperative cholinesterase activity in order to assess a potential implication on the development of POD. In contrast to the study of Muller et al. we only included patients undergoing cardiac surgery in order to homogenize the patient cohort.

Due to the far-reaching consequences of a POD, it is of great importance to identify patients at risk for the development of such a disorder. Our study investigated the extent to which changes in bed-side enzyme activity of cholinesterases correlates with the development of POD in cardiac surgery patients and to identify possible factors influencing the development of POD.

Material and methods

This manuscript includes data gained during a prospective observational study at the University Hospital Frankfurt. The institutional review board approved the conduct of the study prior to its initiation (428/12 of 19 December 2012).

Participants

Patients were included between February 2013 and February 2014. Over this period, 150 patients who received elective cardiac surgery at the University Hospital Frankfurt were screened for inclusion. The participating patients were informed about the study verbally and in writing. Only patients with written consent were included in the study.

Potential patients had to meet the following inclusion criteria: elective cardiac surgery with and without the use of a cardiopulmonary bypass (CPB) and age over 18 years. Exclusion from the study was based on: preoperatively existing delirium; preoperatively sedated patients with Richmond Agitation and Sedation Scale (RASS) < -2; no proficiency of the German or English language or missing patient consent.

Design

After obtaining consent, patients were examined preoperatively and on the first and second postoperative day. Patients were examined for the presence of a POD using the confusion assessment method for the intensive care unit (CAM-ICU) clinical test[25]. In brief, the CAM-ICU assesses and scores clinical features associated with delirium. Depending on the results from CAM-ICU, a patient was assigned to either the postoperative delirium group (POD) or the no postoperative delirium (no POD) group. The patient was assigned to the POD group if a delirium was diagnosed at least once as per the CAM-ICU. If a patient was either under too much sedation or the examiner was not able to apply the CAM-ICU, the patient was not included for analysis.

Assessment of parameters

All included patients were scheduled for elective surgery and assessed directly before surgery at 7am to determine the presence of delirium. First, the RASS score was obtained, then blood samples were taken for the assessment of butyryl- and acetylcholinesterase activity. Further, blood samples were analyzed for AChE and BChE activity as measured with the ChE Check mobile ® (Securetec Detektions-Systeme AG, Neubiberg, Germany). Both, BChE and AChE activity were assessed using the ChE Check Mobile® as per the manufacturer's instruction. Preoperatively, blood samples were drawn from the fingertip (10µL). Postoperatively, blood samples (1mL) were obtained via an arterial line. As two enzymes were determined in different measurements, two blood samples were taken at different times and analyzed independently. To provide consistency between assessments, measurement of AChE was always performed first, followed by assessment of BChE activity. Measurements were about 10 min apart. As animal data on the circadian changes of cholinesterase reveal an relevant increase during the sleep phase, we have hence taken samples at the same time preoperatively (±1 hour) to ensure consistency of measurements.[26]

The ChE-Check mobile device incorporates a variety of factors contributing to a more precise analysis of cholinesterase activity.[27] Working conditions and technical data for this device are published online.[28] Previously, detailed information on the accuracy of this device have been published before having demonstrated acceptable reliability for the measurement of cholinesterases.[29] Further, this device has been used in the context of POD before.[23 24] Resulting from the incorporation of these measures, the results obtained from this device can be considered to be highly reproducible and reliable, when compared to a reference method for determining choline esterase activity.[30]

Data collection

Basic demographic data, medication, hospitalization period, the length of stay on the intensive care unit, ventilation time as well as postoperative medication, transfusion, information about secondary diagnoses, weight, laboratory values as well as obtained scores were extracted from the patient data management system. Further, the EuroSCORE[31] was calculated for each patient. The EuroSCORE (European System for Cardiac Operative Risk Evaluation) is a risk model that facilitates a calculation of the risk of death after heart surgery. The model asks for 17 parameters about the patient, the condition of the heart and the proposed surgery and calculates the risk of death. The EuroSCORE has become the most widely used risk index for cardiac surgery, potentially improving the results of cardiac surgery. Medication was considered to be anticholinergic based on the study by Ancelin et al.[32] The duration of anesthesia, intraoperative medication, aortic clamping time (APC) and the duration of CPB were extracted from the anesthesia and premedication protocols. The data and results were inserted and maintained in an Excel database.

Statistics

All data were tested for normality using the D'Agostino and Pearson omnibus normality test. Data comparisons of patient characteristics were made using Mann-Whitney U- or $\chi 2$-test, where applicable. To compare activities of cholinesterases between different days, a Wilcoxon signed rank test was used. Univariate analysis was performed using the $\chi 2$-test. Non binary-parameters were stratified by the median. Parameters with a p-value less than 0.1 were included for multivariate analysis, as carried out by binary logistic regression.

Length of ventilation was defined as the time of intubation until extubation; length of stay on the intensive care unit (ICU) was defined as the time from surgery to the discharge from the postoperative ICU; length of stay in the hospital was defined as the time from surgery to discharge from the primary care hospital. For survival analysis, groups were compared using a log rank test and pointwise 95% confidence intervals (CI). A multivariate Cox's proportional hazards regression

backward stepwise model (likelihood ratio) was performed to find independent predictors for outcome parameters.

Results with $p<0.05$ were considered to be statistically significant. All calculations/analyses were performed with SPSS (Version 25, Chicago, IL) or Graphpad Prism (Version 5.0, La Jolla, CA).

Patient and public involvement

No patient involved.

Results

Of the 150 patients screened for this study, 13 were excluded due to cancelled surgery and 23 were excluded due to an unavailability for assessment of delirium resulting from prolonged sedation thus leaving 114 patients available for analysis. Of the 114 patients included within our study, 31 patients (27.2%) developed a postoperative delirium (POD), while 83 patients (72.8%) did not show signs of a POD.

Baseline characteristics

No statistical differences were observed for sex, BMI, in-hospital death, preoperative incidence of alcohol abuse, the preoperative prescription of anticholinergic drugs or the performed procedure (Table 1). Of note, none of the patients without previous history of anticholinergic medication received anticholinergic medication throughout the ICU stay. However, patients who went on to develop a POD had a significantly higher EuroSCORE ($p=0.02$). Further, patients who developed POD were significantly older than patients without the development of POD ($p<0.01$).

Outcome dependent on the development of POD

Patients without the development of POD displayed a significantly shorter length of ventilation ($p=0.02$), shorter length of stay in the ICU ($p<0.01$) and shorter length of hospitalization ($p<0.01$) (Table 1). No differences were observed in regard to mortality, when comparing patients with or without the development of POD.

Assessment of cholinesterases

In the overall study population, the butyrylcholinesterase (BChE) decreased significantly over time, when comparing mean BChE activity on postoperative days one ($p<0.01$) and two ($p<0.01$) with the preoperative BChE activity (Figure 1A). Further, the mean acetylcholinesterase

(AChE) activity increased over time, when comparing the AChE activity on postoperative day two with the preoperative AChE activity ($p<0.05$) (Figure 1B).

No significant preoperative difference in BChE activity was observed in patients with or without POD (Figure 2A). Significant differences were observed in regard to the activity of BChE on postoperative day one ($p=0.03$) (Figure 2B), when comparing patients from the POD and the no-POD groups. However, no significant difference in BChE activity was observed on postoperative day 2 (Figure 2C) between patients with or without POD. Further, patients with the development of POD displayed significantly lower levels of AChE activity preoperatively ($p<0.01$) and on postoperative days one ($p<0.01$) and two ($p<0.01$) (Figure 2D-F).

Parameters associated with POD

To identify parameters associated with the development of POD in patients undergoing cardiac surgery, we performed a univariate analysis and identified age > 71 years, EuroSCORE ≥ 4, anticholinergic premedication and a preoperative AChE activity of < 44.3 U/g Hb (Table 2). To rule out potential confounding variables we performed a multivariate analysis and confirmed age > 71 years, EuroSCORE ≥4, preoperative anticholinergic medication and preoperative AChE activity of < 44.3 U/g Hb as parameters independently associated with the development of POD.

Parameters associated with length of stay on the ICU

Survival analysis demonstrated that patients with POD after cardiothoracic surgery displayed significantly longer LOS in the intensive care unit (Figure 3). To identify further parameters associated with prolonged stay in the ICU following cardiothoracic surgery, we performed various univariate analyses and identified EuroSCORE ≥ 4, preoperative anticholinergic medication, length of ventilation, transfusion of PRBCs, reduced AChE activity on postoperative day one, reduced postoperative BChE activity on postoperative day one and the development of POD as potentially associated (Table 3). To identify confounders, we performed

a multivariate analysis and identified length of ventilation, reduced BChE activity on postoperative day one and the development of POD as independently associated with prolonged length of stay in the ICU.

Discussion

The purpose of this study was to analyze a potential correlation between AChE and BChE activities and the incidence of POD in cardiosurgical patients and to identify further possible predictors for the development of POD.

The incidence of POD in our study population is in line with the literature.[3 4] Our results show that a preoperative AChE activity was significantly lower in patients who went on to develop POD than in patients without the development of POD. Further, BChE activity was significantly lower in patients with POD on the first postoperative day. Our data revealed that the patients who developed a POD were significantly older than those who did not suffer from a POD. These patients were more frequently on anticholinergic medication. Further, the EuroSCORE was higher in such patients and they were longer ventilated. In addition, patients with POD stayed significantly longer in the intensive care unit and were discharged significantly later for follow-up treatment.

Patients who went on to develop POD showed lower preoperative AChE activity compared to patients without the development of POD. This finding is in agreement with the current hypothesis that a reduction in AChE activity is associated with POD. It is hypothesized that due to this deficit, cholinesterase cannot efficiently cleave the neurotransmitter ACh in the synaptic cleft. As a consequence, the stimulus transmission cannot be terminated, and ultimately a new stimulus transmission cannot be initiated.[33]

In a recently published study, Cerejeira et al. measured AChE and BChE activities pre- and postoperatively in patients who had undergone elective hip surgery and examined patients for the development of a POD using CAM-ICU.[22] They came to the conclusion that patients with POD after surgery showed reduced preoperative AChE activity. As in our results, preoperative BChE activity was decreased in patients with POD. Contrary to their findings however, in our patient population groups with or without the development of POD did not differ significantly in preoperative BChE activity. This discrepancy might be attributed to different assays measuring enzyme activities. Most importantly, these findings need to be discussed in light of the 2017

publication by John et al.[23] This group did not find any differences regarding both AChE and BChE activity between patients with or without the development of POD. However, there are some considerable differences in the study design: no preoperative samples were collected in the study by John et al. Further, some samples were refrigerated before analysis, thereby potentially altering the measured enzyme activity. Zivkovic et el., however, have also identified a reduced BChE activity following surgery.[34] They suggested a cholinergic modulation of the inflammatory response that is independent of POD. This finding of a postoperatively decreased BChE activity and a potential association with POD as observed within our study needs to be addressed in further studies specifying the potential impact of cholinesterases in the development of POD, also in the context of inflammation. In a recently published manuscript, Muller et al. found that peri-operative peripheral cholinesterase activities may be related to the development of POD.[24] In this study, cholinesterase activities were measured in surgical patients of various specialties. However, the authors of the above-named study stated the lack of a subgroup analysis discriminating between surgical procedures as a limitation of their study. In our study comprised of patients undergoing cardiac surgery, we were able to find comparable results, potentially indicating an importance of cholinesterase activity in the development of POD.

In a study conducted in 2008, Hubbard et al. were able to show that a higher age was associated with deficits in the anticholinergic system.[35] Photometric determination of AChE revealed no significant difference for BChE activity between younger and older age, but a significantly lower activity of cholinesterases in the older people displaying a significant amount of frailty. They suspected that age was associated with changes in enzyme activity. While a deficit in cholinesterase activity may be observed in elderly patients, a significant correlation with age could not be demonstrated.[36-38] The association between age and the development of POD observed for our patient population fits well with the literature that described such association before.[39] In our cohort, patients with a history of anticholinergic medication suffered from a POD significantly more often than patients in the comparison group. This result supports the assumption

that the anticholinergic predisposition has an influence on the development of the POD. It reduces the function of ACh and might also attribute to a cholinergic deficit. Anticholinergic medication is used when patients are regularly treated with antidepressants (e.g. amitriptyline, doxepin), anticonvulsants (e.g. gabapentin) or for Parkinson's disease (benserazide, L-DOPA). These drugs all have in common that they reduce ACh activity through direct and indirect anticholinergic action. In a study conducted in 2015, Naja et al. investigated geriatric patients with regard to the treatment with anticholinergic drugs before and during hospitalization and the incidence of delirium. They came to the conclusion that the anticholinergic burden was associated with the occurrence of delirium and that anticholinergic exposure correlated with the incidence of delirium and increased mortality.[40]

When interpreting effect sizes of the above-named potential risk factors for the development of POD, preoperative anticholinergic medication had the highest odds ratio for the development of such condition. Further, age was identified to display a high odds ratio with a potential association of POD. A comparable effect on the development of POD was identified for the preoperative EuroSCORE. However, such finding needs to be interpreted with caution, as age is one of the parameters utilized for the calculation of the EuroSCORE. A reduced preoperative AChE activity had the smallest effect (as per an odds ratio of 3.1) of all significant parameters on the development of POD. These findings both demonstrate the importance of a cholinergic deficit and of age as risk for the development of POD. However, when interpreting these findings in an external framework, other parameters which have not been assessed in the present study may be of importance: most importantly, frailty has a demonstrated high impact on the development of POD. In a recently published meta-analysis the OR of frailty for the development of POD was higher (OR > 9) than any of the parameters studied within this trial. Hence, future studies need to address the importance of the factors identified within this study and other factors such as frailty or cognitive impairment.

Patients with POD had a significantly longer duration of anesthesia and were also operated on for longer periods of time. Long-lasting surgery is associated with many other risk factors such as hypoxemia, pain and disturbance of the sleep-wake rhythm.[39 41] The anesthesia itself interferes with various neuronal processes in the brain. It interacts with ion channels, such as the nicotinic acetylcholine receptors, neurotransmitters and second messengers, as well as metabolic processes.[42] The factors mentioned may have influenced the development of POD.

The effects of a POD are far-reaching. In our study, patients with POD not only stayed longer in the ICU, they also spent significantly more days in hospital postoperatively. These observations may be attributed to multiple factors such as delayed mobilization and physiotherapy.[43] Patients with POD require more intensive care from nurses and physicians, so that a transfer to the normal ward is only possible with delay and resulting in higher costs.[44] In a study published in 2004, Ely et al. showed that delirium is an independent predictor of significantly higher 6-month mortality and prolonged hospitalization in ventilated patients in the ICU.[45] Our patients did not show an increased in-hospital mortality in patients with POD while we, however, did not follow up patients for 6 months. Conclusions on associations between long-term mortality and cholinesterase activity may therefore not be drawn from the results of our study.

To determine the diagnosis of delirium, the Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) was used, which is recommended by clinical guidelines.[46] While the CAM-ICU test is a tool for the diagnosis of delirium with the benefits of rapid assessment and no requirement for verbal communication with the patient, the CAM-ICU test does not provide information about motor subtypes of delirium.[47] We believe that future studies addressing this question are potentially of value to help understanding the pathology of this disease.

Strengths and limitations

Our study has several limitations that must be considered when evaluating the results. This study comprises exclusively cardiac surgery patients. Whether these data can be extrapolated to

other patient cohorts remains unclear and warrants further validation. On a statistical note, we have not performed multiple comparison for the assessment of enzyme activities with a consecutive potential increase of the alpha error.

While the literature proposes a myriad of risk factors for the development of POD, differences in the methodology based on different definitions of delirium, differences in assessment of both risk factors and delirium and others, do not allow for a definitive list of risk factors. In conclusion, confounding by potential risk factors not addressed within this study (e.g. frailty or cognitive impairment) may limit the application of the results found within this study.

One limitation may be found in the lack of a consensus on a single classification system for anticholinergic medication. While several classification systems exist (as reviewed by Duran et al. [48]), the true effects of preoperative anticholinergic medication may differ depending on the classification system applied for analysis.

It is known that delirium can fluctuate strongly and occur acutely during the course of the day.[49] In this study, only one measurement was performed in the morning of the day of measurement. Thus, it is possible that not all patients who developed a delirium were detected with the applied screening method. One limitation of our study might be the short duration of two days measurement, which might have led to patients with postoperative delirium not being diagnosed with delirium. Further, a substantial variation of results was observed within the study, potentially limiting the conclusions drawn from the results.

The patient population was reduced from a total of 150 patients to 114, who were ultimately included for analysis. One reason for the exclusion of patients was excessive sedation at postoperative days one and two and thus an exclusion criterion for the CAM-ICU. Future studies should cover a longer observation period in order to be able to include such patients for analysis and to enable further conclusions to be drawn about the temporal development of POD.

Conclusions

We demonstrated that the development of POD after cardiac surgery correlates with postoperative decrease of BChE activity. In addition, patients who developed POD in the course of surgery showed significantly lower preoperative AChE activity as compared to patients without POD. We were able to identify a low preoperative AChE activity, an anticholinergic pre-medication, an increased EuroSCORE and a higher age as predictors for development of POD. In addition, patients with POD differed from their peers by a longer postoperative ventilation time, an extended stay at the ICU and prolonged hospitalization.

Our data show that the cholinergic deficit hypothesis may be of importance for the development of POD. Anticholinergic medication may intervene in this pathophysiological system and may influence AChE and BChE activity resulting in neuroinflammation.

There are various studies investigating the risk factors for the occurrence of POD. Some correlations in the development of POD have been identified. However, the molecular basis of multifactorial POD has not yet been sufficiently understood. Nonetheless, this is necessary in order to develop preventive measures. Further studies are needed to investigate the exact pathomechanisms of risk factors for such disease.

Acknowledgements

None.

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Table and Figure legends

Table 1. Patient characteristics.

	No postoperative delirium (n=83)	Postoperative delirium (n=31)	
Age (y[IQR])	69 (58 – 74)	74 (71-78)	<0.01*
Female sex (n[%])	22 (26.5)	9 (29)	0.79
EuroSCORE (n[%])			0.02
1-5	59 (71.1)	13 (41.9)	
6-10	22 (26.5)	16 (51.6)	
11-15	2 (2.4)	2 (6.5)	
Body Mass Index (kg/m^2[SD])	27.6 (±4.8)	28 (4.8)	0.7*
Alcohol abuse (n[%])	2 (2.4)	0	1
Anticholinergic premedication (n[%])	8 (9.9)	10 (32.3)	<0.01
Procedure (n[%])			0.3
ACVB	33 (39.8)	15 (48.4)	
AVR	24 (28.9)	6 (19.4)	
Combined Procedure	10 (12)	6 (19.4)	
TAVI	4 (4.9)	3 (9.7)	
MVR	6 (7.2)	1 (3.1)	
Other	6 (7.2)	0	
Length of ventilation (min[SD])	471 (±159)	1427 (±3565)	0.02*
Length of stay on ICU (h[SD])	20.1 (±20.1)	93.5 (±183)	<0.01*
Length of stay in hospital (d[SD])	13.1 (±5)	20.9 (13.9)	<0.01*
In-hospital death (n[%])	1 (1.2)	1 (3.2)	0.47*
Preop BChE activity (U/g Hb[median, SD])	2773 (2740±885)	2734 (2891±922)	0.83
PO day 1 BChE activity (U/g Hb [median, SD])	1966 (1971±588)	1674 (1752±730)	0.03
PO day 2 BChE activity (U/g Hb [median, SD])	1870 (1868±564)	1694 (1715±596)	0.16
Preop AChE activity (U/g Hb [median, SD])	45.4 (45±5.7)	42.2 (41.5±6.3)	<0.01*
PO day 1AChE activity (U/g Hb [median, SD])	45.1 (44.1±5.1)	41.8 (42±5.5)	<0.01*
PO day 2 AChE activity (U/g Hb [median, SD])	45.5 (45.6±4.6)	42.7 (42.8±5.8)	<0.01*

Table 1. Patient characteristics. Data are given as means except for age which is presented as the median and as indicated. Data comparisons were made with the *t*-test or the χ^2-test, where applicable. * denotes the use of a non-parametric test due to non-normal distribution of data. ICU = intensive care unit, CABG = coronary artery bypass grafting, AVR = aortic valve replacement, TAVI = transcatheter aortic valve replacement, MVR = mitral valve replacement, BChE =

butyrylcholinesterase, AChE = acetylcholinesterase, PO = postoperative. IQR indicates interquartile range.

Table 2. Univariate and multivariate analysis of parameters associated with the development of postoperative delirium.

	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	*p* value	OR (95% CI)	p value
Age > 71 years	4.48 (1.74 – 11.54)	<0.01	3.02 (1.06 – 8.62)	0.04
BMI > 27.5	1.31 (0.57 – 2.99)	0.67		
Male sex	1.13 (0.45 - 2.84)	0.82		
EURO-Score ≥ 4	5.43 (1.74 – 16.91)	<0.01*	3.68 (1.04 – 12.99)	0.04
Known alcohol abuse	**	1.0*		
Anticholinergic premedication	6.02 (1.96 – 18.52)	<0.01	5.09 (1.51 – 17.23)	<0.01
Length of ventilation > 456 min	1.56 (0.68 -3.6)	0.29		
Transfusion of PRBC	2.26 (0.96 – 5.31)	0.06		0.28
Preop AchE activity of < 44.3 U/g Hb	2.74 (1.15 – 6.54)	0.02	3.1 (1.14 – 8.46)	0.03
Preop BchE activity of < 2762 U/g Hb	1.31 (0.57 – 2.99)	0.53		

Table 2. Univariate and multivariate analysis of parameters associated with the development of postoperative delirium. Data comparisons were made with χ2-test for univariate analysis, binary logistic regression with stepwise exclusion was used for multivariate analysis. BMI = body mass index, PRBC = Packed red blood cells, BChE = butyrylcholinesterase, AChE = acetylcholinesterase. OR indicates odds ratio, CI indicates confidence interval. For multivariate analysis OR is only displayed in significant outcome parameters/where applicable.

Table 3. Univariate and multivariate analysis of parameters associated with length of stay in the ICU.

	Univariate Analysis		Multivariate Analysis		
	Median (95% CI)	*p* value	HR	(95% CI)	*p* value
Age		0.97			
Age > 71 years	0.75 (0.65 – 0.86)				
Age < 71 years	0.79 (0.56 -1.03)				
BMI		0.24			
BMI > 27.5	0.79 (0.68 – 0.91)				
BMI ≤ 27.5	0.71 (0.48 – 0.94)				
Sex		0.89			
Male	0.75 (0.55 – 0.95)				
Female	0.75 (0.64 – 0.86)				
EURO-Score		<0.01			0.33
EURO-Score ≥ 4	0.79 (0.65 – 0.94)				
EURO-Score < 4	0.42 (0.11 – 0.72)				
Known alcohol abuse		0.76			
Present	0.75 (0.66 – 0.84)				
Absent	0.38 (-)*				
Anticholinergic premedication		0.05			0.39
Present	0.75 (0.59 – 0.91)				
Absent	0.75 (0.64 – 0.86)				
Length of ventilation		<0.01	2.77 (1.83 – 4.2)		<0.01
Length of ventilation > 456 min	1.04 (0.87 – 1.2)				
Length of ventilation < 456 min	0.33 (0.28 – 0.39)				
Transfusion of PRBC		0.04			0.98
Present	0.92 (0.76 – 1.07)				
Absent	0.5 (0.28 – 0.72)				
PO day 1 AchE activity		0.03			0.47
PO day 1 AchE activity of < 44.3 U/g Hb	0.79 (0.66 – 0.93)				
PO day 1 AchE activity of > 44.3 U/g Hb	0.71 (0.44 – 0.98)				
PO day 1 BchE activity		<0.01	1.84 (1.24 – 2.75)		<0.01

PO day 1 BchE activity of < 2762 U/g Hb	1 (0.84 – 1.16)			
PO day BchE activity of > 2762 U/g Hb	0.5 (0.29 – 0.71)			
Delirium		<0.01	1.79 (1.1 – 2.91)	0.02
Present	1.08 (0.48 – 1.69)			
Absent	0.71 (0.51 – 0.91)			

Table 3. Univariate and multivariate analysis of parameters associated with length of stay in the ICU. Data comparisons were made with Kaplan-Meier estimates for univariate analysis. Column median indicates median of parameter displayed. Cox-regression analysis with stepwise exclusion was used for multivariate analysis. BMI = Body mass index, EuroSCORE = European System for Cardiac Operative Risk Evaluation, PRBC = Packed red blood cells, BChE = butyrylcholinesterase, AChE = acetylcholinesterase, PO = postoperative. HR indicates hazard ratio, CI indicates confidence interval. For multivariate analysis HR is only displayed in significant outcome parameters/where applicable.

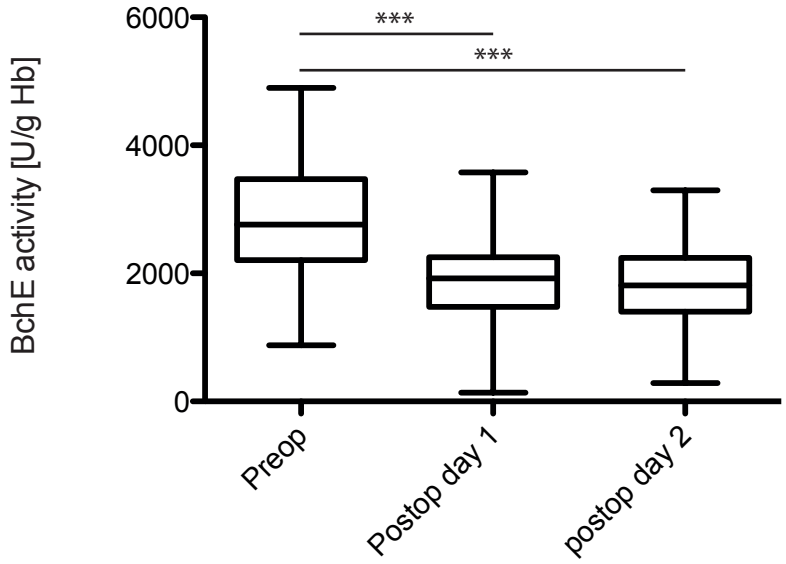
Figure 1. Activity of BChE and AChE in the overall patient population. Activity of A) butyrylcholinesterase (BChE) and B) acetylcholinesterase (AChE) were assessed preoperatively and on postoperative days one and two. *** indicates a p-value of <0.01; * indicates a p-value of <0.05.

Figure 2. Activity of BChE and AChE in patients without or with the development of POD. Activity of butyrylcholinesterase (BChE) was assessed A) preoperatively and on postoperative days B) one and C) two. Activity of acetylcholinesterase (AChE) were assessed D) preoperatively and on postoperative days E) one and F) two. * indicates a p-value of <0.05

Figure 3. Kaplan-Meier estimate. Time to discharge from ICU (logrank test $\chi^2 = 14.88$, $p < 0.01$)

Figure 1

A



B

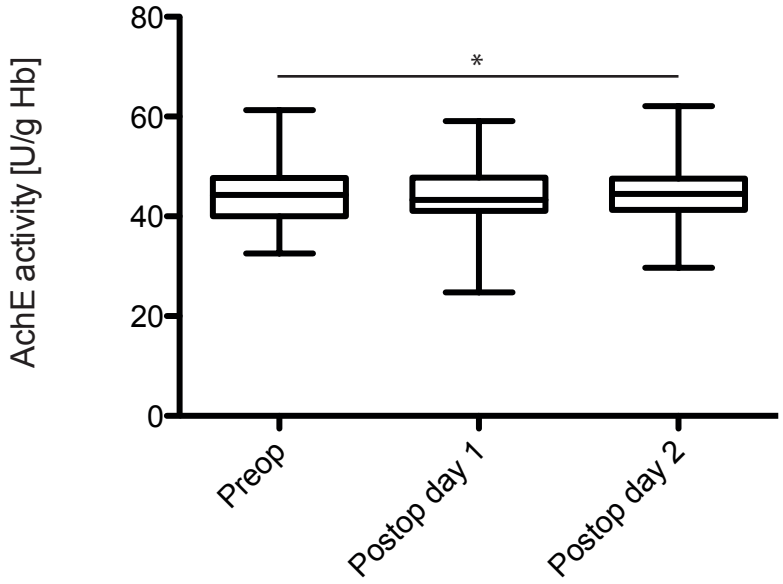
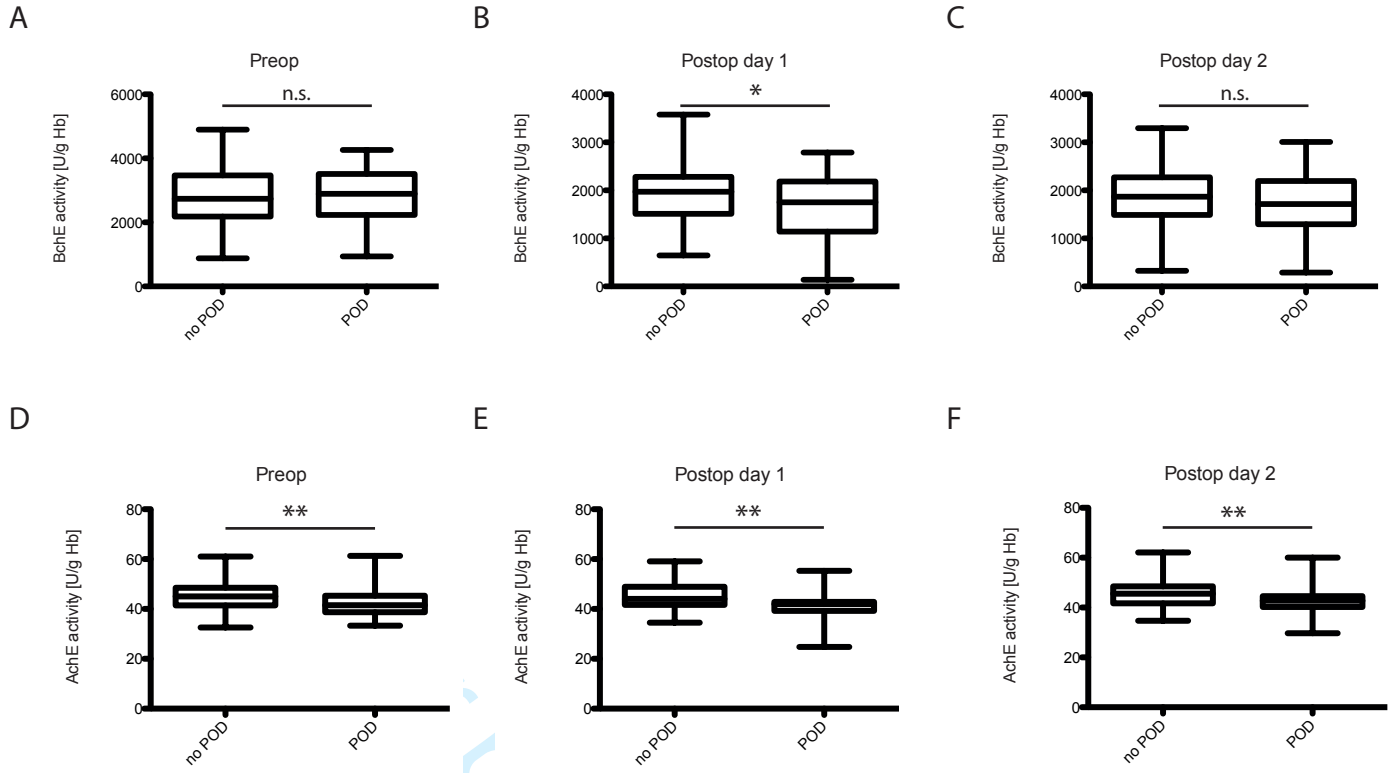
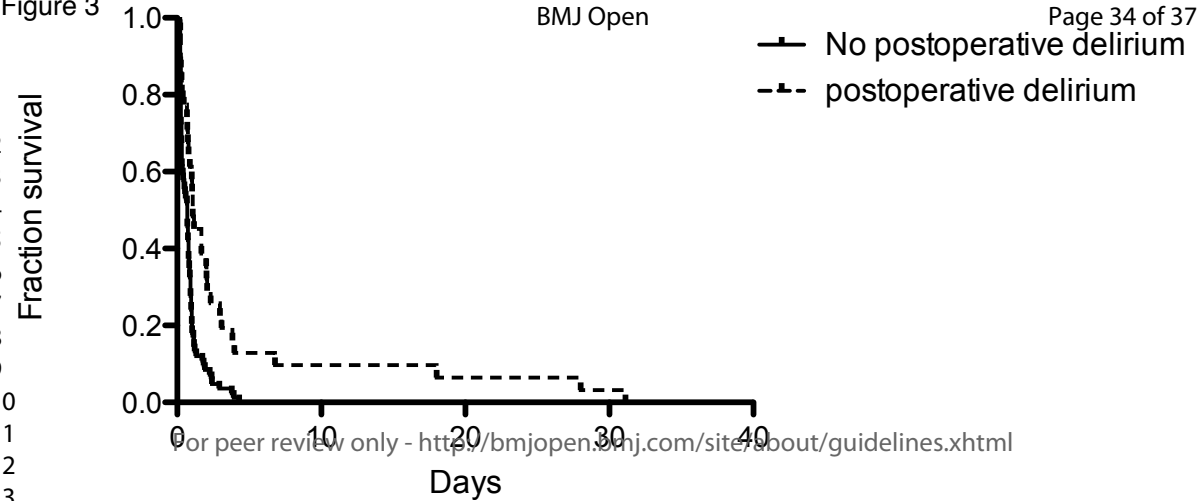


Figure 2





STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Section and Item	Item No.	Recommendation	Reported on Page No.
Title and Abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/Rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	8
Methods			
Study Design	4	Present key elements of study design early in the paper	9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	9
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	9
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9, 10

Section and Item	Item No.	Recommendation	Reported on Page No.
Data Sources/ Measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9, 10
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study Size	10	Explain how the study size was arrived at	n/a
Quantitative Variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11
Statistical Methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11, 12
		(*b*) Describe any methods used to examine subgroups and interactions	11, 12
		(*c*) Explain how missing data were addressed	n/a
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	n/a
		(*e*) Describe any sensitivity analyses	17
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12
		(b) Give reasons for non-participation at each stage	12
		(c) Consider use of a flow diagram	n/a
Descriptive Data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12
		(b) Indicate number of participants with missing data for each variable of interest	n/a
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	n/a
Outcome Data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	n/a
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	

Section and Item	Item No.	Recommendation	Reported on Page No.
Main Results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12, 13
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other Analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12, 13
Discussion			
Key Results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	6

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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BMJ Open

Cholinesterase alterations in delirium after cardiosurgery: a German monocentric prospective study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-031212.R4
Article Type:	Original research
Date Submitted by the Author:	09-Dec-2019
Complete List of Authors:	Adam, Elisabeth; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt, Haas, Victoria; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt Lindau, Simone; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt Zacharowski, Kai; University Hospital Frankfurt, Clinic of Anesthesiology, Intensive Care Medicine and Pain Therapy Scheller, Bertram; Evangelisches Krankenhaus Düsseldorf
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Anaesthesia
Keywords:	Postoperative delirium, Cardiac surgery < SURGERY, Cholinesterease, Acetylcholinesterase, Butyrylcholinesterase

Cholinesterase alterations in delirium after cardiosurgery: a German monocentric prospective study

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Short title: Cholinesterases and postoperative delirium

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Author contributions

EA: wrote the manuscript, analyzed and interpreted the data

VH: conceived the study idea and collected data

SL: collected data, provided critical feedback and contributed to the final version of the manuscript

KZ: supervised the project and contributed to the final version of the manuscript

BS: conceived the study idea, analyzed the data and contributed to the final version of the manuscript

All authors read and approved the final version of the manuscript.

Author Disclosure Statement

The authors have reported no conflicts of interest.

Word count

3416

Data statement

Deidentified participant data are available from the corresponding author upon reasonable request

Abstract

Objectives

Postoperative delirium (POD) is a common complication after elective cardiac surgery. Recent evidence indicates that a disruption in the normal activity of the cholinergic system may be associated with delirium.

Design

Prospective observational study

Setting

Single-center at a European academic hospital.

Primary and secondary outcome measures

In our study the enzyme activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were determined preoperatively as well as on the first and second postoperative day. The confusion assessment method for the intensive care unit (CAM-ICU) was used to screen patients for the presence of POD.

Results

A total of 114 patients were included in the study. POD was associated with a decrease in BChE activity on postoperative day one (p=0.03). In addition, patients who developed POD, had significantly lower preoperative AChE activity than patients without POD (p<0.01). Multivariate analysis identified a preoperatively decreased AChE activity (OR 3.1; 95%CI 1.14-8.46), anticholinergic treatment (OR 5.09; 95%CI 1.51-17.23), elevated EuroSCORE (OR 3.68; 95%CI 1.04-12.99) and age (OR 3.02; 95%CI 1.06-8.62) to be independently associated with the development of POD.

Conclusions

We conclude that a reduction in the acetylcholine hydrolyzing enzyme activity in patients undergoing cardiac surgery may correlate with the development of POD.

Strengths and limitations of this study

- One strength of this study results from the prospective nature
- Another strength is the data acquisition from a high-volume center
- A limitation is the inclusion limited to cardiac surgery patients as it remains unclear whether the results can be extrapolated to other patient cohorts.
- As the symptoms of delirium may vary over time, there may be a possibility that not all patients with delirium were detected, due to a single assessment of delirium per day.

Keywords

Postoperative delirium

Cardiac surgery

Cholinesterase

Acetylcholinesterase

Butyrylcholinesterase

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Introduction

Delirium is a complex neuropsychiatric syndrome that is clinically characterized by sudden onset and fluctuating course. Clinical symptoms according to the actual definition of the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-5[1]) include disturbances in attention, awareness and another cognitive domain. Delirium is characterized as an etiologically unspecific cerebro-organic syndrome representing a decompensation of cerebral function.[2] The duration of delirium varies greatly and the severity ranges from mild to serious conditions.

The causes for delirium are multifactorial. Risk factors include dehydration, sleep deprivation, age, hypoxia, substance intoxication, anemia and hypoglycemia In the general population, the incidence is below 0.4%, in hospitalized patients between 15-22%.[3 4] Particularly after surgical interventions, patients are at risk of developing postoperative delirium (POD). The incidence is described to be as high as 52%.[5] The consequences of a POD are very different and range from prolonged hospital stay, increased risk of wound infections, reduced quality of life, more frequent discharge into nursing homes to increased mortality in the first year after surgery.[6-9] . Recent literature suggests an significant association between frailty and the development of POD with an OR of almost 10, while limitations are considerable due to notable methodological heterogeneity between the methods of studies on such associations.[10] Another risk factor for the development of POD may be a preoperative cognitive impairment, as observed in patients undergoing vascular surgery with a demonstrated OR of greater than 2.[11]

Higher age, longer duration of surgery as well as a reduced preoperative cognitive condition are frequently found in cardiac surgery patients and increase the risk for development of POD in this group of patients.[4] In the literature, the incidence of POD after cardiac surgery varies from 8 to 52%.[4 5 9 12] The duration of the POD in such patients varies widely, lasting three days on average.[6 13] Patients with POD are at risk for developing chronic postoperative cognitive dysfunction (POCD) over time and for suffering from severe long-term cognitive deficits.[14]

There are different hypotheses about the molecular mechanisms involved in the development of delirium.[15] The most common hypothesis for the development of POD is based on a central cholinergic deficit resulting from a deficit of Acetylcholine (ACh): Pathologies at the presynapse, in the synaptic cleft or at the postsynaptic receptor may trigger a central cholinergic deficit. Acetylcholinesterase (AChE) is an enzyme which cleaves ACh in the synaptic cleft and terminates the transmission of a stimulus, a prerequisite for generating a new impulse. If the AChE is restricted in its function ACh remains in the synaptic cleft and blocks a new stimulus transmission.[16] However, several authors have found data challenging this hypothesis as they did not identify an association of preoperative serum anticholinergic activity with the development of POCD[17] or a therapeutic effect of rivastigmine for the prevention of POD.[18] Other hypotheses (e.g. brain injury, metabolic abnormalities) are based on localized or general brain energy deprivation critical to attentional processes such as the caudate nucleus or frontal cholinergic pathways.[17] Systemic inflammation may cause alterations including pro-inflammatory cytokines and prostaglandins mediated by humoral and neural signaling pathways leading to symptoms of delirium.[18]

Butyrylcholinesterase (BChE) is an enzyme which splits choline compounds as well as other esters.[19] For a long time BChE was thought to have a less important function, but recent literature demonstrated that BChE may in part and with a significantly slower rate and affinity act as a substitute in the absence of AChE with a relevant role in the development of a cholinergic deficit.[20 21]

A recently published study identified a significant decrease in the enzyme activity of AChE and BChE in patients with POD after hip surgery.[22] However, the impact of a choline esterase deficit in patients remains unclear. Previously published manuscripts on the impact of cholinesterase activity on POD in surgical patients reach different conclusions. While postoperative measurement of AChE and BChE did not discern between patients with and without POD in a study published by John et al., Muller et al. found a potential relationship between

cholinesterase activity and the development of POD.[23] [24] While John et al. only studied postoperative cholinesterease activity, we sought to incorporate preoperative cholinesterase activity in order to assess a potential implication on the development of POD. In contrast to the study of Muller et al. we only included patients undergoing cardiac surgery in order to decrease heterogeneity.

Due to the far-reaching consequences of a POD, it is of great importance to identify patients at risk for the development of such a disorder. Our study investigated the extent to which changes in bed-side enzyme activity of cholinesterases correlates with the development of POD in cardiac surgery patients and to identify possible factors influencing the development of POD.

Material and methods

This manuscript includes data gained during a prospective observational study at the University Hospital Frankfurt. The institutional review board approved the conduct of the study prior to its initiation (428/12 of 19 December 2012).

Participants

Patients were included between February 2013 and February 2014. Over this period, 150 patients who received elective cardiac surgery at the University Hospital Frankfurt were screened for inclusion. The participating patients were informed about the study verbally and in writing. Only patients with written consent were included in the study.

Potential patients had to meet the following inclusion criteria: elective cardiac surgery with and without the use of a cardiopulmonary bypass (CPB) and age over 18 years. Exclusion from the study was based on: preoperatively existing delirium; preoperatively sedated patients with Richmond Agitation and Sedation Scale (RASS) < -2; no proficiency of the German or English language or missing patient consent.

Design

After obtaining consent, patients were examined preoperatively and on the first and second postoperative day. Patients were examined for the presence of a POD using the confusion assessment method for the intensive care unit (CAM-ICU) clinical test[25]. In brief, the CAM-ICU assesses and scores clinical features associated with delirium. Depending on the results from CAM-ICU, a patient was assigned to either the postoperative delirium group (POD) or the no postoperative delirium (no POD) group. The patient was assigned to the POD group if delirium was diagnosed at least once as per the CAM-ICU. If a patient was either under too much sedation or the examiner was not able to apply the CAM-ICU, the patient was not included for analysis.

Assessment of parameters

All included patients were scheduled for elective surgery and assessed directly before surgery at 7am to determine the presence of delirium. First, the RASS score was obtained, then blood samples were taken for the assessment of butyryl- and acetylcholinesterase activity. Further, blood samples were analyzed for AChE and BChE activity as measured with the ChE Check mobile ® (Securetec Detektions-Systeme AG, Neubiberg, Germany). Both, BChE and AChE activity were assessed using the ChE Check Mobile® as per the manufacturer's instruction. Preoperatively, blood samples were drawn from the fingertip (10µL). Postoperatively, blood samples (1mL) were obtained via an arterial line. As two enzymes were determined in different measurements, two blood samples were taken at different times and analyzed independently. To provide consistency between assessments, measurement of AChE was always performed first, followed by assessment of BChE activity. Measurements were about 10 min apart. As animal data on the circadian changes of cholinesterase reveal an relevant increase during the sleep phase, we have hence taken samples at the same time preoperatively (±1 hour) to ensure consistency of measurements.[26]

The ChE-Check mobile device incorporates a variety of factors contributing to a more precise analysis of cholinesterase activity.[27] Working conditions and technical data for this device are published online.[28] Previously, detailed information on the accuracy of this device have been published before having demonstrated acceptable reliability for the measurement of cholinesterases.[29] Further, this device has been used in the context of POD before.[23 24]

Data collection

Basic demographic data, medication, hospitalization period, the length of stay on the intensive care unit, ventilation time as well as postoperative medication, transfusion, information about secondary diagnoses, weight, laboratory values as well as obtained scores were extracted from the patient data management system. Further, the EuroSCORE[30] was calculated for each

patient. The EuroSCORE (European System for Cardiac Operative Risk Evaluation) is a risk model that facilitates a calculation of the risk of death after heart surgery. The model asks for 17 parameters about the patient, the condition of the heart and the proposed surgery and calculates the risk of death. The EuroSCORE has become the most widely used risk index for cardiac surgery, potentially improving the results of cardiac surgery. Medication was considered to be anticholinergic based on the study by Ancelin et al.[31] The duration of anesthesia, intraoperative medication, aortic clamping time (APC) and the duration of CPB were extracted from the anesthesia and premedication protocols. The data and results were inserted and maintained in an Excel database.

Statistics

All data were tested for normality using the D'Agostino and Pearson omnibus normality test. Data comparisons of patient characteristics were made using Mann-Whitney U- or χ2-test, where applicable. To compare activities of cholinesterases between different days, a Wilcoxon signed rank test was used. Univariate analysis was performed using the χ2-test. Non binary-parameters were stratified by the median. Parameters with a p-value less than 0.1 were included for multivariate analysis, as carried out by binary logistic regression.

Length of ventilation was defined as the time of intubation until extubation; length of stay on the intensive care unit (ICU) was defined as the time from surgery to the discharge from the postoperative ICU; length of stay in the hospital was defined as the time from surgery to discharge from the primary care hospital. For survival analysis, groups were compared using a log rank test and pointwise 95% confidence intervals (CI). A multivariate Cox's proportional hazards regression backward stepwise model (likelihood ratio) was performed to find independent predictors for outcome parameters.

Results with $p<0.05$ were considered to be statistically significant. All calculations/analyses were performed with SPSS (Version 25, Chicago, IL) or Graphpad Prism (Version 5.0, La Jolla,

CA). No correction for multiple comparisons were performed for secondary outcome analysis. Hence results on secondary outcomes are to be considered exploratory.[32]

Patient and public involvement

No patient involved.

Results

Of the 150 patients screened for this study, 13 were excluded due to cancelled surgery and 23 were excluded due to an unavailability for assessment of delirium resulting from prolonged sedation thus leaving 114 patients available for analysis. Of the 114 patients included within our study, 31 patients (27.2%) developed a postoperative delirium (POD), while 83 patients (72.8%) did not show signs of a POD.

Baseline characteristics

No statistical differences were observed for sex, BMI, in-hospital death, preoperative incidence of alcohol abuse, the preoperative prescription of anticholinergic drugs or the performed procedure (Table 1). Of note, none of the patients without previous history of anticholinergic medication received anticholinergic medication throughout the ICU stay. However, patients who went on to develop a POD had a significantly higher EuroSCORE ($p=0.02$). Further, patients who developed POD were significantly older than patients without the development of POD ($p<0.01$).

Outcome dependent on the development of POD

Patients without the development of POD displayed a significantly shorter length of ventilation ($p=0.02$), shorter length of stay in the ICU ($p<0.01$) and shorter length of hospitalization ($p<0.01$) (Table 1). No differences were observed in regard to mortality, when comparing patients with or without the development of POD.

Assessment of cholinesterases

In the overall study population, the butyrylcholinesterase (BChE) decreased significantly over time, when comparing mean BChE activity on postoperative days one ($p<0.01$) and two ($p<0.01$) with the preoperative BChE activity (Figure 1A). Further, the mean acetylcholinesterase

(AChE) activity increased over time, when comparing the AChE activity on postoperative day two with the preoperative AChE activity (p=0.03) (Figure 1B).

No significant preoperative difference in BChE activity was observed in patients with or without POD (Figure 2A). Significant differences were observed in regard to the activity of BChE on postoperative day one (p=0.03) (Figure 2B), when comparing patients from the POD and the no-POD groups. However, no significant difference in BChE activity was observed on postoperative day 2 (Figure 2C) between patients with or without POD. Further, patients with the development of POD displayed significantly lower levels of AChE activity preoperatively (p<0.01) and on postoperative days one (p<0.01) and two (p<0.01) (Figure 2D-F).

Parameters associated with POD

To identify parameters associated with the development of POD in patients undergoing cardiac surgery, we performed a univariate analysis and identified age > 71 years, EuroSCORE ≥ 4, anticholinergic premedication and a preoperative AChE activity of < 44.3 U/g Hb (Table 2). To rule out potential confounding variables we performed a multivariate analysis and confirmed age > 71 years, EuroSCORE ≥4, preoperative anticholinergic medication and preoperative AChE activity of < 44.3 U/g Hb as parameters independently associated with the development of POD.

Parameters associated with length of stay on the ICU

Survival analysis demonstrated that patients with POD after cardiothoracic surgery displayed significantly longer length of stay in the intensive care unit (Figure 3). To identify further parameters associated with prolonged stay in the ICU following cardiothoracic surgery, we performed various univariate analyses and identified EuroSCORE ≥ 4, preoperative anticholinergic medication, length of ventilation, transfusion of PRBCs, reduced AChE activity on postoperative day one, reduced postoperative BChE activity on postoperative day one and the development of POD as potentially associated (Table 3). To identify confounders, we performed

a multivariate analysis and identified length of ventilation, reduced BChE activity on postoperative day one and the development of POD as independently associated with prolonged length of stay in the ICU.

Discussion

The purpose of this study was to analyze a potential correlation between AChE and BChE activities and the incidence of POD in cardiosurgical patients and to identify further possible predictors for the development of POD.

The incidence of POD in our study population is in line with the literature.[3 4] Our results show that a preoperative AChE activity was significantly lower in patients who went on to develop POD than in patients without the development of POD. Further, BChE activity was significantly lower in patients with POD on the first postoperative day. Our data revealed that the patients who developed a POD were significantly older than those who did not suffer from a POD. These patients were more frequently on anticholinergic medication. Further, the EuroSCORE was higher in such patients and they were longer ventilated. In addition, patients with POD stayed significantly longer in the intensive care unit and were discharged significantly later for follow-up treatment.

Patients who went on to develop POD showed lower preoperative AChE activity compared to patients without the development of POD. This finding is in agreement with the current hypothesis that a reduction in AChE activity is associated with POD. It is hypothesized that due to this deficit, cholinesterase cannot efficiently cleave the neurotransmitter ACh in the synaptic cleft. As a consequence, the stimulus transmission cannot be terminated, and ultimately a new stimulus transmission cannot be initiated.[33]

In a recently published study, Cerejeira et al. measured AChE and BChE activities pre- and postoperatively in patients who had undergone elective hip surgery and examined patients for the development of a POD using CAM-ICU.[22] They came to the conclusion that patients with POD after surgery showed reduced preoperative AChE activity. As in our results, preoperative BChE activity was decreased in patients with POD. Contrary to their findings however, in our patient population groups with or without the development of POD did not differ significantly in preoperative BChE activity. This discrepancy might be attributed to different assays measuring enzyme activities. Most importantly, these findings need to be discussed in light of the 2017

publication by John et al.[23] This group did not find any differences regarding both AChE and BChE activity between patients with or without the development of POD. However, there are some considerable differences in the study design: no preoperative samples were collected in the study by John et al. Further, some samples were refrigerated before analysis, thereby potentially altering the measured enzyme activity. Zivkovic et el., however, have also identified a reduced BChE activity following surgery.[34] They suggested a cholinergic modulation of the inflammatory response that is independent of POD. This finding of a postoperatively decreased BChE activity and a potential association with POD as observed within our study needs to be addressed in further studies specifying the potential impact of cholinesterases in the development of POD, also in the context of inflammation. In a recently published manuscript, Muller et al. found that peri-operative peripheral cholinesterase activities may be related to the development of POD.[24] In this study, cholinesterase activities were measured in surgical patients of various specialties. However, the authors of the above-named study stated the lack of a subgroup analysis discriminating between surgical procedures as a limitation of their study. In our study comprised of patients undergoing cardiac surgery, we were able to find comparable results, potentially indicating an importance of cholinesterase activity in the development of POD.

In a study conducted in 2008, Hubbard et al. were able to show that a higher age was associated with deficits in the anticholinergic system.[35] Photometric determination of AChE revealed no significant difference for BChE activity between younger and older age, but a significantly lower activity of cholinesterases in the older people displaying a significant amount of frailty. They suspected that age was associated with changes in enzyme activity. While a deficit in cholinesterase activity may be observed in elderly patients, a significant correlation with age could not be demonstrated.[36-38] The association between age and the development of POD observed for our patient population fits well with the literature that described such association before.[39] In our cohort, patients with a history of anticholinergic medication suffered from a POD significantly more often than patients in the comparison group. This result supports the assumption

that the anticholinergic predisposition has an influence on the development of the POD. It reduces the function of ACh and might also related to a cholinergic deficit. Anticholinergic medication is used when patients are regularly treated with antidepressants (e.g. amitriptyline, doxepin), anticonvulsants (e.g. gabapentin) or for Parkinson's disease (benserazide, L-DOPA). These drugs all have in common that they reduce ACh activity through direct and indirect anticholinergic action. In a study conducted in 2015, Naja et al. investigated geriatric patients with regard to the treatment with anticholinergic drugs before and during hospitalization and the incidence of delirium. They came to the conclusion that the anticholinergic burden was associated with the occurrence of delirium and that anticholinergic exposure correlated with the incidence of delirium and increased mortality.[40]

When interpreting effect sizes of the above-named potential risk factors for the development of POD, preoperative anticholinergic medication had a medium effect[41] for the development of such condition. Further, age was identified to display a medium effect on the potential development of POD. A comparable effect on the development of POD was identified for the preoperative EuroSCORE. However, such finding needs to be interpreted with caution, as age is one of the parameters utilized for the calculation of the EuroSCORE. A reduced preoperative AChE activity also had a medium effect on the development of POD. These findings both demonstrate the importance of a cholinergic deficit and of age as risk for the development of POD. However, when interpreting these findings in an external framework, other parameters which have not been assessed in the present study may be of importance: most importantly, frailty has a demonstrated high impact on the development of POD. In a recently published meta-analysis the OR of frailty for the development of POD was higher (OR > 9) than any of the parameters studied within this trial. Hence, future studies need to address the importance of the factors identified within this study and other factors such as frailty or cognitive impairment.

Patients with POD had a significantly longer duration of anesthesia and were also operated on for longer periods of time. Long-lasting surgery is associated with many other risk factors such

as hypoxemia, pain and disturbance of the sleep-wake rhythm.[39 42] The anesthesia itself interferes with various neuronal processes in the brain. It interacts with ion channels, such as the nicotinic acetylcholine receptors, neurotransmitters and second messengers, as well as metabolic processes.[43] The factors mentioned may have influenced the development of POD.

The effects of a POD are far-reaching. In our study, patients with POD not only stayed longer in the ICU, they also spent significantly more days in hospital postoperatively. These observations may be attributed to multiple factors such as delayed mobilization and physiotherapy.[44] Patients with POD require more intensive care from nurses and physicians, so that a transfer to the normal ward is only possible with delay and resulting in higher costs.[45] In a study published in 2004, Ely et al. showed that delirium is an independent predictor of significantly higher 6-month mortality and prolonged hospitalization in ventilated patients in the ICU.[46] Our patients did not show an increased in-hospital mortality in patients with POD while we, however, did not follow up patients for 6 months. Conclusions on associations between long-term mortality and cholinesterase activity may therefore not be drawn from the results of our study.

To determine the diagnosis of delirium, the Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) was used, which is recommended by clinical guidelines.[47] While the CAM-ICU test is a tool for the diagnosis of delirium with the benefits of rapid assessment and no requirement for verbal communication with the patient, the CAM-ICU test does not provide information about motor subtypes of delirium.[48] We believe that future studies addressing this question are potentially of value to help understanding the pathology of this disease.

Strengths and limitations

Our study has several limitations that must be considered when evaluating the results. This study comprises exclusively cardiac surgery patients. Whether these data can be extrapolated to other patient cohorts remains unclear and warrants further validation. On a statistical note, we

have not performed multiple comparison for the assessment of enzyme activities with a consecutive potential increase of the alpha error.

While the literature proposes a myriad of risk factors for the development of POD, differences in the methodology based on different definitions of delirium, differences in assessment of both risk factors and delirium and others, do not allow for a definitive list of risk factors. In conclusion, confounding by potential risk factors not addressed within this study (e.g. frailty or cognitive impairment) may limit the application of the results found within this study.[10 11]

One limitation may be found in the lack of a consensus on a single classification system for anticholinergic medication. While several classification systems exist (as reviewed by Duran et al. [49]), the true effects of preoperative anticholinergic medication may differ depending on the classification system applied for analysis.

It is known that delirium can fluctuate strongly and occur acutely during the course of the day.[50] In this study, only one measurement was performed in the morning of the day of measurement. Thus, it is possible that not all patients who developed delirium were detected with the applied screening method. One limitation of our study might be the short duration of two days measurement, which might have led to patients with postoperative delirium not being diagnosed with delirium. Further, a substantial variation of results was observed within the study, potentially limiting the conclusions drawn from the results.

The patient population was reduced from a total of 150 patients to 114, who were ultimately included for analysis. One reason for the exclusion of patients was excessive sedation at postoperative days one and two and thus an exclusion criterion for the CAM-ICU. Future studies should cover a longer observation period in order to be able to include such patients for analysis and to enable further conclusions to be drawn about the temporal development of POD.

Conclusions

We demonstrated that the development of POD after cardiac surgery correlates with postoperative decrease of BChE activity. In addition, patients who developed POD in the course of surgery showed significantly lower preoperative AChE activity as compared to patients without POD. We were able to identify a low preoperative AChE activity, an anticholinergic pre-medication, an increased EuroSCORE and a higher age as predictors for development of POD. In addition, patients with POD differed from their peers by a longer postoperative ventilation time, an extended stay at the ICU and prolonged hospitalization.

Our data show that the cholinergic deficit hypothesis may be of importance for the development of POD. Anticholinergic medication may intervene in this pathophysiological system and may influence AChE and BChE activity resulting in neuroinflammation.

There are various studies investigating the risk factors for the occurrence of POD. Some correlations in the development of POD have been identified. However, the molecular basis of multifactorial POD has not yet been sufficiently understood. Nonetheless, this is necessary in order to develop preventive measures. Further studies are needed to investigate the exact pathomechanisms of risk factors for such disease.

Acknowledgements

None.

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Table and Figure legends

Table 1. Patient characteristics.

	No postoperative delirium (n=83)	Postoperative delirium (n=31)	
Age (y[IQR])	69 (58 – 74)	74 (71-78)	<0.01*
Female sex (n[%])	22 (26.5)	9 (29)	0.79
EuroSCORE (n[%])			0.02
1-5	59 (71.1)	13 (41.9)	
6-10	22 (26.5)	16 (51.6)	
11-15	2 (2.4)	2 (6.5)	
Body Mass Index (kg/m^2[SD])	27.6 (±4.8)	28 (4.8)	0.7*
Alcohol abuse (n[%])	2 (2.4)	0	1
Anticholinergic premedication (n[%])	8 (9.9)	10 (32.3)	<0.01
Procedure (n[%])			0.3
ACVB	33 (39.8)	15 (48.4)	
AVR	24 (28.9)	6 (19.4)	
Combined Procedure	10 (12)	6 (19.4)	
TAVI	4 (4.9)	3 (9.7)	
MVR	6 (7.2)	1 (3.1)	
Other	6 (7.2)	0	
Length of ventilation (min[SD])	471 (±159)	1427 (±3565)	0.02*
Length of stay on ICU (h[SD])	20.1 (±20.1)	93.5 (±183)	<0.01*
Length of stay in hospital (d[SD])	13.1 (±5)	20.9 (13.9)	<0.01*
In-hospital death (n[%])	1 (1.2)	1 (3.2)	0.47*
Preop BChE activity (U/g Hb[median, SD])	2773 (2740±885)	2734 (2891±922)	0.83
PO day 1 BChE activity (U/g Hb [median, SD])	1966 (1971±588)	1674 (1752±730)	0.03
PO day 2 BChE activity (U/g Hb [median, SD])	1870 (1868±564)	1694 (1715±596)	0.16
Preop AChE activity (U/g Hb [median, SD])	45.4 (45±5.7)	42.2 (41.5±6.3)	<0.01*
PO day 1AChE activity (U/g Hb [median, SD])	45.1 (44.1±5.1)	41.8 (42±5.5)	<0.01*
PO day 2 AChE activity (U/g Hb [median, SD])	45.5 (45.6±4.6)	42.7 (42.8±5.8)	<0.01*

Table 1. Patient characteristics. Data are given as means except for age which is presented as the median and as indicated. Data comparisons were made with the *t*-test or the χ^2-test, where applicable. * denotes the use of a non-parametric test due to non-normal distribution of data. ICU = intensive care unit, CABG = coronary artery bypass grafting, AVR = aortic valve replacement, TAVI = transcatheter aortic valve replacement, MVR = mitral valve replacement, BChE =

butyrylcholinesterase, AChE = acetylcholinesterase, PO = postoperative. IQR indicates interquartile range.

Table 2. Univariate and multivariate analysis of parameters associated with the development of postoperative delirium.

	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	*p* value	OR (95% CI)	p value
Age > 71 years	4.48 (1.74 – 11.54)	<0.01	3.02 (1.06 – 8.62)	0.04
BMI > 27.5	1.31 (0.57 – 2.99)	0.67		
Male sex	1.13 (0.45 - 2.84)	0.82		
EURO-Score ≥ 4	5.43 (1.74 – 16.91)	<0.01*	3.68 (1.04 – 12.99)	0.04
Known alcohol abuse	**	1.0*		
Anticholinergic premedication	6.02 (1.96 – 18.52)	<0.01	5.09 (1.51 – 17.23)	<0.01
Length of ventilation > 456 min	1.56 (0.68 -3.6)	0.29		
Transfusion of PRBC	2.26 (0.96 – 5.31)	0.06		0.28
Preop AchE activity of < 44.3 U/g Hb	2.74 (1.15 – 6.54)	0.02	3.1 (1.14 – 8.46)	0.03
Preop BchE activity of < 2762 U/g Hb	1.31 (0.57 – 2.99)	0.53		

Table 2. Univariate and multivariate analysis of parameters associated with the development of postoperative delirium. Data comparisons were made with χ2-test for univariate analysis, binary logistic regression with stepwise exclusion was used for multivariate analysis. BMI = body mass index, PRBC = Packed red blood cells, BChE = butyrylcholinesterase, AChE = acetylcholinesterase. OR indicates odds ratio, CI indicates confidence interval. For multivariate analysis OR is only displayed in significant outcome parameters/where applicable.

Table 3. Univariate and multivariate analysis of parameters associated with length of stay in the ICU.

	Univariate Analysis		Multivariate Analysis		
	Median (95% CI)	*p* value	HR	(95% CI)	*p* value
Age		0.97			
Age > 71 years	0.75 (0.65 – 0.86)				
Age < 71 years	0.79 (0.56 -1.03)				
BMI		0.24			
BMI > 27.5	0.79 (0.68 – 0.91)				
BMI ≤ 27.5	0.71 (0.48 – 0.94)				
Sex		0.89			
Male	0.75 (0.55 – 0.95)				
Female	0.75 (0.64 – 0.86)				
EURO-Score		<0.01			0.33
EURO-Score ≥ 4	0.79 (0.65 – 0.94)				
EURO-Score < 4	0.42 (0.11 – 0.72)				
Known alcohol abuse		0.76			
Present	0.75 (0.66 – 0.84)				
Absent	0.38 (-)*				
Anticholinergic premedication		0.05			0.39
Present	0.75 (0.59 – 0.91)				
Absent	0.75 (0.64 – 0.86)				
Length of ventilation		<0.01	2.77 (1.83 – 4.2)		<0.01
Length of ventilation > 456 min	1.04 (0.87 – 1.2)				
Length of ventilation < 456 min	0.33 (0.28 – 0.39)				
Transfusion of PRBC		0.04			0.98
Present	0.92 (0.76 – 1.07)				
Absent	0.5 (0.28 – 0.72)				
PO day 1 AchE activity		0.03			0.47
PO day 1 AchE activity of < 44.3 U/g Hb	0.79 (0.66 – 0.93)				
PO day 1 AchE activity of > 44.3 U/g Hb	0.71 (0.44 – 0.98)				
PO day 1 BchE activity		<0.01	1.84 (1.24 – 2.75)		<0.01

PO day 1 BchE activity of < 2762 U/g Hb	1 (0.84 – 1.16)			
PO day BchE activity of > 2762 U/g Hb	0.5 (0.29 – 0.71)			
Delirium		<0.01	1.79 (1.1 – 2.91)	0.02
Present	1.08 (0.48 – 1.69)			
Absent	0.71 (0.51 – 0.91)			

Table 3. Univariate and multivariate analysis of parameters associated with length of stay in the ICU. Data comparisons were made with Kaplan-Meier estimates for univariate analysis. Column median indicates median of parameter displayed. Cox-regression analysis with stepwise exclusion was used for multivariate analysis. BMI = Body mass index, EuroSCORE = European System for Cardiac Operative Risk Evaluation, PRBC = Packed red blood cells, BChE = butyrylcholinesterase, AChE = acetylcholinesterase, PO = postoperative. HR indicates hazard ratio, CI indicates confidence interval. For multivariate analysis HR is only displayed in significant outcome parameters/where applicable.

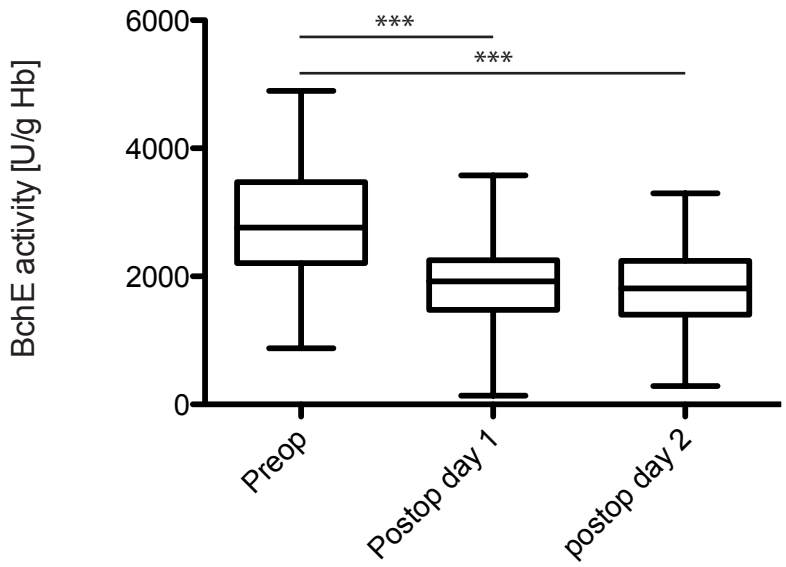
Figure 1. Activity of BChE and AChE in the overall patient population. Activity of A) butyrylcholinesterase (BChE) and B) acetylcholinesterase (AChE) were assessed preoperatively and on postoperative days one and two. *** indicates a p-value of <0.01; * indicates a p-value of 0.04.

Figure 2. Activity of BChE and AChE in patients without or with the development of POD. Activity of butyrylcholinesterase (BChE) was assessed A) preoperatively and on postoperative days B) one (* indicates a p-value of 0.03) and C) two. Activity of acetylcholinesterase (AChE) were assessed D) preoperatively and on postoperative days E) one and F) two. * indicates a p-value of <0.01

Figure 3. Kaplan-Meier estimate. Time to discharge from ICU (logrank test $\chi^2 = 14.88$, $p < 0.01$)

Figure 1

A



B

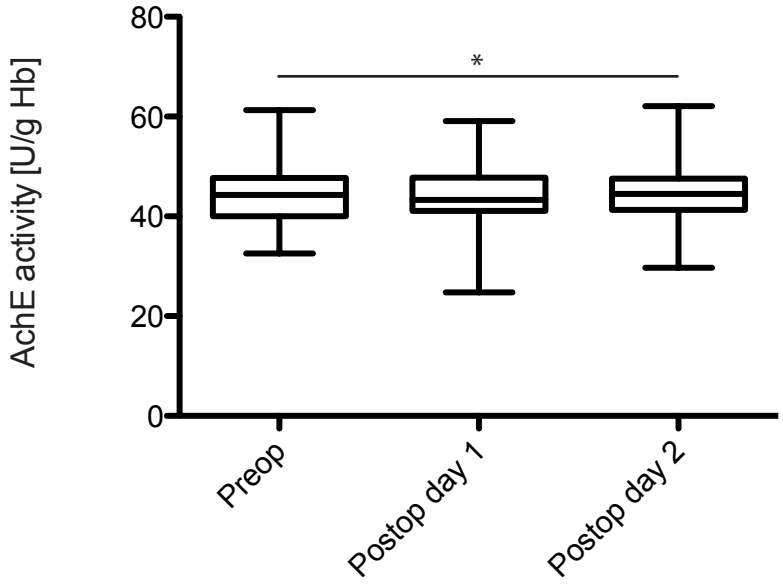
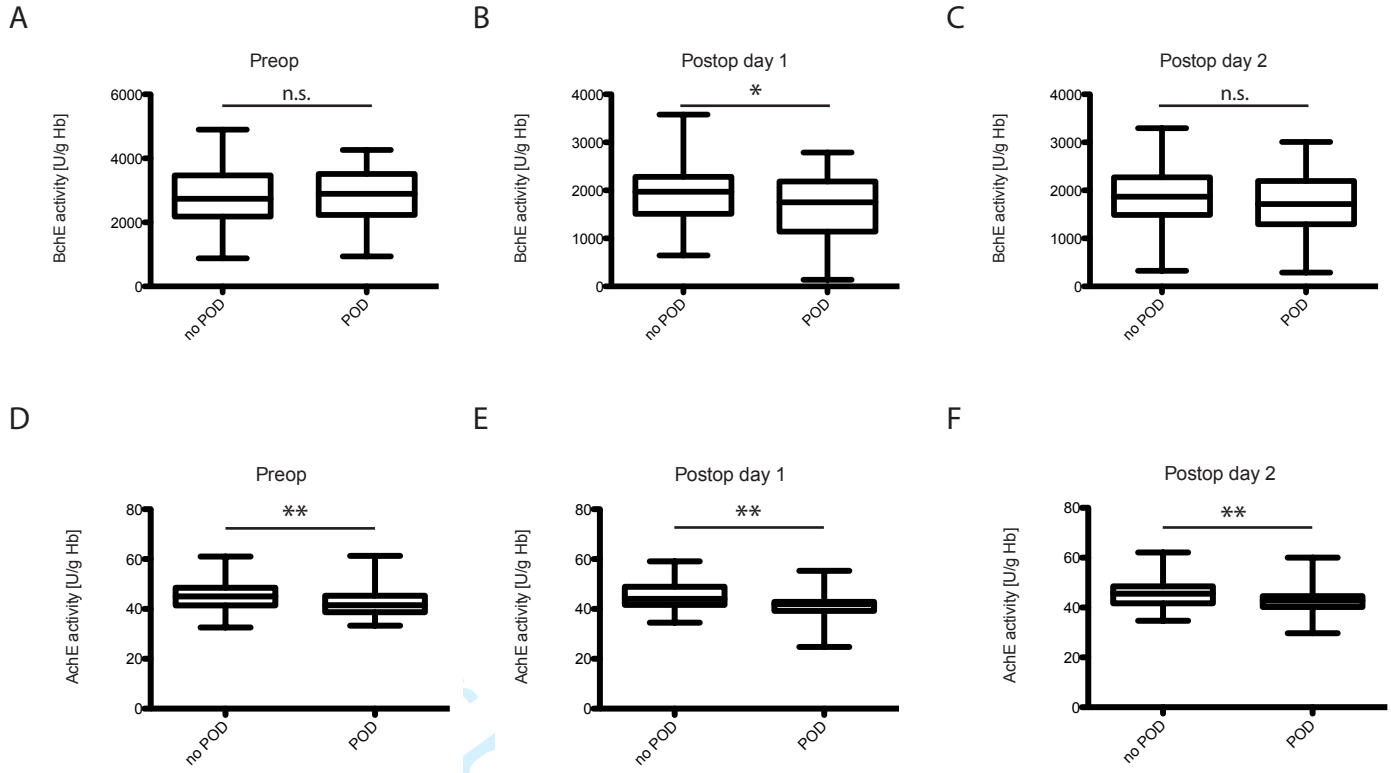
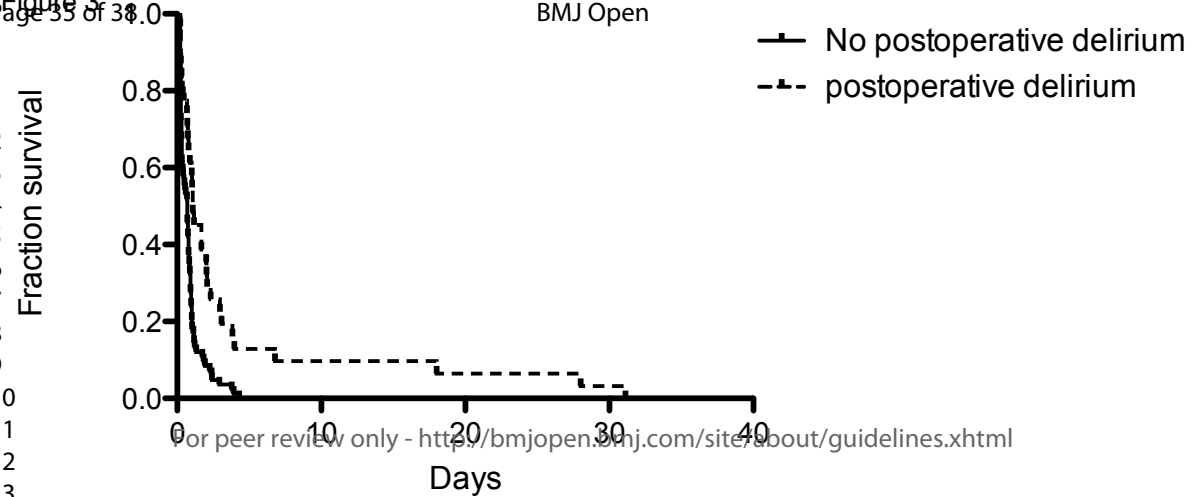


Figure 2





STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Section and Item	Item No.	Recommendation	Reported on Page No.
Title and Abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/Rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	8
Methods			
Study Design	4	Present key elements of study design early in the paper	9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	9
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	9
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9, 10

Section and Item	Item No.	Recommendation	Reported on Page No.
Data Sources/ Measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9, 10
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study Size	10	Explain how the study size was arrived at	n/a
Quantitative Variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11
Statistical Methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11, 12
		(*b*) Describe any methods used to examine subgroups and interactions	11, 12
		(*c*) Explain how missing data were addressed	n/a
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	n/a
		(*e*) Describe any sensitivity analyses	17
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12
		(b) Give reasons for non-participation at each stage	12
		(c) Consider use of a flow diagram	n/a
Descriptive Data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12
		(b) Indicate number of participants with missing data for each variable of interest	n/a
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	n/a
Outcome Data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	n/a
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	

Section and Item	Item No.	Recommendation	Reported on Page No.
Main Results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12, 13
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other Analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12, 13
Discussion			
Key Results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	6

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Once you have completed this checklist, please save a copy and upload it as part of your submission. DO NOT include this checklist as part of the main manuscript document. It must be uploaded as a separate file.