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Complete List of Authors:	Michels, Bernhard; University Hospital Regensburg Holzamer, Andreas ; Universitatsklinikum Regensburg, Department of Cardiothoracic Surgery Graf, Bernhard; University Hospital Regensburg, Anaesthesiology Bredthauer, Andre; University Hospital Regensburg, Anaesthesiology Petermichl, Walter; University Hospital Regensburg, Anaesthesiology Müller, Anika; Charité Universitätsmedizin Berlin, Anaesthesiology Zausig, York; Universitatsklinikum Regensburg, Department of Anaesthesiology Bitzinger, Diane; Universitatsklinikum Regensburg, Department of Anaesthesiology
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Butyrylcholinesterase as a perioperative complication marker in patients after transcatheter aortic valve implantation?

Bernhard Michels[1], Andreas Holzamer[2], Bernhard Graf[3], Andre Bredthauer[3], Walter Petermichl[3], Anika Müller[4], York Zausig[5], Diane Bitzinger[3]

[1] *Department of Gastroenterology, University Hospital Regensburg, Regensburg, Germany*

[2] *Department of Cardiothoracic Surgery, University Hospital Regensburg, Regensburg, Germany*

[3] *Department of Anesthesiology, University Hospital Regensburg, Regensburg, Germany*

[4] *Department of Anesthesiology and Intensive Care Medicine Campus Charité Mitte and Campus Virchow-Klinikum, Charité-Universitätsmedizin Berlin, Berlin, Germany*

[5] *Department of Anesthesiology and Intensive Care Medicine, Hospital Aschaffenburg-Alzenau, Aschaffenburg, Germany*

Corresponding author:

PD Dr. med. Diane Bitzinger

University of Regensburg

Department of Anesthesiology

Franz-Josef-Strauss-Allee 11

93053 Regensburg, Germany

Phone: +49-941-944 7801

Fax: +49-941-9447802

Email: diane.bitzinger@ukr.de

Abstract

Objectives: Transcatheter aortic valve implantation (TAVI) is performed in elderly patients with severe aortic valve stenosis and increased operative risks. We tested the hypothesis that acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) have a predictive value for prevalent complications after TAVI and could serve as indicators of systemic inflammation in the early postoperative period.

Design: Prospective observational study.

Setting: This study is a secondary analysis of multi-center CESARO-study.

Participants: 48 TAVI patients were included, 43 obtained the complete assessment.

Primary and secondary outcome measures: Patients clinical parameters, demographic data, peripheral AChE and BChE activities and routine blood markers were assessed throughout the perioperative period using bedside point-of-care measurements for AChE and BChE. Postoperative complications screening was conducted up to the 3rd postoperative day and included infections, delirium and heart-rhythm disturbances. After assessment the patients were divided into complication and non-complication group.

Results: Of 43 patients, 24 developed postsurgical complications (55.8%). Preoperative assessment showed no significant differences regarding demographic data and laboratory markers, but preoperative BChE levels were significantly lower in patients who developed postoperative complications (complication group 2589.2 ± 556.4 vs. non-complication group 3295.7 ± 628.0, $p < 0.05$). In complication group we observed an early, sustained reduction in BChE activity from preoperative to postoperative period. In complication group BChE levels were significantly lower at each time point compared to non-complication group. AChE activity showed no significantly difference between both groups. Complication group also had longer stay in hospital overall.

Conclusion: BChE could be a useful perioperative biomarker to identify patients with a higher risk for postoperative complications after TAVI. By using point-of-care measurements the levels of BChE are fast available and can lead to an early targeted therapy. Predicting the length of the hospital stay might play an important role in staff and resources management for these patients.

Trial registration: NCT01964284

Key words: cardiac surgery, TAVI, inflammation, delirium, butyrylcholinesterase, acetylcholinesterase

Strengths and limitations of this study

This study is a secondary analysis of the prospective observational multi-centre CESARO-study.

Our study included 48 cardiosurgical patients with an observation time of three days.

BChE could be a useful perioperative biomarker to identify patients with a higher risk for postoperative complications after TAVI.

By using point-of-care measurements the levels of BChE are fast available and can lead to an early targeted therapy.

Predicting the length of the hospital stay might play an important role in staff and resources management for these patients.

Introduction:

Recently, transcatheter aortic valve implantation (TAVI) has become the therapeutic standard for medical treatment in elderly, multimorbid patients with severe aortic valve stenosis and increased operative risks (1, 2). TAVI involves the implantation of a prosthetic valve, which is introduced with a catheter through transfemoral (TF), transapical (TA) or direct transaortic access. Usually, the TF approach is preferred, because thoracotomy and penetration of the myocardium are not needed. The TA approach is common, if severe artherosclerotic disease does not allow retrograde insertion of the catheter. In patients with severe aortic stenosis, who could not undergo a surgical replacement of the aortic valve, TAVI significantly reduced the rates of death at any cause, compared to standard therapy (3). However, previous studies have shown that pneumonia, acute renal failure, indication for a permanent pacemaker and delirium were the most frequently recorded complications after TAVI (4). Covello et. al. reported a pneumonia rate of 7-8 % after TAVI (5). The incidence of delirium after TAVI is reported as 29 % in literature (6).

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are a focus of current research. Recent studies have shown that AChE and BChE serve as diagnostic markers of low-grade systemic inflammation (7–9). Rapid changes in cholinesterase activity have also been reported in patients after acute trauma, infections, burns and critical illness (10–14). Both enzymes may serve as indicators of systemic inflammation and have remarkable predictive value for mortality in critically ill patients. Zivkovic et. al. showed that reduced serum activity of BChE indicates severe systemic inflammation in criticall ill patients (13). Furthermore, a recent study showed, that a sustained reduction in serum cholinesterase enzyme activity predicts patient outcome following sepsis (15).

Other studies postulate low preoperative plasma cholinesterase activity as a risk marker of postoperative delirium in elderly patients (16). A recently published study on cholinesterase activity in cardiac surgical patients showed no postoperative differences in cholinesterase activities between delirious and non-delirious patients, but showed a perioperative decrease of BChE which was potentially caused by cardiopulmonary bypass (17). However, due to high variability in the etiology and progress of clinical conditions, it was difficult to determine whether the changes in the enzyme activity correlated with the emergence of disease or was affected by concomitant factors such as cardiopulmonary bypass.

This is the first study to investigate the roles of AChE and BChE as inflammatory markers in cardiac surgical patients under standardized perioperative conditions without using cardiopulmonary bypass. Our aim of the present study is to evaluate if there is a predictive association of perioperative determination of AChE and BChE activity and the occurrence of postoperative complications after TAVI.

Material and Methods:

Study design and patient population:

This work is a secondary analysis of the prospective observational multi-center CESARO-study, powered for the detection of postoperative delirium. The CESARO study was initiated at Charité – Universitätsmedizin Berlin, Department of Anesthesiology and Operative Intensive Care Medicine (Clinicaltrials.gov ID: NCT01964284) and approved by the local independent Charité Ethics Committee, Charité – Universitätsmedizin Berlin, Germany (ref.: EA1/220/13). After further approval of the local ethics board of the University of Regensburg a total of 48 patients were included into the study between March 2014, and June 2016 at University Hospital of Regensburg. Written informed consent was obtained from each patient (Figure 1).

Inclusions criteria: minimum age of 18 years, admission to intensive care unit (ICU) following elective TAVI in general anesthesia.

Exclusion criteria: missing consent, patients with a known pseudocholinesterase deficiency, patients with language, visual or hearing impairments.

Preoperative variables:

Preoperative data included demographic data, such as age, sex, height, weight, regular use of alcohol and nicotine, American Society of Anesthesiologists (ASA) class, logistic Euro Score (European System for Cardiac Operative Risk Evaluation), New York Heart Association (NYHA) class, long-term medication and left ventricular ejection fraction (EF). The patients' previous medical history was examined for conditions such as chronic kidney disease, cerebrovascular events, including stroke and transient ischemic attacks, myocardial infarction, chronic obstructive pulmonary disease, diabetes mellitus and pre-existing cardiac arrhythmias. Every patient was screened for preoperative delirium, using the nursing delirium screening scale (NU-DESC). Preoperative assessment of AChE, BChE, CRP, leukocytes, haemoglobin and creatinine were performed (table 1).

Intraoperative variables:

Key elements of intraoperative data included the selected access type, anesthetic procedure, transfusion of erythrocyte concentrates and extubation rate as well as the procedure duration.

Postoperative variables:

Postoperative data included the patient's stay in the ICU and the stay in hospital in general. Next to the sampling of laboratory markers, every patient was screened for delirium with NU-DESC for the first 3 days after surgery. Patients were daily assessed for pain, using the numeric rating scale (NRS score: 0 = no pain – 10 = maximum pain). Furthermore, any complication in recovery time was noticed. Mortality reasons are divided into cardiac, acute kidney injury, cardiovascular events and infections.

Variables:

Delirium:

Delirium screening was conducted perioperatively using a validated screening tool (NU-DESC) (18). NU-DESC assesses five dimensions: orientation, behaviour, communication, illusion/hallucination and psychomotor retardation. The symptoms are rated on a three-point scale, whereas a score of two or more cumulative points indicated delirium. Delirium assessment was performed one day prior to the operation, on admission to ICU and daily up to the third postoperative day. Patients with Richmond Agitation Sedation Scale (RASS) ≤ -2 were excluded for the current testing.

Laboratory parameters:

Blood samples were taken from every patient at following points: one day before operation (screening), shortly before anesthetic induction, on admission to ICU, one day after surgery and two days after surgery (Figure 2). The measurements included the determination of AChE and BChE. Both were measured in 10 µl whole blood, using *ChE check mobile*, a point-of-care testing device (ChE check mobile®, Securetec Detektions-Systeme AG, Neubiberg, Germany) by following the manufacturer's instructions. Also, blood count, C-reactive protein (CRP), creatinine were measured at each time point. Creatine kinase (CK) and heart enzymes (CK-MB) were measured on the first postoperative day in the normal laboratory control. Brain natriuretic Peptide (NT-proBNP) was measured at the screening day.

Postoperative complications:

Since delirium, pneumonia, heart rhythm disturbances and acute renal failure are the most frequently reported complications after TAVI, we have screened all patients until the discharge of the hospital. Infection was defined as an increase in CRP, fever and diagnosed infection-focus (pneumonia, urinary tract infection, other infections). Delirium was diagnosed by using NU-DESC. Postoperative heart rhythm disturbances occurred by AV-block and atrial fibrillation. Patients were divided into two groups: those who did not develop any postoperative complications (non-complication group) and patients who showed one of these complications within 3 days after TAVI (complication group).

Operation procedure:

All patients were admitted and evaluated at least one day before the operation. TAVIs were performed by the cardiac team (cardiac surgeon, cardiologist, and cardiac anesthesist) in a hybrid operating theatre. All procedures were performed with the patients placed under general anesthesia. In all patients, monitoring consisted of pulsoximetry, 5-channel electrocardiogram, invasive blood pressure, central venous pressure, urinary output and bladder temperature. The maintenance of normothermia was accomplished by a heating blanket placed beneath the patient. The patients received right ventricular pacemakers for rapid ventricular pacing during balloon aortic valvuloplasty and valve-expansion. Pre-oxygenation was performed with pure oxygen using a facemask. Anesthesia was induced with etomidate (Etomidat-Lipuro®, B. Braun Melsungen AG, Melsungen, Germany), remifentanil (Ultiva®, GlaxoSmithKline GmbH & Co. KG, Munich, Germany) and rocuronium (Rocuronium Inresa®, Inresa Arzneimittel GmbH, Freiburg, Germany) and maintained with sevoflurane (Sevorane®, AbbVie Deutschland GmbH & Co.KG, Wiesbaden, Germany). Piritramide and metamizole were used as additional pain medication. PONV prophylaxis was used intraoperatively, depending on the patient's risk. Cardiovascular drugs (e.g. norepinephrine, and dobutamine) were administered, as needed. A prophylactic antibiotic (1.5 g, Cefuroxim Hikma®, Hikma Pharma GmbH, Gräfelfing, Germany) was administered to each patient. In the operating theatre, the patient was connected to a defibrillator, and a TEE probe was introduced. After preparing the access points and anticoagulation

with heparin (Ratiopharm GmbH, Ulm, Germany; mean given dose 5293 ± 2643 IU), the native valve was opened under rapid ventricular pacing, and the prothesis was implanted. The position and function of the prothesis was verified with TEE. Extubation of the patient was the goal at the end of each procedure. After surgery, patients were monitored for at least 12 hours in the ICU. Following this period, patient care continued either in the ICU or in the general ward. There was no use of heart lung machines.

Patient and public involvement

Patients were not involved in the study.

Statistics:

The data were electronically gathered and stored by using Excel. Data analysis was performed by using SPSS (Version 22.0; SPSS Inc., Chicago, IL, USA). Data are presented as mean with standard deviation. Shapiro-Wilk test was used to verify Gaussian distribution of the study groups. Statistical significance between the patient groups was tested using t-test, Mann-Whitney U test, analysis of variance and chi-quadrat. A p value < 0.05 indicated statistical significance.

Results:

Baseline data

A total of 48 patients were included, and 43 patients completed the assessment battery (figure 1). The mean age was 79.5 +/- 5.71 years and the mean BMI was 27.93 +/- 5.36. There were almost equally men and women (22 (51.2 %) vs. 21 (48.8 %), table 1). All patients received elective TAVI in general anesthesia. TF access was selected for 32 (74 %), with TA chosen for 11 (26 %) patients. There was no use of heart-lung-machines. The demographic data and pre-existing conditions are shown in table 1. 32 (74.4 %) had an ASA-class of three, 11 (25.6 %) of four. Except of four, every patient was extubated immediately after operation and brought to ICU. One high risk patient was still intubated when brought to ICU and died two days after operation by multiorgan failure. Another patient was extubated on the first postoperative day. Two patients were extubated a few hours after brought to ICU. Patients were discharged to a normal ward after one day and left the hospital after 13.28 +/- 6.2 days.

Postoperative complications

24 patients (55.8 %) had postoperative complications as defined above. One multimorbid and high-risk patient died due to multiorgan-failure at ICU two days after surgery.

Of 43 patients, 12 developed postoperative delirium (27.9 %). Most patients developed their delirium on the first day after surgery.

Of 43 patients, 2 developed pneumonia. However, in 3 patients with raised infection markers and suspected infection no focus was found. All of them received antibiotics.

There were 7 patients with postoperative indication for pacemaker (16.3%). Overall 12 patients developed heart rhyhtm disturbances (27.9%). Some of the patients developed more than one complication, e.g. delirium or infection.

Comparison between complication and non-complication group

Preoperative variables

Preoperative assessment showed no significant differences regarding demographic data and laboratory routine markers like haemoglobin, leukocytes, CRP, NT-proBNP and creatinine ($p = n. s.$). Preoperative BChE levels were significantly lower in patients who developed postoperative complications (D 1 complication group 2589.2 ± 556.4 vs. D 1 non-complication group 3295.7 ± 628.0 $p < 0.05$, table 2). Preoperative AChE enzyme activity in contrast did not show any difference between complication and non-complication group. There was also no difference regarding alcohol ($p = 0.23$) or nicotine ($p = 0.8$) consumption. Men showed a higher incidence of postoperative complications ($p = 0.09$).

Postoperative variables

All patients were postoperatively admitted to the ICU extubated and hemodynamic supported by catecholamines. Two patients did not meet the extubation criteria in the operation room and were extubated a few hours later at ICU. One high risk patient died at ICU due to multiorgan-failure. One patient was extubated on the first postoperative day.

Complication group showed an early, sustained and statistically significant decrease in BChE activity from the preoperative to the first postoperative measurement (D 0: 2784.0 ± 534.9 vs POD 0: 2379.6 ± 525.1, $p < 0,05$, figure 3). In contrast in patients without postoperative complications we observed a delayed decrease in BChE activity (D 0: 3072.6 ± 652.1 vs POD 2: 2713.5 ± 510.6, $p < 0.05$). In all time points a significantly lower BChE activity was observed in patients with complications compared to patients without postoperative complication (figure 3).

Both groups showed a moderate decrease in AChE activity after preoperative screening measurement (figure 4). From anesthesia induction to the second postoperative measurement we observed no significant changes in AChE activity over time in both groups. There were no significant differences in AChE activity between patients with and without complication in any time point (figure 4).

Patients, who developed postoperative complications had a significantly longer stay in hospital in general (complication-group: 15.2 ± 6.3 vs. non-complication-group: 11.1 ± 5.5 days, $p < 0.05$). There was no difference regarding the stay on ICU (complication group vs. non-complication group p = n.s.) Complication-group also showed a higher Nu-DESC score on the first postoperative day (complication-group: 2.1 ± 2.4 vs. non-complication group: 0.58 ± 0.51, $p < 0.05$). Patients with postoperative delirium showed highest NU-DESC score on the first postoperative day (delirium: 3.3 ± 2.6 vs. non-delirium: 0.27 ± 0.79). The preoperative score of NU-DESC was 0.42 ± 0.67 within patients, who developed postoperative delirium. Routine laboratory markers like haemoglobin, leukocytes, CRP, CK, CK-MB and creatinine did not show any difference (complication group vs. non-complication group p = n. s., table 2).

Furthermore, there was no difference in Euroscore regarding on complication (complication group vs. non-complication group p = n. s., table 1)).

TA vs. TF

Patients, who underwent TA approach declared postoperative higher pain levels measured by NRS ($p < 0.05$). They also showed higher CRP levels on POD 2 (88.8 ± 44.5 vs. 161.6 ± 70.2, $p < 0.05$, table 2) and higher levels of CK (110.8 ± 134.5 vs. 398.7 ± 139.0, $p < 0.05$) and CK-MB (8.3 ± 11.8 vs. 29.8 ± 14.7, $p < 0.05$) on the first postoperative day. There were no further differences between patients with TF and TA approach, especially regarding on complications or BChE and AChE enzyme levels.

Discussion:

TAVI has become the therapeutic standard for medical treatment in elderly patients with severe aortic valve stenosis and increased operative risks. The primary objective of the present investigation was to evaluate the roles of AChE and BChE as predictive markers for prevalent complications in cardiosurgical patients after TAVI.

Previous studies assumed an interaction of the immune and cholinergic system (19) and identified AChE and BChE as useful biomarkers for early detection of patients with emerging inflammation (16). Rapid changes in cholinesterase activity have been reported in patients after acute trauma, infections, delirium and critical illness (10–14). Both enzymes may serve as indicators of systemic inflammation and have a remarkable predictive value for mortality in critically ill patients. Zivkovic et al. showed that bedside-measurement of BChE activity predicts patient morbidity and length of ICU stay following major traumatic injury (20). Another study with patients undergoing venoarterial extracorporeal membrane oxygenation therapy after cardiac surgery revealed BChE as a strong predictor of all-cause and cardiovascular mortality (10).

In our present study patients with postoperative complications after TAVI had significant lower preoperative levels of compared to the non-complication group. This finding suggests that BChE activity, used in combination with common preoperative evaluation procedures, could serve as a useful predictive indicator to identify high-risk patients.

Due to high variability in the onset, aetiology and progress of clinical conditions among patients, determining whether changes in the enzyme activity are correlated with the emergence of disease or are affected by concomitant factors is difficult. John et al. tested the hypothesis that AChE and BChE have an impact on patients after cardiac surgery with postoperative delirium. They showed that AChE increased and BChE decreased within the first 3 days after surgery but did not discern between patients with and without delirium. The authors supposed that the perioperative change of AChE and BChE activity might possibly be explained by an interaction of AChE and BChE and the use of a cardiopulmonary bypass (17). In our present study we evaluated the role of AChE and BChE activity in cardiosurgical patients after TAVI, as a standardized operative procedure. We could show that complication group shows a significantly perioperative decrease of BChE within the first 3 days after TAVI, despite the fact that there was no use of heart-lung machines in our patients. Furthermore, there was no use of blood products in the present study, so we can rule out a possible interaction of AChE and BChE with blood products as well.

The pathophysiologic mechanism behind the BChE activity change and the systemic inflammation presumably involves the non-neuronal anti-inflammatory activity of the cholinergic system. Conventional markers like CRP and leucocytes did not differ in both groups.

Delirium is a complex symptom which is very common in operative and non-operative disciplines in the course of hospital stay. The incidence is especially high among patients undergoing heart surgery (21). The incidence in this patients population has been described to be from 30 up to 80 % (22). The incidence of delirium after TAVI is reported as 29 % in literature (6). Delirium occurred significantly more frequently following TA procedures (23). In the present study 26,7 % of the patients were diagnosed with delirium overall. There was no difference depending on TA or TF approach. Perioperative measurement of AChE and BChE did not discern between patients with and without delirium, which is in accordance with the findings by John et al.

The present study highlights the validity of BChE measurements for early detection of high-risk patients after TAVI. Surprisingly, the BChE assessment proved more effective than the EURO-Score in discriminating between the patient groups making it a valuable biomarker for the early detection of high-risk patients. Euro-Score is a well-established clinical assay for the patient mortality analysis (24) and requires documenting multiple and diverse datasets. The datasets are in most cases readily available; however, in some cases, a particular set of data might not be accessible, delaying or making the scoring impossible. By using a POCT system for a single BChE measurement, the results of an equally efficient outcome assessment tool are readily available at the bedside.

Prompt assessment of the systemic immune response with an immediate, rapid and affordable bedside measurement of the BChE activity might improve risk evaluation and help optimize postoperative management and therapy of patients after TAVI. Predicting the length of the hospital stay might play an important role in staff and resources management for these patients.

Limitations

Limitations of the present study might be the short duration of 3 days' measurement. Blood was taken from each patient; in case the analysis could not be performed immediately (during anesthesia induction), the sample was cooled down in a refrigerator. Maybe values of AChE and BChE changed in combination with lower temperature. Furthermore, it was only one measurement performed with each sample, so no control values could be achieved.

A further limitation of this study is the low number of included patients. However, even with the low sample, the described test demonstrated high sensitivity, particularly in the initial time period, as compared to the benchmark methods, suggesting a rapid, effective, and simple patient outcome assay.

There was no comparison between the aetiology of inflammation. A larger, possibly multicentre study would be needed to validate our findings.

Characteristic	Total sample (n = 43)
Age (years) [M (SD)]	79.47 (5.71)
Sex [n (%)]	
Male	22 (51.2)
Female	21 (48.8)
BMI [M (SD)]	27.93 (5.36)
ASA – PS [n (%)]	
3	32 (74.4)
4	11 (25.6)
Operative procedure [n (%)]	
Transapicale TAVI	11 (25.6)
Transfemorale TAVI	32 (74.4)
Relevant comorbidities [n (%)]	
hypertension	37 (86)
diabetes	16 (37.2)
Congestive heart failure	36 (83.7)
Congestive kidney failure	20 (46.5)
Coronary heart disease	31 (72.1)
Cardiac arrhythmias	25 (58.1)
Stroke/TIA	8 (18.6)
nicotine	19 (44.2)
alcohol	8 (18.6)
hypothyreosis	10 (23.3)
hypercholesterinemia	14 (32.6)
Euroscore [n (%)]	
low	15 (34.9)
middle	18 (41.9)
high	10 (23.3)

Table 1 Description of baseline data

Variables	complication	N	M	SD	p-value
Euroscore	Yes no	24 19	21.8 22.8	15.4 13.5	0.82
Weight [kg]	Yes no	24 19	78.3 74.9	15.5 18.0	0.50
BMI [kg/m²]	Yes no	24 19	28.1 27.7	4.7 6.2	0.86
Age [years]	Yes no	24 19	79.9 78.9	5.3 6.3	0.57
NT-proBNP [pg/ml]	Yes no	24 19	6244.8 4806.6	6773.1 7809.4	0.56
Hemoglobin D 0 [g/dl]	Yes no	24 19	12.1 12.2	1.9 1.4	0.92
Hemoglobin POD 0 [g/dl]	Yes no	24 19	10.7 10.9	1.5 1.4	0.57
Hemoglobin POD 1 [g/dl]	Yes no	24 19	10.1 10.1	1.4 1.1	0.99
Hemoglobin POD 2 [g/dl]	Yes No	24 19	10.1 10.3	1.1 1.2	0.67
Hemoglobin POD 3 [g/dl]	Yes No	24 19	9.6 10.0	1.0 1.3	0.27
Creatinine D 0 [mg/dl]	Yes no	24 19	1..4 1.1	0.7 0.4	**0.09**
Creatinine POD 1 [mg/dl]	Yes No	24 19	1.2 1.1	0.4 0.4	0.35
Creatinine POD 2 [mg/dl]	Yes no	24 19	1.5 1.2	0.8 0.4	0.19
Creatinine POD 3 [mg/dl]	Yes No	24 19	1.5 1.1	1.0 0.4	0.24
Leukocytes D 0 [/nl]	Yes no	24 19	7.8 7.3	2.1 1.6	0.38
Leucoytes POD 1 [/nl]	Yes No	24 19	9.6 8.8	4.3 2.1	0.50
Leukocytes POD 2 [/nl]	Yes No	24 19	9.9 9.4	2.7 3.5	0.62
Leukocytes POD 3 [/nl]	Yes No	24 19	8.8 7.0	3.1 1.5	**0.08**
CRP D 0 [mg/l]	Yes no	24 19	16.3 9.7	17.8 11.9	0.26
CRP POD 1 [mg/l]	Yes No	24 19	31.8 22.0	21.1 18.9	0.18
CRP POD 2 [mg/l]	Yes No	24 19	116.3 101.3	52.9 78.2	0.52
CRP POD 3 [mg/l]	Yes No	24 19	115.3 72.7	68,5 76,0	0.11
BChE D 0 [U/l]	Yes no	24 19	2784.0 3072.6	534,9 652,1	0.12
BChE D 1 [U/l]	Yes no	24 19	2589.2 3295.7	556,4 628,0	**0.001**

BChE POD 0 [U/l]	Yes No	24 19	2379.6 2972.5	525.1. 599.2	**0.001**
BChE POD 1 [U/l]	Yes No	24 19	2300.3 2936.2	561.0 523.1	**< 0.005**
BChE POD 2 [U/l]	Yes No	24 19	2166.7 2713.5	537.0 510.6	**0.002**
AChE D 0 [U/gHb]	Yes no	24 19	45.0 43.3	8.1 6.0	0.45
AChE D 1 [U/gHb]	Yes no	24 19	42.0 39.2	10.0 4.7	0.26
AChE POD 0 [U/gHb]	Yes No	24 19	42.9 37.6	10.0 6.4	**0.051**
AChE POD 1 [U/gHb]	Yes No	24 19	41.5 38.4	9.2 5.2	0.2
AChE POD 2 [U/gHb]	Yes No	24 19	41.2 36.5	8.2 6.8	**0.058**
CK POD 1 [U/l]	Yes No	24 19	189.6 186.1	186.8 190.5	0.95
CK-MB POD 1 [ng/ml]	Yes No	24 19	15.5 15.9	18.2 14.3	0.95
CK-Index POD 1	Yes No	24 19	7.2 8.5	3.6 3.8	0.31
Stay in hospital [days]	Yes no	24 19	15.2 11.1	6.3 5.5	**0.03**

Table 2 Perioperative laboratory markers

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AVAILABILITY OF DATA AND MATERIAL

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

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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CONTRIBUTIONS

DB, YZ, AM and BM were responsible for study design, statistical analyses and drafting of the manuscript. WP, AB, BM and BG performed the experiments and drafted the manuscript. BM and AM were responsible for statistical analysis. All authors read and approved the final manuscript.

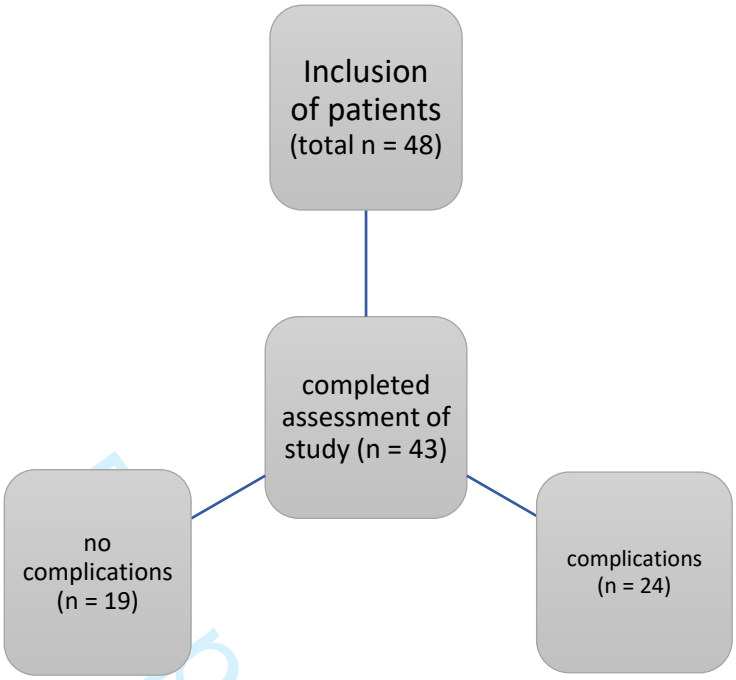

Figure 1 Study design: a total of 48 patients were screened. 5 patients were not included. 43 patients completed the assessment of the study. 19 patients showed no complications. 24 patients developed postoperative complications.

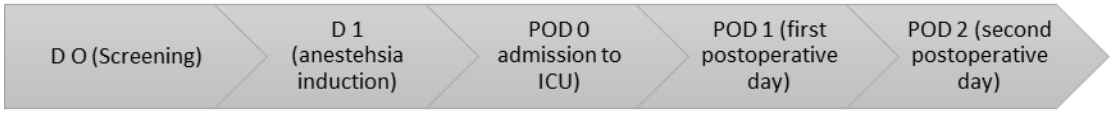

Figure 1 Timeline of measurements of BChE and AChE: blood samples were taken one day preoperative (D O), shortly before anesthetic induction (D 1), on admission to ICU (POD 0), one day after surgery (POD 1) and two days after surgery (POD 2). If the measuremnets could not be conducted immediatley, the samples have been cooled in a refridgerator and the measurement was performed up to 2 hours later.

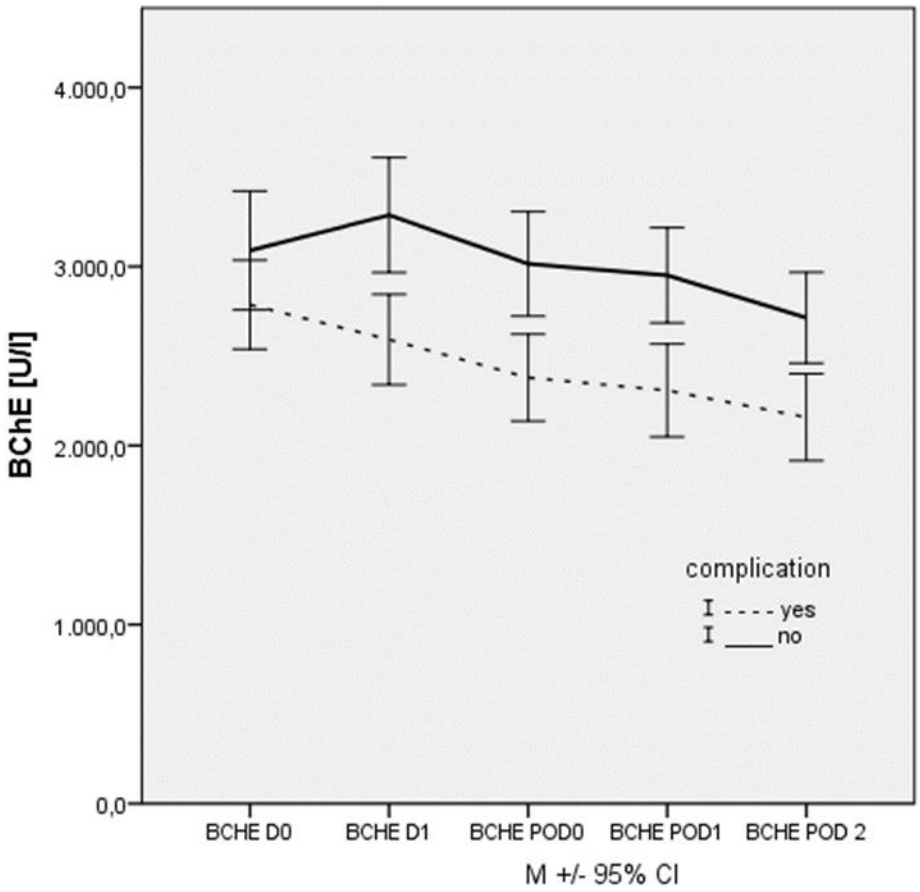

Figure 3 Perioperative levels of BChE activty in complication and non-complication group: Complication group showed an early, sustained and statistically significant decrease in BChE activity from the preoperative to the first postoperative measurement (analysis of variance; D 0: 2784.0 ± 534.9 vs POD 0: 2379.6 ± 525.1, $p < 0.05$). Patients without postoperative complications showed a delayed decrease in BChE activity (analysis of variance D 0: 3072.6 ± 652.1 vs POD 2: 2713.5 ± 510.6, $p < 0.05$). In all time points a significantly lower BChE activity was observed in patients with complications compared to patients without postoperative complication (t-test).

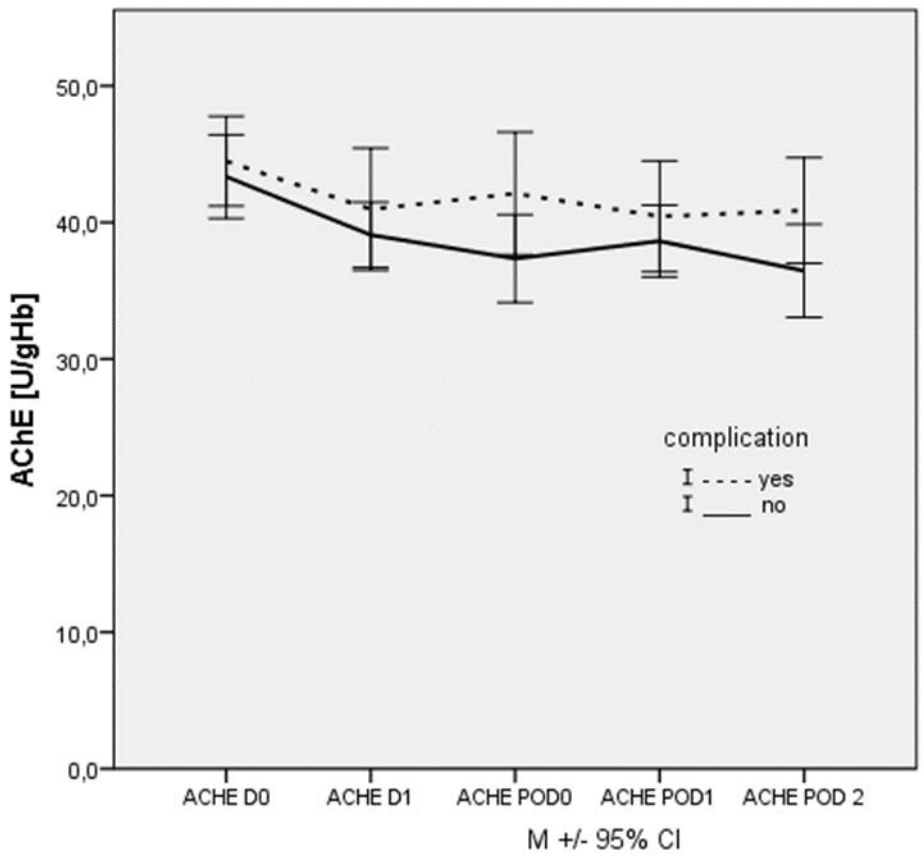

Figure 4 Perioperative levels of AChE activity of complication and non-complication group: From anesthesia induction to the second postoperative measurement we observed no significant changes in AChE activity over time in both groups (analysis of variance, p = n.s.). There were no significant differences in AChE activity between patients with and without complication in any time point (t-test, p = n.s.). However, analysis of variance showed a significantly decrease over time referred to the first measurement on D 0.

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Butyrylcholinesterase as a perioperative complication marker in patients after transcatheter aortic valve implantation: a prospective observational study

Bernhard Michels[1], Andreas Holzamer[2], Bernhard Graf[3], Andre Bredthauer[3], Walter Petermichl[3], Anika Müller[4], York Zausig[5], Diane Bitzinger[3]

[1] *Department of Gastroenterology, University Hospital Regensburg, Regensburg, Germany*

[2] *Department of Cardiothoracic Surgery, University Hospital Regensburg, Regensburg, Germany*

[3] *Department of Anesthesiology, University Hospital Regensburg, Regensburg, Germany*

[4] *Department of Anesthesiology and Intensive Care Medicine Campus Charité Mitte and Campus Virchow-Klinikum, Charité-Universitätsmedizin Berlin, Berlin, Germany*

[5] *Department of Anesthesiology and Intensive Care Medicine, Hospital Aschaffenburg-Alzenau, Aschaffenburg, Germany*

Corresponding author:

PD Dr. med. Diane Bitzinger

University of Regensburg

Department of Anesthesiology

Franz-Josef-Strauss-Allee 11

93053 Regensburg, Germany

Phone: +49-941-944 7801

Fax: +49-941-9447802

Email: diane.bitzinger@ukr.de

Abstract

Objectives: Transcatheter aortic valve implantation (TAVI) is performed in elderly patients with severe aortic valve stenosis and increased operative risks. We tested the hypothesis that acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) have a predictive value for prevalent complications after TAVI and could serve as indicators of systemic inflammation in the early postoperative period.

Design: Prospective observational study.

Setting: This study is a secondary analysis of multi-center CESARO-study.

Participants: 48 TAVI patients were included, 43 obtained the complete assessment.

Primary and secondary outcome measures: Patients clinical parameters, demographic data, peripheral AChE and BChE-activities and routine blood markers were assessed throughout the perioperative period using bedside point-of-care measurements for AChE and BChE. Postoperative complications screening was conducted up to the 3rd postoperative day and included infections, delirium and heart-rhythm disturbances. After assessment the patients were divided into complication and non-complication group.

Results: Of 43 patients, 24 developed postsurgical complications (55.8%). Preoperative assessment showed no significant differences regarding demographic data and laboratory markers, but preoperative BChE-levels were significantly lower in patients who developed postoperative complications (complication group 2589.2 ± 556.4 vs. non-complication group 3295.7 ± 628.0, Cohen's $r = 0.514$, $p < 0.001$). In complication group we observed an early, sustained reduction in BChE-activity from preoperative to postoperative period. In complication group BChE-levels were significantly lower at each time point compared to non-complication group. AChE-activity showed no significantly difference between both groups. Complication group also had longer stay in hospital overall.

Conclusion: BChE could be a useful perioperative biomarker to identify patients with a higher risk for postoperative complications after TAVI. By using point-of-care measurements the levels of BChE are fast available and can lead to an early targeted therapy. Predicting the length of the hospital stay might play an important role in staff and resources management for these patients.

Trial registration: NCT01964274

Key words: cardiac surgery, TAVI, inflammation, delirium, butyrylcholinesterase, acetylcholinesterase

Strengths and limitations of this study

- This study is a secondary analysis of the prospective observational multi-center CESARO-study.
- Our study included 48 cardiosurgical patients with an observation time of three days.
- BChE could be a useful perioperative biomarker to identify patients with a higher risk for postoperative complications after TAVI.
- By using point-of-care measurements the levels of BChE are fast available and can lead to an early targeted therapy.
- Predicting the length of the hospital stay might play an important role in staff and resources management for these patients.

Introduction:

Recently, transcatheter aortic valve implantation (TAVI) has become the therapeutic standard for medical treatment in elderly, multimorbid patients with severe aortic valve stenosis and increased operative risks (1, 2). TAVI involves the implantation of a prosthetic valve, which is introduced with a catheter through transfemoral (TF), transapical (TA) or direct transaortic access. Usually, the TF approach is preferred because thoracotomy and penetration of the myocardium are not needed. The TA approach is common, if severe artherosclerotic disease does not allow retrograde insertion of the catheter. In patients with severe aortic stenosis, who could not undergo a surgical replacement of the aortic valve, TAVI significantly reduced the rates of death at any cause, compared to standard therapy (3). However, previous studies have shown that pneumonia, acute renal failure, indication for a permanent pacemaker and delirium were the most frequently recorded complications after TAVI (4). Covello et. al. reported a pneumonia rate of 7-8 % after TAVI (5). The incidence of delirium after TAVI is reported as 29 % in literature (6).

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are a focus of current research. Recent studies have shown that AChE and BChE serve as diagnostic markers of low-grade systemic inflammation (7–9). Rapid changes in cholinesterase activity have also been reported in patients after acute trauma, infections, burns and critical illness (10–14). Both enzymes may serve as indicators of systemic inflammation and have remarkable predictive value for mortality in critically ill patients. Zivkovic et. al. showed that reduced serum activity of BChE indicates severe systemic inflammation in critical ill patients (13). Furthermore, a recent study showed, that a sustained reduction in serum cholinesterase enzyme activity predicts patient outcome following sepsis (15).

Other studies postulate low preoperative plasma cholinesterase activity as a risk marker of postoperative delirium in elderly patients (16). A recently published study on cholinesterase activity in cardiac surgical patients showed no postoperative differences in cholinesterase activities between delirious and non-delirious patients, but showed a perioperative decrease of BChE which was potentially caused by cardiopulmonary bypass (17). However, due to high variability in the etiology and progress of clinical conditions, it was difficult to determine whether the changes in the enzyme activity correlated with the emergence of disease or was affected by concomitant factors such as cardiopulmonary bypass.

This is the first study to investigate the roles of AChE and BChE as inflammatory markers in cardiac surgical patients under standardized perioperative conditions without using cardiopulmonary bypass. Our aim of the present study is to evaluate if there is a predictive association of perioperative determination of AChE and BChE activity and the occurrence of postoperative complications after TAVI.

Material and Methods:

Study design and patient population:

This work is a secondary analysis of the prospective observational multi-center CESARO study, powered for the detection of postoperative delirium. The CESARO study was initiated at Charité – Universitätsmedizin Berlin, Department of Anesthesiology and Operative Intensive Care Medicine (Clinicaltrials.gov ID: NCT01964274) and approved by the local independent Charité Ethics Committee, Charité – Universitätsmedizin Berlin, Germany (ref.: EA1/220/13) on 14 August 2013. After further approval of the local ethics board of the University of Regensburg a total of 48 patients were included into the study between March 2014, and June 2016 at University Hospital of Regensburg. Written informed consent was obtained from each patient.

Inclusions criteria: minimum age of 18 years, admission to intensive care unit (ICU) following elective TAVI in general anesthesia.

Exclusion criteria: missing consent, patients with a known pseudocholinesterase deficiency, patients with language, visual or hearing impairments.

Data

Data were acquired from anesthetic charts (Medlinq V.1.3, Hamburg, Germany), the patient document system used in the ICU (Metavision, iMDsoft, Tel Aviv, Israel) and medical reports from the electronic hospital information system (SAP, Walldorf, Germany) from the preoperative, intraoperative and postoperative periods until the patients were discharged from the hospital.

Preoperative variables:

Preoperative data included demographic data, such as age, sex, height, weight, regular use of alcohol and nicotine, American Society of Anesthesiologists (ASA) class, logistic EuroSCORE (European System for Cardiac Operative Risk Evaluation), New York Heart Association (NYHA) class and left ventricular ejection fraction (EF). The patients' previous medical history was examined for conditions such as chronic kidney disease, cerebrovascular events, including stroke and transient ischemic attacks, myocardial infarction, chronic obstructive pulmonary disease, diabetes mellitus and pre-existing cardiac arrhythmias. Every patient was screened for preoperative delirium, using the nursing delirium screening scale (NU-DESC). Preoperative assessment of AChE, BChE, CRP, leukocytes, haemoglobin and creatinine were performed (table 1).

Intraoperative variables:

Key elements of intraoperative data included the selected access type, anesthetic procedure, transfusion of erythrocyte concentrates and extubation rate as well as the procedure duration.

Postoperative variables:

Postoperative data included the patient's stay in the ICU and the stay in hospital in general. Next to the sampling of laboratory markers, every patient was screened for delirium with NU-DESC for the first 3 days after surgery. Patients were daily assessed for pain, using the numeric rating scale (NRS score:

0 = no pain – 10 = maximum pain). Furthermore, any complication in recovery time was noticed. Mortality reasons are divided into cardiac, acute kidney injury, cardiovascular events and infections.

Variables:

Delirium:

Delirium screening was conducted perioperatively using a validated screening tool (NU-DESC) (18). NU-DESC assesses five dimensions: orientation, behaviour, communication, illusion/hallucination and psychomotor retardation. The symptoms are rated on a three-point scale, whereas a score of two or more cumulative points indicated delirium. Delirium assessment was performed one day prior to the operation, on admission to ICU and daily up to the third postoperative day. Patients with Richmond Agitation Sedation Scale (RASS) ≤ -2 were excluded for the current testing.

Laboratory parameters:

Blood samples were taken from every patient at following time points: one day before operation (screening), shortly before anesthetic induction, on admission to ICU, one day after surgery and two days after surgery (figure 1). The measurements included the determination of AChE and BChE. Both were measured in 10 µl whole blood, using *ChE check mobile*, a validated point-of-care testing device (ChE check mobile®, Securetec Detektions-Systeme AG, Neubiberg, Germany; In-Vitro-Diagnostics Guideline 98/79/EG; DIN EN ISO 18113-2 and -3) by following the manufacturer's instructions. Also, blood count, C-reactive protein (CRP), creatinine were measured at each time point. Creatine kinase (CK) and heart enzymes (CK-MB) were measured on the first postoperative day in the normal laboratory control. Brain natriuretic Peptide (NT-proBNP) was measured at the screening day. To deal with missing values, we included three defined measurements (time points) into the analysis.

Postoperative complications:

Since delirium, pneumonia, heart rhythm disturbances and acute renal failure are the most frequently reported complications after TAVI (4), we have screened all patients until the discharge of the hospital. Infection was defined as an increase in CRP, fever and diagnosed infection-focus (pneumonia, urinary tract infection, other infections). Delirium was diagnosed by using NU-DESC. Postoperative heart rhythm disturbances occurred by AV-block and atrial fibrillation. Patients were divided into two groups: those who did not develop any postoperative complications (non-complication group) and patients who showed one of these complications within 3 days after TAVI (complication group).

Operation procedure:

All patients were admitted and evaluated at least one day before the operation. TAVIs were performed by the cardiac team (cardiac surgeon, cardiologist, and cardiac anesthesist) in a hybrid operating theatre, according to the German guidelines for TAVI procedures. All procedures were performed with the patients placed under general anesthesia. In all patients, monitoring consisted of pulsoximetry, 5-channel electrocardiogram, invasive blood pressure, central venous pressure, urinary output and bladder temperature. The maintenance of normothermia was accomplished by a heating blanket placed beneath the patient. The patients received right ventricular pacemakers for rapid ventricular pacing during balloon aortic valvuloplasty and valve-expansion. Pre-oxygenation was performed with

pure oxygen using a facemask. Anesthesia was induced with etomidate (Etomidat-Lipuro®, B. Braun Melsungen AG, Melsungen, Germany), remifentanil (Ultiva®, GlaxoSmithKline GmbH & Co. KG, Munich, Germany) and rocuronium (Rocuronium Inresa®, Inresa Arzneimittel GmbH, Freiburg, Germany) and maintained with sevoflurane (Sevorane®, AbbVie Deutschland GmbH & Co.KG, Wiesbaden, Germany). Piritramide and metamizole were used as additional pain medication. PONV prophylaxis was used intraoperatively, depending on the patient's risk. Cardiovascular drugs (e.g. norepinephrine, and dobutamine) were administered, as needed. A prophylactic antibiotic (1.5 g, Cefuroxim Hikma®, Hikma Pharma GmbH, Gräfelfing, Germany) was administered to each patient. In the operating theatre, the patient was connected to a defibrillator, and a TEE probe was introduced. After preparing the access points and anticoagulation with heparin (Ratiopharm GmbH, Ulm, Germany; mean given dose 5293 ± 2643 IU), the native valve was opened under rapid ventricular pacing, and the prothesis was implanted. The position and function of the prothesis was verified with TEE. Extubation of the patient was the goal at the end of each procedure. After surgery, patients were monitored for at least 12 hours in the ICU. Following this period, patient care continued either in the ICU or in the general ward. There was no use of heart lung machines.

Patient and public involvement

Patients were not involved in the study.

Statistics:

The data were electronically gathered and stored by using Excel (Excel 2013, Microsoft Corporation, Redmond, Washington, USA). Data analysis was performed by using SPSS (Version 22.0; SPSS Inc., Chicago, IL, USA). Frequency distributions and percentage rates were used for the categorical variables. Data are presented as mean with standard deviation and with Cohen's r effect size. Shapiro-Wilk test was used to verify Gaussian distribution of the study groups. Statistical significance between the patient groups was tested using t-test, Mann-Whitney U test, analysis of variance and chi-quadrat. Friedman analysis of ranks was performed to compare the cholinesterase activity change over time in each group. A multivariate logistic regression analysis was performed to investigate the association between cholinesterase activity and postoperative complications. A p-value < 0.05 indicated statistical significance.

Results:

Baseline data

A total of 48 patients were included, and 43 patients completed the assessment battery. The mean age was 79.5 +/- 5.71 years and the mean BMI was 27.93 +/- 5.36. There were almost equally men and women (22 (51.2 %) vs. 21 (48.8 %), table 1). All patients received elective TAVI in general anesthesia. TF access was selected for 32 (74 %), with TA chosen for 11 (26 %) patients. There was no use of heart-lung-machines. The demographic data and pre-existing conditions are shown in table 1. 32 (74.4 %) had an ASA-class of three, 11 (25.6 %) of four. Except of four, every patient was extubated immediately after operation and brought to ICU. One high risk patient was still intubated when brought to ICU and died two days after operation by multiorgan failure. Another patient was extubated on the first postoperative day. Two patients were extubated a few hours after brought to ICU. Patients were discharged to a normal ward after one day and left the hospital after 13.28 +/- 6.2 days.

Postoperative complications

24 patients (55.8 %) had postoperative complications as defined above. One multimorbid and high-risk patient died due to multiorgan-failure at ICU two days after surgery.

Of 43 patients, 12 developed postoperative delirium (27.9 %). Most patients developed their delirium on the first day after surgery.

Of 43 patients, 2 developed pneumonia. However, in 3 patients with raised infection markers and suspected infection no focus was found. All of them received antibiotics.

There were 7 patients with postoperative indication for pacemaker (16.3%). Overall 12 patients developed heart rhythm disturbances (27.9%). Some of the patients developed more than one complication, e.g. delirium or infection.

Comparison between complication and non-complication group

Preoperative variables

Preoperative assessment showed no significant differences regarding demographic data and laboratory routine markers like haemoglobin ($p = 0.917$), leukocytes ($p = 0.383$), CRP ($p = 0.716$), NT-proBNP ($p = 0.563$) and creatinine ($p = 0.089$). Preoperative BChE levels were significantly lower in patients who developed postoperative complications (D 1 complication group 2589.2 ± 556.4 vs. D 1 non-complication group 3295.7 ± 628.0 Cohen's $r = 0.514$, $p < 0.001$, table 2). Preoperative AChE enzyme activity in contrast did not show any difference between complication and non-complication group. There was also no difference regarding alcohol ($p = 0.226$) or nicotine ($p = 0.807$) consumption. Men showed a higher incidence of postoperative complications ($p = 0.095$).

Postoperative variables

All patients were postoperatively admitted to the ICU extubated and hemodynamic supported by catecholamines. Two patients did not meet the extubation criteria in the operation room and were extubated a few hours later at ICU. One high risk patient died at ICU due to multiorgan-failure. One patient was extubated on the first postoperative day.

Complication group showed an early, sustained and statistically significant decrease in BChE activity from the preoperative to the first postoperative measurement (D 0: 2784.0 ± 534.9 vs POD 0: 2379.6 ± 525.1, $p < 0.001$, figure 2). In contrast in patients without postoperative complications we observed a delayed decrease in BChE activity from the preoperative to postoperative period (D 0: 3072.6 ± 652.1 vs POD 2: 2713.5 ± 510.6, $p < 0.001$, figure 2). In all time points a significantly lower BChE activity was observed in patients with complications compared to patients without postoperative complication (figure 2).

Both groups showed a moderate decrease in AChE activity after preoperative screening measurement (figure 3). From anesthesia induction to the second postoperative measurement we observed no significant changes in AChE activity over time in both groups. There were no significant differences in AChE activity between patients with and without complication in any time point (figure 3).

Further analysis showed high effect sizes for the perioperative measurements of BChE. In contrast, effect sizes for AChE were much lower, which affirms the results above (table 2).

Patients, who developed postoperative complications had a significantly longer stay in hospital in general (complication-group: 15.2 ± 6.3 vs. non-complication-group: 11.1 ± 5.5 days, Cohen's $r = 0.325$, $p = 0.033$). There was no difference regarding the stay on ICU (complication group vs. non-complication group Cohen's $r = 0.132$, $p = 0.379$). Patients with postoperative delirium showed highest NU-DESC score on the first postoperative day (delirium: 3.3 ± 2.6 vs. non-delirium: 0.27 ± 0.79). The preoperative score of NU-DESC was 0.42 ± 0.67 within patients, who developed postoperative delirium. Routine laboratory markers like haemoglobin, leukocytes, CRP, CK, CK-MB and creatinine did not show any difference (complication group vs. non-complication group p = n. s., table 2).

Furthermore, there was no difference in EuroSCORE regarding on complication (complication group vs. non-complication group Cohen's $r = 0.034$, $p = 0.824$, table 1)).

TA vs. TF

Patients, who underwent TA approach declared postoperative higher pain levels measured by NRS ($p < 0.001$). They also showed higher CRP levels on POD 2 (88.8 ± 44.5 vs. 161.6 ± 70.2, Cohen's $r = 0.574$, $p < 0.001$) and higher levels of CK (110.8 ± 134.5 vs. 398.7 ± 139.0, Cohen's $r = 0.728$, $p < 0.001$) and CK-MB (8.3 ± 11.8 vs. 29.8 ± 14.7, Cohen's $r = 0.650$, $p < 0.001$) on the first postoperative day. There were no further differences between patients with TF and TA approach, especially regarding on complications or BChE and AChE enzyme levels.

Discussion:

TAVI has become the therapeutic standard for medical treatment in elderly patients with severe aortic valve stenosis and increased operative risks. The primary objective of the present investigation was to evaluate the roles of AChE and BChE as predictive markers for prevalent complications in cardiosurgical patients after TAVI.

Previous studies assumed an interaction of the immune and cholinergic system (19) and identified AChE and BChE as useful biomarkers for early detection of patients with emerging inflammation (16). Rapid changes in cholinesterase activity have been reported in patients after acute trauma, infections, delirium and critical illness (10–14). Both enzymes may serve as indicators of systemic inflammation and have a remarkable predictive value for mortality in critically ill patients. Zivkovic et al. showed that bedside-measurement of BChE activity predicts patient morbidity and length of ICU stay following major traumatic injury (20). Another study with patients undergoing venoarterial extracorporeal membrane oxygenation therapy after cardiac surgery revealed BChE as a strong predictor of all-cause and cardiovascular mortality (10).

In our present study patients with postoperative complications after TAVI had significant lower preoperative levels of BChE compared to the non-complication group. Effect sizes were particularly high for BChE measurements in this homogeneous patient group, which may rule out a great variance of enzyme activities. In combination with common preoperative evaluation procedures, BChE activity may serve as a useful predictive indicator to identify high-risk patients. Future studies are needed to clarify clinical implications.

Due to high variability in the onset, aetiology and progress of clinical conditions among patients, determining whether changes in the enzyme activity are correlated with the emergence of disease or are affected by concomitant factors is difficult. John et al. tested the hypothesis that AChE and BChE have an impact on patients after cardiac surgery with postoperative delirium. They showed that AChE increased and BChE decreased within the first 3 days after surgery but did not discern between patients with and without delirium. The authors supposed that the perioperative change of AChE and BChE activity might possibly be explained by an interaction of AChE and BChE and the use of a cardiopulmonary bypass (17). In our present study we evaluated the role of AChE and BChE activity in cardiosurgical patients after TAVI, as a standardized operative procedure without cardiopulmonary bypass. We could show that complication group showed a significantly perioperative decrease of BChE within the first 3 days after TAVI, despite the fact that there was no use of heart-lung machines in our patients. Furthermore, there was no use of blood products in the present study, so we can rule out a possible interaction of AChE and BChE with blood products as well. While in the CESARO study a wide spectrum of operative disciplines has been analysed and the perioperative enzyme activities showed small effect sizes, we can show high effect sizes for BChE in this secondary analysis of a homogeneous patient group with standardized operative procedure.

BChE activity could be regarded as an inflammatory parameter in this context. In literature, lower levels of BChE activity have already been described during inflammatory processes, stress and malnutrition. Therefore, lower levels of BChE activity in complication group might reflect perioperative inflammation, which is known to promote complications like delirium or infections. Conventional markers like CRP and leucocytes did not differ in both groups.

Delirium is a complex symptom which is very common in operative and non-operative disciplines in the course of hospital stay. The incidence is especially high among patients undergoing heart surgery (21). The incidence in this patients population has been described to be from 30 up to 80 % (22). The

incidence of delirium after TAVI is reported as 29 % in literature (6). Delirium occurred significantly more frequently following TA procedures (23). In the present study 26,7 % of the patients were diagnosed with delirium overall. There was no difference depending on TA or TF approach. Perioperative measurement of AChE and BChE did not discern between patients with and without delirium, which is in accordance with the findings by John et al.

The present study highlights the validity of BChE measurements for early detection of high-risk patients after TAVI. Surprisingly, the BChE assessment proved more effective than the EuroSCORE in discriminating between the patient groups making it a valuable biomarker for the early detection of high-risk patients. EuroSCORE is a well-established clinical assay for the patient mortality analysis (24) and requires documenting multiple and diverse datasets. The datasets are in most cases readily available; however, in some cases, a particular set of data might not be accessible, delaying or making the scoring impossible. By using a POCT system for a single BChE measurement, the results of an equally efficient outcome assessment tool are readily available at the bedside and may complete conventional assessments. Further studies with a greater patient population siare needed to investigate the clinical implications.

Prompt assessment of the systemic immune response with an immediate, rapid and affordable bedside measurement of the BChE activity might improve risk evaluation and help optimize postoperative management and therapy of patients after TAVI. Predicting the length of the hospital stay might play an important role in staff and resources management for these patients.

Limitations

Limitations of the present study might be the short duration of 3 days' measurement. Blood was taken from each patient; in case the analysis could not be performed immediately (during anesthesia induction), the sample was cooled down in a refrigerator. Maybe values of AChE and BChE changed in combination with lower temperature. Furthermore, it was only one measurement performed with each sample, so no control values could be achieved.

The study protocol required daily cholinesterase activity measurements in the postoperative period, without specifying time or requesting multiple daily measurements. Therefore, circadian fluctuations in enzyme activities could not be considered.

The biggest limitation of the present study is the low number of included patients. Further studies with larger patient groups and with focus on the underlying mechanisms of the different complications would be needed to validate our findings and the clinical implications.

Characteristic	Total sample (n = 43)
Age (years) [M (SD)]	79.47 (5.7)
Sex [n (%)]	
Male	22 (51.2)
Female	21 (48.8)
BMI [M (SD)]	27.93 (5.4)
ASA – PS [n (%)]	
3	32 (74.4)
4	11 (25.6)
Operative procedure [n (%)]	
Transapicale TAVI	11 (25.6)
Transfemorale TAVI	32 (74.4)
Relevant comorbidities [n (%)]	
Hypertension	37 (86)
Diabetes	16 (37.2)
Congestive heart failure	36 (83.7)
Congestive kidney failure	20 (46.5)
Coronary heart disease	31 (72.1)
Cardiac arrhythmias	25 (58.1)
Stroke	8 (18.6)
Nicotine	19 (44.2)
Alcohol	8 (18.6)
Hypothyreosis	10 (23.3)
Hypercholesterinemia	14 (32.6)
EuroSCORE [n (%)]	
low	15 (34.9)
middle	18 (41.9)
high	10 (23.3)

Table 1 Description of baseline data; all data are presented as n (number) and (%). ASA, American Society of Anaesthesiologists Classification; BMI, body mass index

Variables	Complication	N	M	SD	p-value	Cohens' r
EuroSCORE	Yes	24	21.8	15.4	0.824	0.034
	no	19	22.8	13.5		
Weight	Yes	24	78.3	15.5	0.504	0.102
[kg]	no	19	74.9	18.0		
BMI	Yes	24	28.1	4.7	0.860	0.037
[kg/m²]	no	19	27.7	6.2		
Age	Yes	24	79.9	5.3	0.556	0.086
[years]	no	19	78.9	6.3		
NT-proBNP	Yes	24	6244.8	6773.1	0.563	0.099
[pg/ml]	no	19	4806.6	7809.4		
Hemoglobin	Yes	24	12.1	1.9	0.917	0.029
D 0 [g/dl]	no	19	12.2	1.4		
Hemoglobin	Yes	24	10.7	1.5	0.565	0.068
POD 0 [g/dl]	no	19	10.9	1.4		
Hemoglobin	Yes	24	10.1	1.4	0.986	0.000
POD 1 [g/dl]	no	19	10.1	1.1		
Hemoglobin	Yes	24	10.1	1.1	0.673	0.087
POD 2 [g/dl]	No	19	10.3	1.2		
Hemoglobin	Yes	24	9.6	1.0	0.272	0.173
POD 3 [g/dl]	No	19	10.0	1.3		
Creatinine D	Yes	24	1.4	0.7	0.089	0.247
0 [mg/dl]	no	19	1.1	0.4		
Creatinine	Yes	24	1.2	0.4	0.347	0.124
POD 1 [mg/dl]	No	19	1.1	0.4		
Creatinine	Yes	24	1.5	0.8	0.188	0.223
POD 2 [mg/dl]	no	19	1.2	0.4		
Creatinine	Yes	24	1.5	1.0	0.240	0.244
POD 3 [mg/dl]	No	19	1.1	0.4		
Leukocytes	Yes	24	7.8	2.1	0.383	0.131
D 0 [/nl]	no	19	7.3	1.6		
Leucoytes	Yes	24	9.6	4.3	0.496	0.113
POD 1 [/nl]	No	19	8.8	2.1		
Leukocytes	Yes	24	9.9	2.7	0.616	0.081
POD 2 [/nl]	No	19	9.4	3.5		
Leukocytes	Yes	24	8.8	3.1	0.079	0.336
POD 3 [/nl]	No	19	7.0	1.5		
CRP	Yes	24	16.3	17.8	0.716	0.094
D 0 [mg/l]	no	19	19.8	18.2		
CRP	Yes	24	31.8	21.1	0.177	0.236
POD 1 [mg/l]	No	19	22.0	18.9		
CRP	Yes	24	116.3	52.9	0.516	0.114
POD 2 [mg/l]	No	19	101.3	78.2		
CRP	Yes	24	115.3	68,5	0.113	0.284
POD 3 [mg/l]	No	19	72.7	76,0		
BChE	Yes	24	2784.0	534,9	0.118	0.238
D 0 [U/l]	no	19	3072.6	652,1		
BChE	Yes	24	2589.2	556,4	**<0.001**	0.514
D 1 [U/l]	no	19	3295.7	628,0		
BChE	Yes	24	2379.6	525.1.	**<0.001**	0.469
POD 0 [U/l]	No	19	2972.5	599.2		
BChE	Yes	24	2300.3	561.0	**<0.001**	0.504

POD 1 [U/l]	No	19	2936.2	523.1		
BChE	Yes	24	2166.7	537.0	**0.002**	0.462
POD 2 [U/l]	No	19	2713.5	510.6		
AChE	Yes	24	45.0	8.1	0.446	0.118
D 0 [U/gHb]	no	19	43.3	6.0		
AChE	Yes	24	42.0	10.0	0.263	0.172
D 1 [U/gHb]	no	19	39.2	4.7		
AChE	Yes	24	42.9	10.0	0.051	0.295
POD 0 [U/gHb]	No	19	37.6	6.4		
AChE	Yes	24	41.5	9.2	0.196	0.198
POD 1 [U/gHb]	No	19	38.4	5.2		
AChE	Yes	24	41.2	8.2	0.058	0.294
POD 2 [U/gHb]	No	19	36.5	6.8		
CK	Yes	24	189.6	186.8	0.953	0.009
POD 1 [U/l]	No	19	186.1	190.5		
CK-MB	Yes	24	15.5	18.2	0.946	0.012
POD 1 [ng/ml]	No	19	15.9	14.3		
CK-Index	Yes	24	7.2	3.6	0.314	0.174
POD 1	No	19	8.5	3.8		
Stay in	Yes	24	15.2	6.3	**0.033**	0.325
hospital [days]	no	19	11.1	5.5		

385 Table 2 Perioperative laboratory markers; all data are presented as number (n) or as mean ± SD

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Figure 1 Timeline of measurements of BChE and AChE: blood samples were taken one day preoperative (D O), shortly before anesthetic induction (D 1), on admission to ICU (POD 0), one day after surgery (POD 1) and two days after surgery (POD 2). If the measuremnets could not be conducted immediatley, the samples have been cooled in a refridgerator and the measurement was performed up to 2 hours later.

Figure 2 Time trajectories of BChE activities in TAVI-patients (n = 43). Pre-operative (DO), shortly before anesthetic induction (D 1), on admission to ICU (POD 0), one day after surgery (POD 1) and two days after surgery (POD 2) measurements in patients with (dashed) and without (solid) complication. Data are presented as median ± standard deviation. * Difference between groups; # Difference within groups.

Figure 3 Time trajectories of AChE activities in TAVI-patients (n = 43). Pre-operative (DO), shortly before anesthetic induction (D 1), on admission to ICU (POD 0), one day after surgery (POD 1) and two days after surgery (POD 2) measurements in patients with (dashed) and without (solid) complication. Data are presented as median ± standard deviation.

Contributorship statement

DB, YZ, AM, AH and BM were responsible for study design, statistical analyses and drafting of the manuscript. WP, AB, BM and BG performed the experiments and drafted the manuscript. BM and AM were responsible for statistical analysis. All authors read and approved the final manuscript.

Competing interests

The authors declare no conflict of interest.

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Data sharing statement

No additional data are available.

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Figure 1

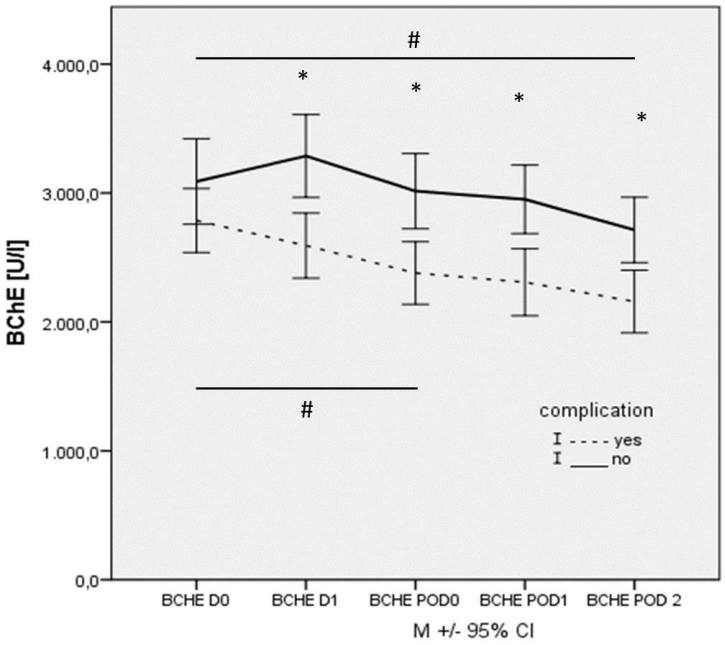

Figure 2

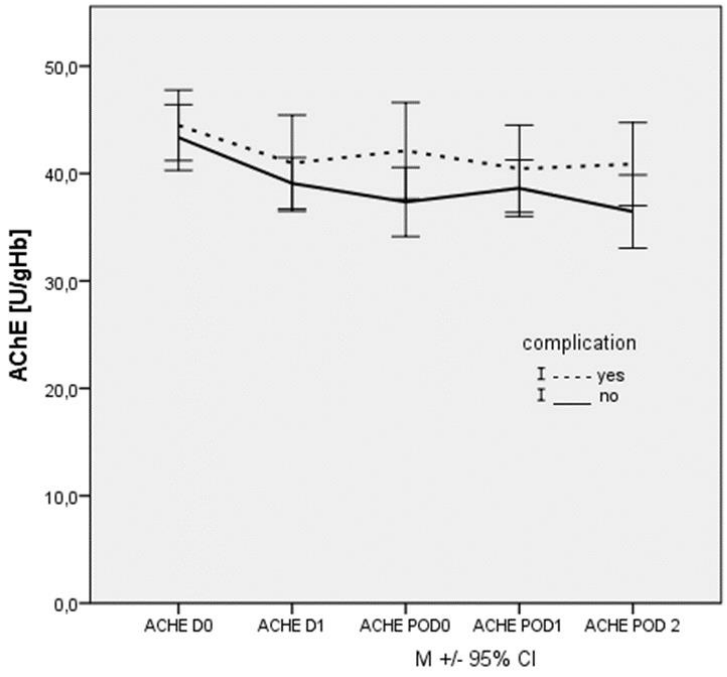

Figure 3

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract [line 34-36]
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found [line 30-55]
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported [line 73-100]
Objectives	3	State specific objectives, including any prespecified hypotheses [line 73-105]
Methods		
Study design	4	Present key elements of study design early in the paper [line 108-119]
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection [line 122-125]
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up [line 116-119] *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
		(*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 [line 127-177]
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 [line 122-125]
Bias	9	Describe any efforts to address potential sources of bias [line 159-168]
Study size	10	Explain how the study size was arrived at [line 108-115]
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why [line 127-177]
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding [line 208-217]
		(*b*) Describe any methods used to examine subgroups and interactions [not included]
		(*c*) Explain how missing data were addressed [line 166-168]
		(*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
		(*e*) Describe any sensitivity analyses [not included]

Continued on next page

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 [line 221-230]
		(b) Give reasons for non-participation at each stage [line 221-230]
		(c) Consider use of a flow diagram → removed (as suggested by reviewer)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders [line 221-230]
		(b) Indicate number of participants with missing data for each variable of interest [line 233-241]
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) [line 226-230]
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time [line 226-230]
		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
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 [line 232-289]
		(*b*) Report category boundaries when continuous variables were categorized [line 232-289]
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period [line 232-289]
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses [not applicable]
Discussion		
Key results	18	Summarise key results with reference to study objectives [line 294-351]
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 [line 356-373]
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence [line 294-351]
Generalisability	21	Discuss the generalisability (external validity) of the study results [line 294-351]
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 [line 461-463]

*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.

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.

BMJ Open

Butyrylcholinesterase as a perioperative complication marker in patients after transcatheter aortic valve implantation: a prospective observational study

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Keywords:	Adult intensive & critical care < ANAESTHETICS, Anaesthesia in cardiology < ANAESTHETICS, Adult cardiology < CARDIOLOGY, Valvular heart disease < CARDIOLOGY

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Butyrylcholinesterase as a perioperative complication marker in patients after transcatheter aortic valve implantation: a prospective observational study

Bernhard Michels[1], Andreas Holzamer[2], Bernhard Graf[3], Andre Bredthauer[3], Walter Petermichl[3], Anika Müller[4], York Zausig[5], Diane Bitzinger[3]

[1] *Department of Gastroenterology, University Hospital Regensburg, Regensburg, Germany*

[2] *Department of Cardiothoracic Surgery, University Hospital Regensburg, Regensburg, Germany*

[3] *Department of Anesthesiology, University Hospital Regensburg, Regensburg, Germany*

[4] *Department of Anesthesiology and Intensive Care Medicine Campus Charité Mitte and Campus Virchow-Klinikum, Charité-Universitätsmedizin Berlin, Berlin, Germany*

[5] *Department of Anesthesiology and Intensive Care Medicine, Hospital Aschaffenburg-Alzenau, Aschaffenburg, Germany*

Corresponding author:

PD Dr. med. Diane Bitzinger

University of Regensburg

Department of Anesthesiology

Franz-Josef-Strauss-Allee 11

93053 Regensburg, Germany

Phone: +49-941-944 7801

Fax: +49-941-9447802

Email: diane.bitzinger@ukr.de

Abstract

Objectives: Transcatheter aortic valve implantation (TAVI) is performed in elderly patients with severe aortic valve stenosis and increased operative risks. We tested the hypothesis that acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) have a predictive value for prevalent complications after TAVI and could serve as indicators of systemic inflammation in the early postoperative period.

Design: Prospective observational study.

Setting: This study is a secondary analysis of multi-center CESARO-study.

Participants: 48 TAVI patients were included, 43 obtained the complete assessment.

Primary and secondary outcome measures: Patients clinical parameters, demographic data, peripheral AChE and BChE-activities and routine blood markers were assessed throughout the perioperative period using bedside point-of-care measurements for AChE and BChE. Postoperative complications screening was conducted up to the 3rd postoperative day and included infections, delirium and heart-rhythm disturbances. After assessment the patients were divided into complication and non-complication group.

Results: Of 43 patients, 24 developed postsurgical complications (55.8%). Preoperative assessment showed no significant differences regarding demographic data and laboratory markers, but preoperative BChE-levels were significantly lower in patients who developed postoperative complications (complication group 2589.2 ± 556.4 vs. non-complication group 3295.7 ± 628.0, Cohen's $r = 0.514$, $p < 0.001$). In complication group we observed an early, sustained reduction in BChE-activity from preoperative to postoperative period. In complication group BChE-levels were significantly lower at each time point compared to non-complication group. AChE-activity showed no significantly difference between both groups. Complication group also had longer stay in hospital overall.

Conclusion: BChE could be a useful perioperative biomarker to identify patients with a higher risk for postoperative complications after TAVI. By using point-of-care measurements the levels of BChE are fast available and can lead to an early targeted therapy. Predicting the length of the hospital stay might play an important role in staff and resources management for these patients.

Trial registration: NCT01964274

Key words: cardiac surgery, TAVI, inflammation, delirium, butyrylcholinesterase, acetylcholinesterase

Strengths and limitations of this study

- This study is a secondary analysis of the prospective observational multi-center CESARO-study.
- Our study included 48 cardiosurgical patients with an observation time of three days.
- BChE could be a useful perioperative biomarker to identify patients with a higher risk for postoperative complications after TAVI.
- By using point-of-care measurements the levels of BChE are fast available and can lead to an early targeted therapy.
- Predicting the length of the hospital stay might play an important role in staff and resources management for these patients.

Introduction:

Recently, transcatheter aortic valve implantation (TAVI) has become the therapeutic standard for medical treatment in elderly, multimorbid patients with severe aortic valve stenosis and increased operative risks (1, 2). TAVI involves the implantation of a prosthetic valve, which is introduced with a catheter through transfemoral (TF), transapical (TA) or direct transaortic access. Usually, the TF approach is preferred because thoracotomy and penetration of the myocardium are not needed. The TA approach is common, if severe artherosclerotic disease does not allow retrograde insertion of the catheter. In patients with severe aortic stenosis, who could not undergo a surgical replacement of the aortic valve, TAVI significantly reduced the rates of death at any cause, compared to standard therapy (3). However, previous studies have shown that pneumonia, acute renal failure, indication for a permanent pacemaker and delirium were the most frequently recorded complications after TAVI (4). Covello et. al. reported a pneumonia rate of 7-8 % after TAVI (5). The incidence of delirium after TAVI is reported as 29 % in literature (6).

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are a focus of current research. Recent studies have shown that AChE and BChE serve as diagnostic markers of low-grade systemic inflammation (7–9). Rapid changes in cholinesterase activity have also been reported in patients after acute trauma, infections, burns and critical illness (10–14). Both enzymes may serve as indicators of systemic inflammation and may have a predictive value for mortality in critically ill patients. Zivkovic et. al. showed that reduced serum activity of BChE indicates severe systemic inflammation in critical ill patients (13). Furthermore, a recent study showed, that a sustained reduction in serum cholinesterase enzyme activity predicts patient outcome following sepsis (15).

Other studies postulate low preoperative plasma cholinesterase activity as a risk marker of postoperative delirium in elderly patients (16). A recently published study on cholinesterase activity in cardiac surgical patients showed no postoperative differences in cholinesterase activities between delirious and non-delirious patients, but showed a perioperative decrease of BChE which was potentially caused by cardiopulmonary bypass (17). However, due to high variability in the etiology and progress of clinical conditions, it was difficult to determine whether the changes in the enzyme activity correlated with the emergence of disease or was affected by concomitant factors such as cardiopulmonary bypass.

This is the first study to investigate the roles of AChE and BChE as inflammatory markers in cardiac surgical patients under standardized perioperative conditions without using cardiopulmonary bypass. Our aim of the present study is to evaluate if there is a predictive association of perioperative determination of AChE and BChE activity and the occurrence of postoperative complications after TAVI.

Material and Methods:

Ethics approval statement and patient population:

This work is a secondary analysis of the prospective observational multi-center CESARO study, powered for the detection of postoperative delirium. The CESARO study was initiated at Charité – Universitätsmedizin Berlin, Department of Anesthesiology and Operative Intensive Care Medicine (Clinicaltrials.gov ID: NCT01964274) and approved by the local independent Charité Ethics Committee, Charité – Universitätsmedizin Berlin, Germany (ref.: EA1/220/13) on 14 August 2013. After further approval of the local ethics board of the University of Regensburg a total of 48 patients were included into the study between March 2014, and June 2016 at University Hospital of Regensburg. Written informed consent was obtained from each patient.

Inclusions criteria: minimum age of 18 years, admission to intensive care unit (ICU) following elective TAVI in general anesthesia.

Exclusion criteria: missing consent, patients with a known pseudocholinesterase deficiency, patients with language, visual or hearing impairments.

Data

Data were acquired from anesthetic charts (Medlinq V.1.3, Hamburg, Germany), the patient document system used in the ICU (Metavision, iMDsoft, Tel Aviv, Israel) and medical reports from the electronic hospital information system (SAP, Walldorf, Germany) from the preoperative, intraoperative and postoperative periods until the patients were discharged from the hospital.

Preoperative variables:

Preoperative data included demographic data, such as age, sex, height, weight, regular use of alcohol and nicotine, American Society of Anesthesiologists (ASA) class, logistic EuroSCORE (European System for Cardiac Operative Risk Evaluation), New York Heart Association (NYHA) class and left ventricular ejection fraction (EF). The patients' previous medical history was examined for conditions such as chronic kidney disease, cerebrovascular events, including stroke and transient ischemic attacks, myocardial infarction, chronic obstructive pulmonary disease, diabetes mellitus and pre-existing cardiac arrhythmias. Furthermore, we evaluated the preoperative anticholinergic burden using the anticholinergic drug scale (18). This scale ranges from zero (no anticholinergic activity) to three (highest anticholinergic activity). Each long-term drug was screened for its anticholinergic activity and for each patient the number of points was assessed. Every patient was screened for preoperative delirium, using the nursing delirium screening scale (NU-DESC). Preoperative assessment of AChE, BChE, CRP, leukocytes, haemoglobin and creatinine were performed (table 1).

Intraoperative variables:

Key elements of intraoperative data included the selected access type, anesthetic procedure, transfusion of erythrocyte concentrates and extubation rate as well as the procedure duration.

Postoperative variables:

Postoperative data included the patient's stay in the ICU and the stay in hospital in general. Next to the sampling of laboratory markers, every patient was screened for delirium with NU-DESC for the first 3 days after surgery. Patients were daily assessed for pain, using the numeric rating scale (NRS score: 0 = no pain – 10 = maximum pain). Furthermore, any complication in recovery time was noticed. Mortality reasons are divided into cardiac, acute kidney injury, cardiovascular events and infections.

Variables:

Delirium:

Delirium screening was conducted perioperatively using a validated screening tool (NU-DESC) (19). NU-DESC assesses five dimensions: orientation, behaviour, communication, illusion/hallucination and psychomotor retardation. The symptoms are rated on a three-point scale, whereas a score of two or more cumulative points indicated delirium. Delirium assessment was performed one day prior to the operation, on admission to ICU and daily up to the third postoperative day. Patients with Richmond Agitation Sedation Scale (RASS) ≤ -2 were excluded for the current testing.

Laboratory parameters:

Blood samples were taken from every patient at following time points: one day before operation (screening), shortly before anesthetic induction, on admission to ICU, one day after surgery and two days after surgery (figure 1). The measurements included the determination of AChE and BChE. Both were measured in 10 µl whole blood, using *ChE check mobile*, a validated point-of-care testing device (ChE check mobile®, Securetec Detektions-Systeme AG, Neubiberg, Germany; In-Vitro-Diagnostics Guideline 98/79/EG; DIN EN ISO 18113-2 and -3) by following the manufacturer's instructions. Also, blood count, C-reactive protein (CRP), creatinine were measured at each time point. Creatine kinase (CK) and heart enzymes (CK-MB) were measured on the first postoperative day in the normal laboratory control. Brain natriuretic Peptide (NT-proBNP) was measured at the screening day. To deal with missing values, we included three defined measurements (time points) into the analysis.

Postoperative complications:

Since delirium, pneumonia, heart rhythm disturbances and acute renal failure are the most frequently reported complications after TAVI (4), we have screened all patients until the discharge of the hospital. Infection was defined as an increase in CRP, fever and diagnosed infection-focus (pneumonia, urinary tract infection, other infections). Delirium was diagnosed by using NU-DESC. Postoperative heart rhythm disturbances occurred by AV-block and atrial fibrillation. Patients were divided into two groups: those who did not develop any postoperative complications (non-complication group) and patients who showed one of these complications within 3 days after TAVI (complication group).

Operation procedure:

All patients were admitted and evaluated at least one day before the operation. TAVIs were performed by the cardiac team (cardiac surgeon, cardiologist, and cardiac anesthesist) in a hybrid operating theatre, according to the German guidelines for TAVI procedures. All procedures were performed with the patients placed under general anesthesia. In all patients, monitoring consisted of pulsoximetry, 5-channel electrocardiogram, invasive blood pressure, central venous pressure, urinary output and

bladder temperature. The maintenance of normothermia was accomplished by a heating blanket placed beneath the patient. The patients received right ventricular pacemakers for rapid ventricular pacing during balloon aortic valvuloplasty and valve-expansion. Pre-oxygenation was performed with pure oxygen using a facemask. Anesthesia was induced with etomidate (Etomidat-Lipuro®, B. Braun Melsungen AG, Melsungen, Germany), remifentanil (Ultiva®, GlaxoSmithKline GmbH & Co. KG, Munich, Germany) and rocuronium (Rocuronium Inresa®, Inresa Arzneimittel GmbH, Freiburg, Germany) and maintained with sevoflurane (Sevorane®, AbbVie Deutschland GmbH & Co.KG, Wiesbaden, Germany). Piritramide and metamizole were used as additional pain medication. PONV prophylaxis was used intraoperatively, depending on the patient's risk. Cardiovascular drugs (e.g. norepinephrine, and dobutamine) were administered, as needed. A prophylactic antibiotic (1.5 g, Cefuroxim Hikma®, Hikma Pharma GmbH, Gräfelfing, Germany) was administered to each patient. In the operating theatre, the patient was connected to a defibrillator, and a TEE probe was introduced. After preparing the access points and anticoagulation with heparin (Ratiopharm GmbH, Ulm, Germany; mean given dose 5293 ± 2643 IU), the native valve was opened under rapid ventricular pacing, and the prothesis was implanted. The position and function of the prothesis was verified with TEE. Extubation of the patient was the goal at the end of each procedure. After surgery, patients were monitored for at least 12 hours in the ICU. Following this period, patient care continued either in the ICU or in the general ward. There was no use of heart lung machines.

Patient and public involvement

Patients were not involved in the study.

Statistics:

The data were electronically gathered and stored by using Excel (Excel 2013, Microsoft Corporation, Redmond, Washington, USA). Data analysis was performed by using SPSS (Version 22.0; SPSS Inc., Chicago, IL, USA). Frequency distributions and percentage rates were used for the categorical variables. Data are presented as mean with standard deviation and with Cohen's r effect size. Shapiro-Wilk test was used to verify Gaussian distribution of the study groups. Statistical significance between the patient groups was tested using t-test, Mann-Whitney U test, analysis of variance and chi-quadrat. Bonferroni correction was done in case of repeat-measurements to rule out alpha error accumulation. Friedman analysis of ranks was performed to compare the cholinesterase activity change over time in each group. A multivariate logistic regression analysis was performed to investigate the association between cholinesterase activity and postoperative complications. A p-value < 0.05 indicated statistical significance.

Results:

Baseline data

A total of 48 patients were included, and 43 patients completed the assessment battery. The mean age was 79.5 +/- 5.71 years and the mean BMI was 27.93 +/- 5.36. There were almost equally men and women (22 (51.2 %) vs. 21 (48.8 %), table 1). All patients received elective TAVI in general anesthesia. TF access was selected for 32 (74 %), with TA chosen for 11 (26 %) patients. There was no use of heart-lung-machines. The demographic data and pre-existing conditions are shown in table 1. 32 (74.4 %) had an ASA-class of three, 11 (25.6 %) of four. Except of four, every patient was extubated immediately after operation and brought to ICU. One high risk patient was still intubated when brought to ICU and died two days after operation by multiorgan failure. Another patient was extubated on the first postoperative day. Two patients were extubated a few hours after brought to ICU. Patients were discharged to a normal ward after one day and left the hospital after 13.28 +/- 6.2 days.

Postoperative complications

24 patients (55.8 %) had postoperative complications as defined above. One multimorbid and high-risk patient died due to multiorgan-failure at ICU two days after surgery.

Of 43 patients, 12 developed postoperative delirium (27.9 %). Most patients developed their delirium on the first day after surgery.

Of 43 patients, 2 developed pneumonia. However, in 3 patients with raised infection markers and suspected infection no focus was found. All of them received antibiotics.

There were 7 patients with postoperative indication for pacemaker (16.3%). Overall 12 patients developed heart rhythm disturbances (27.9%). Some of the patients developed more than one complication, e.g. delirium or infection.

Comparison between complication and non-complication group

Preoperative variables

Preoperative assessment showed no statistically significant differences regarding demographic data and laboratory routine markers like haemoglobin ($p = 0.917$), leukocytes ($p = 0.383$), CRP ($p = 0.716$), NT-proBNP ($p = 0.563$) and creatinine ($p = 0.089$). Preoperative BChE levels were significantly lower in patients who developed postoperative complications (D 1 complication group 2589.2 ± 556.4 vs. D 1 non-complication group 3295.7 ± 628.0 Cohen's $r = 0.514$, $p < 0.001$, table 2). Preoperative AChE enzyme activity in contrast did not show any statistically significant difference between complication and non-complication group. There was no statistically significant difference regarding pre-existing anticholinergic medication ($p = 0.153$). There was also no statistically significant difference regarding alcohol ($p = 0.226$) or nicotine ($p = 0.807$) consumption. Men or women did not show a significantly higher incidence of postoperative complications ($p = 0.095$). There was no statistically significant difference between complication and non-complication group regarding the anticholinergic burden ($p = 0.229$).

Postoperative variables

All patients were postoperatively admitted to the ICU extubated and hemodynamic supported by catecholamines. Two patients did not meet the extubation criteria in the operation room and were extubated a few hours later at ICU. One high risk patient died at ICU due to multiorgan-failure. One patient was extubated on the first postoperative day.

Complication group showed an early, sustained and statistically significant decrease in BChE activity from the preoperative to the first postoperative measurement (D 0: 2784.0 ± 534.9 vs POD 0: 2379.6 ± 525.1, $p < 0.001$, figure 2). In contrast in patients without postoperative complications we observed a delayed decrease in BChE activity from the preoperative to postoperative period (D 0: 3072.6 ± 652.1 vs POD 2: 2713.5 ± 510.6, $p < 0.001$, figure 2). In all time points a significantly lower BChE activity was observed in patients with complications compared to patients without postoperative complication (figure 2).

Further analysis involving partial correlation and regression analysis showed, that there was no influence of pre-operative anticholinergic medication on BChE results (p = n. s.).

Both groups showed a moderate decrease in AChE activity after preoperative screening measurement (figure 3). From anesthesia induction to the second postoperative measurement we observed no significant changes in AChE activity over time in both groups. There were no significant differences in AChE activity between patients with and without complication in any time point (figure 3).

Further analysis showed large effect sizes for the perioperative measurements of BChE. In contrast, effect sizes for AChE were much lower, which affirms the results above (table 2).

Patients, who developed postoperative complications had a significantly longer stay in hospital in general (complication-group: 15.2 ± 6.3 vs. non-complication-group: 11.1 ± 5.5 days, Cohen's r = 0.325, p = 0.033). There was no statistically significant difference regarding the stay on ICU (complication group vs. non-complication group Cohen's r = 0.132, p = 0.379). Patients with postoperative delirium showed highest NU-DESC score on the first postoperative day (delirium: 3.3 ± 2.6 vs. non-delirium: 0.27 ± 0.79). The preoperative score of NU-DESC was 0.42 ± 0.67 within patients, who developed postoperative delirium. Routine laboratory markers like haemoglobin, leukocytes, CRP, CK, CK-MB and creatinine did not show any statistically significant difference (complication group vs. non-complication group p = n. s., table 2). he

Furthermore, there was no statistically significant difference in EuroSCORE regarding on complication (complication group vs. non-complication group Cohen's r = 0.034, p = 0.824, table 1).

TA vs. TF

Patients, who underwent TA approach declared postoperative higher pain levels measured by NRS ($p < 0.001$). They also showed higher CRP levels on POD 2 (88.8 ± 44.5 vs. 161.6 ± 70.2, Cohen's r = 0.574, $p < 0.001$) and higher levels of CK (110.8 ± 134.5 vs. 398.7 ± 139.0, Cohen's r = 0.728, $p < 0.001$) and CK-MB (8.3 ± 11.8 vs. 29.8 ± 14.7, Cohen's r = 0.650, $p < 0.001$) on the first postoperative day. There were no further statistically significant differences between patients with TF and TA approach, especially regarding on complications or BChE and AChE enzyme levels.

Discussion:

TAVI has become the therapeutic standard for medical treatment in elderly patients with severe aortic valve stenosis and increased operative risks. The primary objective of the present investigation was to evaluate the roles of AChE and BChE as predictive markers for prevalent complications in cardiosurgical patients after TAVI.

Previous studies assumed an interaction of the immune and cholinergic system (20) and identified AChE and BChE as useful biomarkers for early detection of patients with emerging inflammation (16). Rapid changes in cholinesterase activity have been reported in patients after acute trauma, infections, delirium and critical illness (10–14). Both enzymes may serve as indicators of systemic inflammation and may have a predictive value for mortality in critically ill patients. Zivkovic et al. showed that bedside-measurement of BChE activity predicts patient morbidity and length of ICU stay following major traumatic injury (21). Another study with patients undergoing venoarterial extracorporeal membrane oxygenation therapy after cardiac surgery revealed BChE as a strong predictor of all-cause and cardiovascular mortality (10).

In our present study patients with postoperative complications after TAVI had significant lower preoperative levels of BChE compared to the non-complication group. Effect sizes were particularly large for BChE measurements in this homogeneous patient group. In combination with common preoperative evaluation procedures, BChE activity may serve as a useful predictive indicator to identify high-risk patients. Future studies are needed to clarify clinical implications.

Due to high variability in the onset, aetiology and progress of clinical conditions among patients, determining whether changes in the enzyme activity are correlated with the emergence of disease or are affected by concomitant factors is difficult. John et al. tested the hypothesis that AChE and BChE have an impact on patients after cardiac surgery with postoperative delirium. They showed that AChE increased and BChE decreased within the first 3 days after surgery but did not discern between patients with and without delirium. The authors supposed that the perioperative change of AChE and BChE activity might possibly be explained by an interaction of AChE and BChE and the use of a cardiopulmonary bypass (17). In our present study we evaluated the role of AChE and BChE activity in cardiosurgical patients after TAVI, as a standardized operative procedure without cardiopulmonary bypass. We could show that complication group showed a significantly perioperative decrease of BChE within the first 3 days after TAVI, despite the fact that there was no use of heart-lung machines in our patients. Furthermore, there was no use of blood products in the present study, so we can rule out a possible interaction of AChE and BChE with blood products as well. While in the CESARO study a wide spectrum of operative disciplines has been analysed and the perioperative enzyme activities showed small effect sizes, we can show large effect sizes for BChE in this secondary analysis of a homogeneous patient group with standardized operative procedure.

BChE activity could be regarded as an inflammatory parameter in this context. In literature, lower levels of BChE activity have already been described during inflammatory processes, stress and malnutrition (9, 11, 22). Therefore, lower levels of BChE activity in complication group might reflect perioperative inflammation, which is known to promote complications like delirium or infections (20, 23). Conventional markers like CRP and leucocytes did not differ in both groups.

Delirium is a complex symptom which is very common in operative and non-operative disciplines in the course of hospital stay. The incidence is especially high among patients undergoing heart surgery (24). The incidence in this patients population has been described to be from 30 up to 80 % (25). The incidence of delirium after TAVI is reported as 29 % in literature (6). Delirium occurred significantly more frequently following TA procedures (26). In the present study 26,7 % of the patients were diagnosed with delirium overall. There was no difference depending on TA or TF approach.

Perioperative measurement of AChE and BChE did not discern between patients with and without delirium, which is in accordance with the findings by John et al.

The present study highlights the validity of BChE measurements for early detection of high-risk patients after TAVI. Surprisingly, the BChE assessment proved more effective than the EuroSCORE in discriminating between the patient groups making it a valuable biomarker for the early detection of high-risk patients. EuroSCORE is a well-established clinical assay for the patient mortality analysis (27) and requires documenting multiple and diverse datasets. The datasets are in most cases readily available; however, in some cases, a particular set of data might not be accessible, delaying or making the scoring impossible. By using a POCT system for a single BChE measurement, the results of an equally efficient outcome assessment tool are readily available at the bedside and may complete conventional assessments. Further studies with a greater patient population are needed to investigate the clinical implications.

Prompt assessment of the systemic immune response with an immediate, rapid and affordable bedside measurement of the BChE activity might improve risk evaluation and help optimize postoperative management and therapy of patients after TAVI. Predicting the length of the hospital stay might play an important role in staff and resources management for these patients.

Limitations

Limitations of the present study might be the short duration of 3 days' measurement. Blood was taken from each patient; in case the analysis could not be performed immediately (during anesthesia induction), the sample was cooled down in a refrigerator. Maybe values of AChE and BChE changed in combination with lower temperature. Furthermore, it was only one measurement performed with each sample, so no control values could be achieved.

The study protocol required daily cholinesterase activity measurements in the postoperative period, without specifying time or requesting multiple daily measurements. Therefore, circadian fluctuations in enzyme activities could not be considered.

The biggest limitation of the present study is the low number of included patients and the related statistical power. Further studies with larger patient groups and with focus on the underlying mechanisms of the different complications would be needed to validate our findings and the clinical implications. A larger, possibly multicenter study would be needed to evaluate more postoperative complications and the roles of BChE and AChE in particular complications.

Characteristic	Total sample (n = 43)
Age (years) [M (SD)]	79.47 (5.7)
Sex [n (%)]	
Male	22 (51.2)
Female	21 (48.8)
BMI [M (SD)]	27.93 (5.4)
ASA – PS [n (%)]	
3	32 (74.4)
4	11 (25.6)
Operative procedure [n (%)]	
Transapicale TAVI	11 (25.6)
Transfemorale TAVI	32 (74.4)
Relevant comorbidities [n (%)]	
Hypertension	37 (86)
Diabetes	16 (37.2)
Congestive heart failure	36 (83.7)
Congestive kidney failure	20 (46.5)
Coronary heart disease	31 (72.1)
Cardiac arrhythmias	25 (58.1)
Stroke	8 (18.6)
Nicotine	19 (44.2)
Alcohol	8 (18.6)
Hypothyreosis	10 (23.3)
Hypercholesterinemia	14 (32.6)
EuroSCORE [n (%)]	
low	15 (34.9)
middle	18 (41.9)
high	10 (23.3)
Pre-operative anticholinergic drugs	16 (37.2)

Table 1 Description of baseline data; all data are presented as n (number) and (%). ASA, American Society of Anaesthesiologists Classification; BMI, body mass index

Variables	Complication	N	M	SD	p-value	Cohens' r
EuroSCORE	Yes	24	21.8	15.4	0.824	0.034
	no	19	22.8	13.5		
Weight	Yes	24	78.3	15.5	0.504	0.102
[kg]	no	19	74.9	18.0		
BMI	Yes	24	28.1	4.7	0.860	0.037
[kg/m²]	no	19	27.7	6.2		
Age	Yes	24	79.9	5.3	0.556	0.086
[years]	no	19	78.9	6.3		
NT-proBNP	Yes	24	6244.8	6773.1	0.563	0.099
[pg/ml]	no	19	4806.6	7809.4		
Hemoglobin D	Yes	24	12.1	1.9	0.917	0.029
0 [g/dl]	no	19	12.2	1.4		
Hemoglobin	Yes	24	10.7	1.5	0.565	0.068
POD 0 [g/dl]	no	19	10.9	1.4		
Hemoglobin	Yes	24	10.1	1.4	0.986	0.000
POD 1 [g/dl]	no	19	10.1	1.1		
Hemoglobin	Yes	24	10.1	1.1	0.673	0.087
POD 2 [g/dl]	No	19	10.3	1.2		
Hemoglobin	Yes	24	9.6	1.0	0.272	0.173
POD 3 [g/dl]	No	19	10.0	1.3		
Creatinine D	Yes	24	1.4	0.7	0.089	0.247
0 [mg/dl]	no	19	1.1	0.4		
Creatinine	Yes	24	1.2	0.4	0.347	0.124
POD 1 [mg/dl]	No	19	1.1	0.4		
Creatinine	Yes	24	1.5	0.8	0.188	0.223
POD 2 [mg/dl]	no	19	1.2	0.4		
Creatinine	Yes	24	1.5	1.0	0.240	0.244
POD 3 [mg/dl]	No	19	1.1	0.4		
Leukocytes	Yes	24	7.8	2.1	0.383	0.131
D 0 [/nl]	no	19	7.3	1.6		
Leucoytes	Yes	24	9.6	4.3	0.496	0.113
POD 1 [/nl]	No	19	8.8	2.1		
Leukocytes	Yes	24	9.9	2.7	0.616	0.081
POD 2 [/nl]	No	19	9.4	3.5		
Leukocytes	Yes	24	8.8	3.1	0.079	0.336
POD 3 [/nl]	No	19	7.0	1.5		
CRP	Yes	24	16.3	17.8	0.716	0.094
D 0 [mg/l]	no	19	19.8	18.2		
CRP	Yes	24	31.8	21.1	0.177	0.236
POD 1 [mg/l]	No	19	22.0	18.9		
CRP	Yes	24	116.3	52.9	0.516	0.114
POD 2 [mg/l]	No	19	101.3	78.2		
CRP	Yes	24	115.3	68.5	0.113	0.284
POD 3 [mg/l]	No	19	72.7	76.0		
BChE	Yes	24	2784.0	534.9	0.118	0.238
D 0 [U/l]	no	19	3072.6	652.1		
BChE	Yes	24	2589.2	556.4	**<0.001**	0.514
D 1 [U/l]	no	19	3295.7	628.0		
BChE	Yes	24	2379.6	525.1	**<0.001**	0.469
POD 0 [U/l]	No	19	2972.5	599.2		
BChE	Yes	24	2300.3	561.0	**<0.001**	0.504

POD 1 [U/l]	No	19	2936.2	523.1		
BChE POD 2 [U/l]	Yes No	24 19	2166.7 2713.5	537.0 510.6	**0.002**	0.462
AChE D 0 [U/gHb]	Yes no	24 19	45.0 43.3	8.1 6.0	0.446	0.118
AChE D 1 [U/gHb]	Yes no	24 19	42.0 39.2	10.0 4.7	0.263	0.172
AChE POD 0 [U/gHb]	Yes No	24 19	42.9 37.6	10.0 6.4	0.051	0.295
AChE POD 1 [U/gHb]	Yes No	24 19	41.5 38.4	9.2 5.2	0.196	0.198
AChE POD 2 [U/gHb]	Yes No	24 19	41.2 36.5	8.2 6.8	0.058	0.294
CK POD 1 [U/l]	Yes No	24 19	189.6 186.1	186.8 190.5	0.953	0.009
CK-MB POD 1 [ng/ml]	Yes No	24 19	15.5 15.9	18.2 14.3	0.946	0.012
CK-Index POD 1	Yes No	24 19	7.2 8.5	3.6 3.8	0.314	0.174
Stay in hospital [days]	Yes No	24 19	15.2 11.1	6.3 5.5	**0.033**	0.325
Anticholinergic burden	Yes No	24 19	0.82 0.42	1.191 0.838	0.229	0.189

Table 2 Perioperative laboratory markers; all data are presented as number (n) or as mean ± SD

Figure 1 Timeline of measurements of BChE and AChE: blood samples were taken one day preoperative (D O), shortly before anesthetic induction (D 1), on admission to ICU (POD 0), one day after surgery (POD 1) and two days after surgery (POD 2). If the measuremnets could not be conducted immediatley, the samples have been cooled in a refridgerator and the measurement was performed up to 2 hours later.

Figure 2 Time trajectories of BChE activities in TAVI-patients (n = 43). Pre-operative (DO), shortly before anesthetic induction (D 1), on admission to ICU (POD 0), one day after surgery (POD 1) and two days after surgery (POD 2) measurements in patients with (dashed) and without (solid) complication. Data are presented as median ± standard deviation. * Difference between groups; # Difference within groups.

Figure 3 Time trajectories of AChE activities in TAVI-patients (n = 43). Pre-operative (DO), shortly before anesthetic induction (D 1), on admission to ICU (POD 0), one day after surgery (POD 1) and two days after surgery (POD 2) measurements in patients with (dashed) and without (solid) complication. Data are presented as median ± standard deviation.

Contributorship statement

DB, YZ, AM, AH and BM were responsible for study design, statistical analyses and drafting of the manuscript. WP, AB, BM and BG performed the experiments and drafted the manuscript. BM and AM were responsible for statistical analysis. All authors read and approved the final manuscript.

Competing interests

The authors declare no conflict of interest.

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Data sharing statement

No additional data are available.

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Figure 1

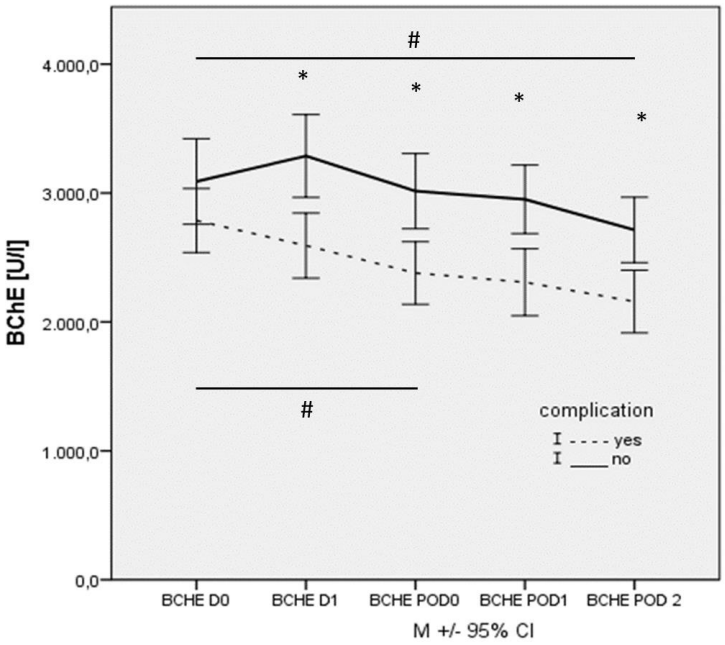

Figure 2

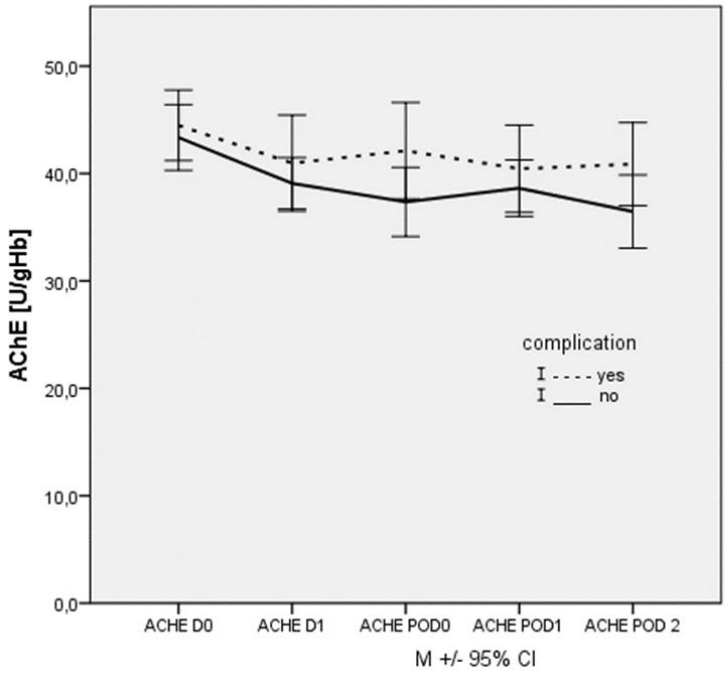

Figure 3

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract [line 34-36, page 2]
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found [line 30-55, page 2]
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported [line 73-105, page 4]
Objectives	3	State specific objectives, including any prespecified hypotheses [line 73-105, page 4]
Methods		
Study design	4	Present key elements of study design early in the paper [line 108-119, page 5]
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection [line 122-125, page 5]
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up [line 116-119, page 5] *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
		(*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 [line 127-180, page 5-6]
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 [line 122-125, page 5]
Bias	9	Describe any efforts to address potential sources of bias [line 108-208, page 5-7]
Study size	10	Explain how the study size was arrived at [line 108-115, page 5]
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why [line 127-180, page 5-6]
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding [line 211-221, page 7]
		(*b*) Describe any methods used to examine subgroups and interactions [line 211-221, page 7]
		(*c*) Explain how missing data were addressed [line 170-171, page 6]
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed [line 106-180, page 5-6] *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
	(*e*) Describe any sensitivity analyses [211-221, page 7]

Continued on next page

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 [line 225-234, page 8]
		(b) Give reasons for non-participation at each stage [line 225-234, page 8]
		(c) Consider use of a flow diagram → removed (as suggested by reviewer)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders [line 225-234, page 8 and 12]
		(b) Indicate number of participants with missing data for each variable of interest [225-234, page 8]
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) [line 230-234, page 8]
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time [line 237-245, page 8]
		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
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 [line 236-302, page 8-9]
		(*b*) Report category boundaries when continuous variables were categorized [line 236-302, page 8-9]
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period [line 236-302, page 8-9]
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses [211-221, page 7]
Discussion		
Key results	18	Summarise key results with reference to study objectives [line 305-366, page 10-11]
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 [line 371-383, page 11]
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence [line 305-366, page 10-11]
Generalisability	21	Discuss the generalisability (external validity) of the study results [line 305-366, page 10-11]
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 [line 433-436, page 16]

*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.

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.