Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Chemico-Biological Interactions 381 (2023) 110557

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Serum butyrylcholinesterase as a marker of COVID-19 mortality: Results of the monocentric prospective observational study

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ARTICLE INFO

Keywords:
COVID-19
Butyrylcholinesterase
Biomarker
SARS-CoV-2
C-reactive protein
Interleukin-6

ABSTRACT

The COVID-19 pandemic represents an excessive burden on health care systems worldwide and the number of patients who require special care in the clinical setting is often hard to predict. Consequently, there is an unmet need for a reliable biomarker that could predict clinical outcomes of high-risk patients. Lower serum butyrylcholinesterase (BChE) activity was recently linked with poor outcomes of COVID-19 patients. In line with this, our monocentric observational study on hospitalized COVID-19 patients focused on changes in serum BChE activity in relation to disease progression. Blood samples from 148 adult patients of both sexes were collected during their hospital stay at the Clinics of Infectiology and Clinics of Anesthesiology and Intensive Care, Trnava University Hospital in alignment with routine blood tests. Sera were analyzed using modified Ellman's method. Patient data with information about the health status, comorbidities and other blood parameters were collected in pseudonymized form. Our results show a lower serum BChE activity together with progressive decline of BChE activity in non-survivors, while higher stable values were present in discharged or transferred patients requiring further care. Lower BChE activity was associated with higher age and lower BMI. Moreover, we observed a negative correlation of serum BChE activity with the routinely used inflammatory markers, C-reactive protein and interleukin-6. Serum BChE activity mirrored clinical outcomes of COVID-19 patients and thus serves as a novel prognostic marker in high-risk patients.

1. Introduction

The global pandemic of coronavirus disease (COVID-19), caused by the SARS-CoV-2 virus, has significantly affected life worldwide. It has created an excessive burden on health care systems and has additional social and economic impacts [1]. With over 750 million cumulative cases and over 6.8 million deaths [2], COVID-19 continues to constitute a public health emergency of international concern [3].

Even if the majority of the infected patients are asymptomatic or show just mild symptoms, in some cases, silent hypoxemia and respiratory failure can develop without preceding dyspnea [4]. The severe form of COVID-19 is characterized by hypoxic respiratory failure, while the patient's condition can rapidly deteriorate, up to the onset of septic shock with subsequent multi-organ failure [5,6]. In addition to respiratory distress, other complications may arise, such as neurological, gastrointestinal, musculoskeletal disorders, skin lesions, cardiovascular disorders and blood clotting [1,7–9].

Based on the latest report of the International Health Regulations (2005) Emergency Committee regarding the COVID-19 pandemic, the WHO recommends to enhance access to COVID-19 vaccines, diagnostics and therapeutics as well as to support research to improve vaccines, to understand the full spectrum, incidence and impact of the post COVID-19 condition, and to develop relevant integrated care pathways [3].

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https://doi.org/10.1016/j.cbi.2023.110557
Received 31 March 2023; Received in revised form 15 May 2023; Accepted 18 May 2023
Available online 19 May 2023

Abbreviations

AChE	acetylcholinesterase
ALP	alkaline phosphatase
ALT	alanine transaminase
ARDS	acute respiratory distress syndrome
AST	aspartate transaminase
BChE	butyrylcholinesterase
BMI	body mass index
CCI	Charlson Comorbidity Index
CRP	C-reactive protein
HFNO	high-flow nasal oxygen
GMT	gamma-glutamyl transferase
IL-6	interleukin 6
MODS	multiple organ dysfunction syndrome
MV	mechanical ventilation
NIV	non-invasive ventilation
PCR	polymerase chain reaction

Along these lines, there is an extensive search for risk factors and diagnostic markers of the severe COVID-19. So far, older age, male sex, obesity, cardiovascular disease and other associated diseases have been recognized [7,10–13]. Several multi-omics studies have uncovered proteomic and metabolomics changes associated with clinical outcome but have not yet been translated to clinical practice [14–17]. Recently, a pilot study with 26 COVID-19 positive patients revealed an association between severity and mortality and serum butyrylcholinesterase (BChE) activity [18], leading the authors to propose BChE activity as a predictor of severity and mortality for COVID-19 pneumonia.

BChE is an esterase broadly expressed throughout the body, exceeding the other cholinesterase, acetylcholinesterase (AChE), by ten times [19]. The highest BChE levels in humans are detected in plasma and the liver [19]. The liver is also the organ with the highest BChE mRNA content [20]. Although no endogenous substrate has been discovered for BChE so far, BChE participates in the regulation of the activity of the cholinergic system by the breakdown of acetylcholine [21]. Also, BChE hydrolyzes the hunger hormone octanoyl ghrelin to desacyl ghrelin [22]. Increased levels of active ghrelin in BChE knock-out mice were proposed to explain the high fat diet-induced obesity in these mutants [23,24]. Additionally, BChE is an important scavenging and/or metabolizing enzyme of numerous xenobiotics, including drugs [19].

Plasma BChE does not change with age [25,26]. Rather stable serum BChE activity is however reduced in pregnancy [27], but also during certain pathologies, such as hyperlipidemia, type 2 diabetes mellitus, obesity [23,28–30], inflammatory conditions, liver disease, sepsis and cardiovascular diseases [31–34], thus the pathologies also associated with severe COVID-19 disease.

For the above reasons, routine determination of BChE activity in patients could be of clinical benefit in the management of the COVID-19 pandemic and beyond.

2. Materials and methods

2.1. Study design

This monocentric prospective observational study was conducted from October 2021 to May 2022 with approval by the local ethics committees of the Faculty of Pharmacy of Comenius University in Bratislava (Ethics Committee Statement 03/2021) and Trnava University Hospital. The study was carried out according to The Code of Ethics of the World Medical Association (Declaration of Helsinki). During the beginning of our study, the delta variant of the SARS-CoV-2 virus was predominant in Slovakia. The omicron variant started to be predominant (59% of samples identified after sequencing) in mid-January, as stated in the press release of the Public Health Authority of the Slovak Republic [35]. Patients from the Clinic of Infectiology and the Clinic of Anesthesiology and Intensive Medicine were enrolled in the study. These two departments were during pandemic period allocated primarily for the treatment of high-risk patients with severe COVID-19 thus associated with high mortality rate. The patients were enrolled in the study after signing an informed consent form and agreeing to participate in the study. Patients with the SARS-CoV-2 virus confirmed by polymerase chain reaction (PCR) test of both sexes and aged >18 years were included in the study.

2.2. COVID-19 treatment

Patients were treated according to The Standardized Preventive, Diagnostic and Therapeutic Procedure in Care for Critically Ill with COVID-19 (last update: 2.12.2021) developed at that time by the Ministry of Health of the Slovak Republic. Additional oxygen support was provided by high flow nasal oxygen (HFNO) or non-invasive ventilation (NIV) available at the Clinic of Infectiology, or by mechanical ventilation (MV) at the Clinic of Anesthesiology and Intensive Medicine. HFNO provides a *trans*-nasal humidified rapid-insufflation ventilatory exchange [36]. NIV is another non-invasive technique, a positive pressure driven delivery of oxygen into the lungs [37]. MV is an assisted ventilation that provides exchange of the air in the lungs [38]. For selected patients, prone positioning was used to improve gas exchange.

2.3. Data collection

Demographic data and clinical measurements were accessed from the hospital's electronic health record system in pseudonymized form. Information on age, sex, weight, height, BMI, smoking, preexisting comorbidities, vaccination status, chronic pharmacotherapy, treatment during hospitalization, and clinical outcome of the individual patient was collected from hospital discharge or death summaries. The present comorbidities were evaluated for their burden using the Charlson Comorbidity Index (CCI) [39] and classified into the corresponding organ system group.

Clinical parameters were observed throughout hospitalization and collected from the day of patient enrollment to the end of hospitalization. Altogether, 25 clinical parameters were followed, including standard serum biochemical parameters, inflammatory markers, muscle damage and cardiac markers, parameters of iron metabolism, hematological profile, and markers of glomerular filtration. For the list of the collected parameters, see *Supplementary data*.

2.4. Butyrylcholinesterase activity measurement

The whole blood samples were collected according to their routine morning blood test using 7.5 ml of S-Monovette Serum-Gel tubes (SARSTEDT, product no. 01.1602.001) and centrifuged at 2500×*g* for 10 min at room temperature. The supernatants representing sera were separated and freshly analyzed and subsequently stored at −80 °C for re-analysis.

A modified Ellman's method was used to detect BChE activity in serum according to a protocol optimized in our laboratory [40]. Each sample was analyzed in triplicates on two separate occasions; thus, each sample was analyzed six times. Ellman's reaction is based on the enzymatic hydrolysis of butyrylthiocholine (BTC) yielding thiocholine, which then reacts with the Ellman's reagent, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), generating a yellow color. BChE activity was determined by reaction of 2.5 μl of serum sample with 1 mM BTC iodide (Sigma-Aldrich, product no. B3253) in 5.0 mM HEPES buffer, pH = 7.5 (Sigma-Aldrich, product no. H3375), containing 0.5 mM DTNB (Sigma-Aldrich, product no. B8130) at room temperature. HEPES buffer was

used to increase the stability of DTNB; 0.1 M HEPES buffer and a 1 M phosphate buffer, pH = 7.5, were used to prepare a stock solution of 20 mM DTNB (stored in the dark at 4 °C) [40]. Before the enzymatic reaction, preincubation was carried out for 15 min with 0.5 mM DTNB solution protected from light to reduce the background arising from free SH groups in sera [40]. The following reaction was measured at 405 nm using a BioTek ELx800 spectrophotometer for 20 min at 46 s intervals. BChE activity is expressed as $\Delta mO.D._{405nm}$ using the slope of the linear phase of the saturation curve.

2.5. Data analysis

Data were analyzed using GraphPad PRISM (Version 9.3.1., 2021) and JMP Statistical Software (Version 16.1). The significance level for all analyses was set at $p < 0.05$. The patients were divided into subgroups according to their clinical outcomes of hospitalization as discharged, transferred, and non-survivors. The clinical parameters and serum BChE activity of the patients were analyzed over time with the end point at the end of hospitalization, i.e., at discharge, transfer to another department, or death. Demographic data and clinical parameters (age, sex, BMI, vaccination, smoking status, BChE activity, and other biochemical measurements) were expressed as mean with interquartile range (IQR) or median with IQR. Continuous clinical data, BChE activity, and individual changes between groups were analyzed at the beginning of hospitalization (during the first 5 days, median = day 1) and at the end of hospitalization (during the last 5 days, median = day 0). For patients with 3 or more blood sample collections during hospital stay, percent change of BChE activity was calculated. Patients were divided into subgroups according to sex (male/female), age (<65 years/ ≥ 65 years) and BMI (BMI <30/BMI ≥30). One-way analysis of variance (ANOVA) followed by post hoc tests and unpaired t tests for parametric continuous data or Wilcoxon/Kruskal-Wallis tests for data with non-normal distributions were used to compare data between the groups. The Spearman rank correlation test was used to evaluate the relationship between BChE and relevant clinical parameters.

3. Results

3.1. Demographics and clinical characteristics

A total of 148 patients were included in the study, 76 patients (51.4%) hospitalized at the Clinic of Infectiology and 72 patients hospitalized at the Clinic of Anesthesiology and Intensive Medicine (48.6%). Within the cohort, clinical status of 46 patients (31%) improved to the point that they were discharged from the hospital, 49 patients (33%) improved enough to be transferred to a more specialized unit and 53 patients (36%) died (Table 1). The median age was 66 years, while age was equally represented in the patient groups ($p = 0.061$). There was no significant difference in sex or BMI between patient groups ($p = 0.499$, $p = 0.341$, respectively). Thirty patients (20.3%) of the entire cohort of patients were vaccinated with at least one dose. The lowest vaccination rate was observed in the group of non-survivors (11.3%; $p = 0.279$). Fully vaccinated individuals included 10 (21.7%) discharged patients, 9 (18.4%) transferred patients and 6 (11.3%) non-survivors ($p = 0.365$). The patients did not differ in smoking rates ($p = 0.442$) and in the number of organ systems affected by current comorbidities ($p = 0.289$). The median value of CCI for all patients was 4 (IQR 2–7), with a difference observed between transferred patients and non-survivors ($p = 0.021$).

The median hospital stay was 10 days, with the longest stay for transferred patients with a condition that required further follow-up in another clinic ($p = 0.014$). All patients received oxygen support: 52 (35.1%) patients received HFNO ($p = 0.0003$), 146 (97.8%) patients received NIV ($p = 0.106$) and 66 (44.6%) patients received MV ($p < 0.0001$). The prone position was used in 51 (34.5%) patients ($p < 0.0001$). Together in all groups of patients, 48 (32.4%) patients suffered bacterial superinfection during hospitalization ($p = 0.499$). Sepsis was present during hospitalization in 23 patients (15.5%), with the highest rate in non-survivors ($p = 0.010$). ARDS and MODS were present only in transferred patients and non-survivors ($p < 0.0001$). Detailed results are shown in Table 1.

Table 1
Demographics and clinical characteristics of the patient groups.

Characteristics	Discharged = 46 (31%)	Transferred = 49 (33%)	Non-survivors = 53 (36%)	p value
Sex (female, %)	21 (45.7%)	24 (49.0%)	20 (37.8%)	0.500
Age (median, IQR)	70 (54.3–76.0)	63 (50.0–71.0)	67.0 (58.0–77.5)	0.061
BMI (mean, IQR)	30.1 (24.9–31.9)	30.8 (24.7–35.2)	31.1 (27.7–34.6)	0.341
Vaccination (at least one dose) (n, %)	11 (23.9%)	12 (24.4%)	7 (11.3%)	0.279
Smoking (n, %)	5 (12.8%)	3 (6.3%)	7 (12.2%)	0.442
Number of comorbidities (median, IQR)	4 (2–5)	3 (2–4)	4 (2–5)	0.289
CCI (median, IQR)	5 (2–8)	3 (1–5)	4 (2–8)	**0.016**
Days of hospitalization (median, IQR)	9.5 (6–12.5)	22 (4–37)	9 (6–18)	**0.014**
MV (n, %)	0 (0%)	32 (65.3%)	34 (64.2%)	**< 0.0001**
HFNO (n, %)	6 (13%)	19 (38.8%)	27 (50.9%)	**0.0003**
NIV (n, %)	44 (95.7%)	49 (100%)	53 (100%)	0.106
Prone position (n, %)	0 (0%)	27 (55.1%)	24 (45.3%)	**< 0.0001**
Superinfection (n, %)	13 (28.3%)	19 (38.8%)	16 (30.2%)	0.500
Sepsis (n, %)	1 (2.2%)	10 (20.4%)	12 (22.6%)	**0.010**
Pneumonia (n, %)	30 (65.2%)	36 (73.5%)	44 (83%)	0.128
ARDS (n, %)	0 (0%)	17 (34.7%)	17 (32.1%)	**< 0.0001**
MODS (n, %)	0 (0%)	3 (6.1%)	18 (34%)	**< 0.0001**

Data are characterized as mean or median with IQR. For comparison between groups, chi-square tests were used for categorical data and one-way ANOVA or Wilcoxon/Kruskal-Wallis tests for continuous data, $p < 0.05$. IQR – interquartile range, BMI – body mass index, CCI – Charlson Comorbidity Index, MV – mechanical ventilation, HFNO – high-flow nasal oxygen, NIV – non-invasive ventilation, ARDS – acute respiratory distress syndrome, MODS – multiple organ dysfunction syndrome.

3.2. Serum butyrylcholinesterase activity

Serum BChE activity was measured in 148 patients throughout hospitalization at multiple time points (805 measurements in total; Fig. 1), where we observed group differences in BChE activity according to outcome. BChE activity had an increasing trend during hospitalization for discharged patients (Fig. 1A), while transferred patients (Fig. 1B) and non-survivors (Fig. 1C) showed a flat trendline.

BChE activity measured near admission (i.e., during the first five days of the hospitalization, median day = 1), was compared to BChE activity measured at the end (i.e., during the last 5 days of the hospitalization, median day = 0). The results revealed unchanged values for groups of discharged and transferred patients, although transferred patients had significantly lower BChE activity at the end of hospitalization as compared to discharged ones. In non-survivors, BChE activity was significantly lower at the end of hospitalization compared to admission, and BChE activity was also reduced at the end of hospitalization relative to discharged patients (Fig. 1D).

The individual percent change of BChE activity was studied in patients in which values for BChE activity at the beginning and end of hospitalization were available (n = 71). While changes in serum BChE activities of discharged and transferred patients are close to 0, the

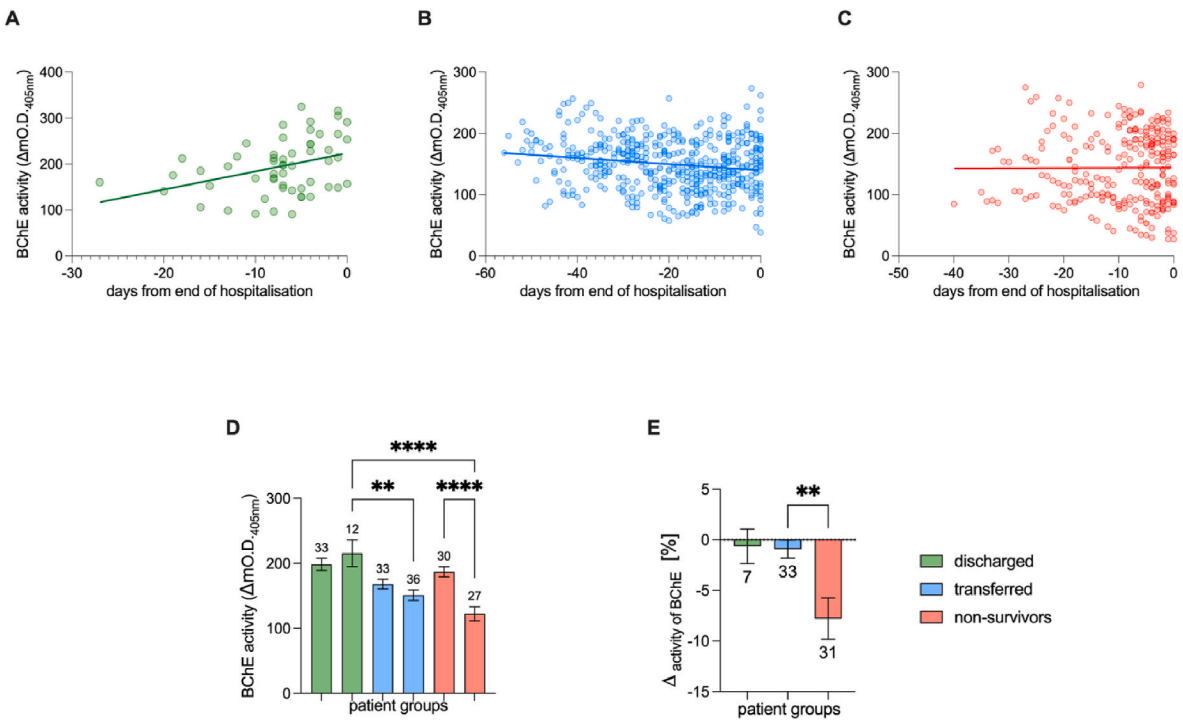

Fig. 1. Serum butyrylcholinesterase (BChE) activity in patients during hospitalization based on clinical outcomes. (A–C) Points represent individual measured values within a group of discharged (A), transferred (B) patients, and non-survivors (C) over time. The negative value of the horizontal axis retrospectively reflects the days from the end of hospitalization in the clinic (median day = 0). (D) Change in BChE activity in patients at baseline (median day = 1 from start; on left) and at the end (median day = 0 from end; on right) of hospitalization according to their clinical results (mean ± SEM). (E) Percent decrease of baseline BChE activity (median day = 1) by the end of hospitalization (median day = 0), calculated for the individual patient and expressed as mean ± SEM, $p < 0.05$. Sample size for each subgroup is indicated above the bars.

percent change in serum BChE activity in non-survivors decreased by −7.8% (range −42.9%–5.35%; Fig. 1E).

3.3. Serum butyrylcholinesterase activity regards to sex, age, and BMI

Several authors report that serum BChE activity depends on sex [26], age [25], and BMI [30]. Therefore, we analyzed the change in serum BChE in COVID-19 patients within subgroups and also between subgroups with respect to clinical status and time during hospitalization.

We observed no sex-dependence in BChE activity in the whole patient cohort or within the patient groups of discharged and transferred patients at the beginning or at the end of the hospitalization (Table 2). However, in non-survivors at the beginning of hospitalization, sex-related difference in BChE activity was observed ($p = 0.003$), while this difference disappeared by the end of hospitalization.

Although BChE activity did not correlate with the age of the studied patients ($r_s = -0.0778$, $p = 0.454$), significantly lower values were observed at the start and at the end of hospitalization in patients older than 65 years ($\Delta mO.D._{405\ nm}$: 174.8 ± 43.2 vs. 141.2 ± 57.5, $p = 0.003$ median day = 1; 197.1 ± 52.1 vs. 160.9 ± 62.5, $p = 0.024$, median day

Table 2
Differences in serum BChE activity based on sex, age, and BMI, including clinical outcomes and time of hospitalization.

Patient groups	Time of hospitalization	SEX			AGE			BMI		
			ΔmO.D.	p value		ΔmO.D.	p value		ΔmO.D.	p value
Whole cohort	Start	M	177.6 ± 46.6	0.084	<65	197.1 ± 52.1	**0.024**	<30	167.5 ± 44.1	**<0.0001**
		F	194.8 ± 50		≥65	174.8 ± 43.2		≥30	206.9 ± 45.5	
	End	M	145.6 ± 59.2	0.182	<65	160.9 ± 62.5	0.152	<30	148.6 ± 55.1	0.506
		F	164 ± 62.7		≥65	141.2 ± 57.5		≥30	157.6 ± 66.9	
Discharged	Start	M	200.2 ± 52.2	0.853	<65	230.3 ± 41.1	**0.004**	<30	177.5 ± 46.4	**0.004**
		F	196.6 ± 56.8		≥65	177.8 ± 56.5		≥30	230.7 ± 48.9	
	End	M	210.4 ± 67	0.783	<65	235.5 ± 69.3	0.355	<30	188.9 ± 69.2	0.061
		F	222.7 ± 83.1		≥65	195.7 ± 72.9		≥30	268.8 ± 40	
Transferred	Start	M	160.9 ± 47.5	0.231	<65	182.4 ± 44.5	0.073	<30	154.6 ± 47.5	**0.035**
		F	180.3 ± 44.8		≥65	153.1 ± 45.4		≥30	187.8 ± 40.1	
	End	M	147.4 ± 46.3	0.522	<65	166.7 ± 46.1	**0.012**	<30	151.6 ± 46.5	0.933
		F	158.6 ± 54.6		≥65	122.2 ± 44.6		≥30	153 ± 53.7	
Non-survivors	Start	M	171.8 ± 34	**0.003**	<65	186.9 ± 47	0.981	<30	168.8 ± 35.8	**0.009**
		F	217.8 ± 41.8		≥65	187.3 ± 40.2		≥30	208.0 ± 40.3	
	End	M	124.4 ± 53.5	0.220	<65	131.9 ± 58.8	0.916	<30	128 ± 47.3	0.606
		F	147.6 ± 53.9		≥65	133.9 ± 49.3		≥30	137.5 ± 61.1	

Values are expressed as mean ± SD within each group; $p < 0.05$. Start, value of serum BChE activity at the beginning of the hospitalization (median day = 1); End, value of serum BChE activity at the end of the hospitalization (median day = 0); <65, patients younger than 65 years; ≥65, patients with age 65 and older; <30, patients with BMI lower than 30; ≥30, patients with BMI 30 and more.

= 0, respectively) (Table 2). We observed no change in BChE activity at the beginning and the end of hospitalization in discharged or transferred patients regardless of age, while in non-survivors both age groups showed decreased BChE activity at the end of the hospitalization (Fig. 2).

Serum BChE activity correlated with BMI of hospitalized patients (r_s = 0.4922, $p < 0.0001$). The BMI also had an impact on the BChE change during the hospitalization. While patients with BMI <30 had BChE activities comparable at the beginning and end of hospitalization (ΔmO.D. $_{405\ nm}$: 167.5 ± 44.1 vs. 148.6 ± 55.1; p = 0.066), obese patients with a BMI ≥30 had a higher baseline serum BChE activity compared to the end of hospitalization (ΔmO.D. $_{405\ nm}$: 206.9 ± 45.5 vs. 157.6 ± 66.9, p = 0.0001) (Table 2).

When comparing the results in the view of clinical progression, BChE activity was preserved in the group of discharged patients regardless of BMI (Fig. 3A and B). Transferred patients showed the same activity only in the group of BMI <30 (Fig. 3C) but decreased BChE activity in patients with high BMI (Fig. 3D). We observed a statistically significant decrease in serum BChE activity in non-survivors with BMI <30 (Fig. 3E) and equally with BMI ≥30 (Fig. 3F).

3.4. Correlation of serum BChE activity with clinical markers

Among the studied clinical parameters and markers, only mediators of inflammation differed between the groups within the first 5 days of hospitalization. Patients who were later transferred during hospitalization and required additional care and non-survivors had significantly higher levels of white blood cells (p = 0.036), neutrophils (p = 0.004) and neutrophil-lymphocyte ratio ($p < 0.0001$), together with lower levels of lymphocytes (p = 0.001), eosinophils (p = 0.0002) and basophils (p = 0.017) (**Supplementary data 1**). Within five days of the end of hospitalization, in non-survivors, higher levels of white blood cells (p = 0.001), neutrophils ($p < 0.0001$), C-reactive protein ($p < 0.0001$), and neutrophil-lymphocyte ratio ($p < 0.0001$) were observed, together with lower levels of red blood cells (p = 0.025), hemoglobin (p = 0.003), hematocrit (p = 0.006), lymphocytes ($p < 0.0001$), eosinophils (p = 0.001), and basophiles (p = 0.029) (**Supplementary data 2**).

Results from the correlation analysis revealed a moderate correlation of serum BChE activity with RBC (r_s = 0.563; $p < 0.0001$), HGB (r_s = 0.555; $p < 0.0001$) and HCT (r_s = 0.554; $p < 0.0001$). Serum BChE activity was most strongly correlated with serum total protein (r_s = 0.657; p = 0.0007) and serum albumin level (r_s = 0.742; $p < 0.0001$). Among inflammatory markers, a moderate negative correlation of serum BChE activity was observed with serum levels of C-reactive protein (r_s = −0.498; $p < 0.0001$) and a strong negative correlation with interleukin-6 (r_s = −0.867; p = 0.002) levels was observed. Interestingly, BChE activity did not correlate with markers of liver damage. We did not observe a correlation between serum BChE and ALT (r_s = −0.063; p = 0.756), ALP (r_s = 0.042; p = 0.837), AST (r_s = −0.207; p = 0.300) or GMT (r_s = 0.073; p = 0.707) (**Supplementary data 3**).

4. Discussion

In the presented research, changes in serum BChE activity of 148 hospitalized patients with severe COVID-19 during hospitalization were studied. These results support a growing body of evidence showing a relationship between BChE activity and clinical status. The added value of this study includes assessing BChE activity in one of the largest cohorts of patients with severe COVID-19 during the whole period of hospitalization, rather than a single time point at admission, thus providing insights into the dynamics of BChE activity in relation to clinical progression and outcome. Additionally, to the best of our

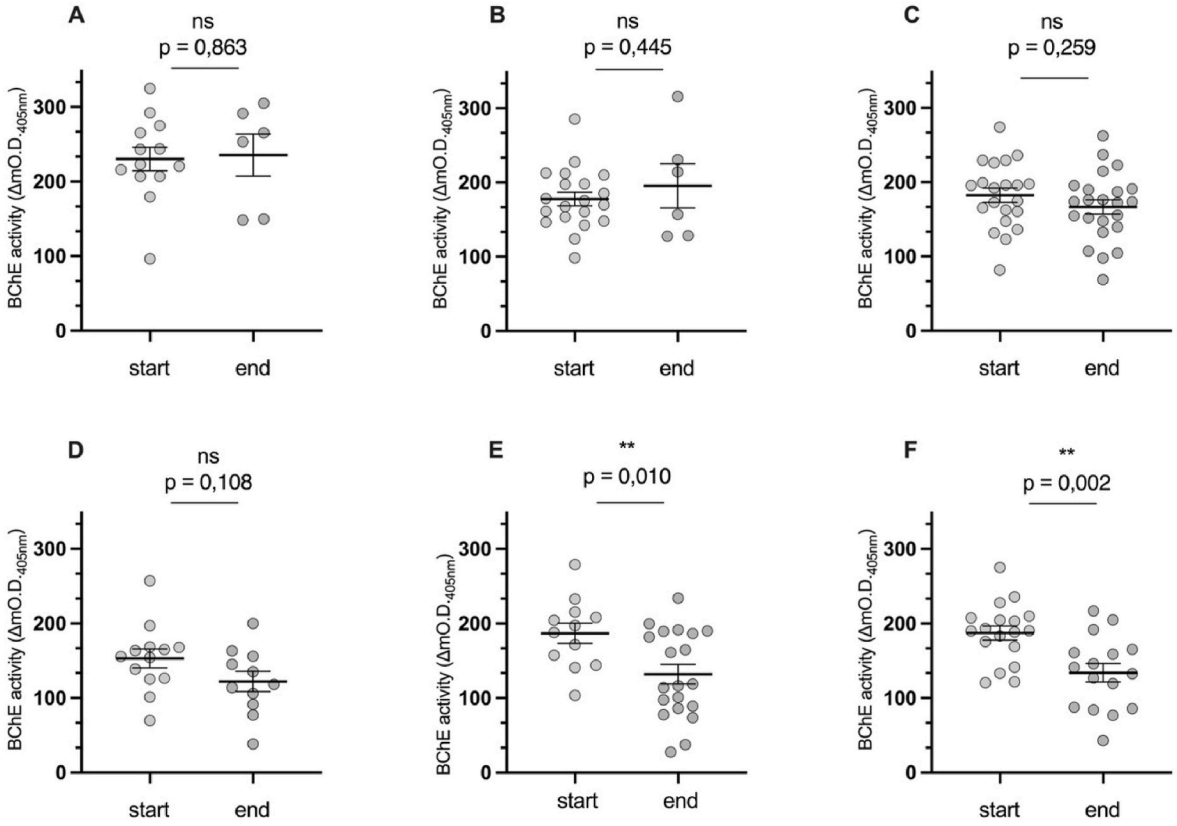

Fig. 2. Serum BChE activity in patients according to age. Comparison of BChE values at the beginning (left; median day = 1) and the end (right; median day = 0) of the hospitalization in the discharged patients of age <65 years (A) and ≥65 years (B), patients transferred to different wards of age <65 years (C) and ≥65 years (D) and non-survivors of the age <65 years (E) and ≥65 years (F). Values are expressed as individual values with mean ± SEM within each group; $p < 0.05$.

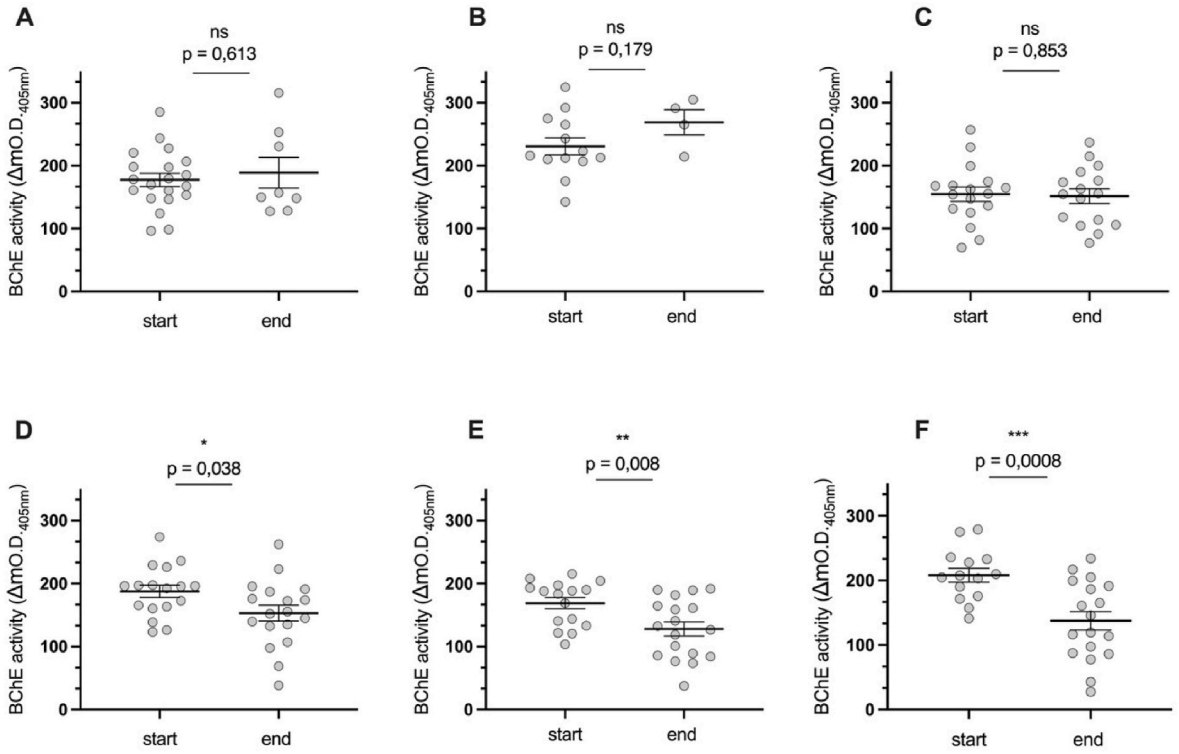

Fig. 3. Serum BChE activity in patients according to BMI. Comparison of BChE values at the beginning (left; median day = 1) and the end (right; median day = 0) of the hospitalization in the discharged patients of BMI <30 (A) and BMI ≥30 (B), patients transferred to different wards of BMI <30 (C) and BMI ≥30 (D) and non-survivors of the BMI <30 (E) and BMI ≥30 (F). Values are expressed as individual values with mean ± SEM within each group; $p < 0.05$.

knowledge, this is the first study to investigate the association of clinical status of patients with severe COVID-19 and BChE activity with respect to BMI, age, and other clinical markers that may impact serum BChE activity.

Our results complement and extend the work of other research groups that have proposed serum BChE as a predictor of severity and mortality of patients with COVID-19. Nakajima et al. first described alterations of BChE activity in 26 COVID-19 patients hospitalized in 2020 [41]. Patients with severe COVID-19 had significantly lower serum BChE activities at hospital admission compared to the mild-to-moderate group, together with lower activities in the non-survivors compared to the survivors group. These findings were confirmed in a larger cohort of 147 patients, thus highlighting the importance of BChE as a marker of COVID-19 severity [42]. Nevertheless, the prognostic role of BChE activity on admission was uncertain because the significant effect on mortality was only shown in the univariant but not multivariant analysis [42].

In line with this, a study of 96 Tunisian patients with COVID-19 hospitalized in 2020 showed no association between serum BChE activity measured during admission to the ICU and prognosis. Nevertheless, the authors saw an association with a fatal outcome for serum BChE lower than 5000 IU/L measured during the hospitalization [43]. A follow-up report from this research group in 137 patients with COVID-19 in the ICU confirmed their previous results but also showed lower serum BChE activity of non-survivors compared to survivors even at the day of admission [44]. Similarly, Espeter et al. showed on 54 critically-ill COVID-19 patients longer survival of patients with serum BChE activity greater than 1.557 U/I measured within the first 24 h after admission [45].

Our results from 148 patients with severe COVID-19 support the role of BChE as a prognostic marker. Nevertheless, this is not based on the serum activity obtained during admission. We observed no difference in serum BChE activity within the first 5 days after admission between discharged, transferred patients, and non-survivors. The discrepancy between our results and results of other groups could be based on different diagnostic protocols and the pandemic situation at the sites of admission. All patients included in our study were the most severe COVID-19 cases admitted to the hospital thus all of them were of high mortality risk, which is reflected in the comparable serum BChE activities at admission. Nevertheless, the change in their clinical status during hospitalization was captured in the dynamics of BChE activity, resulting in an increase or stabilization of serum BChE activity in surviving patients and decrease in serum BChE activity in deceased patients. The results of Bahloul et al. also showed a lower nadir value (lowest value recorded sometimes during hospitalization) of serum BChE activity in non-survivors compared to survivors [44]. Unfortunately, their work does not provide information about the exact time of analysis of the lowest BChE values in regard to the clinical status or the time relative to the final outcome. In this study, BChE activity was followed throughout the whole hospitalization, allowing us to make insights about dynamic changes in BChE activity during clinical progression. Our results, in line with the observation of the pilot study described above [41], showed that serum BChE activity is not a constant value and is progressively changing with the clinical status of each patient, with the highest percentage decrease in non-survivors. We observed the lowest serum BChE activity values in patients with a fatal course of the disease compared to patients transferred to another department or discharged to outpatient care at the end of the hospital stay. Furthermore, in non-surviving patients we observed a statistically significant decrease in serum BChE activity during hospitalization, along with the highest percentage decrease in activity. At the same time, in discharged patients, a trend of increased serum BChE activity was seen. Transferred patients requiring further medical care had significantly lower serum BChE activity compared to discharged patients, and the values were stable during hospitalization. Given these findings, we propose an important role of monitoring serum BChE activity not only at hospital admission, but also throughout the hospital stay due to its ability to assess the prognosis of the disease.

Serum BChE activity was also proposed to act as a 'negative' acute-phase protein in the inflammatory response [41]. A decrease in other relevant biomarkers, such as lymphocytes, eosinophils, and basophils, was observed in non-survivors and transferred patients, together with a positive correlation between serum BChE activity, albumin, and total proteins. On the contrary, a negative correlation was found between serum BChE activity and 'positive' acute phase proteins, namely C-reactive protein and IL-6. The results are consistent with previous COVID-19 studies [44,45] and studies focused on the role of serum BChE activity as a marker of clinical outcome in critically-ill patients [31,46,47]. Other groups have reported impairments of the cholinergic anti-inflammatory pathway in COVID-19 or possible therapeutic targets for the human α7-nicotinic acetylcholine receptor, suggesting the use of cholinesterase inhibitors or M1-muscarinic agonists to increase brain cholinergic activity and vagal efferent activity, consequently reducing the production of pro-inflammatory cytokines [48–51]. In contrast, in our work, non-survivors had the lowest serum BChE activity together with decreased lymphocytes, which are known to produce acetylcholine that plays an important role in immune response to viral infection [52]. Our results are thus in agreement with the previously proposed theory of Espeter et al. on a depleted cholinergic anti-inflammatory immune response in this group of patients [45].

Variability of serum BChE activity was previously reported in a relationship with age [25,53], sex [50], BMI [30], liver dysfunction [54], and other chronic underlying conditions [54–56]. Regarding age, we observed lower BChE activity in older patients (≥65 years) compared with younger patients (<65 years), while no statistically significant correlation was observed between serum BChE activity and age. However, in both age groups, a consistent decrease in serum BChE activity was observed in non-surviving patients. On the contrary, for BMI, we observed a higher activity of BChE in obese patients with BMI ≥30 compared to patients with BMI <30, with a decrease in serum BChE activity in fatal cases. A positive correlation of serum BChE activity and increased BMI was also observed in patients. The sex of the patient did not affect serum BChE activity. Although changes in serum BChE activity were observed in both age and BMI of patients, the role of serum BChE activity and its changes that reflect the clinical outcomes of patients were independent of them. Despite the fact that serum BChE activity was previously described as a marker of liver dysfunction [54–56], we did not observe any significant correlation between serum BChE activity and ALT, ALP, AST or GMT in patients with liver dysfunction included in our study, supporting previously reported results on critically-ill patients [46]. The role of serum BChE as a marker of multiple chronic conditions has been proposed in recent years. To assess the possible influence of current chronic comorbidities on serum BChE activity, the CCI was calculated for each patient. In our study, no correlation was observed between CCI and serum BChE activity, and CCI also did not reflect the clinical results of hospitalized patients. A multivariate analysis of serum BChE activity, age, BMI and CCI, however, identified a positive correlation between age and CCI, together with the previously mentioned correlation of serum BChE activity and BMI. These results and discrepancies in the role of CCI and its previously proposed role in the stratification of COVID-19 patients [57–59] could be explained by the fact that the majority of patients enrolled in the study were polymorbid and in severe condition and there might have been other factors that influenced clinical results.

Our research also has some limitations. Due to the strain of the COVID-19 pandemic and limited resources of healthcare workers at the study sites, it was not possible to collect blood samples from each enrolled patient with the same frequency or days of hospitalization, resulting in the categorization of values measured for each group within the first 5 days of hospitalization as a 'start value' and the last 5 days of hospitalization as an 'end value', while changes in serum BChE activity of each individual are also evaluated as a percentage change from the 'start value' as a baseline. Not all other blood parameters were analyzed with the same frequency and at the same time as serum BChE activity. Data on other international scales evaluating the severity of the clinical status of the patient were not available, such as the SOFA (Sequential Organ Failure Assessment) score, GCS (Glasgow Coma Scale) score, and the SAPS (Simplified Acute Physiology score) II. Espeter and colleagues show a positive correlation between the SOFA score and serum BChE activity, reflecting its potential to evaluate disease severity [45]. More research on serum BChE activity and its superior prognostic value, also in combination with other biomarkers and scores used routinely in clinical practice, would be beneficial. Interindividual variability of serum BChE activity may also explain discrepancies between research groups and should also be considered in future research.

5. Conclusion

Our results are consistent with preliminary data indicating changes in the cholinergic system in patients with COVID-19. Serum BChE activity reflects changes in the clinical status of hospitalized patients with severe COVID-19. In patients with improved health status, this trend is expressed as an increase in BChE activity. Stable lower BChE values can be observed in long-term ill COVID-19 patients, while in non-surviving patients we observed the most dramatic decrease in serum BChE activity together with the lowest measured values, and these findings are the most notable in patients with older age and lower BMI. Serum BChE activity is also positively correlated with serum albumin and total protein, and a negative correlation is observed in relation to inflammatory markers, indicating the involvement of the cholinergic system in the immune response.

Taken together, changes in serum BChE activity mirror the clinical course of the disease and thus continuous monitoring can be used as a relevant marker of the fatal course of severe COVID-19.

Funding statement

This work was supported by The Ministry of Education, Science, Research and Sport of the Slovak Republic [grant numbers VEGA 1/0815/21 to A.H, VEGA 1/0283/22 to D.D]; The Slovak Research and Development Agency [grant number SK-FR-19-0005 to A.H.]; The European Regional Development Fund [National infrastructure for technology transfer support in Slovakia II – NITT SK II to A.H.]; Comenius University Bratislava [UK/355/2022 to L.M.].

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank the medical and nursing team at the Clinic of Infectiology and the Clinic of Anesthesiology and Intensive Care for their involvement in the blood samples collection during the whole period of the study, AnalytX, Ltd. for their assistance with samples centrifugation and Pavilion of Clinical and Laboratory Disciplines, University of Trnava in Trnava for the provision of a laboratory for all necessary measurements during the research. We express our gratitude to Evan Paul for his proofreading of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbi.2023.110557.

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