

Original article

Exploratory associations between APOE genotype, biochemical markers, and postoperative cognitive outcomes after colorectal cancer surgery: preliminary results from Bosnia and Herzegovina

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Summary

Introduction. Postoperative cognitive dysfunction (POCD) is a common complication after major surgery. Genetic susceptibility, particularly the APOE $\epsilon 4$ allele, and systemic metabolic status may influence its development. This study investigated associations between biochemical markers of hepatic and renal function and postoperative cognitive outcomes according to APOE $\epsilon 4$ status.

Methods. This prospective multicenter study included 113 cognitively healthy adults undergoing elective colorectal cancer surgery under general anesthesia. Cognitive function was assessed using the Mini-Mental State Examination (MMSE) preoperatively, on postoperative day 7, and at three months. APOE genotyping was performed by PCR-RFLP. Urea, creatinine, AST, ALT, GGT, ALP, CK, LDH, and amylase were measured preoperatively and on postoperative day 7. POCD was defined as an MMSE score ≤ 23 . Associations were assessed using nonparametric tests and multivariate logistic regression adjusted for age, sex, education, and baseline MMSE.

Results. Genotyping was successful in 109 patients. Early POCD occurred in 105 patients (92.9%), and persistent POCD at three months in 45 (39.8%). Among genotyped patients, 94 (86.2%) were APOE $\epsilon 4$ carriers and 15 (13.8%) were non-carriers. In non-carriers, preoperative urea, creatinine, and amylase were independently associated with persistent POCD. In $\epsilon 4$ carriers, postoperative creatinine and GGT were independently associated with persistent POCD. Early POCD was associated with preoperative CK and LDH and postoperative ALT in non-carriers.

Conclusion. Biochemical correlates of POCD may differ according to APOE genotype. Baseline metabolic status may be more relevant in non-carriers, whereas postoperative disturbances may be more important in $\epsilon 4$ carriers. Larger studies using standardized POCD definitions are warranted.

Keywords: postoperative cognitive dysfunction, APOE $\epsilon 4$, colorectal surgery, renal function, gammaglutamyl transferase

Introduction

Postoperative cognitive dysfunction (POCD) is a frequent neurological complication after major surgery, associated with reduced quality of life and increased mortality [1, 2]. Its etiology is multifactorial, involving inflammatory responses, oxidative stress, and neurovascular alterations induced by surgical trauma [3]. Activation of the immune system during surgery leads to release of peripheral cytokines that compromise the blood-brain barrier, trigger microglial activation, and cause hippocampal dysfunction [4].

Systemic metabolic integrity, particularly renal and hepatic function, may modulate postoperative neurocognitive outcomes [2, 5]. Even subtle deviations in urea, creatinine, or gamma-glutamyl transferase (GGT) could indicate cognitive vulnerability [6]. Genetic predisposition, notably the APOE $\epsilon 4$ allele, is an additional determinant. APOE $\epsilon 4$ carriers have reduced cerebral blood flow, impaired amyloid- β clearance, and blood-brain barrier dysfunction, which may exacerbate surgery-induced cognitive decline [7, 8].

To date, no study from Bosnia and Herzegovina has examined the interaction between APOE $\epsilon 4$ and perioperative biochemical markers in relation to POCD after colorectal cancer surgery. This study aimed to investigate whether biochemical markers of hepatic and renal function were associated with postoperative cognitive outcomes and whether these associations differed between APOE $\epsilon 4$ carriers and non-carriers. We emphasize that POCD was defined using an absolute MMSE cut-off (≤ 23), not the recommended ISPOCD criteria, which resulted in a higher observed incidence and limited comparability with most published studies.

Methods

Study design and participants

A prospective, multicenter clinical study was conducted. A total of 113 cognitively healthy adult patients undergoing elective colorectal cancer surgery under general anesthesia between 2021 and 2023 were enrolled. Exclusion criteria: pre-existing neurological disorders (Alzheimer's, Parkinson's), chronic hepatic or renal insufficiency, or preoperative MMSE ≤ 23 .

Cognitive assessment

Cognitive function was evaluated using the Mini-Mental State Examination (MMSE) [9] preoperatively, on postoperative day 7, and after three months. POCD was defined as an absolute postoperative MMSE score ≤ 23 [9]. This definition differs from the recommended ISPOCD criteria (which require a reliable decline from baseline) and was adopted as a pragmatic, clinically oriented threshold. Consequently, the reported incidence of POCD in this study was higher than in studies using change-based definitions and should be interpreted accordingly.

Biochemical analyses

Biochemical parameters (urea, creatinine, AST, ALT, GGT, ALP, CK, LDH, amylase, CRP, lipid profile) were measured preoperatively and on postoperative day 7 using standard automated methods.

APOE genotyping

DNA was isolated from whole blood using a commercial kit (GeneJET Genomic DNA Purification Kit, Thermo Fisher Scientific). APOE genotyping was performed by PCR-RFLP with HhaI restriction enzyme [10]. Based on

$\epsilon 4$ allele presence, patients were categorized as carriers ($\epsilon 2/\epsilon 4$, $\epsilon 3/\epsilon 4$, $\epsilon 4/\epsilon 4$) or non-carriers ($\epsilon 2/\epsilon 2$, $\epsilon 2/\epsilon 3$, $\epsilon 3/\epsilon 3$).

Statistical analysis

Data were analyzed separately for $\epsilon 4$ carriers and non-carriers. Mann-Whitney U test was used for group comparisons. Variables with $p < 0.10$ in univariate analysis were entered into multivariate logistic regression adjusted for age, sex, education, and baseline MMSE. Significance was set at $p < 0.05$. Given the small number of non-carriers ($n=15$), results for this subgroup were statistically unstable and should be considered exploratory.

Results

Patient characteristics and genotyping

Of 113 patients, 51 (45.1%) were women; mean age was 66.6 ± 11.3 years; 74 (65.5%) had >12

years of education. APOE genotyping was successful in 109 patients. Among these, 94 (86.2%) carried at least one $\epsilon 4$ allele ($\epsilon 3/\epsilon 4$: 67, $\epsilon 2/\epsilon 4$: 27), and 15 (13.8%) were non-carriers ($\epsilon 3/\epsilon 3$: 14, $\epsilon 2/\epsilon 2$: 1). Four samples (3.5%) could not be analyzed. Early POCD (day 7) occurred in 105 patients (92.9%), and persistent POCD (three months) in 45 patients (39.8%).

Univariate associations

Table 1 summarizes the univariate associations (Mann-Whitney U test p -values) between biochemical parameters and POCD, separately for early (day 7) and persistent (three months) POCD, stratified by APOE $\epsilon 4$ carrier status.

Multivariate associations

Table 2 presents the independent predictors of early POCD (postoperative day 7) identified by multivariate logistic regression, adjusted for age, sex, education, and baseline MMSE.

Table 1. Biochemical correlates of POCD - univariate analysis (Mann-Whitney U p -values)

Parameter	Time	APOE $\epsilon 4$ carriers ($n=94$)	APOE $\epsilon 4$ non-carriers ($n=15$)
Early POCD (day 7)			
CK – preoperative	Pre	0.412	0.050
LDH – preoperative	Pre	0.594	0.032
ALT – postoperative	Day 7	0.877	0.050
Persistent POCD (3 months)			
Urea – preoperative	Pre	0.203	0.021
Creatinine – preoperative	Pre	0.694	0.032
Amylase – preoperative	Pre	0.281	0.049
Creatinine – postoperative	Day 7	0.046	0.165
GGT – postoperative	Day 7	0.023	0.202

Significant $p < 0.05$ in bold.

Table 2. Multivariate associations with early POCD (day 7)

Predictor	APOE ε4 status	OR	95% CI
Preoperative CK	Non-carriers	1.48	1.02–2.14
Preoperative LDH	Non-carriers	1.63	1.08–2.45
Postoperative ALT	Non-carriers	1.41	1.01–1.97
Postoperative ALT	Carriers not entered	–	

p-values: CK $p=0.038$; LDH $p=0.019$; ALT $p=0.044$. No independent associations with early POCD were identified in ε4 carriers.

Due to the very high proportion of patients with early POCD (92.9%), the model for early POCD has limited discriminative ability; the odds ratios reported above should be interpreted with particular caution.

Table 3 shows the independent predictors of persistent POCD (three months postoperatively) from the multivariate logistic regression model.

Table 3. Multivariate associations with persistent POCD (three months)

Predictor	APOE ε4 status	OR	95% CI	p
Preoperative urea	Non-carriers	1.89	1.17–3.05	0.009
Preoperative creatinine	Non-carriers	1.72	1.09–2.71	0.020
Preoperative amylase	Non-carriers	1.54	1.03–2.30	0.035
Postoperative creatinine (day 7)	Carriers	1.81	1.12–2.93	0.015
Postoperative GGT (day 7)	Carriers	2.14	1.28–3.57	0.004

Other indicators

CRP, complete blood count, and lipid profile showed no significant associations with POCD in any group.

Discussion

To our knowledge, this is the first study from Bosnia and Herzegovina examining the interaction between APOE ε4 and biochemical markers in relation to POCD after colorectal cancer surgery. Given the methodological limitations (non-standard POCD definition, small non-carrier subgroup, unusually high ε4 frequency), all findings should be considered hypothesis-generating and require independent replication.

Incidence of POCD. The early POCD rate (92.9%) was considerably higher than figures commonly reported in noncardiac surgery studies (e.g., 25–40% at one week) [11]. As noted throughout, this was primarily because we used an absolute MMSE cutoff (≤ 23) rather than the ISPOCD criteria (decline of ≥ 1 –2 standard deviations from baseline). Our definition identified a mixture of pre-existing impairment, perioperative decline, and measurement variability. The decrease to 39.8% after three months was consistent with the literature on resolution of early cognitive changes [11].

APOE ε4 distribution. The 86.2% carrier rate among successfully genotyped patients was unusually high – a frequency not observed in any European general population (where

the $\epsilon 4$ allele frequency typically ranged from 10% to 25%). This extreme distribution immediately raises concerns about sampling bias, potential genotyping error, or a highly selected patient population (e.g., an unrecognized association with colorectal cancer susceptibility). No population-based data from eastern Bosnia are available for comparison, but the observed carrier rate far exceeds expected values. Consequently, our findings cannot be generalized to any known reference population, and the results from the $\epsilon 4$ carrier subgroup should be viewed as exploratory only. A recent meta-analysis by Hsiao et al. [13] confirmed that the APOE $\epsilon 4$ allele was associated with short-term POCD, which was consistent with our stratified observations, but that study used standard POCD definitions and population-typical $\epsilon 4$ frequencies.

Biochemical associations in non-carriers. In this very small subgroup ($n=15$), preoperative urea, creatinine, and amylase were independently associated with persistent POCD. This may suggest that baseline renal and pancreatic metabolic integrity is important for cognitive resilience in patients without the $\epsilon 4$ allele. Elevated preoperative CK and LDH (markers of cellular stress) were associated with early POCD in non-carriers. However, given the extremely small sample size, these associations were statistically unstable, and the logistic regression models were at high risk of overfitting (especially with multiple predictors relative to the number of events). Therefore, these results should be considered no more than exploratory.

Biochemical associations in $\epsilon 4$ carriers. In carriers, only postoperative markers (creatinine and GGT) were associated with persistent POCD, while no preoperative markers reached significance. Postoperative GGT showed the highest odds ratio (2.14) in this sample. GGT is not only a cholestasis marker but also an indicator of oxidative stress [6]. One hypothesis is that APOE $\epsilon 4$ carriers may have a reduced capacity to neutralize surgically induced oxidative stress, making GGT a more sensitive

marker in this subgroup [7, 8]. However, this interpretation remains speculative until replicated in larger cohorts with appropriate event-to-variable ratios.

Comparison with literature. Our stratified analysis does not directly test the $\epsilon 4$ -POCD association but shows that biochemical associations differ by $\epsilon 4$ status. This complements meta-analyses that report a short-term effect of $\epsilon 4$ on POCD [12, 13].

Limitations. This study has several major limitations. First, the sample size is small, and the non-carrier subgroup ($n=15$) is critically small, rendering subgroup comparisons and logistic regression results unstable. Second, the number of events per variable in the logistic regression models is low (especially in the non-carrier group), which substantially increases the risk of overfitting; the reported odds ratios may be inflated or spurious. Third, the unusually high frequency of the APOE $\epsilon 4$ allele (86.2%) strongly suggests sampling bias or possible genotyping error; no population-based data from eastern Bosnia are available for direct comparison, but the observed frequency is incompatible with known European allele distributions. Fourth, the use of an absolute MMSE cutoff (≤ 23) rather than a reliable change index overestimates early POCD incidence and does not conform to current nomenclature recommendations [1]; moreover, as noted, the near-universal occurrence of early POCD makes predictive modeling for this outcome inherently limited. Fifth, we have not collected data on anesthesia duration, intraoperative hypotension, postoperative opioid use, or infectious complications – all known to influence POCD. Sixth, four samples (3.5%) could not be genotyped. Seventh, we have not assessed which specific cognitive domains (memory, attention, processing speed) were most affected. Finally, the findings may not be generalized to laparoscopic surgery, to other populations, or even to the general surgical population of Bosnia and Herzegovina given the extreme $\epsilon 4$ frequency.

Conclusion

In this preliminary, exploratory study, preoperative urea, creatinine, and amylase were associated with persistent POCD in APOE ϵ 4 non-carriers, whereas postoperative creatinine and GGT were associated with persistent POCD in ϵ 4 carriers. Postoperative GGT showed the

highest odds ratio among carriers in this sample (OR 2.14). These findings suggest that distinct metabolic pathways may contribute to postoperative cognitive outcomes depending on APOE genotype. Larger multicenter studies using standardized POCD definitions are needed to determine whether perioperative biochemical monitoring has clinical predictive value.

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Ethical approval. The Ethics Committee of the University of East Sarajevo, Faculty of Medicine Foča, Republic of Srpska, Bosnia and Herzegovina, approved the study

and informed consent was obtained from all individual respondents. The research was conducted according to the Declaration of Helsinki.

Conflicts of interest. The authors declare no conflict of interest.

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Istraživačke povezanosti između APOE genotipa, biohemijskih markera i postoperativnih kognitivnih ishoda nakon operacije kolorektalnog karcinoma: preliminarni rezultati iz Bosne i Hercegovine

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Uvod. Postoperativna kognitivna disfunkcija (POCD) česta je komplikacija nakon velikih hirurških zahvata. Genetska predispozicija, posebno alel APOE ε4, i sistemski metabolički status mogu uticati na njen razvoj. Cilj istraživanja bio je ispitati povezanost biohemijskih pokazatelja funkcije jetre i bubrega sa postoperativnim kognitivnim ishodima u zavisnosti od statusa APOE ε4.

Metode. Prospektivnim multicentričnim istraživanjem obuhvaćeno je 113 kognitivno zdravih odraslih osoba podvrgnutih elektivnom hirurškom liječenju kolorektalnog karcinoma u opštoj anesteziji. Kognitivne funkcije procjenjivane su Mini-Mental State Examination (MMSE) testom preoperativno, sedmog postoperativnog dana i nakon tri mjeseca. Genotipizacija APOE izvršena je metodom PCR-RFLP. Urea, kreatinin, AST, ALT, GGT, ALP, CK, LDH i amilaza određivani su preoperativno i sedmog postoperativnog dana. POCD je definisana vrijednošću MMSE ≤23. Povezanosti su analizirane neparametrijskim testovima i multivarijantnom logističkom regresijom prilagođenom za dob, pol, obrazovanje i početnu vrijednost MMSE.

Rezultati. Genotipizacija je uspješno urađena kod 109 ispitanika. Rana POCD utvrđena je kod 105 (92,9%), a perzistentna POCD nakon tri mjeseca kod 45 (39,8%) ispitanika. Među genotipizovanim ispitanicima, 94 (86,2%) su bili nosioci APOE ε4, dok 15 (13,8%) nisu bili nosioci. Kod osoba koje nisu bile nosioci ε4, preoperativne vrijednosti uree, kreatinina i amilaze bile su nezavisno povezane sa perzistentnom POCD. Kod nosilaca ε4, postoperativne vrijednosti kreatinina i GGT bile su nezavisno povezane sa perzistentnom POCD. Rana POCD bila je povezana sa preoperativnim vrijednostima CK i LDH, te postoperativnim vrijednostima ALT kod osoba koje nisu bile nosioci ε4.

Zaključak. Biohemijski korelati POCD mogu se razlikovati u zavisnosti od APOE genotipa. Metabolički status prije operacije može biti značajniji kod osoba koje nisu nosioci ε4, dok postoperativni poremećaji mogu imati veći značaj kod nosilaca ε4. Potrebna su veća istraživanja uz primjenu standardizovanih definicija POCD.

Ključne riječi: postoperativna kognitivna disfunkcija, APOE ε4, kolorektalna hirurgija, bubrežna funkcija, gama-glutamil transferaza