Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
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Three-vessel coronary artery disease may predict changes in biochemical brain injury markers after off-pump coronary artery bypass grafting[#]

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https://doi.org/10.1631/jzus.B1700553

Neurological injury is a frequent and important complication of coronary artery bypass grafting (CABG). Several risk factors for this type of sequela have been identified, among them aortic arch atherosclerosis. Our previous study indicated that atherosclerotic burden in coronary arteries may likewise predict postoperative neurological complications (Pawliszak et al., 2016b). We assessed the severity of this condition by using the SYNTAX score calculator. However, diagnosing angiographic three-vessel coronary artery disease (3VD) could be an even simpler method of achieving this goal.

Postoperative neurological injury is a diagnostic challenge. Comprehensive evaluation of its various clinical manifestations requires time, specialized equipment, and qualified personnel, and therefore is never performed outside of a research setting. Consequently, clinicians could greatly benefit from devising an indicator of brain damage that is sensitive, specific only to brain tissue, and easily measurable in patients' blood shortly after surgery. Such requirements may be met by the three following state-of-the-art biochemical markers: glial fibrillary acidic protein (GFAP), neuroserpin (NSP), and phosphorylated axonal neurofilament subunit H (PNFH). They were identified on the basis of recently published pilot studies and have never before been used in cardiac surgery (Rodríguez-González et al., 2011; Singh et al., 2011; Luger et al., 2017). The aim of this study was to assess the correlation between 3VD and changes in levels of these three novel biochemical brain injury markers following off-pump CABG.

This prospective observational cohort study was approved by the Bioethics Committee of the Collegium Medicum in Bydgoszcz, Poland, completed according to the standards established in the Helsinki Declaration, and based at Antoni Jurasz University Hospital No. 1 in Bydgoszcz, Poland. Patients aged 60–70 years scheduled for elective CABG were recruited. Patients with a known history of neurologic or psychiatric illness, previous cardiac surgery or angioplasty, left ventricle ejection fraction of ≤30%, and/or carotid artery stenosis of ≥70% were excluded. Participants were divided into two groups based on

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[#] Electronic supplementary materials: The online version of this article (https://doi.org/10.1631/jzus.B1700553) contains supplementary materials, which are available to authorized users

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the presence or absence of 3VD in preoperative coronary angiography.

All surgical interventions were performed by the same operator and under the same anesthetic protocol. Each procedure was carried out through a median sternotomy using an Octopus coronary stabilizer (Medtronic Inc., MN, USA) for distal anastomosis and a side-biting clamp on the aorta for proximal anastomosis. Choice of graft vessels was left to the surgeon's discretion. Blood samples were collected just before the first incision of the skin and just after stitching of the skin. They were drawn from the ulnar vein into 5-ml tubes containing a clot activator and gel. Serum was obtained by blood centrifugation (2500*g* for 20 min at room temperature), divided into aliquots, and stored at −80 °C until analyzed. Levels of GFAP, NSP, and PNFH were determined using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (BioVendor-Laboratorni medicina a.s., Brno, Czech Republic). The inter- and intra-assay coefficients of variation for all tests were ≤11%.

All statistical analyses were performed using Statistica® 13.1 (Dell Software Inc., Round Rock, TX, USA). Data were assessed for normality using the Shapiro-Wilk test, and homogeneity of variance was checked using the Brown-Forsythe test. Since our data did not meet the assumption of parametric statistics, the Mann-Whitney *U* test was employed for independent samples and the Wilcoxon test for paired samples. For categorical data analysis, Fisher's exact test was used. All correlations were analyzed with Spearman's nonparametric correlation coefficient. A *P* value of less than 0.05 was considered statistically significant.

After eligibility assessment, 35 consecutive patients were included in the study. A 3VD group consisting of 26 patients was compared with a non-3VD group of 9 patients. Participants' pre-, intra-, and postoperative characteristics (Table 1) did not differ between the groups. Postoperatively, patients from the 3VD group presented statistically significant changes in the levels of GFAP, NSP, and PNFH, while patients from the non-3VD group presented a statistically significant change only in the level of NSP (Fig. 1). We did not observe any significant differences in the preoperative levels of GFAP, NSP, or PNFH between the sex and comorbidity groups (Figs. S1–S3). Correlations between other clinical parameters and the preoperative levels of the biochemical brain injury markers are presented in Table S1.

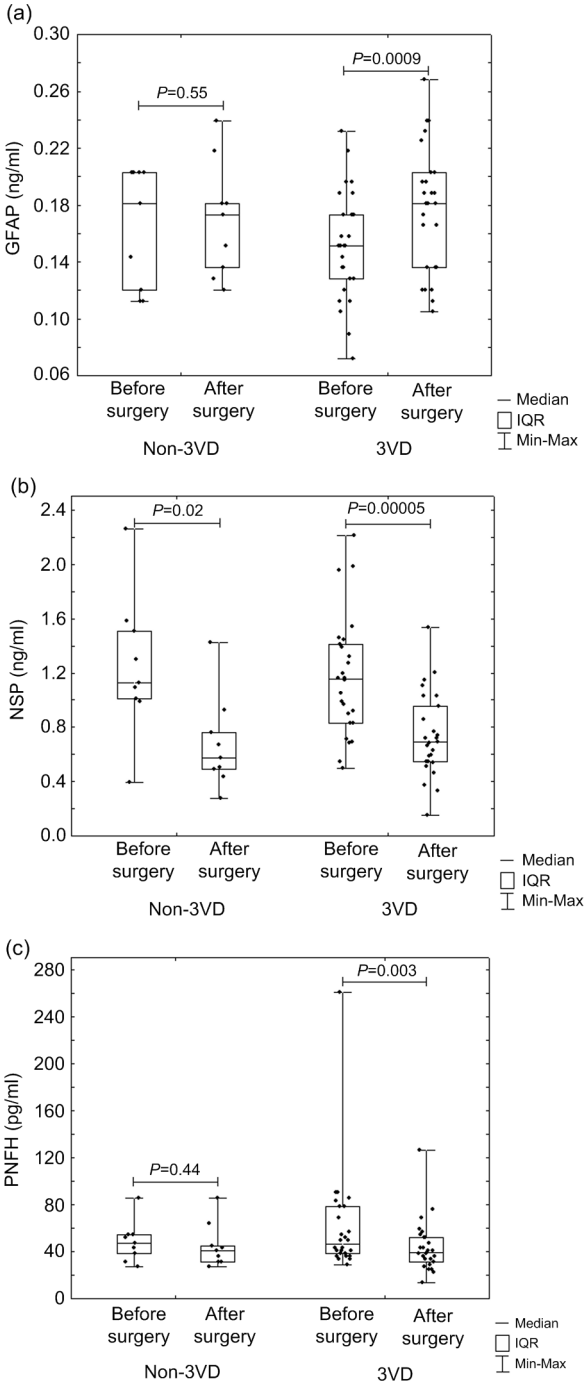

Fig. 1 Comparisons of GFAP (a), NSP (b), and PNFH (c) levels between off-pump CABG patients stratified according to the presence or absence of 3VD

GFAP: glial fibrillary acidic protein; NSP: neuroserpin; PNFH: phosphorylated axonal neurofilament subunit H; IQR: interquartile range

Table 1 Pre-, intra-, and postoperative parameters

Parameter	Type of disease		
	Non-3VD (*n*=9)	3VD (*n*=26)	*P*
Age (year)	68 (64–69)	65 (61–68)	0.36
Male gender	9 (100%)	19 (73%)	0.15
Previous MI	5 (56%)	13 (50%)	0.99
Hypertension	8 (89%)	19 (73%)	0.65
Diabetes mellitus	5 (56%)	10 (38%)	0.45
Dyslipidemia	4 (44%)	9 (35%)	0.70
BMI (kg/m^2)	29 (28–32)	29 (27–31)	0.57
LVEF (%)	67 (58–67)	66 (49–72)	0.84
EuroSCORE	1.02 (0.92–2.44)	1.59 (1.11–2.20)	0.40
Pre-operative sCr (mg/dl)	0.77 (0.73–0.84)	0.85 (0.78–0.98)	0.21
Duration of surgery (min)	200 (180–240)	210 (198–240)	0.34
Number of bypass grafts	2 (2–2)	3 (2–3)	0.12
Intubation time (h)	12 (10–12)	11 (9–15)	0.94
RR-S min (mmHg)	90 (80–90)	80 (70–90)	0.20
RR-D min (mmHg)	40 (40–50)	40 (30–50)	0.99
HGB (g/L)	108 (107–120)	103 (94–113)	0.25
HCT min (%)	33 (33–37)	33 (29–35)	0.36
Days in intensive care unit (d)	2 (1–2)	2 (1–2)	0.79
GFAP (ng/ml)			
Before surgery	0.18 (0.12–0.20)	0.15 (0.13–0.17)	0.39
After surgery	0.17 (0.14–0.18)	0.18 (0.14–0.20)	0.62
NSP (ng/ml)			
Before surgery	1.13 (1.01–1.51)	1.16 (0.83–1.41)	0.55
After surgery	0.57 (0.49–0.76)	0.69 (0.55–0.95)	0.38
PNFH (pg/ml)			
Before surgery	47.42 (38.20–54.39)	46.26 (38.20–78.00)	0.64
After surgery	40.49 (31.37–45.10)	39.35 (31.37–52.06)	0.85

The data are presented as number of cases (percentage) or median (interquartile range). MI: myocardial infarction; BMI: body mass index; LVEF: left ventricular ejection fraction; sCr: serum creatinine; RR-S min: minimal level of systolic blood pressure during surgery; RR-D min: minimal level of diastolic blood pressure during surgery; HGB: hemoglobin; HCT: hematocrit; GFAP: glial fibrillary acidic protein; NSP: neuroserpin; PNFH: phosphorylated axonal neurofilament subunit H

The main finding of this pilot study was that there was a greater change in postoperative levels of the biochemical brain injury markers after off-pump CABG in patients with 3VD than in patients without 3VD. Presumably, this is because a greater atherosclerotic burden in coronary arteries predicts stenosis in other vascular zones that promote postoperative brain damage, especially the ascending aorta. Its surgical manipulation was shown to markedly increase the risk of various neurological complications (Szwed et al., 2014, 2017; Pawliszak et al., 2016a). All biochemical brain injury markers introduced for the first time in the current study showed a great value in this clinical setting by providing tangible results in small groups of patients. For this reason, they deserve a thorough evaluation in further research. The fact that only levels of NSP changed significantly in patients both with and without 3VD may seem unexpected, especially considering that NSP and PNFH are derived from the same type of brain cells (neurons). However, unlike PNFH, NSP is not phosphorylated which makes it susceptible to proteolytic breakdown in blood, which may lower its diagnostic potential. High preoperative levels of GFAP were also unexpected. Nevertheless, biological variability in GFAP levels is very high even among healthy individuals, and depends on both the kind of material tested and the test methodology (Table S2).

The strengths of this study include its innovative nature and cutting-edge biochemical methodology, as well as its prospective design and consistent cohort of patients operated on by a single surgeon. At the same time, we acknowledge that due to its preliminary nature, the number of participants was small and blood samples were collected at only two time points. Summing up, patients with 3VD undergoing elective

off-pump CABG present a greater change in postoperative levels of biochemical brain injury markers than patients without 3VD. This observation may help clinicians to optimize risk evaluation and the clinical management of patients undergoing off-pump CABG. Furthermore, the pioneering biochemical data yielded by this study provide both encouragement and a reference point for future research using GFAP, NSP, and PNFH.

Compliance with ethics guidelines

Wojciech PAWLISZAK, Krzysztof SZWED, Artur SŁOMKA, Natalia PIEKUŚ-SŁOMKA, Magdalena SZWED, Mariusz KOWALEWSKI, Ewa ŻEKANOWSKA, and Alina BORKOWSKA declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

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List of electronic supplementary materials

Fig. S1 Serum levels of GFAP before surgery in patients stratified by sex and comorbidities

Fig. S2 Serum levels of NSP before surgery in patients stratified by sex and comorbidities

Fig. S3 Serum levels of PNFH before surgery in patients stratified by sex and comorbidities

Table S1 Correlations of preoperative GFAP, NSP, and PNFH serum levels with clinical parameters in whole cohort and in the non-3VD and 3VD groups

Table S2 Comparison of median or mean levels of GFAP among healthy controls

中文概要

题　目：三支冠脉病变可预测非体外循环下冠状动脉旁路移植手术后颅脑损伤生化标志物的变化

目　的：研究冠状动脉旁路移植手术（CABG）后患者的胶质纤维酸性蛋白（GFAP）、神经源性丝氨酸蛋白酶抑制素（NSP）及磷酸化轴突神经丝亚基H（PNFH）水平与三支冠脉病变（3VD）的相关性。

方　法：招募计划进行 CABG 的 60~70 岁病人作为研究对象，根据术前冠状动脉造影显示是否患有 3VD 分为两组。排除患有精神疾病、做过心脏手术或心血管成形手术以及左室射血分数≤30%和（或）颈动脉狭窄率≥70%的病人。

结　论：在本研究中，术后 3VD 组患者的 GFAP、NSP 和 PNFH 水平存在统计学上的显著变化，而非 3VD 组患者仅在 NSP 水平上出现统计学上的显著变化。同时发现，3VD 患者在非体外循环 CABG 后颅脑损伤生化标志物的水平变化比没有 3VD 的患者更明显。

关键词：三支冠脉病变；磷酸化轴突神经丝亚基H；胶质纤维酸性蛋白；神经源性丝氨酸蛋白酶抑制素；血管造影诊断