Examination of surgical stress during carotid endarterectomy in regional anaesthesia

PhD thesis

Peter Szabó MD

Supervisors: Tihamer Molnár, MD, PhD

Sandor Márton, MD, PhD

Program Leader: Prof. Andras Vereczkei, MD, PhD

Doctoral School Leader: Prof. Lajos Bogár, MD, PhD

Department of Anaesthesiology and Intensive Therapy, University of Pécs, Medical School

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I. INTRODUCTION

Carotid endarterectomy (CEA) is a challenging surgical and anaesthesiological procedure. These operations can be performed either in general or regional anaesthesia. The increased peri-operative stress is often pronounced as weakness of CEA performed in regional anaesthesia. Surgical stress induces release of adrenocorticotropic hormone (ACTH) resulting in elevation of cortisol level in the systemic circulation. This stress response is commonly associated with increased myocardial oxygen consumption, glycemic disturbances, infectious complications due to immunosuppressive effects and impaired wound healing, etc.

The multicentre GALA-study, including *3.526* patients with symptomatic or asymptomatic carotid stenosis randomly assigned to surgery under general or regional anaesthesia, has not shown a definite difference in outcomes such as stroke, myocardial infarct or death within 30 post-operative days. However, local anaesthesia positively influenced early post-operative neurocognitive outcomes. Eventually, the anaesthesiologist and surgeon, in consultation with the patient, should decide which anaesthetic technique is preferable on an individual basis.

Ideally, the combination of adequate stress relief and awake surgery allowing an appropriate neuromonitoring under local anaesthesia would provide the most advantages for CEA. Titrating mild sedation, the contact with the patient can be kept throughout the surgery, while anxiety and threats are effectively suppressed. Several medications are used for this purpose such as benzodiazepines, opiates, propofol, α-2 agonists as dexmedetomidine and clonidine. Recently, numerous papers were published regarding anaesthesia-induced neuroprotection of such medications. Importantly, propofol reduces cerebral metabolic rate and has antioxidant and anti-inflammatory properties exerting neuroprotective effects in various tissue cultures or animal models.

In our centre, regional cervical block is the preferred technique for CEA preventing peri-operative neurological complications. However, procedural and emotional stress may adversely influence patient's satisfaction and comfort. Theoretically, the increased cerebral oxygen consumption and hypertensive periods might be associated with higher incidence of complications and worse outcome. We hypothesized that an appropriate awake sedation is able to prevent such complications.

The diameter of vessels is regulated by the L-arginine nitric oxide (NO) pathway. NO is produced by the conversion of Larginine into L-citrulline by nitric oxide synthase (NOS).1 The synthesis of NO can be blocked with guanidino-substituted analogs of L-arginine, such as asymmetric dimethylarginine (ADMA), by inhibition of the NOS active site. ADMA and symmetric dimethylarginine (SDMA) are protein breakdown products of L-arginine. SDMA competes with arginine uptake and antagonizes the effects of L-arginine. Recently, arginine-rich peptides were found

to be neuroprotective in animal models. In contrast, circulating ADMA levels have been assessed in a variety of systemic cardiovascular diseases, and are increased in conditions associated with hypoxia, ischaemic stroke, and carotid stenosis. An increased serum level of ADMA is associated with endothelial dysfunction and more pronounced atherosclerosis. In addition, ADMA promotes subclinical atherosclerosis in a site-specific manner, with a greater proatherogenic influence at known vulnerable sites in the arterial tree. Carotid restenosis after endarterectomy is also associated with elevated concentrations of ADMA in serum.

S100β in the peripheral blood is a sensitive marker of both blood brain barrier (BBB) dysfunction and ischaemic brain damage, even during clinically uneventful carotid endarterectomy (CEA). During carotid cross-clamp, the jugulo-arterial (j-a) blood gases and acid base variables mirror the degree of cerebral hypoperfusion. In particular, the P(j-a)CO2 (CO2 gap), and the ratio of P(j-a)CO2/C(a-j)O2 may be useful markers of critical brain hypoperfusion.

II. OBJECTIVES

We have performed three investigations for answering our questions.

II.1. We aimed to explore the stress response of patients randomized according to different sedation protocols: (i) per os premedication only with alprazolam (BDZ group) or (ii) premedication with alprazolam combined with target control infusion (TCI) of propofol (BDZ+TCI group) for awake CEA.

II.2. A. We tested the hypothesis that L-arginine pathway metabolites correlate with cerebral metabolic responses and blood brain barrier integrity/ischaemic brain damage during awake CEA.

B. A second aim was to explore markers predicting the need for an intra-operative shunt.

II.3. Finally we tested the perioperative periods demographic, clinical and laboratory parameters effect on long term outcome, especially on the restenosis of the operated artery, progression of contralateral stenosis and mortality.

III. METHODS

This prospective, randomized clinical study was approved by the Clinical Centers Regional and Institutional Research Ethics Committee, Chair: Professor G Kosztolányi, on 23 March 2013, *N°4820*. Informed consent was obtained from each patient.

III.1. Study population

Fifty-five consecutive patients with significant carotid stenosis (symptomatic, n: 32) were recruited into this prospective study at the Department of Vascular Surgery, University of Pecs, Hungary. Exclusion criteria were end stage renal disease and refusing to participate in the study. The diagnosis of significant carotid stenosis was based on both carotid duplex ultrasound and computed tomography angiography. Patients scheduled for CEA were enrolled following carotid duplex scan and neurological examination at the outpatient clinic of the Department of Neurology, University of Pecs. Patients with a focal deficit such as transient ischaemic attack or minor stroke pertaining to the index carotid stenosis were defined as symptomatic. Duplex scanning was performed bilaterally with a Philips HD7XE with a 5-10 MHz linear probe. Duplex ultrasound examination included gray scale and color and was performed in the transverse and sagittal planes. Duplex scan measurements of internal carotid artery (ICA) peak systolic velocity (PSV), ICA end-diastolic velocity, and the ratio of ICA PSV to common carotid artery (CCA) PSV were analyzed to identify a 70-99% ICA stenosis. The Carotid Consensus Panel criteria were used to diagnose significant carotid artery stenosis (PSV > 230 cm/second, end-diastolic velocity > 100 cm/second, ICA/CCA ratio > 4). In agreement with international guidelines, significant carotid atherosclerosis was defined by 70-99% stenosis of the ICA determined by duplex scan.

Medical history, including hypertension, dyslipidemia, diabetes mellitus, stroke or transient ischaemic attack, current cigarette smoking, and oral medication, was obtained from all patients.

III.2. Protocol of premedication and sedation

All patients took 0.5 mg alprazolam *per os* 30 minutes prior to surgery. In target-controlled infusion (TCI) group, propofol was started right before regional anaesthesia according to Schnider-protocol (Injectomat TIVA Agilia, Fresenius Kabi, Germany). Starting effect site concentration (0.5 ng ml^{-1})

was adjusted until Ramsay 2-3 level of sedation was achieved. No additional sedatives were given to patients in the control group.

III.3. Regional anaesthesia, shunt insertion

All patients underwent an awake CEA under regional anaesthesia. Deep and superficial cervical plexus blocks were performed in each patient. Additional local anaesthetic was given by the vascular surgeon if it was necessary. Verbal contact was maintained and contralateral hand motor function was observed as neuro monitoring. A decision about shunt insertion was made if verbal or motor functions declined after carotid clamping.

III.4. Blood sampling and laboratory measurements

An arterial line was inserted by the anaesthetist in the operating theatre. The internal jugular vein was cannulated by the vascular surgeon, and the tip of the catheter was positioned in the jugular bulb. Jugular and arterial blood samples, for blood gas analysis and lactate measurement by Radiometer Abl 800 flex, were taken in parallel four times intra-operatively, before and after carotid clamp, then before and after release of clamp.

Plasma samples were taken right at the beginning of the operation (T_1), before release of carotid clamp (T_2), after release of clamp (T_3), 2 hours post-operatively (T_4) and 1 day after surgery (T_5).

The samples were frozen within 60 min and stored at -80 C until analysis.

Serum levels of S100β were examined by automated electrochemiluminescent immunoassay (Liaison Sangtec 100 system; DiaSorin, Bromma, Sweden).

The amino acid content of the blood serum samples was retrieved by the solid-phase extraction (SPE) method and was quantified by high performance liquid chromatography after derivatization. SPE of the analytes was performed as previously described. Arginine and homoarginine were detected at ʎ=337 nm, ʎ = 520 nm, and ʎ = 454 nm was used for ADMA and SDMA.

Analysis of plasma cortisol levels were performed at the Department of Laboratory Medicine (Medical School, University of Pécs, Hungary) by a commercially available solid phase, competitive chemiluminescent immunoassay (Immulite 2000 Siemens, Healthcare Diagnostics, Germany). All measurements were performed in duplicate.

A validated method was developed in the Department of Forensic Medicine, University of Pecs, for measuring plasma alprazolam concentration by supercritical fluid chromatography-tandem mass spectrometry (SFC-MS/MS) at the beginning and the end of surgery (T_1 and T_3 respectively). Separation were performed by SFC (Waters UPC^2) using an Acquity UPC^2 Torus Diol (2.1 mm x 100mm, 1,7 mm) column and analyzed on a Xevo TQ-S triple quadrupole MS with electron spray ionization. For alprazolam five MRM transitions were collected in order to confirm identity.

Although 55 patients were recruited into this study, L-arginine pathway metabolites were measured in only 49. The data of six patients were not available because of a failed blood sampling procedure.

III.5. 5 years follow-up

The long-term outcome of enrolled patients was analysed based on telephone interview and electronic records of the Medical Information System used at the University of Pecs, Medical School. Vascular surgery events, such as more than 50% restenosis of the operated carotid artery, new onset of significant contralateral carotid stenosis, lower limb amputations and mortality within 5 years were evaluated.

III.6. Statistical analysis

Data were evaluated using SPSS (version 20.0; IBM, Armonk, NY, USA). Categorical data were summarized by means of absolute and relative frequencies (counts and percentages). Quantitative data are presented as mean and 95% confidence interval (CI), as well as mean ± SD. The Kolmogorov-Smirnov test was applied to check for normality. The chi-square test for categorical data and Student t-test for continuous data were used for analysis of demographic and clinical factors. The non-parametric Mann-Whitney test was used for S100β, lactate, L-arginine, ADMA, and SDMA analyses. Non-normally distributed data are presented as median and interquartile range. Binary logistic regression analysis was applied to confirm independent predictors. The cutoff value of L-arginine (n=49) to predict the need for shunt was calculated by receiver operator curve (ROC) analysis. Correlation analysis was performed calculating Spearman's correlation coefficient (r). A p-value < .05 was considered statistically significant.

IV. RESULTS

IV.1. Demographic and clinical characteristics

Demographic and clinical parameters of the total study population and TCI vs control groups are shown in **Table I.**

	TCI n=24	**control n=31**	**total population n=55**
shunt	**2**	**4**	**6**
age	**63,29±8,26**	**67,74±7,5**	**65,80±8,08**
male	**17**	**27**	**44**
female	**7**	**4**	**11**
BMI	**27,47±5,72**	**27,23±3,46**	**27,33±4,53**
right sided operation	**11**	**15**	**26**
left sided operation	**13**	**16**	**29**
operated stenosis (%)	**85,42±5,88**	**84,03±6,64**	**84,64±6,22**
contralateral stenosis (%)	**44,09±21,64**	**45,96±28,78**	**45,1±25,51**
clamp time	**22,13±6,76**	**22,58±6,61**	**22,38±6,61**

IV.2. Plasma cortisol concentration in TCI vs control groups

The temporal profile of plasma cortisol concentrations in the two sedation groups are shown in **Figure 1**. Plasma cortisol reached its peak in the plasma at T_3 and then gradually decreased at T_4 and T_5. There was no significant difference between the two groups (TCI vs control) in baseline cortisol level

(T_1). In contrast, significantly higher plasma cortisol level was observed at T_2, T_3 and T_4 in the BDZ group (at T_2 median: *24.5*, IQR: *23.0 – 28.05* vs. median: *14.2*, IQR: *8.64 – 17.7*; at T_3 median: *26.0*, IQR: *24.3 – 31.85* vs. median: *15.1*, IQR: *8.29 – 18.1*; at T_4 median: *22.7*, IQR: *15.6 – 27.7* vs. median: *8.88*, IQR: *5.81 – 14.7*) Moreover, the plasma concentration of cortisol was even lower at T_4 compared to the baseline level (T_1) in the TCI group, while its increased level persisted in the control group. This difference postoperatively disappeared by 24 hours (T_5).

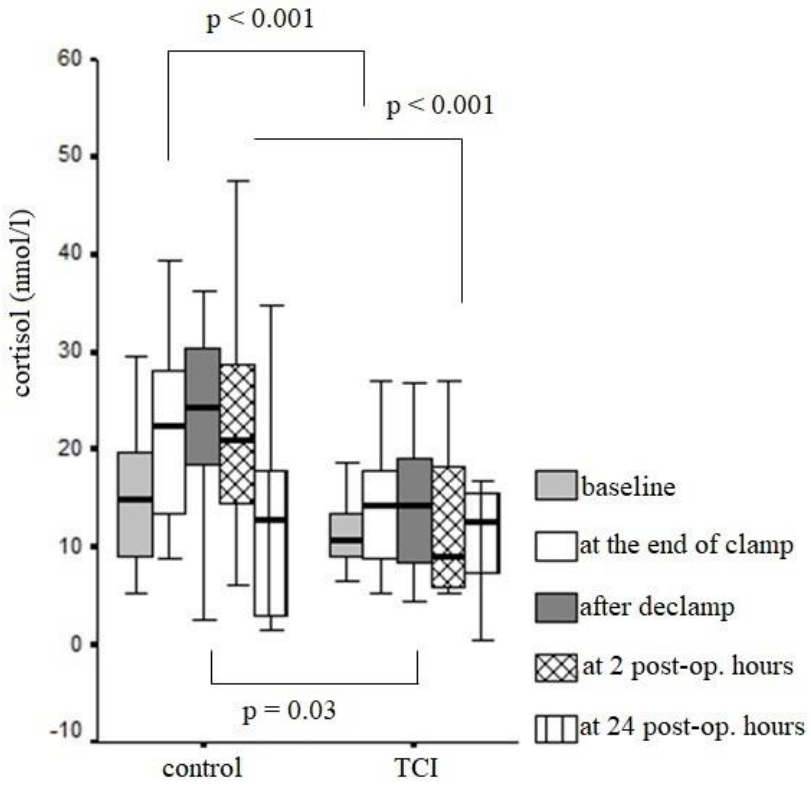

Figure 1. Changes of plasma cortisol concentration in the sedation groups

IV.3. Plasma alprazolam concentration and cortisol

Alprazolam plasma concentrations were measured at the beginning (T_1) and at the end (T_3) of the procedure. Neither T_1, nor T_3 alprazolam level showed any significant correlation with plasma cortisol concentration measured at any time point.

IV.4. Clamp time, shunt

The clamp time showed no significant difference between the asymptomatic and symptomatic subgroups (23±6 min vs. 22±6 min).

Acute neurological signs occurred in six patients just after carotid cross-clamp, which disappeared after successful shunting. Shunt insertion was necessary in *2/24* cases in the TCI group and *4/31* cases in BDZ group. The mean±SD shunt time was 20 ± 8 min in the shunted subgroup.

IV.5. Pre-operative correlation

The serum concentration of pre-operative arterial L-arginine was significantly lower in patients requiring an intraoperative shunt (n = 6) compared with patients not requiring one (n=43) (median 30.3 mmol/L, interquartile range [IQR] 24.4-34.4 mmol/L vs. median 57.6 mmol/L [IQR 42.3-74.5 mmol/L]; p = 0.002).

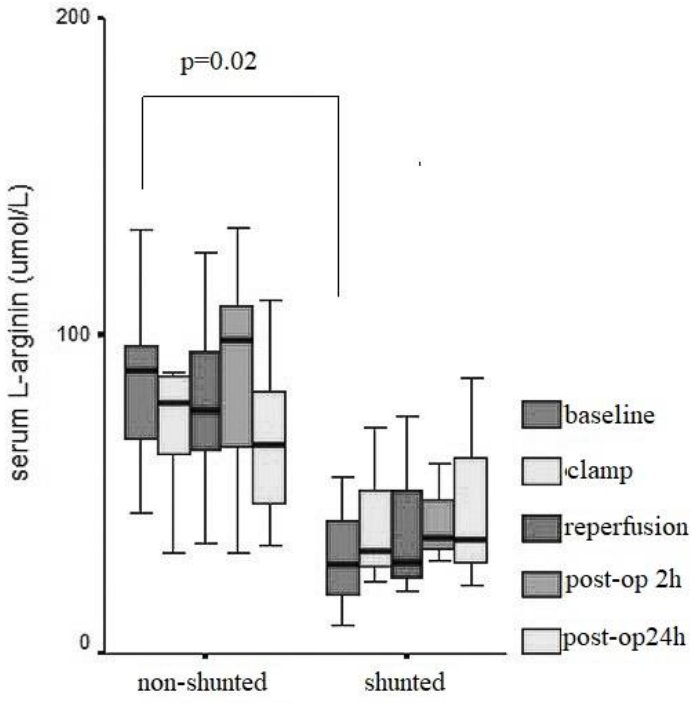

Figure 2. L-arginine plasma concentrations in shunted and non-shunted patients

Other parameters were not different between the two groups. The preoperative arterial concentration of serum L-arginine also showed an inverse correlation with pre-operative arterial lactate levels (r=-0.447, p=0.002). There was no correlation between arterial lactate concentration and ADMA or SDMA levels.

IV.6. Temporal profile of markers

Next the correlation between pre-operative arterial L-arginine pathway metabolites with the anaerobic cerebral metabolism indicator P(j-a)CO2/C(a-j)O2 ratio and jugular lactate measured during the most critical stages (after carotid clamp, and before and after release of clamp) of awake carotid endarterectomy was explored. An inverse correlation was found between the pre-operative arterial concentration of L-arginine and jugular lactate, which persisted both during cross-clamp and reperfusion (r =-0.757 [p =0.002] and r = -0.678 [p = 0.008], respectively). In contrast, the pre-operative arterial concentration of ADMA was positively correlated with the anaerobic cerebral metabolism indicator P(j-a)CO2/C(a-j)O2 both during clamp and reperfusion (r = 0.701 [p = 0.005] and r = 0.644 [p = 0.01], respectively) (Table 3). The pre-operative arterial concentration of SDMA was not correlated with P(j-a)CO2/C(a-j)O2. Neither ADMA nor SDMA levels correlated with jugular lactate concentrations at any time points.

Whether the pre-operative arterial L-arginine pathway metabolites correlate with jugular S100β, a marker of both BBB disruption and ischaemic brain damage during clamp and reperfusion, was also examined. The pre-operative arterial concentration of L-arginine, but not ADMA and SDMA, showed an inverse correlation with jugular S100B measured during reperfusion (r =0.702, p = 0.005).

Finally the correlation between P(j-a)CO2/C(a-j)O2 ratio and both jugular (intra-operative) and arterial (pre- and postoperative) S100B concentrations was examined. The ratio of P(j-a)CO2/C(a-j)O2 during reperfusion showed a significant positive correlation with arterial concentration of S100β measured at 2 hours (0.365; p = 0.016) but not 24 hours after surgery.

IV.7. Predictive role of pre-operative arterial L-arginine

Observing inverse correlations between pre-operative L-arginine and jugular lactate concentrations, and presuming that NO donor L-arginine might have a protective role to compensate cerebral perfusion during carotid clamp, ROC analysis was used to determine the predicted cut off level for pre-operative arterial L-arginine.

A concentration of pre-operative arterial L-arginine < 35 mmol/L (area: 0.904; sensitivity: 95.0%; specificity: 83.3% [p = 0.002]) predicted the need for an intra-operative shunt (positive predictive value = 83%, negative predictive value = 95%). Using this cutoff value for L-arginine, the patients

were categorized into two subgroups: patients with pre-operative arterial L-arginine < 35 mmol/L as a high-risk population (n = 7), and 35 mmol/L as a low risk subgroup (n = 42). Independent predictor for shunt insertion Based on a binary logistic regression analysis, including age, comorbidities, severity of contralateral carotid stenosis and baseline biomarkers, only the previously determined cutoff point for L-arginine independently predicted the need for shunt insertion after cross-clamp of the carotid artery (odds ratio 0.061, 95%, CI 0.004 - 0.866; p = 0.039). This indicates that patients with a pre-operative arterial L-arginine concentration higher than the cut off value had a significantly lower risk of shunt insertion.

IV.8. Comparison of subgroups at high risk versus low risk for shunt

Comparing the pre-operative arterial L-arginine based subgroups, jugular lactate concentration was significantly increased in the high risk versus the low risk subgroup after de-clamp (median 1.8 mmol/L [IQR 1.5-2.2 mmol/L] vs. 1.3 mmol/L [IQR 1.0 -1.6 mmol/L]; p = 0.03).

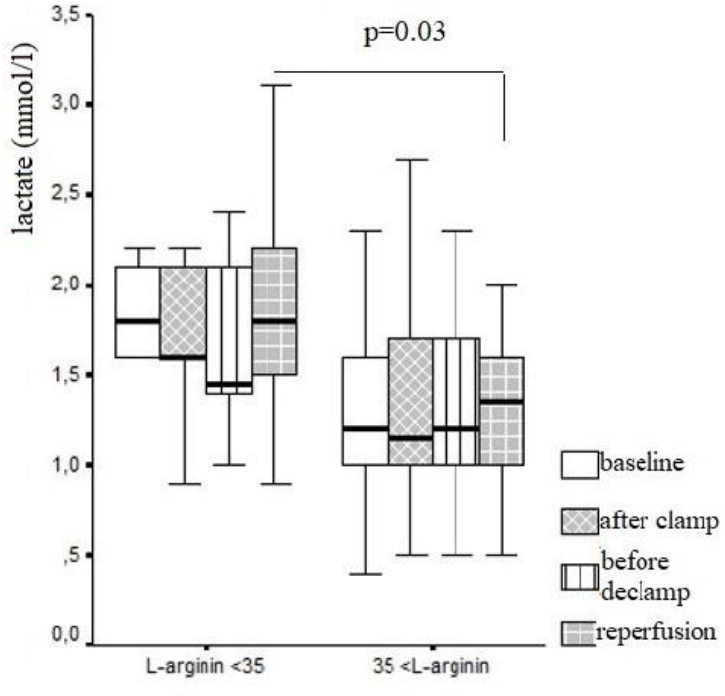

Figure 3. Temporal profile of lactate levels in high risk versus low risk patients

Similarly, jugular S100β concentration was significantly higher in the high risk population 24 hours post-operatively (median 53.9 nmol/L [IQR 43.9-57.0 nmol/L] vs. 12.4 nmol/L [IQR 1.7-34.0 nmol/L); p = 0.02).

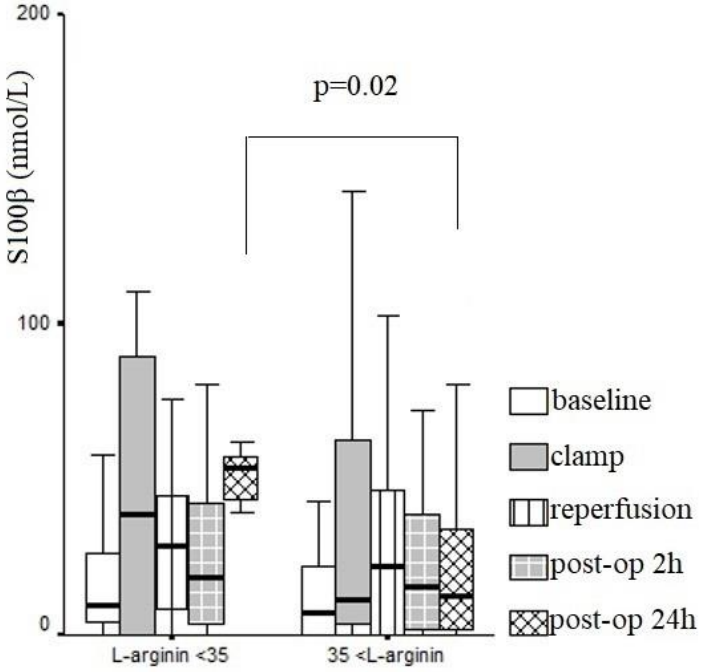

Figure 4. Temporal profile of plasma S100β levels in high risk versus low risk patients

However, when patients were not dichotomized according to the pre-operative L-arginine threshold level, neither jugular lactate and S100B nor blood gas-derived parameters were statistically different between shunted and non-shunted subgroups.

IV.9. Anaerobic metabolism indicators

In the subgroup of patients without the need of shunts, the relationship between clamp time and anaerobic indicators, such as arterial and jugular lactate, and P(j-a)CO2/C(a-j)O2, were analyzed. Clamp time only showed an inverse correlation (0.296; p = 0.035) with P(j-a)CO2/C(a-j)O2 ratio before de-clamp. The concentration of jugular lactate and P(j-a)CO2/C(a-j)O2 ratio showed an inverse correlation only before clamp (0.345; p = 0.013). Similarly, the preoperative concentration of arterial lactate also showed an inverse correlation (0.332; p = 0.017) with the P(j-a)CO2/C(a-j)O2 ratio before clamp, and it persisted during reperfusion (0.442; p = 0.002).

IV.10. 5 years follow-up

In our retrospective analysis, we have found information about the outcome of 54 patients. Within 5 years, seven patients (13%, mean age: 61±11.33, 6 males) suffered from more than 50% restenosis of the operated carotid artery. Five from the seven patients had to be stented, one patient's carotid was already occluded by the control examination. One patient's stenosis remained asymptomatic and was less than 70%.

In 13 patients (24%), a significant, contralateral carotid stenosis developed over 5 years. In 14 patients (25.9%), a peripheral arterial disease was diagnosed, from whom 5 (9.3%) had femoral or crural amputation. An acute coronary syndrome occurred in 18 patients (33.3%). Finally, 13 patients (24%) died in 5 years.

Importantly, significantly higher postoperative plasma cortisol concentration at T_4 was retrospectively observed in those patients who later suffered from acute coronary syndrome.

Binary logistic regression analysis including demographic, clinical and laboratory parameters such as age, gender, body mass index, degree of the ipsilateral and contralateral carotid stenosis, comorbidities, perioperative glucose and cortisol concentrations was performed to find independent predictors for follow-up endpoints. Accordingly, only plasma cortisol concentration 2 hours after the operation (T_4) proved to be an independent predictor of carotid restenosis within 5 years (OR: 1.67, 95% CI: 1.02-2.73, p=0.04). Similarly, plasma cortisol concentration at T_4 is an independent predictor of the onset of symptomatic acute coronary syndrome in 5 years (OR: 1.11, 95% CI: 1.02-1.21, p=0.01).

Based on receiver operating characteristic (ROC) analysis, the plasma cortisol concentration at T_4 was proved to be an independent predictor of later onset restenosis (cut-off level: cortisol≥ 19.45 μg ml^{-1}, sensitivity: 67%, specificity: 76%, area: 0.708, 95% CI: 0.651-0.958, p=0.01).

There was a strong statistical connection between mortality (n=13) and lower limb arterial disease (n=8/13) (p=0.01). Those patients who died within 5 years (n=13, 9 males), were older during surgery (age: 70±8.7 vs 64±7.4 years, p=0.05). Older age determined higher risk of mortality in 5 years (OR: 1.25, 95% CI: 1.01-1.54, p=0.041).

DISCUSSION

V.1. Effect of premedication and propofol sedation on surgical distress

Here, we compared the effectiveness of two anaesthetic procedures for stress relief in patients undergoing CEA in a randomized study: (i) CEA in regional block following alprazolam premedication and (ii) the previous technique combined with intraoperative propofol sedation. The temporal profile of plasma cortisol as stress marker was explored in the two cohorts. Our findings suggest the superiority of combination of alprazolam premedication with intraoperative propofol sedation compared to premedication alone in attenuation of stress related to CEA. Importantly, neither the baseline cortisol levels nor the relevant clinical parameters (such as blood pressure, intra-operative blood loss or the degree of contralateral carotid stenosis) showed significant differences between the two groups prior to surgery. Thus, the increased plasma cortisol level in the alprazolam only group at the time of reperfusion and even few hours later might be attributed to intra-and post-operative stress. This intra-operative stress response was presumably alleviated with the use of sedative dose of propofol. In addition, the plasma concentration of cortisol was even lower at the second postoperative hours (T_4) in the TCI group compared to its baseline level. All of these suggest that propofol has beneficial pleiotropic effects. Recently, propofol anaesthesia was found to be associated with decreased indices of ischemic cerebral damage and improved cognitive performance after CEA, although propofol itself has little impact on adrenocortical function.

Surprisingly, the universally used oral dose of 0.5 mg alprazolam resulted in extremely altered plasma concentration of the substance. Accordingly, no correlation was found between plasma cortisol and alprazolam level at any time point, neither in the total study population, nor in the subgroups respectively.

Finally, fewer patients required intra-operative shunt insertion in the TCI group compared to the alprazolam only group, nevertheless statistical conclusion could not be drawn due to small sample size here.

Based on our results, regional anaesthesia combined with intra-operative propofol sedation is a safe technique for CEA and provides stress relief without hampering mental status evaluation. We suppose that any attempts on preventing increased cortisol response has a prolonged beneficial effect for high risk vascular surgery patients.

V.2. Association between L-arginine pathway and cerebral ischaemia in CEA

It was found that the basal serum concentration of L-arginine was able to predict the need for an intra-operative shunt during CEA. This association may be just an observation and not necessary a cause effect phenomenon. This is also a hypothesis generating fact requiring further study and clarification, particularly comparison with other non-invasive tests such as stump pressure. Some studies have attempted to explore predictors of carotid clamping intolerance during endarterectomy. Maximal decrement in middle cerebral artery (MCA) mean flow velocity after carotid compression measured by transcranial Doppler (TCD) has been proven as a predictor of carotid clamping intolerance. In another clinical study, blood flow velocity was monitored by TCD during CO2 and L-arginine stimulation both in basal conditions and 3 months after carotid surgery. A significantly lower reactivity to L-arginine on the stenotic side was found in the pre-operative phase: this asymmetric reactivity was not observed after CEA. This lower reactivity to L-arginine is probably related to endothelium dysfunction due to the carotid pathology, as the abnormalities disappeared 3 months after endarterectomy. It suggests the physiologic role of L-arginine in hemodynamic compensatory mechanisms in patients with carotid artery disease. The number and volume of diffusion-weighted imaging (DWI) lesions after CEA are highly predictive of brain infarction. As shunt dependence in conscious patients with CEA is highly associated with the development of new DWI brain lesions compared with non-shunted patients, a significant increase in the brain damage marker S100β protein was expected, both in the jugular and systemic circulation. Nevertheless the concentration of jugular S100β was unable to predict shunt dependency in this cohort. The concentration of baseline L-arginine was the only marker, which independently predicted shunt dependency. Therefore, it was considered that the L-arginine pathway may affect cerebral perfusion through regulating the vascular tone. In accordance, pre-operative arterial L-arginine levels showed a negative correlation with jugular lactate concentration before clamp, also during the reperfusion phase suggesting the beneficial metabolic role of NO-donor L-arginine. Besides, concentration of S100β in the systemic circulation 24 h postoperatively was lower among patients with higher preoperative arterial L-arginine levels, suggesting a neuroprotective property of L-arginine.

This activation of the L-arginine pathway might be part of ischaemic preconditioning in atherosclerotic patients. Indeed, L-arginine pre-treatment could prevent hypoxic brain injury in experimental stroke. Moreover, L-arginine significantly attenuated ischaemia/reperfusion induced injury and mimicked the neuroprotective effect of postconditioning. Taking that into consideration, an increased bioavailability of L-arginine might be an adaptive response to chronic ischaemic states, as it was previously reported in chronic hypoxia.

In contrast to L-arginine, the pre-operative arterial concentration of ADMA showed a positive correlation with the anaerobic cerebral metabolism indicator P(j-a)CO2/C(a-j)O2 ratio but not with jugular lactate during carotid cross-clamp and reperfusion. It indicates that an increased ADMA level is associated with poor cerebral perfusion, presumably as a result of its vasoconstrictor property. Indeed, infusion of ADMA increased vascular stiffness and decreased cerebral perfusion in healthy subjects. The ratio of P(j-a)CO2/C(a-j)O2 during reperfusion was positively correlated with the systemic concentration of S100β measured 2 h after surgery. This may indicate that reperfusion with higher j-a CO2 gap or lower cerebral oxygen extraction, or both, might be associated with alteration of BBB integrity. The appearance of ischaemic brain damage is not supported by the S100β trend because it normalized by 24 h post-operatively in the low risk subgroup. Accordingly, all operations were clinically uneventful. Nevertheless, an important limitation of this study is that brain imaging was not performed to reveal silent ischaemic lesions in apparently uneventful patients after CEA. Observing inverse correlations between pre-operative L-arginine and jugular lactate concentrations and presuming the protective role of the NO donor L-arginine, the cut off level for pre-operative arterial L-arginine was determined to predict intra-operative shunt dependency. Using this cut off value for pre-operative arterial L-arginine, a subgroup of patients with a serum L-arginine level < 35 mmol/L were selected as a high-risk population for an uncompensated cerebral circulation after carotid cross-clamping. Importantly, this threshold value seems extremely low compared with healthy subjects. Similarly, L-arginine ranging from 10 to 60 mmol/l in the elderly carried an excess risk of death associated with a 0.1 mmol/l increase in ADMA ranging from +68 to +16%, while such an effect was abolished for plasma L-arginine values > 60 mmol/L. Interestingly, the severity of contralateral carotid stenosis was not a predictor of shunt dependency in multiple regression analysis. Data obtained from L-arginine based subgroup analysis showed that patients with lower than the threshold level of L-arginine were more vulnerable to anaerobic metabolism, indicated by increased production of jugular lactate and BBB dysfunction or silent ischaemia reflected by an increased level of jugular S100β, even in clinically uneventful CEA. Recently, the following factors were found to be associated with a carotid shunt use: older age, female sex, occlusion of the contralateral carotid artery, and a lower mean flow in the contralateral CCA by duplex scan. Moreover, a factor against the likelihood of a carotid shunt was the history of contralateral carotid surgery. The present study is not comparable to this prospective database, because the sample size and the mean age of the present cohort was lower; in addition, more male patients were enrolled without occlusion of the contralateral carotid artery. In addition, pre-operative endothelial dysfunction L-arginine pathway markers were focused on instead of duplex scan parameters. The current findings indicate that the measurement of arterial L-arginine and ADMA concentration as a part of pre and intra-operative assessment might be clinically worthwhile, to prevent ischaemic incidents during CEA. Based on the impressive positive

and negative predictive values, this pilot study may suggest that patients with a preoperative arterial L-arginine level > 35 mmol/l have a low likelihood of shunt dependency during surgery. However, the small number of shunted patients is a limitation in calculating accurate predictive values. In accordance with other authors, the difficulties in studies on the need for a shunt are emphasized. Accordingly, a higher risk for shunt can be predicted, but it cannot be determined which patients definitely need a shunt or which can be safely operated on without a shunt. In addition, although this study was prospectively planned, some of the results are based on a low number of patients (i.e. only six patients were shunted). Therefore, another prospective longitudinal study with a larger sample size is required to determine the role of L-arginine as a prospective biomarker of shunt dependency and its effect on clinical outcome in patients with CEA. Moreover, the value of L-arginine measurements in the prediction of shunt need should be tested in patients undergoing surgery under general anaesthesia as well.

V.3. Future perspectives

In patients with a complete circle of Willis, that is adequate to supply the ipsilateral cerebral circulation during clamping, an impaired autoregulation may be less important. Therefore, additive information about the pre-operative L-arginine level and the adequacy of the circle of Willis either by measuring MCA velocity or ICA stump back pressure may provide a more accurate approach to determine if a patient is at risk of intra-operative cerebral ischaemia. Further studies should also clarify whether patients with a preoperative L-arginine level lower than the threshold, benefit from L-arginine supplementation prior to surgery.

V.4. 5 years follow-up

Five-year mortality showed the strongest coincidence with the presence of peripheral arterial disease. Out of all perioperative parameters only cortisol plasma concentration measured 2 hours after surgery was able to predict high risk of carotid restenosis and symptomatic coronary disease. These results suggest that surgical distress may contribute to a worse clinical outcome on the long-term, possibly via the increased sympatho-adrenal activity and other factors exacerbating atherogenesis. Whether this phenomenon could be explained by harmful long-term effects of intra-operative stress, or frail patients have both higher stress response and worse outcome, needs further clarification. The beneficial effect of propofol sedation on late vascular complications should also be the subject of further investigations.

VI. NOVEL FINDINGS

- Awake sedation with propofol is able to decrease peri-operative stress response without impairing neuromonitoring.
- Cortisol kinetics could not confirm alprazolam premedication's stress lowering effect in a clinically relevant degree.
- Positive correlation between higher preoperative ADMA concentration and $P_{j\text{-}a}CO_2/C_{a\text{-}j}O_2$ (reflecting anaerobic brain metabolism) during carotid clamp and reperfusion indicates the association of ADMA and decreased cerebral perfusion reserve.
- Low preoperative L-arginine concentrations are able to predict the need of shunt insertion after carotid clamp, which suggests the protective role of the NO-donor L-arginine.
- An association was found between the grade of surgical stress response and the onset of restenosis in the operated carotid artery within five years.
- Elevated procedural stress is related to increased risk of coronary events within five years after CEA.
- According these findings, decreasing operational distress in CEA may influence the long-term outcome of patients suffering of vascular disease beneficially.

VII. LIST OF PUBLICATIONS

VII.1. Publications related to the thesis

Peter Szabo, Janos Lantos, Lajos Nagy, Sandor Keki, Eva Volgyi, Gabor Menyhei, Zsolt Illes, Tihamer Molnar. L-arginine pathway metabolites predict need for intraoperative shunt during carotid endarterectomy. *Eur J Vasc Endovasc Surg* 2016;52(6):721-728.

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VII.2. Presentations related tot he thesis

Szabó Péter, Völgyi Éva, Szabó Zoltán, Tóth Krisztina, Molnár Tihamér, Márton Sándor. Agyi oxigénfelhasználás és anaerob metabolizmus vizsgálata regionális érzéstelenítésben végzett carotis endarterectomia során, változásuk a szedáció függvényében. A Magyar Aneszteziológiai és Intenzív Terápiás Társaság XLII. Kongresszusa, Siófok, 2014 május 22-24.

Molnár Tihamér, Völgyi Éva, Szabó Zoltán, Márton Sándor, **Szabó Péter.** Jugularis S100B és laktát vizsgálata regionális érzéstelenítésben végzett carotis endarterectomia során. A Magyar Aneszteziológiai és Intenzív Terápiás Társaság XLII. Kongresszusa, Siófok, 2014 május 22-24.

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VII.3. Other publications

Mühl D, Füredi R, **Szabó P**: Thrombocyta (TCT) funkciók és hemostaseológiai paraméterek változása ulra-high dózisú streptokinase (UH-SK) kezelésben. *Aneszteziológia és Intenzív Terápia* 2003; 33(1): 27.

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VII.4. Other presentations

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