

INTERDISCIPLINARY DOCTORAL SCHOOL

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**ENDOTHELIAL DYSFUNCTION AND AUTONOMIC NERVOUS SYSTEM
ALTERATIONS IN ISCHEMIC CARDIOMIOPATHY**

SUMMARY

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ABBREVIATIONS

BMI=body mass index

CABG=coronary artery bypass grafting

CAD=coronary artery disease

CRP=C reactive protein

CT=computed tomography

EKG=electrocardiogram

eNOS=endothelial nitric oxide synthase

FMD=flow mediated dilation

HIF 1 α =hypoxia induced factor 1 α

hs CRP=high sensitivity C reactive protein

ICA=internal carotid artery

IL 1 β =interleukine 1 β

IL 6=interleukine 6

INOCA=ischemia with non obstructive coronary arteries

LDL=low density lipoproteins

MBG=myocardial blush grading

MRI=magnetic resonance imaging

NE=norepinephrine

NO=nitric oxide

oxLDL=oxidized low density lipoproteins

PAD=peripheral artery disease

PAT=peripheral artery tonometry

ROS=reactive oxygen species

SOD =superoxide dismutase

SNS= sympathetic nervous system

SYNTAX I score= Synergy Between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery

5-HT=5-hydroxytryptamine

BRIEF ABSTRACT

Introduction: Cardiovascular diseases share a common pathologic path, with similar risk factors and major global impact on morbidity and mortality. Regarding ischemic cardiomyopathy, recent research emphasizes the atherosclerotic process, aiming at identifying new molecular targeted therapies.

Objective: The present research studies the intricate interaction between endothelial dysfunction, inflammation, oxidative stress and the autonomic nervous system in patients diagnosed with ischemic heart disease scheduled for coronary artery bypass grafting (CABG).

Material and methods: We selected 88 adult patients, enrolled between January 2020 -June 2021. Clinical and ancillary tests were used to assess the subjects (lab tests, EKG, echocardiography, vascular ultrasonography, coronary angiogram, and CT). 3 studies were performed. The first one evaluated flow mediated dilation (FMD) and SYNTAX I score (indicative of coronary atherosclerosis), as well as the correlation between FMD and systemic atherosclerosis (carotid artery stenosis and peripheral artery disease). The second study highlighted the impact of inflammation and oxidative stress biomarkers (IL 1 β , IL 6, HIF 1 α , LOX-1, SOD 1) on the autonomic nervous system (the circulating levels of norepinephrine). The third study investigated the impact of diabetes mellitus on endothelial dysfunction and oxidative stress. Specific statistical analysis was employed to analyze the results.

Results: Statistically significant associations were observed between FMD and SYNTAX I score. Furthermore, FMD was correlated with carotid artery stenosis severity and peripheral artery disease. SYNTAX I score was also associated with high serum norepinephrine levels. The studied inflammation and oxidative stress biomarkers were linked to serum norepinephrine. Also, diabetic patients exhibited a more severe atherosclerotic burden (higher SYNTAX I score), augmented endothelial dysfunction (lower FMD values), and elevated serum serotonin levels.

Discussion: The research we conducted investigated endothelial dysfunction, oxidative stress, inflammation and the autonomic nervous system in a less studied population, subjects scheduled for CABG. The first study demonstrated a persistent strong association between FMD and SYNTAX I score, proving that FMD can be used as a method to evaluate peripheral, as well as coronary endothelial dysfunction. Additionally, FMD was linked to carotid artery stenosis and

peripheral artery disease, results in line with previous research. Ischemic heart disease is currently considered a pathology in which inflammation, oxidative stress and autonomic nervous system activation overlap. IL 1 β and IL 6 play a central role in local inflammation and systemic inflammation, a series of recent studies investigating these molecules as potential therapeutic targets in atherosclerosis. Modulating the HIF 1 α pathway, as inflammatory and oxidative stress mediator, is especially important as it could limit the deleterious effects of ischemia/reperfusion (present during CABG). The antioxidant properties of SOD 1 could lead to therapies for ischemia/reperfusion prevention. LOX 1, the receptor for oxidized LDL, undergoes a process of cardiac upregulation after CABG, suggesting it as a potential therapeutic target. Moreover, after comparing the present research with previous studies, we concluded that we used a unique combination of inflammation and oxidative stress biomarkers in a rarely studied population. We also observed that this particular combination is associated with norepinephrine circulating levels, indicative of autonomic system activity. Literature data states that diabetic patients suffer from a more severe coronary atherosclerotic burden, an observation validated by the third study we conducted. However, the research we performed was the first to enroll patients scheduled for CABG which compared endothelial dysfunction (FMD) according to the presence of diabetes mellitus. Regarding serotonin, the most important finding of recent research is that it acts synergistically with diabetes, augmenting hyperglycemia induced endothelial dysfunction. In the studied population serum serotonin was associated with diabetes. Another interesting conclusion was the lack of association of other oxidative stress biomarkers (LOX-1 and SOD 1) and diabetes mellitus. The results we obtained can be attributed to an already elevated oxidative stress due to severe atherosclerotic burden, regardless of the presence of diabetes.

Conclusion: FMD can be used as a surrogate for coronary, carotid and peripheral atherosclerotic disease. In patients scheduled for CABG, SYNTAX I score, as well as inflammatory and oxidative stress biomarkers, are associated with circulating norepinephrine. Diabetic patients suffer from a more severe atherosclerotic coronary burden and a reduced FMD compared with patients without diabetes. Serotonin, a marker of endothelial dysfunction and neurotransmitter, was considerably elevated in patient with diabetes, suggesting it as a potentially more sensitive biomarker or as a therapeutic target in patients with severe ischemic heart disease scheduled for CABG.

PART I-THEORETICAL BACKGROUND

Coronary atherosclerosis represents the main cause of myocardial ischemia. Ischemic cardiomyopathy can be regarded as a continuum, with stable (chronic coronary syndrome) and unstable (acute coronary syndromes) phases.

Chronic coronary syndrome is marked by episodes of reversible myocardial ischemia, due to the unbalance between coronary supply and demand. Angina pectoris is a clinical diagnosis in 90 % of cases. The personal history of cardiovascular disease and the presence of cardiovascular risk factors support the diagnosis. Ancillary basic investigations consist of laboratory measurements, EKG, EKG Holter monitoring, echocardiography, and magnetic resonance. After performing the basic investigations and assessing the pretest probability, inducible ischemia is tested for. The purpose is to identify patients who would benefit most from myocardial revascularization.

Evaluating the SYNTAX I score is a method applied both in research and clinical practice. The European Revascularization Guide recommends calculating it in order to assess for long term survival and adverse major cardiovascular events after percutaneous coronary angioplasty.

In case of non obstructive coronary artery ischemia (INOCA) , stenoses under 50 % are identified in epicardial coronary arteries and ischemia is due to spasm, endothelial dysfunction of the micro-/macro-circulation, or myocardial bridging.

New concepts define atherosclerosis as a status of active chronic inflammation associated to lipid intimal deposits.

In conclusion, the diagnosis of ischemic cardiomyopathy is based on clinical and ancillary investigations, with inflammatory parameters being added by recent research.

The vascular endothelium is the main factor for maintaining vascular homeostasis. Endothelial dysfunction disrupts coronary blood flow and favours the development of atheroma.

Coronary angiography remains the gold standard to evaluate endothelial function in the epicardial circulation. To assess coronary microcirculation invasive and non invasive methods are employed. Non invasive methods include radionuclids (SPECT and PET), contrast myocardial echocardiography, cardiac CT, and cardiac MRI. Invasive methods are: coronary angiography (with evaluation of the TIMI flow and MBG), coronary Doppler flow measurements, coronary flow reserve, and thermodilution.

Nitric oxide is the most important vasodilation substance generated by the vascular endothelium. Nitric oxide is synthesized in the endothelial cell from L-arginine, the reaction being catalyzed by eNOS. Besides NO induced vasodilation, the compound also exerts antiagregant, anti-inflammatory, antiproliferative, and antioxidant effects.

Considering that coronary endothelial function evaluation methods are expensive and difficult to apply, non invasive peripheral reactivity techniques (FMD and peripheral arterial tonometry-PAT) are being used as a surrogate for endothelial coronary function.

Inflammation plays a central role in all stages of atherosclerosis, from subclinical endothelial dysfunction to severe atheroma burden and acute cardiovascular events. IL 1 β , IL 6, C reactive protein (CRP), as well as hypoxia inducible factor 1 α (HIF 1 α) have recently been studied. Oxidative stress is also involved in atherosclerosis, with excess generation of reactive oxygen species (ROS), and oxidized low density lipoproteins (ox LDL) formation. OxLDL are further connected with LOX-1 endothelial receptor. Superoxide dismutase 1 (SOD 1) and the SOD family enzymes protect cells from oxidative stress by neutralizing superoxide radicals. High levels of circulating serotonin (5-hydroxytryptamine-5-HT) are associated with vasoconstriction and accelerated atherosclerosis. FMD is influenced by the sympathetic nervous system as well. Previous research underlined different responses of endothelial dependent vasodilation and FMD. Autonomic dysfunction accompanies cardiovascular disease, the interest for neuromodulation as a potential therapy remaining high. Norepinephrine (NE), as neurotransmitter, exerts its action through alpha- and beta-adrenergic receptors. The immune system and the sympathetic nervous system seem to be connected at immune cytokine level. 5-HT is also a neurotransmitter involved in autonomic modulation. Although mainly produced by intestinal enterochromaffine cells, 5-HT is engulfed and released by sympathetic neural endings.

Ischemic cardiomyopathy is associated with autonomic dysfunction. Moreover, it is crucial to outline the connections between endothelial dysfunction, inflammation, oxidative stress, and autonomic dysfunction, theory demonstrated by various serum biomarkers.

PART II THE RESEARCH

Research purpose and objectives

CABG patients represent a distinct category of ischemic cardiomyopathy. Besides the generally severe atherosclerotic burden, these subjects also undergo a process of ischemia/reperfusion associated with the CABG surgery.

The purpose of the research was to study endothelial dysfunction, inflammation, oxidative stress, and autonomic nervous system activation in patients diagnosed with ischemic cardiomyopathy scheduled for CABG, and to underline the connections between these processes.

The objectives of the study consisted of:

1. Evaluating the selected patients regarding the distribution of cardiovascular risk factors (body mass index, smoking status, type 2 diabetes mellitus, dyslipidemia, and arterial hypertension).
2. Reporting the presence of prior myocardial infarction and the systolic function of the left ventricle in the studied population.
3. Diagnosing the presence of atherosclerosis in other arterial territories (namely the internal carotid artery-ICA and lower extremities-peripheral artery disease-PAD).
4. Reporting the values of SYNTAX I score and FMD.
5. Measuring serum concentrations of routine parameters (haemoglobin, thrombocytes, leucocytes, lipids, basal glycemia), as well as hsCRP and troponin.
6. Determining the circulating concentrations of certain inflammatory, endothelial dysfunction, oxidative stress, and sympathetic nervous system (SNS) biomarkers (HIF-1 α , IL-1 β , IL-6, NE, 5-HT, SOD-1 și LOX-1).
7. Studying the correlation between FMD and: coronary atherosclerosis (evaluated through the SYNTAX score), carotid artery disease, and PAD.
8. Assessing the association between plasma NE concentration, as a marker for SNS activity, and the severity of ischemic cardiomyopathy, evaluated through the SYNTAX score.
9. Investigating potential correlations between inflammatory (hsCRP, IL-1 β , IL-6 și HIF-1 α) and oxidative stress biomarkers (LOX-1 și SOD-1) and the circulating levels of NE.

10. Establishing if endothelial dysfunction (evaluated through FMD and serum biomarkers- 5-HT, SOD-1, and LOX-1) is more severe in diabetic patients scheduled for CABG surgery versus patients undergoing the intervention, but without diabetes.

Patient selection

We conducted a longitudinal prospective study which selected 88 adult patients diagnosed with stable coronary artery disease (CAD) scheduled for CABG surgery, between January 2020 and June 2021. The patients were admitted in the Cardiovascular Surgery ward of the ICCO Clinic in Brasov. The study protocol was approved by the Ethical Board of the Transylvania University of Brasov (registration number 1/2.03.2019) and was in line with the ethical principles of the Declaration of Helsinki and The Code for Good Clinical Practice.

The inclusion criteria was as follows:

- Severe CAD-patient with stable CAD which presented effort angina or an angina equivalent (CCS class III or IV) scheduled for CABG surgery;
- Age over 18 years;
- Signed informed consent;
- The presence of cardiovascular risk factors (smoking, dyslipidemia, arterial hypertension, type 2 diabetes mellitus).

The exclusion criteria were:

- Acute coronary syndromes (in the prior month or at admission);
- Severe valvular disease requiring surgical correction;
- Severe liver failure;
- Severe kidney failure;
- Recent (the prior month) or active bleeding;
- Coagulation disorders;
- Active cancer;
- Carcinoid syndrome;
- Inflammatory disease (infections or autoimmune disease);
- Pheochromocytoma;
- Severe psychiatric illness;

- Treatment with selective serotonin reuptake inhibitors and monoamin oxidase inhibitors;
- Alpha blocker treatment.

The study initially enrolled 88 patients, but 4 were later excluded (2 of them presented acute coronary events after being admitted, and 2 withdrawn because of personal reasons).

Methodology

The patients enrolled in the study were evaluated clinically, biologically, ultrasonography was used to measure FMD, to perform echocardiography, and to evaluate ICA stenosis and PAD), lower limb arteries CT was performed to confirm and grade PAD, and coronary angiography. To calculate the SYNTAX I score we used an online tool (<https://syntaxscore.org/>).

Serum/plasma biomarkers were measured from peripheral venous blood, drawn à jeune, 3 days prior to CABG surgery. Samples were centrifuged and the supernatant was preserved at -80 degrees Celsius. The concentrations of the studied biomarkers (IL 1 β , IL 6, HIF 1 α , LOX-1, SOD-1, and NE) were determined using the ELISA method, with commercially available kits.

Characteristics of the studied population

The research was conducted on a predominantly male population (67 subjects of the 84). The average age was of 65.09 years (the study including patients aged 46 to 88). Cardiovascular risk factors were distributed as follows: body mass index had an average of 28.36 kg/m², the study included 17 smokers, 33 diabetic patients, 80 patients with dyslipidemia, and 79 hypertensive subjects.

The majority of the study had no prior myocardial infarction (51 patients). The global systolic performance of the left ventricle was assessed using the left ventricle ejection fraction evaluated with the Simpson method. Most patients had an ejection fraction of 40 to 50 % (38 patients), followed by those with 50 to 60 % ejection fraction (32 subjects). The study also included patients with an ejection fraction below 40 %, but their number was limited (14 patients).

According to the NASCET criteria for ICA stenosis grading, the study participants were divided into three groups. 20 of the subjects did not have significant ICA stenosis, 34 patients were diagnosed with 50-69 % ICA stenosis, and 30 patients had severe (70-99 %) ICA stenosis. Regarding PAD, only 11 patients in the study had lower limb artery stenosis.

The SYNTAX I score had a median of 29.75, with a minimum of 17 and a maximum of 54. FMD measurements revealed that the patients had a median value of 2.79 % (minimum of 0.95 % and maximum of 6.75%).

All patients were treated with a beta blocker, an antiaggregant, and had dyslipidemia treatment. The beta blocker used was bisoprolol (43 patients), nebivolol (21 subjects), and metoprolol (20 patients). Regarding antihypertensive drugs, 46 patients were treated with renin-angiotensin-aldosterone inhibitors, 7 subjects only with a thiazide diuretic, 11 patients required treatment with a calcium channel blocker, and 14 subjects were treated with a combination of the three drugs. The majority of the patients were treated with a statin (73 subjects), 2 patients received a fibrate, and 8 individuals were treated with a statin and a fibrate. 48 patients received aspirin, 17 aspirin plus clopidogrel, and 19 clopidogrel and an anticlotting agent. The studied population also included patients under diuretic treatment (2 subjects treated with a loop diuretic, 12 subjects with an aldosterone inhibitor, and 32 subjects received a combination of the two).

Study 1-Flow mediated dilation and its correlations with coronary and systemic atherosclerosis in patients scheduled for surgical myocardial revascularization

Objectives

The purpose of the study was to evaluate endothelial dysfunction (using FMD) in patients with severe CAD (evaluated with the SYNTAX I score) undergoing CABG surgery. Additionally, carotid artery disease and PAD was assessed. Also, we tested the hypothesis considering that FMD was associated with CAD and systemic atherosclerosis (diagnosed in the ICA and lower limb arteries).

Methodology

We enrolled 84 patients, which fulfilled the inclusion criteria and signed the informed consent. SYNTAX I score was calculated using the coronary angiograms. To measure FMD Doppler ultrasonography was applied. FMD evaluation consisted of measuring the brachial artery diameter before (D1) its occlusion with a sphingomomanometer cuff and after the obstacle is released (D2- maximal dilation diameter). FMD was calculated using the formula $FMD = \frac{(D2 - D1)}{D1} \times 100$. Distal arterial occlusion is used (in the forearm), thus stimulating the release of NO and NO-dependent vasodilation. To standardize examinations and compare the results of the research several guidelines were issued, and a value of $FMD < 6.5\%$ was considered indicative of endothelial dysfunction.

Doppler ultrasonography was also used to quantify ICA stenosis according to the NASCET criteria: mild ($< 50\%$), moderate (50-69 %), and severe (70-99%). PAD was diagnosed on clinical and ultrasonographic aspects and its severity was graded using angio-CT. The patients were divided into 6 groups based on the CT scan: 0-no PAD; 1-mild stenosis ($< 49\%$); 2-moderate stenosis (50-74 %); 3-severe stenosis (75-99 %); 4-occlusion.

Statistical analysis

Collected data was gathered using Microsoft Office Excel 2019 and analysed using JASP 0.19. Patient characteristics were compared using the T test, Chi square test, and Mann-Whitney U test. To evaluate associations the Pearson correlation (for normally distributed variables), the Spearman correlation (for skewed variables), and the One way ANOVA test were employed. The p value was considered statistically significant at $p < 0.05$.

Results

The study included patient aged 46 to 88, 79.76 % being males. The average age was 64.25 ± 7.24 in males and 68.41 ± 10.44 in females. The majority of the subjects was overweight (BMI -28.07 kg/m^2 in males and 29.48 kg/m^2 in females). Hypertension and Dyslipidemia were the most frequent cardiovascular risk factors. In the male population 22.38 % were smokers and 38.8 % had type 2 diabetes mellitus, and the female group consisted of 11.76 % smokers and 41.17 % diabetic patients. The median FMD value was of 2.79 % (minim of 0.95 % and maximum of 6.75 %). FMD values did not correlate with age in the studied population ($p > 0.05$; $r = -0.072$).

Regarding the SYNTAX I score, the median value was of 29.75 (minimum of 17 and maximum of 54). SYNTAX I score did not correlate with age either ($p > 0.05$; $r = 0.09$).

FMD and SYNTAX I score were associated with a statistically significant p ($r = 0.898$; $p < 0.001$). Also, FMD was ststistically associated with ICA stenosis ($p < 0.001$; p using One Way ANOVA). Furthermore, FMD and PAD were also correlated, the difference between patients with PAD versus those without PAD being significant ($p < 0.001$; p calculated using One Way ANOVA).

Discussion

Clinical characteristics of the patients were similar according to gender. Although no statistically significant differences were observed when comparing males and female, some aspects are worth mentioning. First, the average age in the female group was 68.41 years versus 64.25 years in the male group, possibly due to estrogen cardiovascular protection in women. The BMI was slightly higher in females. Smokers were mainly male, and diabetes was encountered equally in men and women. ICA stenosis was diagnosed in 52 males and 12 females, gender distribution similar to other populations undergoing CABG surgery. PAD was observed in 11 patients, all of them being male, literature data confirming the late diagnosis of PAD and atypical presentation of PAD in women.

Males and females both had FMD values under the cutoff of 6.5 %, diagnostic for endothelial dysfunction. Although lower FMD values were registered in males (median of 2.55 % versus 4.46 % in women), the difference was not statistically significant ($p = 0.214$). The study enrolled menopausal women (the average age was 68.41 ± 10.44), explaining the similar FMD values in the elderly, results that were similar to other research.

However, a lower FMD value was present with older age, reflecting a more pronounced endothelial dysfunction, although no statistical significance could be attributed to the finding. In contrast, other researchers reported a reduction of FMD with older age.

We observed a median of 30.5 of the SYNTAX I score in males and a median of 26 in females, reflecting a more severe CAD in men undergoing CABG surgery. However, the difference was not statistically significant.

We noted significant correlations between FMD and SYNTAX I score ($r=0.898$ and $p<0.001$), underlining that arterial vasodilation is reduced in patients with severe CAD. Previous studies concluded that FMD and SYNTAX I score were associated in populations with cardiovascular risk factors but no diagnosed cardiovascular disease. Patients with severe CAD were less studied. The present research was the first to evaluate FMD in patients scheduled for myocardial surgical revascularization, and to demonstrate that a strong association between FMD and SYNTAX I score was still present even in this category.

Furthermore, endothelial dysfunction assessed through FMD was associated with the presence and severity of ICA stenosis, a lower FMD being indicative of ICA atherosclerosis. Similar results were observed by other studies evaluating endothelial dysfunction in patients with ICA stenosis.

To this moment, FMD was rarely used in vascular surgery, although previous research established FMD as a reliable tool for assessing endothelial dysfunction in PAD. The present study found connections between FMD and severe PAD.

Several limitations to the study need mentioning. First, FMD measurement can be biased by multiple factors, but a part of them can be controlled before the investigation. Second, we included only a few patients with PAD, which could affect the results regarding PAD and its correlation with endothelial dysfunction. Third, results obtained on PAD are difficult to expand on the female gender since no females with PAD were identified in the studied population.

Efforts to standardize FMD measurement are targeted towards rendering it a useful tool to evaluate therapeutic interventions, such as assessing endothelial dysfunction in CABG patients undergoing procedures to improve endothelial function (such as antioxidant agents or stimulating NO release after ischemic preconditioning). As it is a non-invasive method, FMD can be used

repeatedly, without side effects, and it could be used for an early detection of experimental therapies.

Conclusions

In patients scheduled for CABG surgery characteristics were similar in males versus females.

The present study observed a statistically significant association between FMD and SYNTAX I score, proving that peripheral endothelial dysfunction (assessed through FMD) reflects coronary atherosclerotic burden.

Additionally, FMD was associated with ICA stenosis and PAD, and thus can be regarded as a marker for systemic atherosclerosis.

As a clinical application, FMD, as a non invasive method to evaluate endothelial dysfunction, could be used as an earlier identifier of the effects of experimental therapies targeting endothelial function.

Study 2- The impact of inflammation and oxidative stress biomarkers on the sympathetic nervous system in severe coronary atherosclerosis

Objectives

The objectives of the study were to evaluate the association between plasma NE, as a marker for SNS activation, and the severity of CAD, evaluated through the SYNTAX I score. Furthermore, we investigated potential correlations between inflammatory (hsCRP, IL-1 β , IL-6 and HIF-1 α) and oxidative stress (LOX-1 and SOD-1) markers, and SNS activity (evaluated through circulating NE levels), in patients scheduled for CABG surgery.

Methodology

84 individuals were selected for the study (after signing the informed consent and applying the inclusion/exclusion criteria). Clinical, biological (hsCRP, IL 1 β , IL 6, HIF 1 α , SOD-1, LOX-1, NE), ultrasonographical, and angiographical evaluations (coronary angiograms in order to calculate the SYNTAX I score) were performed.

Statistical analysis

Categorical variables were expressed as frequencies (number and percentage-n and %). Continuous variables were characterised by average (and standard deviation)-for normally distributed data, and median (with minimum and maximum values)-for data with skewed distribution. The distribution of the variables was tested using the Shapiro-Wilk test. Pearson correlation (for normally distributed variables) and Spearman correlation (for data with skewed distribution) were applied. Statistical significance was set at $p < 0.05$. Statistical analysis was performed in Microsoft Excel 2019 and Jasp 0.19.

Results

CAD severity, reflected by a high SYNTAX I score, was linked to high levels of NE, used a surrogate for SNS activity.

Statistically significant correlations were observed between 1 β and NE ($p < .001$; $r 0.49$), IL 6 and NE ($p = 0.003$; $r 0.32$), HIF 1 α and NE ($p 0.049$; $r 0.21$).

Oxidative stress biomarkers (LOX 1 and SOD 1) also correlated to circulating levels of NE ($p.004$ and $r 0.31$ for LOX 1 and NE; $p 0.016$ and $r 0.26$ for SOD 1 and NE).

Considering that the studied population had cardiovascular risk factors (BMI, smoking, Dyslipidemia, hypertension, diabetes) that could have influenced the noted associations, we tested if they were individually related to NE or any of the biomarkers. No significant associations were identified.

Discussion

Interleukines, as inflammatory biomarkers, was recently studied in cardiovascular disease. IL 1 β mediated the generation of IL 6, which, in turn, stimulates the systemic activation of cytokines and acute phase proteins. Recent research was centered on inflammatory cytokines as therapeutic targets. The largest of them, the CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcome Study) trial tested Canakinumab, a selective monoclonal antibody for 1 β , enrolled patients with diagnosed CAD and inflammation. The study observed a significant reduction of acute cardiovascular events and of cardiovascular mortality. On the other hand, research conducted on Tocilizumab, selective antibody for 6, did not reveal clinical benefit for now. In patients undergoing CABG surgery, high preoperative IL 6 levels were associated with early graft occlusion and late cardiovascular events.

The HIF pathway modulation could be of interest as it could limit the effects of ischemia/reperfusion (entity present during CABG surgery). However, recent evidence suggests that maintaining the intracellular homeostasis of HIF 1 α might be preferred over completely deleting the effects of this signaling pathway. Some of these approaches might reveal new strategies to prevent and treat organ lesions in patients undergoing surgical procedures.

In cases of increased SOD activity, such as states accompanied by ischemia/reperfusion, the antioxidant effect seems to be erased and cellular lesion occurs. Aortic clamping during CABG surgery causes important ischemia/reperfusion lesions and a significant increase in oxidative stress. SOD enzymes might prove an essential therapeutic pathway to alter the oxidant/antioxidant balance.

Increased ROS generation also causes LDL oxidation, with oxLDL formation, contributing to the atherosclerotic process via LOX 1 receptors. LOX 1 is upregulated in the heart following an ischemia/reperfusion event, such as CABG, suggesting that it might be a potential therapeutic target.

The study we conducted used a unique combination of inflammatory and oxidative stress biomarkers. Each of these parameters correlated with the increased NE circulating level, neurohormone indicative of SNS activation. Moreover, comparing the present research with existent studies, we concluded that it was the first evaluation of this combination of biomarkers in patients undergoing CABG surgery-procedure accompanied by major ischemia/reperfusion. The trial underlined a statistically significant relation between these inflammatory and oxidative stress markers, on one hand, and NE circulating levels, on the other. Autonomic dysfunction accompanies cardiovascular disease, the interest for neuromodulation remaining increased. NE acts through alpha and beta adrenergic receptors. Nevertheless, the consequences of eliminating the effects of the SNS on atherosclerosis through renal denervation procedures require further investigation as previous research revealed serious adverse effects.

Several limitations of the study need mentioning. First, the studied inflammatory and oxidative stress biomarkers were only several of many molecules involved in atherosclerosis. Second, the serum concentrations of the studied parameters were determined only once, while serial measurements might establish stronger associations.

Conclusions

Patients scheduled for CABG surgery had increased serum levels of the tested inflammatory markers. Also, oxidative stress and SNS activity showed increased levels.

CAD severity, evaluated through the SYNTAX I score, was correlated with SNS activity, indicated by plasma NE.

Additionally, NE associated with inflammation and oxidative stress, suggesting that inflammation, oxidative stress and the SNS are part of an intricate network.

No relations were identified between the cardiovascular risk factors and the tested parameters, hence their presence did not bias the results.

The present study has important clinical implications. The development of a risk score encompassing inflammatory and oxidative stress markers might be useful for identifying patients that need a more aggressive treatment strategy to reduce inflammation, oxidative stress, and SNS activation. Such a scoring system might be needed especially in the case of CABG surgery in order to personalize treatment options and improve outcome.

Study 3- The impact of diabetes mellitus on endothelial dysfunction and oxidative stress in patients with severe coronary artery disease

Objective

The purpose of the study was to establish whether endothelial dysfunction is more severe in patients undergoing CABG surgery that have concomitant type 2 diabetes mellitus, compared to patients scheduled for CABG but without diabetes. To evaluate endothelial dysfunction ultrasonography (brachial FMD) and serum markers measurements (5-HT, SOD-1, and LOX-1) were used.

Methodology

The study enrolled 84 patients with severe CAD scheduled for CABG surgery, after signing the informed consent and applying the inclusion/exclusion criteria. The SYNTAX I score was calculated after the coronary angiography was performed, FMD was measured according to existing guidelines, and serum markers were determined using the ELISA method.

Statistical analysis

Categorical variables were expressed as frequencies (number and percentage-n and %). Continuous variables were characterised by average (and standard deviation)-for normally distributed data, and median (with minimum and maximum values)-for data with skewed distribution. The distribution of the variables was tested using the Shapiro-Wilk test. Pearson correlation (for normally distributed variables) and Spearman correlation (for data with skewed distribution) were applied. Statistical significance was set at $p < 0.05$. Statistical analysis was performed in Microsoft Excel 2019 and Jasp 0.19.

Results

Patients with diabetes had higher SYNTAX I scores ($p=0.050$), reflecting a more severe coronary atherosclerotic burden in that category.

We noted lower FMD values in patients with diabetes compared to those without diabetes ($p=0.079$), even if the result was only borderline statistically significant.

The most interesting result of the study was that serum 5-HT concentrations were significantly higher in patients with diabetes versus those without ($p < 0.001$).

In the studied population we observed similar oxidative stress levels (serum SOD 1 and LOX 1 concentrations) when comparing groups according to the presence of diabetes.

Statistically significant correlations were noted between diabetes and SYNTAX I score ($p=0.029$; $r=0.238$), diabetes and FMD ($p=0.042$; $r=-0.223$), and diabetes and 5-HT ($p<0.001$; $r=0.423$).

Discussion

In patients with diabetes coronary atherosclerosis is more diffuse and severe, hence the SYNTAX I score is higher, an observation validated by the present study as well. A significant difference ($p=0.05$) was also noted between patients with CAD and diabetes versus patients with CAD without diabetes.

The vascular endothelium is the main modulator of vascular tonus and homeostasis, endothelial dysfunction being accompanied by vasoconstriction and pro atherogen/pro thrombotic states. The methods of evaluating endothelial dysfunction in clinical practice are based on inducing reactive hyperemia due to NO generation. FMD is a validated tool to assess endothelial function and it can also be used as a surrogate for coronary endothelial dysfunction. All the patients enrolled in the present trial exhibited FMD values under the cutoff of 6.5 % set by guidelines, regardless of the presence of diabetes.

FMD was influenced by the presence of diabetes, proving reduced vasodilation, even if the results were marginally statistically significant ($p=0.042$; $r=-0.223$). After conducting a review of the literature we concluded that the study was the first to enroll patients undergoing CABG surgery and to compare FMD based on the presence of diabetes.

Few studies compared endothelial dysfunction in subjects with CAD and diabetes versus those with CAD but without diabetes. We found that 2 previous trials also concluded that endothelial dysfunction (through FMD) was more severe if individuals associated type 2 diabetes mellitus.

High circulating 5-HT levels were associated with a prothrombotic status caused by the proaggregant effects of 5-HT. The most important observation of recent research was that 5-HT acted in synergy with diabetes, augmenting endothelial dysfunction induced by hyperglycemia and resulting in accelerated atherosclerosis. Patients diagnosed with CAD already exhibit disruptions of the endothelial function, therefore their cardiovascular system is easily affected by elevated 5-HT. Reverse correlations were identified between circulating levels of 5-HT and endothelial function in

smokers, proved by FMD and peripheral arterial tonometry, surrogate methods to evaluate endothelial dysfunction.

Oxidative stress could represent a common pathway in endothelial dysfunction. Additionally, 5-HT and oxidative stress appear to act synergistically in the atherosclerotic process.

The studied population exhibited a statistically significant correlation between 5-HT and diabetes ($p < 0.001$; $r = 0.423$). Moreover, we observed a significant difference ($p < 0.001$) when comparing serum 5-HT concentrations in patients with diabetes versus those without diabetes. The study we conducted was the first to evaluate serum 5-HT in patients with CAD undergoing CABG surgery and compare the results based on the presence of diabetes.

Selective serotonin reuptake inhibitors are an efficient treatment for depression. Evidence from clinical trials supports the idea that depression and cardiovascular disease share common pathways such as inflammation and endothelial dysfunction. Recent clinical trials were centered on cardiovascular effects of those antidepressants, but with conflicting results.

Sarpogrelate, which is a 5-HT_{2A} and 5-HT_{2B} receptor antagonist, inhibits serotonin dependent platelet aggregation. In the search for efficient therapies to reduce the burden of severe atherosclerosis, especially in type 2 diabetes mellitus, sarpogrelate treatment showed a reduction of atheroma plaques. Moreover, 5-HT seems to be involved in vascular smooth muscle cell proliferation, the main cause for graft occlusion after CABG surgery. In vitro studies conducted on isolated porcine veins, human isolated saphenous vein, and human isolated internal mammary artery concluded that sarpogrelate could prevent graft vasospasm and early graft occlusion. Further research is needed to define the clinical profiles of patients that would benefit the most from sarpogrelate treatment.

Another interesting conclusion of the present study was the lack of association between SOD-1 and LOX-1, as oxidative stress markers, and diabetes. Increased SOD-1 activity was considered to be linked with antioxidant effects in CAD, but recent trials failed to find significant associations between SOD-1 and CAD severity. A possible explanation could be that in CAD SOD-1 is neutralized by the elevated ROS.

The present study included subjects with an already elevated level of oxidative stress due to severe coronary atherosclerosis, regardless of the presence of diabetes.

Limitations of the study include the reduced standardization of FMD as a surrogate to evaluate endothelial dysfunction, although it is used in research and clinical practice. Additionally, the marginally statistically significant difference of SYNTAX I score and FMD according to the presence of diabetes might have been influenced by the small patient samples.

Conclusions

The present study observed that the coronary atherosclerotic burden (SYNTAX I score) is more severe in patients diagnosed with diabetes, even in those undergoing CABG surgery.

Even if FMD and circulating 5-HT levels indicate a more pronounced endothelial dysfunction in patients with associated diabetes compared with patients without diabetes, SOD-1 and LOX-1 serum concentrations, markers of oxidative stress, were not influenced by diabetes.

Serotonin (5-HT) could serve as a more sensitive biomarker or as a therapeutic target in patients with CAD and diabetes, but further research is needed to validate our results. Measuring 5-HT before CABG surgery could identify subjects with a higher risk of graft spasm or early graft occlusion.

Of note is that the studied population (patients with severe CAD scheduled for CABG surgery) was a special category, summing up the effects of endothelial dysfunction, oxidative stress, and inflammation. As endothelial dysfunction seems reversible in any stage of atherosclerosis, even after atherothrombotic events occur, therapeutic pathways aiming involved in these pathologic processes are worth further exploring.

ORIGINALITY OF THE RESEARCH

The present research was the first to evaluate FMD in patients with severe CAD scheduled for CABG surgery and the first to reveal that FMD (as a surrogate for peripheral endothelial function) correlates to coronary atherosclerotic burden assessed through the SYNTAX I score. Furthermore, FMD reflects systemic atherosclerosis, as it was associated with ICA stenosis and PAD.

Furthermore, it was the first study to use this combination of inflammatory, oxidative stress, and SNS activation biomarkers. It might be of use to develop a scoring system including these markers in order to identify patients in need for a more aggressive treatment in order to lower inflammation, oxidative stress, and excessive activation of the SNS.

Moreover, the present study compared patients with severe CAD scheduled for CABG surgery based on the presence of type 2 diabetes mellitus, observing similar oxidative stress levels (SOD-1 and LOX-1 serum concentrations), but increased circulating 5-HT in patients with concomitant diabetes. Hence, serotonin (5-HT) could serve as a more sensitive biomarker for oxidative stress or as a potential therapeutic target in patients with severe CAD associating diabetes, but further research is required to validate present results.

LIST OF PUBLISHED PAPERS

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