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# 11-DEHYDRO THROMBOXANE B2 LEVELS AFTER PERCUTANEOUS TRANSLUMINAL ANGIOPLASTY IN PATIENTS WITH PERIPHERAL ARTERIAL OCCLUSIVE DISEASE DURING A ONE YEAR FOLLOW-UP PERIOD

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The aim of our study was to determine if the generation of thromboxane is altered in patients with peripheral arterial occlusive disease following percutaneous transluminal angioplasty (PTA) during a one year follow-up period. In this study, 175 patients diagnosed with peripheral arterial occlusive disease (PAOD) and demonstrating short-distance claudication or ischemic rest pain, requiring PTA in either the iliac, femoral, or popliteal arteries, were enrolled. The excretion of 11-dehydro thromboxane B2 ($TXB_2$) was measured in urine samples by high-performance liquid chromatography-mass spectrometry and recalculated based on the creatinine concentration. The urine samples were collected the morning prior to PTA, immediately following PTA and the day after PTA. All of the study subjects were then observed for a period of 12 months. Urine samples were also collected during the follow-up visits, and the levels of 11-dehydro $TXB_2$ were measured at 1 month (1458.1 pg/mg creatinine ± 1240.8), 3 months (1623.3 pg/mg creatinine ± 1362.2), 6 months (1314.8 pg/mg creatinine ± 1378.7) and 12 months (1473.2 pg/mg creatinine ± 1455.2) after the PTA procedure. All of the patients were taking 75 mg of aspirin per day throughout the course of the study, as well as 75 mg of clopidogrel for six weeks following PTA. Overall, the mean $TXB_2$ values immediately after PTA were significantly higher than either before the procedure (1524.4 pg/mg creatinine ± 1411.1 vs. 2098.1 pg/mg creatinine ± 1661.8; P = 0.00002), the day after PTA, or at any other point during the study. Moreover, preoperative $TXB_2$ levels correlated well with the composite endpoints of death, myocardial infarction and stroke during the follow-up period (OR 7.42 [CI 95% = 1.2-48.8]; P = 0.02). Our findings suggest that clinicians should consider the use of $TXA_2$ synthase inhibitors and receptor antagonists in combination with peripheral percutaneous transluminal angioplasty in patients with peripheral arterial occlusive disease.

Key words: *angiology, thromboxane, percutaneous transluminal angioplasty, peripheral arterial occlusive disease, inflammation, prostacyclin*

## INTRODUCTION

Atherosclerosis is an inflammatory vascular disease of the arteries (1), and its prevalence is increasing in developed countries. Among the many inflammatory cells and their mediators, there is a specific class of lipids that act as mediators of inflammation. Thromboxane A2 ($TXA_2$) is a member of the family of lipids known as eicosanoids (2). $TXA_2$ is a vasoconstrictor and a potent hypertensive agent. It also directly activates platelet aggregation (3, 4). The major $TXA_2$ metabolites are thromboxane B2 ($TXB_2$) and 11-dehydro-$TXB_2$, the latter of which is an inactivation product of $TXA_2$ that forms in the plasma from its parental compound by hydrolysis within a matter of several seconds. Typically, 11-dehydro-$TXB_2$ is almost completely cleared through the urine; however, its levels may increase due to the activation of platelets during blood sampling or intravascular procedures. Thus, 11-dehydro-$TXB_2$, which has a half-life of approximately 45 minutes, has been proposed as a tool to monitor the systemic production of thromboxanes in cardiovascular disorders (5). We wanted to know if thromboxane levels in patients with peripheral arterial occlusive disease (PAOD) were changed following percutaneous transluminal angioplasty (PTA). In PAOD, the treatment of patients with aspirin plays an important role. Thromboxane metabolism in platelets is inhibited by aspirin, which irreversibly blocks the enzyme cyclooxygenase-1 in platelets (5).

## MATERIALS AND METHODS

This study enrolled 175 patients with peripheral arterial occlusive disease (PAOD), all below the age of 75 years, demonstrating short-distance claudication or ischemic rest-pain (Rutherford 3 or 4) who required percutaneous transluminal

angioplasty (PTA) in either the iliac, femoral, or popliteal arteries (*Table 1*). A careful evaluation was performed to exclude patients with signs of inflammation, ischemic ulceration or trophic lesions, acute limb ischemia, myocardial infarction or stroke (within the last 6 months), chronic kidney disease, and neoplastic disease. Thus, the patients in this study group corresponded to stage 3 or stage 4 of the Rutherford PAOD classification. The patients received aspirin (75 mg) and either atorvastatin or simvastatin (20 or 40 mg, according to individual treatment goals) throughout the course of the study. Clopidogrel was administered for 6 weeks following the PTA procedure.

The study was accepted by the local Bioethics Committee at Jagiellonian University. All of the patients signed an informed consent form.

The excretion of 11-dehydro-$TXB_2$ was measured in urine samples by high-performance liquid chromatography-mass spectrometry and was then recalculated based on the creatinine concentration. Urine samples were collected the morning prior to PTA ($T_0$), immediately following PTA ($T_1$), and the day after PTA ($T_2$). All of the study subjects were then observed for a period of 12 months. Urine samples were collected during follow-up visits and the levels of 11-dehydro-$TXB_2$ were measured at 1 month ($T_3$), 3 months ($T_4$), 6 months ($T_5$) and 12 months ($T_6$) following the PTA procedure. The method for measuring 11-dehydro-$TXB_2$ has been previously validated and compared with the results of a commercial enzyme-linked immunoassay (ELISA) in a population of asthmatics (6). Briefly, urine samples were collected and immediately frozen in 1 mL aliquots and stored at –70°C. After thawing on ice, the samples were centrifuged for 10 minutes at 10,000 × g at 4°C, and 0.5 mL of supernatant was used for the extraction. Each sample was mixed with the chemically identical internal deuterated standard 11-dehydro-thromboxane $B_2$-d4 (9α,15S-dihydroxy-11-oxothromba-5Z,13E-dien-1-oic-3,3,4,4-d4 acid, Cayman Chemical Co, Ann Arbor, MI, 2 ng). This allowed for the compensation of the variable recovery ratio (90 – 110%) and the analysis of other analytical errors. After the samples were acidified with acetic acid (pH = 3.5), a two-step extraction with 0.5 mL tert-buthyl-ether: methanol (80:20 V/V) was performed. The upper organic phase was separated by a short centrifugation and then dried under nitrogen at 37°C. The extract was dissolved in 60 µL methanol, and 10 µL was then used for high-performance liquid chromatography (HPLC) separation and mass spectrometry measurements. HPLC, equipped with autosampler (Shimadzu Sil-2-AC, Shimadzu Scientific Instruments, Inc. Columbia, MD, USA) and Zorbax Eclipse XDB C-18 column (100 × 3.0 mm, Agilent Technologies, Inc. Santa Clara, CA, USA), was used for the measurements. The following separation gradient was used: A, acetonitrile/water/acetic acid (20/80/0.0001) and B, acetonitrile/iso-propanol/acetic acid (55/45/0.0001, v/v). All of the reagents were of HPLC grade from JT Baker (Center Valley, PA). During the mobile phase binary linear gradient separation (1 min 8% B, 9.5 min 8 – 95% B, 0.5 min 95% B, 0.5 min 95 - 100% B, 2 min 100% B), the retention time for 11-hydroxy-dehydro-$TXB_2$ was 7.29 minutes. Analytes were measured using multiple reaction monitoring mode (MRM) tandem mass spectrometry (Qtrap 4000, AB Sciex, Concord, Ontario) equipped with electrospray ion source and operating in negative ionization mode. The urinary thromboxane metabolite had the pseudomolecular ion 367 Mz and monitored ion 161 Mz (371 and 165 Mz for the deuterated standard).

Quantifications were performed using a stable isotope dilution method from the area under the peak. The concentration of creatinine in the urine was measured using a standard enzymatic analytical assay on a Vitros 350 analyzer (Ortho Diagnostics, Raritan, NJ). The urinary excretion of the thromboxane metabolite was recalculated to pg per mg creatinine to compensate for a variable urine concentration. This assay was validated using pure chemical standards for intra- or inter-assay coefficients of variance (3.8% and 10.9%), and the lowest limit of quantification was determined to be 1.84 pg/mL with an accuracy of 97.95% (7).

During the follow-up visits, the clinical status of each patient was evaluated according to Rutherford's scale. The ankle-brachial index (ABI) test and Doppler ultrasound of target

*Table 1.* Characteristics of the patients.

| Categories | Whole group n = 175 | Without restenosis n = 125 | With restenosis n = 50 | P | Without SAE n = 164 | With SAE n = 11 | P |
|---|---|---|---|---|---|---|---|
| **Average Age** | 64.76 ± 8.94 | 64.2 ± 8.85 | 66.47 ± 9.1 | 0.2078 | 65,7 ± 9.05 | 60.0 ± 5.29 | 0.0317 |
| **Male** | 120 (69%) | 96 (77%) | 24 (48%) | 0.0002 | 111 (68%) | 9 (82%) | 0.5208 |
| **Female** | 55 (31%) | 29 (23%) | 26 (52%) | | 53 (32%) | 2 (18%) | |
| **Rutherford 3** | 112 (64%) | 88 (70%) | 24 (48%) | 0.0062 | 106 (65%) | 6 (54%) | 0.7260 |
| **Rutherford 4** | 63 (36%) | 37 (30%) | 26 (52%) | | 58 (35%) | 5 (46%) | |
| **Average Duration of PAOD [years]** | 3.81 ± 4.41 | 3.83 ± 4.81 | 3.76 ± 3.51 | 0.9708 | 3.79 ± 4.37 | 4.01 ± 5.69 | 0.9238 |
| **Diabetes Mellitus I** | 6 (3%) | 4 (3%) | 2 (4%) | 0.7374 | 5 (3%) | 1 (9%) | 0.8334 |
| **Diabetes Mellitus II** | 55 (31%) | 40 (32%) | 15 (30%) | 0.7968 | 50 (30%) | 5 (46%) | 0.4841 |
| **Hipertention** | 128 (73%) | 91 (73%) | 37 (74%) | 0.8926 | 119 (73%) | 9 (82%) | 0.0006 |
| **Heart Failure** | 21 (12%) | 14 (11%) | 7 (14%) | 0.5792 | 20 (12%) | 1 (9%) | 0.8630 |
| **Coronary Artery Disease** | 77 (44%) | 55 (44%) | 22 (44%) | 1 | 74 (45%) | 3 (27%) | 0.4005 |
| **Current Smokers** | 47 (27%) | 36 (29%) | 11 (22%) | 0.3461 | 44 (27%) | 3 (27%) | 0.7495 |
| **Smoking in the past** | 106 (61%) | 71 (57%) | 35 (70%) | 0.1117 | 101 (62%) | 5 (46%) | 0.4586 |
| **Average Pack-years of Smokers** | 35.01 ± 16.44 | 34.4 ± 16.91 | 36.92 ± 14.96 | 0.7022 | 34.58 ± 16.40 | 42.75 ± 16.39 | 0.14191 |
| **Hypercholesterolaemia** | 73 (42%) | 54 (43%) | 21 (42%) | 0.9038 | 70 (43%) | 3 (27%) | 0.4917 |

PAOD, peripheral arterial occlusive disease; SAE, serious adverse event.

arteries were performed. For the assessment of restenosis or reocclusion, a compound parameter of decreased ABI and confirmatory USG imaging with color Doppler was utilized. Additionally, a composite endpoint of myocardial infarction, stroke and death was assessed for the study group.

*Statistical analysis*

Quantitative features were characterized using the mean value ± standard deviation, while qualitative features were presented as frequencies and percentages. To determine if the quantitative data were normally distributed, the Shapiro-Wilk test was applied. Because the distribution of 11-dehydro-$TXB_2$ levels was not normal, these values were log(10)-transformed to calculate descriptive statistics and perform multivariate analyses. Measurements of 11-dehydro-$TXB_2$ in samples collected at the control visits after revascularization were analyzed by a repeated measures ANOVA to evaluate their change over time in relation to the effects of revascularization after 12 months. Graphs were generated to show the distribution of 11-dehydro-$TXB_2$ at each time point and at control visits. The patients were grouped depending on the occurrence of restenosis or reocclusion. Logistic regression analysis was used to evaluate the association between 11-dehydro-$TXB_2$ levels and the occurrence of restenosis or reocclusion, or the association between the composite endpoint, the occurrence of restenosis or reocclusion and the following factors: age, duration of disease, gender, presence of diabetes, hypertension, history of stroke, cigarette use (current and past), coronary artery disease, history of heart infarction, and hypercholesterolemia (*Table 2*). The odds ratios (ORs) and 95% confidence intervals (95% CI) for the significant associations were calculated. The qualitative variables were compared using the $\chi^2$ test, and Yates correction was applied to smaller samples.

The statistical analyses were performed with STATISTICA version 10 with MedPack (StatSoft Inc., Tulsa, OK, USA). P values < 0.05 were considered to be statistically significant.

## RESULTS

In total, 175 patients with PAOD who required endovascular treatment were enrolled in the study (demographic data are shown in *Table 1*). No signs of inflammation were observed in any of patients at the time of the PTA procedure. In all of the patients, body temperature was below 37°C and leukocytosis and CRP levels in the peripheral blood were normal. Ischemic symptoms graded as a 3 by Rutherford's scale were observed in 112 subjects and graded as a 4 in 63 patients. PTA was only performed in 41 cases (23.4%), whereas 134 patients (76.6%) required both PTA and stent implantation. All of the patients completed their 12 month follow-up.

During the 12-month follow-up period, we observed 50 cases of either restenosis or reocclusion. No events were observed at 1 month, but there were 13 events at 3 months, 16 at 6 months, and 21 at 12 months. Other serious follow-up events (not related to angioplasty) included 2 deaths by 6 months and 2 additional deaths by 12 months. Two cerebrovascular incidents (CVA) were noted at the 3 month time point and another subject had a CVA by 6 months. At 6 months, there was also a single case of myocardial infarction, and an additional two were observed at 12 months. During the follow-up period, no limb amputations were performed.

All of the patients were systematically evaluated for ABI values during the follow-up visits. Patients with restenosis or reocclusion demonstrated average ABI values at the target limb of 0.56 ± 0.039, 0.51 ± 0.043 and 0.60 ± 0.032 at the 3 month, 6 month and 12 month time points, respectively. In contrast, the ABI values in patients without restenosis or reocclusion were 0.80 ± 0.022, 0.77 ± 0.028 and 0.76 ± 0.028, respectively.

Overall, the mean values of 11-dehydro-$TXB_2$ immediately following PTA ($T_1$) were significantly higher than either before PTA ($T_0$) for the entire study group (1524.4 pg/mg creatinine ± 1411.1 vs. 2098.1 pg/mg creatinine ± 1661.8; P = 0.00002), on the day after PTA ($T_2$), or at any other time point during the study (*Fig. 1*).

*Table 2.* Univariate and multivariate analyses of risk factors for restenosis and serious adverse event (SAE).

| | **Restenosis (0/1)** | | | | **SAE (0/1)** | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariate analysis | | Multivariate analysis | | Univariate analysis | | Multivariate analysis | |
| | **OR (95%CI)** | **P** | **OR (95%CI)** | **P** | **OR (95%CI)** | **P** | **OR (95%CI)** | **P** |
| **Average Age** | 1.02 (0.99 – 1.1) | 0.21 | 1.01 (0.97 – 1.05) | 0.54 | 0.94 (0.87 – 1.0) | 0.07 | 0.93 (0.85 – 1.0) | 0.08 |
| **Gender** | 1.44 (0.73 – 2.9) | 0.28 | 1.42 (0.68 – 2.96 | 0.35 | 0.47 (0.09 – 2.3) | 0.33 | 0.25 (0.03 – 1.7) | 0.16 |
| **Diabetes** | 1.34 (0.68 – 2.6) | 0.39 | 1.06 (0.51 – 2.2) | 0.88 | 2.4 (0.71 – 8.4) | 0.15 | 2.7 (0.61 – 11.9) | 0.19 |
| **Hypertension** | 1.14 (0.43 – 3.0) | 0.79 | 0.96 (0.29 – 3.2) | 0.94 | 0.72 (0.09 – 6.0) | 0.76 | 1.20 (0.08 – 17.4) | 0.89 |
| **History of stroke** | 1.25 (0.43 – 3.6) | 0.68 | 1.14 (0.36 – 3.6) | 0.82 | 0.92 (0.11 – 7.84) | 0.94 | 0.51 (0.04 – 7.3) | 0.62 |
| **Current Smokers** | 0.52 (0.23 – 1.1) | 0.10 | 0.48 (0.14 – 1.7) | 0.25 | 1.02 (0.26 – 4.0) | 0.97 | 0.21 (0.02 – 1.77) | 0.15 |
| **Smoking in the past** | 1.30 (0.66 – 2.5) | 0.44 | 0.89 (0.31 – 2.5) | 0.83 | 0.51 (0.15 – 1.8) | 0.30 | 0.17 (0.02 – 1.3) | 0.08 |
| **Coronary Artery Disease** | 1.01 (0.51 – 2.0) | 0.97 | 1.02 (0.41 – 2.6) | 0.96 | 0.45 (0.11 – 1.8) | 0.25 | 0.67 (0.07 – 6.8) | 0.73 |
| **Heart Failure** | 0.93 (0.44 – 1.94) | 0.85 | 0.84 (0.29 – 2.4) | 0.74 | 0.59 (0.12 – 2.9) | 0.51 | 1.08 (0.08 – 14.2) | 0.95 |
| **Hypercholesterolaemia** | 0.84 (0.44 – 1.6) | 0.61 | 0.77 (0.37 – 1.6) | 0.47 | 1.99 (0.5 – 7.8) | 0.3 | 0.47 (0.09 – 2.6) | 0.38 |
| **Preoperative log**[$TXB_2$] | 1.37 (0.55 – 3.4) | 0.50 | 1.48 (0.68 – 2.96) | 0.42 | 7.8 (1.25 – 48.9) | 0.03 | 9.25 (1.12 – 76.2) | 0.037 |

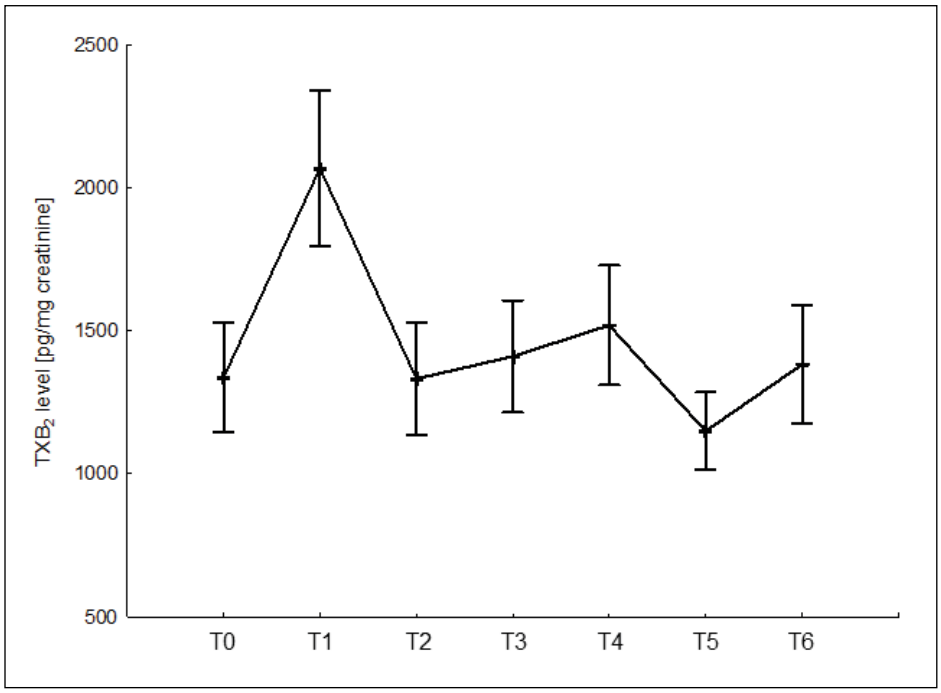


*Fig. 1.* An outburst of 11-dehydro-$TXB_2$ follows the percutaneous transluminal angioplasty (PTA) procedure (T1) in all subjects of the study. Significant increase of thromboxane B2 ($TXB_2$) was observed only 1 day after PTA (P = 0.00002).

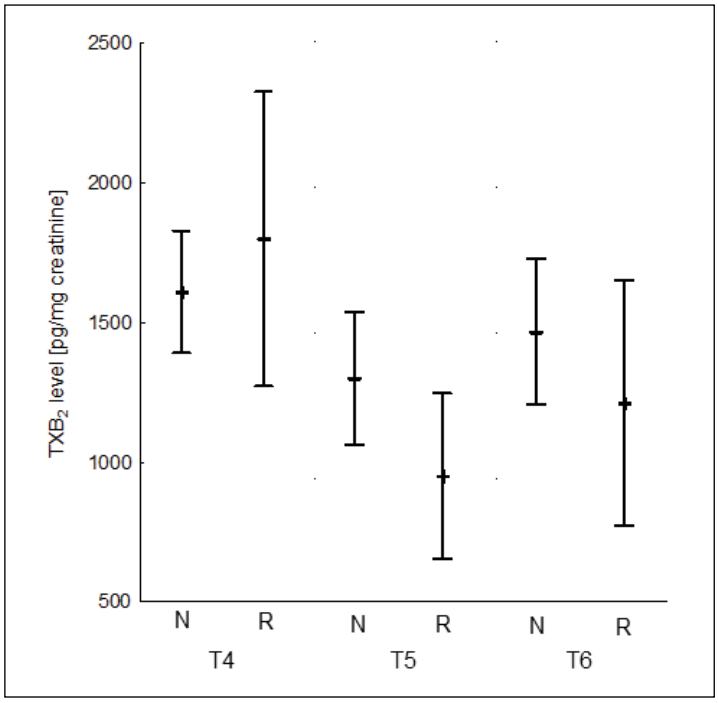


*Fig. 2.* A level of 11-dehydro-$TXB_2$ at 3rd month (T4 P > 0.05), 6th month (T5 P > 0.05) and at 12th month (T6 P > 0.05) follow up visit patients for restenosis/reocclusion. N, no restenosis or re-occlusion; R, restenosis or re-occlusion.

In the group of patients with restenosis or reocclusion during the follow-up time period, 11-dehydro-$TXB_2$ levels (T4, T5, T6) were comparable to patients without restenosis or reocclusion (*Fig. 2*). Moreover, perioperative 11-dehydro-$TXB_2$ levels ($T_0$, $T_1$, $T_2$) did not correlate with restenosis or reocclusion in the follow-up period. The levels of 11-dehydro-$TXB_2$ at a given follow-up were not predictive of the occurrence of restenosis or reocclusion in subsequent months.

However, preoperative 11-dehydro-$TXB_2$ levels ($T_0$) correlated well with the composite endpoint of death, myocardial infarction (MI), and stroke during the follow-up period (OR 7.42 [CI 95% = 1.2 – 48.8]; P = 0.02) (*Fig. 3*). These serious adverse events (SAE) recorded during the follow-up period were compared to the 11-dehydro-$TXB_2$ values in *Fig. 3*.

Classical risk factors of atherosclerosis progression (age, duration of disease, diabetes, hyperlipidemia, hypertension, tobacco smoking) did not affect restenosis or reocclusion events.

## DISCUSSION

In our study, we found that, following PTA, the level of thromboxane is transiently increased. This result is in agreement with Parmar *et al.* (8). Percutaneous transluminal angioplasty

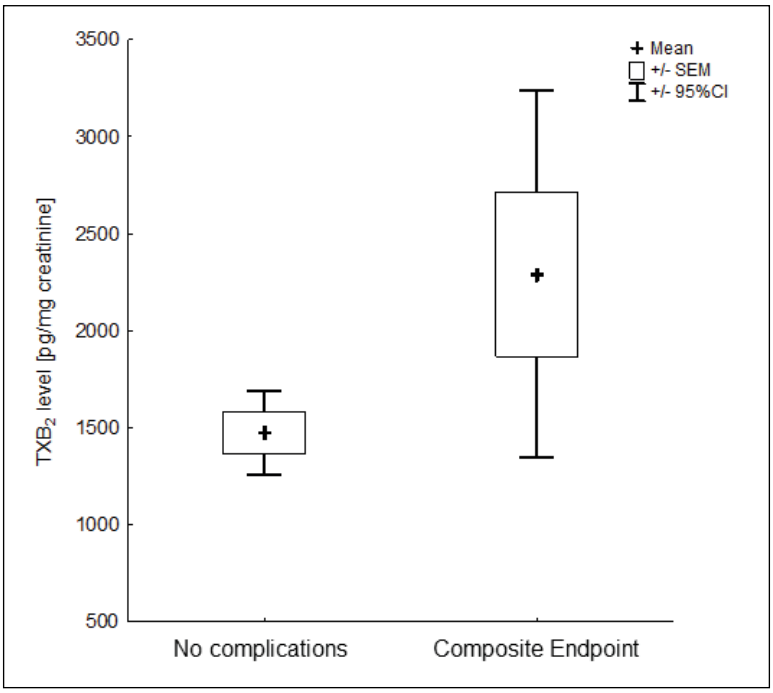


*Fig. 3.* There is significant dependence between preoperative 11-dehydro-$TXB_2$ level ($T_0$) and composite endpoints (OR 7.42 [CI 95% = 1.2 – 48.8], P = 0.02).

(PTA) is an acute, local stimulus to platelets in which activation is regarded as an important factor for a later restenosis. PTA has been shown to induce endothelial injury with subsequent platelet aggregation, activation of macrophages, and smooth muscle cells (9).

We also found that preoperative 11-dehydro-$TXB_2$ levels ($T_0$) correlated well with the composite endpoint of death, myocardial infarction, and stroke during a 12 month follow-up period. Therefore, the level of $TXB_2$ is a risk factor for fatal composite endpoints. In 2012, Niccoli *et al.* (10) showed that the platelet count, multivessel coronary disease, and coronary atherosclerosis extent index were independent predictors of the $TXB_2$ upper quartile level. In 2013, Vazzana *et al.* (11) showed that urinary 8-iso-$PGF_{2\alpha}$ and 11-dehydro-$TXB_2$, both *in vivo* markers of oxidative stress and platelet activation, were potential contributors to increased cardiovascular risk in patients with a low-HDL phenotype.

Thrombosis and restenosis remain frequent complications after PTA in patients with peripheral arterial disease. Vascular smooth muscle cell proliferation, increased extracellular matrix volume, thrombosis and vascular remodeling all contribute to the pathogenesis of restenosis (12). Restenosis at the site of a previously successful PTA is an important limitation to the long-term benefits of this procedure (13). Platelet activation is regarded as an important stimulus for a later restenosis (14). The balance between the production of prostacyclin (PGI2) and thromboxane A2 ($TXA_2$) is of great importance because of their opposite actions on vascular tone and platelet reactivity. The eicosanoids (PGI2 and $TXA_2$) are derived from arachidonic acid metabolism, and they are found at the sites of inflammation. $TXA_2$, a potent smooth muscle constrictor (15) and platelet aggregator (16), and PGI2, an effective vasodilatator and platelet antiaggregator (17) are the major products formed in the cyclooxygenase-catalyzed arachidonic acid metabolic pathway in platelets (18) and endothelial cells (19), respectively.

The balance between PGI2 and $TXA_2$ is shifted toward the formation of $TXA_2$ in several cardiovascular diseases (20-26). The influence of peripheral PTA on PGI2 and $TXA_2$ synthesis has been studied *in vitro* in rabbits by Mattsson *et al.* (27). In this study, the authors found that the procedure decreased the release of PGI2 from a dissected vessel wall but did not change the formation of $TXA_2$. Additionally, a previous clinical study in patients undergoing PTA for peripheral arterial disease used urinary $TXB_2$ and $PGF_{2\alpha}$ to demonstrate platelet activation. They showed a twofold increase in $TXA_2$ but no significant change in PGI2 production after PTA, which shifted the PGI2/$TXA_2$ balance toward $TXA_2$ formation (28). Increased $TXA_2$ release was also seen in PTA of coronary arteries (29).

Thrombosis and restenosis are frequent complications after either PTA or surgical revascularization in peripheral arterial occlusive disease. There is accumulating evidence to suggest that oxidative stress in the vessel wall is involved in atherosclerosis (30). In particular, oxidative stress can occur after balloon angioplasty and this may contribute to the development of restenosis (31). Several pieces of experimental data indicate that the modulation or suppression of the inflammatory response after endovascular treatment may exert beneficial effects on the outcome of the patient. In patients with high C-reactive protein levels after endovascular coronary intervention, immunosuppressive therapy with prednisolone resulted in a reduction of clinical events and angiographic stenosis rate (32).

However, one must remember that, while platelets can be a source of urinary $TXB_2$, young platelets or other inflammatory cells can also produce $TXB_2$. Thus, the sources of urinary $TXA_2$ metabolite excretion cannot be differentiated.

In conclusion, our report describes the levels of 11-dehydro-$TXB_2$ after PTA in PAOD patients during a 12 month follow-up period. We also showed that the preoperative 11-dehydro-$TXB_2$ levels ($T_0$) correlated well with the composite endpoint of death, myocardial infarction and stroke during this follow-up period. These findings suggest that clinicians should consider the use of $TXA_2$ synthase inhibitors and receptor antagonists in combination with peripheral percutaneous transluminal angioplasty. This combination may help to prevent thrombosis and restenosis. This study also highlights that a low dose of acetylsalicylic acid and intermittent clopidogrel are not sufficient to protect atherosclerosis patients against major cardiovascular events in those patients which have elevated thromboxane biosynthesis. Further research in this area is recommended.

We also recognize the limitations of observational design, use of a single center setting, and are aware of the possibility of other existing confounders.

*Abbreviations:* ABI, ankle-brachial index; CVA, cerebrovascular incident; MI, myocardial infarction; PAOD, peripheral arterial occlusive disease; PGI2, prostacyclin; PTA, percutaneous transluminal angioplasty; SAE, serious adverse event, $TXA_2$, thromboxane $A_2$; $TXB_2$, thromboxane $B_2$.

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