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Association of plasma concentrations of salicylic acid and high on ASA platelet reactivity in type 2 diabetes patients

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Abstract

Background: *The objective of this study was to investigate the association between plasma concentrations of salicylic acid (SA) and other minor acetylsalicylic acid (ASA) metabolites and high on ASA platelet reactivity assessed with different methods in type 2 diabetic patients (T2DM).*

Methods: *Study cohort consisted of 293 T2DM patients on chronic ASA therapy. Platelet function inhibition was analyzed using measurements of serum thromboxane B_2 (S-TxB_2), VerifyNow Aspirin and Platelet Function Analyzer (PFA)-100 assays. The concentration of ASA metabolites in plasma was measured with a high-performance liquid chromatography (HPLC).*

Results: *In logistic regression analysis both ASA dose/kg of body weight and plasma SA concentration were found to be predictive of S-TxB_2 concentrations above 0.72 ng/mL cut-off point (OR 16.9, 95% CI 2.29–125.8, $p = 0.006$ and OR 5.34, 95% CI 2.67–10.68, $p < 0.001$, respectively). When using the VerifyNow Aspirin Assay, the concentrations of SA were significantly lower ($p = 0.007$) in the group with high on ASA platelet reactivity when compared with the group with normal on ASA platelet reactivity. In logistic regression analysis plasma SA concentration was found to be predictive of VerifyNow Aspirin Reaction Units (ARU) ≥ 550 (OR 3.86, 95% CI 1.86–8.00, $p < 0.001$).*

Conclusions: *Our study suggests that disturbances of pharmacokinetic mechanisms might contribute to lower plasma SA levels, and subsequently incomplete inhibition of thromboxane A_2 synthesis as measured with S-TxB_2 concentrations and increased platelet reactivity measured with VerifyNow in T2DM patients.* (Cardiol J 2013; 20, 2: 170–177)

Key words: aspirin, diabetes, salicylic acid, platelet reactivity, platelet function testing

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Introduction

Acetylsalicylic acid (ASA) inhibits platelet aggregation through irreversible acetylation of platelet cyclooxygenase (COX)-1 and subsequent inhibition of the transformation of arachidonic acid (AA) into thromboxane A_2 (TxA_2), a potent vasoconstrictor and aggregating agent [1]. ASA selectively and rapidly acetylates the COX-1 enzyme, and is, in this respect, 166 fold more effective in inhibiting COX-1 than COX-2 enzyme. In addition, immediate ASA-degradation product, salicylic acid (SA), weakly binds to a COX-1 second binding site at Arg120 and may contribute to ASA antiplatelet effect [2]. Despite its well-documented therapeutic benefits, ASA treatment does not provide complete protection against cardiovascular events, which can be related to increased platelet reactivity. The reasons for such variability in the therapeutic response are poorly understood, though several studies have evaluated the effect of ASA to explain high platelet reactivity particularly in diabetic individuals on ASA therapy [3–7]. ASA is an effective inhibitor of platelet TxA_2 production, nevertheless is often considered as overall relatively weak platelet inhibitor because of its limited effects on aggregation in the presence of high concentrations of such agonists as adenosine diphosphate (ADP) or collagen. This may account for some of the variability of the response to these stimulants, which activate platelets through both COX-dependent and COX-independent pathways [8–13]. In addition, increased hydrolysis of circulating ASA, which corresponds to faster elimination of the drug from the circulation by degradation into SA and acetate, may also cause an altered response to ASA [6, 14].

The goal of this study has been to evaluate, in exclusively type 2 diabetes mellitus (T2DM) population treated with ASA, whether an association could be observed between plasma concentrations of SA and other minor ASA metabolites and high on ASA platelet reactivity assessed with different methods. For this purpose TxA_2 metabolites were measured in serum in order to obtain a specific measure of ASA-induced COX-1 inhibition. We also assessed the VerifyNow Aspirin Assay, which demonstrates very high sensitivity to COX-1 dependent ASA effects [8]. In addition, shear dependent platelet functions were measured with less COX-1 inhibition dependent device — Platelet Function Analyzer (PFA)-100.

Methods

Patient population and study design

The local ethics committee of the Medical University of Warsaw approved both the study protocol and the informed consent form. The study was conducted in accordance with the current version of the Declaration of Helsinki at the time when the study was designed, and informed written consent was obtained from all patients. The study subjects were recruited consecutively from patients with T2DM participating in a multi-center, prospective, randomized, and open-label AVOCADO [Aspirin Vs/Or Clopidogrel in Aspirin-resistant Diabetics inflammation Outcomes] study presenting to the outpatient clinic of the Central Teaching Hospital of the Medical University of Warsaw. The full characterization of the study population, including the inclusion and exclusion criteria were published previously [15]. Briefly, 304 subjects with T2DM were recruited who, at the time of enrollment, had been taking ASA tablets at the dose of 75 mg per day for at least 3 months for primary or secondary prevention of myocardial infarction (MI). No clopidogrel or antiplatelet drugs other than ASA were used in any of the investigated patients. All patients had been taking oral antidiabetic agents and/or insulin for at least 6 months; diet-controlled diabetic patients were not included. Compliance to ASA therapy was determined at the study entry based upon the patient's own statement and serum thromboxane B_2 ($S\text{-}TxB_2$) level measurement.

Blood sample and assay procedures

Blood samples were collected in the morning, after an overnight fast and 2–3 hours after the last ASA dose. Blood was obtained from the antecubital vein, and the initial 2 mL of blood were discarded to avoid spontaneous platelet activation. Blood was drawn into tubes containing 3.2% sodium citrate for VerifyNow measurements, 3.8% sodium citrate for PFA-100 measurements. All blood samples were processed within 2 h of collection. Whole blood for $S\text{-}TxB_2$ was allowed to clot at 37°C for 1 h before separating serum by centrifugation. Serum was obtained from venous blood by centrifugation at 1000 g for 15 min at 4°C, and aliquots were stored at -80°C for further analysis.

High-performance liquid chromatography method for determination of ASA and its metabolites in plasma. The concentration of ASA

and its metabolites in plasma was measured by method described by Krivosikova et al. [16]. A high--performance liquid chromatograph (HPLC) system (Knauer, Germany) was equipped with UV variable wavelength diode array detector and C18 reverse phase column 150 × 3.9 mm (Waters). The mobile phase consisted of water-85% phosphoric acid--butanol-tetrabutylamoniumhydroxide-methanol (134:1:1:63). The flow rate was 0.9 mL/min, the system was heated to 45°C, the wavelength of detector was set at 237 nm. Salicylic acid, gentisic acid (GA) (Sigma) and salicyluric acid (SUA) (all from Roth) were used as calibration standards. The chromatograms analysis was performed with ClarityChrom Software (Knauer, Germany) [16].

Serum thromboxane-B_2 (S-TxB_2). S-TxB_2 was measured also with an enzyme immunoassay kit according to the manufacturer's instructions (Cayman Chemicals, Ann Arbor, MI, USA). Samples with results outside the standard curve were re-assayed with appropriate dilutions. An optimal compliance was confirmed by S-TxB_2 levels below 7.2 ng/mL in all patients as described previously in a diabetic population by Mortensen et al. [5].

Analysis of platelet functions. Platelet reactivity was measured with VerifyNow Aspirin Assay (Accumetrics, San Diego, CA, USA) and PFA-100 assay (Dade-Behring International, Inc., Newark, DE, USA). These assays were performed as described in detail previously [15]. In current study, normal platelet reactivity on ASA therapy with a PFA-100 was defined as collagen/epinephrine closure time (CEPI-CT) ≥ 193 s (the manufacturer's lower limit of the normal range for aspirin-free healthy controls) and with VerifyNow Aspirin Assay as Aspirin Reaction Units (ARU) ≥ 550 [15].

Statistical analysis

Continuous variables are presented as mean ± ± standard deviation and categorical variables are presented as frequencies and percentages. All variables were tested for non-normality by the Kolmogorov-Smirnov test. Continuous variables were compared using the Student T-test or the nonparametric Mann-Whitney U test, and categorical variables were compared using the χ^2 or Fisher exact test, when applicable. Spearman's Kendall and Pearson correlation coefficients were obtained to evaluate the association of ASA and metabolites levels and various phenotypic factors, including among others ASA dose in mg/kg of body weight, as well as residual platelet reactivity and S-TxB_2 concentrations. A two-sided P-value of < 0.05 was considered significant. To determine parameters linked to ASA metabolism and residual platelet reactivity, recursive partitioning and logistic regression models were used to build binary classification trees and to estimate odds ratios (OR) and 95% confidence intervals (CI) for each splitting factor. We applied the Classification Tree routine in the SPSS software to construct a classification tree accounting for potential confounders. As an alternative to logistic models, tree-based models rectify classification analysis, and they are useful for non-linear dependent and for visualizing complex interactions. Since recursive partitioning is exploratory and not hypothesis testing, multiple comparison tests were not applied. The end results of recursive partitioning were terminal nodes representing combinations of independent factors associated with an increased likelihood of high on ASA platelet reactivity. Analyses were performed with SPSS 20.0 for Windows (SPSS Institute, IL, USA). In all cases two-tailed p values < 0.05 were considered statistically significant.

Results

From the initially enrolled 304 patients, complete clinical data and blood samples became available for 293 patients. Subsequently, 8 patients were eliminated from the analysis based on the suspected ASA non-compliance (S-TxB_2 concentrations > 7.2 ng/mL). Demographic characteristics, clinical data, and results of platelet function tests are summarized in Table 1. The variability of plasma concentrations of SA, GA and SUA in T2DM patients are illustrated in Figure 1. There was no statistically significant correlation observed between independent binary variables (e.g., disease conditions or concomitant medications) and the plasma concentrations of SA or other minor metabolites. No statistically significant correlation was observed between plasma concentrations of SA, GA and SUA and continuous variables (Table 2).

The measured plasma concentrations of S-TxB_2 varied widely between 0 and 6.9 ng/mL (median 0.153 ng/mL, interquartile range [IQR] 0.05–0.61 ng/mL) in the investigated T2DM patients taking ASA for more than 3 months. No simple linear relationship was established between plasma concentrations of SA and S-TxB_2 (data not shown). However, both bivariate correlation coefficients (Kendall's tau b and Spearman's rho between –0.23 and –0.33) showed low correlation for S-TxB_2 with the plasma SA concentration at the $p = 0.01$ significance level. In order to further analyze the relationship between S-TxB_2 and SA

Table 1. Demographic and clinical characteristics of the study patients (n = 293).

Demographic	
Age [years]	67.5 ± 8.7
Female gender	138 (47.1%)
Duration of diabetes [years]	10.2 ± 8.8
BMI [kg/m^2]	31.19 ± 11.9
Dyslipidemia	240 (81.9%)
Hypertension	271 (92.5%)
CAD	164 (56.0%)
Prior MI	88 (30.0%)
Prior stroke	24 (8.2%)
Heart failure	109 (37.2%)
History of smoking	167 (57.0%)
Current smoking	29 (9.9%)
Concurrent medications	
Oral hypoglycemic	252 (86.1%)
Insulin	93 (31.7%)
Beta-blockers	209 (71.3%)
ACE inhibitors	192 (65.5%)
Statins	210 (71.7%)
PPI	72 (24.9%)
Biochemical parameters	
HGB [mmol/L]	8.6 ± 0.8
HCT	0.41 ± 0.04
RBC [10^{12}/L]	4.6 ± 0.46
WBC [10^9/L]	7.1 ± 2.14
PLT [10^9/L]	226.1 ± 57.8
MPV [fl]	9.9 ± 1.2
eGFR [mL/min/1.73 m^2]	71.0 ± 20.5
Creatinine [μmol/L]	88.4 ± 26.52
Fasting glucose [mmol/L]	7.5 ± 2.1
HbA1c [%]	7.0 ± 1.3
hsCRP [mg/L]	4.2 ± 5.8
Fibrinogen [μmol/L]	12.56 ± 3.1
Total cholesterol [mmol/L]	4.3 ± 1.0
Triglycerides [mmol/L]	1.52 ± 0.76
HDL-C [mmol/L]	1.26 ± 0.35
LDL-C [mmol/L]	2.29 ± 0.8
vWF%	136.7 (101.9–178.7)
S-TxB$_2$ [ng/mL]	0.153 (0.049–0.61)
Verif yNow [ARU]	458 (417–518)
CEPI-CT [s]	265.00 (171.00–300.00)
CADP-CT [s]	97.00 (78.5–129.5)

Data are expressed as mean ± SD or N (%) or median (interquartile range); BMI — body mass index; CAD — coronary artery disease; MI — myocardial infarction; ACE — angiotensin-converting enzyme; PPI — proton pump inhibitors; HGB — hemoglobin; HCT — hematocrit; RBC — red blood cells; WBC — white blood cells; PLT — platelet count; MPV — mean platelet volume; eGFR — estimated glomerular filtration rate; HbA1c — glycosylated hemoglobin; hsCRP — high sensitivity C-reactive protein; HDL-C — high density lipoproteins cholesterol; LDL-C — low density lipoproteins cholesterol; vWF — von Willebrand factor; S-TxB$_2$ — serum thromboxane-B$_2$; ARU — aspirin reaction units; CEPI-CT — collagen/epinephrine closure time by PFA-100 method; CADP-CT — collagen/adenosine diphosphate closure time by PFA-100 method

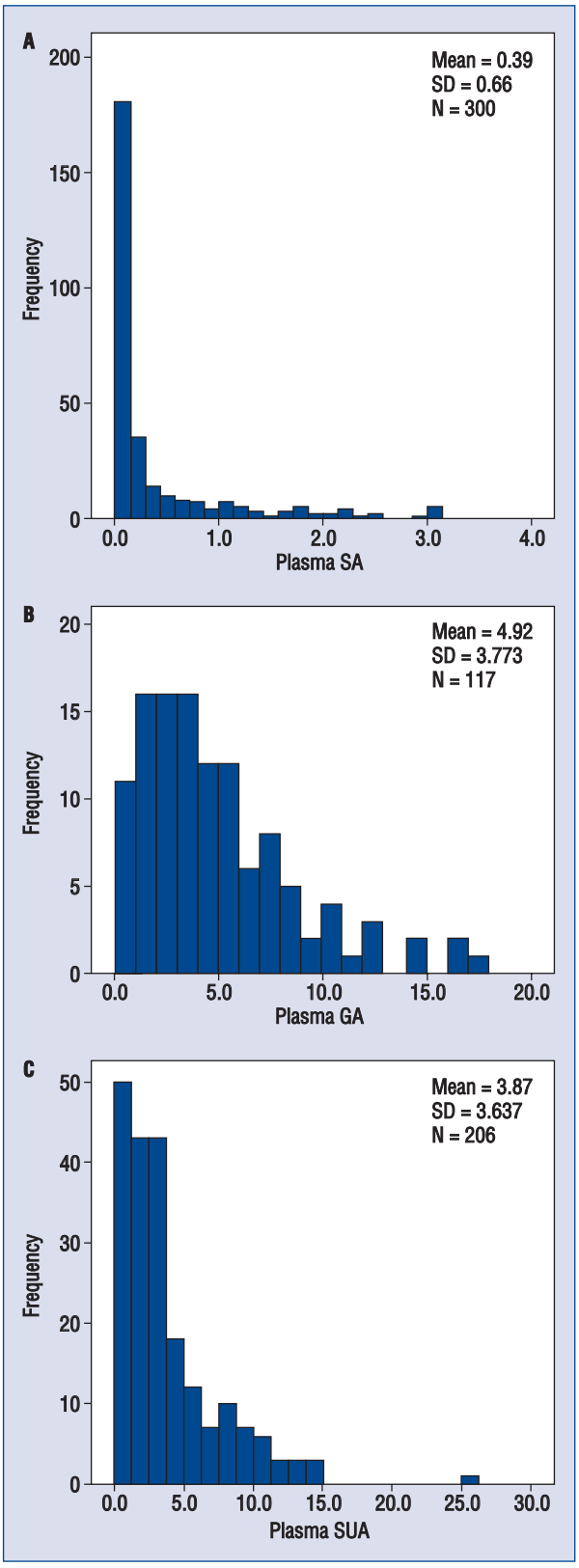

Figure 1. Frequency distribution of plasma concentrations (in ng/μL) of salicylic acid (SA) (**A**), gentisic acid (GA) (**B**) and salicyluric acid (SUA) (**C**) in investigated diabetic population.

Table 2. Plasma concentrations in ng/μL of salicylic acid (SA), gentisic acid (GA) and salicyluric acid (SUA) in the population of diabetic patients during chronic acetylsalicylic acid (ASA) treatment.

		SA [ng/μL]	GA [ng/μL]	SUA [ng/μL]
Mean		0.385120	4.9151	3.8675
Variance		0.436	14.237	13.225
Median		0.088763	3.9740	2.7315
Minimum		0.0000	0.47	0.13
Maximum		3.1319	17.25	25.28
Percentile	25th	0.031106	2.2750	1.3113
	50th	0.088763	3.9740	2.7315
	75th	0.393150	6.7720	5.2190

(in binary format), we have established several empirical cut-off criteria (e.g., at 75th percentile — 0.72 ng/mL, and at 95th percentile — 5.1 ng/mL) which divide the investigated patients into 2 categories based on S-TxB_2 concentration (0 = = response below selected cut-off point and 1 = = response above the selected cut-off point). Statistically lower concentrations of SA were observed in the patients above both cut-off values (0.72 and 5.1 ng/mL) of S-TxB_2 concentrations ($p < 0.0001$ and $p = 0.003$, respectively by Mann-Whitney test).

The results of the hierarchical classification tree analysis (recursive partitioning) of the independent factors involved in the different S-TxB_2 concentrations (dependent variable) during prolonged ASA treatment is shown in Figure 2. S-TxB_2 concentrations (at Node 0) were classified as 0 (below cut-off point) and 1 (above cut--off point) based on 75th percentile of observed S-TxB_2 concentrations (0.72 ng/mL). The second level response (Node 1 and Node 2) were based on the calculated cut-off plasma concentration of SA (0.13 ng/μL), which created two different groups of patients with different effects on S--TxB_2 concentrations ($p < 0.001$, χ^2). The third level of response was only observed for Node 1 (i.e. patients with SA plasma concentrations of < 0.13 ng/μL) and based on the ASA dose/kg of body weight (i.e. ≤ 0.862 mg/kg of body weight and > 0.862 mg/kg of body weight), and indicates that these two different groups of ASA/kg of body weight dosing displayed statistically significant ($p = 0.003$, χ^2) differences in effect of plasma SA concentration range which influence S-TxB_2 concentrations. In logistic regression analysis both ASA dose/kg of body weight and plasma SA concentration were found to be predictive of S-TxB_2 concentrations above cut-off point (i.e. 0.72 ng/mL) (OR 16.9, 95% CI 2.29–125.8, $p = 0.006$ and OR

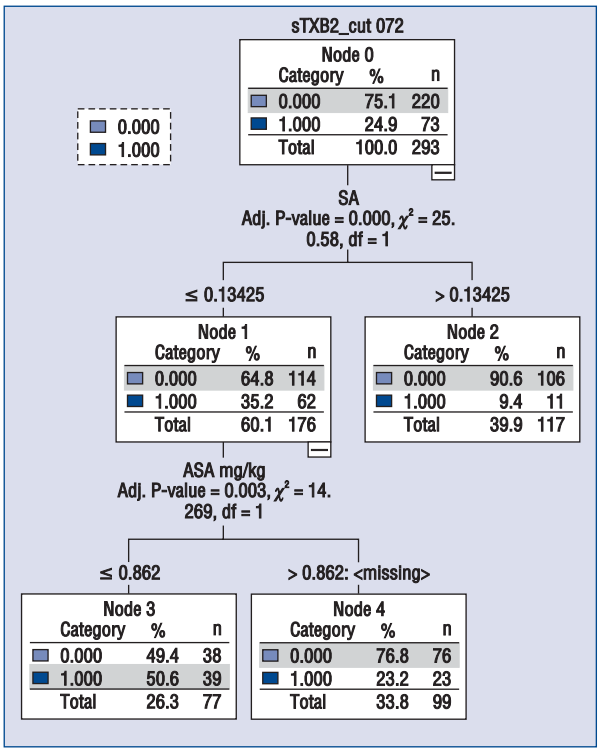

Figure 2. Hierarchical classification tree (recursive partitioning) analysis of the independent factors (salicylic acid [SA] plasma concentrations and acetylsalicylic acid [ASA] dose in mg/kg) involved in the different serum thromboxane-B_2 (S-TxB_2) response (dependent variable) during prolonged ASA treatment. S-TxB_2 concentrations (at Node 0) were classified as 0 (below cut-off point or normal on ASA platelet reactivity) and 1 (above cut-off point, or high on ASA platelet reactivity) based on 75th percentile of observed S-TxB_2 concentrations (0.072 ng/mL). The second level (Node 1 and Node 2) were based on the calculated cut-off plasma concentration of SA (0.13 ng/μL), which created two different groups of patients with different effects on S-TxB_2 ($p < 0.001$, χ^2). The third level was only observed for node 1 (i.e. patients with SA plasma concentrations of < 0.13 ng/μL) and based on the ASA dose/kg of body weight (i.e. ≤ 0.862 mg/kg and > 0.862 mg/kg), and indicates that these two different groups of ASA/kg of body weight dosing displayed statistically significant ($p = 0.003$, χ^2) differences in effect of SA concentration range which influence S-TxB_2 concentration.

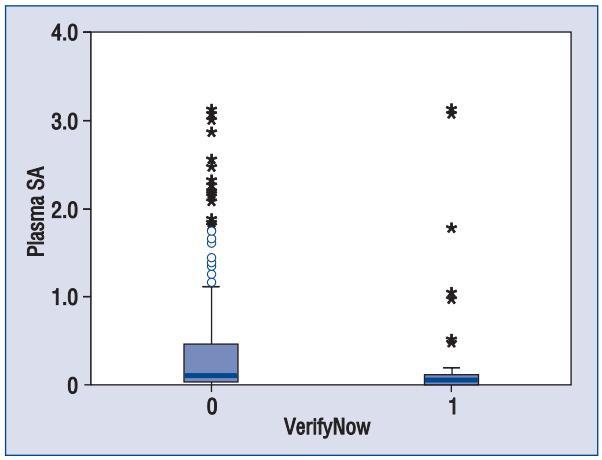

Figure 3. Plasma concentrations of salicylic acid (SA) in relation to platelet activity evaluated using VerifyNow test. VerifyNow = 0 indicates normal on acetylsalicylic acid (ASA) platelet reactivity (Aspirin Reaction Units [ARU] < 550; N = 239 patients; 81.56%) and VerifyNow = 1 indicates high on ASA platelet reactivity (ARU ≥ 550, N = 54 patients; 18.44%). Concentrations of SA was expressed in ng/μL. Statistical significance between groups was analyzed using Mann-Whitney test. Statistical significance was assumed at $p < 0.05$.

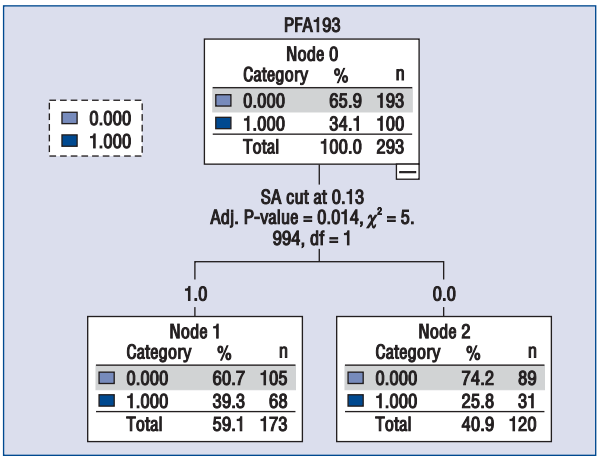

Figure 4. Hierarchical classification tree (recursive partitioning) analysis of the independent factors (i.e. plasma SA concentrations) involved in the collagen/epinephrine closure time (CEPI-CT) ≥ 193 s (dependent variable) during prolonged acetylsalicylic acid (ASA) treatment (see details in text). PFA-100 CEPI-CT (at Node 0) was classified as 0 (CEPI-CT ≥ 193 s) and 1 (CEPI-CT < 193 s). The second level (Node 1 and Node 2) were based on the calculated cut-off plasma concentration of SA (0.13 ng/μL), which created two different groups of patients with different effects on CEPI-CT ($p < 0.014$, χ^2).

5.34, 95% CI 2.67–10.68, $p < 0.001$, respectively). There was low linear correlation between SA concentration and VerifyNow ARU (Pearson –0.162, $p = 0.003$). The concentrations of SA were significantly lower ($p = 0.007$) in the group with high on ASA platelet reactivity defined as ≥ 550 ARU (0.639 ng/mL) when compared with the group with normal on ASA platelet reactivity (0.101 ng/mL) (Fig. 3). In logistic regression analysis plasma SA concentration was found to be predictive of VerifyNow ARU ≥ 550 (OR 3.86, 95% CI 1.86–8.00, $p < 0.001$).

In respect to relation between CEPI-CT (as measured by PFA-100) and SA concentrations, statistically significant logarithmic regression was observed between SA concentrations split at 0.13 ng/μL (Node 0 above, in hierarchical analysis) and CEPI-CT ≥ 193 s (OR 1.88, 95% CI 1.1–3.1, $p < 0.015$). No other analyzed independent variables correlated with CEPI-CT threshold at ≥ 193 s (Fig. 4). Statistically significant correlation (by Pearson, Kendall's tau b and Spearman's rho, $p > 0.05$) could be established between neither plasma concentrations of SA and CADP-CT original values (in [s]), nor between S-TxB_2 and CADP-CT original values (in [s]) (data not shown). Moreover, no non-parametric correlation (Kendall's tau and Spearman's; $p > 0.05$) between HbA1c and CEPI-CT, CADP-CT, VerifyNow ARU, S-TxB_2 and all measured ASA metabolites were found. In logarithmic regression analysis only week correlation ($p < 0.07$) between HbA1c and CEPI-CT < 193 s, but not with CADP-CT, VerifyNow ARU and other measured parameters, was observed.

Discussion

The results of our study demonstrate that in the population of diabetic patients treated with ASA for primary or secondary prevention for the period of at least 3 months, plasma SA levels correlate with point-of-care platelet function assays used for determination of platelet reactivity, and can be to some degree predictive of S-TxB_2 level, and thus platelet's COX-1 inhibition. In the present study all investigated patients received the same dose of ASA, namely 75 mg daily, which is widely used in the clinical practice and, based on previous reports, appears sufficient to fully inhibit COX-1 activity as demonstrated by > 95% inhibition of S-TxB_2, at least in non-diabetic individuals [7, 17, 18].

Based on the cut-off values for S-TxB_2 we excluded from further analysis 8 patients, and as the number of excluded patients was very low it does not appear to affect overall statistic calcula-

tions. This approach is in agreement with current trend observed in lately published studies as lack of reliable method used for the assessment of compliance is consider to be a major limitation of previously published papers in this field [5, 9].

ASA is mainly metabolized by the liver and intestinal human carboxylesterase-2 to acetyl and salicylate moieties, and has a half-life of 15 to 20 min, whereas salicylate has a half-life of 3 to 6 h [19]. SA level measurements have been applied in order to assess compliance to ASA therapy according to numerous recently published studies [20–23]. However there are only few studies that directly evaluated the relationship between SA level and different tests of platelet function [22–24]. In our study S-TxB_2 was < 7.2 ng/mL in vast majority of subjects, with exception of 8 patients, indicating low TxB_2 generation, which confirms the effective inhibitory effect of ASA on the COX-1 activity in platelets from blood samples collected in T2DM population treated with ASA for at least 3 months.

TxA_2 is a potent platelet activator and vasoconstrictor, and S-TxB_2 is a measure of maximum platelet capacity to produce TxA_2 [25]. Once-daily administration of low-dose ASA may be associated with incomplete inhibition of platelet COX-1 activity and thromboxane-dependent function in diabetics [3, 4, 23, 26]. In the present study, despite the lack of simple linear relationship between plasma concentrations of SA and the S-TxB_2, we found lower concentrations of SA in several groups of patients selected based on empirical cut-off points for S-TxB_2. In a further analysis predictive factors involved in the different S-TxB_2 concentrations were low SA plasma concentrations. In contrary to our study Pulcinelli et al. [22] did not find any correlation between plasma SA level and S-TxB_2 production. In addition, in a previously published HPLC in pharmacokinetic study aimed on 24-h variations of biological sensitivity to ASA and platelet reactivity measured with AA induced light transmission aggregometry (LTA) in population of patients with coronary artery disease (CAD) study by Henry at al. [23], no differences were found between SA concentrations and platelet reactivity.

It was previously reported, that an increased body weight has been associated with a lower biochemical responsiveness to ASA, as assessed by S-TxB_2 or platelet function assays, and with a possible lower clinical efficacy of low-dose ASA, although the clinical impact of this phenomenon has never been formally tested in large trials [27–29]. This pharmacokinetic-based mechanism reducing ASA responsiveness in a fraction of patients with obesity may be especially important in T2DM. Based on these considerations, we calculated ASA dose in mg/kg of body weight, and we found that lower dose of ASA based on the ASA dose per kg of body weight can predict higher S-TxB_2 concentrations. As it was suggested by others, the higher doses or twice daily dosing of ASA may overcome the reduced platelet response to ASA, thus increased platelet reactivity, observed in diabetic patients [3, 26].

Moreover, based on our analysis we found the lower plasma concentration of SA in patients with high platelet reactivity based on VerifyNow measurements. In addition, the subsequent analysis showed that low SA plasma concentrations have a predictive value for high on ASA platelet reactivity mesured with this COX-1 specific method. In our study, SA concentrations were also higher in patients with normal on ASA platelet reactivity measured with PFA-100 CEPI-CT assay when compared with patients with high on ASA platelet activity. No correlation could be established in our study between the history of CAD in the investigated cohort of T2DM patients, platelet functions parameters or the plasma concentrations of ASA metabolites. Similarly to our results, in the small study of 10 MI survivors and 10 healthy controls, Ahmed et al. [24] did not find any correlation between SA level and PFA-100 CEPI-CT in survivors of MI taking different doses of ASA ranging from 75 to 300 mg per day. In another study, diabetic patients with CAD had significantly higher levels of both platelet aggregation and activation compared to non-diabetic patients with CAD despite treatment with the same dosage of ASA [5].

Limitations of the study

The inherent limitations of observational design apply to this study. It may be argued that we did not apply an LTA as a standard method for the platelet activation measurements. Instead we applied the measurement of the serum level of TxB_2 as a good confirmation of adequate compliance to a daily ASA therapy, as well platelet COX-1 dependent TxB_2 synthesis inhibition. Pretreatment measurements could not be conducted because all patients included in this study had diagnosed CAD or multiple risk factors for CAD and therefore were on ASA therapy at the time of enrollment. Finally, it needs to be mentioned here that the results obtained must be interpreted with caution as lack of control group without diabetes do not allow to directly assess the impact of diabetes on ASA pharmacokinetics.

Conclusions

In conclusion, our observational study found that disturbances of pharmacokinetic mechanisms associated with diabetes might contribute to lower SA levels in some patients, and thus incompletely inhibited TxA_2 synthesis measured with S-TxB_2 concentrations and increased platelet reactivity measured with VerifyNow. Further pharmacokinetic and pharmacodynamic studies are needed to investigate the relationship between low platelet inhibition and ASA metabolites in diabetic patients, in particular with concomitant CAD.

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Conflict of interest: none declared

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