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The aspirin resistance and platelets' receptors expression in chronic heart failure patients with type 2 diabetes mellitus

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Aim. Chronic heart failure and type 2 diabetes mellitus are closely inter-related conditions through different pathological mechanisms, including platelet activation. The aim of our study was to estimate the prevalence of aspirin resistance in heart failure patients with type 2 diabetes mellitus and to clarify platelets` abnormalities according to glycemic control status.

Methods and results. We studied 65 patients with heart failure and type 2 diabetes mellitus. Platelet function was evaluated using standard light transmission aggregometry. Measurements of CD41, CD41a, CD61 and CD62P were carried out by fluorescent microscopy study. The plasma levels of soluble P-selectin and soluble CD40L were measured by ELISA assays. Among the studied patients, 23.1 % were aspirin-resistant by ADP-induced platelet aggregation test and 36.1 % after the use of epinephrine-induced platelet aggregation test. The poor glycemic control in patients with chronic heart failure was associated with high plasma P-selectin level and high CD41 and CD61 expression.

Conclusion. The study findings confirm the role of glycemic control status in prevention of thrombotic complications in chronic heart failure patients with type 2 diabetes mellitus.

Аспіринорезистентність та експресія рецепторів тромбоцитів у хворих на хронічну серцеву недостатність із цукровим діабетом 2 типу

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Хронічна серцева недостатність і цукровий діабет 2 типу тісно пов'язані спільними патогенетичними механізмами, включаючи активацію тромбоцитів. З метою вивчення залежності тромбоцитарної дисфункції від контролю глікемії у 65 хворих дослідили стан агрегації тромбоцитів, рівень експресії рецепторів CD41, CD41a, CD61 і CD62P методом імунофлуоресценції. Рівень розчинних Р-селектину та ліганду CD40L визначили у плазмі імуноферментним методом. Встановили, що за даними АДФ-індукованої агрегації тромбоцитів поширеність аспіринорезистентності становить 23,1%, а за результатами адреналін-індукованої агрегації тромбоцитів – 36,1%. У пацієнтів із незадовільним контролем глікемії спостерігали вірогідне збільшення рівня розчинного Р-селектину та експресії рецепторів CD41 та CD61. Це свідчить про важливість контролю глікемії у профілактиці тромботичних ускладнень у хворих на хронічну серцеву недостатність із супутнім цукровим діабетом 2 типу.

Ключові слова: *аспіринорезистентність, хронічна серцева недостатність, активація тромбоцитів, цукровий діабет 2 типу.*

Запорізький медичний журнал. – 2014. – №3 (84). – С. 4–8

Аспиринорезистентность и экспрессия рецепторов тромбоцитов у пациентов с хронической сердечной недостаточностью и сахарным диабетом 2 типа

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Хроническая сердечная недостаточность и сахарный диабет 2 типа тесно связаны между собой общими патогенетическими механизмами, включая активацию тромбоцитов. С целью изучения зависимости тромбоцитарной дисфункции от контроля гликемии у 65 пациентов исследовали процесс агрегации тромбоцитов, уровень экспрессии рецепторов CD41, CD41a, CD61 и CD62P методом иммунофлуоресценции. Уровень растворимых Р-селектина и лиганда CD40L определяли в плазме иммуноферментным методом. Установлено, что по данным АДФ-индуцированной агрегации тромбоцитов распространенность аспиринорезистентности составляет 23,1%, а по результатам адреналин-индуцированной агрегации тромбоцитов – 36,1%. У пациентов с неудовлетворительным контролем гликемии наблюдали достоверное увеличение уровня растворимого Р-селектина и экспрессии рецепторов CD41 и CD61. Это свидетельствует о важности контроля гликемии в профилактике тромботических осложнений у пациентов с хронической сердечной недостаточностью и сопутствующим сахарным диабетом 2 типа.

Ключевые слова: *аспиринорезистентность, хроническая сердечная недостаточность, активация тромбоцитов, сахарный диабет 2 типа.*

Запорожский медицинский журнал. – 2014. – №3 (84). – С. 4–8

Chronic heart failure (CHF) is a complex clinical syndrome associated with high mortality rates [1]. The presence of type 2 diabetes mellitus (DM) is an independent unfavourable prognostic factor in heart failure patients. The prevalence of DM in the world will dramatically increase to 439 million adults by 2030 [2]. CHF and DM are often coexist. Overall prevalence of DM in CHF is approximately 20–25% [3]. Diabetic patients account for 25 % of all patients enrolled in large CHF trials.

DM and CHF are inter-related conditions through complex pathophysiological mechanisms, including platelet activation.

It was shown, that platelet reactivity is high in CHF patients with sinus rhythm despite aspirin use [4,5]. The prognostic role of platelets` disorders in CHF remains unclear [6]. The presence of DM in CHF patients can dramatically increase platelet dysfunction. The problem is much complicated by high level of aspirin resistance in diabetic patients [7]. The prevalence of aspirin resistance in CHF with DM has never been studied. Poor glycemic control is thought to be another important cause of increased platelet reactivity [8]. Hyperglycaemia in diabetes can cause glycation of platelets and fibrinogen [9].
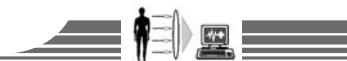
The aim of this study was to estimate the prevalence of aspirin resistance in CHF patients with DM and to clarify platelet function abnormalities according to glycemic control status.

Materials and methods

Study population. The inclusion criteria for study were: a more than 1-year documented history of CHF due to coronary artery disease, sinus rhythm, type 2 diabetes mellitus, no change in medication for > 3 months, daily aspirin use 100 mg > 6 months. All patients had previous history of myocardial infarction. Exclusion criteria were: type 1 DM, patients with type 2 DM on insulin therapy and/or thiazolidinediones, major bleedings in anamnesis, chronic renal failure, chronic obstructive pulmonary disease, autoimmune diseases, significant liver failure, cancer. All patients were Caucasian.

The study protocol was approved by the local Ethics Committee, and all subjects gave written informed consent. The study was conducted in accordance with the Helsinki Declaration.

Laboratory tests. Venous blood samples were taken in the morning following overnight fast and after a supine rest of ≥15 min. Blood samples were taken between 7 and 9 into plasma vacuum tubes containing 7.2 mg di-potassium EDTA. After centrifugation at 1800 x g for 10 min, plasma was collected and frozen at -70 ^{0}C until being investigated.

The plasma glucose and glycated hemoglobin (HbA1$_c$) were measured using the Roche Cobas Integra immunoassay method (Roche, Vienna, Austria). The detection of HbA1$_c$ was certified by the National Glycohemoglobin Standartization Program (NGSP). The optimal glucose control was considered by HbA1c of less than 7%. Insulin resistance was assessed from fasting insulin and glucose levels using homeostasis model assessment (HOMA-IR), thus: HOMA-IR=fasting glucose (mmol/L) x fasting insulin (μU/mL)/22.5.

P-selectin and sCD40L were tested in duplicate using ELISA module sets obtained from Bender MedSystems (Vienna, Austria). Plasma levels of insulin and C-peptide were measured with immunoassays from DRG Diagnostics (Germany). Commercially available assay kit was used for determination of plasma brain natriuretic peptide (BNP) purchased from Biomedica (Austria).

Renal function was expressed as estimated glomerular filtration rate (eGFR, mL/min/1.73 m²) calculated from the Modification in Diet and renal Disease equation [10].

Preparation of washed platelet suspension. The method for isolation of human platelets by centrifugation and washing by Cazenave et al. was used [11]. The procedure was described in details previously [12]. Briefly, the blood from a forearm vein was collected into a conical 15-mL centrifuge tube containing 1 volume of acid-citrate-dextrose (ACD) anticoagulant for 6 volumes of blood (final pH 6.5 and citrate concentration 22 mM). Platelet-rich plasma was prepared by centrifugation at 200 g for 20 minutes. Platelets were then isolated from platelet-rich plasma by centrifugation at 1400 g for 10 minutes in the presence of 50 ng/mL prostaglandin E$_1$ and 10% (v/v) acid citrate/dextrose, pH 4.6, and resuspended at a concentration of $4×10^8$ cells/mL in Tyrode's-HEPES buffer. After three procedures of washing and centrifugation a drop of platelet rich suspension was placed on adhesive slides «Super Frost Plus» from Menzel Gmb & Co

KG (Germany) and the cells were allowed to settle for 20 min at room temperature. The spread cell monolayer was fixed with 100% methanol.

Fluorescent microscopy study. The surface expression of platelet receptors was determined by immunofluorescent technique, using the following monoclonal antibodies against CD41 (alpha IIb integrin, platelet glycoprotein IIb), CD 41a (glycoprotein IIb/IIIa or fibrinogen receptor), CD 61 (beta 3 integrin, platelet glycoprotein IIIa) and CD 62P antigens (P-selectin). All primary antibodies were obtained from Diaclone (France). The FITC-conjugated goat anti-mouse IgG was obtained from Sigma-Chemical (USA). The fixed adherent platelets on slides were transferred onto the primary antibody diluted in a ratio 1:1000 in phosphate-buffered saline, pH 7.4 (PBS). The samples were incubated for 24 hours at + 4°C. After that the platelets were washed multiple times in PBS. Then the samples were transferred to a goat anti-mouse IgG diluted in a ratio 1:64 in PBS. The incubation time was 60 min at +37 ^{0}C. After washing the samples were transferred in the medium containing 90% glycerin and PBS in a ratio 9:1 for fluorescent microscopy investigation. After incubation with antibodies the samples were observed with fluorescence microscope Axioskop (Carl Zeiss, Germany). Carl Zeiss 38HE UW filter (excitation: 470/40 nm; emission: 525/50 nm) was used. The images were recorded with a high-sensitive CCD camera (model COHU-4922, COHU Inc., USA) and were analyzed with automatic digital morphology analysis system VIDAS-386 and VIDAS-2.5 software (Kontron Elektronik, Germany). Microscopic examination was done with a magnification of ×400 (objective: ×40, eye piece: ×10) and oil immersion. Totally 10 microscopic fields were analyzed. Absolute fluorescence was expressed as arbitrary units (pixel units, AU) and represented sum of fluorescence or individual adherent platelet in one defined area.

Platelet aggregation tests. Blood samples were collected in 3.8% sodium citrate tubes in a ratio 9:1 blood to anticoagulant. Whole-blood specimens were centrifuged for 10 min at 200 g to obtain platelet-rich plasma. Platelet-poor plasma was obtained on the remaining specimen by recentrifugation at 2,000 g for 15 min. Platelet count was measured on the platelet-rich plasma and was adjusted to between 200 x $10^3/\mu$l and 300 x $10^3/\mu$l with platelet-poor plasma. Aggregation test was performed with adenosine 5'-diphosphate (ADP) (Sigma, USA) at 10 μM using an optical aggregometer (Solar, Minsk, Belarus). Aspirin resistance was defined by previously reported criteria: ≥ 70% ADP- and ≥ 40% epinephrine-induced aggregation [13,14].

Statistical methods. Statistical analysis was performed using standard commercial software Statistica (Statsoft, Tulsa, USA). All continuous variables were tested for a normal distribution using the Shapiro-Wilk's W test. Continuous variables are presented as mean±standard deviation or median (inter-quartile range) if non-normally distributed. Categorical variables are presented as counts and proportions. All normally distributed parameters were compared using a one-way ANOVA, followed, in case of significance, by a two-side Tukey test for multiple comparisons. Differences in non-normally distributed variables between groups were assessed by Kruskal-Wallis test with *post hoc* Mann-Whitney test. Correlation analysis was performed using Spearman rank correlation.

Results and discussion

Patients' characteristics. During the study period, we prospectively recruited 65 patients with CHF and DM and 35 patients with CHF without DM, whose baseline characteristics are shown in *table 1*.

Baseline characteristics of study population

	Patients with CHF and without DM (n=35)	Patients with CHF and DM with HbA$_{1c}$ < 7 % (n=24)	Patients with CHF and DM with HbA$_{1c}$ > 7 % (n=41)
Age (years)	60±8.6	63.7±8.6	61.7±6.9
Females	10 (29)	11 (46)	9 (22)
BMI (kg·m^{-2}; median and range)	28.3 (26.1–32.5)	30.3 (28.3–31.9)	29.7 (27.4–32)
LFEF(%)	53.1±11.1	49.1±11.8	47.9±12.9
NYHA class, II/III/IV, *n*(%)	22/11/2 (63/31/6)	15/8/1 (63/33/4)	25/14/2 (61/34/5)
eGFR (mL/min/1.73 m^2)	61.4±15.4	58.5±16.1	56.5±10.6
Treated hypertension, *n*(%)	27 (77)*	21 (88)	40 (98)
Cardiovascular treatment			
ACE inhibitors/ARB, *n*(%)	32 (91)	22 (92)	38 (93)
β-blockers, *n*(%)	30 (86)	20 (83)	36 (88)
Spironolactone, *n*(%)	8 (23)	5 (21)	11 (27)
Statins, *n*(%)	31 (89)	20 (83)	34 (83)
Diuretics, *n*(%)	23 (66)	14 (58)	33 (80)
ASA, *n*(%)	35 (100)	24 (100)	41 (100)

NB: CHF, chronic heart failure; DM, type 2 diabetes mellitus; BMI, body mass index; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; eGFR, estimated glomerular filtration rate; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker. Values presented are n (%) or mean ± SD unless otherwise stated. * P<0.05 patients with CHF and decompensated DM vs. patients with CHF without DM.

24 diabetic patients had an HbA1c of less than 7%. Age, sex, severity of CHF, left ventricular ejection fraction (LVEF), estimated glomerular filtration rate and cardiovascular treatment were comparable between three groups. Hypertension rate was slightly frequent in patients with CHF and decompensated DM. The baseline characterisctics of patients related to diabetes are presented in *table 2*.

Baseline characteristics of study population related to diabetes

	Patients with CHF and DM with HbA$_{1c}$ > 7 % (n=41)	Patients with CHF and DM with HbA$_{1c}$ < 7 % (n=24)
Diabetes duration (years; median and range)	3 (1,5–8)	3 (1–5,5)
Laboratory findings		
FPG (mmol/L; median and range)	9.6 (6.4–12.0)	6.1 (4.8–6.8)**
HbA$_{1c}$ (%; median and range)	9.1 (8.0–10.0)	6.1 (5.4–6.3)***
Insulin (mU/mL)	14.5±6.1	14.7±6.4
HOMA-IR (median and range)	5.4 (3.8–7.7)	3.7 (2.5–6.3)*
C-peptide (ng/mL; median and range)	8.8 (5.2–16.3)	5.1 (4.2–10.3)

NB: CHF, chronic heart failure; DM, type 2 diabetes mellitus; FPG, fasting plasma glucose; HbA$_{1c}$, glycated haemoglobin A$_{1c}$; HOMA-IR, Homeostasis Model Assessment – insulin resistance index. Values presented are mean ± SD unless otherwise stated. *P < 0.05; ** P < 0.01; *** P < 0.0001.

Patients with CHF and decompensated DM had higher fasting plasma glucose, HbA$_{1c}$ and HOMA-IR levels. The duration of the diabetes was similar. Glucose lowering treatment on admission was comparable between groups (data not shown).

Aspirin resistance rate. The rate of aspirin resistance was tend to be higher in patients with CHF and DM (by ADP-induced aggregation: 23.1% in diabetic patients vs. 14.3% in non-diabetic patients, P=0.43; by epinephrine-induced aggregation: 36.9% in diabetic patients vs. 31.4% in non-diabetic patients, P=0.66), but the difference was not statistically significant. The compensation of DM didn't influence on aspirin resistance rate also.

Platelet activation markers in CHF patients according to glycemic status. The platelet data are presented in *table 3*. No significant changes in platelets' parameters were observed between CHF patients without DM and patients with CHF and compensated DM.

In contrast, patients with CHF and decompensated DM presented higher platelet surface CD62P, CD41, CD41a and CD61 expression in comparison to CHF patients without DM. Patients with CHF and HbA1$_c$ of more than 7% showed also

Platelet function profile according to glycemic control status

	Patients with CHF and without DM (n=35)	Patients with CHF and compensated DM (n=24)	Patients with CHF and decompensated DM (n=41)
P-selectin (ng/ml)	69±35.4	62.5±28.4	82.3±24.4‡
sCD40L (ng/ml, median and range)	0.53 (0.18–0.86)	0.46 (0.2–1.13)	0.29 (0.18–0.81)
Platelet surface P-selectin expression (arbitrary units, median and range)	1.66 (0.59–2.13)	1.35 (1.11–2.54)	1.88 (1.59–2.63)^ʃ
Platelet surface CD41 expression (arbitrary units, median and range)	1.46 (0.49–2.7)	1.26 (1.0–2.25)	2.45 (1.75–3.08)^{ʃ‡}
Platelet surface CD61 expression (arbitrary units, median and range)	1.41 (0.53–2.23)	1.53 (1.11–2.01)	2.3 (1.53–3.12)^{ʃ‡}
Platelet surface CD41a expression (arbitrary units, median and range)	1.64 (0.61–2.19)	1.76 (1.47–2.19)	2.3 (1.44–2.64)^ʃ
20 μmol/l ADP–LTA (%)	71 ±15	73±13	73±13
5 μmol/l epinephrine–LTA (%)	20 (14–34)	27 (16–52)	23 (13–32)

NB: CHF, chronic heart failure; DM, type 2 diabetes mellitus; sCD40L, soluble CD40 ligand; ADP, adenosine diphosphate; LTA, light transmittance aggregometry. Values presented are mean ± SD unless otherwise stated. ^{ʃ} P<0.05 patients with CHF and decompensated DM vs. patients with CHF without DM; ‡ P<0.05 patients with CHF and decompensated DM vs. patients with CHF and compensated DM.

Correlations between platelet activation markers and glycemic parameters

	FPG		Insulin		HOMA-IR		C-peptide		HbA$_{1c}$	
	ρ	P-value	ρ	P-value	ρ	P-value	ρ	P-value	ρ	P-value
P-selectin	0.216	0.084	0.31	0.012	0.396	0.001	0.347	0.005	0.23	0.061
CD 62P	0.179	0.168	0.17	0.19	0.217	0.092	0.263	0.04	0.185	0.153
CD 41	0.236	0.071	0.037	0.782	0.211	0.108	0.008	0.952	0.471	0.00017
CD 61	0.19	0.149	0.069	0.603	0.226	0.085	0.161	0.224	0.3	0.022
ADP-aggregation	0.065	0.605	0.238	0.057	0.251	0.043	0.107	0.395	0.038	0.746

NB: CHF, chronic heart failure; DM, type 2 diabetes mellitus; FPG, fasting plasma glucose; HbA$_{1c}$, glycated haemoglobin A$_{1c}$; HOMA-IR, homeostasis model assessment – insulin resistance index; CD 62P, P-selectin; CD 41, glycoprotein IIb; CD 61, glycoprotein IIIa.

higher plasma P-selectin and higher platelet surface CD41 and CD61 expression compared with CHF patients with HbA1$_c$ of less than 7%.

Plasma sP-selectin correlated positively with FPG, insulin, C-peptide and HOMA-IR (*table 4*). Platelet surface P-selectin correlated only with C-peptide. Both IIb and IIIa glycoprotein receptors correlated with HbA1$_c$.

Our study was the first where platelet function profiles in specific cohort of patients with CHF and concominant DM on aspirin therapy were estimated. The results showed that in patients with CHF and decompensated DM the platelet activity is high despite aspirin use. Previously it was reported, that patients with CHF have increased platelet activity. In PLUTO-CHF trial 57 % of patients with CHF on aspirin therapy presented high GP IIb/IIIa and P-selectin expression [4]. Gurbel et al. found that platelet activity is heightened in 22% of outpatients with stable heart failure symptoms and is not affected by antecedent aspirin therapy [6].

In recent meta-analysis Krasopoulos et al., who included 2930 patients in 20 studies, it was shown that overall prevalence of aspirin resistance in cardiovascular diseases was approximately 28% and the resistant patients were at a greater risk of clinically important cardiovascular morbidity [15]. In Fateh-Moghadam S. et al. study the prevalence of aspirin resistance was 21.5 % in diabetic patients [7]. The results of our study showed that near one third of CHF patients with DM were aspirin-resistant.

The influence of DM on platelets is multifactorial and complex. The results of BARI 2D substudy suggested that obesity and insulin resistance may influence platelet reactivity in type 2 DM [16]. Increased ADP-stimulated platelet P-selectin expression despite aspirin use was showed in the studied patients. The relationship between insulin resistance and platelet reactivity was of similar magnitude in patients regardless of aspirin use or nonuse. These results confirmed the limited efficacy of aspirin in patients with diabetes. In G.Y. Lip et al. study the plasma level of P-selectin was significantly higher in diabetic patients with cardiovascular disease on aspirin therapy compared to healthy individuals [20]. The reactivity was increased in obese and insulin resistant patients. Potential mechanisms of aspirin resistance in CHF and DM are follow: decreased endothelial nitric oxide production, increased platelet turnover, altered platelet structure as a result of dyslipidaemia and disproportionate increase in intra-platelet calcium concentration [21]. Another possible cause is hypersensitivity of platelets to уADP, which

was shown in *vitro* studies [22]. Hyperglycaemia in diabetes can cause glycation of platelets increasing their reactivity [9].

Use of aspirin in CHF remains complex and controversial problem. Coronary artery disease (CAD) is known to be the primary etiological reason in the majority of patients with CHF. It would be reasonable to expect that aspirin would be beneficial in CHD patients with underlying CAD. Instead, there are some evidence that aspirin is not useful and even possibly harmful. The Warfarin/Aspirin Study in Heart failure (WASH) provides no evidence that aspirin is effective in patients with heart failure compared to the placebo group [17]. In the WATCH study, there was no difference in mortality in aspirin, clopidogrel, and warfarin use [18]. Conversely, the meta-analysis of two studies suggests more hospitalizations for CHF in aspirin group [19]. The potential mechanism is unfavourable interaction between ACE-inhibitors and aspirin.

So, aspirin is not ideal medication for CHF patients with DM and sinus rhythm. From one hand, the results of previous and our studies demonstrated that platelet reactivity remains high in patients on aspirin therapy. From another hand, the WASH and WATCH trials demonstrated that the risk of HF hospitalization was significantly greater in aspirin-treated patients. The use of another antiplatelet agents (clopidogrel) looks potentially beneficial but needs confirmation in clinical trials.

Another finding of our study was the influence of glycemic control on the platelet reactivity. Hyperglycemia accelerates the formation of advanced glycation end products, which are known to cause endothelial dysfunction and thus may be linked to platelet activation in diabetes. Advanced glycation end products induce tissue factor production in human monocytes in vitro and may enhance platelet reactivity [23]. It was reported previously that activation of platelet glycoprotein IIb/IIIa and P-selectin expression was increased similarly after addition of isosmotic concentrations of glucose and mannitol in whole blood samples from diabetic patients [24]. The lower blood thrombogenicity in patients with better glycemic control may have contributed to a reduction in cardiovascular events in these patients. According to the last ESC guidelines for the treatment of CHD metformin should be considered as a first-line agent in overweight patients with type 2 DM and without significant renal dysfunction. Systematic evaluation of the effect of metformin treatment on platelet function is lacking and results are controversial. Decreased sensitivity to platelet-aggregating agents during metformin treatment was demonstrated in Collier et al. study [25]. Metformin

may have platelet stabilizing effects as shown by reduced platelet density and β-thromboglobulin [26]. However, others observed no effect of metformin on spontaneous or ADP-induced platelet aggregation in a placebo-controlled study [27].

Study limitations. A larger sample size may be needed to demonstrate statistically significant difference in aspirin resistance rate between diabetic and non-diabetic CHF patients. The arachidonic acid-induced platelet aggregation was not performed in our study. Finally, urine metabolites of thromboxane were not measured.

Conclusions

Laboratory findings of aspirin resistance are present in CHF patients with DM. The rate of aspirin resistance varies from 23 to 37% depending on tests used.

Poor glycemic control of DM is associated with increased platelet activity in CHF patients.

The perspectives for future research. Antithrombotic therapy with thienopyridines can be alternative strategy for the heart failure patients with type 2 diabetes mellitus. The clinical perspective of this strategy need to be evaluated in future studies.

References

1. Askoxylakis, V., Thieke, C., Pleger, S. T., Most, P., Tanner, J., Lindel, K., et al. (2010) Long-term survival of cancer patients compared to heart failure and stroke: a systematic review. *BMC Cancer,* 10, 105–115. doi:10.1186/1471-2407-10-105.
2. Shaw, J., Sicree, R., & Zimmet, P. (2010) Global estimates of the prevalence of diabetes for 2010 and 2030. *Diab Res and Clin Pract,* 87, 4–14. doi: 10.1016/j.diabres.2009.10.007.
3. Baliga, V., & Sapsford, R. (2009) Diabetes and heart failure: an overview of epidemiology and management. *Diab Vasc Dis Res,* 6,164–171. doi: 10.1177/1479164109338773.
4. Serebruany, V., Malinin, A., Jerome, S., Lowry, D., Morgan, A., Sane, D., et al. (2003) Effects of clopidogrel and aspirin combination versus aspirin alone on platelet aggregation and major receptor expression in patients with heart failure: the Plavix Use for Treatment Of Congestive Heart Failure (PLUTO-CHF) trial. *Am Heart J*, 146, 713–720.
5. Gibbs, C., Blann, A., Watson, R., & Lip, G. (2001) Abnormalities of hemorheological, endothelial, and platelet function in patients with chronic heart failure in sinus rhythm. *Circulation*, 103, 1746–1751.
6. Gurbel, P., Gattis, W., Fuzaylov, S., Gaulden, L., Hasselblad, V., Serebruany, V., & O`Connor, C. (2002) Evaluation of platelets in heart failure: is platelet activity related to etiology, functional class, or clinical outcomes? *Am Heart J*, 143, 1068–1075.
7. Fateh-Moghadam, S., Plöckinger, N., & Cabeza, S. (2005) Prevalance of aspirin resistance in patients with type 2 diabetes. *Acta Diabetol,* 42, 99–103. doi:10.1007/s00592-005-0186-y.
8. Ferroni, P., Basili, S., Falco, A., & Davi, G. (2004) Platelet activation in type 2 diabetes mellitus. *J Thromb Haemost,* 2, 1282–1291. doi: 10.1111/j.1538-7836.2004.00836.x.
9. Dunn, E., Ariens, R., & Grant, P. (2005) The influence of type 2 diabetes on fibrin structure and function. *Diabetologia,* 48, 1198–1206. doi: 10.1007/s00125-005-1742-2.
10. Levey, A. S., Bosch, J. P., Lewis, J. B, Greene, T, Rogers, N., & Roth, D. (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med,* 130, 461–470.
11. Cazenave, J., Hemmendinger, S., Beretz, A., Sutter-Bay, A., Launay, J. (1983) Platelet aggregation: a tool for clinical investigation and pharmacological study. *Methodology Ann Biol Clin,* 41, 167–179.
12. Gibbins, J. M., & Mahaut-Smith, M. P. (2004) Preparation of washed platelet suspensions from human and rodent blood. *Platelets and megakaryocytes.* J. M. Walker (Ed.). Totowa ; New Jersey: Humana Press, (p. 13–28).
13. Gum, P., Kottke-Marchant, K., Welsh, P., White, J., & Topol, E. (2003) A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. *J Am Coll Cardiol,* 41, 961–965. doi:10.1016/S0735-1097(02)03014-0.
14. Stef, G., Csiszar, A., Lerea, K., Ungvari, Z., & Veress, G. (2006) Resveratrol inhibits aggregation of platelets from high-risk cardiac patients with aspirin resistance. *J Cardiovasc Pharmacol,* 48, 1–5.
15. Krasopoulos, G., Brister, S. J., Beattie, W. S., & Buchanan, M. R. (2008) Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. *BMJ,* 336, 195 doi:10.1136/bmj.39430.529549.BE.
16. Schneider, D. J., Hardison, R. M., Neuza, L., Sobel, B. E., & Brooks, M. M. (2009) Association between platelet P-selectin expression and obesity in patients with type 2 diabetes. *Diabetes Care*, 32, 944–949.
17. Cleland, J. G., Findlay, I., Jafri, S., Sutton, G., Falk, R., Bulpitt, C., et al. (2004). The Warfarin/Aspirin Study in Heart Failure (WASH): a randomized trial comparing antithrombotic strategies for patients with heart failure. *Am Heart J,* 148, 157–164.
18. Massie, B. M., Collins, J. F., Ammon, S. E., Armstrong, P. W., Cleland, J. G. F., Ezekowitz, M., et al. (2009) The Warfarin and antiplatelet therapy in chronic heart failure (WATCH) trial. *Circulation,* 119, 1616–1624.
19. Cleland, J. G. F., Ghosh, J., Freemantle, N., Kaye, G. C., Nasir, M., Clark, A. L., & Coletta, A. P. (2004) Clinical trials update and cumulative meta-analyses from the American College of Cardiology: WATCH, SCD-HeFT, DINAMIT, CASINO, INSPIRE, STRATUS-US, RIO-LIPIDS and cardiac resynchronisation therapy in heart failure. *Eur J Heart Fail,* 6, 501–508. doi:10.1016/j.ejheart.2004.04.014.
20. Lim, H. S., Blann, A. D., & Lip, G. Y. H. (2004) Soluble CD40 ligand, soluble P-selectin, interleukin-6, and tissue factor in diabetes mellitus. *Circulation,* 109, 2524–2528. doi: 10.1161/01.CIR.0000129773.70647.94.
21. Ajjan, R., & Grant, P. J. (2006) Coagulation and atherothrombotic disease. *Atherosclerosis,* 186, 240–259.
22. Macchi, L., Christiaens, L., & Severine, B. S. (2002) Resistance to aspirin in vitro is associated with increased platelet sensitivity to adenosine diphosphate. *Thromb Res,* 107, 45–49.
23. Osende, J. I., Badimon, J. J., Fuster, V., Herson, P., Rabito, P., Vidhun, R., et al. (2001) Blood thrombogenicity in type 2 diabetes mellitus patients is associated with glycemic control. *J Am Coll Cardiol ,* 38, 1307–1312. doi:10.1016/S0735-1097(01)01555-8.
24. Keating, F. K., Sobel, B. E., & Schneider, D. J. (2003) Effects of increased concentrations of glucose on platelet reactivity in healthy subjects and in patients with and without diabetes mellitus. *Am J Cardiol*, 92, 1362–1365.
25. Collier, A., Watson, H. H., Patrick, A. W., Ludlam, C. A., & Clarke, B. F. (1989) Effect of glycaemic control, metformin and gliclazide on platelet density and aggregability in recently diagnosed type 2 (non-insulin-dependent) diabetic patients. *Diabete Metab,* 15, 420–425.
26. Gregorio, F., Ambrosi, F., Manfrini, S., Velussi, M., Carle, F., & Testa, R. (1999) Poorly controlled elderly type 2 diabetic patients: the effects of increasing sulphonylurea dosages or adding metformin. *Diabet Med*, 16, 1016–1024.
27. 27. Nagi, D. K., & Yudkin, J. S. (1993) Effects of metformin on insulin resistance, risk factors for cardiovascular disease, and plasminogen activator inhibitor in NIDDM subjects. A study of two ethnic groups. *Diabetes Care*, 16, 621–629.

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